Neural stem cells plasticity and differentiation

Hakima Flici

▶ **To cite this version:**

Hakima Flici. Neural stem cells plasticity and differentiation. Cellular Biology. Université de Strasbourg, 2012. English. NNT : 2012STRAJ118 . tel-01070644

HAL Id: tel-01070644
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UNIVERSITÉ DE STRASBOURG

ÉCOLE DOCTORALE DES SCIENCES DE LA VIE ET DE LA SANTE

Institut de Génétique et de Biologie Moléculaire et Cellulaire

THÈSE présentée par :

Hakima FLICI

soutenue le : **21 Septembre 2012**

pour obtenir le grade de : **Docteur de l'université de Strasbourg**

Discipline/ Spécialité : Biologie du développement

Différenciation et plasticité des cellules souches neurales

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Dedicated to my brother, Sofiane

ACKNOWLEDGMENTS

I would first like to thank my committee members, Prof. **GONZALEZ Cayetano**, Prof. **REICHERT Heinrich**, Prof. **IMLER Jean-Luc**, and Prof. **DAVIDSON Erwin** for their kindness and also for accepting to evaluate my thesis report.

I would also like to thank my advisor, Dr. **Angela Giangrande**, for giving me the opportunity to join her group at IGBM, where I have gained both scientific rigour and the ability to experimentally put my ideas into practice, and for her kind guidance and support.

I am thankful to the directors of IGBMC, **Pr. MORAS Dino** and **Pr. POURQUIE Olivier** for offering me the chance to be part of IGBMC community.

I also thank CERBM GIE and FRM for their financial support and the confidence they have given to my project.

I am grateful for the dedication of **Dr. Pietro LANEVE**. I have highly appreciated your near unrestricted availability and have greatly enjoyed our stimulating discussions. Oh, Pietro, they're no words to say thanks for you. You were and you will be forever a precious example for me, giving without waiting for recompense. Many thanks for correcting the whole manuscript of my thesis, and for your continuous support.

I would also like to thank my first coach **Dr. Orban KOMONIYI**. You were a great teacher. I have highly appreciated working and discussing with you.

Thanks to the current and previous members of Giangrande lab: Orbán KOMONYI, Pietro LANEVE, Damiano PORCELLI, Sára BERZSÉNYI, Arun KUMAR, Ömer Faruk KARATAŞ, Francesca TAGLINI, Tripti GUPTA, Anna POPKOVA, Giulia PAPALINI, Céline DIEBOLD, Carole MURA, Iren KEREKES, Guillaume TREBUCHET, and Giuseppe D'AGOSTINO, H. Bahar SAHIN, Yoshihiro YUASA, Chethan REDDY. It was wonderful to work in a lab with people who are willing to help each other out, and even collect virgins for you on Saturday.

I am thankful for the help of Dr. Leila LAREDJ and Dr. Muhammad SHUAIB for helping me to correct the manuscript of my thesis.

Claude DELAPORTE and Monica BALLARINO for being helpful, lovely and humorous.

I would like to thank all the members with whom I enjoyed to share the fly room: Nadine BROGAST, Gabriella SCATA and Claudine ACKERMANN. Thanks also to Rihab BOUCHAREB and Khalid OUARARHNI for long discussions about science and the joys of life.

For research support, I am indebted to all the members of the imaging facility, Marcel BOEGLIN, Marc KOCH, Yves LUTZ, Jean-Daniel FAUNY, Didier HENTSCH, Pascal KESSLER , Jean-Luc VONESCH, without whom I would not have able to perform my acquisitions. Similarly, the members of fly facility Nadine and Holly.

Furthermore, I would like to thank IGBMC Administration team for providing guidance and support over the course of my PhD.

It is impossible to advance alone, in research as elsewhere. A big thank to everyone who helped me during this period of my life.

Jelina, It was a great pleasure to share three continuous months behind you,… Keep in touch girl.

I would not have been able to complete this work without the emotional support of my family and friends, who closely shared both the frustrations and joys over the course of this work, but always provided support and strong encouragement, for which I am very grateful. Thank you Mom, Dad, Sofiane, Samir, Souad, Zineb, Dahmane, Ferrodja and Samy for your unending love and support.

I would also like to thank all my friends and family that I didn’t mention.

Finally, as we say in fresh “On garde toujours le meilleur pour la fin” So, thank you Aziz, for your love and continuous support.

Dear Jocelyne “Ma grenobloise”, many thanks for your permanent support.

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PAPER II: H. Flici and A. Giangrande (2012). Stem cell aging and plasticity in the *Drosophila* nervous system. **Landes Bioscience**, Fly, Volume 6, Issue 2.

PAPER III: H. Flici, O. Komonyi, P. Laneve, S. Berzsenyi, A. Giangrande. The homeobox transcription factor Repo collaborates with the histone acetyl transferase CBP to regulate the initiation and the maintenance of the glial fate and the cell fate choice of neuronal progenitors. **In preparation**.

PAPER IV: P. Laneve, C. Delaporte, G. Trebuchet, O. Komonyi, **H. Flici,** A. Popkova, G. D'Agostino, F. Taglini, I. Kerekes and A. Giangrande. The Gcm/Glide protein visualized at last: novel hints on its metabolism and a marker for novel cell type. **Submitted to Developmental Biology.**

ABBREVIATIONS

A

A: abdominal

AbdA: Abdominal-A

AbdB: Abdominal-B

Acetyl-CoA: acetyl-coenzyme A

AD: transactivation domain

Ada: transcriptional ADAptor

Ago: Archipelago

Ago1: Argonaute-1

Akt: protein kinase B, PKB

A/P: Anterior/Posterior

Ascl1: Achaete-scute complex-like 1

ATAC: Ada two A containing complex

ATP: Adenosine triphosphate

ATXN: ataxin

B

β-gal: β-galactosidase

bHLH: basic Helix Loop Helix

BMP4: Bone Morphogenic Protein 4

Brm: Brahma

BTB: Bric-à-brac Tramtrack Broad

CBP FL: CBP full-length

CBP FLAD: CBP full-length acetyltransferase deficient

C

CARM1: coactivator-associated arginine methyltransferase 1

Cas: Castor

CB: central brain

CBG: cell body glia

CBP: cAMP response-element binding protein

Cdc25: cell division cycle 25

Cdk2: Cyclin dependent kinase 2

CG: channel glia

CNS: central nervous system

CoREST: co-repressor for element-1 silencing transcription factor

CREB: cAMPresponse element-binding

D

DAPI: 4',6-diamidino-2-phenylindole

DBD: DNA binding domain

dILP: *Drosophila* insulin-like peptides

dInR: *Drosophila* insulin receptor

Dlx2: distal-less homeobox 2

DNA: deoxyribonucleic acid

Dpn: Deadpan

Dpp: Decapentaplegic

E

EED: embryonic ectoderm development

E(z): Enhancer of zeste

Eg: Eagle

EGFR: epidermal growth factor receptor

Elav: Embryonic lethal, abnormal vision
ELP3: Elongation Protein 3
EMS: methanesulfonate
En: Engrailed
ETS: erythroblast transformation specific
ESC: Embryonic stem cell
Esc: Extra sex combs
EZH2: enhancer of zeste homolog 2

F

FACS: fluorescence activated cell sorting
FGF-2: fibroblast growth factor-2

G

Gal4/80: GALactose metabolism 4/80
GB: glioblast
GBS: Gcm binding site
Gcm/Glide: Glial cells missing/glial cell deficient
Gcn5/PCAF: General control nonderepressible 5/p300/CBP associated factor
GFAP: glial fibrillary acidic protein
GFP: Green fluorescent protein
Gcm/Glide: Glial Cell Missing/Glial Cell Deficiency
GLAST: Glutamate astrocyte-specific transporter
GMC: ganglion mother cell
GNAT: Gcn5-related N-Acetyltransferase
Grh: Grainyhead

H

HAT: Histone acetyl-transferase
HBO1: histone Acetyltransferase Binding to ORC-1
HD: Huntington's disease
HDAC: Histone deacetylase
Hkb: Huckebein
HMT: histone methyltransferase
Hox: Homeobox
Hkb: Hunchback
HTLV-1: Human T cell leukemia/lymphoma virus type 1

I

IE: instability element
IG: interface glia
IGF: insulin-like growth factor
IgG: Immunolglobulin G
IHC: immunohistochemistry
INP: intermediate neural progenitor
IP: Immunoprecipitation
IPC: intermediate progenitor cell
ISN: intersegmental nerve
ISNG: intersegmental and segmental nerve root glia
Iswi: Imitation Switch

J

JmjC: Jumanji C domain

K

K: lysine
Kr: Kruppel

L

LG: longitudinal glia

LGB: longitudinal glioblast

Loco: Locomotion defects

LSD1: Lysine specific demethylase 1

Lz: Lozange

M

MAM: methyl methacrylate

MAP2: microtubule-associated protein 2

MB: mushroom bodie

MEF2: Myocyte enhancing factor 2

MG: midline glia

Mira: Miranda

MLL: Mixed-lineage leukemia

MOF: Male absent on the first

MORF: MOZ-related factor

MOZ: Monocytic leukemia zinc finger protein

mRNA: messenger ribonucleic acid

Myc: v-myc myelocytomatosis viral oncogene homolog

MYST: MOZ/YBF2/SAS2/Tip60 NCoR: Nuclear receptor co-repressor

N

NAD: nicotinamide adenine dinucleotide

NAP1: nucleosome assembly protein 1

NB: neuroblast

Neuro: Neurogenin

N-COR: Nuclear receptor corepressors

NGB: neuroglioblasts

NLS: nuclear localization signal

NRG: nerve root glia

NS: nervous system

NSC: Neural stem cell

NuRD: Nucleosome remodeling and deacetylase

O

OL: optic lobe

P

P300: protein of 300 kDa

PAF400: 400kDa PCAF associated factor (synonym of TRRAP)

Par-6: Partition-Defective 6

Pc: Polycomb

PC4: Positive coactivator 4

PcG: Polycomb group

PEST sequence: proline, glutamic acid, serine, threonine rich sequence

Ph: Polyhomeotic

PH3: phospho-Histone H3-Ser10

Pho: Polyhmomeotic

PHD: Plant Homeo Domain

Pho: Pleiohomeotic

PI3K: phosphatidylinositol 3-kinase-Akt

PIWI: P-element induced wimpy testis

PKMT: lysine HMT

PNS: peripheral nervous system

Pnt: Pointed

PolyQ: poly-glutamine

PRC1: Polycomb repressor complex 1

PRC2: Polycomb repressor complex 2

PRMT: arginine HMT

Psc: Posterior sex combs

Q

qPCR: quantitative polymerase chain reaction

R

R: arginine

Rb: Retinoblastoma protein

REST/NRSF: RE1 silencing TF/neural-restrictive silencing factor

RHG: Reaper Hid, and/or Grim

Repo: Reversed polarity

RFP: Red fluorescent protein

RG: radial glia

RNA Pol II: RNA Polymerase II

RNA: ribonucleic acid

RNAi: RNA interference

Rpd3: Reduced potassium dependency 3

Rpr: Reaper Hid, and/or Grim

RTH: Rubinstein-Taybi

S

S: serine

SAGA: Spt-Ada-Gcn5-acetyl-transferase

Sbf1: SET binding factor 1

SC: Stem cell

Sca: scabrous

Sce: sex combs extra

SET domain: Su(var)39, E(z) and trx domain

SGZ: subgranular zone

Sin3: Switch independent

Sir2: Silent information regulator 2

SIRT: Sirtuin type protein

Slimb: Supernumerary limbs

SMRT: silencing mediator for retinoid and thyroid receptors

Sog: Short gastrulation

SOP: sensory organs precursor

SPG: subperineurial glia

Stg: String

STAT: Signal Transducer and Activator of Transcription

SVZ: subventricular zone

Su(z)12/SUZ12: suppressor of zeste 12

T

T: thoracic

T: threonine

TAFII250 : TFIID 250 kDa

TBP: TATA binding protein

TF: transcription factor

TFIIB: Transcription factor II D

Tip60: HIV-1 Tat interacting protein

TM3: Third multiple #3

TOR: target of paramycin

Trx: Trithorax

TrxG: Trithorax group

Ts: temperature sensitive

Ttk: Tramtrack

Tyr: Tyrosine

U

UAS: Uprtream activating sequence

Ubx: Ubithorax

UTR: Untranslated Region

V

VNC: ventral nerve cord

VPA: valproic acid

VUM: Ventral Unpaired Median

VZ: ventricular zone

W

wg: wingless

YBF2/SAS3: Yeast binding factor 2/Something about silencing 3

Z

Zn: zinc

ABSTRACTS

1. En Français/ In French

Différenciation et plasticité des cellules souches neurales

Le cerveau humain est un tissu en renouvellement constant où l'équilibre entre les cellules nouvellement formées et les cellules perdues est maintenu par la division asymétrique des cellules souches neurales (CSN), appelées aussi neuroblastes (NBs), produisant deux cellules filles différentes : l'une possédant la capacité de s'auto-renouveler et l'autre engagée dans la différenciation en neurones et en cellules gliales. Aujourd'hui, le défi fondamental de la neurobiologie est de comprendre la plasticité des CSNs, afin d'expliquer les mécanismes conduisant ces cellules à générer à la fois des neurones et des cellules gliales sains, mais aussi des cellules à identité ambiguë, pathologique. Ceci est particulièrement important lorsque l'on considère la réparation du système nerveux après une lésion ou une maladie en faisant appel aux cellules souches.

Mon projet de thèse porte sur la compréhension des mécanismes clés contrôlant l'élaboration du destin gliale et du destin neuronale *in vivo,* en utilisant comme modèle les CSNs de la corde ventrale de la drosophile (l'équivalent de la moelle épinière chez les vertébrés). Chez ce modèle, le facteur de transcription Glide/Gcm constitue le déterminant glial: son expression ectopique dans les précurseurs neunaux induit de la glie ectopique tandis que son absence induit la glie à adopter le destin neuronal. En utilisant le potentiel gliogénique de ce facteur dans les CSNs, nous avons déterminé plusieurs nouveaux processus impliqués dans la plasticité des CSNs.

Nous avons tous d'abord montré que les précurseurs neunaux peuvent être complètement redirigés pour adopter le destin glial et que cette conversion est stable et complète. Le processus de conversion du destin ne se manifeste pas uniquement par l'expression de marqueurs gliaux mais aussi par des changements spécifiques au niveau de la chromatine. En effet, durant nos analyses nous avons identifié le niveau d'acétylation de l'histone H3 au niveau de la lysine 9 (H3K9ac) comme une nouvelle marque qui distingue les neurones des cellules gliales, les neurones ont des niveaux élevés en H3K9ac contrairement au cellules gliales qui ont des niveau faibles de cette marque épigénétique. D'une manière intéressante, les cellules gliales ectopiques, qui se différencient normalement en neurones, ont

aussi des niveaux faibles en H3K9ac. En analysant le déroulement de la gliogenèse ectopique, nous avons aussi pu montrer que l'établissement du destin glial passe par une étape intermédiaire où les marqueurs de neuroblastes et les marqueurs gliaux sont co-exprimés.

L'on considère les cellules souches comme des précurseurs pouvant se diviser de manière indéfinie *in vitro*. Toutefois, *in vivo*, ces cellules prolifèrent activement lors du développement, puis entrent dans une phase quiescente ou dans un programme apoptotique chez l'adulte. Par des expériences d'expression conditionnelle dans des lignages de CSNs identifiés, nous avons pu montrer que la capacité des CSNs à se convertir en glie après expression forcée de Glide/Gcm décline avec l'âge et que lors de l'entrée en phase quiescente ou apoptotique, elles ne peuvent plus être converties. D'une manière surprenante, nous avons également montré que l'expression ectopique de Glide/Gcm dans les neurones post-mitotiques n'induit pas un changement du destin mais de l'apoptose, alors que les CSNs dont la division a été bloquée peuvent être converties.

Glide/Gcm est le facteur initiateur du destin glial par sa présence transitoire dans tous les précurseurs gliaux, par contre les mécanismes contrôlant sa cascade moléculaire reste fragmentaire. Nos analyses ont permis d'identifier de nouveaux facteurs impliqués dans ce processus et de comprendre comment ces facteurs peuvent affecter le destin des précurseurs générés par les CSNs. Il est connu que Glide/Gcm est capable d'induire l'expression de son propre gène et celui de sa cible directe, *repo*. A ceci nous avons rajouté une nouvelle voie qui est le pouvoir de Repo à induire l'expression de *glide/gcm*. D'une manière intéressante, nous avons aussi montré que la stabilité de la protéine Glide/Gcm est contrôlée par deux voies opposées, où Repo et dCBP (pour *Drosophila* Creb Binding Protein) jouent un rôle majeur. La première voie implique dCBP qui stabilise Glide/Gcm, induisant ainsi l'expression de ces cibles directes, y inclus *glide/gcm* et *repo*. La deuxième voie implique à la fois Repo et dCBP qui, ensemble, s'opposent à la progression de la première boucle en induisant la dégradation de Glide/Gcm. Ces voies suggèrent que la modulation quantitative de facteurs clés est cruciale, à des temps de développement précis, afin de garantir un destin glial complet. En déséquilibrant l'activité de ces facteurs, les cellules restent dans un état intermédiaire, où le destin glial et le destin neuronal se manifestent au même temps. Nous avons aussi pu montrer que cet état intermédiaire existe transitoirement dans les conditions physiologiques.

Nous pensons que l'établissement d'un destin cellulaire déclenche simultanément des voies opposées et que l'intégration des différents partenaires de ces voies est réalisée à des moments différents afin d'assurer l'initiation et la maintenance d'un destin cellulaire défini.

Un tel mécanisme pourrait être largement utilisé dans différentes cellules souches pour générer des types cellulaires distincts durant le développement.

Mots clés: Cellule souche neurale, Gcm, gliogenèse, CBP, Repo, choix du destin cellulaire.

2. En Anglais/In English

Neural stem cells plasticity and differentiation

The human brain is a tissue in constant renewal where the balance between newly formed and lost cells is maintained by the asymmetric division of neural stem cells (NSCs), also called neuroblasts (NBs) in flies, producing two different daughter cells: one having the ability to self-renew and the other engaged in the differentiation into several types of neurons and glial cells. Today, the fundamental challenge of neurobiology is to understand the plasticity of NSCs in order to explain the mechanisms leading these cells to generate both neurons and glia, and to clarify how in some cases the same cells could differentiate into pathologic cells. This is particularly important when considering the nervous system repair after injury or disease using stem cells.

My PhD project aims to unrevel key mechanisms underlying NSC plasticity *in vivo*, using as a model the *Drosophila* embryonic nerve cord (the equivalent of spinal cord in vertebrates). In this model, the Glial cell missing (Gcm/Glide) transcription factor constitutes the glial fate determinant; its overexpression induces ectopic glia while its loss forces glial precursors to adopt the neuronal fate. Using the gliogenic potential of Gcm/Glide in NSCs, we have identified several novel processes implicated in NSC plasticity.

We find that forced Gcm expression could completely redirected neural precursors toward the glial fate and that this conversion is stable and complete. This process of cell conversion is not only manifested by the expression of glial specific markers but also by specific changes at the chromatic level. Indeed, during our analysis we have identified the levels of acetylated histone H3 at lysine 9 residue (H3K9ac) as a novel mark to distinguish neurons from glia: neurons have high levels of H3K9ac compared to glia. Interestingly, ectopic glial cells that normally differentiate into neurons have also low levels of this epigenetic mark. Following the process of ectopic gliogenesis, we have also demonstrated that cell differentiation passes through an intermediate state where NSC markers are co-expressed with glial specific markers before glial fate consolidation.

The stem cells are considered as precursors that can divide indefinitely *in vitro*. However, *in vivo*, these cells actively proliferate during development then enter apoptotic or quiescent programs in the adult. Taking in consideration these two issues, we have demonstrated that NSC plasticity is age dependent as young NSCs are more competent to be converted into glia *via* Gcm than the old ones. In the same context, we have also shown that quiescent or apoptotic NSCs are not competent to be converted into glia. Surprisingly, our

investigations allowed us to show that the ectopic expression of Glide/Gcm in post-mitotic neurons does not induce a cell fate change but apoptosis, while NSCs whose division is blocked can be converted.

Gcm/Glide is the initiating factor of glial fate establishment by its transient presence in all glial precursors, however the molecular cascade that controls *gcm*/Gcm output is poorly understood. Our studies allowed us to identify new factors involved in Gcm molecular cascade control, and how these factors may affect the fate of NSCs. It is known that Gcm/Glide is able to induce the expression of its own gene and that of its direct target, *repo*. To this we added a new pathway that is the power of Repo to induce *gcm/glide* expression. Remarkably, we also showed that the stability of Gcm/Glide protein is controlled by two interlocked and opposing pathways, where Repo and dCBP (for *Drosophila* Creb Binding Protein) play a major role. The first track involves dCBP which stabilizes Gcm/Glide thereby inducing the expression of its direct targets, including *gcm/glide* and *repo*. The second path involves both Repo and dCBP, which together oppose the progress of the first loop by inducing Gcm/Glide degradation. These pathways suggest that the quantitative modulation of key factors is crucial, at precise developmental timing, to ensure a complete glial fate establishment. By disrupting the activity of these factors, the cells remain in an intermediate state, where the glial and neuronal markers are co-expressed. We also revealed that this intermediate state exists transiently during physiological conditions.

We speculate that cell fate choice between neurons and glia simultaneously triggeres opposing feedback loops, and the integration of different partners at different timings to control cell fate initiation and maintenance. Such mechanism is likely widely used throughout the different stem cell to generate cell type diversity in different tissues.

Key words: Neural stem cells, Gcm/Gide, CBP, Repo, cell fate choice.

INTRODUCTION

INTRODUCTION

1. The fundamental questions in developmental biology

According to Aristotle, the first embryologist known to history, science begins with curiosity: "It is owing to wonder that people began to philosophize, and wonder remains the beginning of knowledge". The development of an animal from a fertilized egg was a source of curiosity since antiquity. Therefore one of the fundamental issues in developmental biology was how does a single cell, the zygote give rise to a vast amount of different cell types (Figure 1). Developmental studies over the last few years have provided us with an understanding of cell families. The belief was that fertilized egg is a very plastic cell which divides and gives rise to embryonic stem cells (ESCs) that can progress in one direction along differentiation pathways, from totipotent, pluripotent or multipotent stem cells (SCs) to more differentiated cells: during this process cells became less and less plastic and somatic cell lineages do not differentiate across different embryonic-derived somatic lineages. Developmental biology is not limited to the study of the process by which a multicellular organism develops from one cell into an adult, but also to the mechanisms controlling cell replacement in adults, after injury or cell death.

Since all cells of a given organism contain the same DNA we need to understand how this same set of genetic instructions is regulated to produce this cell diversity and how cell plasticity is regulated. In addition to maternal and zygotic signaling molecules that initiate regulatory cascades, it is transcription factors (TFs) that act as switches to turn on or off gene expression or even modulate the precise expression output of a gene. Combinatorial control by multiple TFs working in performance can also confer cell type-specific regulation of target genes to produce specialized cell types. Interactions between multiple TFs at shared target genes result in complex gene regulatory networks, but understanding and unraveling these networks will help in understanding how different cell types arise and perhaps further the ability to direct cells to adopt a given fate. Towards this aim the basic question I have addressed in this study is how unique cell type, neural stem cell (NSCs) or neuroblast (NB), is specified during the construction of the embryonic central nervous system (CNS) of *Drosophila melanogaster*. Understanding NSC plasticity is of fundamental importance, not only for grasping development itself, but also for comprehending the pathogenesis of neurodevelopmental diseases, the initiation of neural tumors, and the therapeutic potential of

SCs. This is particularly important when considering the repair and regeneration of the nervous system (NS) after damage.

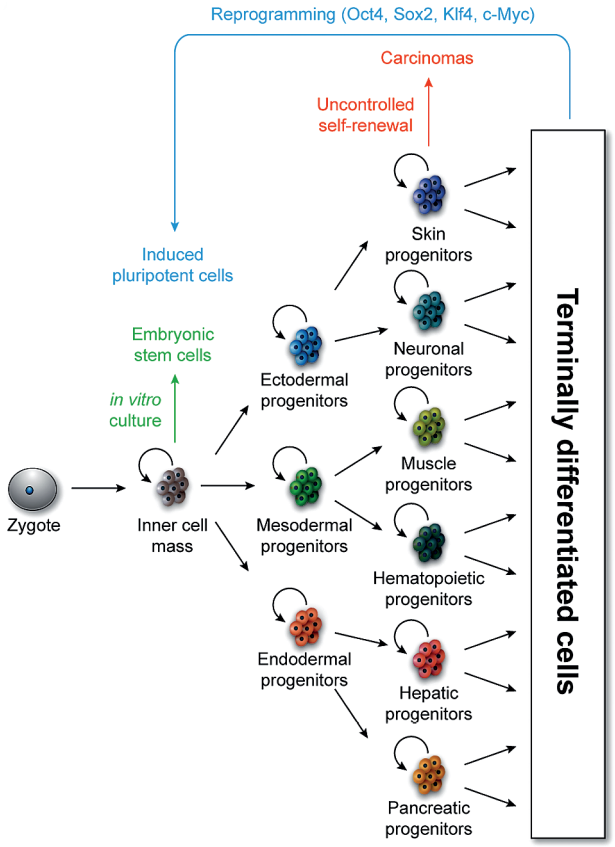

Figure 1. Progenitor cell self-renewal and differentiation contribute to tissue patterning and tumorigenesis.

Schematic representation of the self-renewal and differentiation of progenitor cells, in both normal and oncogenic contexts. Depicted derivatives of the primary germ layers are illustrative rather than inclusive (Ari J. Firestone and James K. Chen, 2009).

2. Cell plasticity

In this section I will discuss successively the concept of cell plasticity and the innovative role of *Drosophila* NSCs in cell plasticity research.

2. 1. General aspects on cell plasticity

In general, cell plasticity refers to the ability of a cell to change its properties in response to intrinsic or extrinsic variations and the interactions between the two in order to generate cells with new properties. This process may be beneficial during development or when considering replacement of cells after injury, or problematic when taking in account their ability to generate cells with pathologic features. This concept is a necessary overall parameter in the definition of SC, which refers to the balance between self-renewal and differentiation but also to the ability of producing different cell populations (Lemischka, 2002).

The classical paradigm of cell plasticity, as described above, holds that all the cell lineages emerge from the most plastic cell known until now, the zygote, which gives rise to pluripotent cells during embryogenesis, and progressively to more restricted cells, in turn giving rise to the specialized cells of the different organs and tissues (Figure 1). In many tissues, self-renewing multipotent SCs are maintained in the adult and serve to replace cells that have a limited life span or to regenerate cells after injury or cell death (Sell, 2004). Such SCs were believed to be restricted in their potential, and limited to generate the types of cells present in the tissue. Through these investigations the family tree for the generation of the major classes of cells in the body are provided. Thus it appears that plasticity is lost progressively as development proceeds and the family trees of cells progress in one direction along these differentiation pathways and to be unable to switch tracks.

Cell plasticity

Dedifferentiation: it involves a terminally differentiated cell reverting back to less differentiated stage from within its own lineage, allowing the cell to proliferate again before re-differentiating, leading to the replacement of those cells that have been lost

Transdifferentiation: this process sees cells regressing to a point where they can switch lineages, allowing them to differentiate into another cell type.

Cell fate conversion: consists the changing the fate of stem cells or their progeny from one fate to another

Reprogramming: aims to induce differentiated cells into reverting to pluripotency. From here, they can differentiate into almost any cell type.

Specification: changes involved in the progressive diversification of the structure and function of cells. It concerns the acquisition of the characteristics that allow different cell types to perform their functions.

2. 2. Cell fate determinants and cell plasticity

The notion of progressive limitation of cell plasticity with development became completely blasphemous, when the role of TFs in lineage specification emerged, starting from 1980s, when Pr. Harold Weintraub's and colleagues discovered that forced expression of MyoD, a TF that determines muscle cell fate, can induce myotube formation in a fibroblast cell line (Davis et al., 1987). Subsequently, cell fusion, consisting the combination of several uninuclear cells to form a multinuclear cell, and nuclear transfer, involving the injection of a defined nucleus in a cell lacking its own nucleus, have shown that the epigenome of differentiated cells can be remarkably plastic (Blau, 1989; Gurdon and Byrne, 2003; Gurdon and Melton, 2008; Hochedlinger and Jaenisch, 2002; Wilmut et al., 1997). Today, cell reprogramming can be obtained simply through ectopic expression or lose of function of defined TFs, known as cell fate determinants or master gene regulators (Davis et al., 1987; Graf and Enver, 2009; Kulessa et al., 1995). Using this strategy, the identity of differentiated cells can be fully reversed and it even makes it possible to produce pluripotent SCs-like from fully differentiated cells "reprogramming", by simply expressing a cocktail of defined TFs (Takahashi and Yamanaka, 2006).

The instructive role of TFs in lineage specification came from the diversity of SC progenitors; the best-studied example is provided by the hematopoietic SCs and their progenitors. For example, the overexpression of the erythroid-megacaryocyte-affiliate TF GATA1 forces macrophage precursors to express erythroid-megacaryocyte lineage markers and to repress the macrophage ones (Kulessa et al., 1995; Visvader et al., 1992). Conversely, the ectopic expression of PU.1 in an erythroid-megacaryocyte cell line induced its conversion into the monocytic lineage (Nerlov and Graf, 1998). The impact of TFs on lineage specification was also demonstrated in the NSCs progenitors. In invertebrates as well as in vertebrates, distinct types of neural progenitor cells generate neurons and glial cells, and in some cases common precursors are shared between the two differentiated cells (Fietz and Huttner, 2011; Fietz et al., 2010; Gotz and Huttner, 2005; Hansen et al., 2010; Lui et al., 2011; Reillo et al., 2011). Interestingly, a single TF specifying a given neural progenitor cell identity can also convert a defined type of neuronal progenitor cell from one type into another. Indeed, the misexpression of T-brain gene-2 forces radial glial (RG) cells, NSCs-like, to produce another type of progenitors, intermediate progenitor cells (IPCs) (Farkas et al., 2008; Sessa et al., 2008). In *Drosophila* CNS, my model of study, one TF Glial Cell Missing/Glial Cell Deficiency (Gcm/Glide, Gcm in the following sections), the glial cell fate determinant, is

able to force NSCs to adopt a glial fate at the expense of the neuronal one, when ectopically expressed in the whole neurogenic region (Bernardoni et al., 1998; Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996).

The facility through which cell fates can be experimentally modified raises the question as to whether such events occur physiologically or in the context of disease. Arguably, in Mammals, Schwann cells possess a natural regenerative capacity called dedifferentiation. Following damages to the nerves they are associated with, Schwann cells dedifferentiate and proliferate (Chen et al., 2007). Another nice example is the transdifferentiation of ectodermal cells into mesodermal cells during gastrulation (Slack, 2007; Yang and Weinberg, 2008). In the case of cell plasticity and pathology, several types of metaplasia have been attributed to transdifferentiation (Slack, 2007), and epithelial mesenchymal transitions may be involved in the formation of metastatic breast cancers (Yang and Weinberg, 2008). Here, as during normal epithelial mesenchymal transitions, the activation of key TFs is essential, like Twist and Snail (Slack, 2007; Yang and Weinberg, 2008).

With the rapidly growing of lineage tracing tools I guess that many more physiological or pathological cell fate conversion events will be discovered in the future.

2. 3. Stem cell plasticity research

SC plasticity research is the most fascinating chapter in the history of biology. Traditionally restricted to the field of developmental biology, SC plasticity has become of increasing interest for biomedical research in more recent years. Indeed, the advances in SC research help us to understand the mechanisms underlying cancerougenesis and neuronal cell degeneration with aging. SC research aims to understand and treat such heavy pathologies. However, a major concern in protocols aiming on cell replacement is safety. This new concept was the subject of controversy because of the possibility of various experimental biases, including the possible presence of contaminating SCs, pluripotent SCs of any adult or cell fusion of SCs with differentiated cells that will be used as a treatment (Lakshmipathy and Verfaillie, 2005). Following this episode, more attention has been paid to the characterization of SCs, their fractionation and the study of their biology.

2. 3. 1. Properties of stem cells

SCs are the foundation for every organ, tissue and cell in the body and are characterized by unique defining properties. As previously mentioned, these cells are

proliferative precursor cells characterized by their ability to self-renew while generating a large number of progeny committed to differentiation. They can either divide symmetrically, producing two identical daughter cells, or asymmetrically producing one "identical" and one more differentiated daughter cell (Lin and Schagat, 1997). SCs are also this population of cells that face the consequence of aging, by changing their potential to differentiate into different cell types and controlling their cell cycling by entering cell cycle arrest, the quiescent phase, or dying under programmed cell death, also called apoptosis. These cells do not only exist during the embryonic life (ESCs), but also in major differentiated tissues of an adult organism (adult SCs), where they play a central role in tissue growth and maintenance (Reya and Clevers, 2005; Yao et al., 2012).

2. 3. 2. *Drosophila* embryonic NSCs as a model system to study cell plasticity

Drosophila melanogaster is an extremely powerful model system for identifying and analyzing complex biological processes in the context of a living organism. It was thoroughly demonstrated that the processes regulating fundamental aspects of animal development and physiology are well conserved, and that insights gained from studies in *Drosophila* can with high likelihood be transferred to other species. For example, developmental genes such as the Hox genes that play essential roles in setting up the vertebrate body axis were originally identified and well characterized in *Drosophila*. About 75 % of known human disease genes have a recognizable match in the genome of fruit flies (Reiter et al., 2001), and 50 % of fly protein sequences have mammalian orthologs. Today, *Drosophila* is used as a genetic model for several human diseases including neurodegenerative disorders. It is also used to study mechanisms underlying aging, immunity, diabetes and cancer, as well as drug abuse.

Specifically, the NSCs of *Drosophila* provide an excellent system to study the mechanisms regulating SC plasticity. As in mammals, the NSC generally called NBs divide to produce new SCs and daughter cells that go through a well-characterized cascade of differentiation steps to develop into neurons or glial cells. In the *Drosophila* embryonic CNS, we know the position and the identity of all NSCs, and their progenies. A variety of molecular markers and tools have been identified to study NSCs, as well as the neurons and glial cells. Most importantly, the genetic tractability of *Drosophila* allows for identifying genes regulating NSC function and plasticity (for more details see Introduction 4. 1.).

2. 4. Broad questions in cell plasticity

Cell fate transformations has changed the way that we view cell plasticity and how we can apply converted cells to regenerative medicine. However, several important questions should be first resolved before using these cells in therapy that I summarized in two questions:

1/ How can transcription factor induce a new program while repressing another?

2/ Is the identity of generated cells completely identical to the desired cell fate?

3. The development of embryonic nervous system

3. 1. The Development of *Drosophila* nervous system

The duration of *Drosophila* life cycle is influenced by the temperature; at 25°C, it takes around 10 days: one day of embryogenesis, which is divided into different seventeen stages where the majority of structures are generated, followed by three successive larval stages, which take around four days, and five days of pupal life where metamorphosis occurs to generate the adult fly.

3. 1. 1. General structure of the nervous system

Drosophila NS is composed of three parts: the CNS which is composed of two parts: the brain and the ventral nerve cord (VNC) (the equivalent of the spinal cord in vertebrates), the peripheral NS (PNS) (Figure 2, left panel) and the stomatogastric NS (Hartenstein et al., 1994). The stomatogastric NS and PNS will not be described.

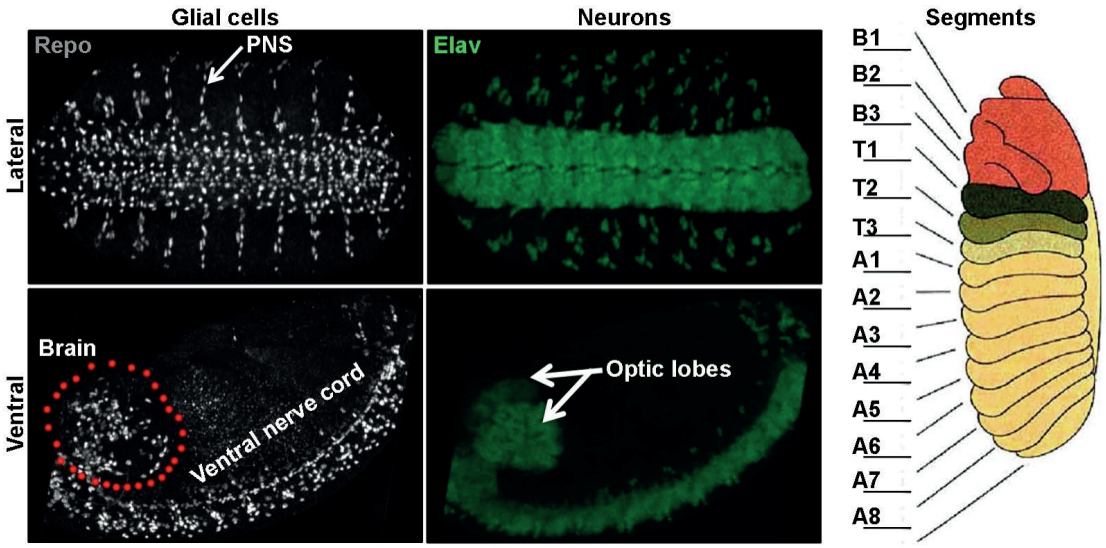

Figure 2. Structure of The Drosophila nervous system

The left and middle panels show the profile of neurons (stained with Elav in green) and glia (stained with Repo in grey) in the nervous system of stage 16 embryos.
The right panel shows the position of the different segments (the cartoon showing the profile of segments is adapted from http://www.zoology.ubc.ca/~bio463/lecture_13.htm).

A large portion of nerve cells is found within the brain and the VNC. The brain contains two optic lobes (OL) and the central brain (CB), including the mushroom bodies (MBs), where learning and memory reside. The VNC serves to connect the brain with the

PNS, which entails all sensory neurons, as well as the neurons controlling the functions of various organs. Like the rest of the arthropod body, the CNS of *Drosophila* is segmented into subunits, called segments or neuromeres. The brain contains three segments B1, B2 and B3, which correspond to the prospective protocerebrum, deutocerebrum, and tritocerebrum, respectively. The VNC contains three thoracic (T1, T2 and T3), and eight abdominal (A1, A2, A3, A4, A5, A6, A7 and A8) segments (Figure 2, Right panel). Due to its simplicity the VNC was so far the principal model for the studies on the molecular mechanism behind cell fate specification in the *Drosophila* CNS, and henceforth it will also be the principal focus of my thesis work.

In the VNC, most neuronal axons are organized in a simple, ladder-like pattern. Within each segment, two horizontal commissural axon tracts (anterior and posterior commissures) cross the midline to connect the two longitudinal connectives, linking adjacent neuromeres to one another along the Anterior/Posterior (A/P) axis. Axon bundles and their associated glia constitute the neuropile. Exiting the longitudinal connectives at each side of the neuromeres there are two peripheral nerves, the segmental (SNR, at the level of the anterior commissure) and the intersegmental (ISNR, posterior to the posterior commissure) nerves, which are made up of peripherally projecting motor axons and centrally projecting sensory axons (Figure 3).

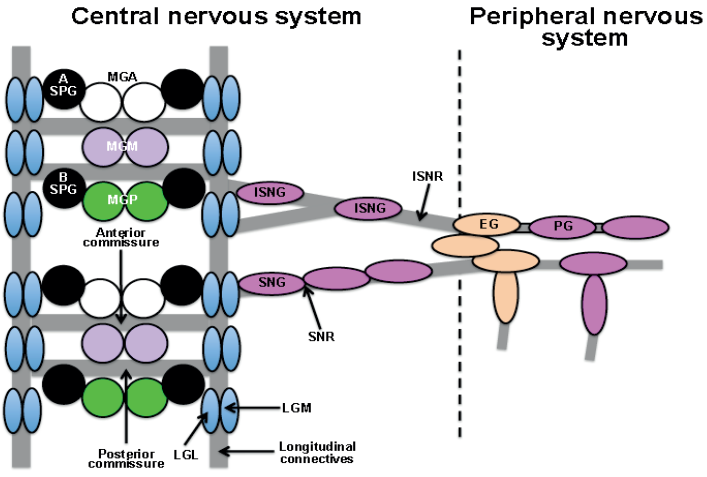

Figure 3. Embryonic CNS and PNS axon pathways and pattern of glial cells.

Midline glia (MG) surround anterior and posterior commissures, longitudinal glia (LGL and LGM, L for lateral and M for medial, respectively) arranged in two parallel rows along the longitudinal connectives. For more details see the text.

3. 1. 2. Early neurogenesis in *Drosophila* embryos

The embryonic life of *Drosophila* starts with the formation of three basic germlayers, the ectoderm, the mesoderm and the endoderm. The NS arises from the ectoderm, a layer that also gives the epidermis, the trachea and the hindgut. At the beginning of embryogenesis, the ectoderm is subdivided into a ventral neurogenic and a dorsal non-neurogenic region by the antagonistic activity of the secreted molecules Decapentaplegic (Dpp) and Short gastrulation (Sog) (Francois and Bier, 1995; Francois et al., 1994). The neurogenic ectoderm (neuroectoderm) starts as a simple epithelium sheet composed of proliferative cells. This region gives rise to the neural progenitors (NSCs or NBs) or sensory organs precursors (SOPs) and to the epidermal precursor cells.

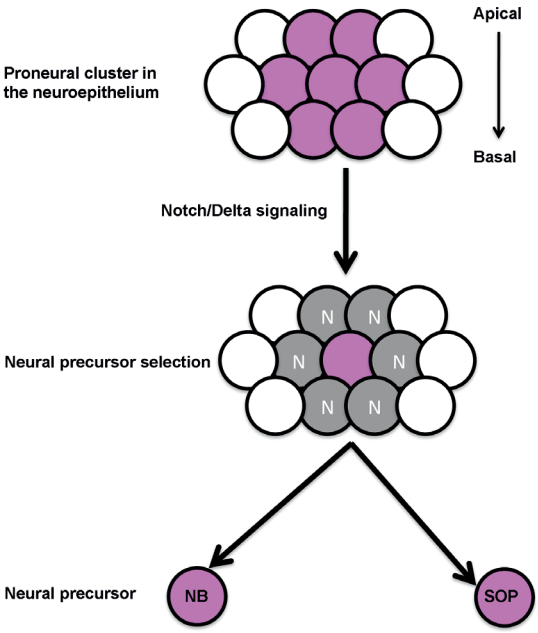

Figure 4. The lateral inhibition process.

After the formation of proneural clusters, neurogenic genes (N) select the future neural precursor: NB or SOP.

The proneural genes control the position and the time at which groups of neuroectodermal cells, called proneural clusters, become competent to form a NSC (Ghysen and Dambly-Chaudiere, 1989), whereas the neurogenic genes control the cell interactions that prevent more than one cell in the group from developing into a NB, this process is called "lateral inhibition" (Figure 4) (Lehman, 1983). The proneural genes codes for TFs having a basic Helix Loop Helix (bHLH) domain (Campuzano et al., 1985; Villares and Cabrera, 1987) and they are represented by the three or the four members of Achate-Scute Complex: Achete, Scute and Letal of Scute (Cabrera et al., 1987; Martin-Bermudo et al., 1991). For the neurogenic genes, two transmembrane proteins have been extensively characterized, Notch,

which represents the receptor, and its ligand Delta. About 30 NBs delaminate from the neuroectoderm per thoracic and abdominal hemisegment (Broadus et al., 1995; Doe, 1992). The remaining cells of the neurogenic region remain superficial and generate the ventral epidermis.

NBs delaminate from the surface in five successive waves (S1-S5) along the M-L and A-P axes in rows and columns in a stereotyped spatiotemporal pattern (Figure 5). NBs are given numerical designations according to their definitive position. The numbers consist of two indexes; the first index indicates the A-P position, and the second one indicates the M-L position of the NB. Thus, for example the NB6-4T is the fourth NB from the ventral midline in row 6 after the formation of all NBs (Figure 5). For more precision, "T" and "A" latters are added to distinguish the thoracic from the abdominal NBs at the same positions, respectively. Even if the name of some NBs is the same in the abdomen and the thorax, the progeny of these cells may differ. For example the thoracic NB6-4T generates neurons and glia, whereas the abdominal one generates only glia (Berger et al., 2005; Schmidt et al., 1997).

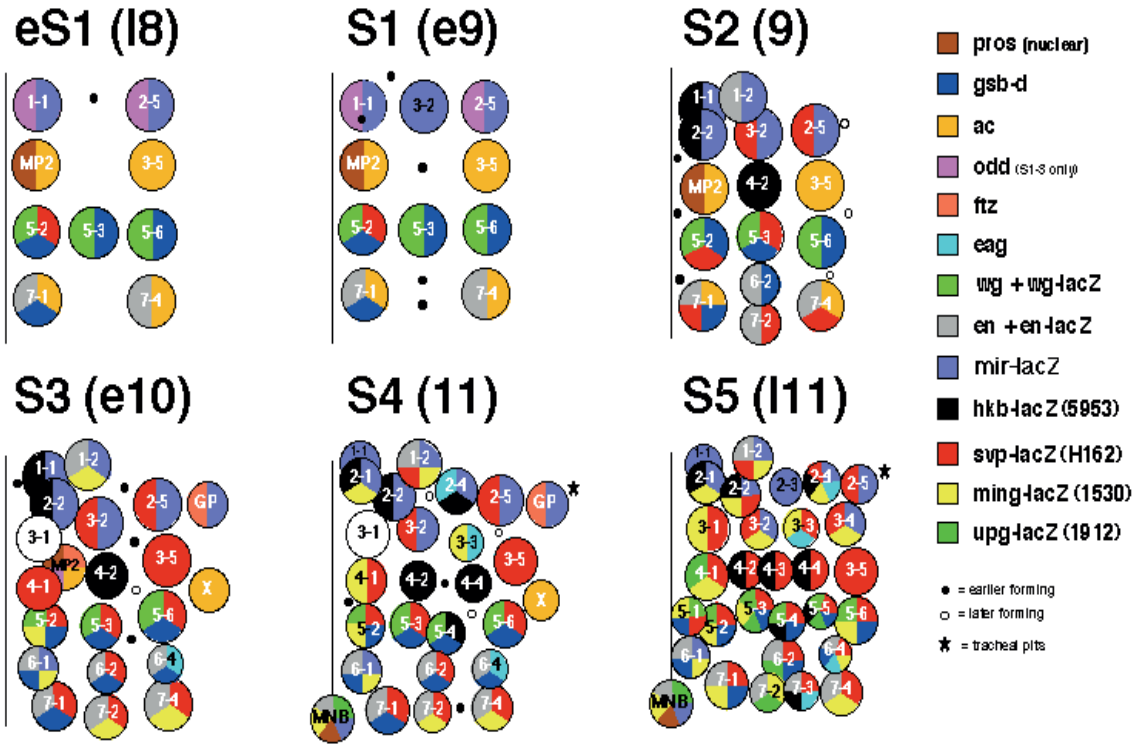

Figure 5: *Drosophila* neuroblasts map

The 30 NBs per hemisegment are generated in five sequential waves. Each NB is generated at a stereotyped time and position, and displays a unique expression profile of molecular markers.
www.neuro.uoregon.edu/doelab/nbmap.html.

By embryonic stage 9-11, approximately 75-80 NBs are formed. To build the CNS, each NB undergoes repeated asymmetric cell divisions to renew themselves while producing intermediate precursors, called ganglion mother cells (GMCs). The GMC divides once more to give two daughter cells that differentiate into neurons and/or glial cells (Chia et al., 2008; Matsuzaki, 2000; Urbach and Technau, 2004; Younossi-Hartenstein et al., 1996). As the neural progenitors produce progeny they change their competence over time in a step-wise manner, generating different types of cells at specific time points. One identified mechanism behind such competence transitions is the progenitor-intrinsic sequential expression of the so-called "temporal genes" (see Introduction 5. 1. 2).

The size of the NBs gradually decreases upon each division and towards the end of embryogenesis, some NBs stop dividing and enter a stage known as quiescence (Truman, 1990), whereas the rest are eliminated *via* programmed cell death (Prokop and Technau, 1991; Truman and Bate, 1988). The only NBs that do not undergo quiescence or programmed cell death at the end of embryonic life are the four OL/MB NBs, which generate very large lineages of 500 neurons each, and one less-well characterized V-L CB NB (Ito and Hotta, 1992; Lee et al., 1999; White and Kankel, 1978).

3. 1. 3. Cells making *Drosophila* CNS

Three cell types compose the CNS, the NBs, the neurons and the glial cells. The NBs are the founding cells of the CNS. Neurons are specialized in transmitting signals between different cell populations within the body, while glia provides insulation to neurons by controlling extracellular homeostasis and acting as NS immune cells.

- Neuroblasts

These cells are first generated during the embryonic life from the neuroectoderm. They are detectable in the VNC and the CB/MB, and serve to generate the CNS of the larva, which contributes to 10 % of neurons in the adult CNS. Contrarily to the VNC and the CB NBs, the OL NBs are generated during the larval life from the neuroepithelial placodes. During the postembryonic life, some reactivated embryonic NBs together with the larval NBs contribute to the generation of the remaining 90 % of adult neurons. (Reviewed by (Egger et al., 2008; Skeath and Thor, 2003; Sousa-Nunes et al., 2010)). See Figure 6 for the distribution of NBs at different developmental stages.

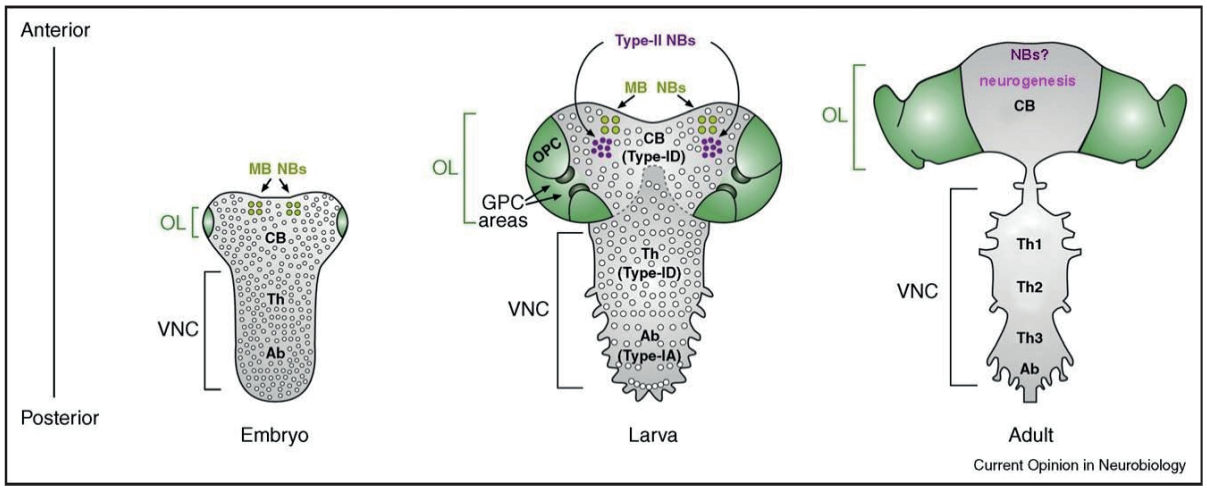

Figure 6. NBs distribution in the developing *Drosophila* CNS.

Representation of embryonic, late-larval and adult CNS, highlighting NBs (circles). The OL (green) is subdivided into the Glial Precursor Cell (GPC) areas and the Outer Proliferation Centre, which generates OPC NBs. For clarity, the Inner Proliferation Centre (IPC) is not shown. The CB/MB contains numerous Type-ID NBs, four Mushroom Body (MB) NBs and eight Type-II NBs. The VNC is subdivided into Thoracic (Th) segments and Abdominal (Ab) segments containing Type-ID and Type-IA NBs respectively. Note that no identifiable NBs are present in the adult CNS. Adapted from (Sousa-Nunes at al., 2010).

While adult NSCs appear to be common in vertebrates, the situation in *Drosophila* is much less clear. It was thought that the NBs that generate the CNS of adult *Drosophila* stop division, undergo apoptosis, or differentiate before eclosion (Bello et al., 2003; Ito and Hotta, 1992; Maurange et al., 2008; Truman and Bate, 1988). However, two recent reports identified small numbers of dividing cells in the adult brain and the majority of these cells express a glial marker, Reversed Polarity (Repo) (Kato et al., 2009; von Trotha et al., 2009). Interestingly, it was also reported in vertebrate that NSCs from the adult hippocampus might eventually differentiate into postmitotic astrocytes (type of glial cells), a process that would explain the loss of SCs and reduction in neurogenesis with age (Encinas et al., 2011). Evidence that astrocytes could hold the capacity to dedifferentiate into RG cells, and even immortalized cells, that could induce gliomas was produced by several labs (Dufour et al., 2009; Jiang and Uhrbom, 2012; Sharif et al., 2007). It was also reported that NSCs have astrocyte characteristics, (reviewed by (Bergstrom and Forsberg-Nilsson, 2012). All together, these results suggest that glial cells and adult NSCs may share the expression of some markers. Might Repo be a shared marker between glia and adult NBs in *Drosophila* brain? Is

it possible that adult NBs express other markers that are not yet identified? I believe that lineage tracers and the simplicity of *Drosophila* NS will soon resolve these questions.

- Glial cells

The term glia means "glue," a reflection of the fact that glial cells really do hold the brain together, occupying the space between neurons. In the developing CNS of the *Drosophila* embryos, glial cells derive from two different germlayers. A small part of these cells, midline glia, derives from mesectodermal progenitors and ensheath the commissural fiber tracts (Bossing and Technau, 1994; Menne and Klambt, 1994). The major part, the lateral glial cells, derives from the neurogenic region of the ectoderm. The first event in the determination of lateral glia is the transient expression of the TF Gcm (see Introduction 5.). Gcm is known to initiate the glial fate by activating downstream targets, which accomplish the differentiation and the maintenance of the glial fate. Among the Gcm target genes is *repo*, which codes for an homeodomain protein "Repo", that is expressed in all lateral glial cells and is used as a general marker for these cells (Figure 7).

The lateral glial cells of the embryonic VNC are subdivided into three categories according to their association with the basic compartments of the CNS: the surface, the cortex, and the neuropile, (Figure 4) (Ito, 1995; Meyer et al., 1987). The group of **surface-associated glia** includes two subgroups: the subperineurial glia (SPG) that lie underneath the outer surface of the CNS, and the channel glia (CG), which are positioned along the dorsoventral channels, demarcating the borders between segmental neuromeres of the VNC. In the category of **cortex-associated glia**, that amalgamates between the neuronal cell bodies in the cortex, only one subtype is described in the embryonic VNC: the cell body glia (CBG). The third category, the **neuropile-associated glia**, includes the glial cells that are associated with axonal structures. Two subtypes were proposed in the embryonic VNC: the nerve root glia (NRG), which is further subdivided into intersegmental and segmental nerve root glia (ISNG and SNG, respectively), and the interface glia (IG), which are associated with the longitudinal connectives and are also called longitudinal glia (LG), (Beckervordersandforth et al., 2008; Ito, 1995). Using a combination of molecular markers, the NSCs generating each lateral glial cell have been identified (Beckervordersandforth et al., 2008).

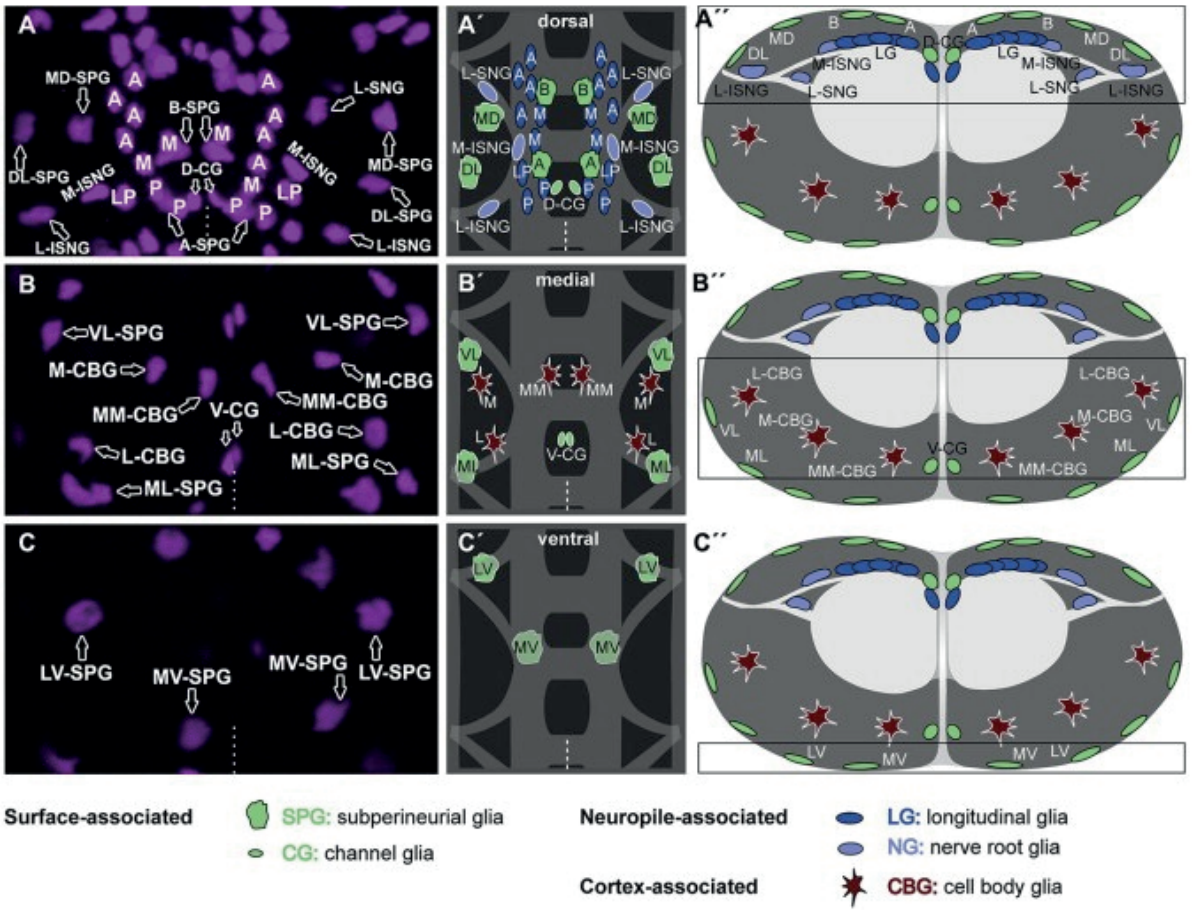

Figure 7. Spatial distribution and classification of glial cells in the VNC.

Pattern of glial cells in an abdominal neuromere at embryonic stage 16. **(A–C)** Horizontal views of a preparation showing nuclear anti-Repo staining (anterior to the top; midline indicated by dashed line), and **(A'–C')** corresponding cartoons at dorsal **(A and A')**, intermediate (B and B'), and ventral layers **(C and C')** as indicated by black frames in cartoons of frontal view (A''–C''; dorsal to the top) (Beckervordersandforth et al., 2008).

- Neurons

Three basic types of neurons are present in the NS of *Drosophila*: moterneurons, interneurons and neurosensory neurons. **Moterneurons** extend axonal projections out into the periphery to innervate the muscles. There are about 30 moterneurons per hemisegment. The **interneurons** extend axons within the CNS to innervate other neurons. To this class belong a total of about 300 interneurons, which can be subdivided into two subclasses: intersegmental interneurons, whose axon projections extend between segments within the CNS, local interneurons with axon projections terminate within their segment of origin in the CNS, and finally, **neurosensory neurons** which extend axons either out into the periphery or into the seat of the CNS to secrete neuropeptides and hormones. A total of about 10 cells have been identified in each hemisegment (Schmid et al., 1999).

3. 2. The development of vertebrate nervous system

The aim of this chapter is not to illustrate the vertebrate neurogenesis but to describe some structures and cell types that will help to understand the following parts. Similar mechanisms involved in fly and vertebrate neurogenesis will be underlined.

3. 2. 1. General structure of the nervous system

The NS of vertebrates has two main divisions: the CNS, consisting of the brain and the spinal cord, and the PNS. The brain consists of three major divisions, organized around the three chambers of the neural tube that develops early in embryonic life: the forebrain, the midbrain, and the hindbrain (Figure 8A).

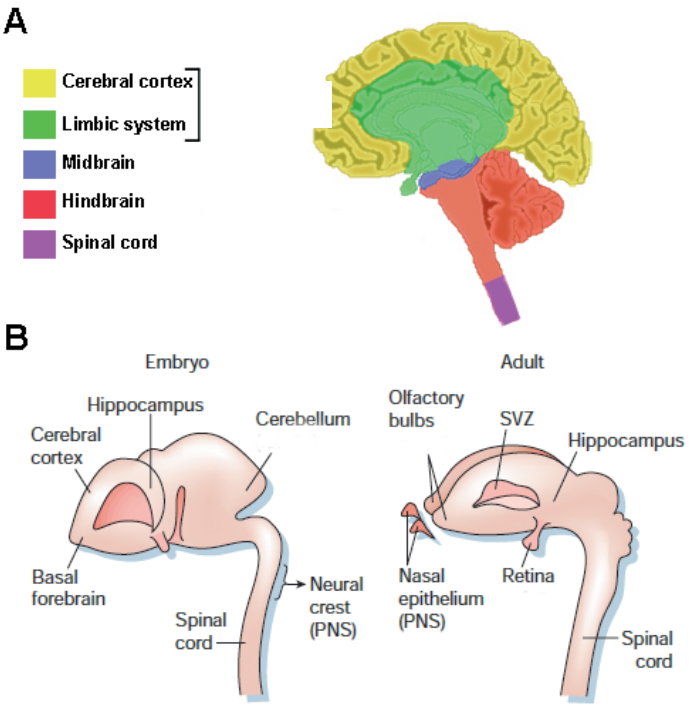

Figure 8.

(A) Structure of vertebrate central nervous system.
(B) The principal regions of the embryonic and adult nervous system from which neural stem cells have been isolated (Temple, 2001).

3. 2. 2. Early neurogenesis in vertebrate embryos

The vertebrate CNS derives from the neural plate, an epithelial sheet that arises from the dorsal ectoderm of the gastrula-stage embryo (Lee and Jessell, 1999). As in *Drosophila*, the vertebrate ectoderm is subdivided into neurogenic and non-neurogenic region by the antagonistic activities of two secreted molecules, Bone Morphogenic Protein 4 (BMP4) and Chordin, the orthologs of Dpp and Sog in Drosophila, respectively (Arendt and Nubler-Jung, 1997; De Robertis and Sasai, 1996; Lichtneckert and Reichert, 2005).

The neural plate is characterized by the expression of neural-specific markers such as members of the Sox gene family (Mizuseki et al., 1998). Clonal analysis has shown that

single cells isolated from the neural tube along the spinal segment are competent to give rise to clones containing both, dorsal, like sensory ganglion neurons, and ventral, like motor neurons, derivatives, indicating that early embryonic neural precursor cells are not restricted in their potential to generate different cell types along the D-V axis (Artinger et al., 1995). Indeed, cellular diversity was shown to depend on signals coming from surrounding tissues that can regulate phenotypic and positional specification of neural precursor lineages along the major axes (Lumsden and Krumlauf, 1996; Tanabe and Jessell, 1996). These signals serves also for reshaping neural plate morphology, upon cell shape changes and cell movements that involve adhesion receptors and cytoskeletal elements (Smith and Schoenwolf, 1997). Subsequently, the neural plate closes to form a neural tube, which becomes patterned along its A-P and D-V axes. Shortly after neural tube closure, a series of vesicles can be clearly distinguished morphologically at the anterior end of the neural tube of the mouse embryo, indicating its pattern along the A-P axis. The most anterior end of the neural tube gives rise to the forebrain, while more posterior regions form the midbrain, the hindbrain (that is further divided into seven midbrain segmental rhombomeres), and the spinal cord.

At the beginning of mouse cortical neurogenesis, a cell type with a specific morphology appears throughout the CNS, the RG cell which arise from the neuroepithelial cells (Kriegstein and Alvarez-Buylla, 2009). As their name indicates, RG share many features with the glial cells, such as expression of the glial marker GFAP (glial fibrillary acidic protein) and GLAST (Glutamate astrocyte-specific transporter) (Campbell and Gotz, 2002), and were originally thought to serve astrocyte-like functions, such as providing the scaffold for the migration of differentiating NSCs. Recently, RG were definitively demonstrated to be the embryonic NSCs using the Cre-recombinase based fate mapping and following the fate of these cells using retroviral-based labeling combined with time lapse imaging in slice culture (Anthony et al., 2004; Noctor et al., 2004b).

The RG divide asymmetrically to produce a post-mitotic neuron or glial cell, as well as another RG cell (Noctor et al., 2004b). The post-mitotic cells migrate along the RG process and complete their differentiation at the appropriate place. Alternatively, the RG cell division may generate an IPC instead of a neuron (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004b). The IPCs are similar to transit amplifying cells, the GMCs of *Drosophila*. These progenitors may be restricted to produce a neuronal or a glial lineage, and can divide symmetrically prior to differentiation. As for *Drosophila* embryonic NSCs, the number of divisions these cells undergo and the type of neurons and glial cells they will generate

depends upon the time and location of their birth within the developing CNS (Okano and Temple, 2009; Qian et al., 2000). Early progenitors will divide massively producing both neurons and glia, while later arising cells may be restricted to a neuronal or glial fate (Falk and Sommer, 2009; Okano and Temple, 2009). In some regions of the NS, like the ventral spinal cord, NSCs will initially generate neuronal derivatives and then later in embryogenesis, produce only glia (Falk and Sommer, 2009). Later in embryogenesis, an additional neurogenic region appears near the VZ (ventricular zone), SVZ (subventricular zone) (Kriegstein and Alvarez-Buylla, 2009). The SVZ continues to produce neurons and glia, as well as IPCs, after the VZ has stopped to do. In the region bordering the lateral ventricles, the SVZ will continue to furnish a neurogenic niche, in the neonatal period and throughout adulthood (Gage, 2002).

3. 2. 3. Cells of the nervous system

- Neural stem cells

NSC is a widely used term, but during development these building blocks for neurons and glia change considerably their gene expression pattern, cytological characteristic and differentiation potential. For example, just when neurogenesis starts neuroepithelial cells are gradually replaced by RG (Malatesta et al., 2000). However, RG are considered as SCs and similar to neuroepithelial cells. Now, it is admitted that both embryonic and adult SCs isolated from the NS that could divide and generate neurons and/or glial cells are NSCs.

In the developing mammalian CNS, isolation of NSCs was performed in early studies from different regions (Cattaneo and McKay, 1990; Kilpatrick and Bartlett, 1993; Reynolds et al., 1992; Temple, 1989). NSCs have been isolated from the basal forebrain, cerebral cortex, hippocampus, cerebellum and the spinal cord. In the PNS, the neural crest holds a population of NSCs that are able to generate Schwan cells (glial cells of the PNS) and sympathetic and sensory neurons (neurons of the PNS) (Stemple and Anderson, 1992). See also (Figure 8B).

While the majority of NSCs will lose their self-renewal capacity and multipontency with time, two germinal zones remain active in the brain throughout adulthood, the subgranular zone (SGZ) in the hippocampus, and the SVZ of the lateral ventricles (Gage, 2000; Lois and Alvarez-Buylla, 1993; McKay, 1997; Rao, 1999; Reynolds and Weiss, 1992). In the most accepted model of adult neurogenesis, the NSC is a radial astrocyte-like, GFAP-positive cell, (reviewed in (Alvarez-Buylla and Lim, 2004). Adult NSCs were also found in

other regions, including the spinal cord and the retina (Figure 8B). Whether neurogenesis occurs in other regions of the adult brain is still debated.

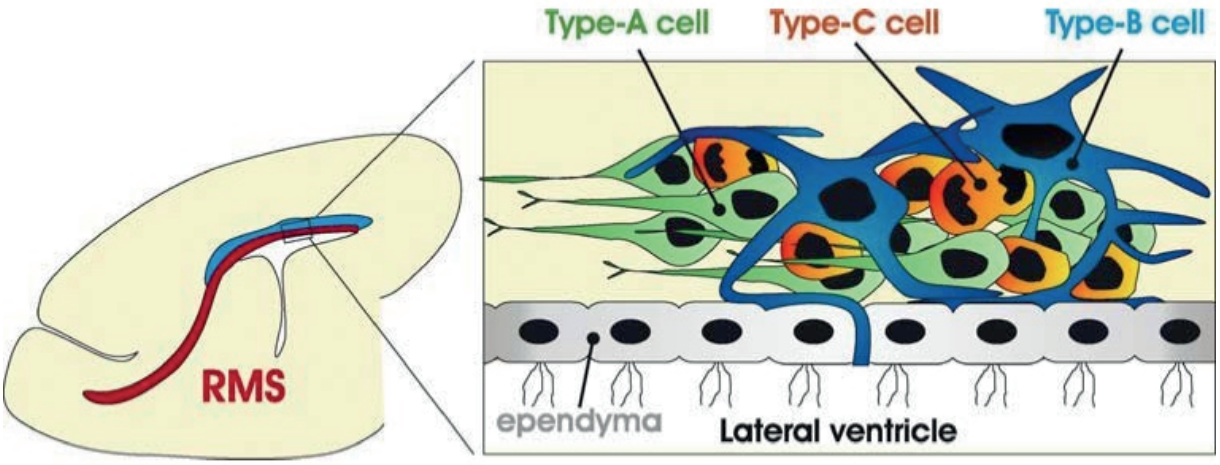

Figure 9. Adult neural stem cell niche.

Astrocyte-like cells (TypeB; blue), lining the ventricle, give rise to progenitor cells (TypeC; orange) that subsequently generate migrating neuroblasts (TypeA cells; green). Early generated neurons reach the olfactory bulb migrating along the rostral migratory stream. (Malatesta et al., 2008).

Adult NSCs are embedded in a peculiar niche and these cells are called differently according to their behavior: **TypeB** cells, also called RG-like precursors, are the mother of all generated NSCs and they are relatively quiescent, **TypeC** cells are the fast-cycling transit-amplifying progenitor cells, and **TypeA** cells are the migrating NBs (Figure 9). The direct evidence for the embryonic origin of TypeB cells comes from *in vivo* lineage tracing analysis, where it was shown that RG convert their morphology by retracting their basal process to produce TypeB cells, this process occur just after birth (Merkle et al., 2004; Ventura and Goldman, 2007). The conversion of RG into TypeB cells involves the loss of the RG morphology and a slowing down of the cell cycle, (reviewed in (Malatesta et al., 2008)).

- Neurons and glia

Three general categories of neurons are commonly recognized: 1/ **Receptors** are highly specialized neurons that act to encode sensory information like the photoreceptors of the eye, 2/ **Interneurons** which receive signals from and send signals to other nerve cells, and 3/ **Effectors or motor neurons** which send signals to the muscles and glands of the body.

There are two types of glial cells in the NS: **macroglia** and **microglia**. Two classes of macroglia were identified in the CNS: **astrocytes** and **oligodendrocytes**. Astrocytes are

believed to provide structural support for the neurons of the brains and help in the repair of neurons following damage. They also regulate the flow of ions and larger molecules in the region of the synapses. A major function of oligodendrocytes is the production of myelin, which surrounds the axons of neurons. In the peripheral nerves, there is another type of supporting cell; **Schwann cells**, which shares many similarities with oligodendrocytes. In the developing NS, the Shwann cell first encircles an axon, and then wraps itself around the neuron, building a myelin sheath. In contrast to macroglia, microglia perform "housekeeping" functions within the CNS. Among their duties is the removal of dead cells within the brain.

4. The mechanism regulating neurodevelopmental plasticity

Studies *in vivo* and *in vitro* showed that the developmental potential of NSCs changes and becomes progressively restricted with time. For in *vitro* cultured NSCs, it is those derived from embryos that exhibit the best developmental potential, and it is clear that such behavior is affected by both, extrinsic and intrinsic mechanisms. In this part of my work I will describe two mechanisms regulating NSC plasticity: 1/ their biology and 2/ epigenetic changes.

4. 1. Regulation of NSC biology

4. 1. 1. Neural stem cell lineage types

While all *Drosophila* neural progenitors are generally called NBs, they can be divided into three different classes depending on if their lineages that comprises only neurons (neuroblasts, NBs), only glia (glioblasts, GBs), or a mix of both (neuroglioblasts, NGBs) (Bossing et al., 1996; Broadus et al., 1995; Doe, 1992; Schmid et al., 1999; Schmidt et al., 1997). In the embryonic VNC there are two GBs; NB6-4A and LGB (longitudinal glioblast), six NGBs; NB1-1A, NB1-3, NB2-2T, NB5-6, NB6-4T, and NB7-4, while the rest of the NBs generate only neurons (Beckervordersandforth et al., 2008).

Drosophila NSCs are also divided according to their molecular identity and their mode of division, into three different types (Figure 10) (Boone and Doe, 2008):

1/ Type I NSCs account for the majority of the SCs in the *Drosophila* CNS, with approximately 180 in the larval brain. The majority of the embryonic NSCs are considered as Type I NSCs due to their mode of division. After each division, these SCs generate a large NSC and a smaller daughter cell, GMC. Type I NSCs express Deadpan (Dpn) and Asence in the nucleus and Prospero in the cytoplasm. Dpn is a bHLH TF (Bier et al., 1992) related to the vertebrate Hes family of TFs, that was described to promote NSCs proliferation (Wallace et al., 2000). Asense is a member of Achete-Scute complex (Gonzalez et al., 1989; Jarman et al., 1993), and ortholog of the vertebrate NSC factor, Achaete-scute complex-like 1 (Ascl1, Mash 1). Unlike the other members of Achete-Scute complex, Asence is not expressed in the proneural clusters in the embryo. Asense expression is initiated in the NSC and is maintained at least in a subset of GMCs (Brand et al., 1993). Prospero, the ortholog of vertebrate Prox1, is a homeodomain TF. After Type I NSC division, Prospero segregates to the GMC where it represses NSC specific genes and activate differentiation genes (Choksi et al., 2006; Southall and Brand, 2009). Thus, the GMC divides only once to produce two post-mitotic cells, neuron

and/or glial cell. Due to this mode of division, Type I NSCs could not produce more than 100 neuronal progeny in its life (Bello et al., 2006; Boone and Doe, 2008; Bowman et al., 2008).

2/ Type II NSCs are less abundant than Type I, they are present in the larval brain and we count only 16 per brain. After each division, these cells give a new NSC and an intermediate neural progenitor (INP). In these NSCs, Dpn is detectable but not Asence and Prospero, this last factor is also undetectable in the INP. Contrarily to GMC, which divides only once, the INP divides four to eight times generating a new INP and a GMC that divides only ones (Bello et al., 2008; Boone and Doe, 2008; Bowman et al., 2008). Thus, Type II NSCs generate much larger cell lineages than Type I NSCs. Recent studies have revealed that the tumor phenotype caused by loss of Brain Tumor (Brat), Numb or Prospero were primarily due to uncontrolled expression of this type of NSCs (Bello et al., 2006; Bowman et al., 2008), clearly showing the proliferative power of these cells.

3/ The third type of NSCs is found in the OL/MB of the larval brain, where NSCs divide symmetrically and gradually converte to asymmetric cell division in response to a wave of proneural gene expression (Egger et al., 2007; Egger et al., 2011). Like Type I NSCs, when MB/OL NSCs start to divide asymmetrically, they self renew and produce a committed cell.

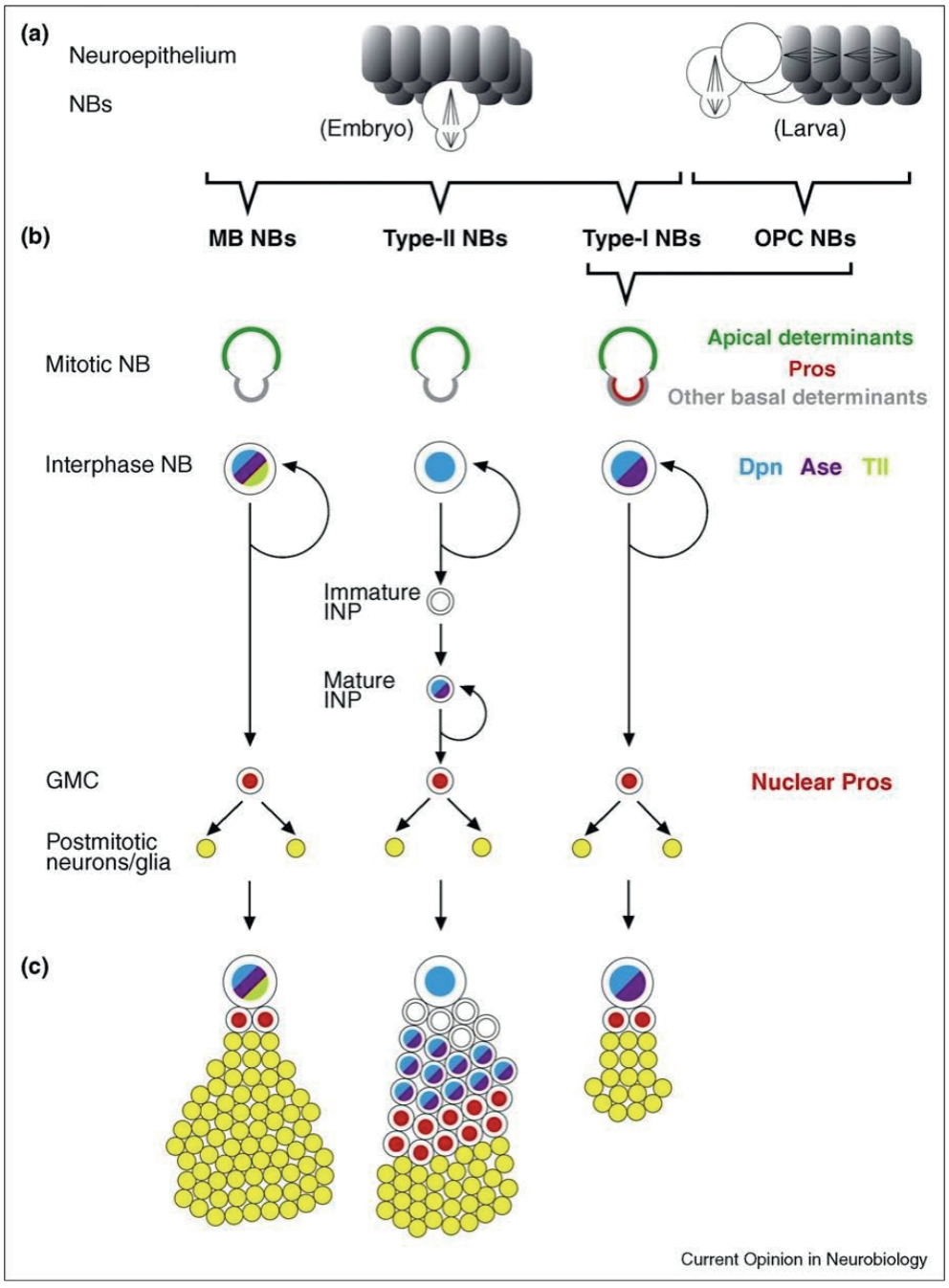

Figure 10. Different modes of neuroblast division.

(a) Schematic representations of NBs (white) delamination from the neuroepithelium (grey). For most NBs this process happens in the early embryo but in the Outer Proliferation Centre (OPC) it occurs in the larva. The OPC neuroepithelium proliferates by symmetric divisions whereas NBs divide asymmetrically. **(b)** Three different molecular signatures and division modes in mitotic and interphase postembryonic NBs. Mushroom Body (MB), Type-I, Type-II and OPC neuroblasts are shown. All NB types and the INPs of Type-II lineages self-renew (curved arrow) but the ganglion mother cell (GMC) does not. The distribution of the basal and apical determinants is shown. **(c)** The lineage sizes of MB and Type-II NBs are larger than those of Type-I NBs. This reflects, at least in part, the absence of a quiescent period in MB neuroblasts and the presence of INPs in Type-II lineages (Sousa-Nunes et al., 2010).

Mammal NCSs can be broadly classified as either embryonic or adult cells. However, their classification according to their molecular identity and mode of division is very hard, due to their heterogeneity. For example, adult PNS SCs appear to have restricted developmental potential and less self-renewal ability compared to their embryonic counterpart (Kruger et al., 2002). In addition, CNS SCs isolated from late embryonic development produce fewer neurons in clonal cultures than those isolated from earlier stages (Qian et al., 1998; Qian et al., 2000). Even NSCs isolated at the same developmental time point but from different regions of the NS exhibit different developmental capacities (Bixby et al., 2002; He et al., 2001).

4. 1. 2. Neural stem cell temporal gene cascade and cell fate specification

During CNS development of vertebrates and invertebrates, many NSCs generate distinct cell types over time, contributing to the vast cellular diversity of the CNS, (reviewed in (Pearson and Doe, 2004)). In *Drosophila* NSCs, a series of TFs, involving Hunchback (Hkb), Kruppel (Kr), Pdm, Castor (Cas), and Grainyhead (Grh), is expressed in a step-wise manner, and have been identified as necessary and sufficient for controlling the specification of neural and glial fates generated within their respective expression window (Brody and Odenwald, 2000; Grosskortenhaus et al., 2006; Kambadur et al., 1998; Novotny et al., 2002; Pearson and Doe, 2003; Sugimoto et al., 2001; Tran and Doe, 2008). Transition in the TF series depend on cell cycle progression and appear to be stabilized by negative cross-regulatory interactions (Isshiki et al., 2001; Kambadur et al., 1998). Since all NSCs express the same cascade of temporal TFs regardless of their time of birth or their position in the CNS (Isshiki et al., 2001), changes in temporal TFs expression are likely not controlled by extrinsic factors. Evidence for this hypothesis was demonstrated by NSCs tissue culture experiments, where NSC undergo the same cascade as *in vivo* (Brody and Odenwald, 2000; Grosskortenhaus et al., 2005).

Importantly, the TF series appears to be used as an internal clock regulating the number of NSC divisions. For example, when the NB7-3 is forced to express the two first TFs of the series, Hb or Kr, this produces an extended lineage, containing many more cells than normal (Isshiki et al., 2001), suggesting that the NSC some how counts the number of its division *via* the TF series and exits the cell cycle only upon it expressing the whole series.

At late embryonic stages, some NSCs enter quiescence before ending the expression of the whole temporal cascade, and they restart it where they left off as they reinter cell cycle at larval stages (Maurange et al., 2008). Interestingly, other NSCs exit the cell cycle and die

under apoptosis before completing the whole temporal cascade, as it is for the abdominal NB5-6A (Baumgardt et al., 2009).

As each GMC continues to express the TF present in its mother cell, it is thought that this expression pattern influences the identity of the generated glial cells and neurons. Genetic loss and gain-of-function experiments performed within these lineages have provided the basis for the understanding of the temporal specification of NSCs (Grosskortenhaus et al., 2006; Isshiki et al., 2001).

Two features might suggest that **mammalian NSCs** use a cascade of TFs to control the fate of its progeny: **1/** the high heterogeneity of mammalian NSCs and, **2/** the ability of the same NSC lineage to generate different cell types over time. Apart from these two interesting properties, there is no convincing evidence that mammalian NSCs use the same mechanism as *Drosophila*. Interestingly, it was shown that Ikaros, the ortholog of the first TF in the temporal series "Hb" in flies, is necessary and sufficient to confer early temporal competence to retinal progenitor cells in mice (Elliott et al., 2008).

4. 1. 3. Neural stem cell mode of division

NSCs undergo multiple self-renewing divisions, a process that can take the form of symmetric cell division, whereby two equal NSCs are generated, or asymmetric cell division, which produces one NSC and one more daughter cell with more restricted developmental potential (Alvarez-Buylla et al., 2001; Temple, 2001). The proper balance between symmetric and asymmetric cell division is crucial for both, to maintain a population of NSC to replace damaged neural cells, and to prevent tumorous overgrowth.

- Symmetric cell division in *Drosophila*

In the *Drosophila* CNS, this mode of cell division is only described in the developing larval brain, where a pool of neuroepithelial cells initially divide symmetrically to expand the pool of proliferating cells, and than the generated NSCs divide asymmetrically to expand the pool of differentiating cells (Egger et al., 2007; Hofbauer, 1990). Several studies have shown that Notch is involved in the process of switching between symmetric and asymmetric division. The first investigation comparing the transcriptional profile of neuroepithelial cells and NBs revealed that transcripts from the Notch signaling pathway are preferentially expressed in neuroepithelial cells (Egger et al., 2010). In addition, neuroepithelial cell lacking Notch are extracted from the neuroepithelium and prematurely express the NB-specific marker, Dpn (Egger et al., 2010; Ngo et al., 2010; Orihara-Ono et al., 2011; Reddy et al.,

2010b; Wang et al., 2011; Yasugi et al., 2010).

- Asymmetric cell division in *Drosophila*

Asymmetric cell division of *Drosophila* NSCs was studied extensively and many components implicated in this process were identified. This type of division passes through three major steps: setting up cell polarity, orientation of the mitotic spindle, and asymmetric segregation of the cell fate determinants.

To perform asymmetric cells division, NSC must first set up the apical-basal axis. This cell polarity, in both embryonic and larval NSCs, is defined by the Par complex, which consists of atypical protein Kinase C, Partition-Defective 6 (Par6) and Bazooka (Petronczki and Knoblich, 2001; Rolls et al., 2003; Schober et al., 1999; Wodarz et al., 2000). The Par complex does not only specify the apical side but it ensures the correct mitotic spindle orientation as well as segregation of cell fate determinants to the basal cortex. To ensure that the cleavage plane of the cells must be orthogonal to the A-P axis formed by the Par complex. To act, Par coordinates with another complex, heteromeric G-protein complex, which consists of Partner of Inscuteable "Pins", G-Protein α subunit-i and Locomotion defects (Schaefer et al., 2000; Yu et al., 2000; Yu et al., 2005). Another protein, Inscuteabale, insures the interaction between the two complexes. Interestingly, larval brains mutant for any of the different genes required for asymmetric division, develop malignant neoplasms due to NBs overgrowth (Castellanos et al., 2008; Caussinus and Gonzalez, 2005).

The ultimate aim of setting cell polarity and aligning mitotic spindle to the apical-basal axis is to ensure that cell fate determinants will be in the basal side and be inherited only by the GMC. These cell fate determinants will inhibit the NSC fate and initiate differentiation (Cabernard and Doe, 2009). Two main groups of cell fate determinants and their adaptors were described. The first group is Numb and its adapter protein, Partner of Numb (Lu et al., 1998; Rhyu et al., 1994). The other group is Prospero and Brain Tumor (Brat) and their adapter protein, Miranda (Miraoui and Marie) (Bello et al., 2006; Choksi et al., 2006; Knoblich et al., 1995). Prospero is the most studied determinant. In the NSC Prospero is cytoplasmic, after division it enters the nucleus of the GMC to repress NSC specific markers, notably genes necessary for self-renewal, and activates genes necessary for the GMC fate (Bello et al., 2008). Indeed, in *prospero* mutant embryos, the GMC adopts the NSC fate (Choksi et al., 2006).

As noted above, GMCs divide only once to generate two post-mitotic cells. When a GMC divides, the cell fate determinant inherited from the NSC, are unequally segregated to

the daughter cells that will adopt different fates. Asymmetric GMC division was well characterized in both, SOP and several NSC lineages (Buescher et al., 1998; Rhyu et al., 1994; Skeath and Doe, 1998; Spana and Doe, 1996; Uemura et al., 1989). The first studied lineage in the VNC is the MP2 lineage, which divides only once to produce two MP2 neurons, the dMP2 and the vMP2. The asymmetric distribution of the protein Numb is crucial for the specification of these two neurons. Indeed, in *numb* mutant embryos, dMP2 neurons are transformed into vMP2 neurons (Spana et al., 1995). Numb action occurs through Notch signaling, since in *Notch; Numb* double mutant embryos MP2 daughter cells acquire the dMP2 fate (Spana and Doe, 1996). As for Numb, Inscuteable has also been found as an important player in asymmetric GMC division. In its absence, Notch fails to form a cortical crescent before mitosis and the mitotic spindle loses its A-P polarity, as a consequence the daughter cells fail to activate Notch depending fate (Buescher et al., 1998).

- Neural stem cell mode of division in mammals

The mechanisms which control *Drosophila* asymmetric NSC division were found to be highly conserved throughout the animal kingdom (Knoblich, 2008). For example, the neuroepithelial to NSCs transition in the *Drosophila* OL bears many similarities to the switch from self-renewing neuroepithelial cells to neurogenic RG cells during NS development in mammals (Farkas and Huttner, 2008; Miyata et al., 2004; Noctor et al., 2004b). In addition, investigations of mammalian cerebral cortex RG cells showed that cortical progenitor cells undergo both symmetric and asymmetric divisions (Chenn and McConnell, 1995; Noctor et al., 2001; Noctor et al., 2004b), that resemble *Drosophila* NSC mode of division. RG cells in this region undergo two types of asymmetric cell division (Noctor et al., 2004a) (Figure 11): one type is neurogenic, during which the RG cell generates a new RG cell and a daughter cell that differentiates into a neuron. This mode of division resembles the division pattern of embryonic *Drosophila* NSCs; the second type, which resembles some larval *Drosophila* NSC mode of division, Type II NSC, generates two daughter cells that both re-enter cell cycle, but one remains in the VZ whereas the other moves to the SVZ and divides only once to generate two neurons.

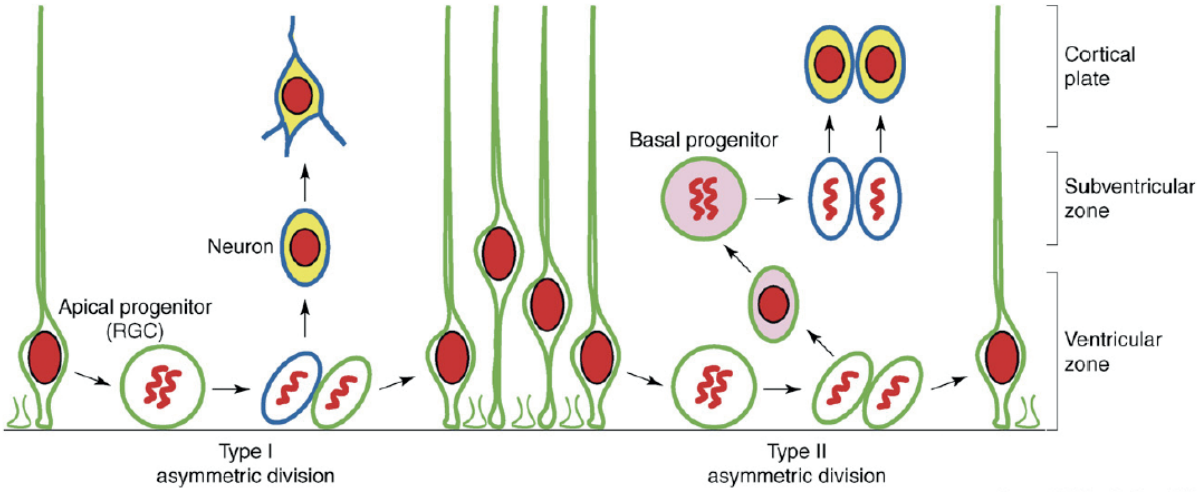

Figure 11.

Neural progenitor cells have been found to undergo at least two different types of asymmetric cell divisions during mammalian neurogenesis. In type I divisions, the RG cell produces another RG cell and a daughter cell that becomes a neuron. In type II, the RG produces two daughter cells that both re-enter the cell cycle (Zhong and Chia, 2008).

It was demonstrated that the majority of genes implicated in *Drosophila* asymmetric cell division are conserved in vertebrate. Despite that, the function of these proteins is only beginning to be elucidated (Doe, 2008; Gotz and Huttner, 2005; Knoblich, 2010; Williams et al., 2011). For example, in the mouse retina, Prox 1, ortholog of Prospero, is important for cell cycle exit and for neuronal differentiation (Dyer et al, 2003). Previously, it was shown that the asymmetric distribution of Numb influences Notch activity in *Drosophila* NSC lineages (Guo et al., 1996). Importantly, in RG daughter cells it was also demonstrated that Partitioning defective protein-3 (Par-3), ortholog of Bazooka, antagonizes the activity of Notch in RG daughter cells. Par-3 acts by promoting the segregation of ubiquitin ligase E3 Mindbomb, modulator of Notch ligands endocytosis, to the apical daughter (Dong et al., 2012).

Despite all these studies, the exact mechanism of how mitotic spindle alignment and asymmetric distribution of cell fate determinants in mammals remain unclear.

4. 1. 4. Neural stem cell quiescence

During *Drosophila* NS development, NSCs proliferate in the embryos to generate neurons and glia that drive larval behaviors. Once embryogenesis completed, most abdominal NSCs are eliminated by programmed cell death (Abrams et al., 1993; Peterson et al., 2002;

White et al., 1994), whereas most of the cephalic and thoracic NSCs enter mitotic quiescence at the embryo-larval transition (Truman, 1990).

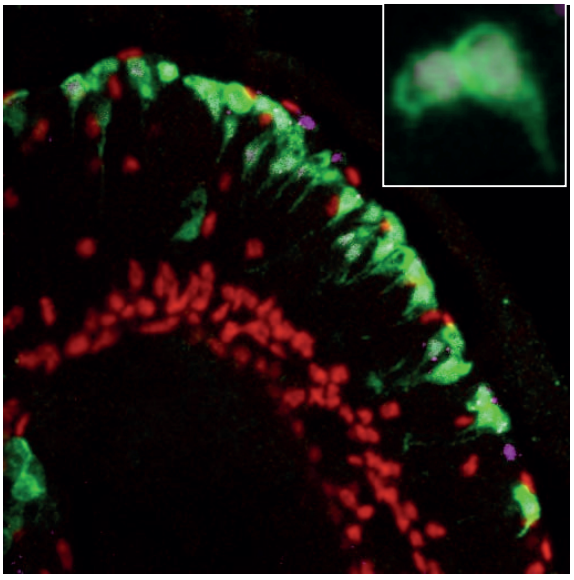

Figure 12. Quiescent *Drosophila* NBs are characterized by an elongated shape and by the presence of a cytoplasmic process moving inward.

A lateral view of the ventral cord of a 17-stage *Drosophila* embryo labelled with the nuclear glial marker anti-Repo (Red) and the NB markers, anti-Dpn (nuclei: magenta) and anti-Mira.
NBs with different mitotic potentials display specific shapes: a quiescent NB sends a long process (right) whereas a proliferating NB (left) has a round shape.

The timing of entry and exit from quiescence is not completely clear. One of the most important features of NSCs is the reduction of their size after each division, an event that seems to be relevant for entering and exiting the G0 phase. Another important singularity of quiescent NSCs is their typical morphology, quiescent NSCs present an elongated shape contrarily to the mitotic ones which are round. This characteristic can be established by immunostaining using anti-Mira antibody (Figure 12).

One of the mot important mechanisms that strongly affect entry into quiescence is the A-P position of the NSC in the CNS. For that it was suggest that **Hox genes** might be implicated on controlling NSC quiescence (Prokop et al., 1998; Truman and Bate, 1988; Tsuji et al., 2008). The role of these genes was demonstrated in the embryonic NB3-3 lineage. At the end of embryogenesis, the NB3-3T enters quiescence whereas the abdominal one continues to proliferate. Importantly, the Hox protein Antennapaedia promotes the NB3-3T to enter quiescence, whereas Abdomanal-A, another Hox protein, acts to prevent the NB3-3A from undergoing cell cycle arrest (Tsuji et al., 2008). The role of the **temporal TFs** series in entry into quiescence was also revealed in the same lineage. The NB3-3 does not express Hb at birth but does sequentially expresses Kr, Pdm and two bursts of Cas as it generates its embryonic lineage. For example, in *pdm* mutant embryos, the NB3-3T enters quiescence earlier. Conversely, loss of Cas function in the same NB inhibits entry into G0 phase (Tsuji et al., 2008). Thus NSCs entry into quiescence is regulated intrinsically by inputs from Hox

genes and the temporal TF series. Whether all NSC lineages adopt the same mechanisms to enter quiescence, is still to be demonstrated.

During larval development, quiescent NSCs exit the G0 phase and enter the proliferative phase to generate neurons of the adult CNS. Upon exit from quiescence, NSCs fist increase their size around two fold before restarting the cell cycle (Chell and Brand, 2010; Truman and Bate, 1988). It is known that this reactivation is regulated by extrinsic influences including nutrition, a glial-cell niche and several mitogenic signals, (reviewed by (Egger et al., 2008; Maurange and Gould, 2005; Sousa-Nunes et al., 2010))**.**

Regarding **nutrition**, it was shown that larval NSCs entered quiescence upon starvation while they were able to re-enter the cell cycle after feeding (Britton and Edgar, 1998). In the same study, it was also demonstrated that the crucial component required for this reactivation are amino acids (Britton and Edgar, 1998). Interestingly, transcriptome analysis of the *Drosophila* VNC revealed that the expression of the *Drosophila* insulin-like peptides (dILP2 and dILP6) parallels NSCs reactivation, and such expression is lost upon amino acid starvation (Chell and Brand, 2010). The *Drosophila* insulin/IGF (insulin-like factor)-like peptides are known to bind a single receptor, dInR (*Drosophila* insulin receptor), activating the PI3K/Akt (phosphatidylinositol 3-kinase/protein kinase B) pathway that leads to cellular growth and proliferation (reviewed in (Goberdhan and Wilson, 2003)). The same pathway was also demonstrated to be crucial for NSC reactivation (Chell and Brand, 2010; Sousa-Nunes et al., 2011). Indeed, the constitutive activation of PI3K/Akt signaling in quiescent NSCs is able to reactivate them in the absence of dietary proteins.

Glial cells seem to be implicated in both, NSCs entry and exit from quiescence. This evidence comes from the study showing that blocking the cell adhesion molecule E-Cadherin, either in glia or NSC, reduces neural proliferation in the CB (Dumstrei et al., 2003). This implication of E-Cadherin in NSC cell cycle regulation could result from effects on exit from quiescence and/or NSC division. Remarkably, it was demonstrated that the *dILP6* promoter drives expression in a set of surface glial cells overlying the NSCs, stelatte glia, suggesting that these glial cells might be the source of the signal that reactivates NSCs (Chell and Brand, 2010). Interestingly, forced expression of insulin/IGF-like peptides in glia is sufficient to drive NSC proliferation in the absence of dietary protein, whereas disrupting vesicle trafficking in glia reduces NSC reactivation (Chell and Brand, 2010; Sousa-Nunes et al., 2011). Therefore, NSCs exist the quiescence phase seems to be regulated by signals coming from the surrounding glial cells (Figure 13).

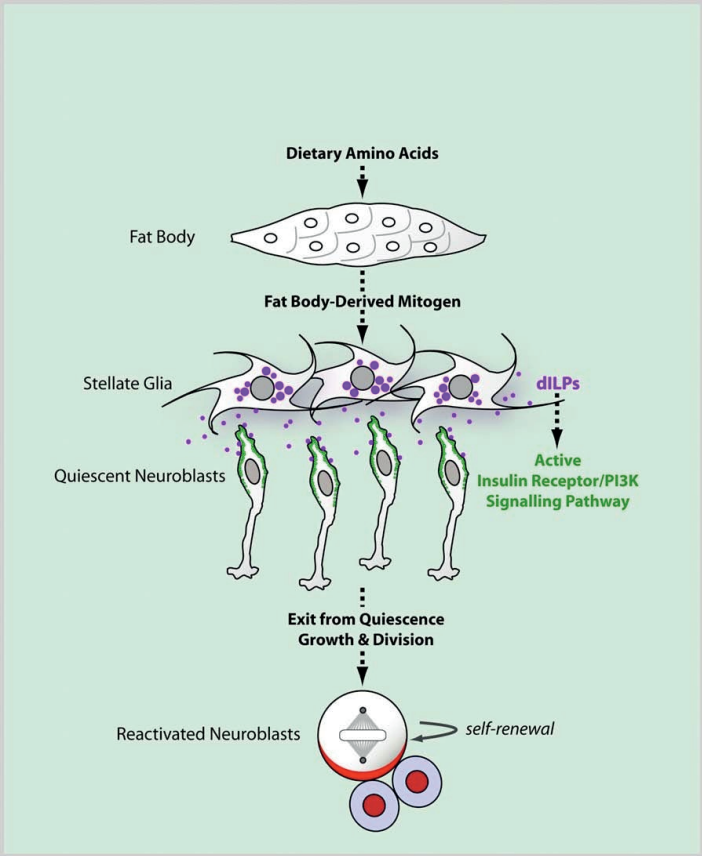

Figure 13. A model for the nutritional control of quiescent NB reactivation.

(Andrea and al., 2011). For more details see the text.

Using co-culture experiments, the role of **fat body**, a *Drosophila* nutrient-sensing organ with adipose and liver like functions, by diffusing mitogenic signals that trigger NSC reactivation was revealed (Britton and Edgar, 1998; Colombani et al., 2003). The nutrient-sensitive TOR (target of paramycin) signaling pathway in the fat body is required to emit this signal, once amino acids are transported into fat body cells by Slimfast (Goberdhan and Wilson, 2003; Sousa-Nunes et al., 2011). Downstream of this signal, Insulin like receptor signaling and the PI3K/TOR network are required in NSCs for exit from quiescence (Figure 13).

Neurogenesis in *Drosophila* is highly patterned along the A-P axis (reviewed by (Maurange and Gould, 2005)). In the CB and in the thorax, NBs continue dividing into the pupal stages (Bello et al., 2003; Truman and Bate, 1988). Whereas in the abdomen, NBs stop dividing in the larva some 2 days earlier, producing less than 12 progeny each (Bello et al., 2003; Truman and Bate, 1988). Interestingly, it was demonstrated that the **temporal TFs series** that continues during post-embryonic neurogenesis, schedules the timing of cell cycle exit, *via* a burst of nuclear **Prospero** (Maurange et al., 2008).

Since the origin of **mammalian** adult NSCs remains controversial, the mechanisms

underling embryonic NSC entry into quiescence is poorly understood and much more importance was given to understand the reactivation and entry into cell cycle arrest in the adult NSCs. The first shared feature between *Drosophila* and mammalian NSC is the increase in the cell size, as it was demonstrated in the mode of NSCs exist from quiescence in the developing mammalian cortex (Alam et al., 2004; Groszer et al., 2006).

Adult NSCs exist primarily in a quiescent state in the adult NS, but can shuttle between quiescent and activated states, or exist as a relatively stable mitotic population (Bonaguidi et al., 2011; Encinas et al., 2011; Lugert et al., 2010). It remains unclear which mechanisms distinguish between return to quiescence and retention in mitosis. However, if we take in consideration the environment of the NSCs, the niche, several similarities with *Drosophila* can be observed. The major cell types of the niche are: endothelial cells, astrocytes, ependymal cells, microglia, mature neurons, and adult NSCs (Figure 9, (Ming and Song, 2011)). It was shown that the regions where the niche resides are highly vascularized, and strong contacts between the neural precursors and the blood vessels occur (Palmer et al., 2000; Shen et al., 2008; Tavazoie et al., 2008), suggesting that blood derived cues are gaining direct access to the adult neural precursors and to their progeny. The blood may serve as a source of nutriments and molecules that control NSC proliferation, which resembles the role of nutriments in *Drosophila* NSC proliferation. Insulin and insulin-like growth factors are important regulators of growth and metabolism. The insulin/IGF pathway is well conserved from invertebrates to mammals (Speder et al., 2011). Interestingly, astrocytes, which are known to be implicated in NSC proliferation (Song et al., 2002), express the pro-proliferative factors fibroblast growth factor-2 (FGF-2) and IGF-1 (Goberdhan and Wilson, 2003; Shetty et al., 2005). IGF-1- expression is induced in stellate astrocytes (astroglia) (Yan et al., 2006; Ye et al., 2004) in response to CNS injuries and is believed to account for the rise in NSC division following cortical ischemia (Yan et al., 2006; Ye et al., 2004).

Altogether, these observations show that the organization of the *Drosophila* NSC "niche" shares attractive similarities with the mammalian one. It will be interesting to investigate how niche cells relay various environmental cues and molecular signaling mechanisms that regulate precursor exit from and return to quiescence. Since the TOR pathway is highly conserved in mammals, it is likely that this pathway controls the same processes that were observed in the *Drosophila* NSCs.

4. 1. 5. Neural stem cell apoptosis

The pattern of embryonic NSC segregation are identical in thoracic and abdominal segments of the VNC (Doe, 1992). However, from the 30 NSCs initially present in each abdominal hemisegment, only three survive into larval life, and these remaining NSCs undergo apoptosis in the larvae (Prokop and Technau, 1991; Truman and Bate, 1988; White et al., 1994). The mechanisms controlling this event are poorly understood.

NSC death is controlled by the activation of pro-apoptotic proteins, Reaper (Rpr), Hid, and/or Grim (RHG) (Bello et al., 2003; Peterson et al., 2002). The RHG proteins act by inducing the caspase-mediated cell death. Loss of Rpr causes extensive hyperplasia due to the presence of ectopic postembryonic NSCs, which proliferate and give rise to extra neurons (Peterson et al., 2002).

NSC apoptosis seems to be regulated by homeotic genes in the embryo and in the larva (Prokop et al., 1998). Indeed, the misexpression of the homeotic gene, Abdominal-A (Abd-A), in the thoracic NSCs induces a drastic decrease in the number of NSCs surviving until the larval stage. However, its mutation maintains the proliferation of abdominal NBs until the larval stage. AbdA is also implicated in regulating larval NSC apoptosis (Bello et al., 2003). The competence of the NSC to undergo this particular AbdA response requires a parallel input from the Grh TF (Almeida and Bray, 2005; Cenci and Gould, 2005).

It seems that similar pathways control apoptosis and quiescence but that slight differences caused by extrinsic factors in addition to the identity of the cell determines whether the cell enters apoptosis or quiescence.

4. 2. Epigenetic regulation of neurodevelopmental plasticity

4. 2. 1. Chromatin organization

The term “chromatin” is used to describe the functional state of eukaryotic genomes, which corresponds to a complex of DNA, histones and non-histone proteins in the cell nucleus. The basic building block of chromatin is the nucleosome, which contains approximately 157 bp of DNA, wrapped around an octamer of the four core histones (H2A, H2B, H3 and H4). Nucleosomes are connected with linker DNA and the resulting structure is further compacted into 30 nm fibers through interaction with linker histones. The higher order chromatin structures are formed upon folding of the 30 nm fibers. Despite this high level of compaction, eukaryotic chromatin is highly dynamic and allows access to the DNA during various essential cellular processes such as DNA replication, DNA repair, and transcription.

Chromatin based epigenetic modifications that affect the transcriptional process are executed *via* different mechanisms such as DNA methylation, histones post-translational modifications, and non-coding RNAs, chromatin remodeling, and incorporation of histone variants. These processes acting alone or in concert combinations allow conformational changes in chromatin structure. They are called "epigenetic regulations" because they dynamically alter the gene activity by modulating protein-DNA interactions, without changing the gene sequence.

Given the purpose and the scope of my thesis work, I mainly discuss histone acetylation and methylation.

4. 2. 2. Histone modifications

Histones are subjected to a variety of post-translational modifications such as acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP ribosylation, deamination and proline isomerization. The most important histone modifications include acetylation and methylation. Acetylation and methylation typically take place on lysine (K) residues, although methylation may also target arginine (R) residues.

- Acetylation/Deacetylation

Acetylation involves the addition of acetyl group(s) to histone and non-histone proteins, and specific acetylation tags, acting alone or in concert, produce distinct outcomes. The balance between the acetylation and deacetylation of these proteins controls gene expression and a variety of cellular processes. Therefore, the aberrant activity of acetytrasferases **(HATs)** and deacetylases **(HDACs)**, the enzymes that catalyze the acetylation and deacetylation of proteins, respectively, was often implicated in several human diseases.

Histone acetylation regulates many cellular processes: **1/** the acetylation of newly synthesized histones is important for their nuclear import and assembly into the nucleosome through the histone chaperones (Tyler et al., 1999; Verreault et al., 1996). In accordance with this idea, *Drosophila* Asf1, a histone chaperone, was found in association with histone H3 that is acetylated at K14 and histone H4 that is acetylated at K5 and K12, a pattern identical to that of newly translated histones in this organism (Tyler et al., 1999), **2/** histone acetylation, such as the acetylation of histone H4 on K16, is essential for the degree of chromatin compaction and folding (Shogren-Knaak et al., 2006), **3/** this modification also regulates the

formation of heterochromatin; indeed, deacetylation of H4 at K16 was shown to be important for amplifying heterochromatin components, whereas acetylation of these sites serves as a barrier to this spreading (Liou et al., 2005), and **4/** it is critical for gene transcription. For example, the acetylation of histone H3 at K9 (H3K9ac) is generally associated with actively transcribed regions (Bhaumik et al., 2007). Interestingly, recent results suggest that it is not the high levels of acetylation that are most important for transcription but rather a high level of acetylation, coupled with rapid deacetylation, (reviewed by (Shahbazian and Grunstein, 2007)), hence, showing that both events, acetylation and deacetylation, are crucial for ensuring appropriate levels of gene transcription.

1) Histone acetyltransferases

HATs are characterized by their ability to acetylate (HAT activity) histone and non-histone proteins, and by their ability to form multi-protein complexes (molecular scaffolding) by recruiting different elements of the transcriptional machinery. HATs catalyze the transfer of acetyl groups from the acetyl-coenzyme A (acetyl-CoA) cofactor to the ε-amino group or the α-amino group of specific K residues in histones and non-histone proteins (Hodawadekar and Marmorstein, 2007; Marmorstein and Roth, 2001).

To date, at least three main families of HATs were described (Sterner and Berger, 2000). They include the **GNAT** superfamily (Gcn5-related N-acetyltrasferases), the **MYST** family (named from its founding members: MOZ,Ybf/Sas3, Sas2, Tip60) and the **p300/CBP** family (protein of 300 kDa and CREB (cAMP response element-binding) binding protein). **Nuclear receptor co-activator**, **TATA binding protein (TBP)-Associated Factor TAFII250** (TFIID 250 kDa)**,** and **TFIIIC** were also described to carry out a HAT activity. All known HATs contain a catalytic domain (called HAT domain), which varies in size between families and appears in association with different sets of effector modules (Marmorstein, 2001).

GNAT family

This group includes the HATs similar to GCN5 (General Control nonderepressible-5) and PCAF (p300/CBP associated factor) (Vetting et al., 2005).

First isolated in *Tetrahymena,* **Gcn5** was the first to be identified as a transcriptional related HAT (Brownell and Allis, 1996; Brownell et al., 1996). *In vitro*, recombinant Gcn5 was found to strongly acetylate histone H3 at K9, K14 and K18, but weakly acetylate histone H4 at K8 and K16 (Kuo et al., 1996). This HAT can efficiently acetylate free histones, but it

is unable to acetylate nucleosomal histones (Grant et al., 1997; Kuo et al., 1996; Ruiz-Garcia et al., 1997), except under special conditions and at high enzyme concentrations (Tse et al., 1998). Only in the presence of multi-subunits native HAT complexes such as SAGA (Spt-Ada-Gcn5-acetyl-transferase) and ATAC (Ada two A containing complex), Gcn5 is able to effectively acetylate nucleosomes, indicating that the influence of other proteins is required to confer this activity. *Gcn5* is conserved from yeast to human, and only one ortholog of yeast *Gcn5* was described in *Drosophila*, *dGcn5* (Smith et al., 1998) that is able to acetylate histone H3 at K9 and K14 (Carre et al., 2005). *dGcn5* mutation induces abnormal pupae development (Carre et al., 2005). In the same study, it was also shown that *dGcn5* is important for cell proliferation in wing imaginal discs. In mammals, both SAGA and ATAC complexes are required for normal embryonic development, and *Gcn5* deletion induces embryonic lethality (Bu et al., 2007). One of the most neurogenic defects in mouse carring a dead Gcn5 HAT activity is a defect in the neural tube closure (Bu et al., 2007; Lin et al., 2008). Another important role of Gcn5 was recently described in mouse models of the Spinocerebellar ataxia type 7. This disorder is a neurodegenerative disease caused by an expansion of a CAG repeat in ATXN7, a component of the SAGA HAT complex, which results in the formation of a polyglutamine tract. Interestingly, it was recently reported that partial loss of Gcn5 functions accelerates both cerebral and retinal degeneration (Chen et al., 2012). In addition, it was also shown that Gcn5 plays an important role in Purkinje cell, a class of GABAergic neurons located in the cerebellar cortex, functions as Gcn5 depletion in these cells leads to mild ataxia (Chen et al., 2012).

PCAF is the homologue of Gcn5 (73% similarity), and is known to interact with p300/CBP (Yang et al., 1996). In a study using a *Drosophila* model of Huntington's disease (HD), a neurodegerative disorder caused by the of polyglutamine tract in the affected protein, authors showed that the aberrant protein, binds to p300/CBP and PCAF and inhibits their activities, leading to a decreased level of histone acetylation (Steffan et al., 2001). Interestingly, the reduced levels of PCAF in HD led to increased degeneration of photoreceptor neurons in the retina (Bodai et al., 2012). Thus, in order to develop methods to intervene in this devastating disease, it becomes crucial to identify how HATS levels increases neuronal cells degeneration.

HAT1 and **HAT2** also belong to the GNAT family. They are known to acetylate newly synthesized histones before their incorporation into the chromatin. In addition, they seem to be implicated in the telomerase regulation (Mersfelder and Parthun, 2008; Verreault,

2000). The last described member of the GNAT family is **ELP3 (**Elongation Protein 3), which was first isolated from yeast (Wittschieben et al., 1999), and conserved through evolution. ELP3 associates with RNA polymerase II and plays an important role in the process of transcriptional elongation.

MYST family

HATs of this family are involved in diverse cellular functions. Tip60 is implicated in DNA repair, cell cycle regulation, apoptosis, and in the activation of some genes (Sapountzi et al., 2006). Ybf2/Sas3 and SAS2 are involved in transcriptional repression, including HML (Hidden MAT Left, a silent mating cassette) loci in yeast, and in maintaining the structure of the heterochromatin of telomeres in yeast (Carrozza et al., 2003). HBO1 (HAT binding to ORC-1), the major HAT of histone H4 *in vivo* on K5 and K12, is present in a complex that is important for the pre-initiation of DNA replication (Miotto and Struhl, 2008, 2010). MOF (Male absent on the first) is known to acetylate the histone H4 at K16 (Smith et al., 2000). This HAT is particularly involved in a phenomenon known as dosage compensation in *Drosophila*, which involves doubling the transcription of genes on the X chromosome in males to compensate the second chromosome present in females (Kind et al., 2008). MOZ (Monocytic leukemia zinc finger protein) and MORF (MOZ-related factor) HATs are highly similar, important for various developmental programs, and are involved in translocations and tumorigenesis process (Yang and Ullah, 2007).

p300/CBP family

CBP and p300 proteins were characterized for the first time at the beginning of 1990, through their interaction with the TF CREB and the adenoviral oncogenic protein E1A, respectively (Chrivia et al., 1993; Eckner et al., 1994). CBP and p300 are usually represented as protein pair CBP/p300 because they share 91% sequence identity and are thought to be functionally equivalent (Arany et al., 1994), Figure 14. Orthologs of CBP and p300 are present in multicellular organisms such as flies, worms and plants. *Drosophila* CBP (dCBP), also called Nejire (Nej), is around 79% similar to human CBP.

CBP/p300 act as transactivation domains and contain modules for protein interaction: a nuclear receptor interacting domain, two cysteine-histidine (CH)-rich domains (CH1 and CH3), a KIX domain (or CREB binding domain) and a glutamine/proline (QP)-rich domain comprising the IBID domain (IFR3-binding domain). The central region of CBP/p300 represents the catalytic core of the protein and contains the HAT domain and two effector

modules: a BRD and a PHD (or CH2) domain (Bedford et al., 2010; Chan and La Thangue, 2001) and (Figure 14). The KIX domain is known to bind the transactivation domain of CREB and other nuclear factors to regulate target gene expression.

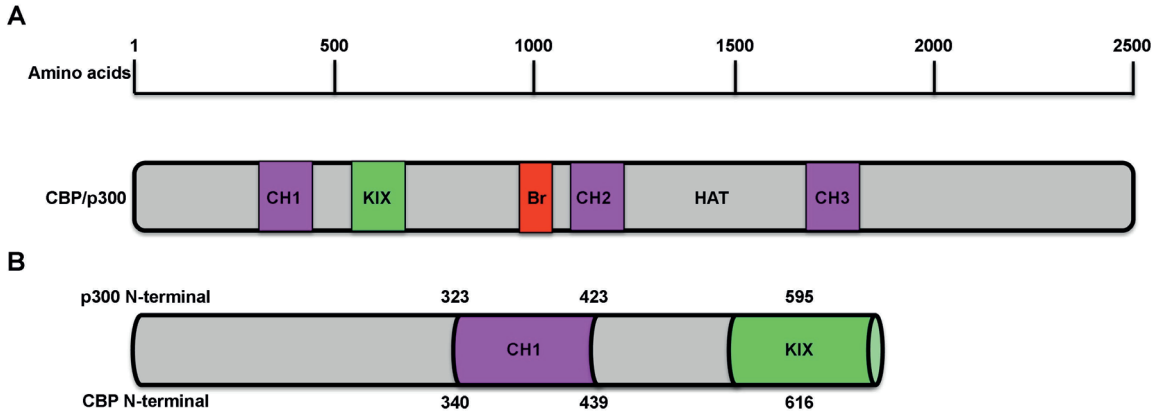

Figure 14. Representation of the different functional domains of CBP and p300.

(A) Localization and size of the functional domains: cysteine/histidine-rich domains CH1, CH2 and CH3, KIX domain, bromodomain (Br), and histone trasferase dmain (HAT). **(B)** The N-terminal of CBP and p300 are indicated. The size of each protein or domain is indicated in number of amino acid residues.

Different roles of CBP

CBP/p300 HAT is an important transcriptional coactivator, which is involved in a wide range of biological processes including DNA transcription, development, the innate immune response and cell cycle regulation (Bedford and Brindle, 2012; Chan and La Thangue, 2001; Goodman and Smolik, 2000). Three major mechanisms of transcriptional activation were described for CBP/p300: **1/** transcriptional activation though histone and non-histone proteins acetylation, **2/** acting as a multivalent scaffold to recruit other cofactors or to allow the assembly of multiprotein complexes, **3/** and serving as a bridge to connect sequence-specific TFs to the components of the basal transcriptional machinery (Chan and La Thangue, 2001).

The primary function of CBP/p300 is to act as a **cofactor for the transcription** of many nuclear proteins. In fact, CBP/p300 protein is essential for the activity of at least forty different TFs. CBP/p300 interacts with the basal TFs TBP and TFIIB and/or forms a complex with the RNA polymerase II (Cho et al., 1998; Yuan et al., 1996). It is also able to interact with known oncogenes (Myc, Jun, Fos), transforming viral proteins (E1A, E6, Tax) and tumor suppressor proteins (p53, E2F, Rb (Retinoblastoma), Smads, RUNX and BRCA1)

(Bannister et al., 1995; Chan and La Thangue, 2001; Eckner et al., 1994). The transcriptional coactivation activity of CBP/p300 is mediated by an intermediary function between the TF binding to the DNA and the transcription machinery.

The second important aspect of CBP/p300 function as a coactivator is related to their ability to **acetylate the nucleosomal histones**, located at gene promoters, allowing easier access to the transcriptional machinery and the recruitment of chromatin remodeling factors. In 1996, two groups demonstrated that CBP/p300 harbors a HAT activity (Bannister and Kouzarides, 1996; Ogryzko et al., 1996), which is primordial. Indeed, the heterozygote inactivation of the catalytic domain (HAT) induces embryonic lethality in mice (Shikama et al., 2003), as well as in *Drosophila*.

Unlike other HATs, which have limited specificity for substrates, CBP/p300 is able to acetylate the four core histones *in vitro*, on different residues: H2A on K5, H2B on K5, K20, K12, K15; H3 on K14, K18, K23, and H4 on K5, K8 and K12 (McManus and Hendzel, 2003; Schiltz et al., 1999). Liu et al. have characterized the structure of the p300/CBP HAT domain, and apart from the central region for the interaction of acetyl-CoA, CBP/p300 differs significantly from the other HAT families (Liu et al., 2008). Interestingly, a structural homology between CBP/p300 HAT domain and the yeast protein RTT109 was described (Tang et al., 2008). RTT109 promotes genome stability and allows the resistance to DNA damaging agents, through its direct acetylation of histone H3 on K56 during the S-phase of the cell cycle. Subsequently, it was demonstrated that CBP/p300 also acetylates H3 at K56, a modification that correlates with genome stability in mammals and flies (Das et al., 2009; Yuan et al., 2009). This histone modification, and that of H3 at K18 appear to be specific for the HAT activity of CBP/p300 (Ferrari et al., 2008). In addition, other studies demonstrated that CBP/p300 is crucial for the acetylation of H3 on K18 in human cultured cells (Horwitz et al., 2008), on K9 and K14 in mice brain (Wang et al., 2010), and acetylation of H4 on K8 in *Drosophila* embryos (Ludlam et al., 2002). The acetylation of H3 on K27 by CBP was also reported, and is conserved from yeast to human (Garcia et al., 2007; Suka et al., 2001; Tie et al., 2009). Interestingly, *via* this histone modification, CBP is able to prevent the gene repression mediated by Polycomb (Pc) group (PcG) activity (Petruk et al., 2001; Tie et al., 2012; Tie et al., 2009). PcG proteins are epigenetic regulators that maintains gene silencing by inducing the methylation of histone H3 at K27 (H3K27-me3), (see Introduction 5. 2. 2. 3).

In addition to histones, CBP/p300 is also able to **acetylate many non-histone proteins.** The other well known substrates of CBP/p300 include the “genome guardian” p53

protein, c-Myb, MyoD, GATA-1, p73 and E2F1 (Boyes et al., 1998; Costanzo et al., 2002; Grossman, 2001; Martinez-Balbas et al., 2000; Polesskaya and Harel-Bellan, 2001; Tomita et al., 2000). Remarkably, CBP/p300 also mediates the acetylation of GCMa, the ortholog of Gcm in humans, acetylation, a modification that induces its stability, and thus increases its transcriptional activity (Chang et al., 2005). Whether CBP/p300 also has a role in regulating the stability and/or the transcriptional activity of *Drosophila* Gcm remains to be determined.

Interestingly, protein acetylation can have two opposing effects, and p53 effectively illustrates this feature, since CBP/p300 acetylates it on many K residues (K370, K372, K373, K381, and K382) and these modifications increase its association with DNA *in vivo* (Luo et al., 2004). Inversely, the acetylation of K residues on HMG(I)Y on its DNA binding domain reduces its transcriptional activity (Munshi et al., 1998). In addition to the above-mentioned effects, acetylation may also affect the protein-protein interactions. For example, the acetylation of the tumor suppressor Rb increases its interaction with the ubiquitin ligase MDM2 (Nguyen et al., 2004).

The presence of more than a hundred interacting partners of CBP/p300, indicates the possible involvement of CBP/p300 in different interconnected signaling pathways. For example, CBP/p300 is both able to stabilize p53 through its acetylation in the nucleus, thus allowing the signaling cascade of p53-dependent apoptosis. On the other hand, CBP/p300 can also induce p53 degradation by stimulating poly-ubiquitination *via* MDM2 in the cytoplasm (Grossman et al., 2001). Thus, CBP/p300 is a multifunctional regulator of p53, and its opposing actions on p53, can be explained by its **ubiquitin ligase activity**, that seems to be cytoplasmic, and the acetylation function that only occurs in the nucleus (Shi et al., 2009). Since, CBP/p300 is able to acetylate a number of factors, it would be interesting to determine whether it is implicated in other interconnected pathways by regulating the protein levels.

CBP is also known to play an important role in the proper function of many complexes, such as trithorax group (TrxG), (see Introduction 5. 2. 2. 3.). CBP/p300 is involved in various cellular processes such as cell cycle regulation *via* its interaction with the complex CyclineE/Cdk2, and also plays a role in DNA replication and repair. In addition, CBP/p300 is involved in **transcriptional repression** in some cases, including that of c-Myc gene, following the cooperation with the HDAC3 (Sankar et al., 2008).

Consequence of p300/CBP dysfunction

Because of its crucial role in many cellular functions, deregulation of CBP/p300 is likely the cause of many human diseases. The archetype human disorder associated with

CBP/p300 is the Rubinstein-Taybi (RTH) syndrome, a congenital developmental disease caused by heterozygous mutations of CBP/p300 in the germline, characterized by growth impairment, mental retardation, as well as distinctive facial and skeletal anomalies (Cantani and Gagliesi, 1998). Interestingly, RTH patients have an increased susceptibility for tumor development (Iyer et al., 2004; Miller and Rubinstein, 1995). However, at present the detailed molecular mechanisms of such effects are not known. In *Drosophila,* as well as in mice CBP/p300 mutation is embryonic lethal (Tanaka et al., 2000; Yao et al., 1998).

Concerning CBP/p300 and its role in the NS, it was reported that its sequestration is the major cause of neurotoxicity in neurodegenerative diseases (Janknecht, 2002). Several studies support this concept starting from *Drosophila* to humans. *dCBP* mutation affects the migration of embryonic peripheral glial cells (Schmidt et al., 2011), as well as the eye development at several stages, including eye determination and photoreceptor cell specification (Kumar et al., 2004). It was later suggested that dCBP effect on the eye implicated it in the EGFR (epidermal growth factor receptor) pathway since mutations of members of this cascade or CBP led to near-identical phenotypes in this tissue. More importantly, a screen aiming to identify CBP targets revealed a direct interaction between CBP and this signaling pathway (Anderson et al., 2005). Although the role of HATs in the aging process is poorly understood, it is known that CBP depletion in *C. elegans* blocks the lifespan extension induced by dietary restriction. Besides, the hypothalamic expression of CBP is remarkably reduced in aging mice (Zhang et al., 2009). Accordingly, CBP/p300 are receiving growing attention as potential therapeutic targets for the treatment of age-related pathologies. CBP is involved in many other processes, including circadian clock regulation, learning and memory, as well as synapse formation (Hung et al., 2007; Marek et al., 2000; Yin et al., 1994). However, the exact molecular mechanisms underlying these different effects are poorly understood.

In addition to the above mentioned consequences, CBP/p300 is also involved in many cancers, following chromosomal translocations of CBP gene, for example, or by abusive use of its function by the oncogenic viral protein E1A (Frisch and Mymryk, 2002). Indeed, CBP/p300 sequestration by E1A results in transcriptional repression of factors normally using p300/CBP as a coactivator (Gallimore and Turnell, 2001). In the context of HTLV-1 virus integration to the genome, it was shown that CBP/p300 was able to promote hyperacetylated histone eviction, and disassembly the nucleosomes near the promoter of the virus. Indeed, when complexed to CREB, the viral protein Tax, responsible for malignant transformation,

with the help of CREB recruits CBP/p300 to the viral promoter, and together with the chaperone NAP1 (nucleosome assembly protein 1) activates transcription by destabilizing nucleosomes locally (Sharma and Nyborg, 2008).

2) Histone deacetyltransferases

HDACs mediate the removal of acetyl-groups from the ε-amino group of K side chains (Hodawadekar and Marmorstein, 2007). Based on sequence similarity and cofactor dependency, HDACs were subdivided into four different classes and two different families. The classical HDAC family is composed of the class I HDACs, including the yeast Rpd3 (Reduced potassium dependency 3) orthologs (HDAC1, -2, -3, -8). The class II corresponds to yeast Hat 1 orthologs, comprising HDAC4, -5, -6, -7, -9, -10, and the class IV is related to human HDAC11. The different classes share sequence similarity within their catalytic domain and require Zn2+ ion as a cofactor for the enzymatic activity. The Sirtuin family contains members of the class III HDACs; which are the yeast Sir2 (Silent information regulator-2) orthologs. Sirtuins do not share sequence homology with the members of the classical HDAC family and use nicotinamide adenine dinucleotide (NAD+) as a cofactor for catalysis (Hodawadekar and Marmorstein, 2007; Yang and Seto, 2007).

Class I HDACs: This class has a highly very conserved catalytic domain (Khochbin et al., 2001). HDAC1 and HDAC2, Rpd3 in *Drosophila* and yeast, represent the catalytic subunits of several conserved transcriptional repressor complexes, including Sin3, NuRD (Nucleosome Remodeling and Deacetylase) and CoREST (co-repressor for element-1-silencing TF) (Knoepfler and Eisenman, 1999; You et al., 2001). In *Drosophila*, the **Sin3** (Switch independent3) complex is required in the absence of activation signals to repress transcription of specific genes within the active domains (Pile and Wassarman, 2000). The **NuRD** complex is known to play a role in the initiation and the maintenance of gene repression by modifying chromatin structure. This is accomplished by first deacetylating histones *via* Rpd3 and then remodeling the chromatin in an ATP dependent manner, *via* the helicase-like ATPases Mi-2 proteins, in order to initiate and maintain the gene repression. Interestingly, it was revealed that Tramtrack (Ttk69), the product of one of Gcm immediate targets and that is involved in neuronal fate suppression, is able to recruit NuRD to its direct target genes (Reddy et al., 2010a), suggesting that Ttk69 may use this mode of action to repress the neuronal genes. Indeed, Mi-2 was identified as an interacting partner of Ttk69, and the two proteins co-localize on many loci on polytene chromosomes (Murawsky et al., 2001).

In addition, **CoREST** was initially identified as a co-repressor of REST/NRSF (for RE1 silencing TF/neural-restrictive silencing factor), another repressing protein (Andres et al., 1999). It was recently shown that REST and CoREST interact with the TF PC4 (positive coactivator 4), and PC4 or REST inactivation induces neuronal gene activation, showing that REST and CoREST act together with PC4 to maintain neuronal gene repression (Das et al., 2010). **HDAC3** was not found within the complex involving HDAC1 and HDAC2, but as part of the core complex of N-CoR (Nuclear receptor corepressors) and SMRT (silencing mediator for retinoid and thyroid receptors) complexes (Khochbin et al., 2001). Interestingly, it was demonstrated that both, HDAC1 and HDAC3, are able to deacetylate GCMa, and HDAC3 was found to regulate the CBP mediated transcriptional activity of GCMa, indicating that CBP and HDACs act together to regulate GCMa activity (Chuang et al., 2006). This work remarkably underlies the functional importance of HDACs on non-histone substrates. HDAC3 is conserved in *Drosophila* (Hdac3). Whether this HDAC plays any role in regulating Gcm activity is not yet established.

Class II HDACs: The members of this class are characterized by their bigger size compared to class I HDACs. They are part of complexes that modulate the repressive effect of TFs such as Myocyte enhancing factor 2 (MEF2) (Khochbin et al., 2001; Yang and Gregoire, 2005). Two HDACs of this class, HDAC-4 and HDAC-5, were described to be implicated in GCMa deacetylation, in cultured placental human cells (Chuang et al., 2006).

Class III HDACs: Class III HDACs (also called Sirtuins) are involved in establishing and maintaining the repressive structure present at the telomeres. In addition, the founder of this protein class, Sir2, is involved in maintaining genome integrity and the process of DNA repair, and it also affects chromatin silencing and life activity, in yeast (Imai et al., 2000). Finally, it has been shown that SIRT6 (Sirtuin type protein 6) specifically deacetylates H3K9ac at the telomeres and H3K56ac during the cell cycle (Michishita et al., 2008; Michishita et al., 2009; Mostoslavsky et al., 2006).

In vivo, dCBP and dSir2 were found in the same immune-complex, and it was suggested that dCBP might affect dSir2 functions, but not during the formation and/or function of heterochromatin, because *dCBP* mutation does not affect this process (Smolik, 2009). Indeed, dCBP is implicated in gene repression inserted in the heterochromatin regions through its acetylation of dSir2 (Figure 15) (Zhao et al., 2009). This implies that there is a dynamic equilibrium between CBP/p300 mediated acetylation and Sir2/SIRT1 mediated deacetylation on some regions in the chromatin. As to aging and HDACs, dSir2

overexpression induces lifespan extension (Rogina and Helfand, 2004), while SIRT6 deletion in mice causes reduction of the lifespan extension (Michishita et al., 2008; Michishita et al., 2009; Mostoslavsky et al., 2006).

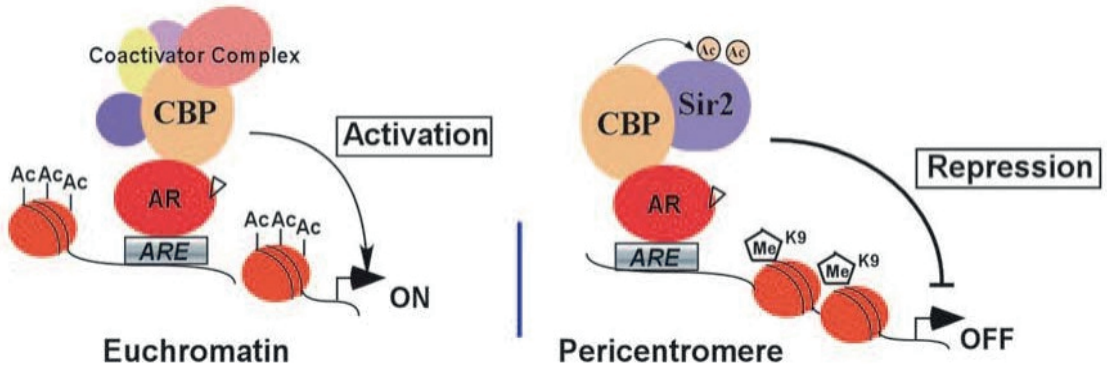

Figure 15. Schematic illustration of CBP coregulator functions depending on the chromatin contexts.

Based on the chromatin states, various transcription factors or coregulator complexes may be recruited to perform different functions in the euchromatic and pericentric regions. In the heterochromatic context, CBP and Sir2 recruited to AR target genes suppress the AR-mediated transactivation. A possible explanation for the repressive effect of CBP at the condensed environment is that CBP contributes to epigenetic silencing by acetylating chromatin proteins, such as Sir2, rather than histone. The CBP-mediated acetylation enhanced Sir2 HDAC activity and led to consecutive histone deacetylation (Zhao et al., 2009).

- Methylation/demethylation

Methylation is a relatively stable epigenetic mark (Trojer and Reinberg, 2006). The incorporation of a methyl group on histones is a complex phenomenon because K residues can be mono-, di- or tri-methylated symmetrically or asymmetrically.

Histones methylation is carried out by histone methyltrasferases (**HMT**): K residues are methylated by K methyltransferases (**PKMT**s) and R residues are methylated by the R methyltransferases (**PRMT**s). The major catalytic domain of these enzymes is the SET (Su(var)3-9, Enhancer-of-zeste, Trithorax) domain. The specificity and the number of added methyl groups depend on the nature of the enzyme but also on its cofactors. For example, the PKMT ESET induces H3K9 dimethylation when it acts alone, but induces the trimethylation of the same residue when it acts together with MAM (methyl methacrylate) (Su and Tarakhovsky, 2006).

In addition to K20 which is specific for histone H4, histone H3 and H4 can be mono-, di-, or tri-methylated in the following K residues: K4, K9, K27, K36 or K79. Unlike

acetylation, methylation may characterize active or silent chromatin. In fact, methylation of H3 on K4, K36 or K79 is generally implicated in transcriptional activation, as well as for trimethylated H3 on K4 and K36, which is involved in transcriptional elongation. However, methylation of H3 on K9, K27 or K20 is associated with gene repression (Kouzarides, 2007). Accordingly, trimethylation of H3 on K9 and K20 is involved in constitutive heterochromatin formation (Hediger and Gasser, 2006; Regha et al., 2007), whereas trimethylation of H3 on K27 is involved in the silencing of Hox genes as well as the maintenance of inactive X chromosome in mammals (Kouzarides, 2007).

The PKMTs EZH2, homolog of E(z) (Enhancer of Zeste) in *Drosophila*, and PR-SET7, which methylate H3K27 and H4K20, respectively, are considered key epigenetic regulators, as these epigenetic marks are trademarks of large chromosomal regions and are passed from generation to generation (Trojer and Reinberg, 2006). This opposes the transient and localized action of PKMTs on H3K4 and H3K36, associated with the initiation and maintenance of gene transcription (Mellor, 2006). Factors methylating R residues, the PRMTs, play a role in the dynamics of gene expression. PRMT1 and CARM1 (coactivator-associated arginine methyltransferase 1) catalyze the dimethylation of the R residues, which is linked to transcriptional activation. However, PRMT5 dimethylates H4R3 in the context of transcriptional repression (Wysocka et al., 2006).

A demethylating activity was attributed to LSD1 (Lysine specific demethylase 1) (Shi et al., 2004), which is able to demethylate H3K4 or H3K9 in different cellular contexts. LSD1 contribute to the transcriptional repressive action of the CoREST complex. However, it is also able to demethylate H3K9, which contributes to transcriptional activation. Finally, the family of proteins with a JmJC (Jumanji C domain) domain was recently found to have a demethylase activity. JHDM1, JHDM2A, JHDM3A and JMJD2A are capable of demethylating K 9 and/or 36 of H3, in a di- or tri-methylated form according to enzyme in consideration (Chen et al., 2006; Whetstine et al., 2006; Yamane et al., 2006).

- Complexes regulating chromatin organization

As mentioned previously, some histone-modifying enzymes if not all, function as catalytic domains of chromatin-remodeling complexes, such as Gcn5 the partner of SAGA and ATAC complexes, the HMT, LDS1, that contributes to the gene repression in the CoREST complex, whereas CBP/p300 antagonizes PcG repression by taking part in the TrxG complex. In the following section I will mainly focus on the mode of action of PcG in gene silencing, because the other complexes go beyond the scope of my thesis work.

The Polycomb group family

PcG genes were initially discovered in *Drosophila* as a gene family that controls fly segmentation by repressing the Hox genes (Lewis, 1978). The homeobox or Hox genes are a set of TFs that are expressed in a spatially restricted manner along the A-P axis during development, which results in the morphological differences between segments from head to tail (Krumlauf, 1994). TrxG, which counteracts PcG activity by activating the Hox genes, was subsequently identified. These two complexes are important to maintain the correct pattern of Hox genes expression. Now, PcG and TrxG proteins are defined as epigenetic regulators of gene expression that are conserved from flies to mammals. Together, they carry out a variety of activities that alter local chromatin structure to promote and maintain, silent and active transcriptional states, respectively.

PcG induces gene repression though the combined activities of the two PcG repressive complexes, the initiator complex PRC2 (Polycomb repressor complex 2), and the maintenance complex PRC1 (Schuettengruber et al., 2007). PRC1 consists of four conserved core components, Pc, Polyhomeotic (Ph), Posterior sex combs and Sex combs extra (Sce). In addition to the four core subunits of PRC1, a number of other proteins were reported to associate with this complex. PRC2 includes two homologues of the EZH1/2, the homologue of EED (extra sex combs embryonic ectoderm development), and the homologue of suppressor of zeste (SUZ12) (Cao et al., 2002; Satijn et al., 2001). Additional components were also described for this complex. A third complex was described in *Drosophila* (PhoRC); which comprises the PRE-binding proteins Pleiohomeotic (Pho) or Pho-like (Phol) that are involved in the recruitment of the PRC2 and PRC1 complexes to target genes by providing sequence-specific DNA binding (Wang et al., 2004), (reviewed in (Schuettengruber et al., 2007)).

To induce gene repression (Figure 16), the PRC2 complex interacts with HDACs to remove the acetylation of H3K9 from transcriptionally active chromatin (Kuzmichev et al., 2002; Tie et al., 2001; van der Vlag and Otte, 1999). In addition to the histone methyltrasferase activity of PRC2, *via* EZH, this protein converts the K27 of H3 to a trimethylated form. This constitutes a unique enzymatic property of the PRC2 complex and is therefore widely regarded as the hallmark of PcG-mediated repression (Cao et al., 2002; Costanzo et al., 2002; Muller et al., 2002). This mark is then recognized by the chromodomain of the Pc protein(s) and facilitates binding of the PRC1 complex (Czermin et al., 2002; Fischle et al., 2003). Following binding, PRC1 catalyzes the ubiquitination of

histone H2A on K119 (H2AK119ub), an essential modification for transcriptional repression (Wang et al., 2004). Further studies have established that components of the RNA interference (RNAi) machinery, such as Argonaut 1 (Ago 1) in *Drosophila* which recruits EZH2, are also involved in PcG gene silencing (Kim et al., 2006).

TrxG complexes include two types of proteins: SET domain proteins such as Trx and Ash1 with a methyltrasferase activity, and ATP-dependent chromatin remodeling proteins like Brahma (Brm) and Imitation Switch (Iswi). Trx methylates H3K4 residue *in vivo* and is present together with dCBP and Sbf1 (SET binding factor 1). It was demonstrated that CBP is required to prevent Pc silencing and maintain robust expression of Pc target genes. CBP acts by increasing the bulk levels of H3K27ac levels, while reducing the bulk of H3K27me3. Recently, it was shown that CBP acts by directly interacting with the TrxG proteins, UTX and Brm. UTX is the *Drosophila* ortholog of the mammalian H3K27-specific demethyles UTX, UTY, and JmiD3 (Tie et al., 2012). It was also demonstrated that CBP/p300 is able to prevent PcG action in human ESCs mutant for CBP (Pasini et al., 2010), suggesting that CBP mode of action to prevent PcG action may be conserved in different species.

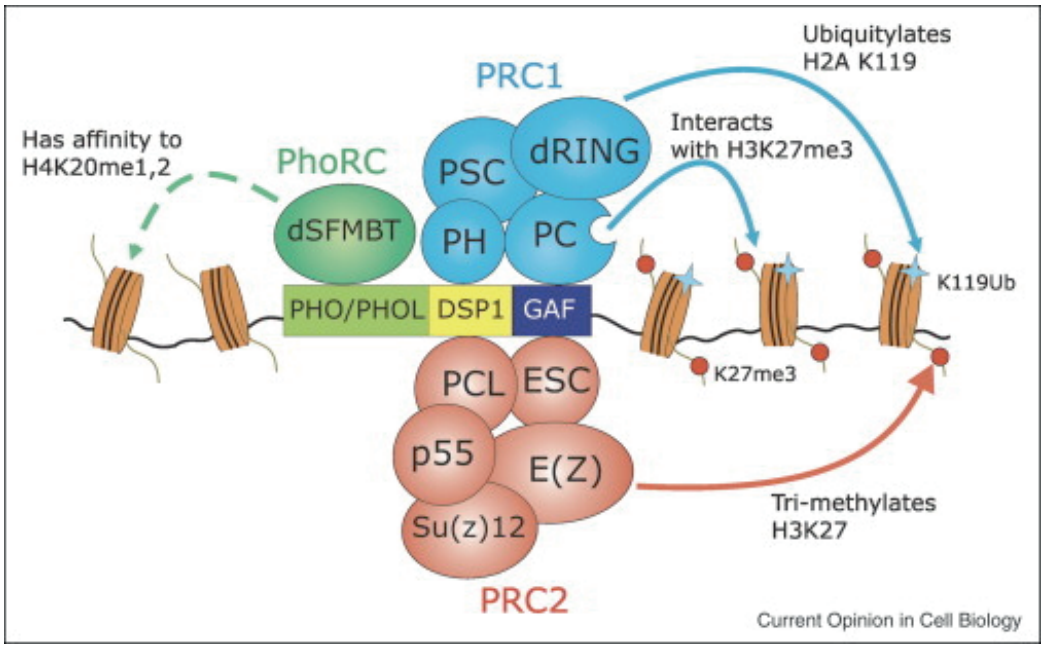

Figure 16. The biochemical activity of the PcG complex.

For more details see the text. (Schwartz and Pirrotta, 2008).

4. 2. 3. Epigenetic control of neural stem cells and their progenies

The ability of NSCs population to be directed to differentiate towards different neuronal pathways requires a certain level of developmental competence. After NSC division,

the decision of the two daughter cells to retain a progenitor cell properties and/or commit to cell cycle exit and neural differentiation is temporally regulated and partly controlled by opposing activities of negative and positive transcriptional regulators. It is becoming increasingly clear that epigenetic regulation is key in the regulation of cell competence, identity and differentiation (Meshorer, 2007). Epigenetic modifications act by dictating the accessibility, and therefore the potential, of genes to be transcribed.

As already mentioned in the part dedicated to NSCs biology, Hox genes and bHLH TFs are both involved in regulating *Drosophila* NSC competence to proliferate, differentiate, or exit the cell cycle (Introduction 4. 1.). The mutation of PcG members strongly affects the competence of NSCs, a process that is probably due to Hox genes deregulation (Introduction 5. 2. 2. 3.). Interestingly, Enok, a member of the HAT MYST family, was identified as a Pc interactor (Strubbe et al., 2011), and its mutation or the inactivation of its HAT activity, induces a MB defect due to an arrest of NBs proliferation (Scott et al., 2001). Since increasing data demonstrate the crucial role of histone and non-histone proteins acetylation in regulating different cell processes, it is possible that Erok acts together with PcG to induce or repress genes implicated in the control of the cell cycle, by acetylating members of the PcG complex. Given that a series of TFs regulates the temporal characteristics of the NB and its offspring (Introduction 4. 1.), it is important to note that increasing data showed the necessity of chromatin remodeling factors for this action. Indeed, the first TF of the temporal series Hb, regulates Hox gene expression *via* its interaction with Mi-2, the ATP dependent helicase found in the chromatin remodeling histone deacetylase complex NuRD together with Rpd3, and which is involved in chromatin remodeling and Pc-mediated repression (Kehle et al., 1998). These data strongly support the idea that the right collaboration between TFs and chromatin remodeling factors ensure the right NB competence at the appropriate time. Interestingly, Ikaros, the mammalian ortholog of Hb, was also described to associate with Mi-2, and to regulate the genomic distribution of the NuRD complex during thymocyte development ((Georgopoulos, 2002), reviewed by (Oestreich and Weinmann, 2012)). Since Ikaros also confers competence to retinal progenitor cells in mice (Elliott et al., 2008), it is possible that the same epigenetic mechanism is used to regulate the aptitude of retinal NSCs.

It is well established that NSCs are different not only by their different ability to generate neurons, glia or both cell types, but also by the timing of their generation and the diversity of their identity. For example, the NB5-6T lineage first generates neurons and glia and then switches to another step where it generates neurons only. NB6-4A only generates glial cells

contrary to the NB1-7A, which solely generates neurons. Even the type of generated neurons or glial cells differs with the progression of the number of cell division. The best-studied example is the NB1-7: in this lineage, the first five divisions generate "U" motoneurons and the five to six last divisions allow the generation of only interneurons. It was thought that the subsequent changes in the developmental plasticity of this lineage were only related to the temporal gene series: U1 is specified by high levels of Hb, U2 by low levels of the same TF, U3 is specified by Kr, U4 by Pdm and U5 by Cas (Grosskortenhaus et al., 2006; Isshiki et al., 2001; Pearson and Doe, 2003). However, it was recently demonstrated that the PcG complex is necessary and sufficient to restrict competence in this lineage; PcG mutation extends the ability of Kr to induce type "U3" motoneurons, and its gain of function causes precocious competence to make this type of neurons (Touma et al., 2012). In the same study it was also demonstrated that mutation of Su(z)12, an essential co-factor of the E(z) H3K27 HMT, extends the competence to generate motoneurons until the end of the lineage, contrary to the mutation of other members of the PcG complex, suggesting that the level of competence restriction may correlate with the level of H3K27 methylation at genes specifying the U3 motoneurons.

The role of epigenetic mechanisms in regulating neurons and glial cells differentiation are poorly documented. A few investigations in this field demonstrated that *dCBP* mutation affects the migration of embryonic peripheral glial cells (Schmidt et al., 2011), synapse formation (Marek et al., 2000), and photoreceptor cell specification (Kumar et al., 2004).

As to the role of methylation, it was shown that Mixed-lineage leukemia (MLL), a member of the TrxG gene family, is primordial for neurogenesis and its depletion from the SVZ NSCs leads to a glial lineage preference (Lim et al., 2009). This phenotype is due to the down regulation of Dlx2 (Distal-less homeobox 2), a key neurogenic regulator, in MLL deficient NSCs, *via* changes in the histone methylation profile, from single high levels of H3K4-me3 to a bivalent poised state marked by both H3K4me3 and H3K27-me3 (Lim et al., 2009).

PcG proteins were also demonstrated to take part in NSC differentiation by inducing the H3K27me3 repressive mark at proneuronal bHLH genes, such as Neurogenin1 (Neurog1) (Hirabayashi et al., 2009). Neurog1 is known to suppress astrocytic differentiation by sequestrating CBP/p300-Smads complex from STAT3 (Signal Transducer and Activator of Transcription 3), leading to the suppression of SMAD3 target genes, a process that promotes glial differentiation.

The role of epigenetic cues on mammalian NSCs specification was nicely documented in the spinal cord motor neurons. In this model, it was proved that extrinsic regulators, retinoic acid signaling, and TF Neurog2 cooperate to recruit CBP to motor neurons specific genes. Remarkably, *CBP* deletion in the developing spinal cord results in a reduced number of motor neuron, whereas the number of interneurons increases (Lee et al., 2009), showing that motor neurons specification needs "high" levels of CBP, and would otherwise differentiate into interneurons.

A lot of efforts were made to understand the factors implicated in NSC plasticity, however little is known about the molecular mechanisms played by these factors to induce neuronal versus glial differentiation.

5. Gcm transcription factors

5. 1. *gcm*/Gcm in *Drosophila*

5. 1. 1. Background

In *Drosophila*, the *gcm* genes consist of two genes, *gcm* and its homolog *gcm2* (Alfonso and Jones, 2002; Kammerer and Giangrande, 2001). The profile expression of the two gene transcripts were first analyzed by *in situ* hybridization, using specific probes for the two mRNAs. During embryogenesis, *gcm* and *gcm2* transcripts are expressed in the same territories, except that *gcm* mRNA is highly expressed compared to its homolog (Alfonso and Jones, 2002; Kammerer and Giangrande, 2001). The two transcripts are first expressed in a cluster of hymocytes precursors in the blastoderm, which correspond to the hemocyte precursors; and then gradually appear in all glial precursors (Figure 17). Note that *gcm* transcripts are also detectable in another type of cells, the tandon cells, and in two asymmetric clusters of unknown cells in the head region (Figure 17, red arrows). At late embryonic stages, *gcm* mRNA is undetectable in the three mentioned territories.

The pattern of Gcm protein expression was less studied due to the difficulty of generating a stable functional antibody against the Gcm antigen. Hence, Gcm expression pattern was analyzed using β-galactosidase (β-gal) staining on transgenic flies carrying a *lac-Z* insertion on the *gcm* promoter (*rA87* strain) (Miller et al., 1998). Indeed, in *rA87* transgenic embryos, β-gal staining faithfully reflects the *gcm* mRNA pattern of expression. Using this tool, it was even possible to follow the fate of cells in which *gcm* is expressed but not maintained, due to β-gal stability. The limitation of this tool for analyzing Gcm is that we do not follow the real behavior of the endogenous Gcm protein. I believe that this constraint can be circumvented using *gcmFlag* transgenic flies that were recently generated in our laboratory, where all *gcm*/Gcm functions, activities and features are maintained, as we will discover in **paper IV** (Results 4.).

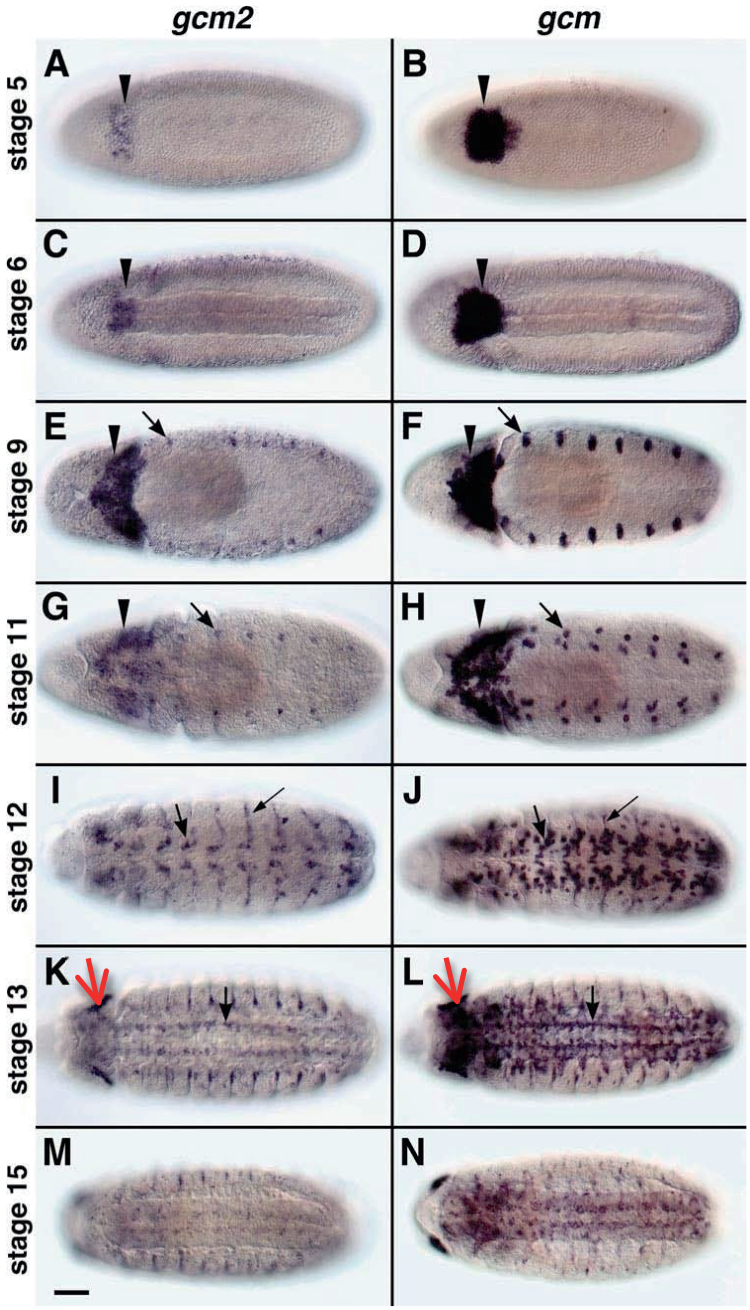

Figure 17. *gcm* and *gcm2* expression pattern at different embryonic stages.

Ventral view of WT embryos stained by in situ hybridization, using *gcm* and gcm2 Dig probes. *gcm2* expression is shown after 6h of staining reaction (left panels). *gcm* expression is shown after 1 h of staining reaction (right panels). At the different stages, *gcm* and *gcm2* expression is showed in the following tissues: procephalic mesoderm (arrowheads, stage 5 and 6), hemocyte precursors (arrowheads, stage9 and 11), in glial precursors in the CNS (arrows, stage 11, 12, 13, 15), in a stripe of lateral ectodermal cells, the tandem cells (thin arrows, stages 12,13,15), and in 2 symmetrical clusters of cells located laterally in the head (red arrow, stage 13). Note that gcm and gcm2 expression fade in the VNC at stage 15. Scale bar: 15um. (Alfonso et al., 2002).

5. 1. 2. Gliogenic potential of Gcm

- Background

During the *Drosophila* embryonic neurogenesis, *gcm* is expressed in all glial precursors (GB and NGBs) and acts as a glial determinant for all lateral glial cells: its mutation forces almost all glial precursors to adopt the neuronal fate; inversely, its ectopic

expression in the ventral ectoderm, the primitive tissue giving rise to the NBs, induces ectopic glial cells at the expense of neurons (Figure 18), (Bernardoni et al., 1998; Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). The gliogenic potential of Gcm is not limited to the neuroectoderm but also to other non neurogenic tissues, such as the mesoderm and the dorsal ectoderm where *gcm* ectopic expression is also sufficient to induce glial differentiation (Akiyama-Oda et al., 1998; Bernardoni et al., 1998; Miller et al., 1998).

The gliogenic potential is not limited to Gcm, but also to its homologue Gcm2. In fact, *gcm2* ectopic expression is also able to trigger the differentiation of additional glial cells (Alfonso and Jones, 2002; Kammerer and Giangrande, 2001). Accordingly, in *gcm* mutant embryos, very few glial cells still detectable, whereas the *gcm-gcm2* double mutant embryos are devoid of glial cells. Interestingly, *gcm2* mutant embryos do not present any major glial defect and they are viable until the adult stage contrary to *gcm* simple mutation or *gcm-gcm2* double mutants, which die at late embryonic stages. Altogether, these findings demonstrate that *gcm* is the major glial regulator.

It was believed, for a long time, that the increase in the glial cell number that occurs between early larval and early pupal development is only due to glial cell division (Colonques et al., 2007; Pereanu et al., 2005). However, increasing evidence demonstrates that this is also due to the division of specific types of NBs, notably the type II lineage, (Hartenstein et al., 2008; Izergina et al., 2009). Interestingly, it was recently demonstrated that Gcm is expressed in some larval glial progenitors, and its expression is necessary and sufficient for the differentiation of INP-derived glial cell formation in type II NBs. The gliogenic potential of Gcm in larval progenitors is not limited to type II NBs, but also to type I NBs, a lineage that does not normally give rise to glia (Viktorin et al., 2011).

Furthermore, it was also demonstrated that Gcm is necessary for the development of wing glial cells (Van De Bor et al., 2000), and for the glial differentiation associated to microchet (Fichelson and Gho, 2003). Gcm is also important for the development of all peripheral glial cells in the PNS. In the optic lobe, *gcm* is expressed in the glia and the lamina neuronal cells (Dearborn and Kunes, 2004).

- Glial fate establishment

A B C

gcm is the master gene regulator for the differentiation of all lateral glial cells and the earliest binary switch known to be necessary for lateral glial cell development in *Drosophila*. However, since *gcm* expression declines before lateral glial differentiation is achieved, the accomplishment of glial differentiation most likely depends most likely on the activity of other factors activated by Gcm. Several genes were identified as downstream targets of Gcm.

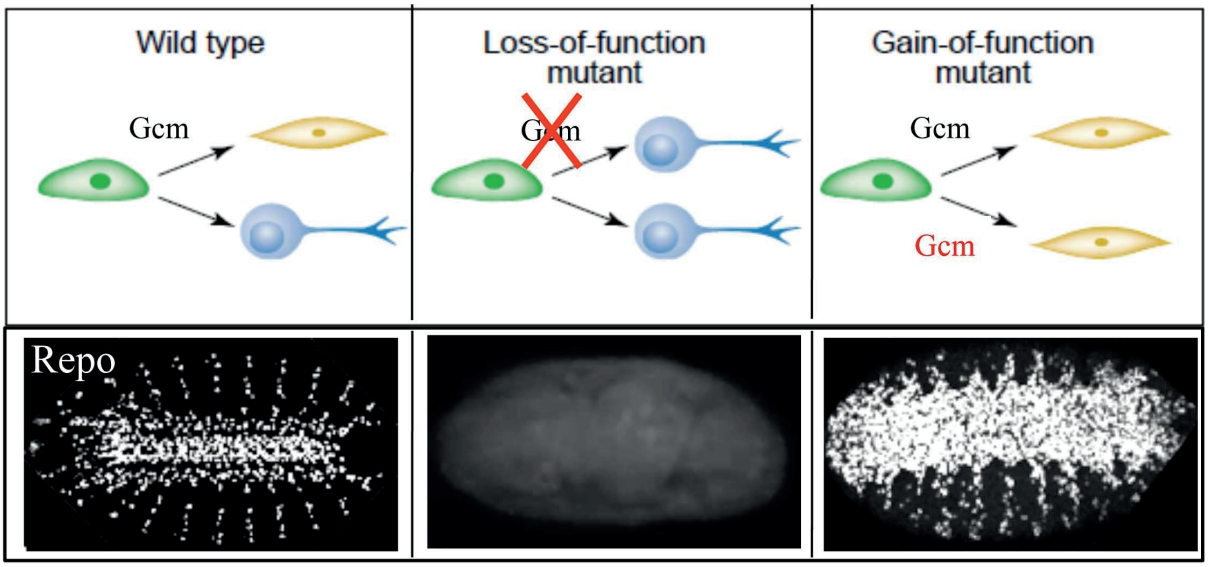

Figure 18. Profile of embryonic glial cells in WT, *gcm* mutant, and *gcm* overexpressing embryos.

(A-C, inferior panels) Ventral view of 16 stage embryos stained with the glial marker anti-Repo.WT embryo contain around 60 glial cells per hemisegment (A), in *gcm* mutant embryos non glial cell is detected (B), *gcm* ectopic expression in the neurogeneic region induces a drastic increase in the number of glial cells (C). **(A-C, superior panels)** Schematic representation of what happens in the different background.

repo: Also called rk2, this consttues the first identified target of Gcm (Halter et al., 1995; Klaes et al, 1994; Klambt et al, 1993). Transient expression of *gcm* in glial precursors is followed by a maintained expression of *repo*. However, while *repo*/Repo is expressed in Gcm positive glia, it is not expressed in Gcm positive hemocytes or tendon cells, suggesting the implication of other factors that act together with Gcm to regulate *repo* expression, that are neural or mesodermal specific. Repo was described as necessary for the maintenance of the glial fate, after its initiation by Gcm. The *repo* mutation does not affect glial cell determination but differentiation as glial cells fail to express late glial markers (Campbell et al., 1994; Halter et al., 1995; Xiong et al., 1994). Repo is a homeodomain transcription factor

(Campbell et al., 1994; Halter et al., 1995; Xiong et al., 1994) and likely exerts its function by directly activating other genes, but the nature of this function is till unknown.

The *repo* promoter carries eleven GBSs and mutations in these regions trigger significant loss of *repo*-reporter gene expression in glia (Akiyama et al., 1996; Lee and Jones, 2005; Schreiber et al., 1997). The analysis of the *cis*-regulatory DNA elements in the *repo* prometer were performed using a *lacZ* reporter fused with different deletions of the *repo*, regulatory sequence. A 4.3 kb DNA region upstream of the *repo* start site is sufficient to reproduce the WT Repo expression pattern. By ectopically expressing *repo*, it was also demonstrated that Repo is able to autoregulate; this process is probably necessary for maintaining *repo* expression after Gcm expression has declined.

pointed1: The *pointed* genes encode two TFs (Pointed 1 and Pointed2). These share the C-terminal region that includes the ETS (for erythroblast transformation specific) DNA-binding domain (Klaes et al., 1994; Klambt, 1993; O'Neill et al., 1994). Gcm induces the expression of one isoform, *pnt1*. As for Repo, it was shown that Pnt1 plays an important role in the terminal differentiation of glial cells. In *pnt1* mutant embryos, LG cells fail to extend cellular processes and to unsheathe the axons. In addition, ectopic expression of Pnt1 in the neurogenic region results in ectopic cells expressing lateral glial markers (Klaes et al., 1994).

Loco (Locomotion defects): Loco encodes two variants, Loco-1 and Loco-2 of the Regulators of G-protein signaling, which act as GTPas-activating proteins (Granderath et al., 1999). *loco 1* expression is restricted to lateral glial cells. In addition, biding sites for both, Gcm and Pnt1, were identified in the *loco* promoter. Thus, *loco* expression is regulated in a biphasic mode. At the beginning of gliogenesis, Gcm activates both *loco* and *pnt1*. Later, Gcm and Pnt1 synergistically activate *loco* in order to mediate high levels of glial specific *loco* expression. In *loco* mutant embryos, glial cells fail to properly unsheathe the longitudinal axons tracts and the glia-glia cell contacts are also affected, resulting in the disruption of the blood-brain barrier.

ttk: The *ttk* gene transcripts are alternatively spliced, giving rise to two isoforms, *ttk69* and *ttk88*. Gcm induces the expression of one isoform *ttk69*, which codes for a TF with a BTB (Bric-à-brac Tramtrack Broad)) zinc-finger. Contrary to the other Gcm targets, *ttk69* is expressed in all glial cells, lateral and midline glia, and in many other tissues, including the muscle and the epidermis (Giesen et al., 1997). *ttk69* expression is induced after that of *gcm*, *pnt1* and *repo* (Badenhorst, 2001). This gene codes for a transcriptional repressor that inhibits the expression of neuronal differentiation genes. In embryos expressing high levels of *ttk69*,

neuronal specific markers were significantly reduced (Giesen et al., 1997). In addition, Ttk69 inhibit the expression of *asence* and *dpn*, two transcription factors that are known to support the neuronal potential in neuronal precursors (Badenhorst, 2001).

Cooperation between the different Gcm targets to reinforce glial differentiation was also described (Yuasa et al., 2003). It was demonstrated that the ectopic expression of *repo* in the neurogenic region is able to induce the expression of the glial specific marker, *M84* (Klambt and Goodman, 1991), whereas the ectopic expression of *ttk69* in the same territory has no effect. Interestingly, the co-expression of the two markers enhances threefold the expression of M84, showing that Ttk69 cooperates with Repo to regulate *M84* gene expression. In addition to that, it was also revealed that Repo and Ttk are both able to repress the neuronal specific marker Elav, when ectopically expressed in the neurogenic region, and the coexpression of the two markers strongly enhances this effect on Elav (Yuasa et al., 2003). Altogether, these findings clearly demonstrate that the different Gcm targets cooperate to establish the glial fate, while repressing the neuronal one.

Other Gcm targets: In addition to the above-mentioned Gcm targets, many others were identified taking advantage of high throughput microarrays analyses (Altenhein et al., 2006; Egger et al., 2002; Freeman et al., 2003). The expression levels of genes were examined in *gcm* mutant embryos, where glial cells are absent, or in embryos overexpressing *gcm*, where numerous neuronal precursors are transformed into glial cells. Bioinformatic analyses were subsequently realized in the selected genes to check for the presence of GBSs (Freeman et al., 2003). Surprisingly, the genes identified in the three different analyses only show moderate overlap. Among the identified genes, some are glial specific, others are specific for other tissues where *gcm* is normally expressed, like hemocytes, and the remaining genes are unknown. To validate some genes, the profile of their expression has been analyzed by *in situ* hybridization or immunohistochemistry (IHC), using WT embryos or embryos mutant for or overexpressing *gcm*. Interestingly, a number of validated genes were only induced in a subtype of glial cells when *gcm* is overexpressed, suggesting the existence of other lineage specific factors that act together with Gcm to establish a defined glial identity, similar to what was shown for Hkb (De Iaco et al., 2006). Microarray analyses identified several direct and indirect Gcm targets. In our laboratory, Dr. A. Popkova has performed a DAM-ID screen to look for direct Gcm targets.

5. 1. 3. *gcm* and hematopoiesis

In *Drosophila* embryos, two types of hemocytes were described: plasmatocytes and

crystal cells (Meister and Lagueux, 2003); hemocytes are the analogues of vertebrate blood cells and key players in the response to immune challenges. Gcm and Gcm2 are required for plasmatocyte development, whereas crystal cell fate needs the expression of another TF, Lozange (Lz) (Lebestky et al., 2000; Waltzer et al., 2003). In a detailed work, Dr. Bataille and colleagues showed that *gcm* is co-expressed with *serpent* in early embryogenesis (stage5), *serpent* codes for a GATA family TF that is expressed in all prohemocytes. Only later (stage6), a cluster of prohemocyte cells downregulate *gcm*, thereby allowing the expression of *lz*. Only 60% of Lz+ cells are able to maintain Lz expression through an autoregulatory loop and acquire a crystal cell fate, the remaining cells become plasmatocytes. The residual Gcm interferes with *lz* expression and promotes plasmatocytes differentiation in the remaining 40% of cells (Bataille et al., 2005). The observation that *gcm*, when ectopically expressed, can induce the differentiation of all prohemocytes into plasmatocytes (Lebestky et al., 2000), and when absent, Lz can transform all prohemocytes into crystal cells, shows that Gcm is a crucial determinant of plasmatocytes fate.

5. 2. *gcm*/Gcm orthologs in vertebrates

There are two orthologs of the *Drosophila* genes in mammals, (*gcm1* and *gcm 2*) in mice and (*GCMa* and *GCMb*) hummans (Akiyama et al., 1996). The products of the human and mouse *gcm* genes share a highly conserved N-terminal region with the *Drosophila* Gcm and Gcm2 proteins. However, the function of Gcm proteins is not conserved in mammals.

5. 2. 1. GCM1

In mammals, *GCMa* is important for placental development (Schreiber et al., 2000). In mouse embryos, *GCM1* is expressed in small clusters of chorionic trophoblast cells that are scattered across the basal surface of the chorion (Anson-Cartwright et al., 2000; Basyuk et al., 1999). This expression is detectable as early as E7.5 within the extra-embryonic ectoderm. Later, *GCM1* continues to be expressed in the labyrinth in the differentiated trophoblast (Basyuk et al., 1999). More specifically, *GCM1* expression is limited to SynT-II cells (Cross et al., 2006). In *GCM1* mutant mice, placental development stalls after chorioallantoic attachment and branching does not begin. As a consequence mutant mice die at between E5.5-E10 stages, due to insufficiency of nutriments and oxygen (Anson-Cartwright et al., 2000; Schreiber et al., 2000). Two GCM1 targets were described: *syncytin* (Yu et al., 2002), a crucial gene for trophoblast cell fusion to form the placental labyrinth, and *aromatase*

(Yamada et al., 1999), coding for a member of the P-450 cytochrome family that is responsible for the synthesis of estrogen (Thompson and Siiteri, 1974).

After birth, the mouse *GCM1* expression was observed in the thymus and in the kidney (Hashemolhosseini et al., 2002). In the thymus, *GCM1* expression is visible after the complete formation of this organ, suggesting that GCM1 is implicated in thymus physiology rather then in its development. Indeed, GCM1 expression is limited to a group of cells that are responsible for the synthesis of the parathyroid hormone, as a second source of this hormone after the parathyroid gland.

5. 2. 2. GCM2

GCM2 is important for the development of the parathyroid gland (Gunther et al., 2000; Kim et al., 1998), because mice deficient for this gene lack parathyroid glands and develop severe hypocalcemia and hyperphosphatemia (Gunther et al., 2000; Kim et al., 1998). Interestingly, the levels of *GCMb* transcripts are higher in human hyperplastic glands compared to normal glands, suggesting that *GCMb* is implicated in parathyroid tumorigenesis (Kebebew et al., 2004). The mechanism by which deregulated *GCM2* gene expression may play a role in parathyroid tumorigenesis is unclear.

5. 2. 3. GCM1 and GCM2 in the nervous system

Despite the important role of the *gcm* genes in *Drosophila* gliogenesis, their role in mammalian neurogenesis still under debate. However, some investigations do not exclude a possible role of *gcm* genes in the mammalian NS.

PCR and *in situ* hybridization analyses revealed the expression of GCM1 in the mouse brain (Iwasaki et al., 2003). In cell culture, GCM1 overexpression in embryonic brain cells is able to induce the expression of some astrocyte markers, such as S100 and GFAP, and the repression of the neuronal marker MAP2 (microtubule-associated protein 2), ((Iwasaki et al., 2003). Interestingly, it was also reported that GCM1 is able to induce gliogenesis, when ectopically expressed in the neurogenic region of the *Drosophila* brain (Kim et al., 1998; Reifegerste et al., 1999), and its expression is able to rescue the *gcm* mutant phenotypes (Kim et al., 1998), suggesting that GCM1 action is context dependent.

GCM2 expression was also detected by RT-PCR using mouse brain mRNA (Kammerer et al., 1999; Kanemura et al., 1999). Hence, unlike GCM1, GCM2 is unable to induce ectopic gliogenesis, when ectopically expressed in the *Drosophila* NS (Kim et al., 1998).

Hes genes are mammalian orthologs of *Drosophila hairy and Enhancer of split*, which encode for bHLH transcriptional repressors. During the development of the neural plate, Hes1 and Hes3 are widely expressed by neuroepithelial cells along the entire neuraxis, but Hes5 is not expressed at this stage (Hatakeyama et al., 2004). As neuroepithial cells gradually convert to RG cells, Hes3 expression is down regulated, whereas Hes5 expression takes place (Hatakeyama et al., 2004). The mechanisms responsible for Hes5 gene activation are poorly understood. Recently, it was demonstrated that *GCM* genes are expressed in the developing NS, and GCM proteins are crucial for the demethylation of CpGs in the promoter region of *Hes5*, a process that is important for the *Hes5* gene activation. It was also proposed that GCM functions as a selective signal to direct neuroepithelial cells of early embryos to acquire SC properties (Hitoshi et al., 2011).

5. 3. *gcm* gene regulation

Despite the crucial role of Gcm in glial and hemocyte fates establishment (Introduction 5. 1. 2. and 5. 1. 3.), the mechanisms governing the initiation and the transient maintenance of its expression in all glial and prohemocyte precursors, is poorly understood. In the NS, four factors were identified as necessary for the maintenance of *gcm* gene expression, Gcm itself, Gcm2, Hkb and Prospero. As we will discover, the action of some factors is lineage specific, which makes *gcm* gene regulation extremely complex.

5. 3. 1. Role of Gcm

The role of Gcm protein in the expression maintenance of its own gene (this process is called *gcm* autoregulation) has first been hypothesized upon finding several Gcm binding sites (GBSs) on the *gcm* promoter (Figure 19), (Kammerer and Giangrande, 2001; Miller et al., 1998; Ragone et al., 2003), and subsequently this has been shown by *in vitro* and *in vivo* analyses demonstrating that Gcm is able to bind and activate its own promoter (Miller et al., 1998). Positive *gcm* autoregulation is probably crucial for the consolidation of its expression, a step that could be necessary for the glial fate decision. Curiously, the ability of Gcm to regulate *gcm* gene expression is functional in the neurogenic region but not in the mesoderm, another tissue where ectopic *gcm* expression is able to transform mesodermal precursors into glia (Bernardoni et al., 1998; Miller et al., 1998), suggesting that *gcm*-autoregulation necessitates neural specific factors.

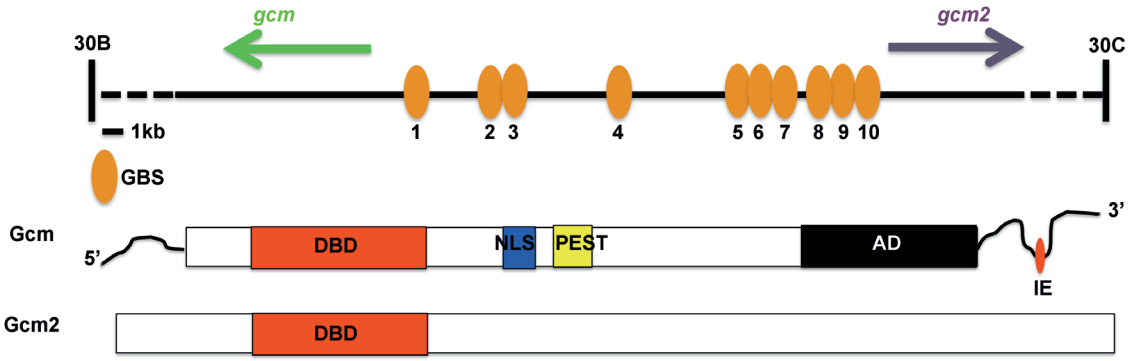

Figure 19. *gcm-gcm2* complex and Gcm transcription factors.

(A) *Drosophila gcm-gcm2* locus. Arrows indicate the transcribed regions. 30B and 30C indicate the localization of *gcm-gcm2* locus in the chromosome 2. **(B)** Gcm and Gcm2 proteins structure: AD, activation domain; DBD, DNA binding domain; NLS, nuclear localization signal; PEST, basic cysteine region that assists Gcm degradation. *gcm* 3'UTR contain an instability element (IE).

5. 3. 2. Role of Gcm2

Gcm and Gcm2 TFs bind the same DNA binding site (the GBS), moreover Gcm2 is able to induce Gcm expression by directly binding to the GBSs present on the *gcm* promoter (Kammerer and Giangrande, 2001). However, Gcm2 is only able to do that in the neurogenic region but not in the mesoderm, suggesting that as for Gcm, Gcm2 also needs cofactors to activate the *gcm* promoter, and these cofactors may be present in the neurogenic region but not in the mesoderm.

5. 3. 3. Role of Prospero

The role of Prospero in *gcm* gene regulation was demonstrated in two specific lineages, the NB-6-4T and the NB7-4. The best-analyzed example is the NB6-4T lineage, where *gcm* transcripts and Gcm protein are expressed (Figure 20), (Akiyama-Oda et al., 1999; Bernardoni et al., 1999; Freeman and Doe, 2001). After NB6-4T division, *gcm* mRNA preferentially segregates to the glial precursor that divides a few more times to generate glial cells only. In the progeny of these glial precursors *gcm* continues to be expressed for a defined period of time that is necessary for glial fate establishment. On the other hand, the low amount of *gcm* mRNA segregated to the second daughter cell, which only generates neurons are eliminated by unknown mechanisms (Figure 20, left panel). These results strongly suggest that gliogenesis needs high levels of *gcm* expression in the glial progenitors to prevent neuronal fate. In fact, in *gcm* mutant embryos, the glial precursors are transformed into neuronal ones (Hosoya et al., 1995). Inversely, *gcm* ectopic expression in the neurogenic

region induces extra glial differentiation at the expense of neurons, in a dose-dependent manner.

In the NB6-4T daughter cells, the Prospero is asymmetrically segregated to the glial precursors. The role of Prospero on *gcm* gene transcription has been identified using *prospero* mutant embryos, where *gcm* transcipts are low in the NB6-4T lineage or in its progeny. Nevertheless, Prospero does not seem to be necessary for the initiation of the *gcm* gene activation, because β-gal derived from the *gcm-lacZ* gene (*rA87* strain) (Hosoya et al., 1995) was detected, although weakly, in NB6-4T progeny cells, mutant for *prospero* (Akiyama-Oda et al., 1999). In addition, in Gcm overexpressing embryos, *prospero* is upregulated, whereas in *prospero* overexpressing embryos, *gcm* expression is not affected. The fact that Prospero does not affect glial cell fate establishment in other glial lineages such as NB6-4A, NB2-5, NB1-1, NB5-6, NB1-3 and GP (Akiyama-Oda et al., 1999), calls for different molecular mechanisms that are Prospero-independent, and which may be used to initiate or maintain *gcm* expression in other glial precursor cells.

5. 3. 4. Role of other factors

Cis-regulatory element: This hypothesis was verified *in vivo* by analyzing the ability of *gcm* cDNA fused with different deletions of *gcm* promoter (9kb to 2kb) to rescue the different glial cell lineages in embryos mutant for *gcm*, where all glial cells are missing (Ragone et al., 2003). Interestingly, the 9kb *gcm* promoter rescues the differentiation of a high number of glial cells, and the number of rescued lineages progressively declines with the reduction of *gcm* promoter length (Ragone et al., 2003). These findings show that the cis-elements seem to carry information for *gcm* expression in specific lineages.

Transcription factor series: The NB1-1A produces six to eight neurons and three glial cells (Bossing et al., 1996; Broadus et al., 1995). Indeed, the GMC generated after the first division gives rise to two neurons, and in the next divisions it produces GMCs that generate a neuron and a glial cell after their divisions (Udolph et al., 2001). Interestingly, *hkb* transcripts are only detectable when gliogenesis starts (Chu-LaGraff et al., 1995), and they colocalize with the *gcm* mRNA (De Iaco et al., 2006). Morover, Gcm and Hkb proteins interact physically and this step is important to enhance *gcm* gene expression *via* its positive autoregulation. This regulation *via* Hkb is important to ensure glial differentiation from the precursors of NB1-1A. Indeed, in *hkb* mutant embryos, the NB1-1A lineage does not generate glia but an increased number of neurons (De Iaco et al., 2006; Udolph et al., 2001).

Hox genes: The NB6-4 lineage shows segment-specific differences in its progeny outcome. The NB6-4T generates neurons and glia, whereas NB6-4A generates only glial cells (Figure 20). The homeotic gene *abd-A* is expressed in the abdominal segments and is required for abdominal segment identity. Interestingly, in *abd-A* mutant embryos carrying a *gcm* reporter (+1.7/+4.5 *gcm-lacZ*) that is known to rescue the NB6-4A lineage, show a complete loss of β -gal expression in abdominal segments, while maintaining expression in the maxillary segment (Jones et al., 2004), showing that one of the segmental differences in the specification of glial precursors are under the control of the homeotic genes. This result highlights the role of Hox genes in *gcm* gene regulation, however the exact mechanim for such control is completely unknown.

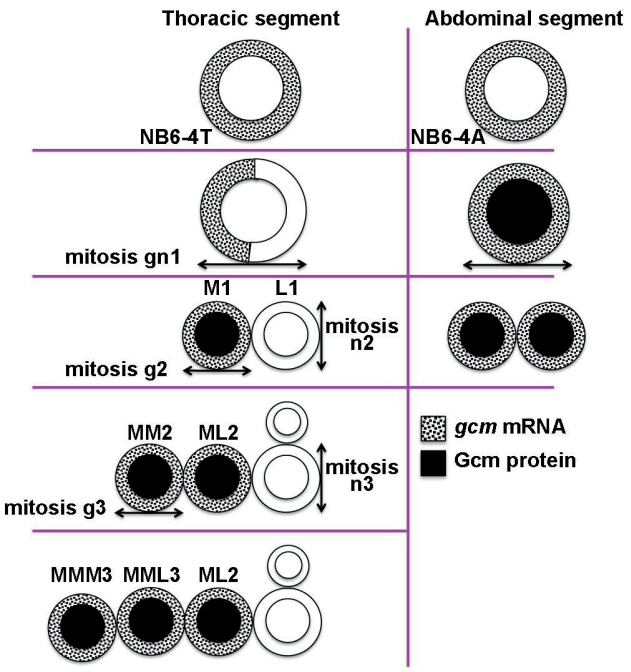

Figure 20. Schematic representation of NB6-4 lineages.

In the thoracic segment **(left column)**, mitosis gn1 occurred in NB6-4T, with the production of M1 (medial) and L1 (lateral) cells. Initially, *gcm* mRNA was evenly distributed, but became localized to the medial half and was inherited primarily by M, which then expressed GCM protein. M1 was a glioblast, which divided twice (mitosis g2 and g3) to give rise to three glial cells, MMM3, MML3 and ML2. L1 generated smaller Prospero-positive ganglion mother cells through mitoses n2 and n3 from the basal side. In the abdominal segment **(right column)**, NB6-4A divided once in the mediolateral orientation, producing two glial cells. During this cell division, *gcm* mRNA was distributed evenly and segregated into both daughter cells. GCM protein was also detected during division and in both daughter cells adapted from modified from (Akiyama-Oda et al., 1999).

Notch signaling: The role of Notch signaling on *gcm* gene regulation was evaluated in adult and embryonic PNS, and Notch effect on *gcm* gene is likely context dependent. Indeed, in *Notch* mutant flies, glial cells are produced at the expence of neurons in the bristale lineage due *gcm* upregulation, while Notch overexpression produces the opposite phenotype (Van De Bor and Giangrande, 2001). Contrary to Notch effect on bristale cells, in the embryonic dorsal bipolar dendritic (dbd) lineage, *Notch* mutation induces a complete loss of *gcm* expression

within the dbd lineage, whereas its expression in other *gcm* positive cells was not affected (Umesono et al., 2002). Altogether, these results indicate that there is a specific requirement of Notch activity for *gcm* expression in different lineages.

In the optic lobe, *gcm* is expressed in the glia and the lamina neuronal cells (Dearborn and Kunes, 2004) and it is required for the differentiation of these cells. Interestingly, in these cells *gcm* is differently regulated; in the lamina *gcm* expression seems to be regulated by Hedgehog, and the glia by Dpp. These results suggest that *gcm* is controlled in different manner in the optic lobe (Yoshida et al., 2005)

Altogether, these findings suggest that *gcm* gene regulation is a complex process that needs the action of several factors, which are also context-dependent.

5. 4. Properties of the *gcm* mRNA

It was previousely demonstrated that the 3'UTR of *gcm* transcript carries an instability element (IE), which is involved in *gcm* RNA degradation (Hosoya et al., 1995; Soustelle et al., 2008). Interestingly, the ectopic expression of a mutant form of *gcm* in this IE, enhances the gliogenic potential of Gcm (Soustelle te al., 2008), showing that *gcm* is regulated transcriptionally and that Gcm threshold levels are crucial for glial fate establishment. In our lab, Dr. P. Laneve thrives to gain more insights into *gcm* mRNA regulation. Various new mechanisms characterizing these transcripts were discovered. Some of them will be included in **Paper IV**.

5. 5. Properties of Gcm protein

The *Drosophila gcm* gene codes for a TF of 504 amino acids, with a distinct nuclear targeting sequence, a DNA binding domain (DBD), a basic cysteine rich region, PEST-like sequences that assist protein degradation, and a transactivation domain (Akiyama et al., 1996; Hosoya et al., 1995; Schreiber et al., 1997), (Figure 19). The *gcm* homologous, *gcm2,* also codes also for a TF that shares several properties with Gcm. The structure of the Gcm2 protein is similar to that of Gcm, with a highly conserved N-terminal Gcm-motif, specifically in the DBD (Kammerer and Giangrande, 2001). Gcm and Gcm2 represent a new class of TFs with a zinc-finger DBD. The latter binds a specific sequence of eight nucleotides 5'-AT(G/A)CGGG(C/T)-3', called GBS, a novel sequence among the known targets of DNA-binding proteins (Akiyama et al., 1996; Cohen et al., 2003; Schreiber et al., 1997).

Althought numerous roles of Gcm on different biological processes such as gliogenesis and hematopoiesis were described, the mechanisms controlling Gcm stability and turnover are poorly understood. The first study that tackled this question demonstrated, using a flagged tagged Gcm (GcmFlag), that Gcm is ubiquitilated by two F-box proteins, Supernumerary limbs (Slimb) and Archipelago (Ago), adaptors of SCF E3 ubiquitin ligases. This modification induces Gcm rapid degradation *via* the proteasome (Ho et al., 2009).

The different role of *gcm* in different cell types development underscores the need for the precise temporal and special regulation of *gcm* gene transcription, as well as of *gcm* products (transcripts and protein). One of the main questions that I addressed during my thesis project is: how *gcm* expression is transiently expressed during gliogenesis?

AIMS

My PhD project constitutes a relevant research axes in Dr. A. Giangrande laboratory, aiming to understand the mechanisms underlying the differentiation of the *Drosophila* nervous system. More precisely, my goel was to analyze the role-played by the fate determinant Gcm TF during physiological gliogenesis and cell fate conversion into glia, and to characterize the necessary factors for directing these events. Within my project, I have addressed the following questions:

1) Is the age and the mitotic state of cells able to influence the gliogenic potential of Gcm? (Results 1 and 2).

2) Is Gcm able to induce a real cellular fate conversion into glia, which is stable and complete, with the same epigenetic properties as those of endogenous glia? (Result 1).

3) Which are the factors that control Gcm transient expression activity? (Result 3).

4) Can we generate a tool that allows us to follow/study the Gcm protein in physiological conditions?

Taking advantage of the gliogenic potential of Gcm TF in the contex of *Drosophila* NSCs, I have lifted the veil on several processes related to cell plasticity and Gcm activity mechanisms.

Since all the work previously performed dealt with the ability of Gcm to convert young NSCs into glia, and since NSCs are able to change their potential to differentiate into different cell types after each cell division, and also to control their proliferation by entering cell cycle arrest (quiescence) or undergoing programmed cell death (apoptosis), in this study I have investigated the effect of age on NSC plasticity and their ability to respond to Gcm cell fate conversion. Initially (first part of results 1.), I will describe the effect of cell aging of NSC plasticity, whereas a second part (the last part of Results 2.) will be dedicated to describe my findings about the Pc role, an HMT and a subunit of PcG complex, in regulating the process of NSC plasticity. I will show that NSC lose progressively their competence to respond to *gcm*-mediated cell fate conversion with aging, that quiescent or apoptotic NSCs lose completely their competence, and that Pc is strongly implicated in such negative response.

Secondary, since none of the previous studies addressed the epigenetic properties of *Drosophila* nerve cells in general, in this study we have focused on studying some epigenetic marks that characterize neurons and glial cells. Section 2 of Results 1 describes our finding concerning the epigenetic marks that characterize neurons and glial cells. Based on that, I have demonstrated that glial cells, endogenous or ectopic, express low levels of H3K9ac while neurons express high levels of this histone modification.

Finally, till now the mechanisms that make *gcm* expression transient during glial fate establishment were completely unclear. To understand this phenomenon, I analyzed the processes that regulate *gcm* gene expression and Gcm protein stability. In the last part of my results, I will show a model that might explain *gcm* transitory expression during gliogenesis. I have demonstrated that auto- and cross-regulatory circuits between Gcm and the homeodomain TF Repo collaborate with the HAT CBP to regulate *gcm* gene expression and Gcm protein accumulation, and I investigated the effect of such control on gliogenesis.

MATERIALS AND METHODS

MATERIALS AND METHODS

The objective of this section is not to describe in detail the experimental procedures, which are included in the relative manuscripts of each part, but to discuss the technical/conceptual principles of the major methods utilized.

1. Methods for *Drosophila melanogaster*

The results obtained using *Drosophila* embryos or larvae were based on two main types of genetic experiments, gain-of-function and loss-of-function.

Gain-of-function experiments concern the induction of gene products (mRNA and protein) expression in tissues where they are (overexpression) or they are not (ectopic expression) normally expressed. This is realized using the **Gal4/UAS system**; Gal4 gene codes for a yeast TF that specifically binds the short section UAS "upstream activation sequence", an enhancer sequence of a yeast promoter to activate transcription of the flanking gene (Brand and Perrimon, 1993). The crossing of driver flies (where Gal4 sequence is inserted at appropriate location of the genome, whose expression is under the control of specific promoter/enhancer sequence and which is specifically activated in a given context) with flies carrying an UAS sequence together with the coding sequence of a defined gene to be over- or ectopically express, (inserted into a suitable and accessible location in the genome), will allow the forced expression of the gene in the context of the promoter controlling the Gal4 gene expression. According to the choice of such promoter, defined gene products can be over or ectopically expressed in different compartments.

Another approach used to induce gene expression was the **Gal80 TARGET system**. Gal80 is another yeast protein, which is known to inhibit the Gal4 activity by binding the same UAS sequence. In flies, the coding sequence of this protein under the control of thermosensitive (ts) promoter is only active at low temperature. Combination of Gal4/UAS system to $Gal80^{ts}$ allows target gene expression at a permissive temperature (29°C), but not at restrictive temperature (18°C). By using this strategy, it is possible to drive specifically gene expression not only in specific compartments, but also at defined time intervals.

Based on the above described strategies, we were able to induce specific gene expression, permanently or transiently, in all or specific neuronal cells, such as NSCs and post-mitotic neurons, during specific intervals of their lifespan.

Loss-of-function experiments refer to complete (null mutant) or partial (hypomorphic mutant) lose of one or several genes functions. In this context, we have specifically analyzed the effects of mutants in which the function of selected genes was completely or partially lost due to alteration in their DNA coding sequence. This kind of transgenic flies derive from three types of screens: **1)** where DNA point mutations were induced by ethyl methanesulfonate (EMS), **2)** where genes were disrupted by the insertion of a transposable element (P element), or **3)** from the imprecise excision of such an element. Using these strategies the activity of a defined gene can be completely or partially lost. The partial loss of a gene activity is generally related to instable gene products, namely the mRNA or the protein.

Using these strategies we were able to evaluate several phenotypes related to mutation or over/ectopic expression of specific genes. The most widely used techniques to evaluate such phenotypes were **immunohistochemistry** and ***in situ* hybridization**, to respectively evaluate the expression profile of proteins or transcripts of genes of interest.

Protein extracts from embryos bearing the Gal4/UAS were also used to investigate the expression level of gene products, mRNA and proteins, using **qPCR and immunoblot**, respectively. Embryonic protein extracts were sometimes used to assess the physical interaction between proteins of interest by **co-immunoprecipitation** analysis.

2. Methods for S2 cells

Cell culture assays were undertaken when fly-based *in vivo* analysis present limits: **1)** to analyze separately the transcriptional activity of some TFs, whose activity is related *in vivo*, **2)** to block the activity of some factors using specific drugs, **3)** to separate inter-locked effects of several factors, and in our case transcriptional from post-translational effects, and **4)** to realize some biochemistry analysis aiming to analyze some post-translational modifications and protein-protein interactions.

S2 cell culture assay is based in the induction of foreign DNA by transfection, to force the cells to express a protein (s) of interest. The coding sequence of such protein is generally inserted into a plasmid, under the control of a promoter that is active in S2 cells, generally the *actin* promoter which is ubiquitously expressed.

To analyze the **transcriptional activity** of TFs, S2 cells were transfected with plasmids bearing the coding sequence of these factors, together with plasmids containing a coding sequence of reporter proteins, like GFP and RFP, under the control of promoters bearing the DNA binding sequence of the TF. The expression of specific proteins reflects the activity of the TF of interest, which can be measured by **immunoblot** or **immunohistochemistry,** by mean of specific antibodies.

Drug treatments were used to block the enzymatic activity of some factors, such as enzymes inducing protein post-translational modifications, in this case acetylation. This kind of experiments allows to determine the role of some modifications in proteins stability and activity, but also to evaluate the role of such modifications in the progression of defined biological processes.

S2 cell culture assay allowed us also to evaluate protein-protein interaction using tagged versions of proteins of interest.

RESULTS

RESULTS

1. 1st part. The gliogenic potential of Gcm and NSC plasticity

1. 1. Background

Previous studies aiming to evaluate the gliogenic potential of *gcm* identified several important mechanisms by which *gcm* controls the glial fate establishment, as well as a number of shared characteristics between endogenous and ectopic glial cells. However, these investigations left many interesting questions unclear.

Drosophila NSCs are not homogeneous and their progeny differ from one type to another (Introduction 4. 1. 1.); indeed, some of them generate only glial cells (GBs), others derive neurons and glia (NGBs), and the large majority exclusively produces neurons (NBs). It was previouely demonstrated that *gcm* ectopic expression in the neurogenic region, using drivers that induce its expression in all NSC types, forces some of them to adopt the glial fate in a *gcm* dosage dependent manner (Bernardoni et al., 1998; Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996). However, whether all ectopic cells derive from NGBs, NBs or both is still unclear. Several lines of evidence suggest that ectopic glial cells may be generated from all NSC types. The first clue is the high number of induced glial cells upon *gcm* ectopic expression, while the second concerns its ability to induce ectopic gliogenesis in other tissues than the NS, such as the mesoderm (Bernardoni et al., 1998; Miller et al., 1998).

Drosophila NSCs, as other types of SCs, actively proliferate during development, and then enter quiescence or die under programmed cell death. All the studies aiming to understand the gliogeneic potential of *gcm* used drivers that induce expression in young NSCs, when these cells are able to cycle: we asked whether the NSCs competence changes with aging and if cell division is a prerequisite to convert them into glia.

Previous studies demonstrated that ectopic glial cells are able to express most, if not all, the known glial markers, *gcm* and non-*gcm* dependent, showing that such cells are able to differentiate. However, none of the earlier studies focused onto the epigenetic marks making ectopic glial cells similar to the endogenous ones. This shortfall is probably due to the lack of adequate studies about the epigenetic marks of endogenous nervous cells. For all these

reasons, we got interested in the epigenetic characteristics of endogenous neurons and glial cells, compared to the ectopic ones.

1. 2. Manuscript I.

Gcm/Glide-dependent conversion into glia depends on neural stem cell age, but not on division, triggering a chromatin signature that is conserved in vertebrate glia

H. Flici, B. Erkosar, O. Komonyi, O. F. Karatas, P. Laneve and A. Giangrande (2011).

Development, 138(19): 4167-78.

Development 138, 4167-4178 (2011) doi:10.1242/dev.070391
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Gcm/Glide-dependent conversion into glia depends on neural stem cell age, but not on division, triggering a chromatin signature that is conserved in vertebrate glia

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SUMMARY

Neurons and glia differentiate from multipotent precursors called neural stem cells (NSCs), upon the activation of specific transcription factors. In vitro, it has been shown that NSCs display very plastic features; however, one of the major challenges is to understand the bases of lineage restriction and NSC plasticity in vivo, at the cellular level. We show here that overexpression of the Gcm transcription factor, which controls the glial versus neuronal fate choice, fully and efficiently converts *Drosophila* NSCs towards the glial fate via an intermediate state. Gcm acts in a dose-dependent and autonomous manner by concomitantly repressing the endogenous program and inducing the glial program in the NSC. Most NSCs divide several times to build the embryonic nervous system and eventually enter quiescence: strikingly, the gliogenic potential of Gcm decreases with time and quiescent NSCs are resistant to fate conversion. Together with the fact that Gcm is able to convert mutant NSCs that cannot divide, this indicates that plasticity depends on temporal cues rather than on the mitotic potential. Finally, NSC plasticity involves specific chromatin modifications. The endogenous glial cells, as well as those induced by Gcm overexpression display low levels of histone 3 lysine 9 acetylation (H3K9ac) and *Drosophila* CREB-binding protein (dCBP) Histone Acetyl-Transferase (HAT). Moreover, we show that dCBP targets the H3K9 residue and that high levels of dCBP HAT disrupt gliogenesis. Thus, glial differentiation needs low levels of histone acetylation, a feature shared by vertebrate glia, calling for an epigenetic pathway conserved in evolution.

KEY WORDS: Neural stem cells, Glia, *Drosophila,* dCBP, Gcm/Glide, Histone acetylation

INTRODUCTION

Glia and neurons, the major cell types of the nervous system, share a common precursor population, the NSCs (Bossing et al., 1996; Delaunay et al., 2008; Doe et al., 1998; Rivers et al., 2008; Schmidt et al., 1997). The multipotency of NSCs and their ability to be redirected towards different fates make these cells a promising tool in regenerative medicine; however, the plastic features of this initially homogeneous population needs to be fully understood. In addition, NSC behavior may rely on the experimental asset: while in vitro NSCs self-renew and may produce multiple fates indefinitely, in vivo, they give rise to specific progenies at distinct developmental stages (Gaspard and Vanderhaeghen, 2011). It therefore becomes important to characterize NSC plasticity at cellular and molecular level in vivo. In particular, can NSCs be completely and stably redirected and, if so, is this a constitutive feature? In addition, as histone modifications characterize and control specific transcriptional and differentiative states (Gibney and Nolan, 2010), what is the impact of fate conversion onto the cellular chromatin state?

Transcription factors play an important role in cell fate induction and, more generally, in plasticity (Graf and Enver, 2009); however, the glial versus neuronal decision in the vertebrate central nervous system (CNS) (Rowitch and Kriegstein, 2010) involves a rather complex gene network, which makes it difficult to assess the role and mode of action of such determinants in vivo (Allen, 2008). The simple *Drosophila* CNS makes it possible to tackle this issue in identified lineages. Moreover, a single transcription factor drives glial differentiation in *Drosophila* embryos: Glial cells missing (Gcm) [also called Glial cell deficient (Glide); referred to as Gcm hereafter] (for a review, see Soustelle and Giangrande, 2007). Gcm is transiently expressed in the lineages that produce glia and acts in the choice between glial and neuronal fates: its loss induces almost complete lack of glia, whereas its overexpression efficiently induces ectopic expression of the *reverse polarity* (*repo*) pan-glial gene (Bernardoni et al., 1998; Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996) and other glial transcripts (Altenhein et al., 2006; Egger et al., 2002; Freeman et al., 2003). The potent gliogenic activity of Gcm therefore provides an ideal asset with which to study lineage restriction and NSC plasticity in vivo.

We here show that *Drosophila* NSCs are stably and completely converted towards the glial fate upon overexpressing Gcm threshold levels. NSCs progressively lose plasticity and can no longer be converted at late embryonic stages, as they enter quiescence. Moreover, NSCs can be converted even in the absence of cell division whereas neurons cannot, showing that plasticity relies on temporal cues rather than on the mitotic potential. Finally, the Gcm pathway triggers low levels of H3K9ac and dCBP, a HAT that triggers H3K9 acetylation. This mark is key for glial development as, increasing H3K9ac levels by specifically overexpressing dCBP in glia, downregulates the expression of glial genes. Thus, a widely expressed HAT is crucial for a cell-specific transcriptional program. Finally, low levels of histone acetylation are conserved in vertebrate glia (Hsieh et al., 2004; Shen et al., 2005), indicating that glial cells need this specific chromatin mark.

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Accepted 28 July 2011

MATERIALS AND METHODS

Flies

Flies were raised at 25°C unless otherwise specified. w^{1118} was the wild type. Transgenic lines were: *UAS-gcm* (one dose: *F18A*: 1XGcm; two doses: *M24A*: 2XGcm) (Bernardoni et al., 1998); *lbe(K)-Gal4,UAS-GFP* (Baumgardt et al., 2009); *mzVum-Gal4* (Landgraf et al., 2003); *apterous-Gal4,UAS-mRFP* (Baumgardt et al., 2007); gcm^{34}*/CyO,twi-LacZ* (Vincent et al., 1996); *UAS-dCBP* and *UAS-dCBP-FLAD* (Kumar et al., 2004); *repo-Gal4/TM3* (Sepp et al., 2001); $repo^{3692}$*/TM3ubx-lacZ* (Halter et al., 1995); *repo-Gal4* (Lee and Jones, 2005); and *UAS-mCD8GFP, UAS-eGFP, elav-Gal4*, *voila-Gal4*, *hs-Gal4, tub-Gal80ts* and stg^{4}*/TM3* (Bloomington Drosophila Stock Center).

Immunohistochemistry and in situ hybridization

Immunolabeling and in situ hybridization on embryos were as described previously (Bernardoni et al., 1998). Primary antibodies were: mouse(m)-α-Repo (1:50), m-α-Engrailed (1:500) and rat(rt)-α-Elav (1:200) from DSHB; chicken-α-GFP (1:1000), rabbit(rb)-α-RFP (1:500), rb-α-Caspase3 (1:500), m-α-H3K9ac (1:500), rb-α-H3K4me3 (1:500) from Abcam; rb-α-GFP (1:500) (Molecular Probes); rb-α-Phospho histone H3 (Ser10) (1:500, Cell Signaling); rb-α-β-gal (1:500, Cappel); rb-α-Eagle [1:500 (Dittrich et al., 1997)]; rb-α-SP2637 and guinea-pig(gp)-α-Nazgul [1:500 (von Hilchen et al., 2010)]; rt-α-Repo [1:1000 (Sen et al., 2005)]; rb-α-Miranda [1:500 (Mollinari et al., 2002)]; gp-α-Deadpan (1:1000, J. Skeath, University of Washington, St Louis, MO); gp-α-dCBP [1:1000 (Lilja et al., 2003)]; rb-α-dTAF-4 [1:100 (Kokubo et al., 1994)]; m-α-Pol II [1:100 (Puvion-Dutilleul et al., 1997)]; rb-α-dGCN5 [1:500 (Lebedeva et al., 2005)]. Secondary antibodies were FITC-, Cy3-, Cy5-conjugated (1:400, Jackson). For in situ hybridization probes were DIG-*pain* [1:100 (Altenhein et al., 2006)] and DIG-*gcm* [1:100 (Bernardoni et al., 1997)]. Larval CNS proteins were immunolabeled as described previously (Ceron et al., 2001).

Imaging and cell counting

Images were taken with SP2 and SP5 Leica confocal microscopes. Image processing used Adobe Photoshop CS3. Cells were counted manually using ImageJ. For cell counting, means and standard errors were calculated and analyzed using Student's *t*-test.

Quantifications

For H3K9ac, H3K4me3 and dCBP relative levels, neurons and glia from the same embryonic VC were subjected to quantification using ImageJ. In brief, masks were generated as a region of interest for each nucleus along the *z*-stack, area (μm^2) and fluorescence intensities (pixel number) were measured and summed for all sections. The density of each nucleus was calculated by dividing the mean intensities over the nucleus volume (pixels/μm^3). For each embryo, values for all cell types were assigned by taking the highest value as reference and distributed in ten intervals from 1 to 10. The percentage of cells distributed in the different intervals was determined for each cell type for each embryo. This approach provided the best internal control. For quantifications, means and standard errors were calculated and samples were subjected to two-sample Kolmogorov-Smirnov test, which is sensitive to differences between cumulative distribution functions of two compared samples.

Northern and western blot analysis

elav-Gal4;UAS-mCD8GFP and *repo-Gal4;UAS-mCD8GFP* 17 stage embryos were used to purify neuronal and glial cell populations, respectively. Embryos were collected in Schneider's medium +3 mM EDTA and dissociated (Wheaton Dounce homogenizer). Single cell suspensions were obtained upon filtering through 70 mm and 40 mm nets (BD Biosciences) and centrifugation (100 ***g***, 5 minutes, 4°C). Cells were washed and collected in Schneider's medium (Gibco BRL), supplemented with 10% FCS (SDMS). GFP+ cells (neurons or glia) were separated with FACSDiVa flow cytometer (Becton Dickinson) (see Fig. S4A in the supplementary material).

For northern blot analyses, RNA was prepared from equal number of neurons and glia using TRIzol Reagent (Invitrogen) following manufacturer's instructions. mRNA levels were determined by northern blot with oligodT probe and using 5S-rRNA as internal control.

For western blot analyses, histone extracts from sorted neurons and glia were obtained as described in Abcam histone extraction protocol. Histone extracts (20 μg) were separated by 15% SDS-PAGE, transferred onto nitrocellulose membrane and probed with the primary antibodies diluted in 1× PBS, 5% bovine serum albumin: m-α-H3K9ac (1:2000), rb-α-H3K4me3 (1:10,000) from Abcam. m-α-H2B (1:10,000, IGBMC) was used for normalization. Signal was detected with Pierce ECL western blotting substrate (Thermo Fisher Scientific, Waltham, MA) using appropriate HRP-conjugated secondary antibodies (1:10,000, Jackson).

Reverse transcription and qRT-PCR

Total RNA was purified from *repo-Gal4;UAS-mCD8GFP* and *repo-Gal4;UAS-mCD8GFP;UAS-dCBP* embryos by TriReagent (MRC), reverse transcribed by SuperScriptII reverse transcriptase (Invitrogen) using a mix of random hexamers (6 μM) and oligodT primers (5 μM), and analyzed by quantitative PCR (qPCR) machine Roche LightCycler480 with Syber Green (Roche) Master mix. For each gene, expression levels were automatically calculated (LightCycler480 Software, release 1.5.0) by calibration to gene-specific standard curves generated on input cDNAs. Collected values, normalized to Actin5C amount, derive from three amplification reactions, each performed on three independent experiments. Primers are described in Table S1 in the supplementary material.

RESULTS

Gcm completely converts fly NSCs towards the glial fate

Previous studies showed that Gcm overexpression in the neuroepithelium prior to NSC birth induces the expression of glial markers through unknown mechanisms (Hosoya et al., 1995; Miller et al., 1998) (see the territory of expression as revealed by GFP labeling in Fig. S1A in the supplementary material). To understand the bases of lineage restriction and NSC plasticity, we specifically overexpressed Gcm with Gal4 drivers active in most (*voila-Gal4*) (Grosjean et al., 2001) (Fig. 1D, see Fig. S1D in the supplementary material) or in subsets of NSCs (*embryonic lethal abnormal vision-Gal4* or *elav-Gal4*) (Berger et al., 2007) (see Fig. S1B,C in the supplementary material). The Repo pan-glial marker is massively induced at ectopic positions, at the expense of the neuronal markers (see Fig. S2E,F in the supplementary material; data not shown), a phenotype that is stronger with the pan-neuroblast *voila-Gal4* driver than with *elav-Gal4* (Campbell et al., 1994) (Fig. 1A-C). Most *Drosophila* CNS glia arise from neuroglioblasts (NGBs) and few from glioblasts (GBs); however, the vast majority of embryonic NSCs only produces neurons [neuroblasts (NBs)] (Bossing et al., 1996; Schmidt et al., 1997). This strongly suggests that the massive number of ectopic glia also arise from precursors that only produce neurons. To verify this hypothesis, we overexpressed Gcm and used two lineage-specific markers, Eagle (Eg) and Engrailed (En), to identify unequivocally pure NBs (Doe, 1992). The so-called Thoracic 2-4 and 3-3 lineages, which are Eg(+),En(–) (Higashijima et al., 1996), clearly show Repo labeling, demonstrating that NBs overexpressing Gcm can produce glia at the expense of neurons (Fig. 1J-O).

We then asked how does Gcm induce glia and found that NSCs overexpressing Gcm lose their stemness, revealed by downregulation of the mitotic marker (PH3) (Fig. 1E) and of the NB markers Miranda (Mira) (Shen et al., 1997) (Fig. 1H,I) and Deadpan (Dpn) (Bier et al., 1992) (data not shown). As overexpression of Gcm begins, few cells express the glial marker ectopically and NBs are still present; however, most of them express both NB and glial markers. Later on, many more cells express the glial marker ectopically and only few cells express the NB marker, most of them also expressing the glial marker (Fig. 1F-I). The progressive increase of ectopic glial cells at the expense of NBs strongly suggests that the NBs initially express their program,

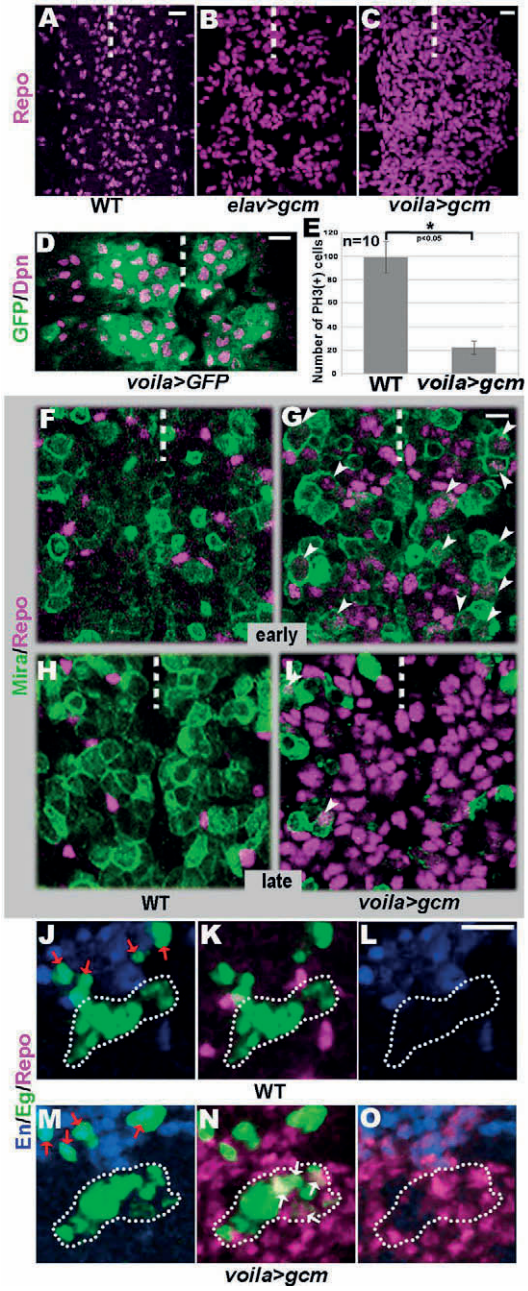

Fig. 1. Gcm redirects NSCs towards the glial fate. (**A-C**) Stage 16 embryos labeled with Repo (magenta) in: (A) control (wild type), (B) *elav-Gal4>UAS-gcm* (*elav>gcm*) and (C) *voila-Gal4>UAS-gcm* (*voila>gcm*) embryos. (**D**) *voila-Gal4>UAS-GFP* (*voila>GFP*) stage 13 embryo labeled with GFP (green) and NB marker Deadpan (Dpn, magenta). (**E**) The number of dividing, phospho-histone H3 (Ser10)-positive cells [PH3(+)], in the ventral cord (VC) of wild-type and *voila>gcm* stage 15 embryos. Data are mean±s.e.m. (**F-I**) Control (wild type) and *voila-Gal4>UAS-gcm* (*voila>gcm*) embryos labeled with the Miranda (Mira, green) NB marker and Repo (magenta) at early (F,G) and late (H,I) stages. Note the presence of Mira(+),Repo(+) cells (arrowheads) in *voila>gcm* (G,I) but not in the control embryo (F,H). (**J-O**) Control (wild type) (J-L) and *voila>gcm* (M-O) stage 14 embryos labeled with lineage-specific markers Eagle (Eg, green) and Engrailed (En, blue). Repo is in magenta. Broken lines indicate the progenies of pure NBs [Eg(+),En(–)],whereas red arrows indicate the progenies of Eg(+),En(+) lineages, one of which is an NGB. White arrows indicate ectopic gliogenesis in pure NBs (N). All panels show confocal projections, anterior is towards the top, broken line indicates midline. Unless specified, all figures show ventral views; error bars indicate ±s.e.m., asterisk indicates statistical significance. Scale bars: 10 μm.

then co-express the glial and the NB ones and finally only express the glial program. NSC conversion is dose-dependent and cell autonomous: it increases when two *UAS-gcm* reporters are used (see Fig. S2A,B in the supplementary material) and is confined to the cells overexpressing Gcm (see Fig. S2I,J in the supplementary material). Finally, the induction of late glial genes [Nazgul, SP2637 (von Hilchen et al., 2010), *pain* (Altenhein et al., 2006), Draper, β-Moody (Freeman et al., 2003)] (see Fig. S2C,D,G,H,K,L in the supplementary material and data not shown) confirms that stable and complete transformation has occurred. Thus, NSCs are fully converted into glia by the Gcm transcription factor.

Gcm cannot convert neurons into glia

Recent data indicate that somatic cells as fibroblast can transdifferentiate into neurons (Vierbuchen et al., 2010) and that one subtype of neurons can transdifferentiate into another (Wright et al., 2010). As the used neuroblast drivers continue to be expressed at late embryonic stages in post-mitotic cells (see Fig. S1E-G in the supplementary material) (Berger et al., 2007), we asked whether the observed phenotype may also arise from neurons, due to transdifferentiation and/or to reversion towards a more immature state.

To address this issue directly, we used Gal4 drivers that are expressed in post-mitotic cells but not in their precursors: *apterous-Gal4 (ap-Gal4)* and *mzVUM-Gal4* are specific to two motoneuron subsets (Baumgardt et al., 2007; Landgraf et al., 2003). These drivers are clearly able to induce Gcm overexpression upon crossing with *UAS-gcm* flies (Fig. 2E-H); however, they never induce ectopic Repo labeling (Fig. 2A-D), regardless of Gcm dose (Fig. 2E-H). Because these drivers are expressed at relatively late embryonic stages, we wondered whether the glial Repo marker might be induced in the larva. Even at that stage, however, no fate conversion was observed (Fig. 2I,J). Interestingly, neurons overexpressing Gcm enter the apoptotic pathway via Caspase 3 activation (Cohen, 1997) (Fig. 2K,L). In summary, neurons cannot be reprogrammed by Gcm.

NSC plasticity decreases during development

We then asked whether NSCs remain plastic throughout their life. The *lbe(K)-Gal4* line (Baumgardt et al., 2009) is expressed in an identified lineage, the so-called 5-6. In the thorax, the (5-6T) NSC is born by stage 9 and divides until stage 15. The TARGET system, based on a ubiquitously expressed thermosensitive Gal80 allele (tub-Gal80ts), makes it possible to repress the Gal4 activity at specific stages (McGuire et al., 2003). *Gal80ts,lbe(K)-Gal4>UAS-gcm* synchronized embryos were raised at the permissive temperature (18°C), shifted to the restrictive temperature (29°C) to induce Gcm expression at different stages (2 hours, 4 hours, 6 hours after egg collection) and let differentiate at that temperature (Fig. 3A-D). Under each condition, we waited for at least 9 hours, in order to allow sufficient time for Gcm activation [Gal80-induced repression is known to rapidly fade away after the shift (McGuire et al., 2003)]. Control animals not expressing Gcm were submitted

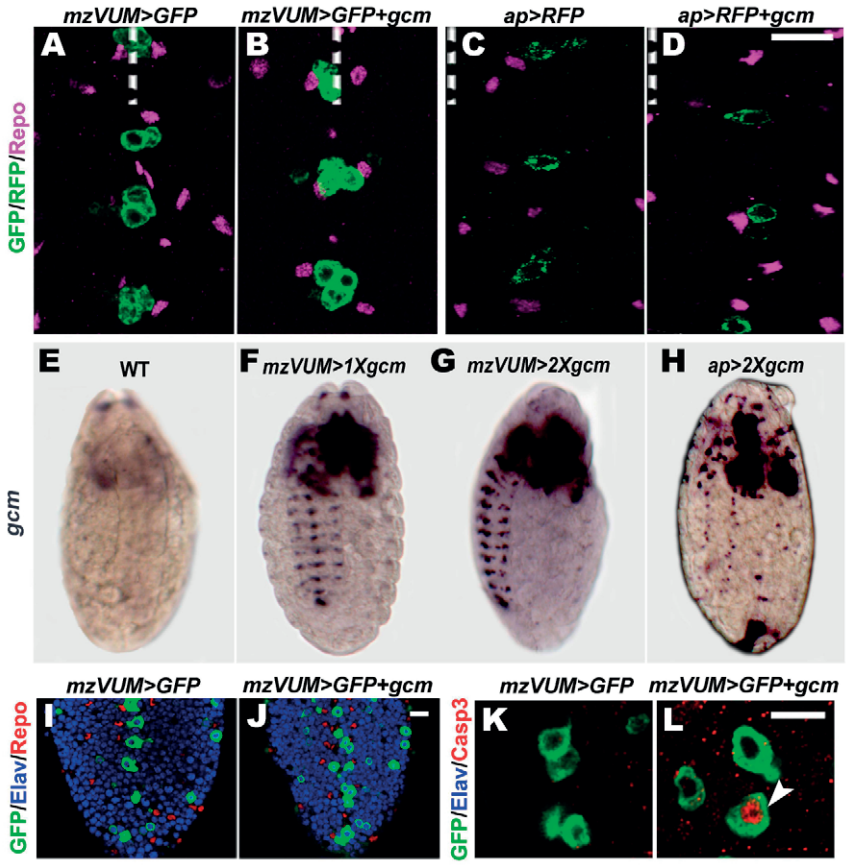

Fig. 2. Gcm cannot reprogram post-mitotic neurons. (**A-D**) Confocal sections of stage 16 embryos. No ectopic Repo (magenta) is observed upon Gcm overexpression using two different post-mitotic drivers, *mzVUM* and *apterous* (*ap*); neurons are visualized with GFP/RFP (green). Compare *mzVUM-Gal4>UAS-GFP* (*mzVUM>GFP*) control embryo (B) with *mzVUM-Gal4>UAS-GFP;UAS-gcm* (*mzVUM>GFP+gcm*) embryo (A) and *ap-Gal4>UAS-RFP* (*aP>RFP*) control embryo (D) with *ap-Gal4>UAS-RFP;UAS-gcm* (*aP>RFP+gcm*) embryo (C). (**E-H**) *gcm* RNA expression pattern in control embryo (wild type) (E) compared with Gcm-overexpressing embryos under *mzVUM-Gal4* and *ap-Gal4* drivers (F-H). *gcm* RNA levels increase when two *UAS-gcm* reporters are used [compare (*mzVUM>1Xgcm*) (F) to (*mzVUM>2Xgcm*) (G)]. (**I**,**J**) Confocal sections of 3rd instar larvae show no ectopic Repo (red) upon Gcm overexpression in post-mitotic neurons; neurons are visualized with Elav (blue), compare *mzVUM>GFP* (I) with *mzVUM>GFP+gcm* (J). (**K**,**L**) mzVUM neurons die by apoptosis upon Gcm overexpression, see the GFP(+),activated Caspase3 (Casp3, red)(+) cells (arrowhead) in L, but not in control animals (K). Scale bars: 10 μm.

to the same regimens for comparison. These results were also compared with those obtained upon constitutive Gcm expression in that lineage (Fig. 3B-D). Clearly, glial induction is most successful when the NSC is challenged with Gcm throughout development. Among the different shifts, the earliest one (2 hours) is more successful than a late one (4 hours) and in the latest shift (6 hours), no ectopic glia can be induced. Thus, the 5-6T NSC becomes less plastic with time.

This particular NSC is eliminated through programmed cell death at stage 15 (Karlsson et al., 2010), whereas others enter the quiescent state and resume proliferation during the larval stages, to build the adult nervous system (Sousa-Nunes et al., 2011). This cellular state is conserved throughout evolution, as quiescent NSCs are typical of the adult mammalian brain (Morshead et al., 1994). We asked whether plasticity decreases with time in all NSCs and induced pulses of Gcm expression at different stages using the heat shock (hs) promoter. We submitted *hs-Gal4>UAS-gcm* synchronized embryos to a 1-hour heat shock at the time NBs first arise and found that most if not all embryonic territories massively produce ectopic glia (see Fig. S3C in the supplementary material). Starting from a 6-7 hours after egg laying (AEL) heat shock, the strong gliogenic potential of Gcm is limited to the neurogenic region (Fig. 3E,F), where a single pulse of Gcm expression is sufficient to induce a stable and complete glial fate (see Fig. S3A,B in the supplementary material). Upon a shock at 8-9 hours AEL, the number of ectopic glia decreases considerably (Fig. 3G), even though in wild-type animals NSCs are still present and actively proliferating at this stage (Fig. 3I,J). Finally, upon a later shock (10-11 hours AEL, which corresponds to embryonic stage 14), almost no cells express ectopic Repo (Fig. 3H), even though Gcm expression is induced (see Fig. S3D in the supplementary material). The rare ectopic Repo(+) cells are also labeled by the Dpn and Mira NB markers, therefore expressing an intermediate fate (Fig. 3K). By these late stages, the number of NBs is still high but that of dividing NBs rapidly decreases (Fig. 3I,J) and that of quiescent NBs, recognized by Mira, Dpn labeling and by morphology [elongated shape and long cytoplasmic extension (Tsuji et al., 2008)], increases significantly (Fig. 3M-Q). Notably, none of the Repo(+),Dpn(+) cells is a quiescent cell (Fig. 3L). Thus, late NSCs can be poorly redirected towards the glial fate. Altogether, these data show that NSC plasticity becomes restricted with time.

NSC plasticity does not require cell division

During development, most novel fates are implemented upon cell division. We therefore asked whether NSC plasticity depends on cell cycle using a mutation in which NBs are generated but cannot divide. Previous studies have shown that NBs mutant for the String (Stg) protein, a Cdc25 phosphatase that activates a cyclin-dependent kinase and therefore mitosis (Edgar and O'Farrell, 1990), are blocked before their first division (Akiyama-Oda et al., 2000; Berger et al., 2010). Strikingly, Gcm overexpression does induce ectopic Repo labeling in *stg* mutant NBs (Fig. 4A-D,H).

We then asked whether the presumptive non-dividing NBs can be fully converted and found that the late glial marker SP2637 is also induced (Fig. 4E). Moreover, similar to wild-type NSCs challenged with Gcm, the NB fate is downregulated in *stg* embryos overexpressing Gcm (Fig. 4F,G). Interestingly, even at these late stages, some non-dividing NBs simultaneously express a glial and a NB marker, a phenotype that is rarely observed in *stg* animals (Fig. 4F,G, arrowheads). Moreover, 14% of the cells that express

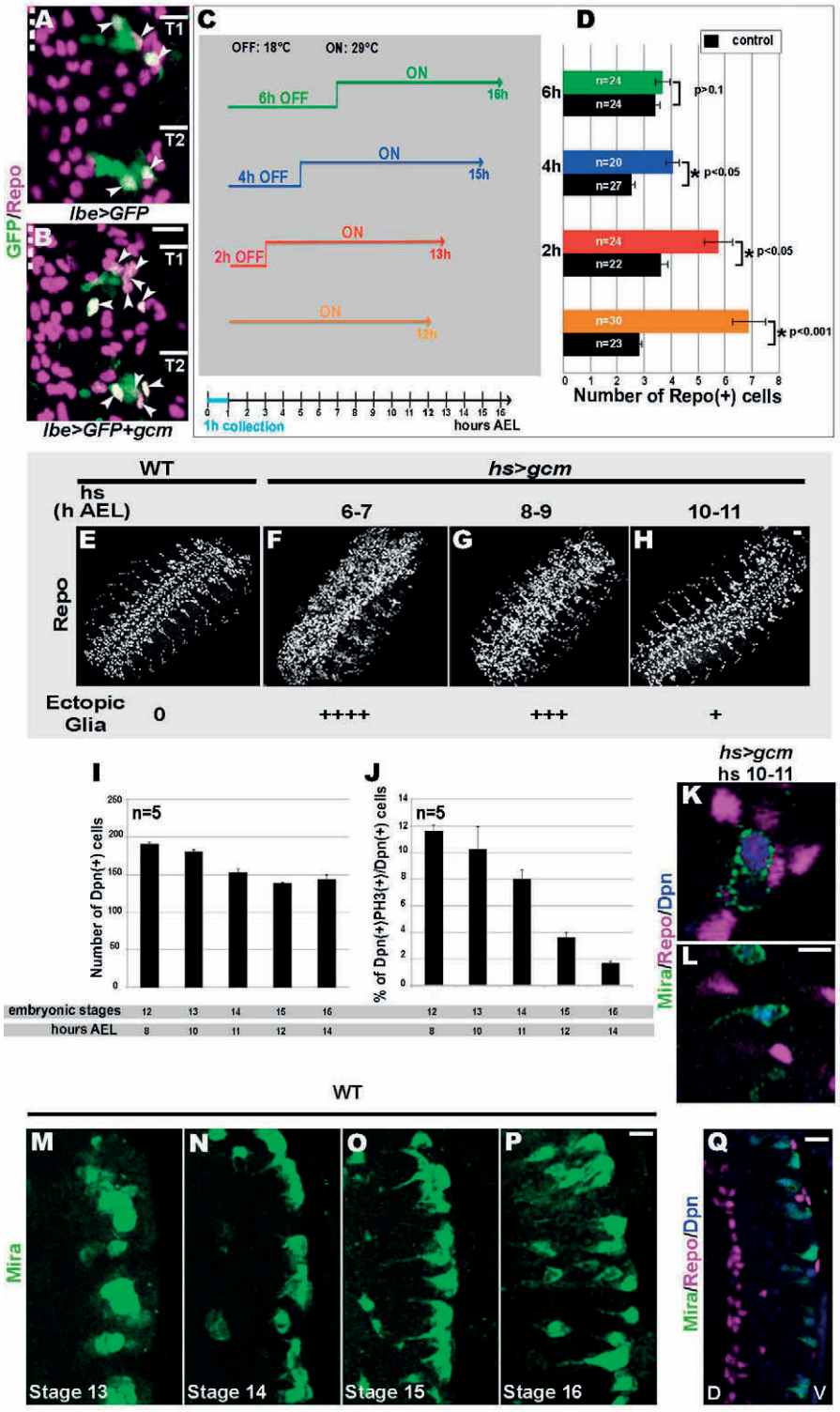

Fig. 3. NSC plasticity decreases during development. (**A**,**B**) Projections of stage 16 embryos show Repo (magenta) expression upon Gcm overexpression in the 5-6T lineage (arrowheads) using the *lbe(K)-Gal4* driver. Compare *lbe(K)-Gal4>UAS-GFP* (*lbe>GFP*) control embryo (A) with *lbe(K)-Gal4>UAS-GFP;UAS-gcm* (*lbe>GFP+gcm*) (B). The 5-6T lineage was visualized by GFP (green). (**C**,**D**) Induction of ectopic Repo upon Gcm overexpression in the 5-6T lineage at different embryonic stages using the TARGET system, compare *Gal80ts,lbe(K)-Gal4>UAS-GFP* (*Gal80ts,lbe>GFP*) control with *Gal80ts,lbe(K)-Gal4>UAS-GFP;UAS-gcm* (*Gal80ts,lbe>GFP+gcm*) animals treated in the same way. Embryos were collected for 1 hour and raised at 18°C (OFF: repressive temperature) then shifted to 29°C (ON: permissive temperature) to induce Gcm expression at different stages, as indicated, until stage 16. Color coding indicates the different times at which the temperature was shifted: 2 hours (red), 4 hours (blue) and 6 hours (green) after egg collection. 0 h indicates embryos kept at 29°C from birth (orange). (D) The number of Repo(+) cells generated from the 5-6T lineage in control (black columns) and Gcm-overexpressing (colored columns) embryos. The number of Repo(+) cells is similar in overexpressing and control embryos upon a temperature shift at 6 hours AEL (green). n indicates the number of 5-6T lineages. (**E-H**) Projections showing Repo (white) in control, *hs-Gal4>W*^{1118} (WT) (E) and *hs-Gal4>UAS-gcm* (*hs>gcm*) embryos (F-H) upon 1 hour heat shock (hs) at the indicated times (hours AEL) then fixed 5 hours after the heat pulse. (**I**,**J**) The graphs show the number of NBs [Dpn(+)] (I) and the percentage of mitotic NBs [PH3(+),Dpn(+)] (J) in the thorax of different embryonic stages. n indicates the number of embryos. Data are mean±s.e.m. (**K**,**L**) *hs>gcm* embryo fixed 5 hours after the hs, showing colocalization between Repo (magenta) and Dpn (blue) in round Mira(+) cells but not in elongated Mira(+) NBs. (**M-Q**) Projections showing thoracic NBs labeled by Mira (green) at the indicated embryonic stages; lateral views, dorsal (D) towards the left, ventral (V). Note the progressive accumulation of quiescent NBs [elongated Mira(+) cells] during development. Scale bars: 10 μm.

Repo also express the neuronal marker Elav, a phenotype that is also very rarely observed in *stg* or wild-type embryos, as well as in wild-type embryos overexpressing Gcm (Fig. 4I and data not shown). Thus, plasticity is not connected to the mitotic potential.

Neurons and glia display different H3K9ac levels

Increasing evidence suggests that transcriptional developmental programs are associated with specific chromatin landmarks (for a review, see Lessard and Crabtree, 2010) and it has been shown that low levels of histone acetylation characterize vertebrate glial cells (Hsieh et al., 2004; Jakob, 2011). We therefore determined the overall histone acetylation profiles of wild-type neurons and glia. The CNS displays high levels of H3K9ac (Qi et al., 2004), which is abundant in euchromatin. We labeled fully differentiated neurons and glia with an anti-H3K9ac antibody and found that glia display lower H3K9ac levels compared with neurons (Fig. 5A-C). To gain quantitative information, we identified ten levels of H3K9ac intensity (see Materials and methods for detailed description) upon

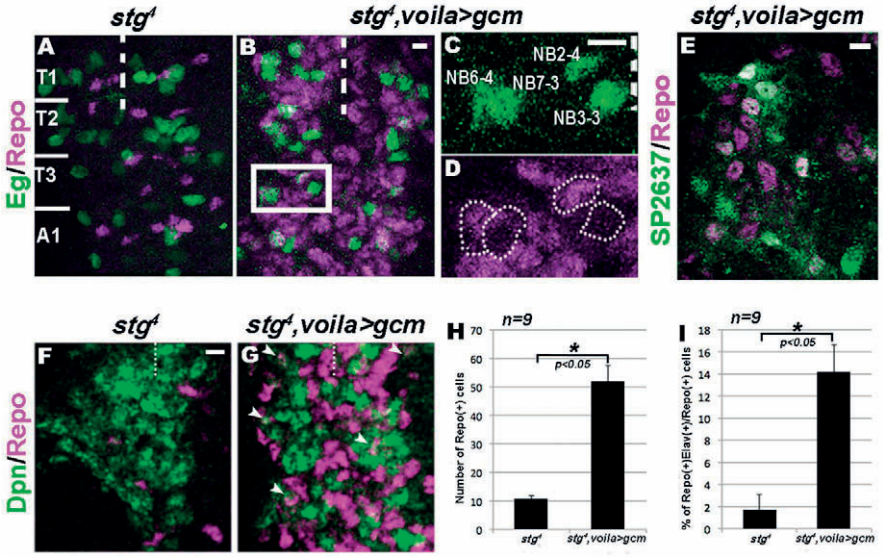

Fig. 4. NSC conversion does not require cell division. (**A-D**) Projections show *stg*^{4} (A) and *stg*^{4}*,voila-Gal4>UAS-gcm* (*stg*^{4}*,voila>gcm*) (B) stage 15 embryos labeled with Eg (green) lineage-specific tracer and Repo (magenta). (C,D) The four Eg-specific lineages present in each thoracic hemisegment (boxed in B; note ectopic gliogenesis in undivided NBs (magenta). (**E**) Confocal section from *stg*^{4}*, voila>gcm* VC shows glial subsets expressing late glial marker SP2637 (green). (**F**,**G**) Confocal sections showing thoracic segments of stage 15 embryos. Note the colocalization between Repo and Dpn in *stg*^{4}*, voila>gcm* but not in *stg*^{4} embryos (G). Scale bars: 10 μm. (**H**,**I**) The graphs show the number of Repo(+) cells (H) and the percentage of Repo(+),Elav(+) cells (I), in the VC of *stg*^{4} and *stg*^{4}*, voila>gcm*$^{-}$ embryos. Data are mean±s.e.m.

comparing glial cells and neurons within the same embryo. Clearly, most glial cells display relatively low levels of H3K9ac compared with neurons (Fig. 5E, embryo *n*=6). Finally, we confirmed this result by western blot on histone extracts from purified neurons and glia (see Fig. S4 in the supplementary material, Fig. 5K). The purity of such populations was validated by using cell-specific markers (see Fig. S4B,C in the supplementary material).

Interestingly, the levels of H3K4me3, which specifically marks transcriptionally active genes (Lessard and Crabtree, 2010), are similar in neurons and glia (embryo *n*=5, *P*>0,08, Fig. 5I), as confirmed by western blot (Fig. 5L). Moreover, H3K9ac partially colocalizes within the cell with H3K4me3 [32.8% colocalization in neurons and 34.4% in glia, *n*=10 (see Fig. S5A-C, Movies 1 and 2 in the supplementary material)], which we confirmed in 3D reconstructions of glial and neuronal nuclei (see Fig. S5A,B in the supplementary material). These data imply that different acetylation levels do not reflect global differences in transcription activity between neurons and glia, and, to further validate this, we showed that the total mRNA levels are not higher in neurons than in glia (Fig. 5J). Finally, we quantified H3K9me3 levels, as a mark of repression and heterochromatin (Lessard and Crabtree, 2010), and found no significant difference between neurons and glia either (embryo *n*=4, see Fig. S5D in the supplementary material). In summary, neurons and glia have distinct properties in terms of chromatin marks, which are independent from the overall transcriptional state.

The Gcm pathway controls the levels of H3K9ac

We then asked whether fate conversion upon Gcm overexpression results in changes in histone acetylation, i.e. whether ectopic glia show low H3K9ac levels. As we wanted to compare the acetylation state of endogenous and ectopic glia within the same animal, we labeled embryos (*elav-Gal4>UAS-GFP;UAS-gcm*) with GFP and Repo [endogenous glia: GFP(–),Repo(+), ectopic glia: GFP(+),Repo(+)] (Fig. 5D) and found that both endogenous and ectopic glia display low H3K9ac levels (embryo *n*=5, Fig. 5F).

Several pieces of evidence indicate that low H3K9ac specifically characterizes glial identity. First, the dose of Gcm impacts onto the number of cells showing low H3K9ac levels [compare the H3K9ac levels upon overexpressing two (Fig. 5F) and one (see Fig. S6G in the supplementary material) dose of Gcm], as it impacts onto the number of ectopic glia. Second, post-mitotic Gcm expression, which does not induce reprogramming, does not change H3K9ac levels (even with two Gcm doses) (see Fig. S6A-C in the supplementary material). Third, if alterations in H3K9ac levels were to reflect fate changes, *gcm* animals, in which presumptive glial cells are converted into neurons, should display opposite changes in the H3K9ac profiles. *gcm*^{34} animals maintain *lacZ* expression from a P-element inserted at the *gcm* locus, thereby allowing us to identify the cells that transform into ectopic neurons [*lacZ*(+),Repo(–),Elav(+)] (Vincent et al., 1996). These cells indeed display high H3K9ac levels, similar to the endogenous neurons (embryo *n*=5) or from wild-type animals (Fig. 5G). Finally, we analyzed the H3K9ac levels in animals that lack the Repo protein, which show no glia to neuron conversion, using a null mutant that maintains *lacZ* expression from a P-element at the *repo* locus (Campbell et al., 1994; Halter et al., 1995). In this mutant, the *lacZ*(+) cells still show low levels of H3K9ac compared with neurons and similar to the wild-type glia (embryo *n*=5) (Fig. 5H). In summary, the Gcm pathway induces global changes in H3K9ac levels.

Neurons and glia display distinct dCBP levels that are controlled by the Gcm pathway

dCBP (Akimaru et al., 1997) constitutes a likely candidate for the above-described histone modification, as its orthologs, CBP/p300 HATs, trigger the acetylation of H3K9 (Wang et al., 2010). We therefore overexpressed dCBP in glia (*repo>dCBP*) and demonstrated for the first time in vivo that it induces high levels of H3K9ac (Fig. 6E,F,H,I,K,L), whereas overexpressing a dCBP mutant form that lacks its HAT activity (dCBP-FLAD) (Kumar et al., 2004) does not (Fig. 6G,J,M). Accordingly, *nej*^{3} embryos, which do not express zygotic dCBP, show reduced levels of H3K9ac, which are due to the maternal load (see Fig. S7A-D in the supplementary material). These data suggest that the dCBP HAT contributes to the different H3K9ac levels observed in neurons and glia.

If neurons and glia display distinct properties in terms of histone acetylation, the HAT responsible for such marking must either act in a different way or accumulate at different levels in these cell

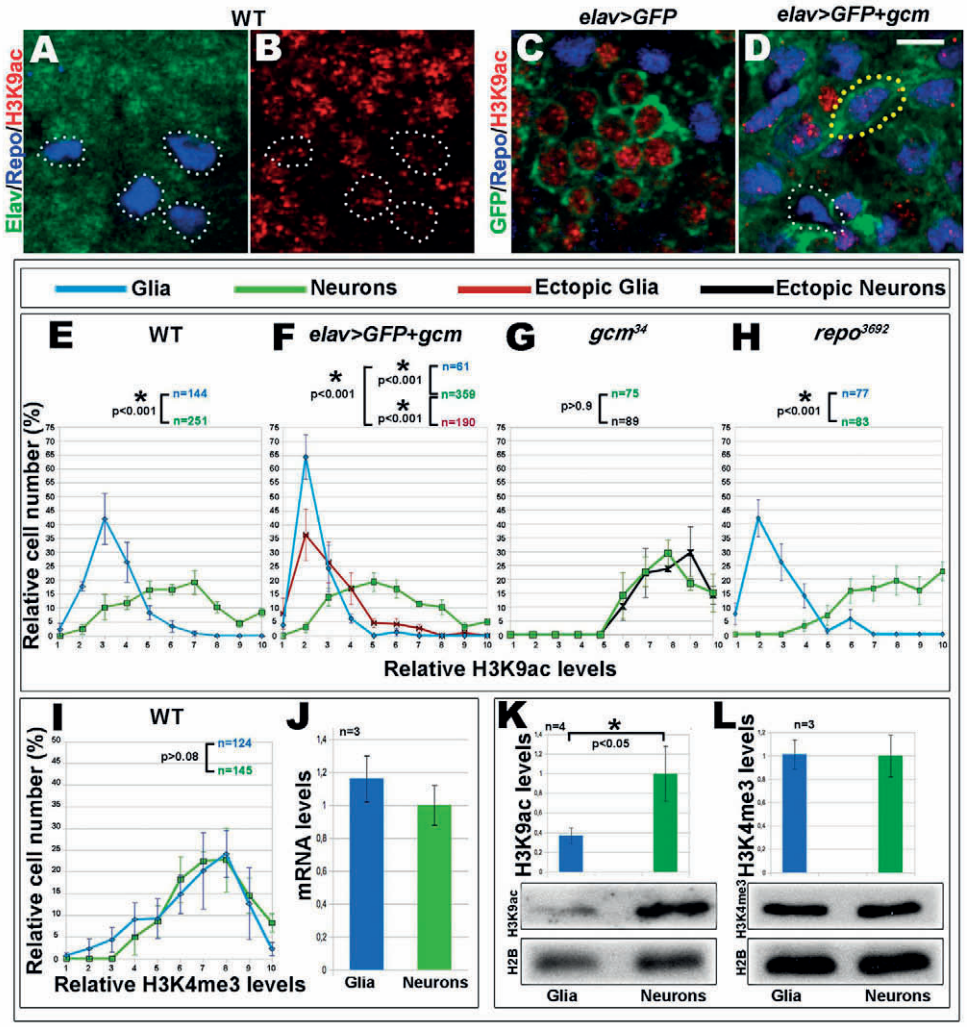

Fig. 5. Levels of chromatin marks in neurons and glia from wild-type, *gcm*, *repo* and Gcm-overexpressing embryos. (**A-D**) Confocal sections (stage 17) showing the profile of H3K9ac (red) in glia (Repo, blue) and neurons (green) of control embryos: wild type (A,B) and *elav-Gal4>UAS-mCD8GFP* (*elav>GFP*) (C). (D) Gcm-overexpressing embryos: *elav-Gal4>UASmCD8GFP;UAS-gcm* (*elav>GFP+gcm*). Neurons are visualized with Elav in A and GFP in C,D. White and yellow dotted circles indicate endogenous [Repo(+)] and ectopic glia [GFP(+),Repo(+)], respectively. (**E-H**) H3K9ac levels in neurons (green), glia (blue), ectopic-glia (red) and ectopic-neurons (black) quantified and plotted for wild-type (E), Gcm-overexpressing (*elav>GFP+gcm*) (F), *gcm* (*gcm*^{34}) (G) and *repo* (*repo*^{3692}) (H) embryos; n indicates the number of cells. (**I**) Quantified neuronal and glial H3K4me3 levels in wild-type embryos. For graphs in E-I, labeling intensity was arbitrarily subdivided into ten levels going from lowest (1) to highest (10) (*x*-axis), *y*-axis indicates the percentage of cells for each intensity (see Materials and methods). Data are mean±s.e.m. (**J**) Comparison of neuronal and glial mRNA levels upon northern blot. mRNA levels were normalized to those of neurons arbitrarily chosen as '1'. (**K**,**L**) Western blot analysis of H3K9ac and H3K4me3 in histone extracts from FACSed glia and neurons. Neurons display higher levels of H3K9ac than glia (K). Neurons and glia show same levels of H3K4me3 (L). Quantifications of western blot results; mean value is shown in graphs ±s.e.m.; n indicates the number of experiments. H2B was used as the loading control. Scale bar: 5 μm.

types. Indeed, most neurons display higher dCBP levels than glia (embryo *n*=4, Fig. 6A). Moreover, and in line with the above data, ectopic glia induced by Gcm overexpression exhibit dCBP levels similar to those of endogenous glia (*n*=5, Fig. 6B); upon ectopic neurogenesis induced by *gcm* loss, dCBP levels are similar to those of endogenous neurons (embryo *n*=5, Fig. 6C). Finally, dCBP levels do not change in *repo* animals (embryo *n*=5) (Fig. 6D) or upon Gcm overexpression in neurons (see Fig. S6D-F in the supplementary material). Thus, like the H3K9ac levels, dCBP levels also change upon the acquisition of specific cell fates. Interestingly, the levels of dGCN5, another major HAT involved in H3K9 acetylation (Carre et al., 2005) are not different between neurons and glia, and dGCN5 overexpression does not affect H3K9ac levels (see Fig. S8A-C,F,G in the supplementary material). Thus, neurons and glia display distinct levels of dCBP, a HAT that affects H3K9ac levels, and this depends on the Gcm pathway.

High dCBP levels affect glial-specific gene expression

The fact that glia display low dCBP and H3K9ac levels and that fate conversion is accompanied by corresponding changes in those levels suggests that low dCBP levels have a physiological relevance in glial differentiation. We therefore determined the consequences of dCBP overexpression in glia and found that this leads to an obvious increase in H3K9ac levels in glial cells and to embryonic lethality. The few larval escapers do not show the

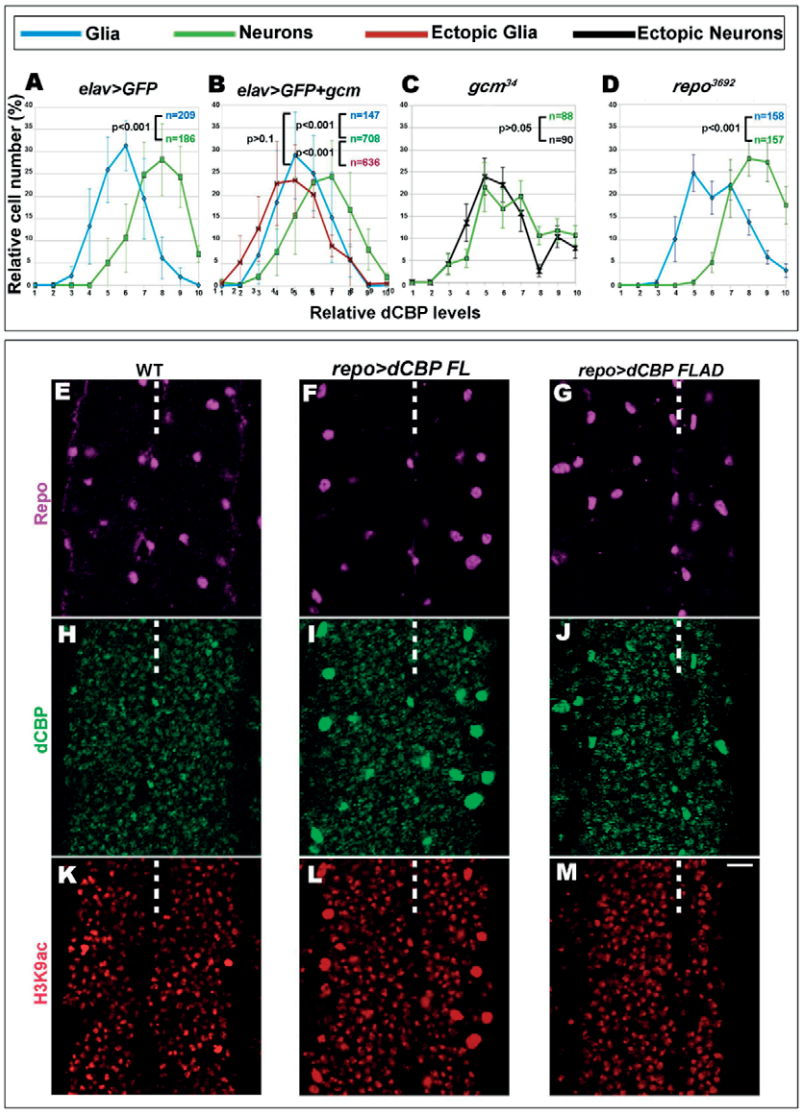

Fig. 6. dCBP levels in neurons and glia. (**A-D**) Graphs show dCBP levels in neurons (green), glia (blue), ectopic glia (red) and ectopic neurons (black) quantified and plotted for control (wild type) (A), Gcm-overexpressing (*elav>GFP+gcm*) (B), *gcm* (*gcm*^{34}) (C) and *repo* (*repo*^{3692}) embryos (D). Data are mean±s.e.m. (**E-M**) Confocal sections from control embryos (wild type), embryos overexpressing wild-type (*repo>dCBP*) or inactive dCBP (*repo>dCBP-FLAD*), labeled with Repo (magenta), dCBP (red) and H3K9ac (cyan). Scale bar: 10 μm.

typical contractions generated by neuronal activity, showing that glial cells cannot sustain high dCBP levels. Such a phenotype depends on the HAT activity, as animals overexpressing the dCBP-FLAD transgene survive until adulthood. The number of Repo(+) cells does not change, nor is the caspase pathway activated, indicating that high dCBP levels do not merely affect the number of glia by inducing glial cell death (see Fig. S7E-G in the supplementary material).

We then asked whether high dCBP levels impact onto the expression of glial-specific genes. Clearly, the levels of *pain*, which is specific to subperineural and nerve root glia (Beckervordersandforth et al., 2008), of Nazgul, which is specific for longitudinal and cell body glia (von Hilchen et al., 2010), and of Repo decrease upon wild-type dCBP, but not upon HAT inactive dCBP overexpression (Fig. 7A-I). The levels of SP2637, a nuclear factor that is specific for surface-associated and nerve root glia (von Hilchen et al., 2010) remain unmodified (Fig. 7J,K). Thus, the expression of most but not all tested glial genes is downregulated by high dCBP levels. By contrast, three ubiquitously expressed genes: DNA Pol II (Puvion-Dutilleul et al., 1997), dGCN5 (Xu et al., 1998) and the dTAF-4 subunit of the TFIID complex that initiates transcription (Kokubo et al., 1994), are not affected (Fig. 7M-R). In order to quantify and extend these findings, we performed quantitative RT-PCR on nine glial-specific transcripts: *repo*, β-*moody*, which is specific to surface glia (Bainton et al., 2005), *draper*, which is specific to lateral glia (Freeman et al., 2003), *pain*, *nazgul*, *SP2637* and three glial transcripts identified by microarrays (Altenhein et al., 2006; Egger et al., 2002; Freeman et al., 2003). As above, dCBP overexpression affects the levels of all transcripts except those of *SP2637* (Fig. 7L). As a negative control, we also analyzed the neuronal *elav* gene, the levels of which are not changed. These data show that high levels of dCBP HAT induce lethality and affect the glial transcriptional program.

DISCUSSION

Understanding the biology and the potential of stem cells of specific origins is a key issue in basic science and in regenerative medicine. We here show that NSCs can be fully and stably redirected towards the glial fate in vivo, via a transient, intermediate, state, upon the expression of a single transcription factor. NSC plasticity is temporally controlled and quiescent NSCs cannot be converted; however, plasticity is independent of cell division. Finally, the acquisition of the glial fate involves low

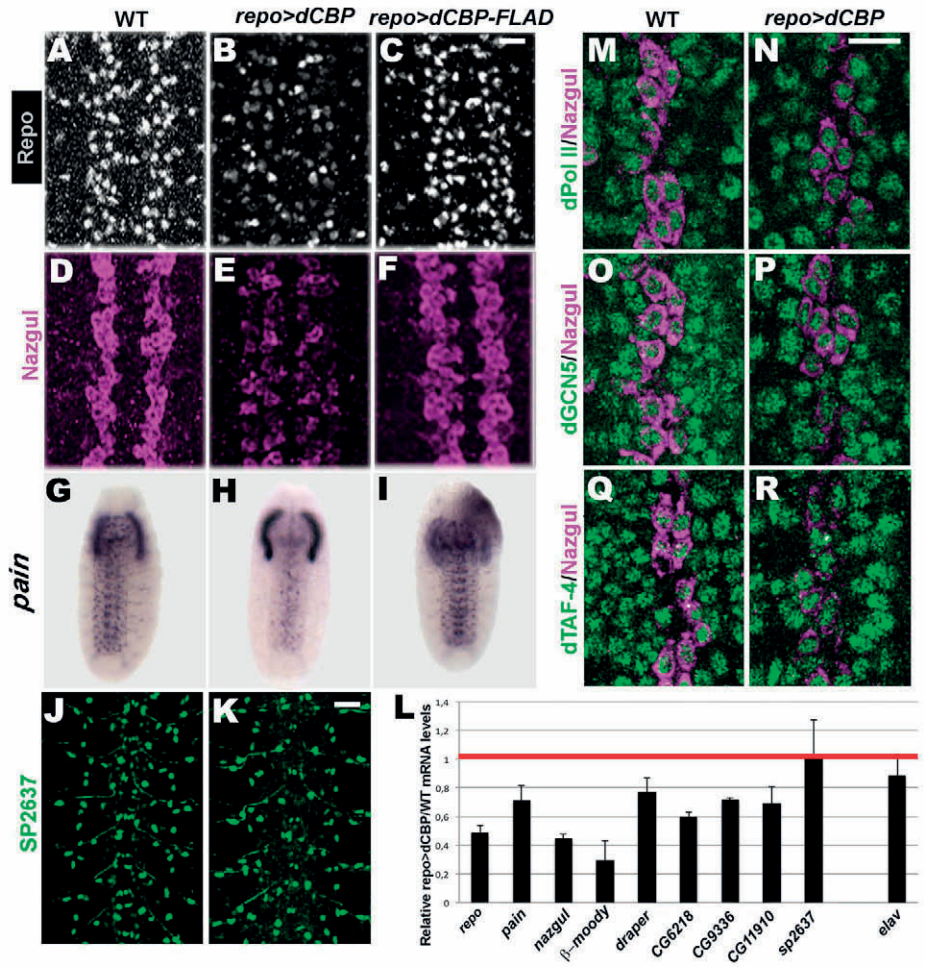

Fig. 7. dCBP overexpression downregulates the expression of glial-specific genes. (**A-I,M-R**) The expression of the glial markers: Repo (white) (A-C), Nazgul (magenta) (D-F,M-R) (same embryos as in A-C) and *pain* (in situ hybridization) (G-I) decrease drastically upon dCBP (B,E,H) but not dCBP-FLAD (C,F,I) overexpression. (**J**,**K**) The glial marker SP2637 (green) and the ubiquitously expressed genes dPol II, dGCN5 and dTAF-4 (green) are not affected upon dCBP overexpression. Compare K with J. Scale bars: 20 μm. (**L**) Relative expression of glial markers upon dCBP overexpression in glia. *elav* is used as a control for the expression of a neuronal gene. For wild-type and dCBP-overexpressing embryos, the amount of each transcript was normalized to that of Actin. The wild-type values were arbitrarily taken as 1 (red line) and, for each transcript, the ratio *repo>dCBP/WT* was determined in three independent experiments (columns show average values±s.e.m.).

histone acetylation, a chromatin modification that is conserved throughout evolution, emphasizing the importance of this mark in glial cells.

NSCs can be fully redirected towards the glial fate in vivo

NSCs produce the different types of neurons and glia that form the nervous system. These precursors can be converted into induced pluripotent cells (Kim et al., 2009) and even into monocytes, a differentiated fate of an unrelated somatic lineage (Forsberg et al., 2010); however, the in vitro behavior may differ markedly from the in vivo situation. For example, the Achaete-Scute Complex homolog-like 1 transcription factor promotes the expression of oligodendrocyte features upon retroviral injection in the dentate gyrus, but promotes neuronal differentiation from the same progenitors in vitro (Jessberger et al., 2008). The use of NB-specific drivers, markers and conditional overexpression protocols, allows us to demonstrate that a single transcription factor can fully convert NSCs into glia in a dose-dependent manner. High Gcm levels probably enable this transcription factor to counteract the endogenous transcriptional program and/or to compensate for the absence of cell-specific co-factors. Quantitative regulation is also required in physiological conditions; for example, the nuclear protein Huckebein enhances the gliogenic potential of Gcm upon triggering its positive autoregulation in a specific lineage (De Iaco et al., 2006). The present study therefore shows for the first time that NSCs can be completely and efficiently redirected in vivo towards a specific fate, also highlighting the importance of quantitative regulation in fate choices.

Temporal control of NSC plasticity

It is widely accepted that NSCs are multipotent precursors; however, their plastic features have not been investigated throughout their life at the cellular level. For example, the existence of a tri-potent NSC with the capacity to generate neurons, astrocytes and oligodendrocytes in the adult brain remains to be demonstrated in vivo (Williams et al., 1991). Our study demonstrates that NSCs are more plastic at early embryonic stages than at the end of embryogenesis. Furthermore, the intrinsically defined program of quiescence is not compatible with fate conversion, even though quiescent cells are subsequently reactivated. As *Drosophila* glia are generated at different stages (Halter et al., 1995) (S. Sorrentino and A.G., unpublished), it is unlikely that a general glial repressor arises late in development and specifically restricts the potential of Gcm. Our data rather imply that temporal cues progressively limit NSC plasticity, a feature that may have important consequences in therapeutic applications.

It will be of great interest to determine whether such irreversible temporal restriction relies on external cues or whether it reflects an internal clock, as it has been shown for the acquisition of temporal identity, the process by which specific progenies are produced at different developmental stages (Doe, 1992).

Finally, our data show that Gcm does not reprogram neurons. Thus, although other somatic (Vierbuchen et al., 2010) and even germ line (Tursun et al., 2011) cells can be reprogrammed into neurons, these post-mitotic cells seem endowed with an efficient brake to fate conversion. Interestingly, dorsal root ganglia neurons can transdifferentiate from one subtype into another in zebrafish, suggesting that, under some conditions, neurons can adopt a different, but closely related, phenotype (Wright et al., 2010). In addition, we cannot formally exclude that a low percentage of immature neurons adopt a glial or a multipotent phenotype upon Gcm overexpression. Nevertheless, our data indicate that neurons are intrinsically different from other cell types, which may reflect a specific chromatin organization and/or expression of an efficient tumor suppressor molecular network (for a review, see Jopling et al., 2011). Transcriptome analyses will help characterizing the molecular signature responsible for the neuronal behavior.

Plasticity and intermediate states

Dedifferentiation and transdifferentiation of somatic cells can occur in the absence of mitosis (Richard et al., 2011), whereas NSCs plasticity has generally been associated to cell division, as a means to erase transcriptional programs and implement new ones. We here show that, like terminally differentiated cells, NSCs can be efficiently redirected in the absence of cell division. The concomitant extinction of the endogenous program and activation of the glial program indicate that conversion occurs via an intermediate state, as has been described in B cell to macrophage experimental transdifferentiation (Xie et al., 2004). The acquisition of an intermediate state (partial reprogramming) has also been proposed for somatic cell reprogramming (Hanna et al., 2009). Our findings raise a more general question as to whether intermediate states are common and unstable features of many plastic process including development. These states may reveal competing molecular pathways that in physiological conditions are alternatively consolidated or switched off in response to cell-specific signals. The development of tools enabling tracing these dynamic states will improve our understanding of cell plasticity under physiological and experimental conditions.

Interestingly, altered tumor suppressor gene expression, which alters the proliferation pathway, leads to ambiguous cell identities, which may reflect the stabilization of intermediate fates (Ma et al., 2007). Similarly, *Drosophila* metastatic cells from brain tumors (Beaucher et al., 2007) and several non-dividing NSC cells challenged with Gcm co-express the neuronal and the glial programs. We propose that the appropriate activation of the mitotic pathway is necessary for efficient consolidation/extinction of specific fates.

Low H3K9ac and dCBP levels characterize glial cells

The interplay of extrinsic signals, transcription factors and chromatin modifications shape the identity of different cell types. The low and high levels of dCBP as well as H3K9ac truly represent a glial and neuronal signature, respectively. They both depend on *gcm*, which controls the fate choice, but not on genes downstream to Gcm, which are not sufficient to implement such choice (compare H3K9ac/dCBP levels in *gcm* embryos, with those in *repo* or *tramtrack* embryos) (Fig. 5H, Fig. 6D; data not shown). Thus, full fate conversion is accompanied by a cell-specific chromatin modification.

Interestingly, whereas dCBP accumulates at different levels in glia versus neurons and its overexpression or loss affects the levels of H3K9ac, the levels of dGCN5, another HAT that is able to acetylate the H3K9 residue in vivo (Carre et al., 2005), are similar in glia and neurons. Moreover, dGCN5 overexpression does not enhance H3K9ac levels nor does it affect the expression of glial genes (see Fig. S8D,E in the supplementary material). These data strongly suggest that the dCBP HAT specifically participates in setting up the H3K9ac signature. It should be noted that dGCN5 is a member of multiprotein complexes (Muratoglu et al., 2003), which may explain why its overexpression cannot produce high HAT activity on its own. The balance between HATs and histone deacetylases (HDACs), enzymes with counteracting activities, is thought to be important in the regulation of histone acetylation levels. Although the investigation of histone deacetylation is not in the focus of this paper, the relevance of HDACs in the control of the glia-neuron histone acetylation signature cannot be excluded.

The tight regulation of histone acetylation in the nervous system seems to be evolutionarily conserved. Human neuronal disorders are frequently connected to downregulation of histone acetylation and HDAC inhibitors are good candidates as therapeutic tools (Lubin et al., 2011). Histone acetylation is instrumental for mammalian memory formation (Lesburgueres et al., 2011; Lubin et al., 2011) and CBP plays an important role in long-term memory processes (Barrett et al., 2011; Valor et al., 2011). Altogether, these data indicate that normal neuronal function requires high levels of histone acetylation.

Our study shows that low HAT activity is necessary for glial differentiation. The increased levels of histone acetylation by overexpression of dCBP cause downregulation of the majority (but not all) of the tested glial genes, whereas the levels of general nuclear factors remain unchanged. The glial cells do not undergo apoptosis, indicating that high dCBP and histone acetylation levels influence specific pathways rather than generally affecting cell viability. The exact molecular mechanisms are not known, yet the behavior is similar in the mammalian CNS. Oligodendrocyte differentiation requires low levels of histone acetylation, resulting from high amounts of HDACs and low amounts of HATs (CBP and P300) (Shen et al., 2005). The role of HDACs was further investigated (Shen et al., 2008; Ye et al., 2009), showing that such enzymes directly repress genes that prevent oligodendrocyte differentiation. The role of HATs was not investigated in these publications, but most probably an appropriate balance between HATs and HDACs is the key factor, which produces low levels of histone acetylation and regulates mammalian as well as *Drosophila* glial differentiation.

The broadly accepted model is that histone acetylation weakens the interaction between positively charged histone tails and negatively charged DNA, thereby contributing to transcriptional activation. Our data contradict this simple model. First, the levels of H3K4me3, a histone mark that is connected to actively transcribed genes (Lessard and Crabtree, 2010), are similar in glia and neurons. Second, the total mRNA levels are not different in the two cell populations. Third, and most importantly, dCBP overexpression in glia specifically causes downregulation of a set of glial genes. It seems that the H3K9ac levels reflect specific functional differences between neurons and glia, rather than simply revealing general gene activation. Maybe neurons require more plastic and dynamic regulation of transcription than other cell types and this process requires higher capacity of histone acetylation. Supporting this theory is the finding that a large number of activity-regulated enhancers bind CBP in cortical neuronal cultures (Kim et al., 2010). The technological breakthrough will be to analyze the transcriptome and the chromatin landscape of a few cells, which will help understanding the mode of action of dCBP and HDACs in the control of *Drosophila* glial and neuronal differentiation.

Acknowledgements
We thank L. Tora, G. Technau, B. Altenhein, A. Mazo, S. G. Georgieva, V. Auld, J. P. Kumar, M. R. Freeman, R. Bainton, J. Skeath, M. Mannervik, V. Rodrigues, J. F. Ferveur, C. Gonzales, S. Thor, the DHSB and the Bloomington Stock Center for reagents and flies. We thank the lab members for comments on the manuscript, and C. Diebold, C. Delaporte and fly, cell separation and imaging facilities for technical assistance.

Funding
Work was supported by INSERM, CNRS, UDS, Hôpital de Strasbourg, ARC, INCA and ANR. B.E. is supported by INSERM/Région Alsace and ARC; O.K. and P.L. are supported by FRM.

Competing interests statement
The authors declare no competing financial interests.

Supplementary material
Supplementary material for this article is available at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.070391/-/DC1

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SUPPLEMENTARY MATERIAL

Supplementary Materials and Methods

List of primers used in qRT-PCR experiments

nazgul	fw5'-AACTGCTGTACGCCAGGACT-3' rev5'-TCATGAACAGCCACATCCAC-3'
draper	fw 5'-CGAGCTAATCGCCTCTCAAC-3' rev 5'-ACACGTCGGCGGTAGTAAAT-3'
β-moody	fw 5'-GGCAACCTGTTGACCGTAGT-3' rev 5'-AGAAGAGCAGGTCGGCAATA-3'
pain	fw 5'-CGACCGCCATACCAGTATCT-3' rev 5'-CCCTAGAGTCAGCCGCATAG-3'
SP2637	fw 5'-CTCCTTTCCAGACCGAGGAC-3' rev 5'-TAAAGGGTCCAATGCGTAGC-3'
CG9336	fw 5'-CCACGCTACCTGCAGAACTT-3' rev 5'-GCAGCCAGCTTGGATATTGT-3'
CG11910	fw 5'-CCGTCTACGGAGTGATCCAT-3' rev 5'-CGACAAATCCAGACTGCTCA-3'
actin5C	fw 5'-TCCAGTCATTCCTTTCAAACC-3' rev 5'-GCAGCAACTTCTTCGTCACA-3'
elav	fw 5'-GGAAGCTGACAACAGCCATT-3' rev 5'-TCTGCATTAGCTGTGCCTGT-3'
repo	fw 5'-AAGCAGCAGCAAGAAGAAGG-3' rev 5'-ATACGGAGCACGTTCAAAGG-3'
CG6218	fw 5'-CCACATTCCTGTTGGGATTC-3' rev 5'-TCTCATCGCAGATGACCAGT-3'

Fly Stocks

Fly stocks were: *UAS-dGCN5* (Carre et al, 2005) and *nej3/FM7,ftzLac-Z* (Bloomington).

Immunohistochemistry and *in situ* hybridization

Following primary antibodies were used for immunolabeling of supplementary information: rb-α-β-Moody and rb-α-Draper (Freeman and al, 2003), rb-α-GCN5 (1:500, (Carre et al., 2005)), m-α-β-Gal (Sigma) 1:200, rb-α-H3K9me3 (Abcam) 1:500.

3D Reconstruction of the nucleus for co-localization analysis

3D Masks were generated for single cell images with Imaris using following algorithm: Enable smooth=true, Surface grain detail=0,1, Enable eliminate background=true, Diameter of largest sphere=5µm, Threshold=50 (may change according to the background and intensity), Quality above 10, Number of voxels above 10. Co-localization analysis was made with Metadata co-localization application.

RT-PCR analysis from separated cells

RNA from separated neurons or glia was prepared using TRIzol Reagent (Invitrogen) following the manufacturer's instructions. First strand cDNA synthesis was performed following the manufacturer's instructions (BioLabs). Intron spanning oligonucleotides (except for CG11910 which has only one exon) are listed bellow. Reactions were conducted in a GeneAmp PCR Systems 9700 (Applied Biosystems). Specific conditions for the RT-PCR included 35 cycles of 45 s at 95 °C, 1 min at 55 °C and 1 min at 72 °C. Extension was at 72 °C for 10 min followed by a 4 °C soak.

List of primers used in RT-PCR experiments

Target gene	**Sequence**	**Expected product size (in bp)**
CG15860	5'-CTGATGCTGCGAGAGGTGT-3' 5'-CGTTGATCTGTTCCTGTTTCC-3'	826
CG3168	5'-ATCGAATTGTGCGGCTATG-3'	993

	5’-CTTGGCCAGGTTGACCAC-3’	
CG9336	5’-CCTGAAGTTCGAGGCTGATG-3’ 5’-TGCTTAACGAACGGCATAGA-3’	225
CG4322	5’-TGCACATCTGCAGCTATATCC-3’ 5’-GGGAGGTTCGATTTGCTTATT-3’	376
CG4844	5’-AAGCAAAGATGGCCAAAATC-3’ 5’-GGGTCTGCTCACAAATGAAG-3’	540
CG11910	5’-ATCGCTATTGCGGTTGAATC-3’ 5’-AGTTGCAGGAAATCGCAGTT-3’	201
CG18318	5’-TGGCCGAGTTCTACGACAAG-3’ 5’-AAACTGCTGGCGATAACCAT-3’	398
elav	5’-CTGTGCCTGTGTGTCTACTC-3’ 5’-CCACTACCTCCACTTCCTAC-3’	214
repo	5’-TCCACGGTGGTTAATGGCAC-3’ 5’-AGTGCTCGTCTTGATGTAGC-3’	384
ttk	5’-AACGATCAAAGAACTCCAAG-3’ 5’-CGTTAGTTTGGGTATGCTG-3’	377

Immunolabeling:

Separated cells were washed in SDMS and cytospinned at 600 rpm for 6 min using CytoTek centrifuge. Cells were fixed in PBT (0.3 % Triton-X in PBS) supplemented with 4% paraformaldehyde for 20 min at room temperature, blocked with PBT supplemented with 5 % normal goat serum for 20 min at room temperature, and incubated overnight at 4°C with the following primary antibodies m-α-Repo, rt-α-Elav, α-chicken-GFP. Cells were then washed three times with PBST and incubated with secondary conjugated antibodies for 1h at room temperature. Finally, cells were washed in PBT supplemented with DAPI (1:10000, Roche) and mounted in Vectashield medium (Vector).

Supplementary figures

Figure S1.

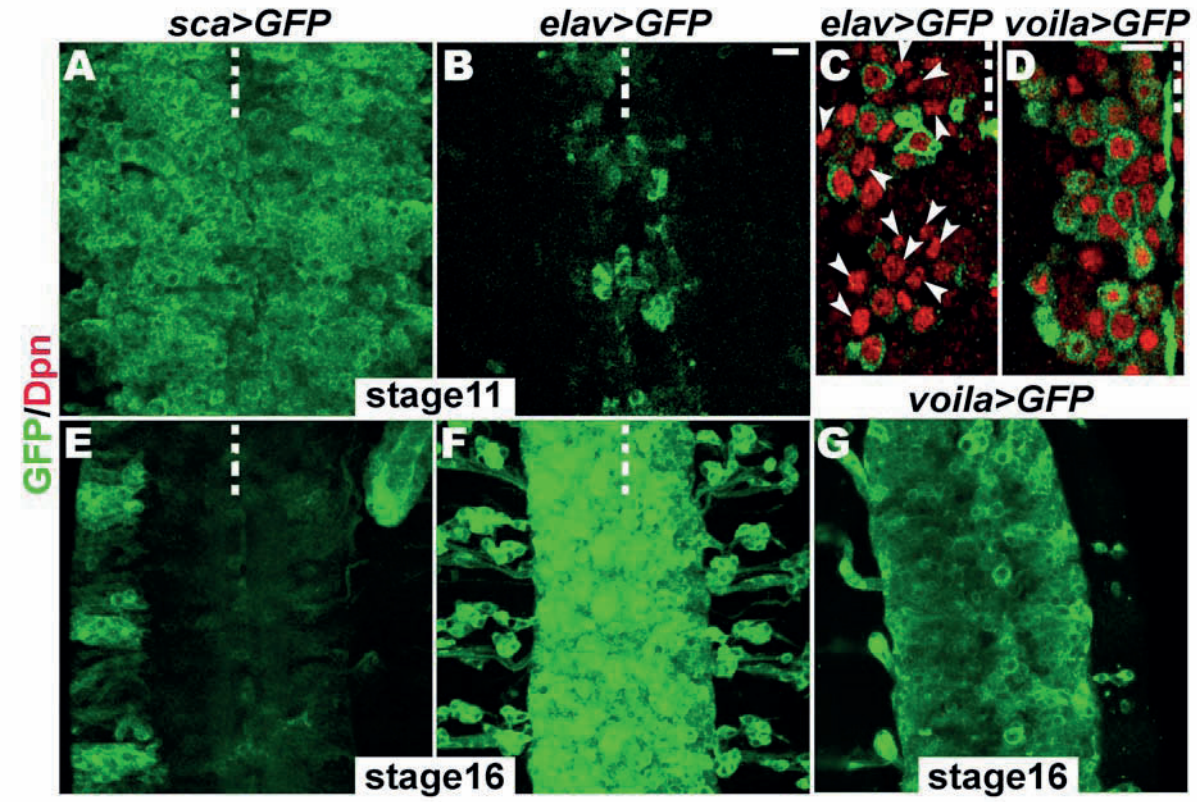

Fig. S1 GFP expression profile of different Gal4 drivers.

Projections of stage 11 **(A,B)** and stage 16 **(E-G)** embryos showing the profile of GFP (green) expression. Note that the *scabrous-Gal4* (*sca*) driver, *sca-Gal4>UAS-mCD8GFP* (*sca>GFP*), is expressed in the whole neuroepithelium (**A**) but not in neurons (**E**), whereas *elav-Gal4>UAS-mCD8GFP* (*elav>GFP*) (**B,F**) and *voila-Gal4>mCD8GFP* (*voila>GFP*) (**C**) do show GFP labeling in neurons. **(C,D)** *elav>GFP* and *voila>GFP* stage 12 embryos labeled with GFP (green) and NB marker Dpn (red). Scale bars=10μm.

Figure S2

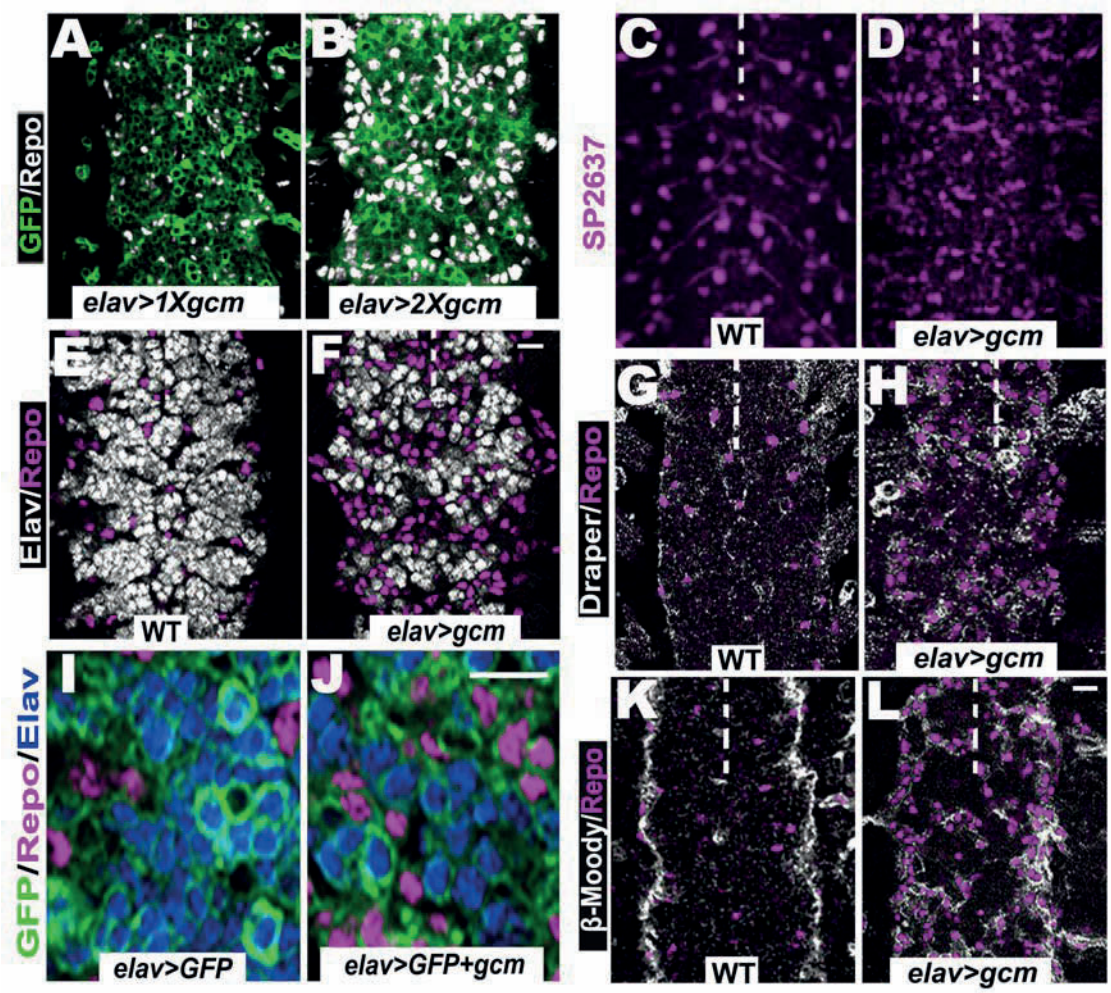

Fig. S2 Gcm induces full fate conversion in a cell autonomous, dosage dependent manner.

Late embryos upon *elav-gcm* overexpression. Except for (**A)**, left panels indicate control and right panels Gcm overexpressing embryos. **(A,B)** Confocal sections from embryos expressing one (*elav>1Xgcm*) or two doses of Gcm (*elav>2Xgcm*). Note the higher number of ectopic glia (Repo, white) in **(B)** than in **(A).** Note that the ectopic glia (Repo (white)) also express the GFP (green) and therefore Gcm. **(C,D)** Projections showing late glial marker SP2637. **(E,F)** Confocal sections show neurons (Elav, white) and glia (Repo, magenta). Note that Elav and Repo are mutually exclusive **(F). (I,J)** Confocal sections from *elav>GFP* **(I)** and *elav>GFP+gcm* **(J)** embryos labeled with Repo (magenta), Elav (blue) and GFP (green) show cell autonomous induction of Repo expression and loss of Elav expression. **(G,H,K,L)** Confocal sections showing that the late markers Draper (white) and β-Moody (white) are expressed in endogenous and ectopic glia (Repo, magenta). Compare **(C,F,G,K)** (*elav>w*^{1118} (WT)) to **(D,E,H,L)** (*elav>gcm*), respectively. Scale bars=10μm.

Figure S3.

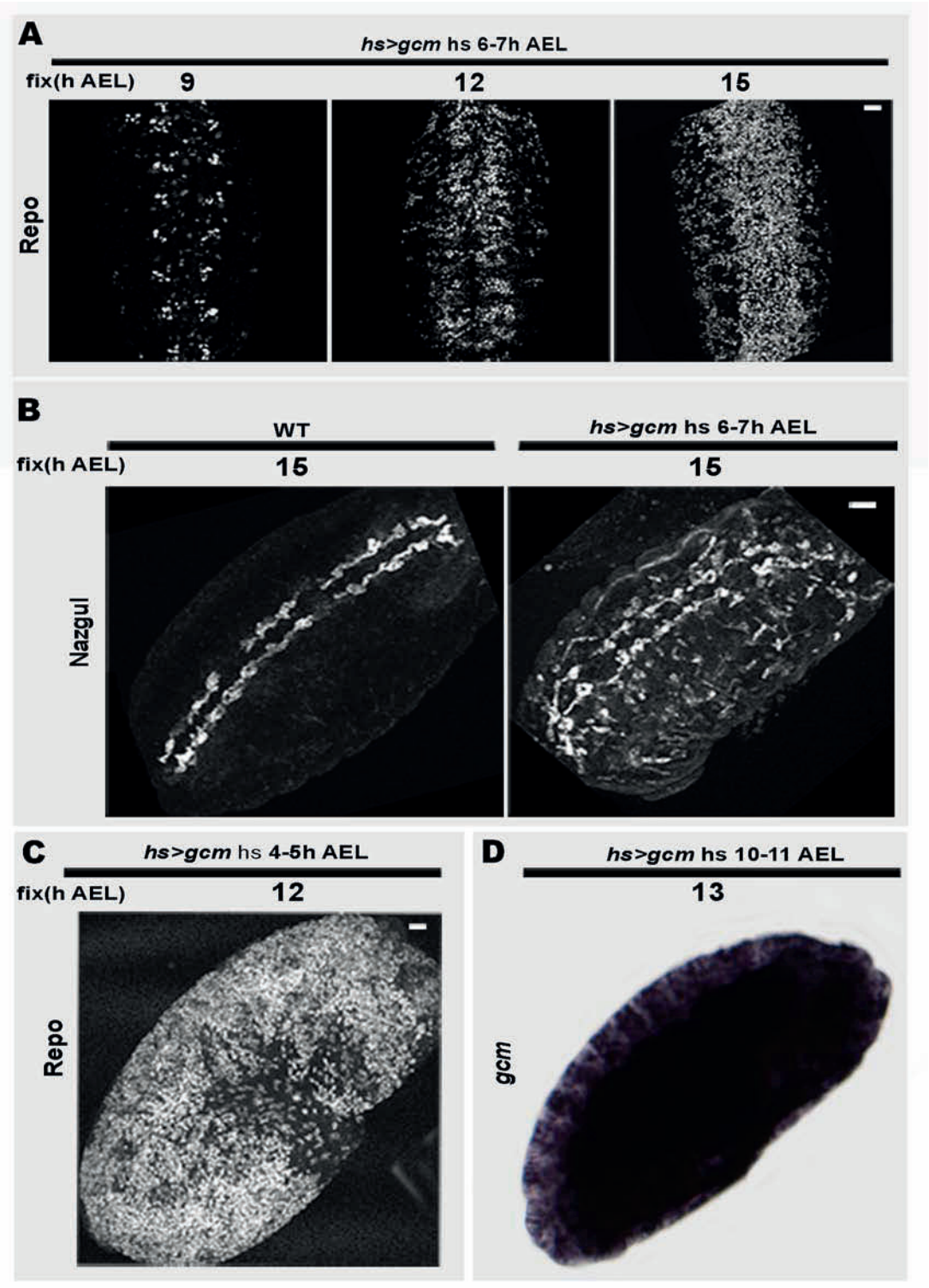

Fig. S3 Gcm overexpression using the heat shock promoter.

(A) Projections of *gcm* overexpressing embryos (*hs>gcm*) upon 1h heat shock pulse at 6-7h AEL and fixation at 9, 12, 15 h AEL. Note that the number of Repo(+) cells (white) does not decrease when animals are fixed at late stages and in fact it progressively increases as they are let differentiate (compare 9, 12 and 15). **(B)** Ectopic glia induced upon a 1h heat shock at 6-7h

AEL and fixation at 15h AEL express the late marker Nazgul**,** compare controls (left) panel and gcm overexpressing embryo (*hs>gcm)* (mid panel) **(C)** The majority of embryonic cells express Repo upon heat shock pulse at 4-5h AEL (*hs>gcm*). **(D)** *gcm* expression in Gcm overexpressing embryos (*hs>gcm*) upon 1h hs pulse at 10-11 AEL and fixation 2h later. Scale bars=10μm.

Figure S4.

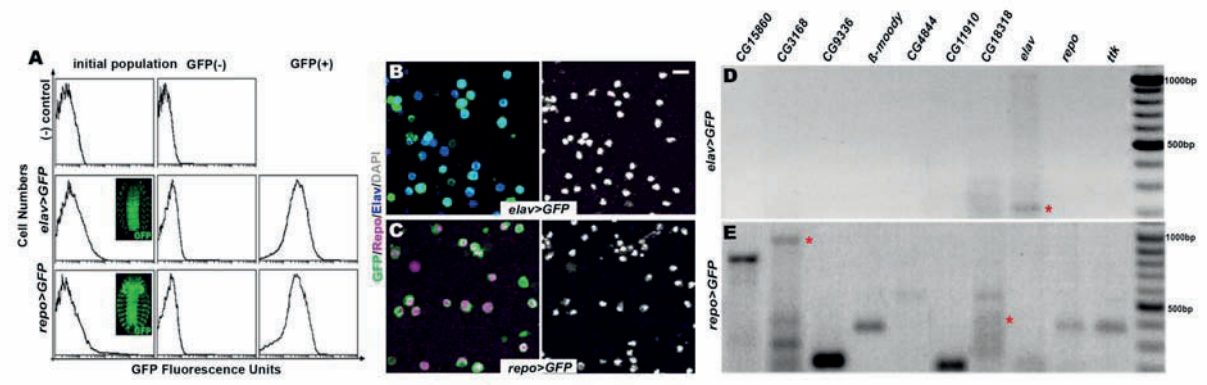

Fig. S4 FACS purification of glia and neurons from dissociated *Drosophila* embryos cells.

(A) FACS profiles of embryonic cells derived from control *UAS-mCD8GFP* (top panel), *elav-Gal4>UAS-mCD8GFP* (mid panel), and *repo-Gal4>UAS-mCD8GFP* (bottom panel) embryos. Insets show the profile expression of GFP (green) in stage 16 embryos using the neuronal driver *elav* and the glial driver *repo*. **(B-E)** The efficiency of cell separation is verified by GFP labeling and neuron/glial cell specific markers. Labeling of separated neurons with GFP (green) and Elav (blue) **(B)** and separated glia with GFP (green) and Repo (magenta) **(C)**. DAPI is in grey. Scale bar=20µm. **(D,E)** RT-PCRs show the profile expression of the glial genes in separated neurons **(D)** and separated glia **(E)**. *elav* is used as a control for the expression of a neuronal gene. Bands are at expected sizes; in case of multiple bands, specific products are marked with red asterisks.

Figure S5.

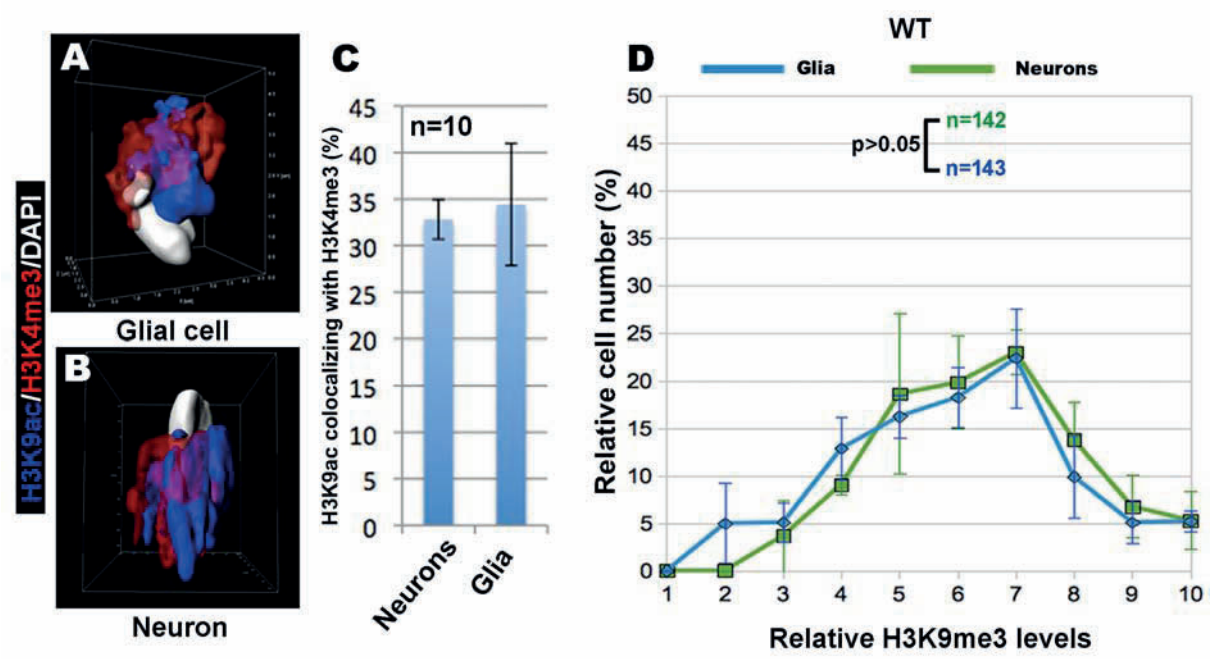

Fig. S5 H3K9ac overlaps with H3K4me3 and H3K9me3 levels are not different between neurons and glia.

(A,B) 3D reconstructions show the nuclei of a neuron **(A)** and a glial cell **(B)**, labeled for H3K9ac (blue), H3K4me3 (red) and DAPI (white). **(C)** The graph shows the percentage of H3K9ac labeling colocalizing with H3K4me3 in neurons and glia, upon quantification and plotting. **(D)** Quantified neuronal (green) and glial (blue) H3K9me3 levels in WT embryos. n indicate the number of analyzed cells.

Figure S6.

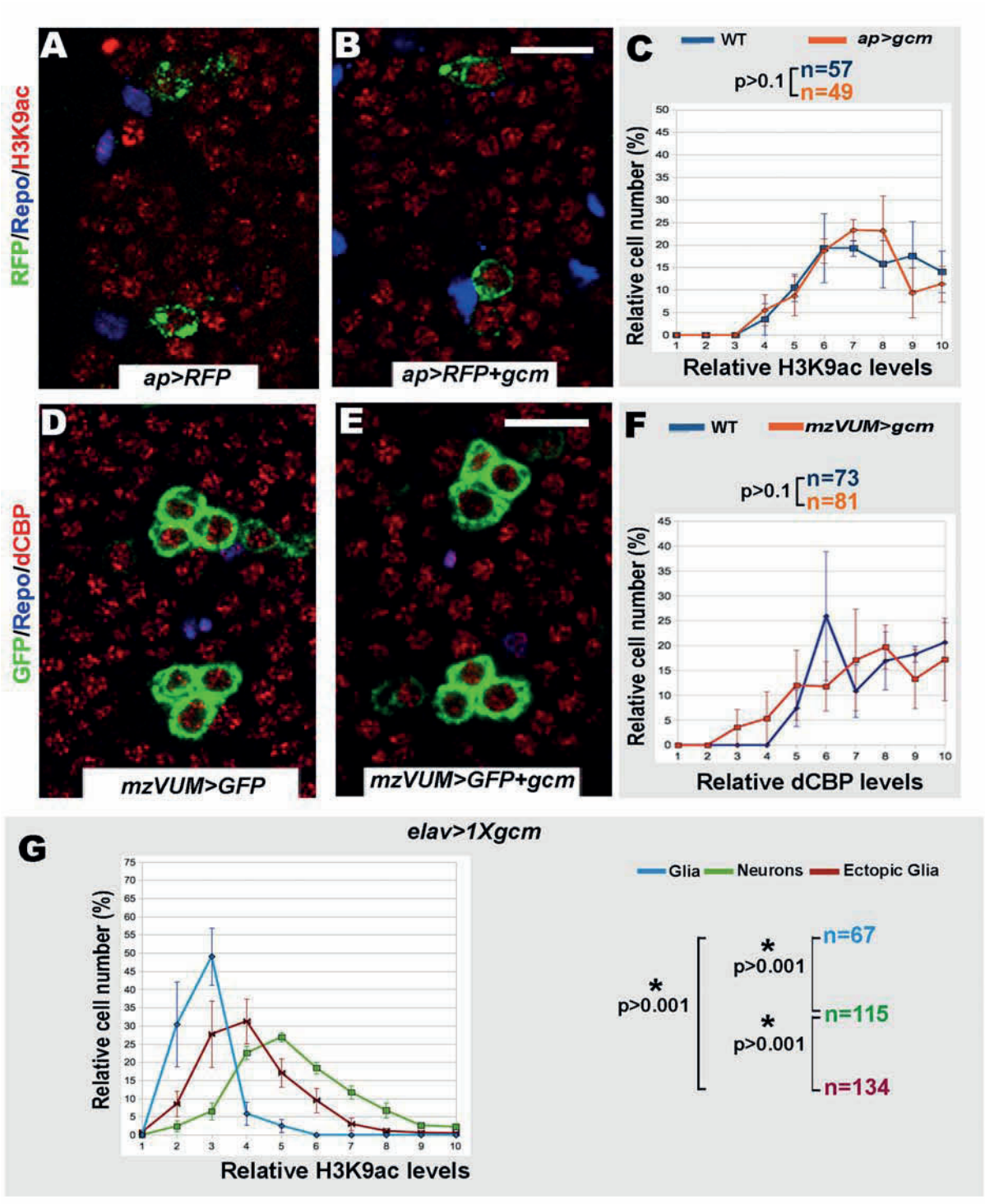

Fig. S6 Gcm postmitotic expression does not convert neurons into glia.

(A,B,D,E) Confocal sections of late stage 16 embryos show in red H3K9ac **(A,B)** or dCBP **(D,E)** labeling in post-mitotic neurons visualized with RFP/GFP (green), upon Gcm overexpression using *ap* and *mzVUM* drivers, respectively. Compare *ap>RFP* **(A)** control embryo to *ap><RFP+gcm* **(B)** and *mzVUM>GFP* **(D)** control embryo to

mzVUM>GFP+gcm **(E)** embryo. **(C,F)** H3K9ac levels from the above genotypes are quantified and plotted. Note that H3K9ac and dCBP levels do not change upon Gcm overexpression in post-mitotic neurons. **(G)** H3K9ac levels in neurons (green), glia (blue) and ectopic-glia (red) quantified and plotted for *elav>1Xgcm*. Note that H3K9ac levels of ectopic glia are lower than those of neurons but higher than those of ectopic glia induced by two doses of Gcm, shown in **Fig. 5F**. Scale bars=10μm.

Figure S7.

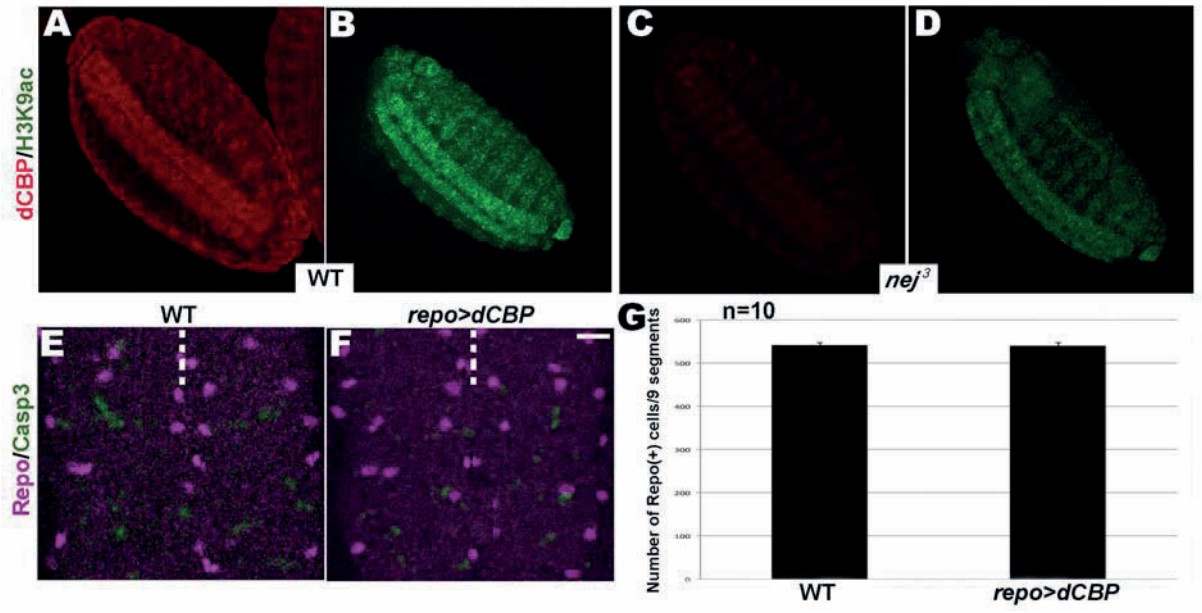

Fig. S7 Glial phenotypes in *nej* and dCBP overexpressing embryos.

(A-D) Projections of stage 17 embryos show dCBP (red) and H3K9ac (green) expression in control (WT) **(A,B)** compared to *nej* **(C,D)** respectively. **(E,F)** Confocal sections showing no colocalization between Repo (magenta) and the apoptotic marker activated-Casp3 (green) in control embryos **(E)** and *repo>dCBP* **(F)**. **(G)** The number of Repo(+) cells is not affected in repo>dCBP embryos, stage 17. n indicates the number of embryos. Scale bar=10μm.

Figure S8.

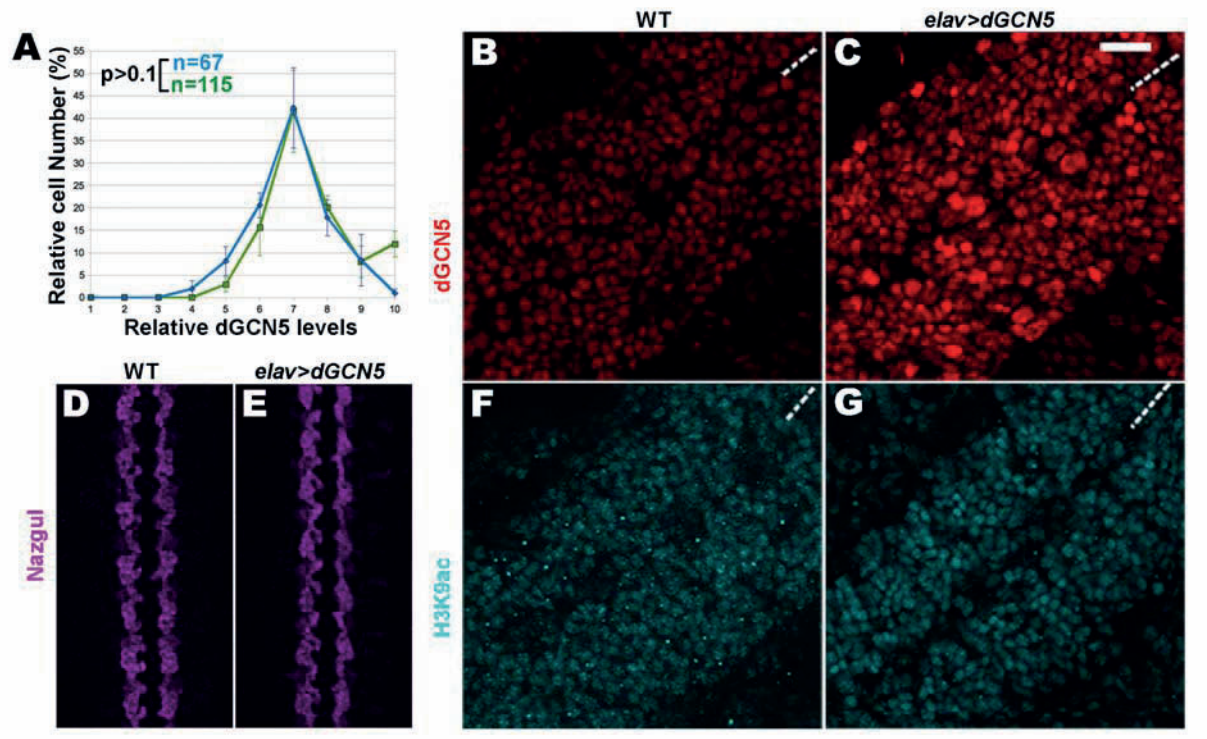

Fig. S8 *dGCN5* overexpressing embryos.

(A) Histogram shows dGCN5 levels in neurons (green) and glia (blue) quantified and plotted for WT embryos. **(B,C,F,G)** Confocal sections of control embryos (WT) and *elav>dGCN5* show that dGCN5 overexpression induces a global increase in dGCN5 (red) but not in H3K9ac (cyan) levels: compare **(B)** to **(C)** and **(F)** to **(G)**, respectively. **(D,E)** Confocal sections of control embryos and *repo>dGCN5* show that dGCN5 overexpression does not affect Nazgul late glial marker labeling, compare **(E)** to **(D)**. Scale bars=10μm**.**

Movie legends

Movie S1. 3D reconstruction of a neuronal nucleus showing H3K9ac and H3K4me3 labeling.

The organization of H3K9ac and H3K4me3 along with DAPI is shown. Note that H3K9ac and H3K4me3 do not colocalize completely.

Movie S2. 3D reconstruction of a glial nucleus showing H3K9ac and H3K4me3 labeling.

The organization of H3K9ac and H3K4me3 along with DAPI is shown. Note that H3K9ac and H3K4me3 do not colocalize completely.

1. 3. Data not shown in the manuscript

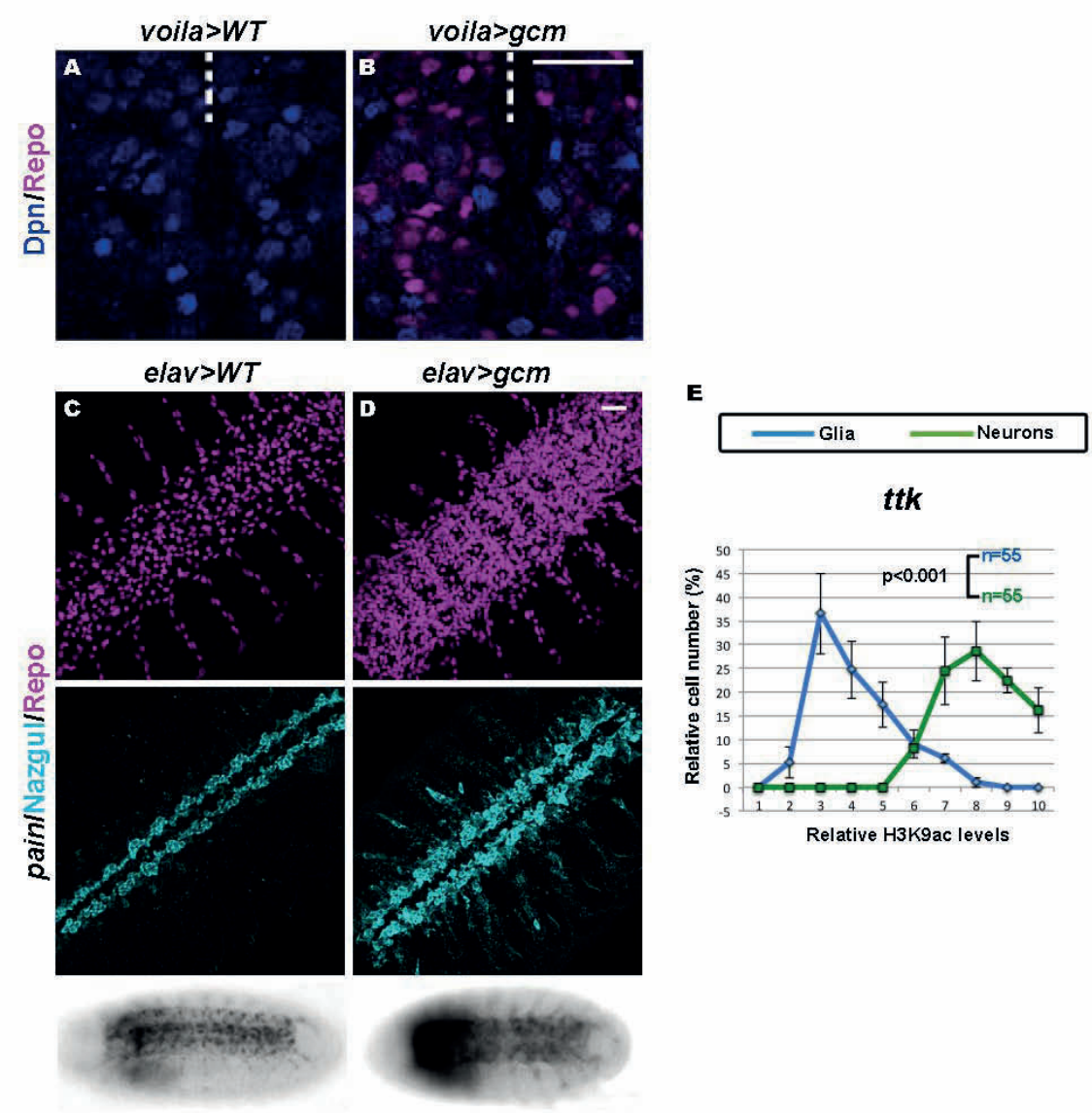

Figure data not shown: Gcm redirects NSCs towards the glial fate.

(A,B) Confocal sections of control (*voila>WT*) and *voila>gcm* early stage embryos labeled with Dpn (blue) and Repo (magenta). Note the presence of Dpn(+),Repo(+) cells in *voila>gcm* **(B)** but not in the control embryo **(A).** Scale bar = 20 μm.

(C,D) Confocal projections of *elav>WT* **(C)** and *elav>gcm* **(D)** stage 16 embryos labeled with the glial markers: Repo (upper panels), Nazgul (cyan, middle panels) (same embryos as for Repo) and *pain* (in situ hybridization, lower panels) increase drastically upon *gcm* overexpression. Scale bar = 20 μm.

(E) H3K9ac levels in neurons (green) and glia (blue) quantified and plotted for *ttk* embryos, n indicates the number of cells. Panel **(E)** was done by O. F. Karatas.

1. 3. Summary of major findings

1. 3. 1. *gcm* overexpression is able to redirect NSCs to adopt the glial fate, a process that goes through an intermediate state

Using *voila-gal4* to drive *gcm* expression in all NSCs at the beginning of neurogenesis, together with lineage-specific markers to recognize the progeny of defined NSC lineages, we clearly demonstrated that the ectopic glial cells not only are generated by NGBs but also by pure NBs that only produce neurons. Using the same driver, together with different NSC and glial markers, we also showed that the glial fate establishment goes through an intermediate state, where NSCs and glial markers are co-expressed. Thus, *gcm* ectopic expression induces gliogenesis from different NSC types, a process that goes through a state in which NSCs and glial markers are co-expressed.

1. 3. 2. Age and mitotic state influence NSC plasticity

Conditional expression of *gcm* in specific NSC lineage, or in all aged NSC (using *heat-shock-gal4* (*hs-gal4*)) driver; allowed us to demonstrate that 1) the ability of these cells to be converted into glia declines with age, and 2) quiescent or apoptotic NSCs lose completely their competence. Using the same strategy, we also revealed that *gcm* ectopic expression in post-mitotic neurons does not induce cell fate conversion but apoptosis, whereas NSCs whose division is blocked do. Thus, NSCs plasticity is age-related but does not dependen on the mitotic potential .

1. 3. 3. Ectopic glial cells display the same epigenetic marks as the endogenous ones

To better understand the characteristic of ectopic glial cells compared to the endogenous one, we first asked what might make glial cells different from neurons at this level. To this aim, we examined several histone modifications in fully differentiated neurons and glial cells, a work that allowed us to show that different levels of H3K9ac characterize neurons and glia: neurons contain high levels of H3K9ac, whereas glial cells have low levels of this histone modification. Interestingly, we also showed that low levels of H3K9ac characterize ectopic glial cells. We proposed that such difference is probably due to dCBP, one of the HATs that are responsible for this kind of histone modification, whose levels which are high in neurons and low in glia. Gcn5, another major HAT that acetylate H3 on K9 residue, accumulates similarly in both cell types. By specifically overexpressing dCBP in glial cells using *repo-gal4* driver, we proved that high levels of dCBP affect glial-specific

gene expression. In sum, *gcm*-mediated NSC conversion occurs not only at the level of gene expression but also at the level of chromatin organization, an event that is necessary to ensure the right levels of glial gene expression.

1. 4. Conclusion

Although we have considerably advanced in our understanding of NSCs; many still unanswered questions remain. What is the *in vivo* potential of a NSC, and how does it diverge from what we see *in vitro*? Our *in vivo* findings provide proof that *gcm* is similarly able to convert all NSC types into glia, despite the identity of their progeny. However, what makes NSCs different is their age. Indeed old NSCs, even from the same lineage, are not responsive to cell conversion by *gcm*, and do not retain their competence for efficient cell fate switch. This progressive loss of plasticity is not related to the mitotic state of NSCs, but most probably to other age dependent factors such as epigenetic changes that makes *gcm* targets inaccessible.

Our investigations also show how glial fate achievement involves low levels of H3K9ac, a marker that seems to be related to the differentiation state of glial cells rather than to their transcriptional status. These data raise a new issue concerning the use of epigenetic modifications to mark different cell types. Central to our results, the defective role of ubiquitously expressed gene levels, like CBP, suggesting that each cell type needs a specific dosage of what we call “ubiquitous gene”, to properly function and differentiate.

Altogether, our work raises an importance point regarding NSC plasticity *in vivo*, which is different from what was already described *in vitro*. Thus, a solid grasp about NSC identity and behavior *in vivo* is important for a future therapeutic purposes.

1. 5. My contribution

- Gcm cannot reprogram post-mitotic neurons; **Figure 2**.
- NSC plasticity decreases during development; all **Figure 3** except for panels F,G which were obtained by O. F. Karatas, and panels I,J by Dr. O. Komonyi.
- Levels of H3K9ac, low in glial cells and high in neurons; **Figure 5** (panels A-F).
- dCBP induces H3K9ac using *repo-gal4* driver; **Figure 6** (E-M).
- dCBP overexpression downregulates the expression of specific glial markers; **Figure 7** (panels A-C, D-F, G-I, and J-K)
- The profile of GFP expression using different gal4 drivers; **Figure S1** (panels A,B,E,F).

- Gcm induces full fate conversion in a cell autonomous, dosage dependent manner; **Figure S2** (panels B,C,D,).
- Gcm overexpression using the heat shock driver; **Figure S3** (panels C,D).
- Gcm post-mitotic expression does not convert neurons into glia, **Figure S6.**
- Gcn5 overexpressing embryos; **Figure S8** except for panels D,E which were done by Dr. O. Komonyi.
- All **Figure data not shown** except for panel **E,** which is from O. F. Karatas
- I prepared all the figures of the manuscript. I wrote the materials and methods. I read and commented the paper.
- I developed the 3D quantification method, using Imaje J tools.

2. 2nd part. Aging and NSC plasticity

2. 1. Introduction

The therapeutic potential of SCs, specifically NSCs, requires a detailed understanding of the mechanisms underlying their plasticity, specifically their ability to produce a functional derived cells, without any cell contaminants, which are not completely converted. *Drosophila* NSCs constitute a powerful model to understand SC plasticity, *in vivo,* since they share several features with the mammalian NSCs, such as their ability to self-renew and differentiate into different types of neurons and glia. We have previously reported that Gcm TF is able to convert NSCs into glia, a process that is age dependent (Results 1.). The aim of this extra-view was to discuss some factors that may make young NSCs more plastic than old ones, and to debate the concept of aging on cell replacement studies. The role of PcG complex in NSC plasticity and biology was also discussed.

2. 2. Manuscript II.

Stem cell aging and plasticity in the *Drosophila* nervous system

H. Flici and A. Giangrande (2012).

Landes Bioscience, Fly, Volume 6, Issue 2.

3. 3rd part. *gcm*/Gcm regulation and cell fate establishment

3. 1. Background

The studies performed on *Drosophila* NSC fate choice identified *gcm* as a master gene regulator of glial versus neuronal fate differentiation, and clarfied a number of important mechanisms by which *gcm* transient expression in glial precursors acts to initiate the glial fate, while repressing the neuronal identity. However, these studies left several interesting questions pertaining to *gcm*/Gcm regulation unanswered. Previously, much attention was paid to the consequences of *gcm* mutations, where glial precursors differentiate into neurons, or on its overexpression, particularly in the neurogenic region, where a number of neuronal precursors differentiate into glia, in a *gcm* dosage dependent manner. The capacity of Gcm to initiate the glial fate while repressing the neuronal one depends on its ability to activate glial promoting genes, such as *repo,* and neuronal repressing genes, like *ttk*. Despite all this progress, what makes *gcm* gene transiently active in all glial precursors is still mysterious. Concerning *gcm* gene activation, it was shown that after its initiation by unknown mechanisms, the maintenance of its expression occurs *via* its own protein "Gcm", a process that is called "*gcm* autoregulation". The right functionality of this mechanism was described to require additional lineage specific cofactors, like Huckebein in the NB1-1A lineage. Whether other factors are required for *gcm* autoregulation in other glial lineages is not known.

3. 2. Manuscript III.

Autoregulatory and cross-regulatory circuits between Gcm and the homeodomain transcription factor Repo collaborate with the histone acetyltransfferase CBP to regulate the glial fate establishment in *Drosophila* nervous system

H. Flici, O. Komonyi, P. Laneve, S. Berzsenyi, A. Giangrande

4. 4th part. The Gcm/Glide protein visualized at last: novel hints on its metabolism and a marker for novel cell type

4. 1. Background

The major and well-described activity of the Gcm TF is its ability to mediate glial fate. However, *gcm* is also expressed in other territories and known to regulate other cell fates, like hemoytes (Alfonso and Jones, 2002; Bernardoni et al., 1997) and tendon cells (Soustelle et al., 2004). In flies, Gcm activity was mostly evaluated by analyzing the profile expression of its direct targets, notably *repo*, while the profile of its expression was studied by *in situ* hybridization using specific probes targeting *gcm* mRNA (Akiyama etal., 1996; Hosoya et al., 1995; Jones et al., 1995; Vincent et al., 1996) or by immunohistochemistry using β-gal or GFP antibodies (Hosoya et al. 1995; Jones at al. 1995; Vincent et al., 1996), in transgenic flies carrying these expression reporters under the control of the *gcm* promoter. These strategies allowed us to have a general view about the *gcm* expression territories or to mark the cells were *gcm* is expressed (Introduction 5. 1. 1.). Unfotunately, few studies sought to understand the behavior of Gcm protein due to the lack of an efficient antibody. To our knowledge, the only study focusing onto the biochemical properties of Gcm was realized in S2 cells using a flag tagged Gcm, where Gcm degradation occuring *via* proteasome was demonstrated (Ho et al., 2009).

Given that increasing evidence show the central role of posttranslational modifications and protein-protein interaction in regulating several biological processes (Polevoda and Sherman, 2002), and in light of the large implication of *gcm* in regulating the identity of several cell types, we found inescapable the development of alternative strategies to evaluate Gcm protein properties *in vivo,* taking advantages of new and efficient approaches.

4. 2. Manuscript IV:

The Gcm/Glide protein visualized at last: novel hints on its metabolism and a marker for novel cell type

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Hakima FLICI

Différentiation et plasticité des cellules souches neurales

Résumé

Différenciation et plasticité des cellules souches neurales

L'étude de la plasticité cellulaire est un puissant outil pour comprendre le choix du destin cellulaire pendant la différenciation et dans les processus cancéreux lors de la transformation d'une cellule normale en une cellule maligne. Chez la drosophile, le facteur de transcription Gcm contrôle la détermination du destin glial. Dans des mutants *gcm*, les cellules qui se développent normalement en glie entrent dans la voie de différenciation neuronale alors que l'expression ectopique de *gcm* dans des progéniteurs neuronaux induit de la glie. Ces données font de Gcm un outil important pour comprendre les bases de la plasticité cellulaire. Mon projet de thèse vise à comprendre les mécanismes contrôlant la plasticité des cellules souches neurales. Nous avons ainsi montré que la capacité des CSNs à se convertir en glie après expression forcée de Glide/Gcm décline avec l'âge et que lors de l'entrée en phase quiescente ou apoptotique, ils ne peuvent plus être convertis. Nous avons aussi découvert que le processus de conversion du destin ne se manifeste pas uniquement par l'expression de marqueurs gliaux mais aussi par des changements spécifiques au niveau de la chromatine. D'une manière intéressante, nous avons aussi montré que la stabilité de la protéine Glide/Gcm est contrôlée par deux voies opposées, où Repo et l'histone acetyltransférase CBP jouent un rôle majeur.

Mots clés: Cellule souche neurale, Gcm, gliogenèse, CBP, Repo, choix du destin cellulaire.

Résumé en Anglais

Neural stem cells plasticity and differentiation

The study of cellular plasticity is a powerful tool to understand the mechanisms directing cell fate choice during differentiation and transformation of a normal cell into a cancerous one. In *Drosophila*, the transcription factor Gcm control glial fate determination. In *gcm* mutants, cells that normally develop into glia enter the path of neuronal differentiation, whereas ectopic expression of *gcm* in neural progenitors induces glia. These properties make *gcm* an important tool for understanding the basics of cellular plasticity. My thesis project aims to understand the mechanisms controlling the plasticity of neural stem cells (NSCs). Based on this aim, we showed that the ability of NSCs to be transformed into glia, after forced expression of Gcm, declines with age and that upon entry into quiescence or apoptosis, they cannot be converted. We also found that the process of fate conversion does not manifest itself only through the expression of glial markers but also by specific changes in the level of chromatin. Remarkably, we also showed that the stability of the protein Gcm is controlled by two opposite and interconnected loops, where Repo and the histone acetyltransferase CBP play a major role.

Key words: Neural stem cells, Gcm/Gide, CBP, Repo, cell fate choice.