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Current Opinion in Neurobiology

Beyond proneural: emerging functions and regulations of proneural proteins

François Guillemot[1] and Bassem A Hassan[2]

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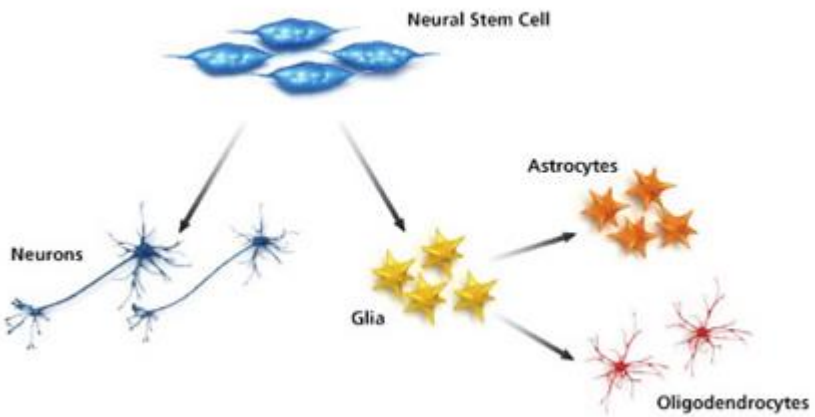
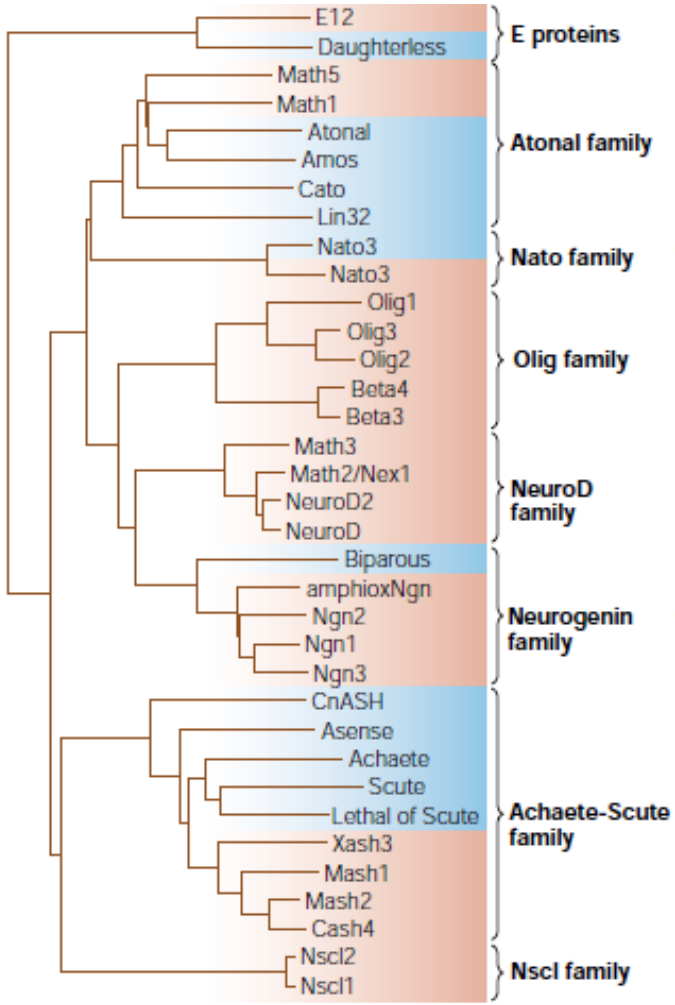
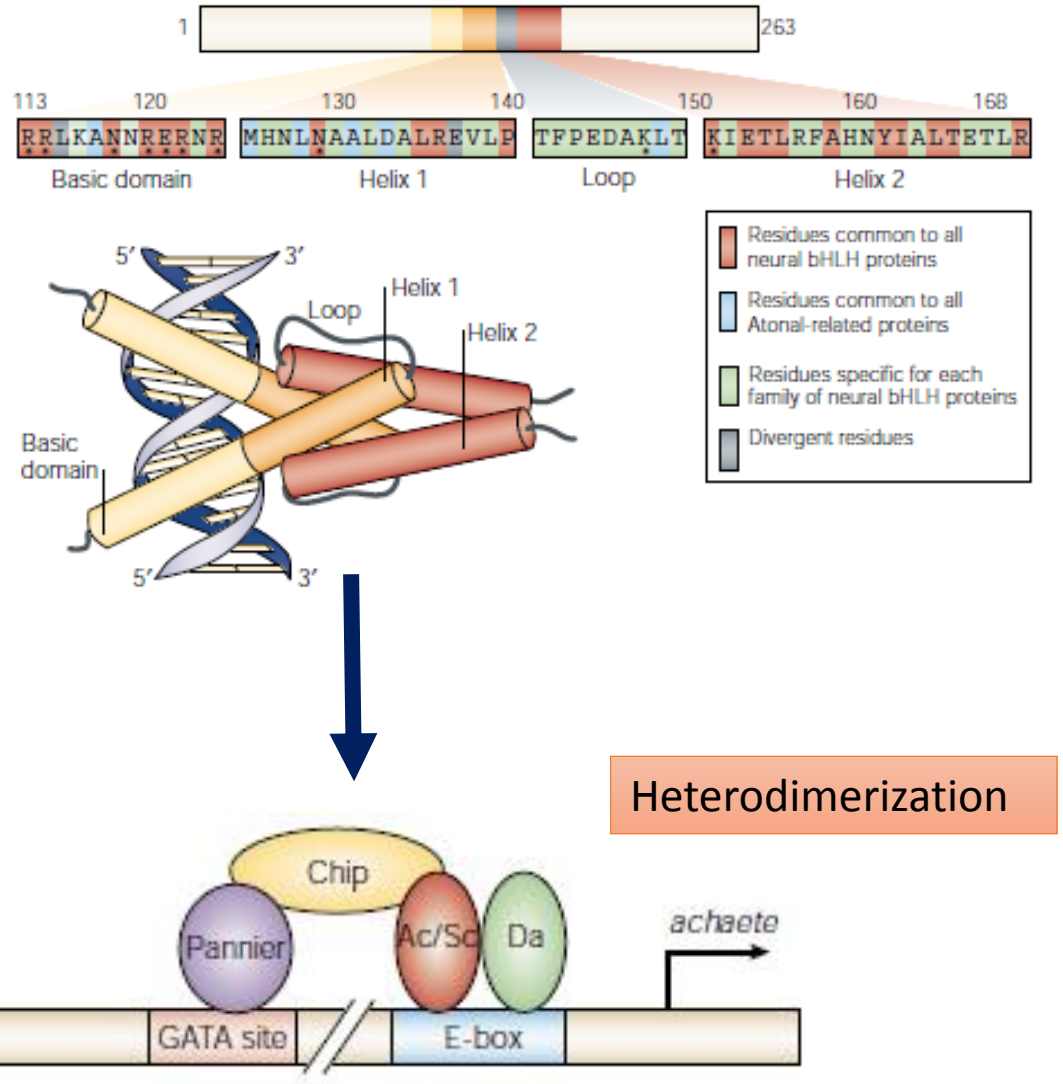


Viviana Canicatti
Celeste Nicola

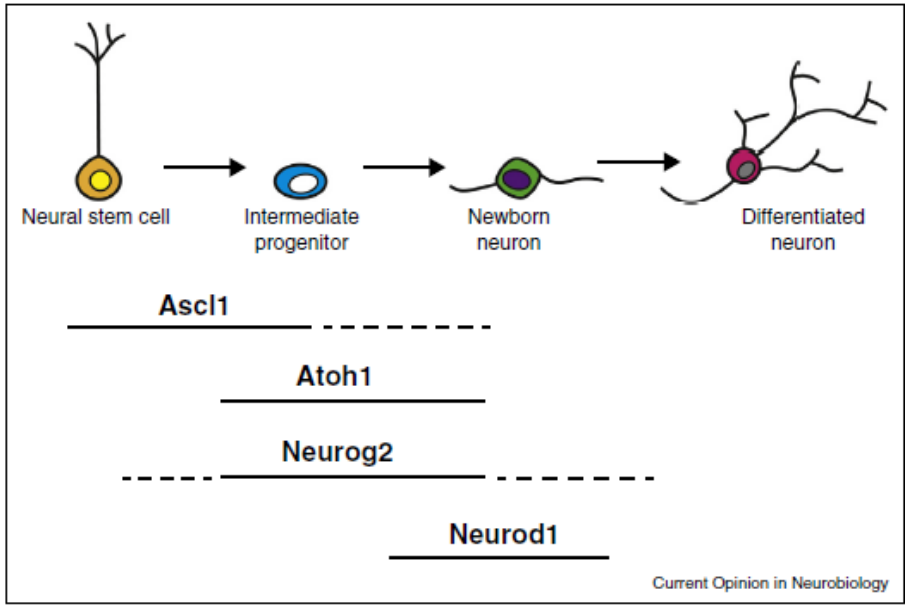
PRONEURAL GENES



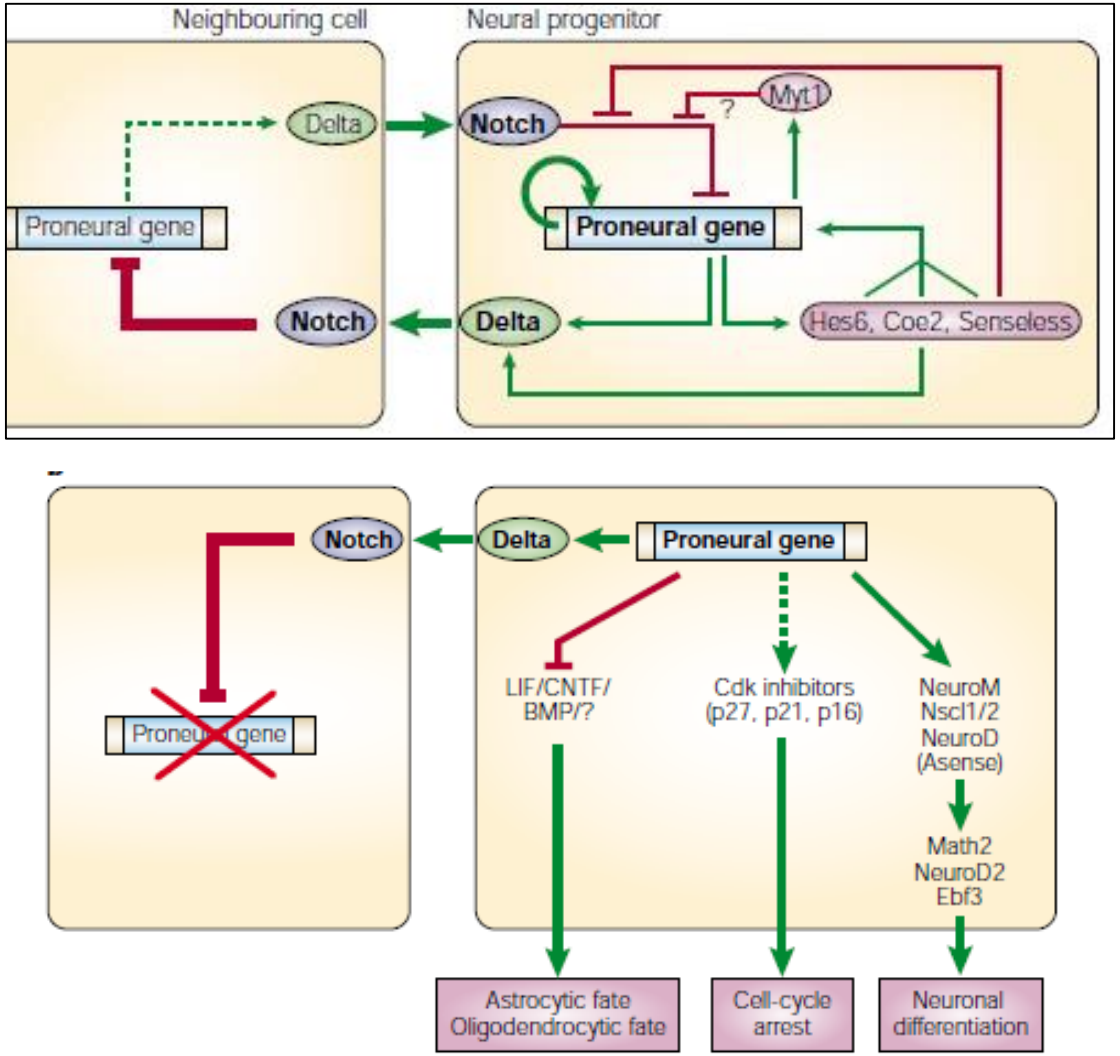


PRONEURAL GENES

Post mitotic neurons (transiently express):
Migration, axon and dendritic growth



Progenitors cells:
Neural-neuronal commitment



New properties and additional roles of proneural factors

- .Regulation of proliferation and differentiation

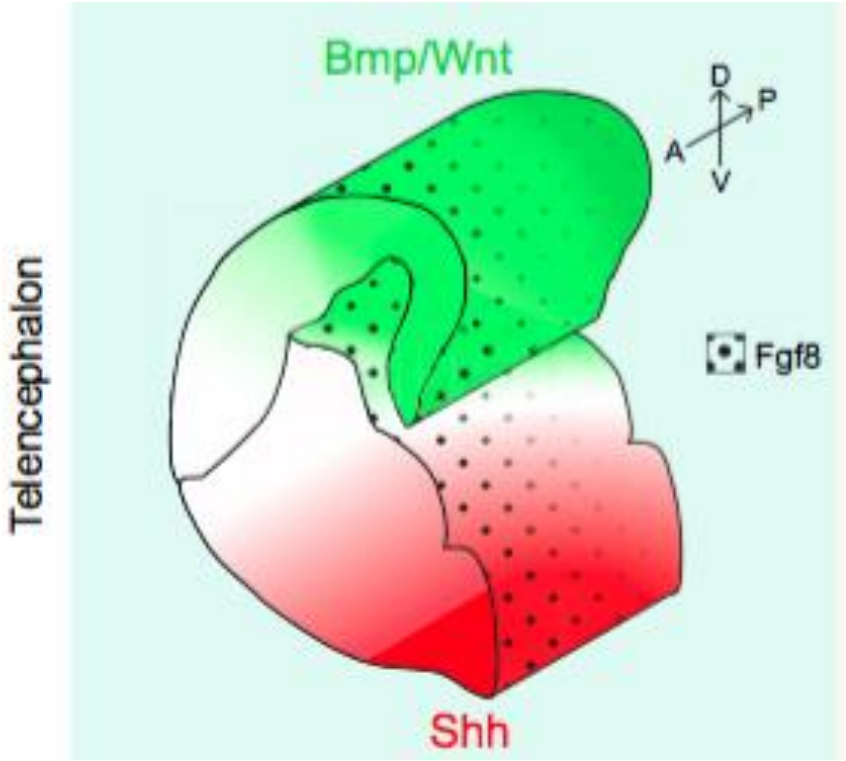
- Transcription of their target genes in different chromatin contexts.

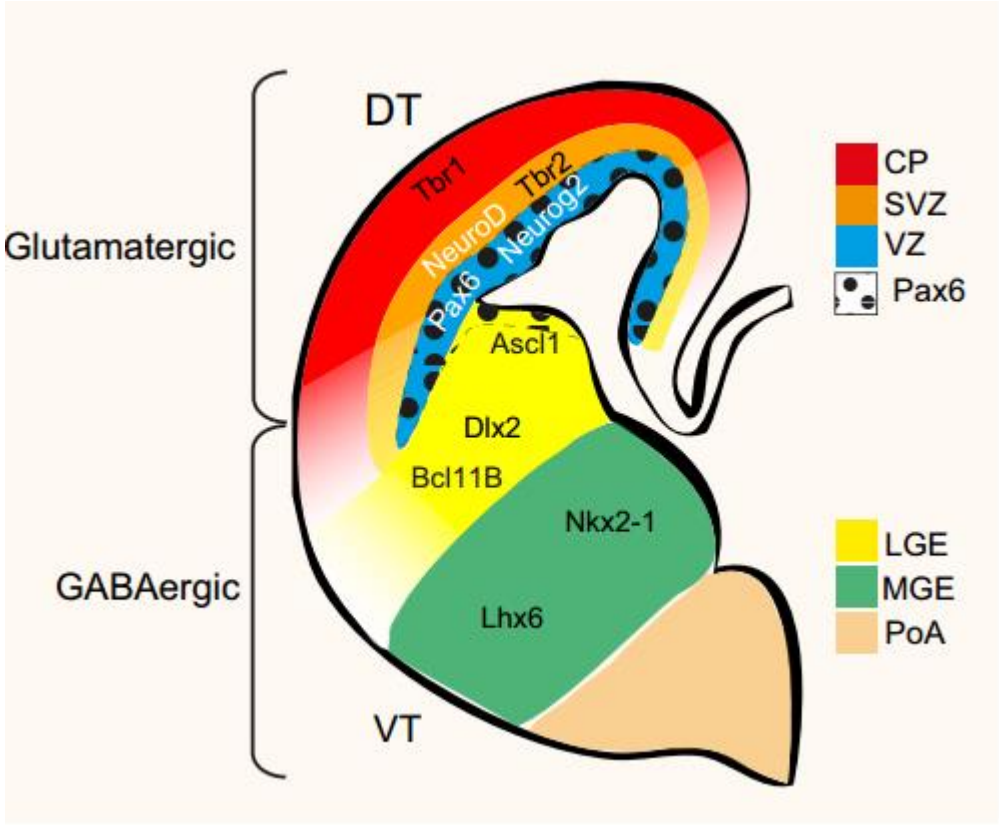
- Capacity to reprogram many types of cells into neurons.

- Post-transcriptional mechanisms that regulate the proneural proteins themselves.

Ascl1 and cortex expansion

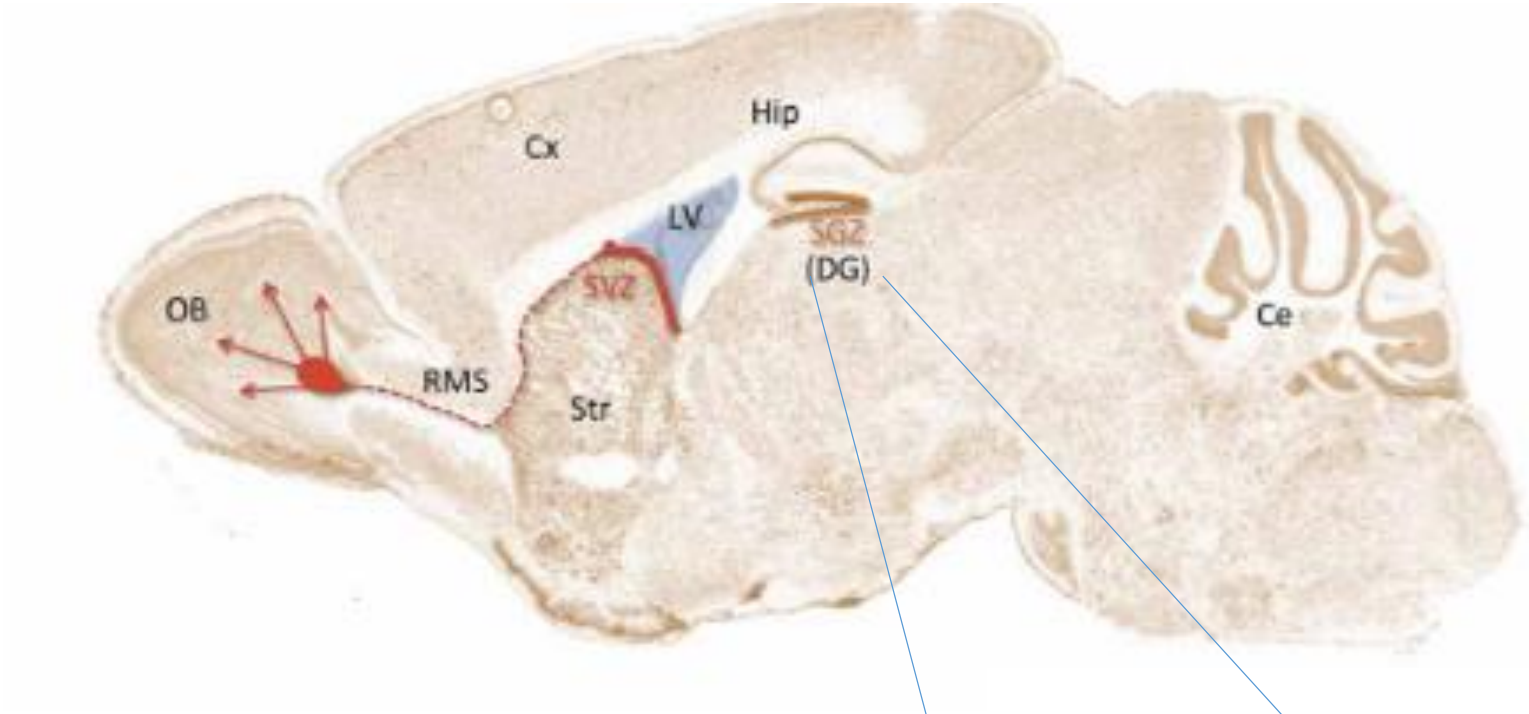
DEVELOPMENT

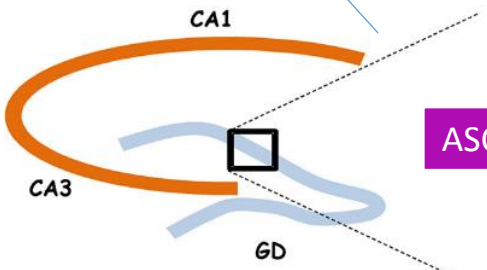




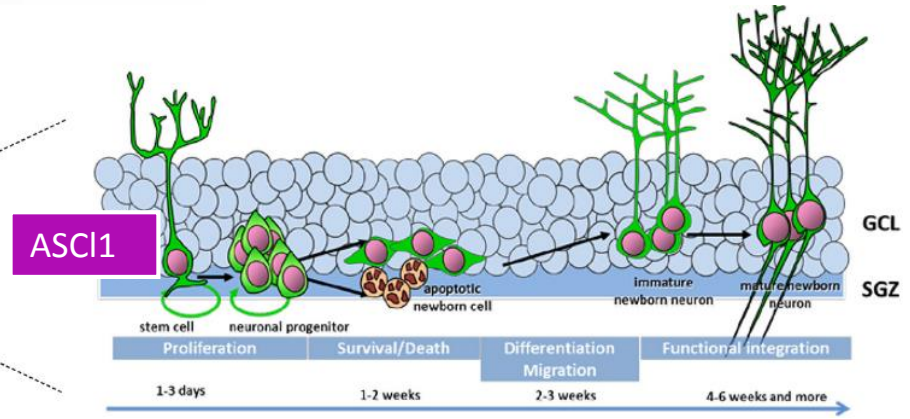
Ascl1 and cell proliferation

ADULT NEUROGENIC REGIONS





ASCl1



Ascl1 and cell proliferation

ADULT NEUROGENIC REGIONS

But, in non-neurogenic regions?

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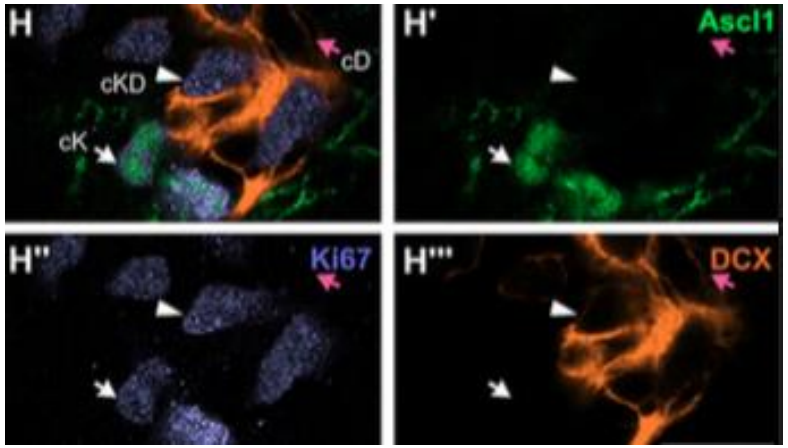
RESEARCH REPORT

STEM CELLS AND REGENERATION

Striatal astrocytes produce neuroblasts in an excitotoxic model of Huntington's disease

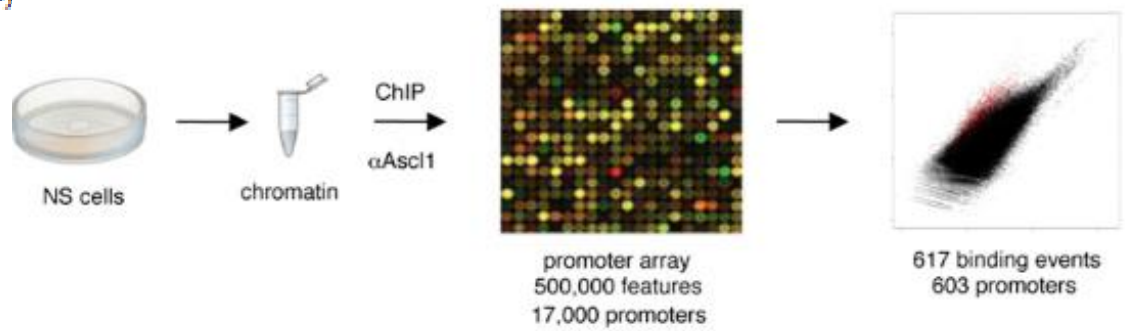
Giulia Nato[1,2], Alessia Caramello[1,2], Sara Trova[1,2], Valeria Avataneo[1,2], Chiara Rolando[3], Verdon Taylor[3], Annalisa Buffo[2,4], Paolo Peretto[1,2,*,‡] and Federico Luzzati[1,2,*,‡]

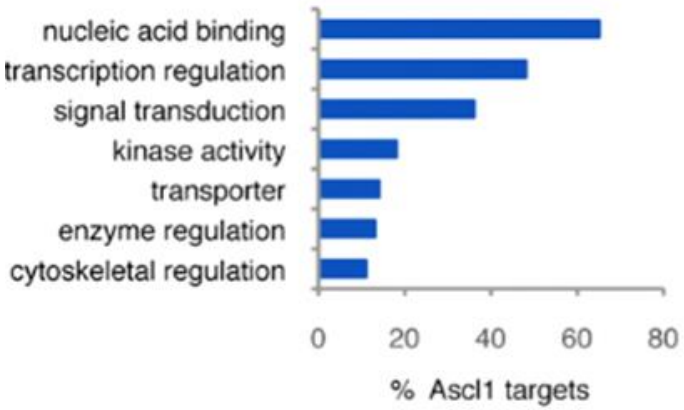


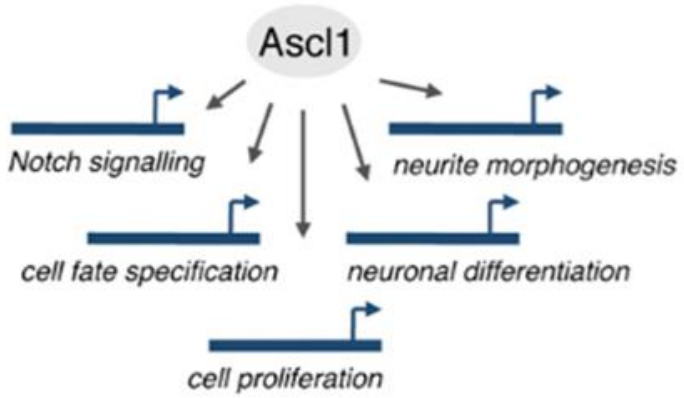
A novel function of the proneural factor Ascl1 in progenitor proliferation identified by genome-wide characterization of its targets

GENOME-WIDE

Diogo S. Castro,[1,5,6] Ben Martynoga,[1] Carlos Parras,[1,2] Vidya Ramesh,[1] Emilie Pacary,[1] Caroline Johnston,[3] Daniela Drechsel,[1] Mélanie Lebel-Potter,[1] Laura Galinanes Garcia,[1] Charles Hunt,[1] Dirk Dolle,[4] Angela Bithell,[3] Laurence Ettwiller,[4] Noel Buckley[3] and François Guillemot[1]



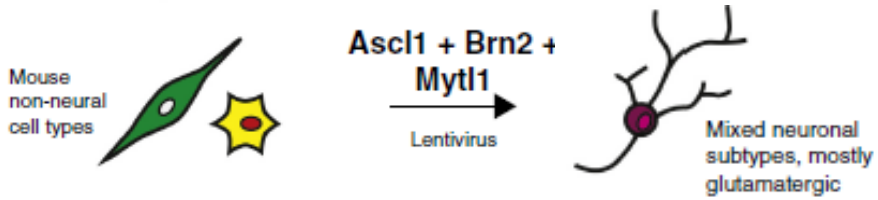




Possibly reflecting this proliferation-promot- ing function, Ascl1 has been implicated in the tumorige- nicity of glioblastoma and other tumours .

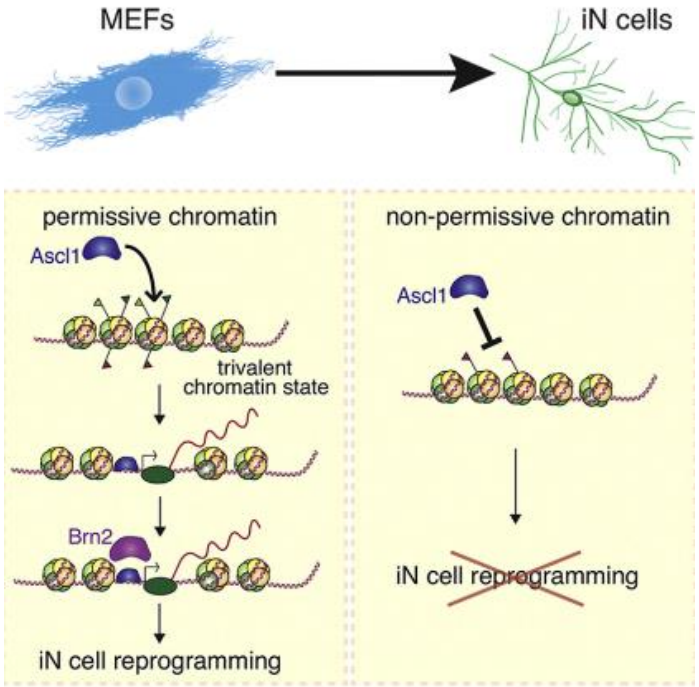
ASCL1 as a PIONEER FACTOR

Hierarchical Mechanisms for Direct Reprogramming of Fibroblasts to Neurons

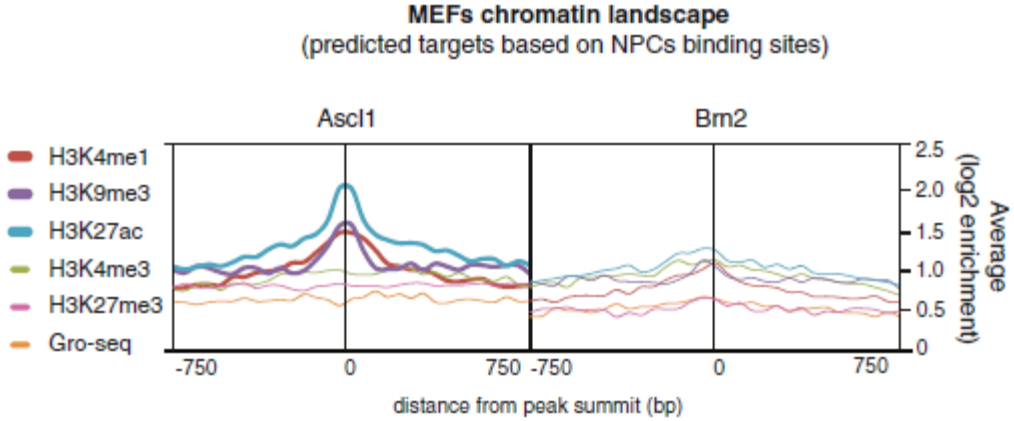


Orly L. Wapinski,[1,2,11] Thomas Vierbuchen,[2,3,4,11] Kun Qu,[1] Qian Yi Lee,[3,4,5] Soham Chanda,[3,4] Daniel R. Fuentes,[2,3,4] Paul G. Giresi,[1] Yi Han Ng,[3,4,6] Samuele Marro,[3,4] Norma F. Neff,[5] Daniela Drechsel,[9] Ben Martynoga,[9] Diogo S. Castro,[10] Ashley E. Webb,[7] Thomas C. Südhof,[8] Anne Brunet,[2,7] Francois Guillemot,[9] Howard Y. Chang,[1,2,*] and Marius Wernig[2,3,4,*]

"On-Target" Pioneer Factor Activity of Ascl1



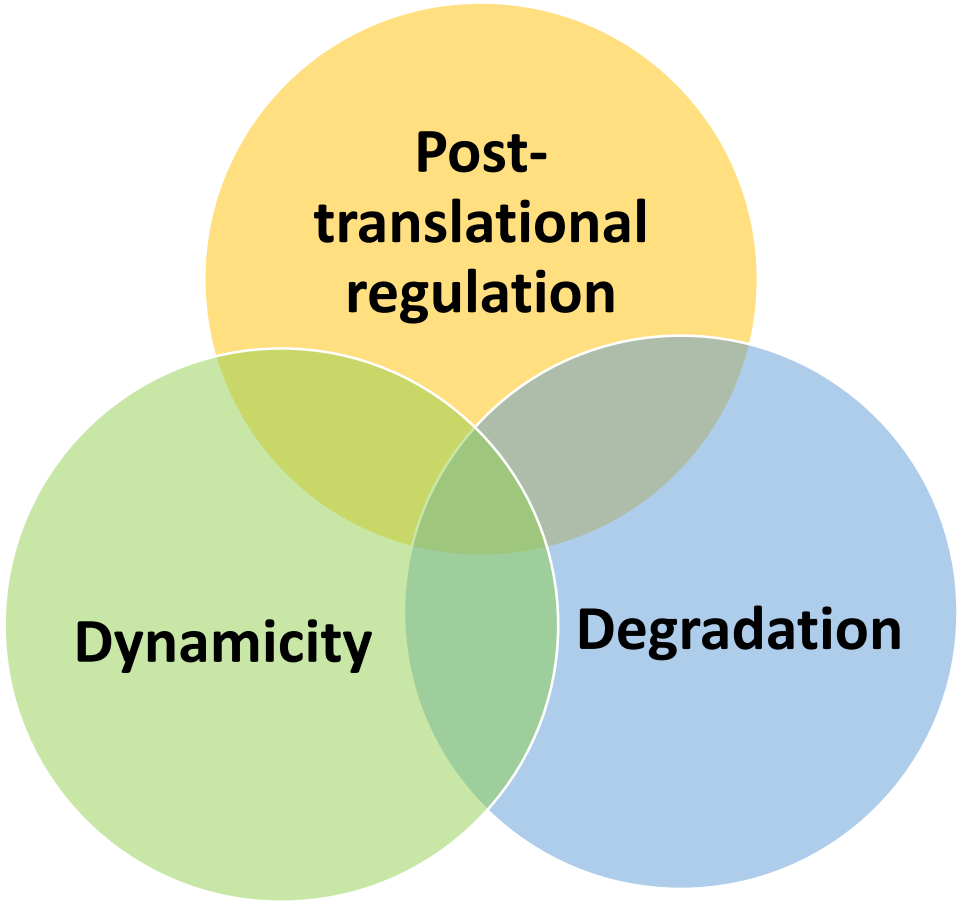
A Chromatin Signature Predicts Reprogramming Capacity



1) How do these genes control both early and late stages of neurogenesis ?

2) Which are the main actors that control the switch of protein activities?

Activities switch: possible controls…



Review

CellPress

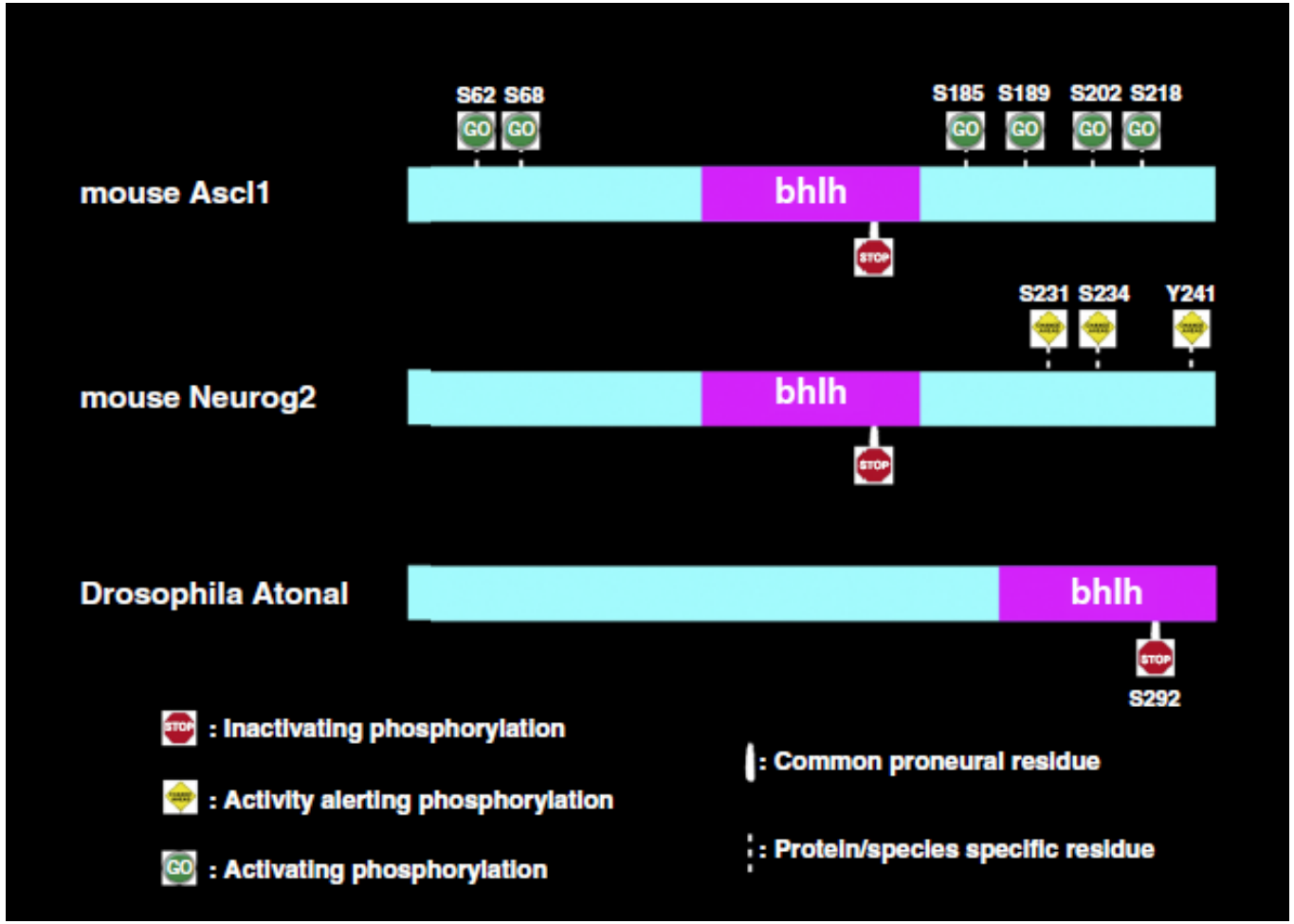
Nervous decision-making:

To divide or to differentiate ?
That is the question..

- **Neurogenesis follows a temporal pattern**, with precursor cells changing their competence and forming different cell types over time: **maintenance of the precursor pool is essential to enable the full repertoire of cell types to form.**

- Highly regulated temporal production of different cell types is conserved throughout amniote evolution, but **modifications to progenitor cell number, location and proliferative capacity has enabled expansion of the mammalian cortex.**

Phosphorylation like switch binary

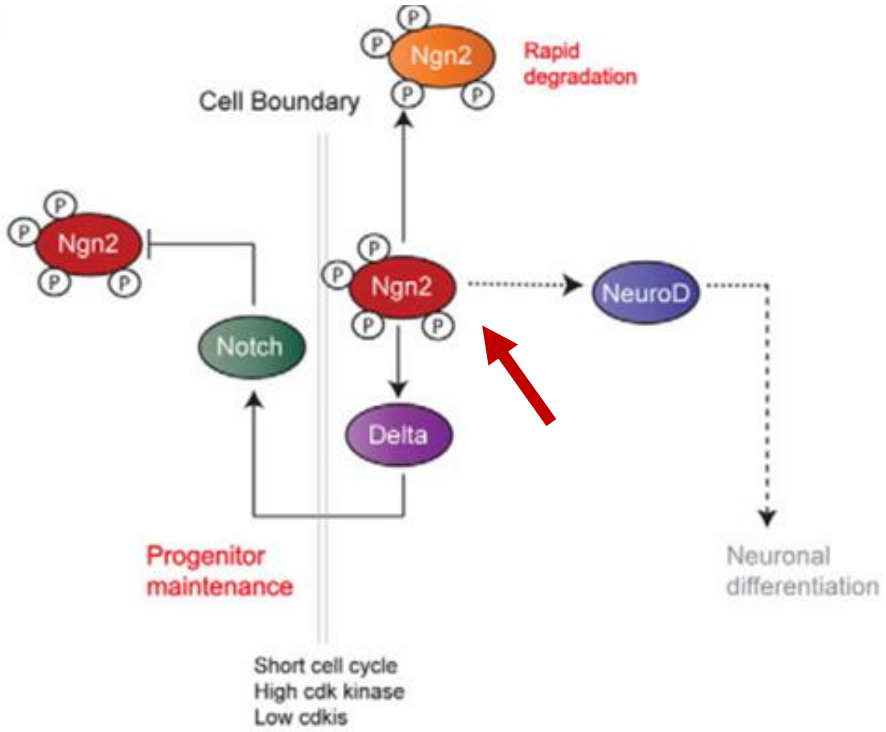


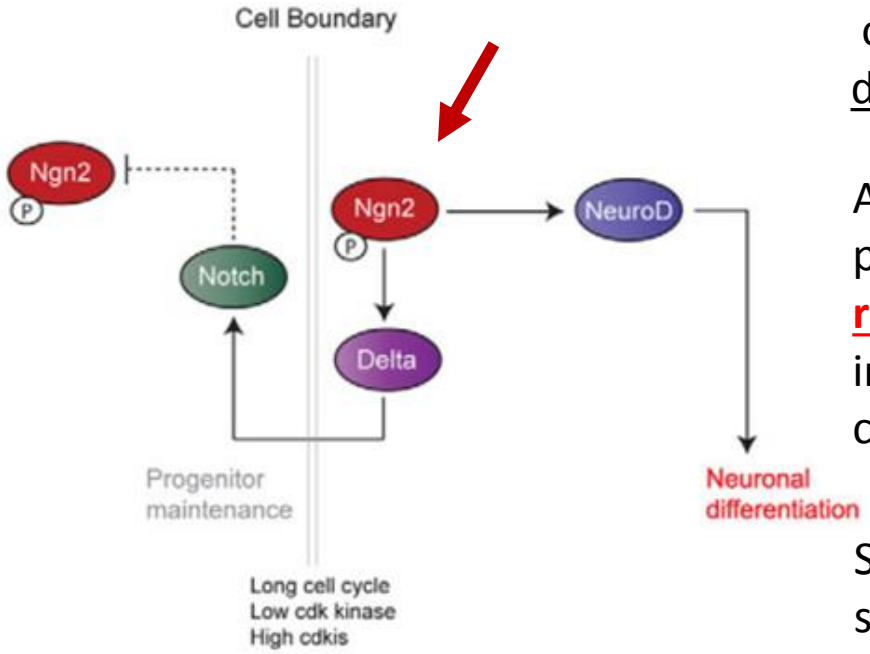
- A conserved post-translationally modified residue controls in a similar way all proneural proteins.

- Modifications of non-conserved residues may fine-tune the context-specific functions of individual proneural proteins.

Phosphorylation like switch binary

Model of cell-cycle dependent post-translational modifications (SP pairs)





<u>Progenitor-associated genes</u> have a more <u>open chromatin</u> state.

<u>Differentiation-associated</u> genes require additional epigenetic remodelling before actvation.

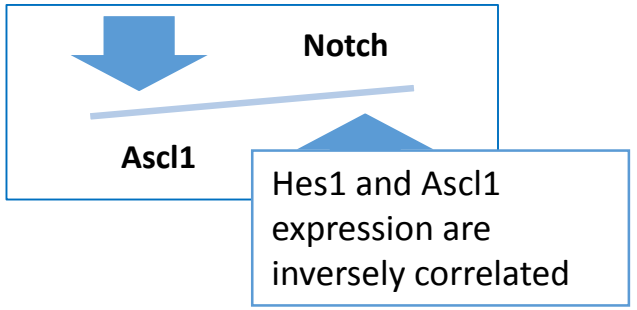
<u>Cdk-dependent phosphorylation coordinates</u> the cell cycle control of <u>precursor maintenance versus differentiation.</u>

A functional response to these phosphorylation events gives a **<u>rheostat-like response</u>** to changes in cyclin-cdk activity during cell cycle and development.

Similar to Ngn2, differential sensitivity of downstream targets to Ascl1 phosphorylation probably results from differences in the requirement for epigenetic remodelling by Ascl1 for activation.

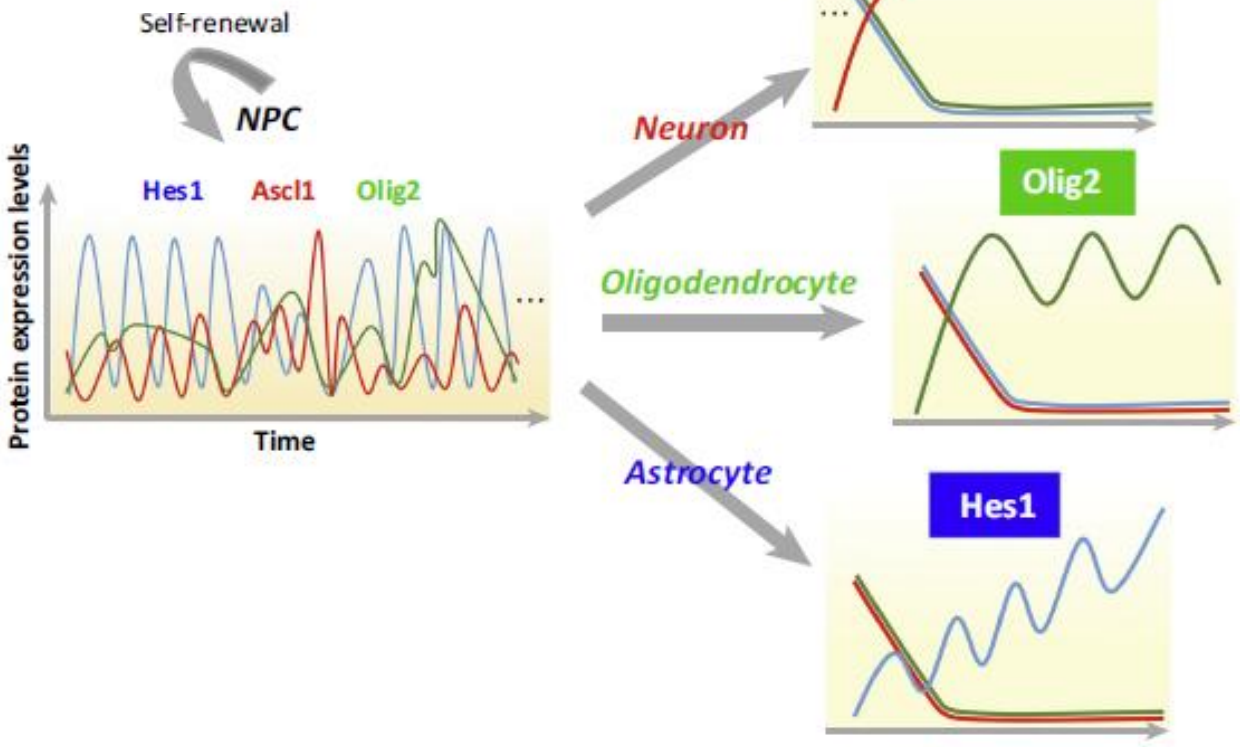
R. Ali *et al.* 2014

Dynamicity



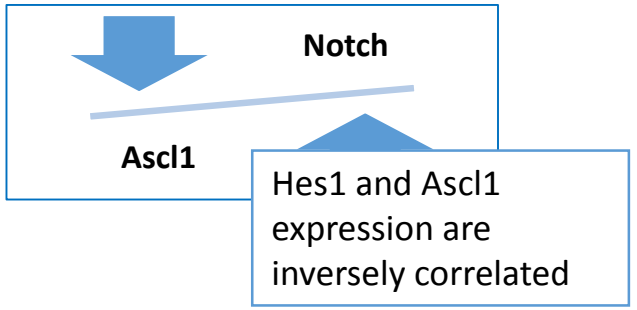
Oscillatory Control of Factors Determining Multipotency and Fate in Mouse Neural Progenitors

Itaru Imayoshi,[1,2,3,4]*† Akihiro Isomura,[1,5]† Yukiko Harima,[1,5] Kyogo Kawaguchi,[6] Hiroshi Kori,[5,7] Hitoshi Miyachi,[1] Takahiro Fujiwara,[3] Fumiyoshi Ishidate,[3] Ryoichiro Kageyama[1,3,5]*



- In ventral telencephalon (perinatal stages) multipotency is characterised by oscillating neurogenic and gliogenic factors.
- The levels of Ascl1 and Neurog2 transcripts and proteins oscillate in neuronal progenitors with periods of 2/3h, as a consequence of repression by oscillating Hes proteins downstream of Notch signaling.
- Proneural proteins expression becomes stabilised when notch signalling is down/regulated and progenitors exit the cell cycle and differentiate.

Dynamicity



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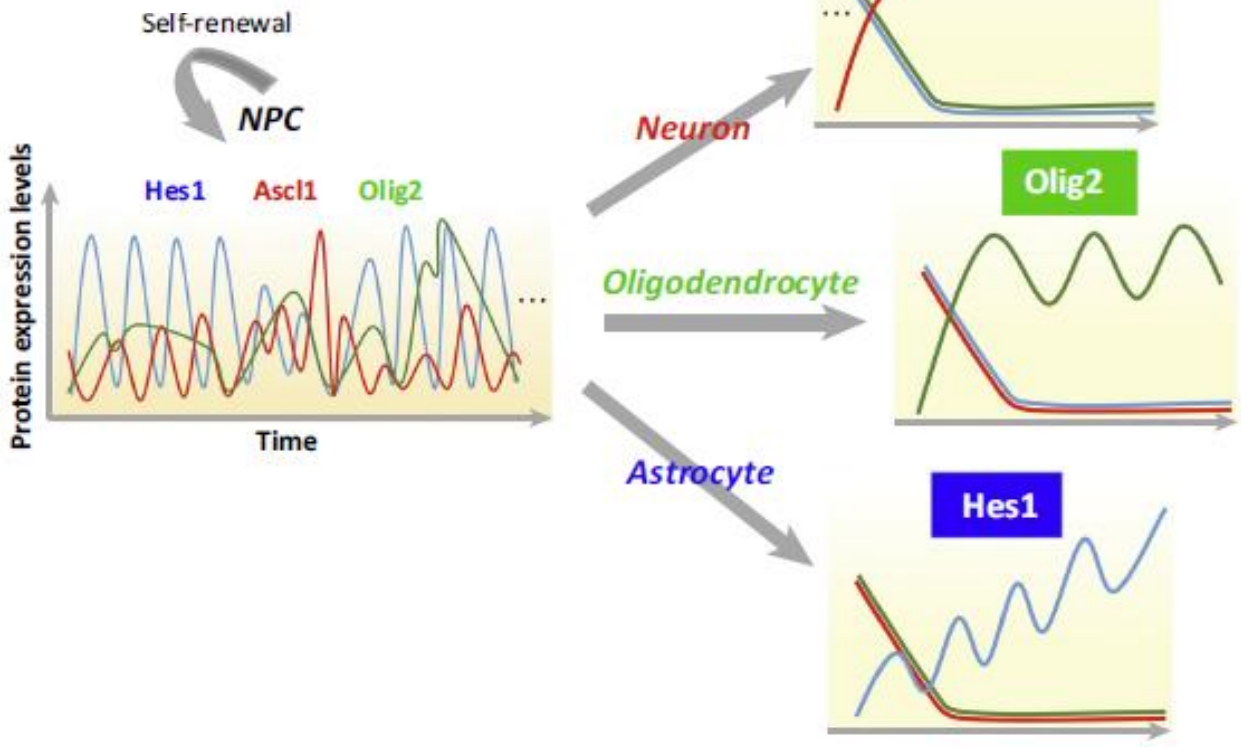
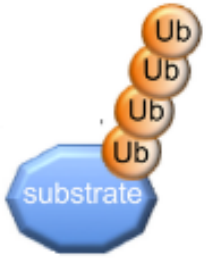
Outstanding questions:

- **What is the mechanism by which oscillatory and sustained expression of bHLH factors differentially regulate downstream gene expression?**
- **Is the Ascl1 expression oscillatory in activated NPCs in the adult brain?**
- **If Hes1 expression does not oscillate in NPCs, what will happen to neural development?**



Regulation of proteins stability



NEURODEVELOPMENT

Return to quiescence of mouse neural stem cells by degradation of a proactivation protein

Noelia Urbán,[1*] Debbie L. C. van den Berg,[1] Antoine Forget,[2,3] Jimena Andersen,[1†] Jeroen A. A. Demmers,[4] Charles Hunt,[1] Olivier Ayrault,[2,3] François Guillemot[1*]

Quiescience

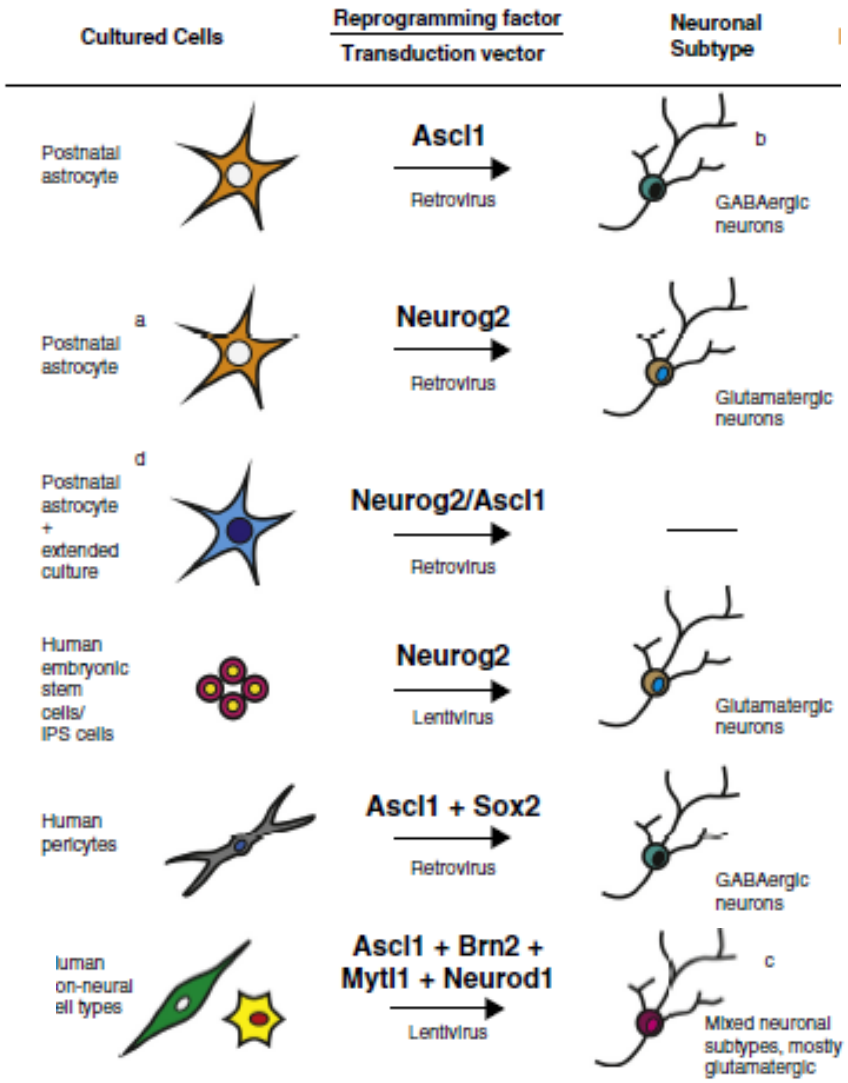
Proliferation

- Ascl1 is an unstable protein that is polyubiquitinylated and targeted to the proteasome by the E3 ubiquitin ligase HUWE1/UREB1/MULE.

- Deletion of Huwe1 in stem cells of the adult hippocampus results in stabilisation of Ascl1 and promotion of cell cycle reentry by inducing the expression of CcnD genes, which prevents the return to quiescence of stem cells and leads eventually to a contraction of the pool of proliferating stem cells.

ABOUT REPROGRAMMING…

IN VITRO

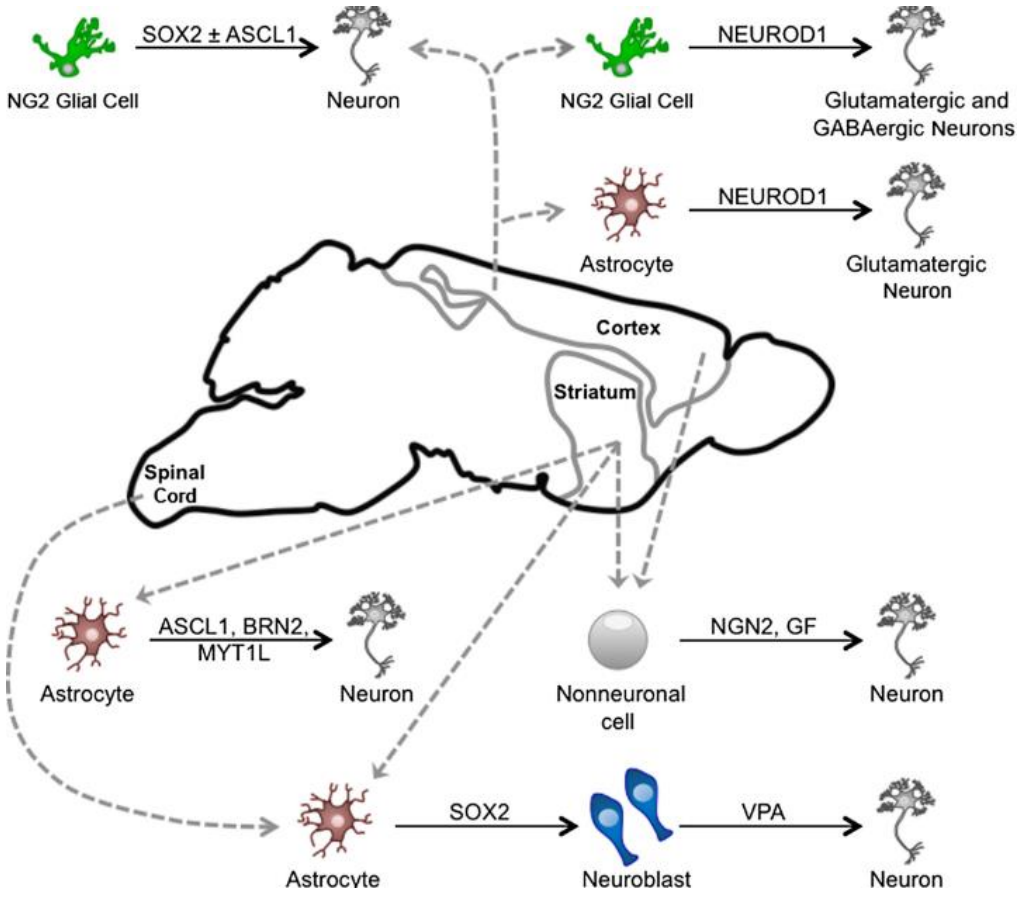


IN VIVO

transduced cell type	reprogramming factor / transduction vector	time between gene transduction and analysis	neuronal features
astrocytes in adult striatum	**Ascl1 + Brn2 + Mytl1**	6 weeks	morphology and molecular markers
	lentivirus		
NG2 glia in injured adult cortex	**Ascl1**	N/A	no reprogramming
	retrovirus		
astrocytes in adult striatum	**Ascl1**	N/A	no reprogramming
	lentivirus		
astrocytes in injured adult spinal cord	**Ascl1**	N/A	no reprogramming
	lentivirus		
activated glial cells in injured striatum and cortex	**Ascl1**	N/A	no reprogramming
	retrovirus		
astrocytes in postnatal and adult brain	**Ascl1**	between 10 and 45 days	morphology, markers, action potentials,synaptic currents
	adeno-associated virus		
activated glial cells in injured striatum and cortex	**Neurog2 + FGF2 + EGF**	7 and 14 days	molecular markers
	retrovirus		
astrocytes and NG2 glia in injured cortex	**NeuroD1**	7 and 14 days	molecular markers, action potentials, synaptic currents
	retrovirus		
NG2 glia in injured adult cortex	**Sox2**	12 and 24 days	morphology, markers, action potentials, synaptic currents
	retrovirus		
astrocytes in adult striatum	**Sox2**	5 weeks	morphology, markers, action potentials, synaptic currents
	lentivirus		requires BDNF+Nogin or Valproic Acid for maturation

ABOUT REPROGRAMMING…

Glia to neuron reprogramming

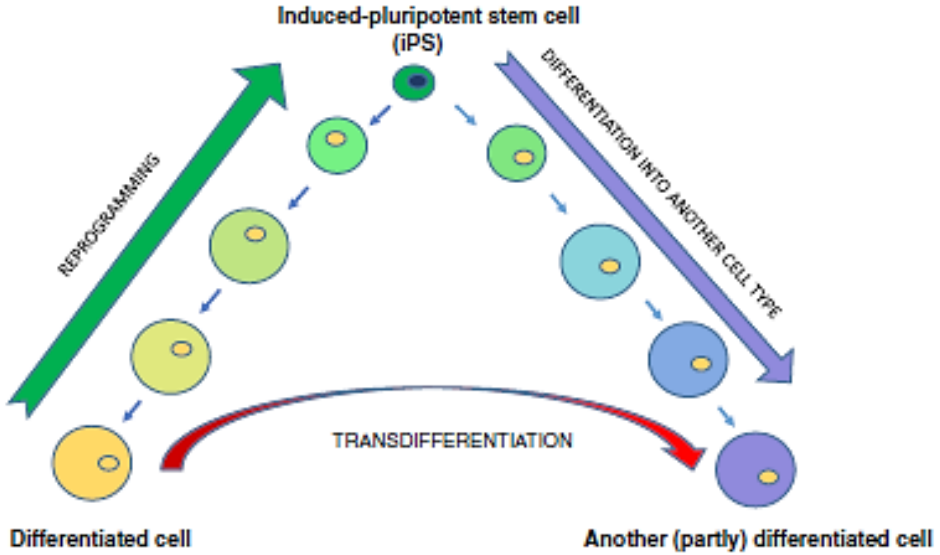


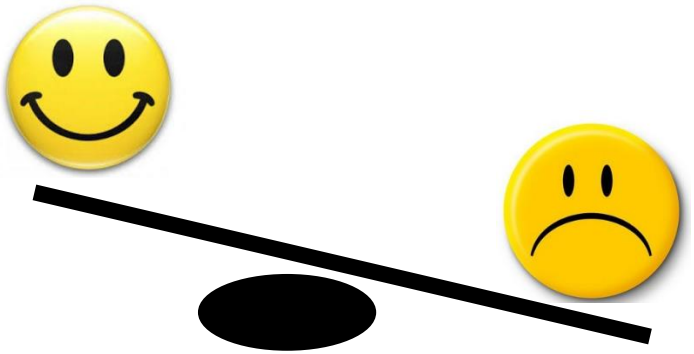
IN VIVO

transduced cell type	reprogramming factor / transduction vector	time between gene transduction and analysis	neuronal features
astrocytes in adult striatum	**Ascl1 + Brn2 + Myt1** lentivirus	6 weeks	morphology and molecular markers
NG2 glia in injured adult cortex	**Ascl1** retrovirus	N/A	no reprogramming
astrocytes in adult striatum	**Ascl1** lentivirus	N/A	no reprogramming
astrocytes in injured adult spinal cord	**Ascl1** lentivirus	N/A	no reprogramming
activated glial cells in injured striatum and cortex	**Ascl1** retrovirus	N/A	no reprogramming
astrocytes in postnatal and adult brain	**Ascl1** adeno-associated virus	between 10 and 45 days	morphology, markers, action potentials,synaptic currents
activated glial cells in injured striatum and cortex	**Neurog2 + FGF2 + EGF** retrovirus	7 and 14 days	molecular markers
astrocytes and NG2 glia in injured cortex	**NeuroD1** retrovirus	7 and 14 days	molecular markers, action potentials, synaptic currents
NG2 glia in injured adult cortex	**Sox2** retrovirus	12 and 24 days	morphology, markers, action potentials, synaptic currents
astrocytes in adult striatum	**Sox2** lentivirus	5 weeks	morphology, markers, action potentials, synaptic currents requires BDNF+Nogin or Valproic Acid for maturation

Direct conversion: a potential regenerative therapy ?

TRANSDIFFERENTIATION VS REPROGRAMMING



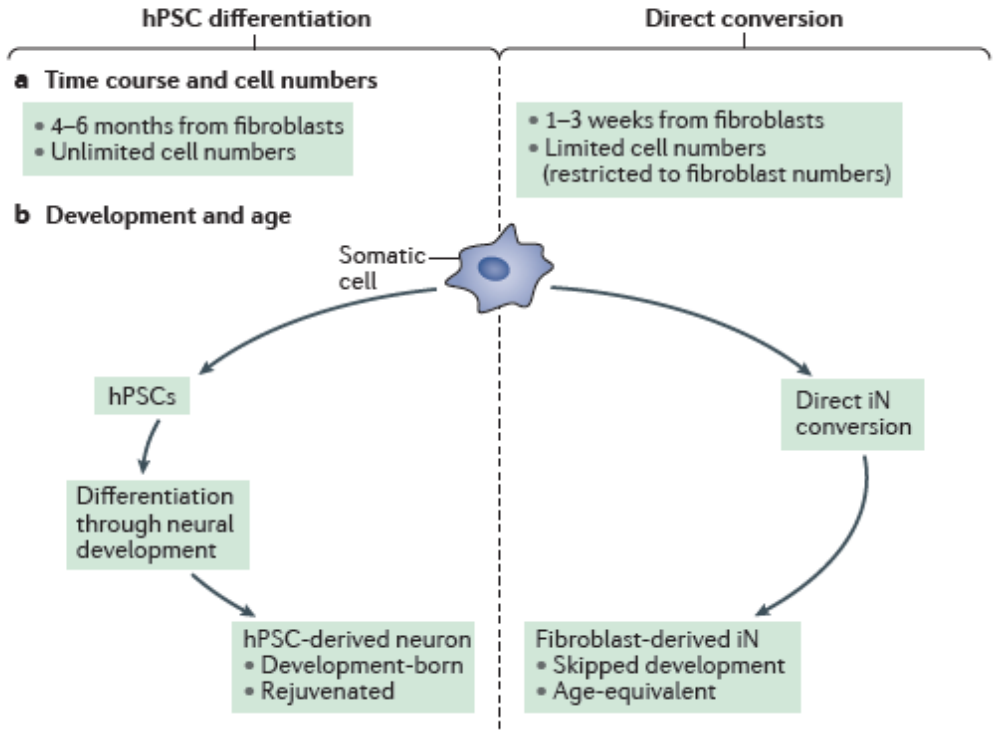


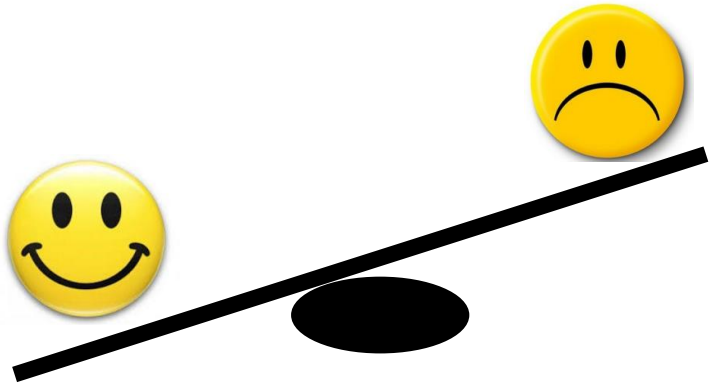
- Transdifferentiation o lineage reprogramming does not carry as high a risk of carcinogenesis

- Are generally cultured for a shorter time than iPS cells and therefore are less susceptible to the accumulation of genetic mutations during in vitro culturing.

Direct conversion: a potential regenerative therapy

TRANSDIFFERENTIATION VS REPROGRAMMING





- **No recapitulation of neurodevelopmental stages**
- **Less expandability and cell numbers.**
- **No rejuvenation.**
- **TFs expression can be temporal and is not continuosly required for iN conversion, so potential risk that permanent overexpression of neurodevelopmental TF might interfere with mature neuronal phenotypes, funcionality of the generated iNs**


Thanks for the attention