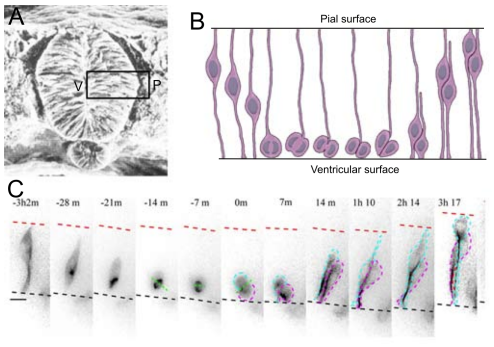
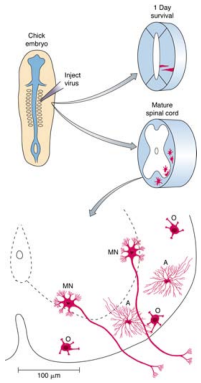
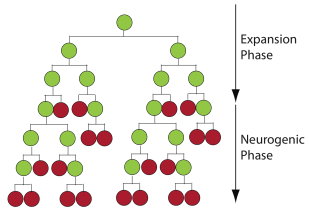
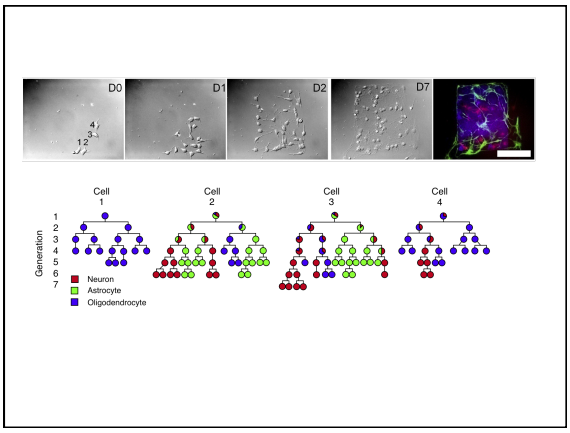
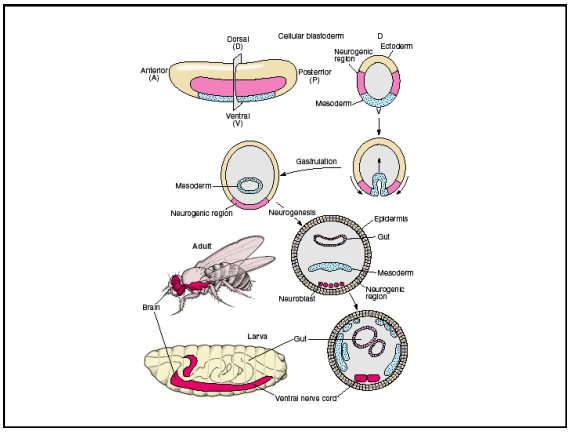
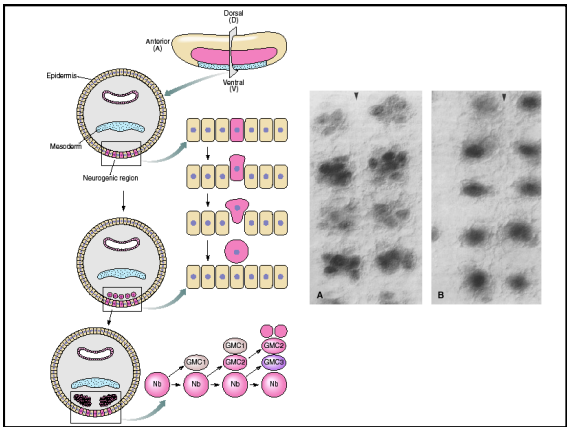

A
V
P
B
Pial surface
Ventricular surface
C
-3h2m
-28 m
-21m
-14 m
-7 m
0m
7m
14 m
1h 10
2h 14
3h 17


1 Day survival
Chick embryo
Inject virus
Mature spinal cord
MN
MN
100 µm


Expansion Phase
Neurogenic Phase

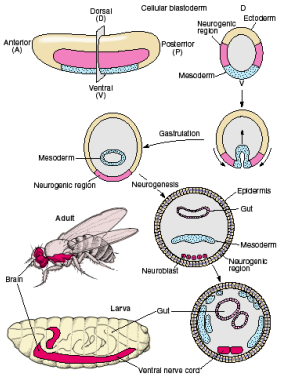

D0
D1
D2
D7
Cell 1
Cell 2
Cell 3
Cell 4
Generation
1
2
3
4
5
6
7
Neuron
Astrocyte
Oligodendrocyte


Dorsal (D)
Cellular blastoderm
D
Anterior (A)
Posterior (P)
Ventral (V)
Neurogenic region
Ectoderm
Mesoderm
Gastrulation
Neurogenic region
Neurogenesis
Epidermis
Gut
Mesoderm
Neuroblast
Neurogenic region
Adult
Brain
Larva
Gut
Ventral nerve cord

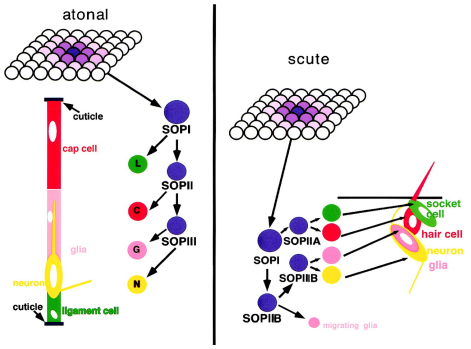

Dorsal (D)
Anterior (A)
Ventral (V)
Epidermis
Mesoderm
Neurogenic region
GMC1
GMC2
GMC3
Nb
A
B

## Fly neurogenesis

- Neurogenic region of embryo
- External sensory organs-chaete
- Internal sensory organs-chordotonal
- Specialized imaginal discs-eg. eye

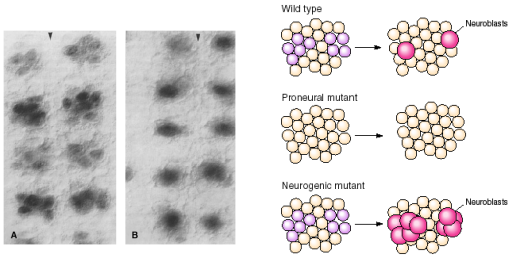


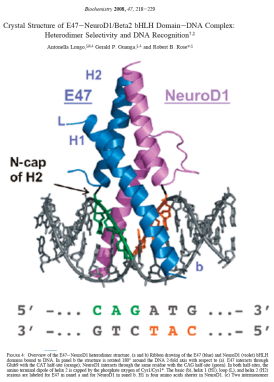

External sense organ development



Mutations in Drosophila led to molecular analysis of neurogenesis

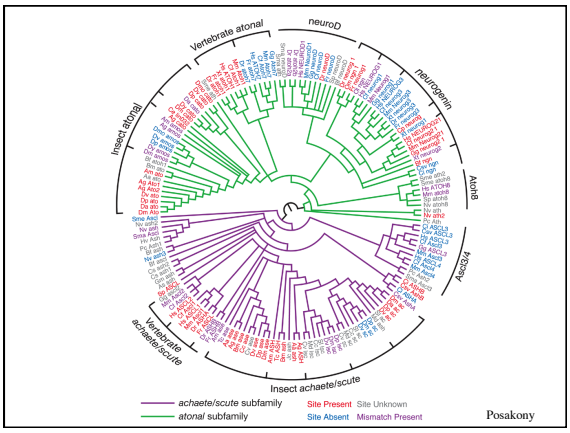
Proneural mutants have no neuroblasts




**bHLH genes**

1. Present in all animals, plants, and yeast, but absent in prokaryotes
2. The bHLH domain is about 60 amino acids long, with a basic DNA binding domain, and a dimerization domain comprised of two alpha-helices separated by a variable loop region.
3. Form several families, Groups A-D, that are involved in cell cycle regulation (c-myc) and tissue specific transcriptional regulation (MyoD1).
4. Group A: tissue specific regulators like proneural genes or myogenic determination genes, bind E-boxes (CANNTG) as heterodimers with other E-proteins (E12 or daughterless)
5. Group B: cell cycle regulators, like Myc and Max, as well as the hairy/Hes proteins, bind to N-boxes (CANNCG)
6. Group C: contain an additional domain, the PAS domain and are called bHLH-PAS proteins; these include Period and the dioxin receptor and bind to NCGTG
7. Group D: these lack a basic domain and act as antagonists to bHLH activity (Id/Emc)



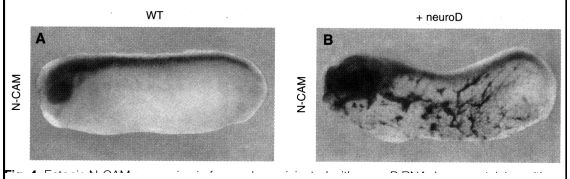

## Vertebrate Proneural Genes

Many different family members.

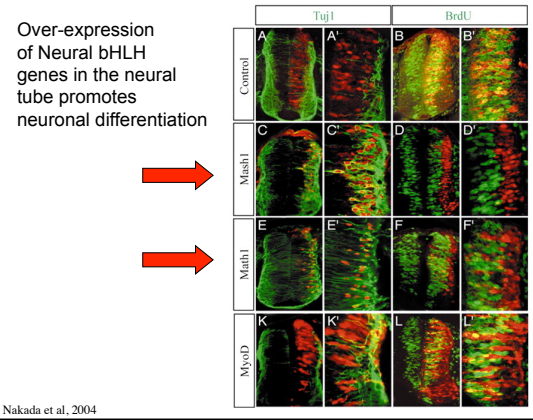
Expressed in both progenitors and differentiated neurons.

Necessary and sufficient for neuronal diversity?

Jackie Lee and Dave Turner showed that overexpression of NeuroD in frog embryos led to expansion of the nervous system



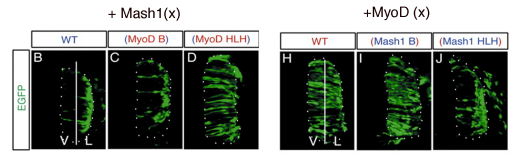
Over-expression of Neural bHLH genes in the neural tube promotes neuronal differentiation




Nakada et al, 2004

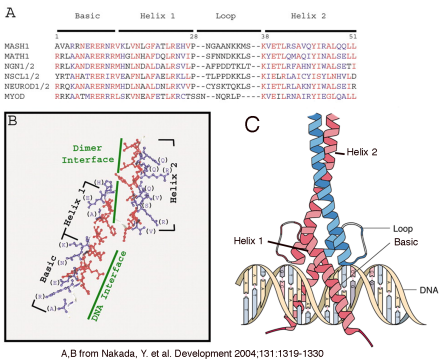
Domain swapping experiments show what part of the bHLH sequence is required for neuronal differentiation

| | Basic | Helix-1 | Loop | Helix-2 | Lateral Phenotype |
|---|---|---|---|---|---|
| Mash1 | RR***NERERNR | VKLVNLGFATLREHV | PNGAANKKMS | KVETLRSAVEYIRAL | + |
| Mash1(MyoD B) | RRKAATMRERRR | VKLVNLGFATLREHV | PNGAANKKMS | KVETLRSAVEYIRAL | + |
| Mash1(MyoD HLH) | RR***NERERNR | LSKVNEAFETLKRCT | SSN*PNQRLP | KVEILRNAIRYIEGL | — |
| Mash1(MyoD H1) | RR***NERERNR | LSKVNEAFETLKRCT | PNGAANKKMS | KVETLRSAVEYIRAL | — |
| Mash1(MyoD L) | RR***NERERNR | VKLVNLGFATLREHV | SSN*PNQRLP | KVETLRSAVEYIRAL | + |
| Mash1(MyoD H2) | RR***NERERNR | VKLVNLGFATLREHV | PNGAANKKMS | KVEILRNAIRYIEGL | + |
| Mash1(NR-AQ) | RR***NERERAQ | VKLVNLGFATLREHV | PNGAANKKMS | KVETLRSAVEYIRAL | — |
| MyoD | RRKAATMRERRR | LSKVNEAFETLKRCT | SSN*PNQRLP | KVEILRNAIRYIEGL | — |
| MyoD(Mash1 B) | RR***NERERNR | LSKVNEAFETLKRCT | SSN*PNQRLP | KVEILRNAIRYIEGL | — |
| MyoD(Mash1 HLH) | RRKAATMRERRR | VKLVNLGFATLREHV | PNGAANKKMS | KVETLRSAVEYIRAL | + |
| MyoD(Mash1 H1) | RRKAATMRERRR | VKLVNLGFATLREHV | SSN*PNQRLP | KVEILRNAIRYIEGL | + |



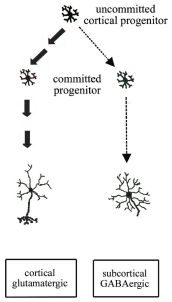

Nakada et al, 2004

Subclass-specific amino acid residues are on an external surface of the dimer



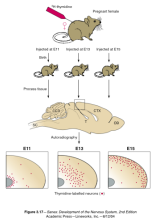

A,B from Nakada, Y. et al. Development 2004;131:1319-1330

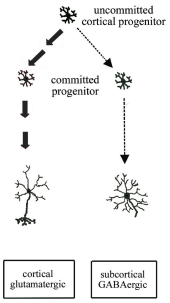
Cerebral cortex histogenesis



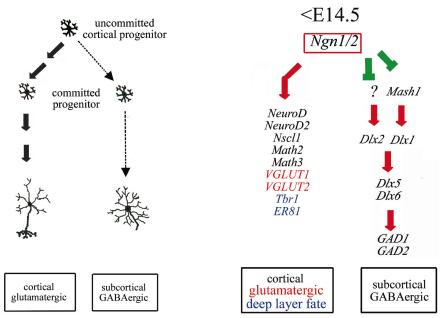

Schuurmans et al, 2004 EMBO J

Correlation between early/late generated neurons and excitatory/inhibitory neurons?

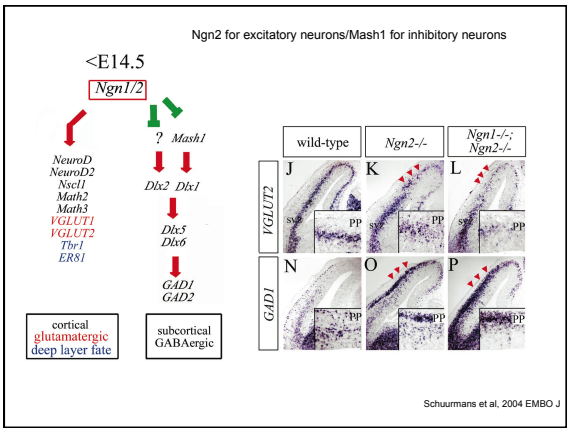


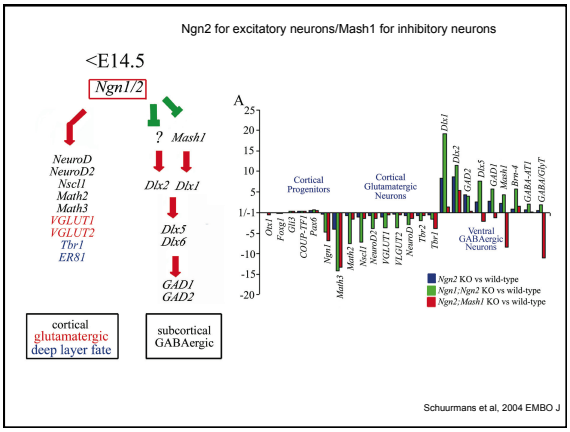


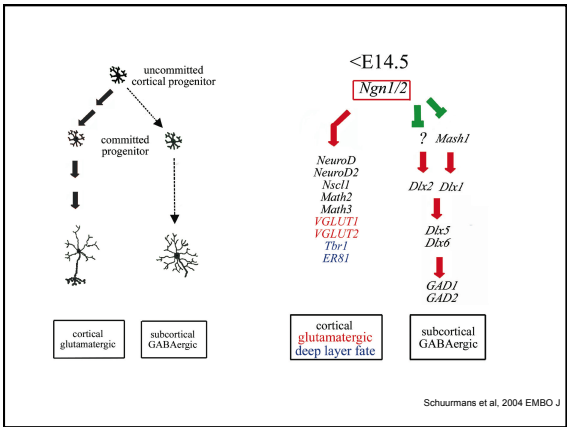

Ngn2 for excitatory neurons/Mash1 for inhibitory neurons




Schuurmans et al, 2004 EMBO J

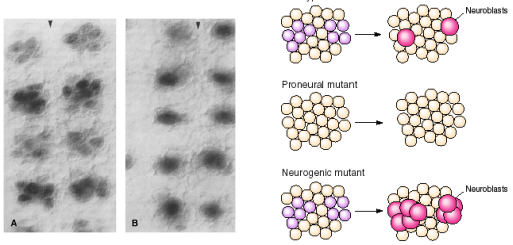

Ngn2 for excitatory neurons/Mash1 for inhibitory neurons
<E14.5
Ngn1/2
? Mash1
NeuroD
NeuroD2
Nscl1
Math2
Math3
VGLUT1
VGLUT2
Tbr1
ER81
Dlx2 Dlx1
Dlx5
Dlx6
GAD1
GAD2
cortical glutamatergic deep layer fate
subcortical GABAergic
wild-type
Ngn2-/-
Ngn1-/-; Ngn2-/-
VGLUT2
GAD1
J
K
L
N
O
P
SVZ
PP
Schuurmans et al, 2004 EMBO J


Ngn2 for excitatory neurons/Mash1 for inhibitory neurons
<E14.5
Ngn1/2
? Mash1
NeuroD
NeuroD2
Nscl1
Math2
Math3
VGLUT1
VGLUT2
Tbr1
ER81
Dlx2 Dlx1
Dlx5
Dlx6
GAD1
GAD2
cortical glutamatergic deep layer fate
subcortical GABAergic
A
Cortical Progenitors
Cortical Glutamatergic Neurons
Ventral GABAergic Neurons
Ngn2 KO vs wild-type
Ngn1;Ngn2 KO vs wild-type
Ngn2;Mash1 KO vs wild-type
Schuurmans et al, 2004 EMBO J


uncommitted cortical progenitor
committed progenitor
cortical glutamatergic
subcortical GABAergic
<E14.5
Ngn1/2
? Mash1
NeuroD
NeuroD2
Nscl1
Math2
Math3
VGLUT1
VGLUT2
Tbr1
ER81
Dlx2 Dlx1
Dlx5
Dlx6
GAD1
GAD2
cortical glutamatergic deep layer fate
subcortical GABAergic
Schuurmans et al, 2004 EMBO J

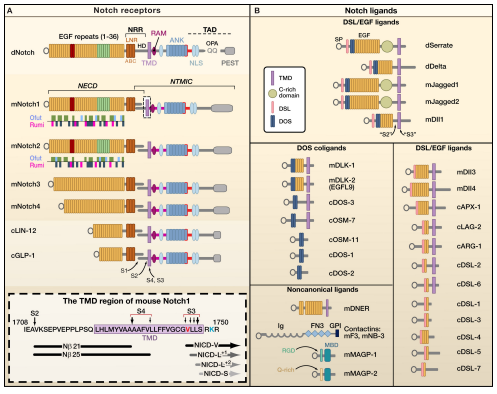
# Notch pathway and neurogenic mutants

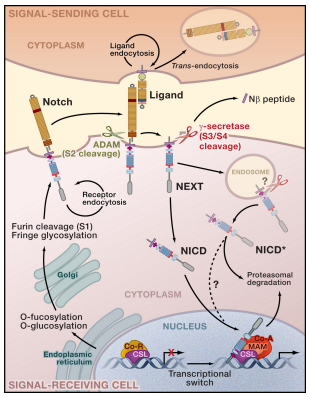
## Neurogenic mutants have too many neuroblasts

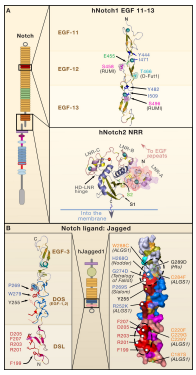








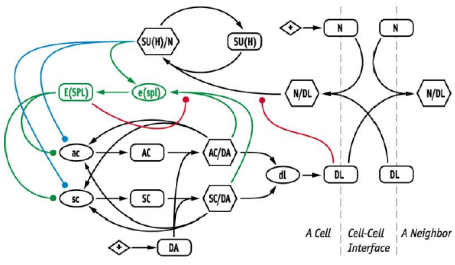


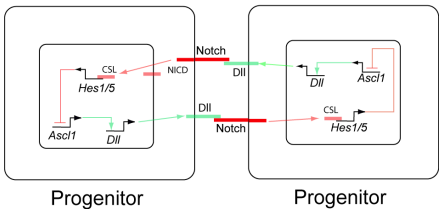

Current Biology, Vol. 12, 778–786, May 14, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0960-9822(02)00839-4

## Robustness, Flexibility, and the Role of Lateral Inhibition in the Neurogenic Network

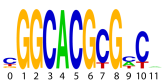
Eli Meir,[1,2,3] George von Dassow,[1,2] Edwin Munro,[1] and Garrett M. Odell[1,2]
[1]Friday Harbor Laboratories

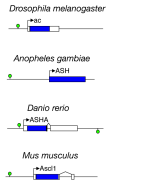


## Notch pathway




## Hes consensus in bHLH promoters of diverse organisms





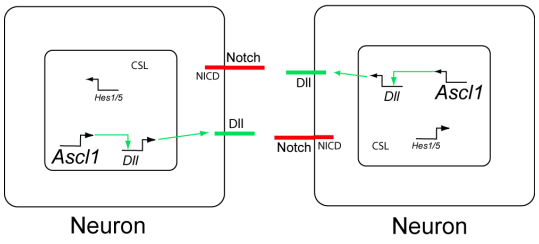

**achaete/scute**

| | |
|---|---|
| Dm | GCAGCC GGCACGCGAC AGGGCC |
| Da | GCAGCC GGCACGCGAC TTCAGT |
| Dp | GCAGCC GGCACGCGAC AGTCCC |
| Dmo | GCAGCC GGCACGCGAC AGCGAC |
| Dv | GGGGCC GGCACGCGAC AGCAAC |
| Ag | GAATGC GGCACGCGAC CGGCTC |
| Aa | TCTGGC GGCACGCGTC CCTGCG |
| Bm | GCGTCC GGCACGCGCC CCACCG |
| Am | AGGGCG GGCACGCGCC TATCCC |
| Am | TCGTCC GGCACGCGCT CCAGCG |
| Tc | GTCTTC GGCACGCGCT CAACCC |
| Cl | ATTGAC GGCACGCGAC ACTCTT |
| Csv | AAGCCG GGCACGCGAC TGACTT |
| Hs | GAGTCC GGCACGCGCC AGGCGC |
| Mm | GAGTCC GGCACGCGCC GGGCGC |
| Cf | GAGTCC GGCACGCGCC AGGCGC |
| Dr | AGGAGC GGCACGCGCC CGGCGT |
| Fr | TCAAGT GGCACGCGTC CGGCGC |
| Sp | TCTGGT GGCACGCGTC TAATAT |
| Nv | GTTTAC GCCACGCGCT CTCTCT |
| Sma | CGATCT GCCACGTGCT ATTTAT |
| Sma | AGCATT GACACGCGTC TTTTTA |

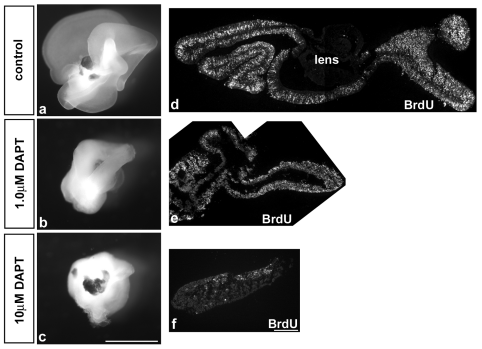
**neurogenin**

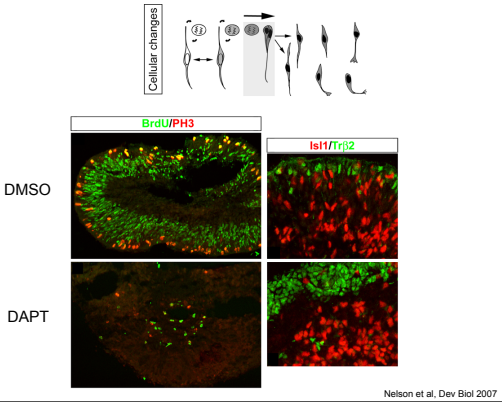
| | |
|---|---|
| Hs | GCTCCT GGCACGCGAC TCCCAG |
| Mm | GCTGCT GGCACGCGAC TCCAAG |
| Cf | GCTCCT GGCACGCGAC TCCCGG |
| Dr | GATAAT GGCACGCGTC TGTCTT |
| Fr | TCAAAT GGCACGCGCC TCTGTG |
| Ol | GACGGC AGCACGCGCC TCGTCC |
| Om | GTACAA GGCACGCGTC TTGCTC |
| Xt | CGTTCT GACACGCGTC CTGCTC |
| Cp | ACAAGG GGCACGCGAC TCCTCT |
| Gg | GGCGCT GGCACGCGCC CGGGCG |
| Bf | GCTGAG GGCACGCGCT GGCTGT |

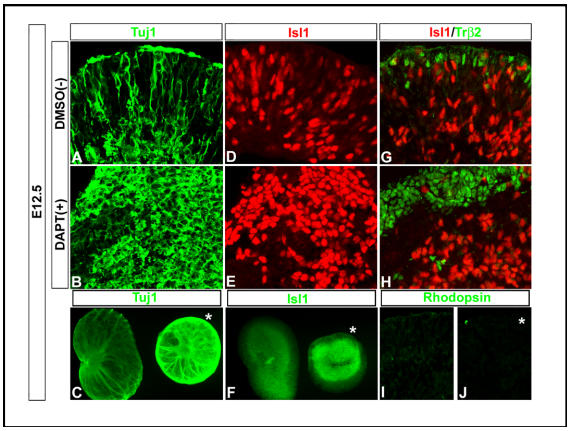
## Notch inhibited: neuronal differentiation



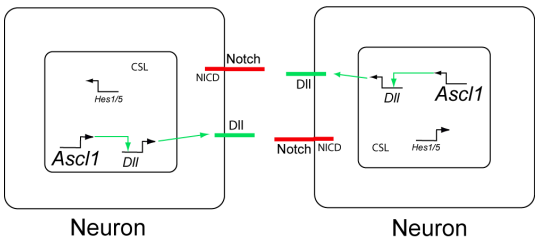
**Inhibition of Notch signaling with DAPT, a gamma-secretase inhibitor (**7 {*N*-[*N*-(3,5-difluorophenacetyl)- l-alanyl]-*S* -phenylglycine *t*-butyl ester)

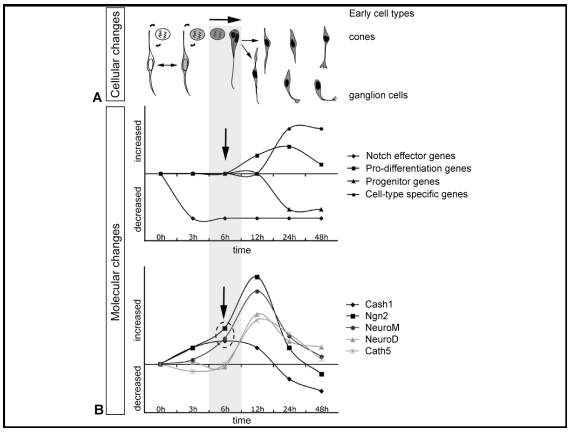


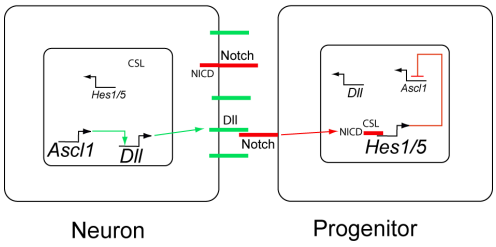



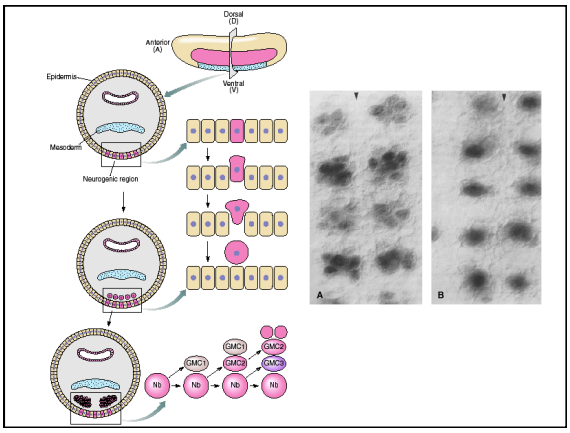
Notch inhibited:
neuronal differentiation

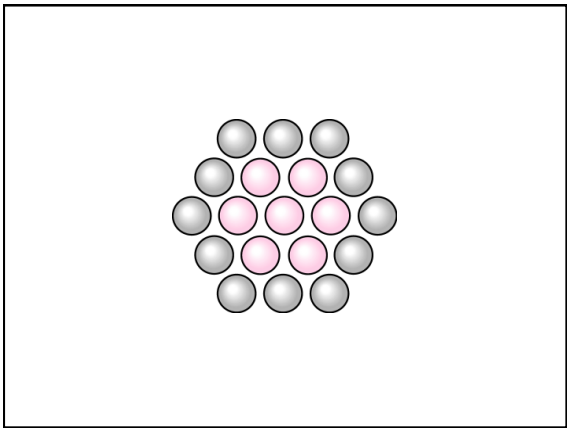


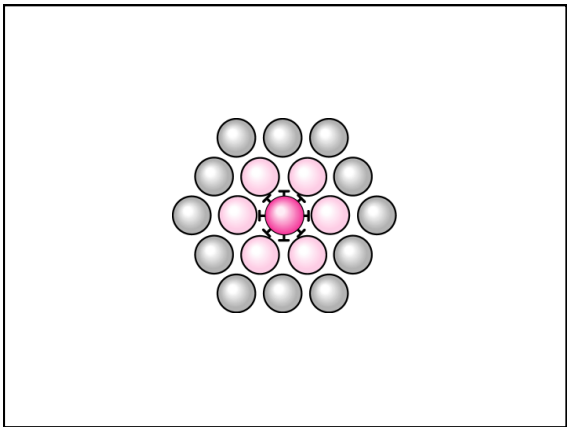



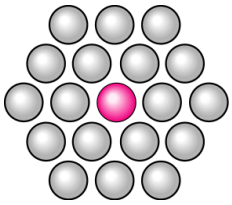

Notch asymmetry



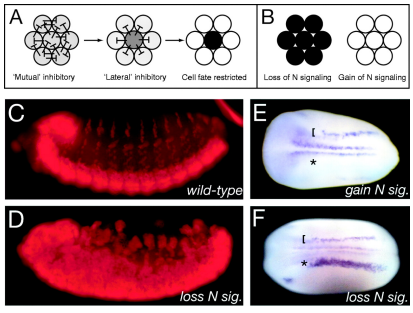

Dorsal
(D)
Anterior
(A)
Ventral
(V)
Epidermis
Mesoderm
Neurogenic region
A
B
GMC1
GMC2
GMC3
Nb







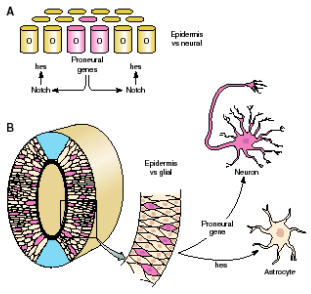
**Inhibitory Notch signaling restricts cell fates**

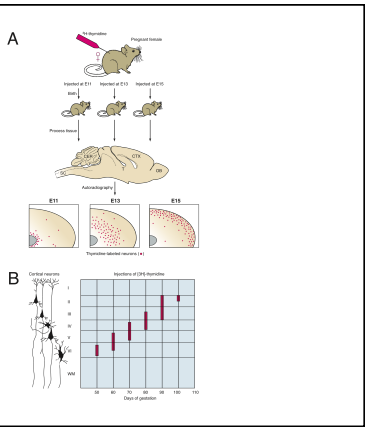


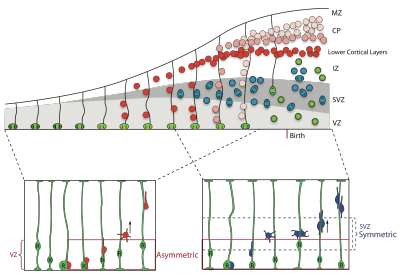

Lai, E. C. Development 2004;131:965-973

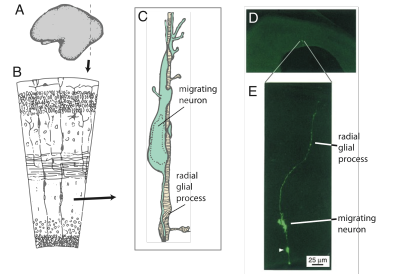
Development

Comparison of Drosophila and vertebrate proneural/Notch pathway




A
B
E11
E13
E15
Cortical neurons
Injections of CH3-thymidine
Days of gestation


MZ
CP
Lower Cortical Layers
IZ
SVZ
VZ
Birth
VZ
Asymmetric
SVZ
Symmetric


A
B
C
migrating neuron
radial glial process
D
E
radial glial process
migrating neuron
25 μm