Control of neuritic growth in Purkinje cells

Belgrade, September 2005
terminal growth (arbour mode)

- constitutive
- no gene expression
- plasticity

long-distance growth (axon mode)

- developmentally regulated
- de novo gene expression
- regeneration
regulation of intrinsic neuronal growth properties

injury-induced molecules (inflammatory cytokines, e.g. LIF)

positive signals

negative signals

injury-induced molecules (scar components)

constitutively-expressed molecules (retrograde cues)
inhibition of regeneration
control of growth
local effect
long-distance effect
intrinsic neuronal growth properties
constitutively-expressed molecules
control of growth
inhibition of regeneration
differential regenerative potential of olivocerebellar and Purkinje axons into embryonic neural transplants
Cell body reaction to axotomy

Survival

Surviving cells (%)

survival time (days)

nr of Pcs/mm

inferior olive

Purkinje cells

Dusart et al., 1999
survival
axon reaction
atrophy
slow cell death

• c-Jun
• JunD
• GAP-43
• NOS
• F3
• Galanin

many neurons respond
regeneration into growth-permissive environment

inferior olivary neurons (LRN, DCN)

no cell body reaction
rare neurons respond close to the lesion

no regeneration
delayed sprouting

Purkinje cells

regeneration into growth-permissive environment

survival
axon reaction

axotomy

Neurite growth-inhibitory molecules

myelin-associated proteins

• Nogo-A
• MAG
• OMgp
• Sema 4D
• Eph B3
• …

extracellular matrix molecules

• NG2
• Proteoglycans (CSPGs)
  - Neurocan
  - Versican
  - Phosphacan
  - Brevican
• Tenascin
• …
Reciprocal distribution of Purkinje axons and myelin in the granular layer of the adult rat cerebellum
neutralising anti-Nogo-A antibodies:
• IN-1
• 472
• 11C7

intact cerebellum
• in vitro
• in vivo
granular layer

IN-1
472
Degradation of CSPGs induces PC axon sprouting
Degradation of CSPGs does not disrupt Purkinje axon myelination
Removal of inhibitory molecules in the CNS environment induces growth

The effect is reversible: outgrowing axons fail to form new endurable connections
Purkinje cell response to injury and regenerative potential change during the second postnatal week. Two major developmental events are taking place during this period:

- formation of Purkinje intracortical plexuses
- myelination of Purkinje axons
Dès les premières phases observables, le cylindre-axe émet des collatérales. Elles sont au nombre de deux, trois ou davantage; il peut y en avoir jusqu’à huit. Mais cette multitude de collatérales e persiste pas; la plupart d’entre elles se resorbent et sur les coupes de cervelet provenant du chat, du chien ou du lapin adultes on en découvre rarement plus de trois.

*Ramón y Cajal, 1911*

does myelin formation contributes to shape the distribution pattern of developing Purkinje cell intracortical plexus?
the corticonuclear projection is already established at birth
cerebellar growth during postnatal development

dorso-ventral

antero-posterior

P1

P4

P8

P15
Sprouting of Purkinje axon collateral branches
Axonal phenotype of Purkinje cells *in vitro*

4 div

20 div

Axon length to terminal arbour (µm)
Axonal phenotype of Purkinje cells transplanted in heterotopic position
Collateral branches of Purkinje axons originate as interstitial sprouts from a precise segment along the intracortical neuritic stem.

This branching pattern is also present in vitro and after transplantation into heterotopic positions suggesting that it is expressed, at least in part, in a cell-autonomous manner.
pruning of Purkinje axon collateral branches
remodelling of Purkinje axon collateral branches during postnatal development

Gianola et al., 2003
myelin formation during cerebellar development

P8  P10  P12  P15

MBP

CaBP/MBP
interaction between Purkinje axons and Nogo-A-positive oligodendrocytes
myelin (Nogo)

azacytidine
anti-Nogo

targets

targets

P5

P15
Azacytidine treatment delays myelination and pruning of Purkinje axon collateral branches.
Application of anti-Nogo antibodies delays myelination and pruning of Purkinje axon collateral branches.
The effect of anti-Nogo antibodies is transient

CaBP/Nogo

CaBP/MBP

injection
observation
P10
P23

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<tbody>
<tr>
<td>number of axon profiles</td>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>total axon length (µm)</td>
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<td>2500</td>
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<tr>
<td>number of grid crossings</td>
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<td>150</td>
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azacytidine
anti-Nogo
targets

myelin
(Nogo)

P5

P15
Uninjured L7/GAP-43 Purkinje cells have normal axon morphology and myelination
Buffo et al., 1997
**WHITE MATTER**

**GRANULAR LAYER**

- L7-GAP-43
- Wild-type

**nr of axon profiles**

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<thead>
<tr>
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<th>7d</th>
<th>60d</th>
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<td>L7-GAP-43</td>
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<td>40</td>
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<td>Wild-type</td>
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<td>20</td>
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**nr of grid crossings**

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<td>L7-GAP-43</td>
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<td>60</td>
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<tr>
<td>Wild-type</td>
<td>15</td>
<td>30</td>
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**WHITE MATTER**

- L7-GAP-43
- Wild-type

**nr of axon profiles**

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<td>L7-GAP-43</td>
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<td>400</td>
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<tr>
<td>Wild-type</td>
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<td>200</td>
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**nr of grid crossings**

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<td>L7-GAP-43</td>
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<td>100</td>
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<tr>
<td>Wild-type</td>
<td>25</td>
<td>50</td>
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The diagram illustrates the number of axons (% of axons) with and without myelin. The bars indicate the percentage distribution for different groups: wt-prox, wt-dist, gap-prox, and gap-dist.
<table>
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<th>Condition</th>
<th>Length (µm ± Standard Error)</th>
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<tbody>
<tr>
<td>wt intact</td>
<td>17.6 µm ± 2.7</td>
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<tr>
<td>GAP-43 intact</td>
<td>18.6 µm ± 3.2</td>
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<tr>
<td>wt injured</td>
<td>35.8 µm ± 15.8</td>
</tr>
<tr>
<td>GAP-43 injured</td>
<td>55.2 µm ± 31.6</td>
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Legend:
- **wild-type**
- **GAP-43**
GAP-43 accumulates along intracortical Purkinje axons

GAP-43 accumulates along intracortical Purkinje axons

- 5.7% GAP-43 +
- 20% GAP-43 -
- 34.5% UNMYEL.
- 39.8% MYEL.

- 2.9% GAP-43 + in axons in white matter
- 7.7% GAP-43 - in axons in white matter
- 89.4% MYEL. in axons in white matter
after axotomy, the intracortical axon of GAP-43 overexpressing Purkinje cells
- lose myelin
- sprout

is GAP-43 responsible for myelin loss along Purkinje axons?
In intact L7/GAP-43 mice axon morphology and myelin are normal. The phenotype is only expressed after injury.

After axotomy, structural changes only occur at precise sites (the transected stump and the torpedo), where GAP-43 accumulates.

Lesion-induced changes (alterations of axoplasmic flow) induce the accumulation of critical quantities of GAP-43 along precise axon segments.
intact GAP-43 mouse

axotomy

sprouting
GAP-43

Rho

LINGO

NgR/P75

Nogo

F3/CASPR

Sema 4D

MAG

OMgp

Nogo

F3/CASPR

GAP-43

disrupted axon-myelin interaction

decreased inhibition

enhanced growth potential
GAP-43
myelin
intact
axotomy
intrinsic
growth
potential
extrinsic
inhibitory
cues
myelin
GAP-43
axotomy
extrinsic
inhibitory
cues
intrinsic
growth
potential
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