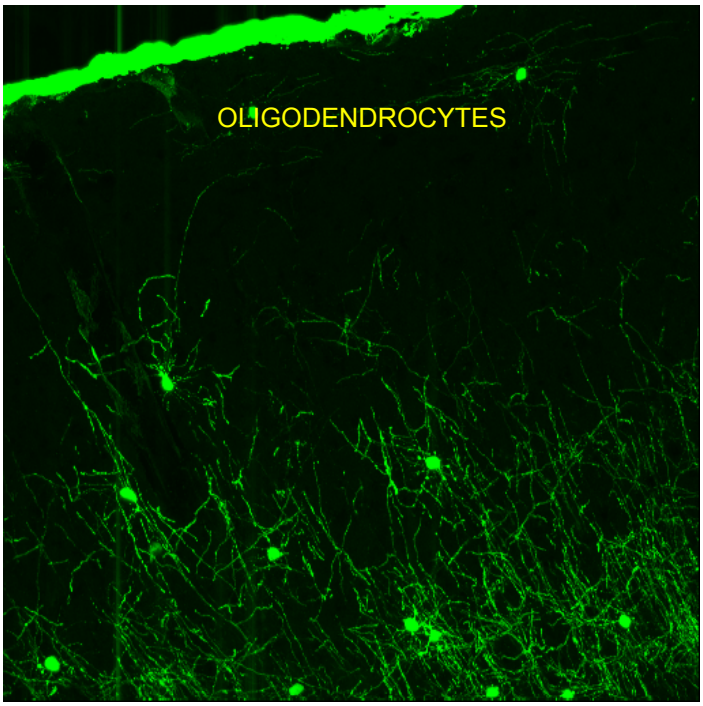
BSc Neuroscience: Module 1

Oligodendrocytes

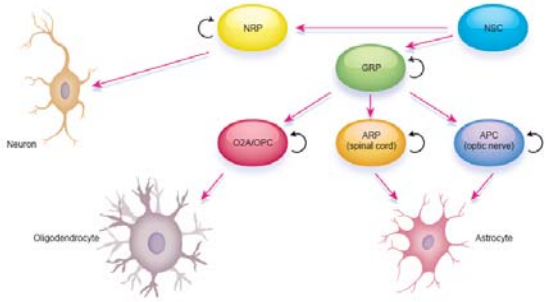
- glial lineage
- the neuron/oligodendroglia switch
- oligodendrocyte development
- myelin formation

Prof R. Reynolds 2012



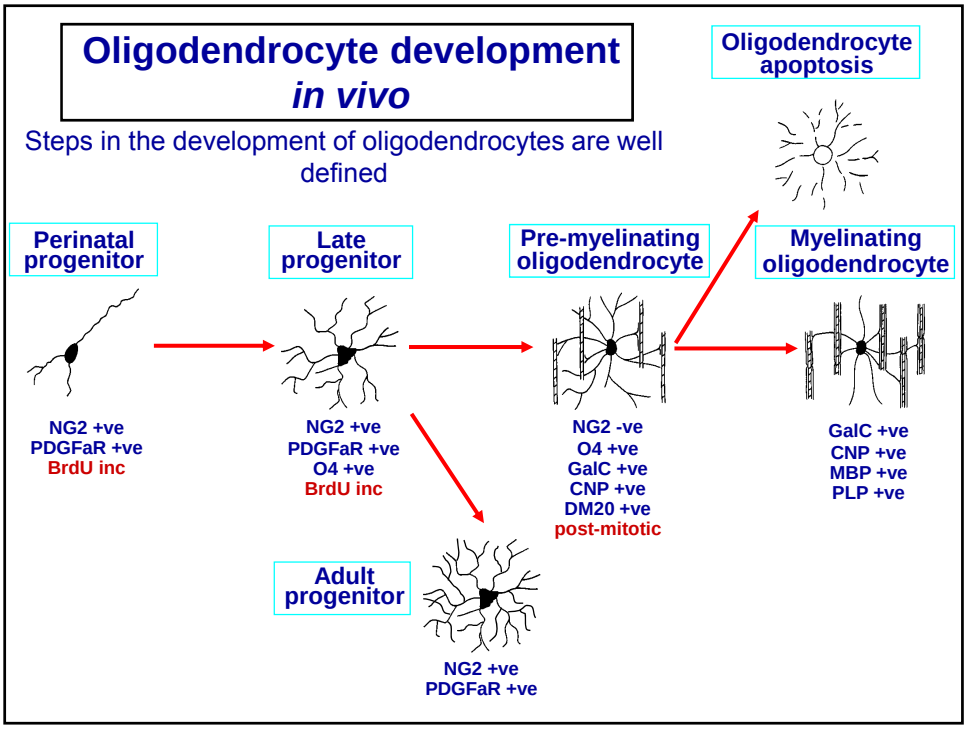
Oligodendrocyte origins

- myelination is vital to the correct functioning of the nervous system.
- correct ratio of oligodendrocytes to axons is essential during development.
- dysmyelination during development usually leads to mental retardation and/or death.
- steps in the development of oligodendrocytes are well defined



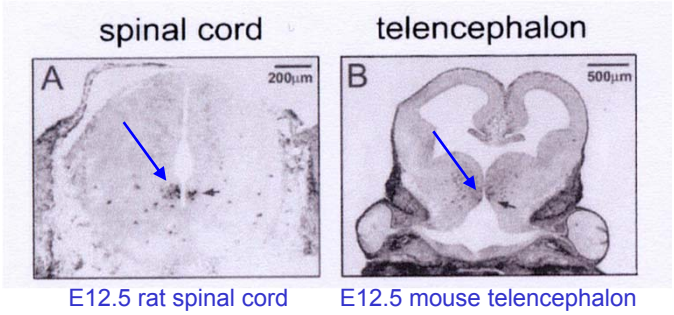
Oligodendrocyte development *in vivo*

Steps in the development of oligodendrocytes are well defined



The origin of oligodendrocytes

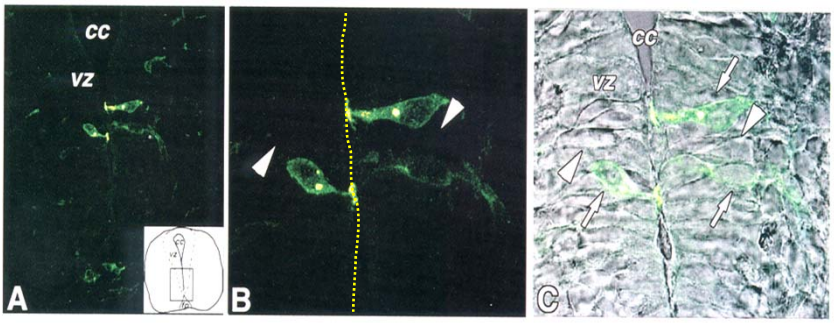
Oligodendrocytes are generated by subventricular zone cells in the brain and spinal cord that give rise to committed oligodendrocyte progenitor cells (OPCs)



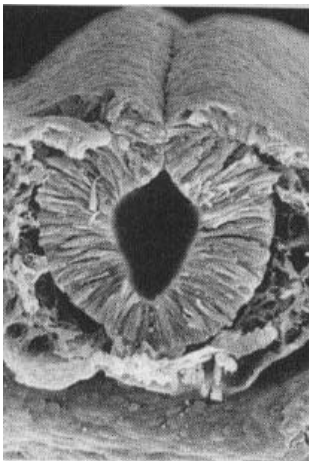
E12.5 rat spinal cord E12.5 mouse telencephalon

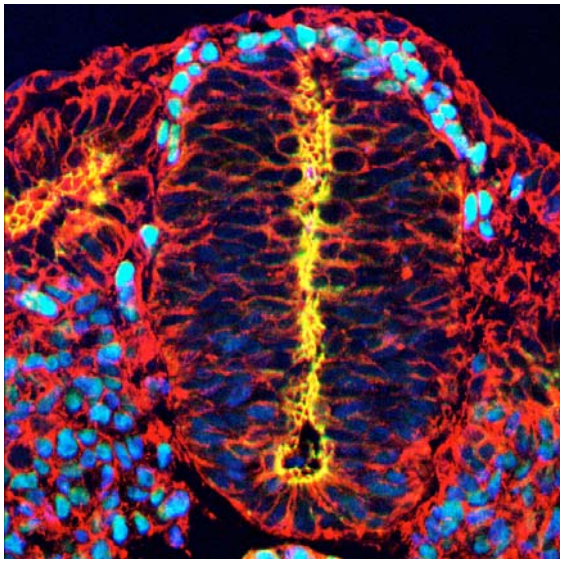
- early ventral ventricular zone restriction in spinal cord and forebrain of rodents
- evidence from appearance of markers (PDGFαR) and culture and transplantation experiments
- pMN domain in the spinal cord

The origin of oligodendrocyte progenitors in the rodent spinal cord

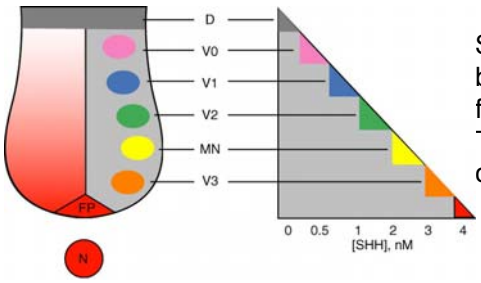


- immunolabeling of mouse E13.5 spinal cord with antibodies to PDGFαR.
- strongly positive cells are found in the ventricular zone, dorsal to the floor plate.
- processes end at the surface of the central canal.





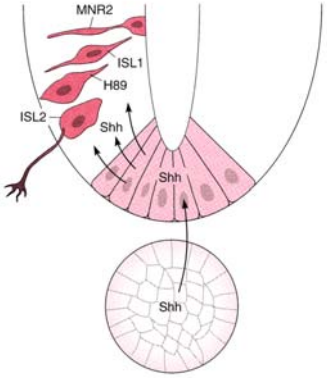
Spinal cord motor neurones



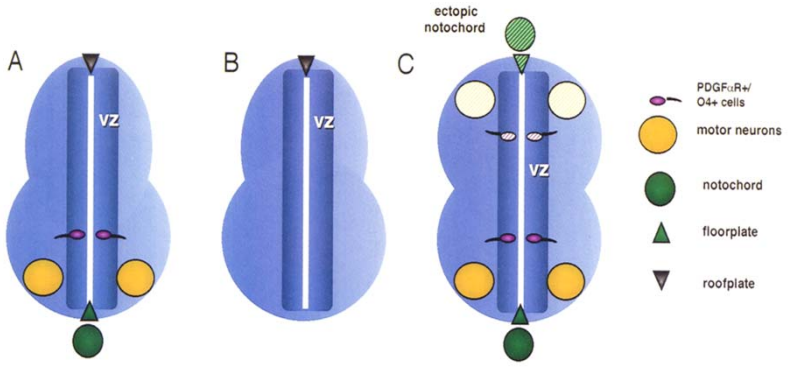
Sonic hedgehog (SHH) is released by the notochord and then the floor plate forming a V to D gradient This graded signal induces different cell types at different D/V positions

SHH induces the expression of LIM homeodomain transcription factors such as Isl1, Isl2, H89 and MNR2.

The expression of these (and other) genes uniquely defines the different motor columns which innervate specific muscles e.g. trunk, limb or autonomic ganglia



Oligodendrocyte induction by ventralizing signals

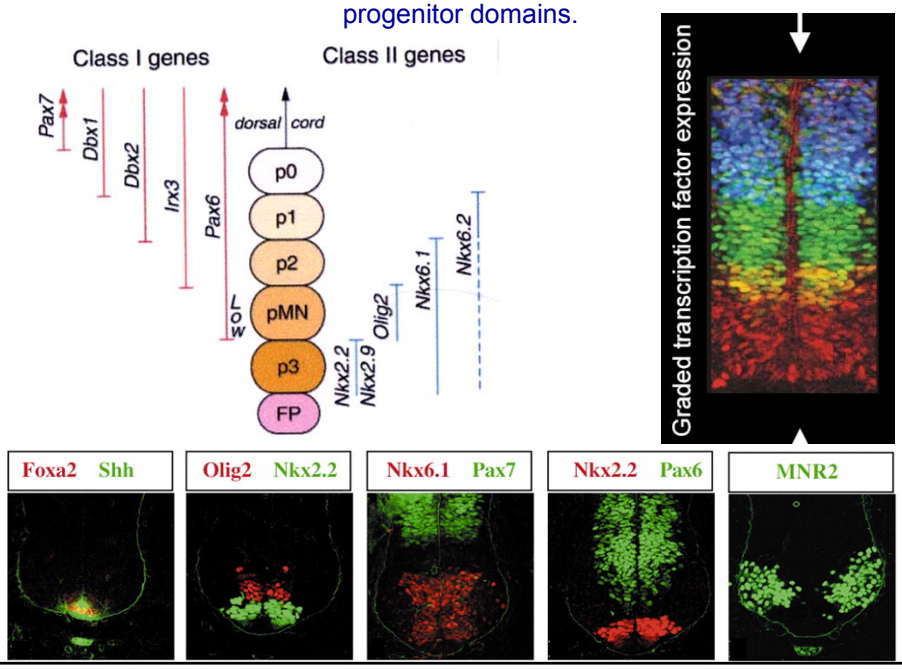


A. During normal spinal cord development, PDGFαR+ cell foci appear in a specific region of the ventral ventricular zone following motoneuron differentiation.

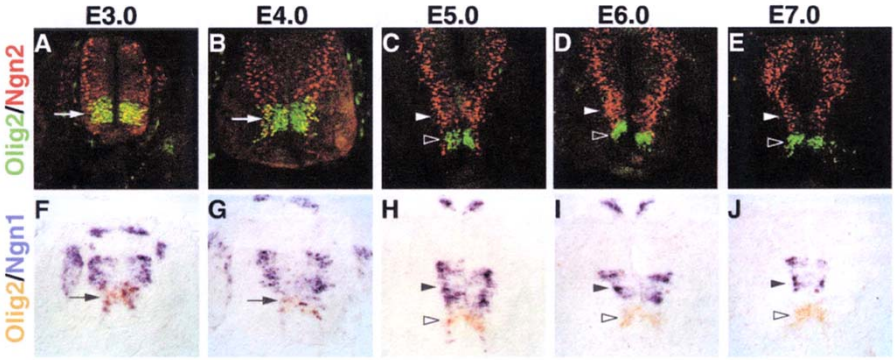
B. Caudal regions of the Danforth short-tail mutant mouse lack a notochord and floor plate, motoneurons and oligodendrocyte precursors fail to develop.

C. Grafting of ectopic notochord induces ectopic floor plate, motoneurons and oligodendrocyte precursors.

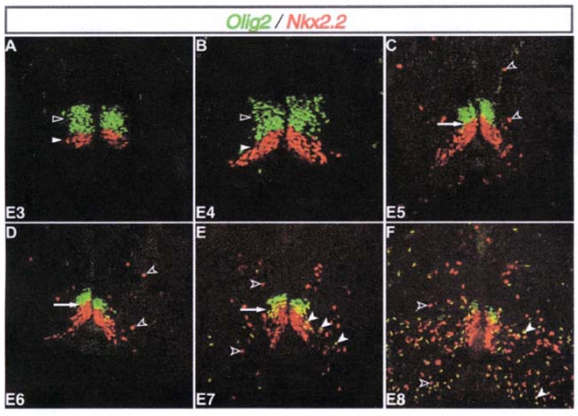
Patterns of transcription factor expression are superimposed on the known progenitor domains.



- shortly after MN production ceases, proneural genes *Ngn1 & 2* are downregulated in the ventral neuroepithelium
- extinction of neurogenins from the *olig2* expressing domain precedes oligodendrocyte progenitor formation



Chick spinal cord



Chick spinal cord

- the domains of *Nkx2.2* and *olig2* expression begin to shift relative to one another to create a region of overlap
- migratory *olig2+Nkx2.2+* progenitors appear within the overlap and disperse throughout the spinal cord

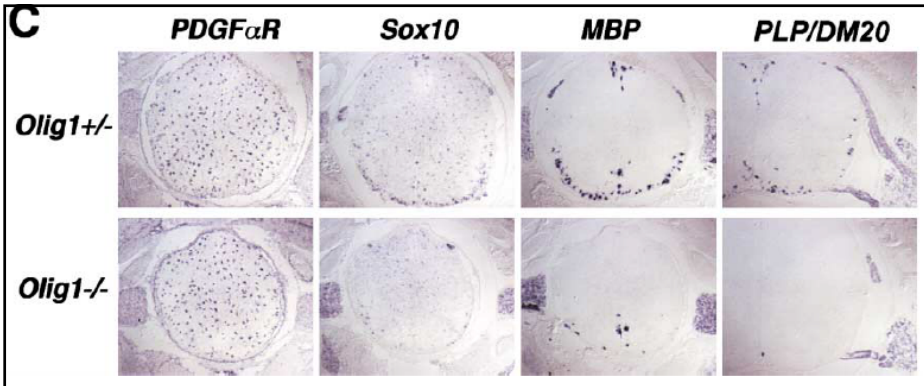
Zhou et al (2001)

The *olig* genes and oligodendrocyte development

David Rowitch

Charles Stiles

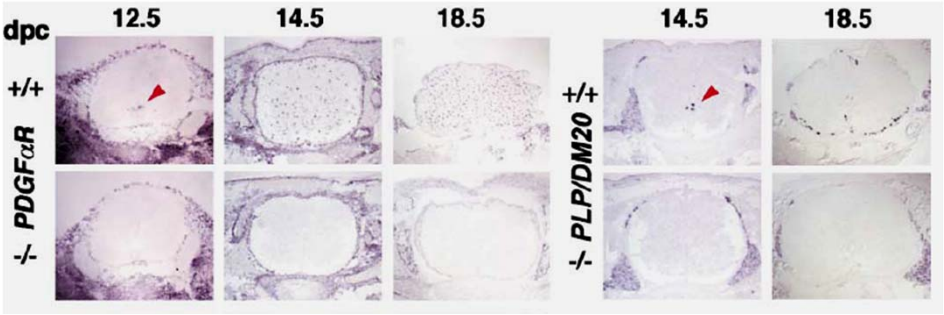
- the *olig* bHLH group of transcription factors, discovered by three groups in 2000, are essential for oligodendrocyte development.
- retrovirus mediated expression of *olig1* in rat neuroepithelial cells in vitro and mouse brain in vivo can drive the production of oligodendrocytes.
- *olig1 & 2* act in conjunction with other transcription factors and homeodomain proteins to specify oligodendrocytes during embryonic development.
- now known to govern multiple aspects of progenitor cell function in the ventral neural tube, including specification of motor neurons.
- *olig1 & 2* expression is regulated by Shh but indirectly via Nkx 6.1 and 6.2.
- all malignant gliomas express *olig2*



Day 0 spinal cord

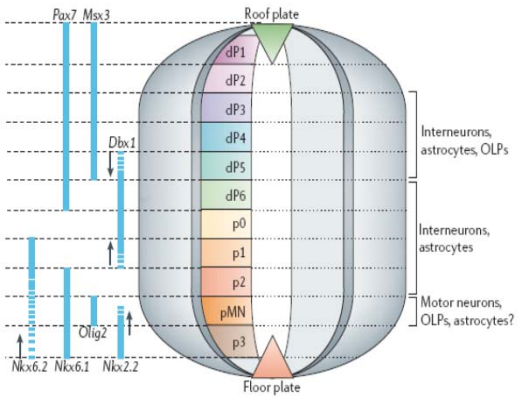
- *Olig1* null mice are viable but show a delay in oligodendrocyte maturation.
- at P14 in wild type and heterozygotes 35% of optic nerve axons are myelinated. In null mutants no myelin is seen.
- oligodendrocytes make contact with axons but do not myelinate them in the brain. Myelinogenesis and axonal recognition uncoupled.
- severely reduced level of myelination in spinal cord.
- at 3 weeks develop severe neurological deficits and die.

Olig2 null spinal cord



- *Olig2* null mice entirely lack oligodendrocyte lineage cells in the spinal cord but not the brain stem.
- *Olig1 & 2* double null mice have no oligodendrocytes anywhere in the CNS.
- *Olig2* necessary for oligodendrogliogenesis
- Motor neurons also lacking in *Olig2* null mice

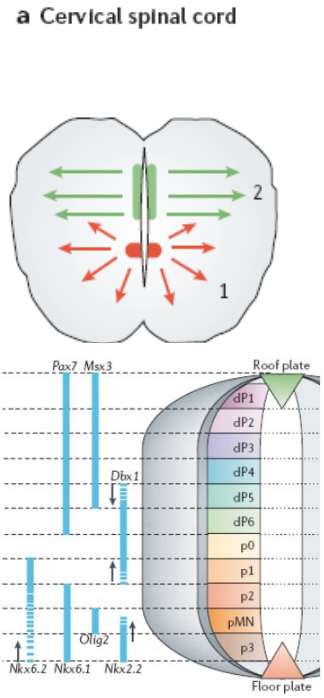
A dual origin for oligodendrocytes !



3 recent studies using cre-lox fate mapping have provided evidence for a multiple origin of oligodendrocyte progenitors in spinal cord, brain stem and forebrain

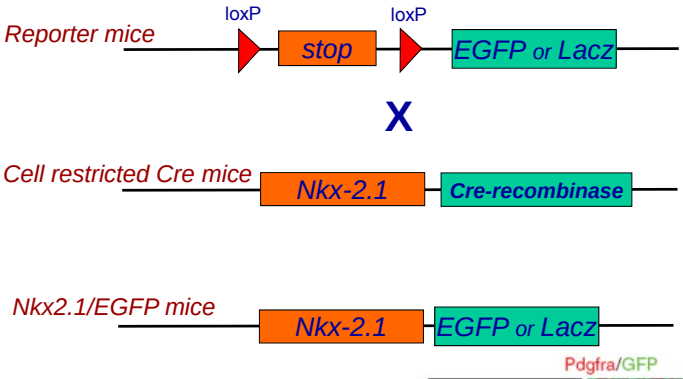
Why should it matter where oligodendrocytes arise during development?

- OPCs generated from pMN domain starting at E12.5
- No ventral OPCs in Nkx6 null mice
- PDGFaR+Olig2+ OPCs still produced in dorsal cord of Nkx6 null mice
- Dorsal OPCs arise from *Pax7 and Msx-3* expressing regions
- Small numbers of dorsal Pax7+ OPCs seen in normal mice but are suppressed by ventral OPCs
- 85% of all spinal cord OPCs generated from ventra domains
- 3% from Dbx1+ domains, 10-15% from Msx+ domains

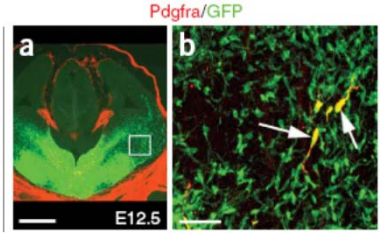


Cai et al, 2005

Cre-lox fate mapping

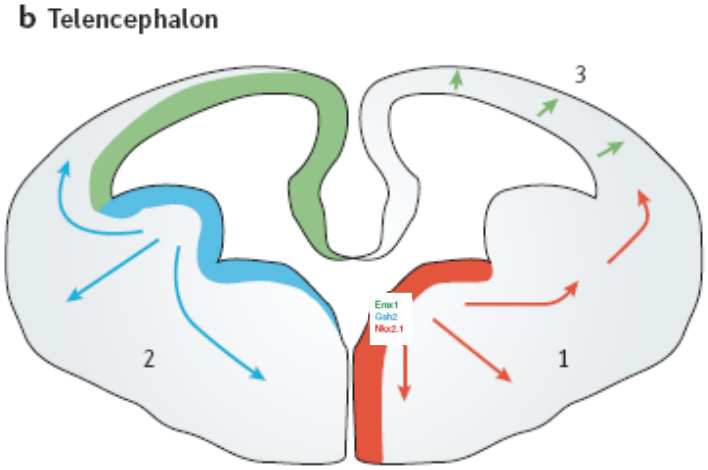


combine with immunohistochemistry to fate map specific cell types

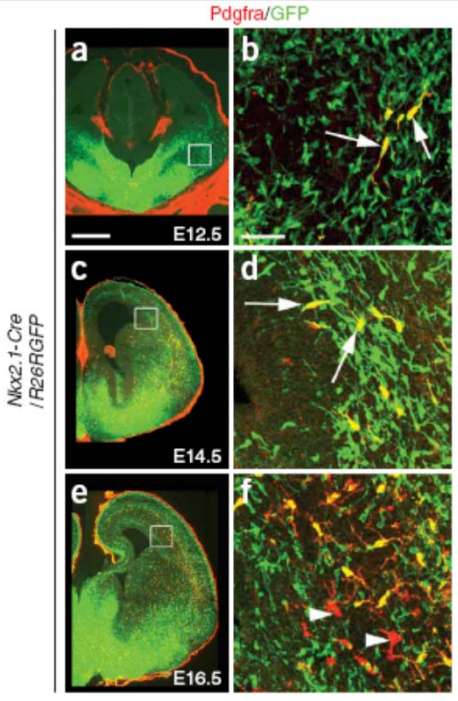


Kessaris et al 2006

Multiple OPCs origins in the forebrain

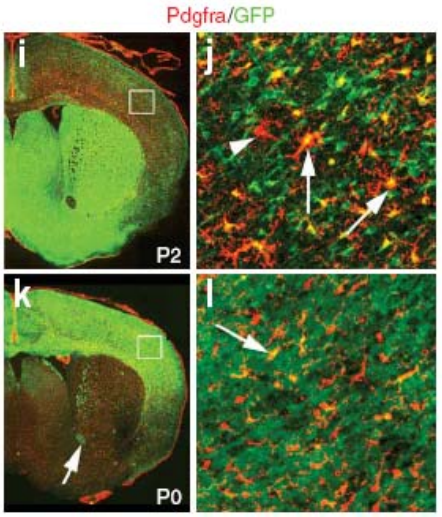


Kessaris et al 2006



Nkx2.1-Cre mice

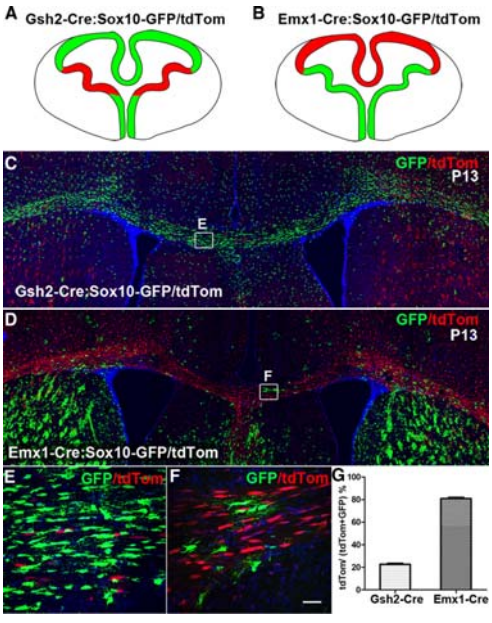
- ventral most precursors produced about E12.5 from medial ganglionic eminence
- populate the entire cortex by E18
- joined by second wave at E16-18 derived from lateral ganglionic eminence
- after E18 ventral contribution starts to decrease

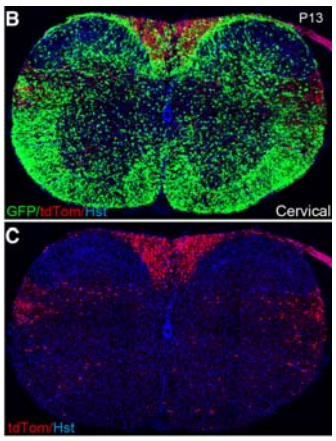


- after E18 another wave of OPCs originates in the cortex itself
- cells derived during wave 1 disappear after birth and are gradually eliminated from all other parts of the brain
- all oligodendrocyte populations appear to function in the same way
- ablation of one population leads to replacement by another with no functional consequences

There are both ventral and dorsal sources of OPCs that arise at different times during development and compete with one another

Dual reporter mouse line generated to colour code ventrally and dorsally derived OPCs





- dorsal and ventral OLPs and OIs
- different migration patterns
- different tract specificities for myelination

Tripathi et al, 2011

OPC migration is guided by regulatory signals

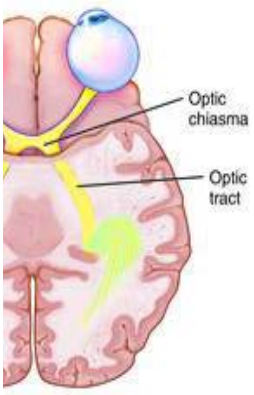
Secreted molecules

1. Growth factors (PDGF, FGFs)
2. Chemotropic molecules (netrins, semaphorins)
3. Chemokines (CXCL1)

Modes of action unclear – concentration gradients?

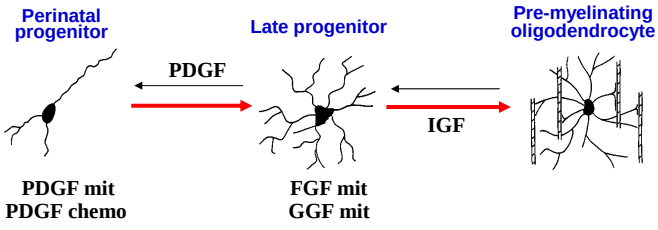
Contact dependent mechanisms

1. Extracellular matrix (fibronectin)
2. Axon surface molecules (NCAM, integrins)
3. Blood vessels



Proliferation

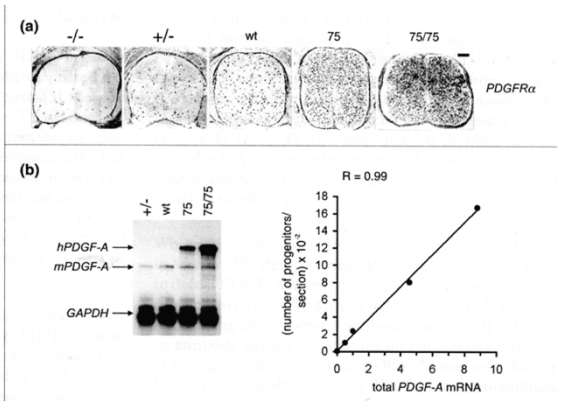
Growth factor effects on oligodendrocyte development



Platelet derived growth factor	- promotes migration, survival & proliferation
Fibroblast growth factor 2	- promotes migration & proliferation - inhibits differentiation
Neuregulin β1 (GGF2)	- promotes proliferation, target dep survival & myelin formation

The role of PDGF in oligodendrocyte development

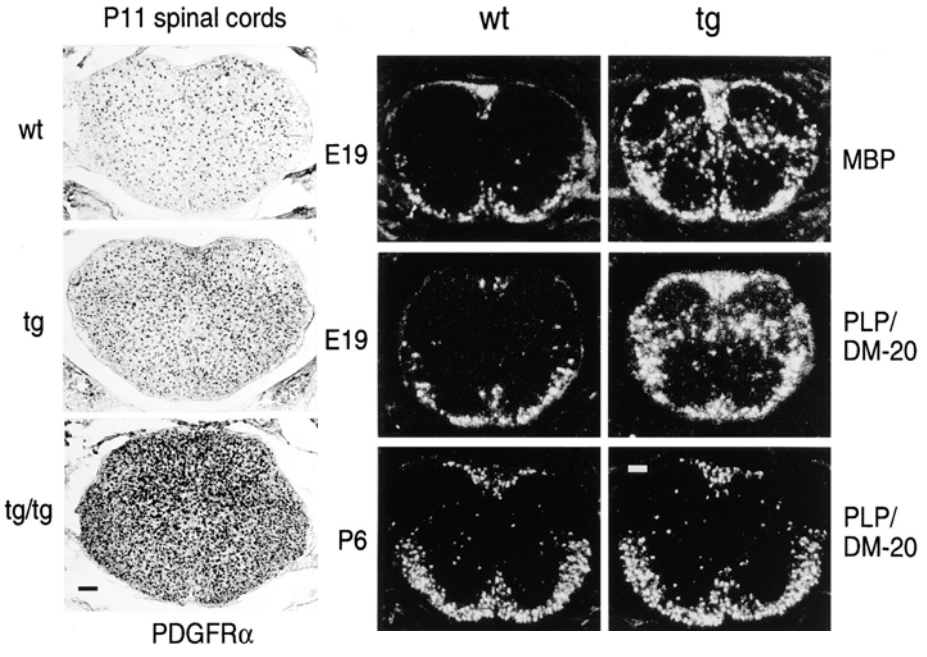
- platelet derived growth factor is a potent mitogen for oligodendrocyte precursors
- there is a clear dose dependent relationship between PDGF and oligodendrocyte progenitor number in the developing spinal cord



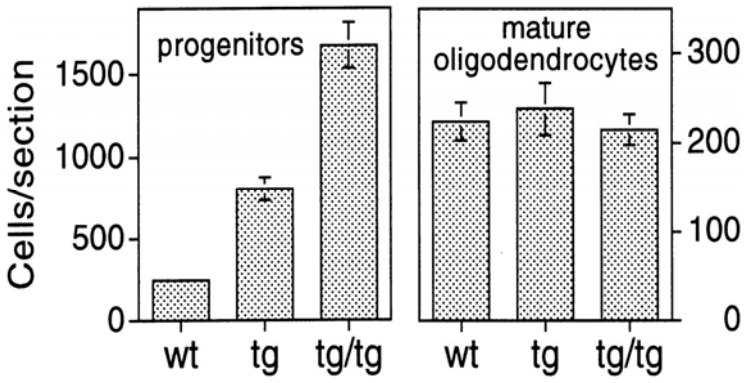
E15 mouse

Calver et al 1998

The role of PDGF in oligodendrocyte development

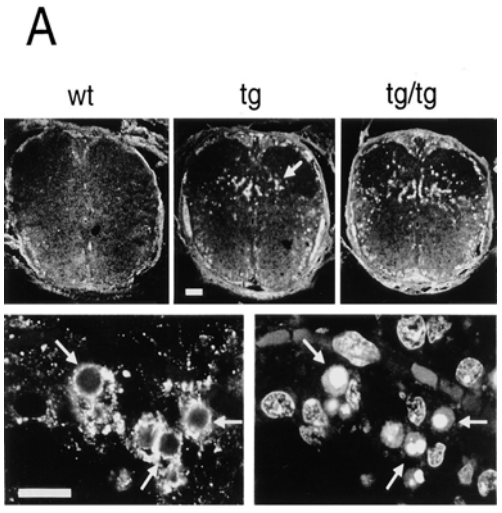


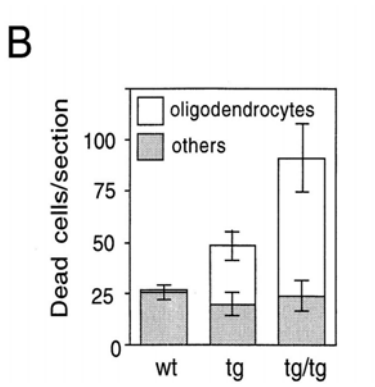
The role of PDGF in oligodendrocyte development



Final oligodendrocyte number is determined by factors other than PDGF. Axonal contact is the most likely factor determining survival of oligodendrocytes.

The role of PDGF in oligodendrocyte development





Oligodendrocytes excess to requirements are removed by apoptosis.

Proliferative arrest and differentiation

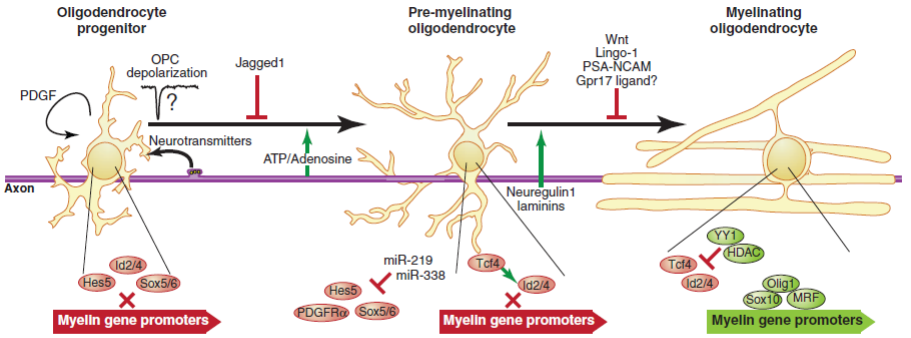


Fig. 3. Schematic of the oligodendrocyte lineage showing some of the intrinsic and extrinsic factors that influence oligodendrocyte differentiation and the myelination of individual axons. Oligodendrocyte differentiation requires the integration of multiple extracellular signals through coordination of multiple intrinsic pathways. Myelination is regulated both at the level of oligodendrocyte differentiation and more subtly at the level of individual axons.

Differentiation of OPCs into oligodendrocytes requires proliferative arrest and activation of transcriptional programme

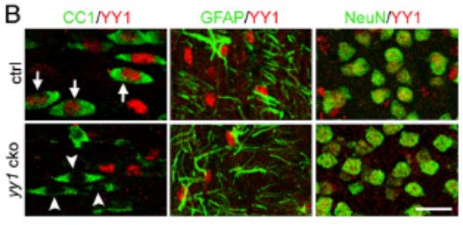
De-repression model of oligodendrocyte differentiation

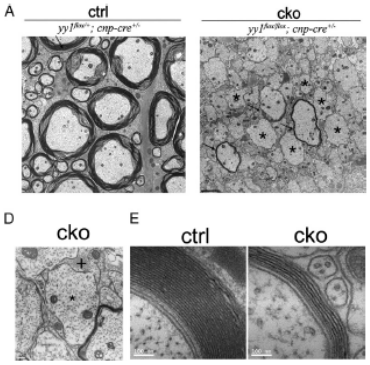
- cell cycle exit not sufficient to induce differentiation
- bioavailability of transcriptional activators & decrease in inhibitors

Activators – Sox10, Olig1/2, HdAc, Mash1
Inhibitors – Hes5, Id4, Tcf4

- transcription factor Yin Yang 1 (YY1) is critical regulator of OPC differentiation
- represses transcriptional inhibitors of myelin genes

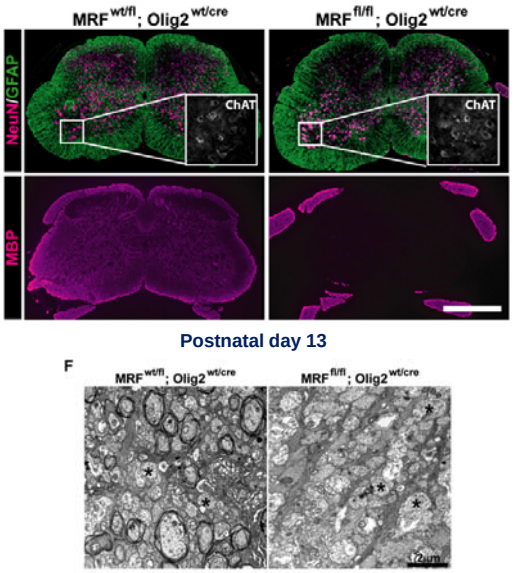
- conditional ablation in oligodendrocytes leads to defective myelination





He et al, 2007

Terminal differentiation



Postnatal day 13

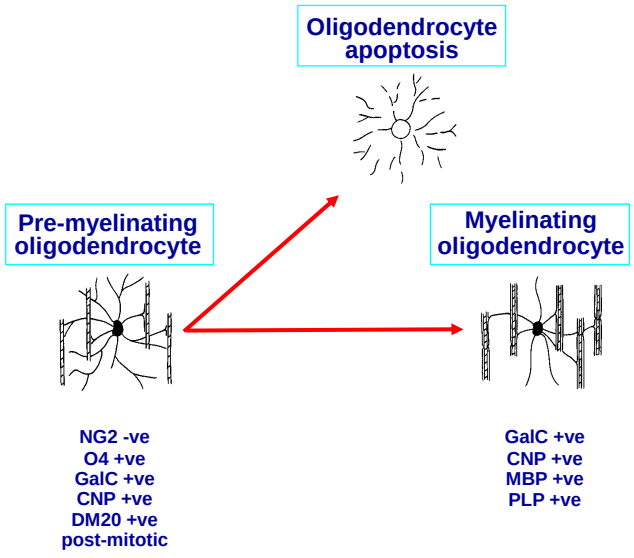
Myelin gene regulatory factor

- nuclear protein
- specifically expressed by post-mitotic oligodendrocytes
- CNS specific
- MRF knockdown in culture led to failure of induction of myelin genes
- KO mice for MRF had severe loss of expression of myelin proteins and no myelin
- severe tremors and ataxia and death at 3 weeks

- master transcriptional regulator with similar role to Krox 20 in PNS

Emery et al, 2009

Axonal contact & survival



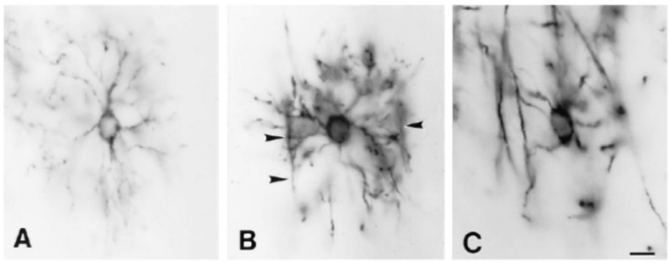
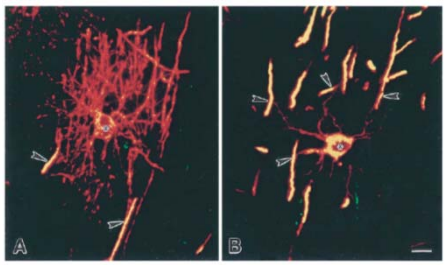


Figure 4. Premyelinating oligodendrocytes differentiate into myelinating oligodendrocytes. DM-20/PLP–positive oligodendrocytes before myelination (*A*), during early stages of axonal ensheathment (*B, arrowheads*), and during active myelination (*C*). Bar, 10 μm.

- developing oligodendrocytes extend numerous radial processes to contact axons.



- as axonal contact is made with an increasing number of axons the oligodendrocyte retracts those processes not required. *Trapp et al, 1997*

- in developing optic nerve, spinal cord & cerebral cortex, a large proportion of newly formed oligodendrocytes die by apoptosis
- only those that successfully contact and ensheath axons survive
- suggests that contact with the axon promotes survival

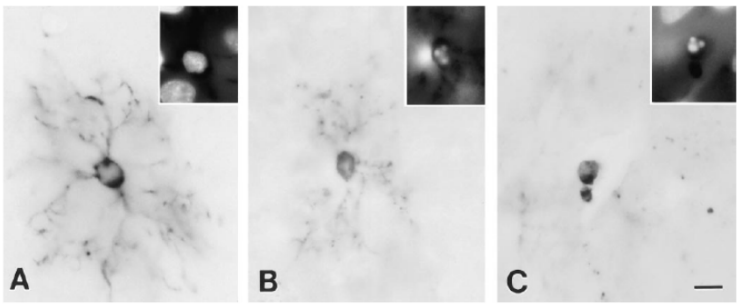
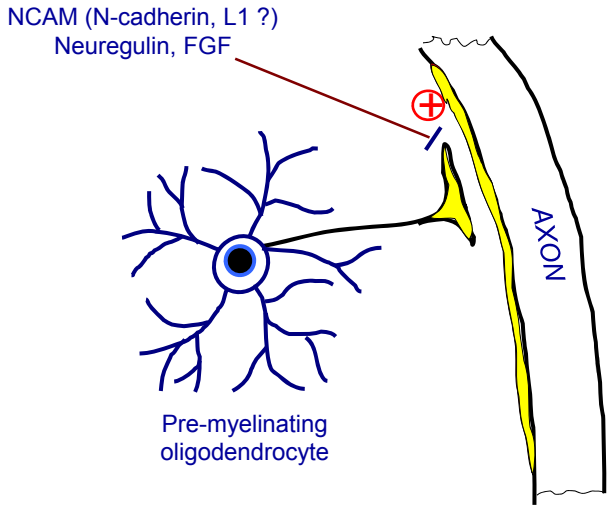
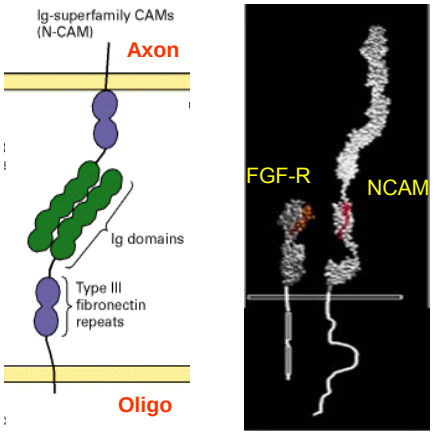


Figure 6. Comparison of DM-20/PLP immunoreactivity and nuclear chromatin staining in developing rat brain. Premyelinating oligodendrocytes with DM-20/PLP immunoreactivity evenly distributed on their surface (*A*) have diffuse nuclear chromatin staining (*A, inset*). Premyelinating oligodendrocytes with fragmented DM-20/PLP staining (*B*) and DM-20/PLP–positive necrotic-appearing cells (*C*) have condensed and fragmented nuclear chromatin (*B* and *C, inset*). *A*, *B*, and *C* were photographed with bright field optics; insets were photographed with ultraviolet optics. Bar, 10 μm.



Cell adhesion molecule interaction



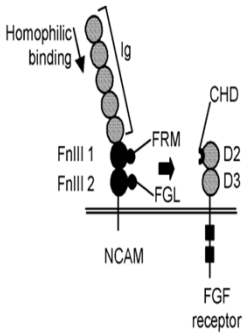
→ **SURVIVAL**

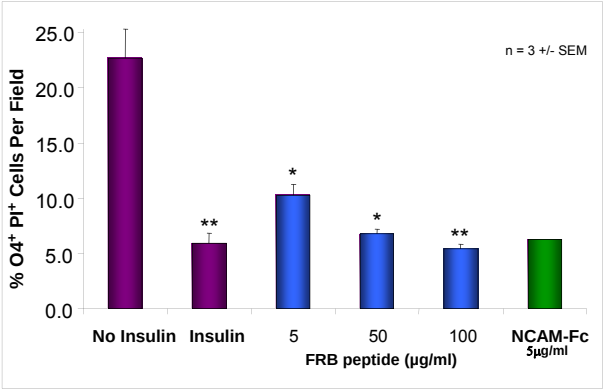
→ **DIFFERENTIATION**

→ **MYELINATION**

- Promote neurite outgrowth and neuronal survival (NCAM, N-cadherin, L1, neurofascin-186)
- NCAM, N-cadherin & neurofascin expressed by axons & oligodendrocytes
- Involved in adhesion of oligodendrocyte to axon & myelin formation
- May provide important axon-derived survival signals

NCAM stimulates oligodendrocyte survival



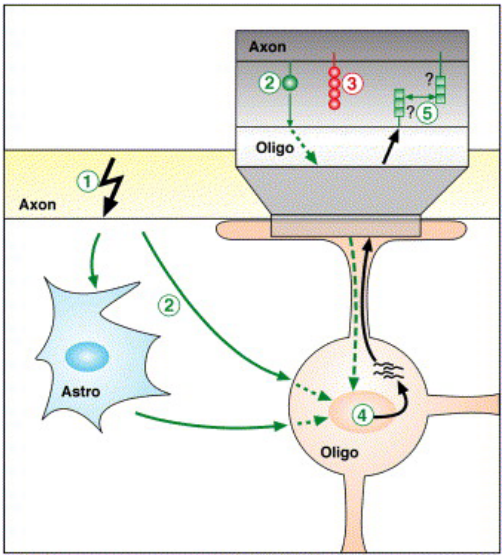


- 10 a. a. peptide corresponding to region of NCAM molecule first fibronectin type III domain DRVEPYSSTA – 4 copies attached to lysine backbone
- Added to medium on day of isolation
- Survival assayed by PI uptake

Palser et al, 2010

Initiation of myelination

The simplest mechanism for determining whether and when an axon becomes myelinated would be the expression of cues on the axon surface



1) Target innervation and electrical activity in axon cause the release of ATP.

2) ATP stimulates astrocytes to produce and secrete LIF (leukaemia inhibitory factor)

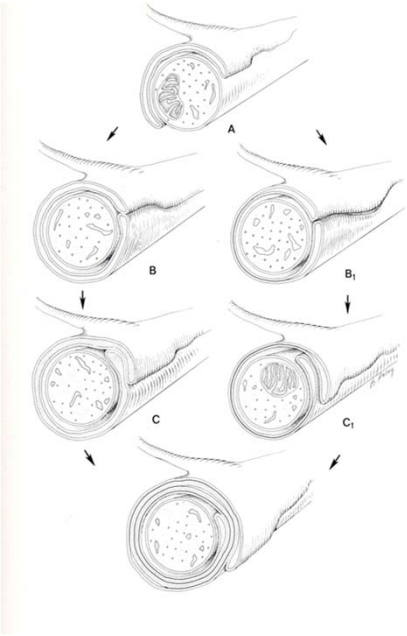
3) Axons can directly stimulate oligodendrocytes through cell adhesion molecules (NCAM, L1)

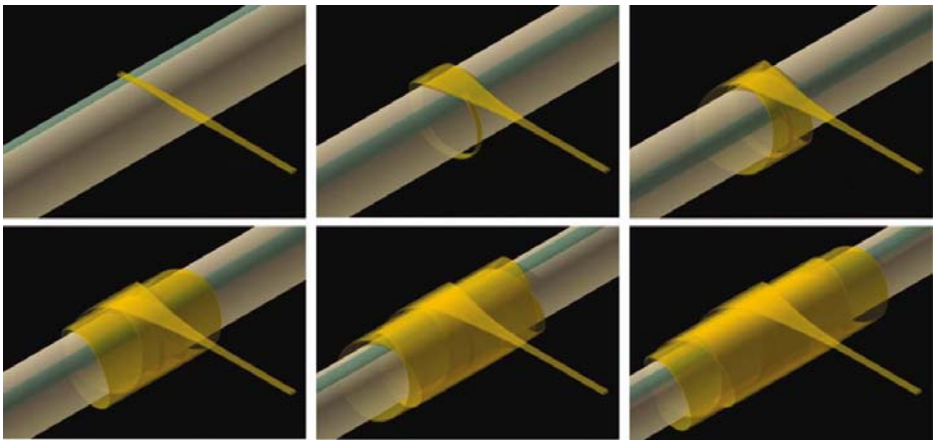
4) Inhibitory molecules are Downregulated (Notch, PSA-NCAM, Lingo-1)

5) Multiple axo-glial signals result in ensheathment

Myelin formation

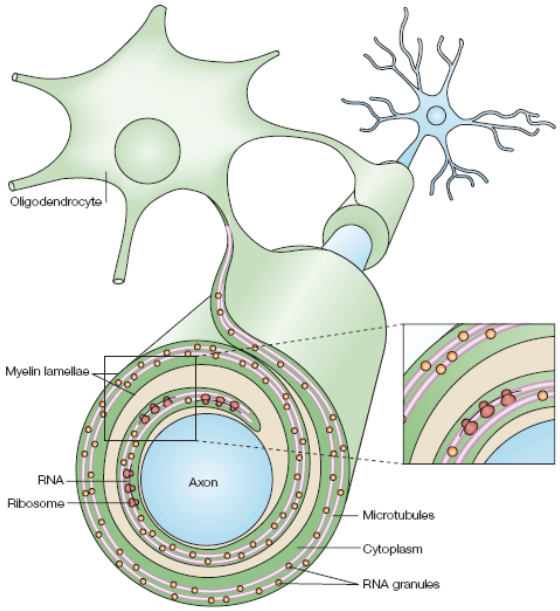
- oligodendrocyte process contacts axon
- leading process tucks under and extends around the axon in multiple wraps
- myelin compaction
- cytoplasm filled areas are inner and outer loops





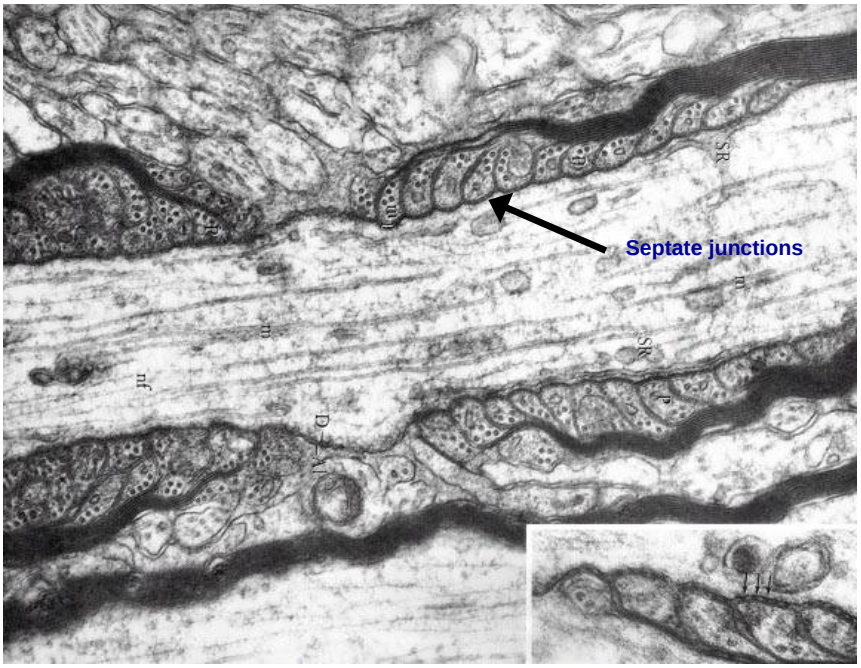
Schematic presentation of the "liquid croissant" model of myelination. We propose that myelin formation occurs by "pouring out" myelin (yellow) into a triangular shaped OLG process that attaches at possible adhesion sites (cyan) to the axon (grey). While this pouring process continues, myelin spreads sideward potentially being guided by axonal membrane proteins that move around the axonal cytoskeleton in a coordinated fashion. Myelin thickening is thus achieved by new layers forming on top of the inner one resulting in a bidirectional coiled turn of myelin layers along the axon reminding of the bidirectional dough edges of a croissant.

Sobottka et al, Glia 2011

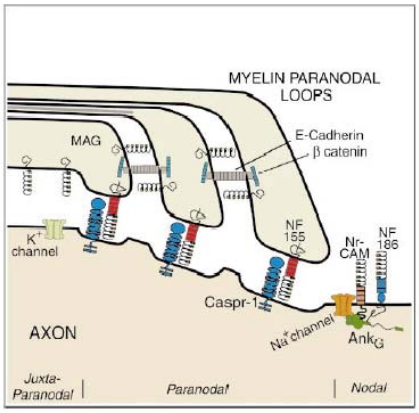


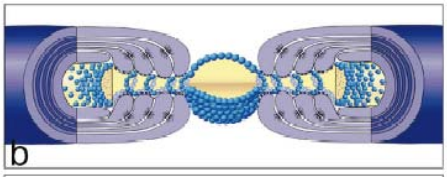
- the oligodendrocyte process has a distinct protein & lipid composition.
- assembly of new myelin membrane occurs distally to the cell body
- ribosomes and RNA granules are transported to the tips of the processes

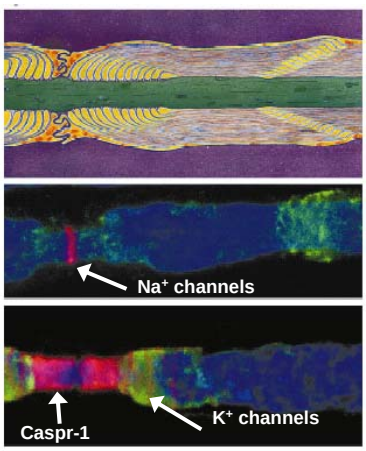
Sherman & Brophy, 2005

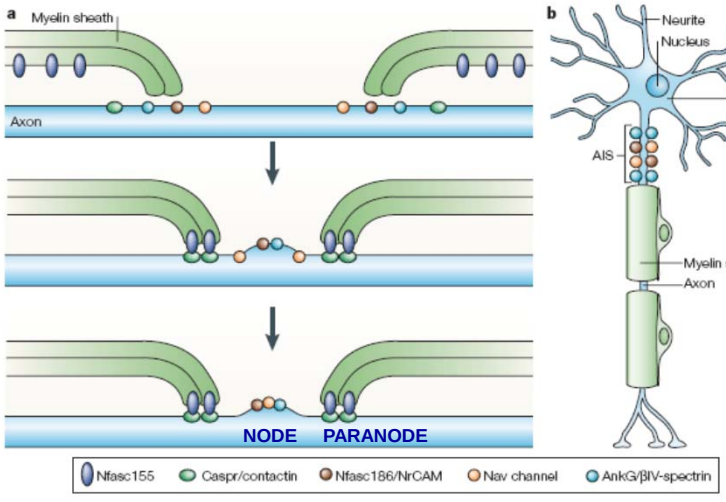


The Node of Ranvier







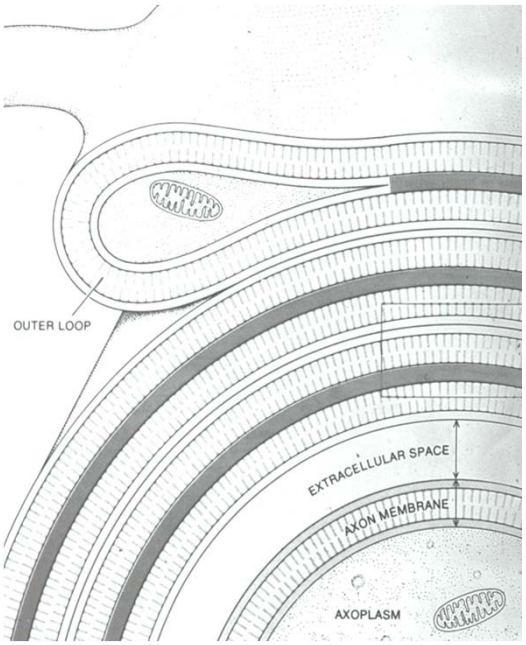


- myelination causes clustering of Na channels at node of Ranvier
- distribution of paranodal and nodal components diffuse before establishment of junctions
- specific junctions formed either side of the node

Myelin structure

- dark lines consist of the compacted intra-cellular faces of the membrane (very protein rich)

- lighter lines consist of the compacted extracellular faces of the membrane



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