

How inflammation promotes regeneration

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Macrophages near neuronal cell bodies can promote regeneration in an otherwise inhibitory environment. Now Yin *et al.* identify oncomodulin as a factor secreted from macrophages that promotes extensive regeneration of lesioned optic nerve axons when applied together with elevated cyclic AMP (cAMP) and mannose.

The inflammation that accompanies injury and disease in the CNS is a “double-edged sword” (ref. 1). It can lead to further damage on the one hand, but on the other hand, immune-mediated inflammation can also support neuroprotection and repair. For example, one or more unknown factors secreted by macrophages promote axonal regeneration of both the optic nerve and spinal sensory axons^{2,3}. Knowing the identity of these factors would not only advance our understanding at the molecular level of how axonal regeneration is promoted, but it would also bring us significantly closer to devising therapeutic approaches to encourage the growth of axons after, say, spinal cord injury. In this issue, Yin *et al.*⁴ identify the first macrophage-derived regeneration-promoting factor, the known calcium-binding protein oncomodulin. Oncomodulin is secreted by macrophages and, in combination with cAMP and the small sugar mannose, effectively promotes regeneration of the optic nerve.

The idea that inflammation and, more specifically, factors secreted from macrophages might enhance axonal regeneration came more than a decade ago². If the peripheral branch of axons from dorsal root ganglion (DRG) neurons is lesioned before injury to the central branch of the same axons, the dorsal spinal axons regenerate^{5,6}. This peripheral conditioning lesion effect can be mimicked either by direct injection of macrophages into the DRG or by induction of inflammation near the neuronal somas². Injury to the lens when the optic nerve is crushed promotes optic nerve regeneration⁷. Again, the effect can be

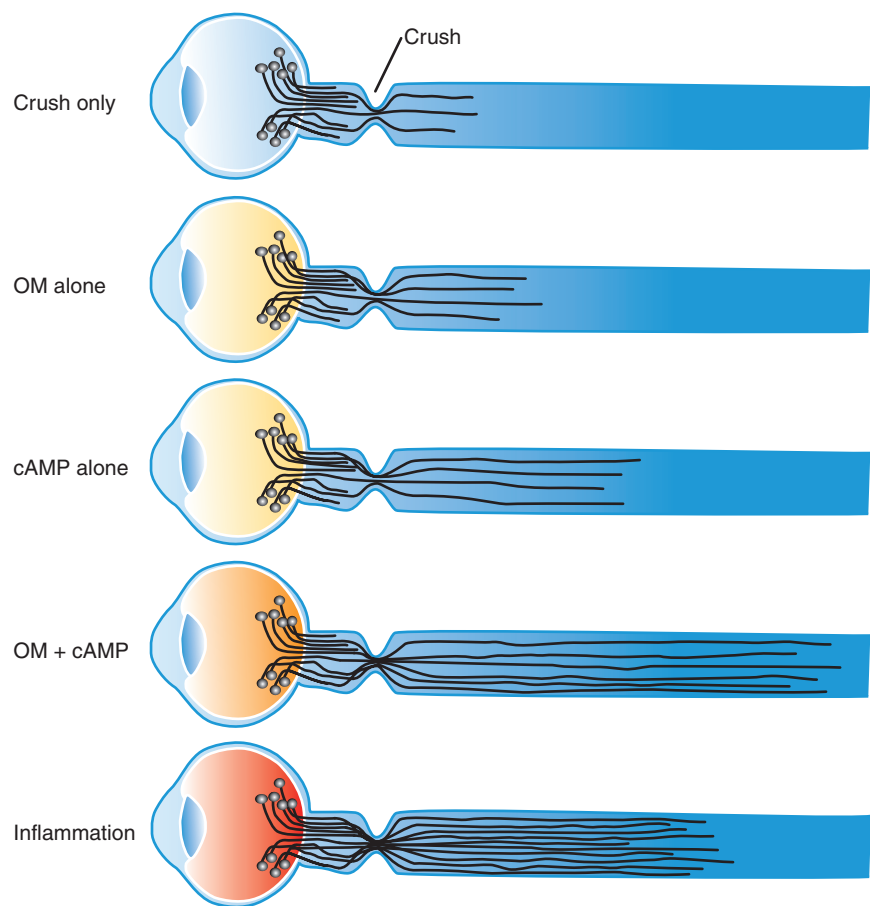


Figure 1 Schematic representation of the number of axons and the distance they regenerated after the various treatments. Mannose is endogenous to the vitreous, and therefore present under all conditions. Oncomodulin (OM) plus cAMP elevation resulted in the longest axons, but inflammation triggered regenerative growth in more axons.

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mimicked by inducing intraocular inflammation without lens injury. Curiously, regeneration is more robust if inflammation is induced three days after the optic nerve crush. In culture, media preconditioned with activated

macrophages promotes retinal ganglion neuron regeneration, demonstrating that direct participation by macrophages is not necessary for the effect. In light of these findings, the race was on to find the soluble factor(s) secreted

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Table 1 Comparison of the distance regenerated and the number of axons regenerated under different conditions

Condition	Number of axons >500 μm	Number of axons >1,000 μm	Longest axon
—	~100	~80	~2 mm
OM	~180	~150	~2.5 mm
cAMP	~200	~200	~4 cm
OM + cAMP	~600	~600	~6.5 cm
Inflammation (zymosan)	~1,100	~1,100	~4.7 cm

The data after induction of inflammation with zymosan are taken from ref. 3. All other data are from the current paper⁴. OM, oncomodulin.

from activated macrophages responsible for the dramatic regeneration of optic neurons.

Yin *et al.* first found that the active component of macrophage-conditioned media migrated with an abundant protein band at 10–15 kDa, and then used mass spectroscopy to unveil oncomodulin as the active component. Notably, oncomodulin alone did not enhance neurite outgrowth of retinal ganglion cells (RGCs) in culture. The same group had noted earlier that a component of the vitreous body promoted process outgrowth in culture when intracellular cAMP levels were elevated⁸. That component turned out to be the simple sugar mannose, and when both mannose and forskolin (to elevate cAMP) were now added together with oncomodulin, process outgrowth *in vitro* increased by almost twofold. Increased intracellular cAMP was necessary for oncomodulin to efficiently bind to RGCs. Comparable growth was seen when macrophage-conditioned media was supplemented with mannose and forskolin. Either mannose or cAMP elevation, combined with oncomodulin, resulted in a substantially less striking effect than the three components together. When macrophage-conditioned media was depleted of oncomodulin, its growth effect on neurites was almost completely lost even in the presence of mannose and elevated cAMP, whereas macrophage-conditioned media alone retained a modest but significant effect on neurite outgrowth. Taken together, these observations suggest that oncomodulin is not the only factor in the conditioned media that can affect neurite outgrowth. If it were, the absence of mannose and cAMP from the conditioned media should abolish its effect. Instead, the authors suggest, this apparent anomaly indicates that other putative growth-promoting factors secreted by macrophages require oncomodulin to be active.

The results from *in vivo* regeneration experiments generally supported the results from *in vitro* studies (Fig. 1 and Table 1). The authors crushed the optic nerve and then injected the various combinations of these key agents, oncomodulin and/or cAMP agonists. Mannose

is endogenous to the vitreous and is therefore present under all conditions. The combination of oncomodulin and cAMP resulted in the longest growth and the largest number of regenerating axons. Following the observation that inflammation was more effective three days after injury than if induced concurrently with the lesion, the authors chose to deliver the individual agents three days after the optic nerve was crushed. One possible explanation for the effect of the three-day delay is that the retinal ganglion neurons are in some way conditioned by the optic nerve lesion to become more responsive to macrophage-secreted factors. The cAMP agonist alone caused some RGC axons to regenerate a considerable distance *in vivo*, though the number of regenerating axons was low. What makes this observation important is that cAMP upregulates a variety of genes that seem to encourage regeneration through an inhibitory environment^{9,10}. Whether oncomodulin is one of these genes remains to be explored.

Previously, the same group reported that macrophage activation in the vitreous, without extra cAMP activation, led to the regeneration of a large number of axons for quite a distance (ref. 3 and Table 1). This observation implies the existence of macrophage-secreted factors, other than oncomodulin, that do not require cAMP to be elevated. At this point, the plot thickens: are these the same factors that require oncomodulin to be active? However, oncomodulin requires elevated cAMP to be effective, as is convincingly demonstrated by the current work: oncomodulin will bind to retinal ganglion neurons only when cAMP is elevated. The clear implication is that cAMP either upregulates the putative oncomodulin receptor or mediates its translocation to the cell surface. The latter is a strong possibility as elevated cAMP induces the translocation of another receptor, the neurotrophin TrkB receptor, to the surface of RGCs (ref. 11). Another mystery that remains to be solved is the role of mannose in the combined effect of cAMP and oncomodulin or macrophage-conditioned media. Whatever it may be, it seems to be independent of energy metabolism or glycoprotein synthesis.

Although the interplay between oncomodulin, cAMP and mannose is currently murky, the combination of these factors clearly has an impressive effect on optic nerve regeneration *in vivo*. The results of this study are difficult to compare directly with other reports on optic nerve regeneration because most preceding studies from other groups used different lesion models, different species and different ways to analyze and present the data. Nevertheless, the treatment combination devised here may well be the best one so far. Three important additional outcomes of the work need mentioning. First, oncomodulin combined with cAMP and mannose improved growth not only in a permissive environment, but also in the inhibitory environment of the damaged CNS. Second, oncomodulin plus the cAMP agonist were effective only when given at the neuronal cell body. This suggests a promising direction for therapeutic research addressing brain or spinal cord injury, as neuronal cell bodies are likely to be more accessible than the injury site itself. Third, the finding that this combination treatment was most effective when delivered three days after injury is serendipitous. Several previously proposed treatments to promote regeneration must be administered at the same time as the injury itself, or even beforehand—a virtually impossible constraint when it comes to treating human injuries.

This study presents us with several important new observations, but a number of outstanding issues inevitably remain, in particular the elucidation of the mechanisms of action. What is the identity of the oncomodulin receptor on RGCs? Most importantly, it remains to be seen whether this trio of agents is effective outside the optic nerve, for example, in the spinal cord. An encouraging step comes from the authors' demonstration that DRG neurons from animals that received oncomodulin *in vivo* grew better on an inhibitory substrate in culture, even without cAMP, than DRG neurons from untreated animals. The study raises the question of whether oncomodulin is involved in the peripheral conditioning-lesion effect on dorsal column regeneration, an effect that can be mimicked by elevation of cAMP (refs. 12,13). Finally, transplantation of activated macrophages into injured spinal cord does result in some degree of functional recovery¹⁴. The authors attribute this effect to unidentified trophic factors secreted by the transplanted macrophages. Although the transplant was at the site of the lesion and not the cell body, one of the relevant factors might still be oncomodulin.

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Chalk one up for ‘nature’ during neocortical neurogenesis

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During development, neurons destined for different neocortical layers are sequentially generated. Shen *et al.* report that this timing is programmed within individual progenitor cells and depends mainly on cell-intrinsic mechanisms.

What do neural stem cells ‘know’ and when do they know it? In the embryonic neocortex, this is a challenging question because different neuronal and glial cell types are generated in a specific temporal pattern from a common stem cell/progenitor pool. This process creates the mature neocortex, six layers of neurons with distinct morphological and functional identities. Cajal-Retzius cells are generated early and are essential for the migration of neurons generated later. Then deep layer neurons are generated, followed by neurons that migrate to increasingly superficial layers, in a so-called ‘inside-out’ pattern. Finally, glia are generated. The regulation of this process is poorly understood, but requires a cell-intrinsic program (‘nature’), gradual changes in cell-extrinsic cues (‘nurture’) or some combination. In this issue, Shen and colleagues¹ take advantage of a powerful *in vitro* system to place cell-intrinsic cues front and center.

The presence of multipotent progenitors in the neocortical germinal zones has been demonstrated by retroviral labeling *in vivo* and clonal analysis *in vitro*². However, cell transplantation indicates that, as development proceeds, the potential of neocortical progenitors gradually becomes restricted. For example, although early progenitors can contribute to upper layer (later) fates when transplanted to older embryos³, late progenitors cannot contribute to deep layer (earlier) fates when transplanted to early embryos⁴.

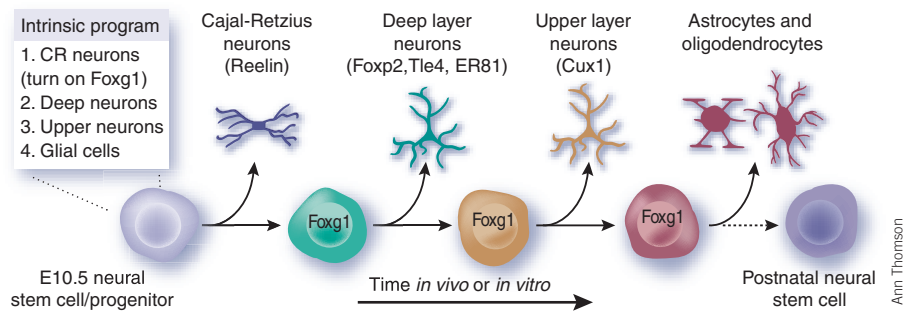


Figure 1 As early as E10.5, neocortical progenitors contain an intrinsic program that specifies the order in which different neuronal and glial cell types will be generated. As these cells mature and the program is executed, their developmental potential becomes restricted to the remaining steps in the program. The transcription factor Foxg1 contributes to this restriction by blocking the production of Cajal-Retzius (CR) neurons and permitting the generation of neocortical neurons (and eventually glia) characteristic of later fates.

Along with other studies^{5–7}, these findings suggest that both cell-intrinsic and cell-extrinsic cues are involved in determining cell fate.

In distinguishing the roles of these signals during neocortical development, the work of Temple and colleagues is among the most informative. They combine adherent progenitor culturing and time-lapse video microscopy to construct family trees of the cells generated from individual progenitors^{8,9}. For lineage analysis, this method is far superior to high-density adherent progenitor cultures and to the clonal ‘neurosphere’ assay in which single progenitors proliferate into balls of cells in suspension. The advantage of the Temple approach is that it permits complete documentation of all cell division, cell migration and cell death, and when combined with subsequent

marker staining, permits the assignment of specific cell fates. Because these cultures are extremely low density, the behavior of each clone is presumed to be encoded by information contained within the initially plated progenitor. With so few cells in a large relative volume, initial cell-cell contact is highly unlikely, nor can secreted molecules reach concentrations sufficient to exert effects.

This research group reported previously that some progenitors produce large clones containing neurons and glia (putative stem cells), and others produce smaller clones containing only neurons (apparent neuroblasts)¹⁰. Furthermore, among the stem cell clones, neurons are generated early during clonal expansion, whereas glia are generated later, mimicking the normal order of cell production *in vivo*. These

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