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Turning On the Machine: Genetic Control of Axon Regeneration by c-Jun

Upregulation of the transcription factor c-Jun has been correlated with axon regeneration after injury in multiple types of neurons. In this issue of *Neuron*, Raivich et al. use a nervous system-specific mutant to provide genetic evidence that c-Jun is necessary for efficient axon regeneration.

Nerve transection provokes a series of complex changes in axotomized neurons, leading to the regeneration of their axons. One important molecular event is the rapid and sustained upregulation of inducible transcription factors such as c-Jun, a component of the AP-1 transcription factor complex. Axotomized peripheral neurons coexpress c-Jun and the growth-associated protein GAP-43 for several weeks during regeneration. In contrast, c-Jun expression is not induced in the CNS when axotomy fails to stimulate a functional regenerative response (see Herdegen et al., 1997, and Herdegen and Leah, 1998 for reviews). While these data support the hypothesis that c-Jun plays an instructive role in axon regeneration, a definitive in vivo functional experiment has been lacking. The study by Raivich et al. in this issue of *Neuron* now provides such an experiment and demonstrates a crucial role for c-Jun in axon regeneration in vivo.

c-jun null mice die at mid-to-late gestation via impaired hepatogenesis, altered fetal liver erythropoiesis, and generalized edema (see references in Raivich et al., 2004). Hence, the authors crossed *c-jun* floxed allele mice (*c-jun*$^{f/f}$) with Nestin-Cre mice to generate nervous system-specific *c-jun* null mice (*c-jun*$^{\Delta n}$). c-*jun*$^{\Delta n}$ mice survived to adulthood and showed no overtly abnormal brain or sciatic nerve morphology. Functionally, the open field behavioral test showed no locomotor- or amygdala-dependent fear-conditioning deficiencies. Hippocampal-dependent learning and memory were also unaltered, as evidenced by the normal performance of *c-jun*$^{\Delta n}$ mice in the Morris watermaze. These data demonstrate a surprising lack of dependence of nervous system development on c-Jun.

In order to study the role of c-Jun during regeneration in vivo, the authors used a facial nerve transection model and quantified behavioral recovery and muscle reinnervation at multiple time points. *c-jun*$^{\Delta n}$ mice had significantly diminished whisker hair motor neuron performance compared to *c-jun*$^{f/f}$ mice at both 4 and 15 weeks following facial nerve transection. Fluorogold retrograde labeling confirmed that markedly fewer motor neurons had reconnected to the whisker pad in *c-jun*$^{\Delta n}$ compared to *c-jun*$^{f/f}$ mice at these same time points. Taken together the results demonstrate that lack of c-Jun markedly impedes the efficiency of motor axon regeneration after nerve injury.

In significant contrast to the drastic effects of c-Jun deletion on axon regeneration, the absence of c-Jun had little effect on axon growth during development. These data, taken together with other studies (see Snider et al., 2002, for a review), support the idea that major differences exist between the genetic program that regulates axon regeneration of adult neurons and the genetic program that controls embryonic axon growth during development. Interestingly, neurotrophins, which are crucial regulators of embryonic axon growth, are unlikely to activate gene transcription via c-Jun during development. On the contrary, c-Jun is activated after NGF withdrawal and is thought to mediate apoptosis (Estus et al., 1994, and see below).

Regenerative axon growth presumably requires both activation of a growth program in the cell body via gene transcription and activation of local signaling cascades that regulate axon assembly (Snider et al., 2002). Nuclear translocation of c-Jun and subsequent gene transcription requires the kinase activity of the Jun N-terminal kinases (JNKs). JNKs are also activated by axotomy concurrent with c-Jun upregulation (Herdegen et al., 1997). Interestingly, in addition to activating c-Jun, JNK1 has been shown recently to phosphorylate several axonal cytoskeletal proteins. JNK1 phosphorylation of MAP-1B or MAP2 enhances their ability to bind to microtubules and thus regulate local microtubule assembly (Chang et al., 2003). In addition, doublecortin (DCX) was recently identified as a substrate of JNKs, and inhibition of JNK-mediated DCX phosphorylation interferes with axon growth (Gdalyahu et al., 2004). Finally, JNK1 regulates rapid cell migration via paxillin, an important local adaptor of focal adhesion and actin filaments (Huang et al., 2003). Taken together, these results raise the possibility that JNKs coordinate c-Jun-mediated gene expression with local axon assembly.

Previous studies, including recent expression profiling screens, have identified a large number of regeneration-associated genes and proteins. However, few of these have actually been demonstrated to be important in regeneration. Among regeneration-associated proteins, GAP-43 and integrins appear to have the most significant roles reported to date. Overexpression of GAP-43 together with a related protein, CAP-23, mimicked the axon growth-promoting effects of a conditioning lesion (Bomze et al., 2001), while targeted mutation of the α7 integrin gene significantly blocked peripheral nerve regeneration in vivo (Werner et al., 2000). Interestingly, both GAP-43 and α7 integrin are potential targets of c-Jun-mediated gene transcription (Herdegen and Leah, 1998; Raivich et al., 2004). Indeed, Raivich et al. showed that, in *c-jun*$^{\Delta n}$ mice, facial motor neurons completely failed to upregulate α7β1 integrin after nerve transection. α7 integrin clearly has the potential to par-

ticipate in local axon assembly. The fact that c-Jun regulates its expression reinforces the notion that c-Jun has an important role in "turning on" the regeneration program after injury.

c-Jun also has a role in regulating neuronal apoptosis. Initial in vitro studies found that c-Jun was activated after NGF withdrawal correlating with neuronal apoptosis. Furthermore, c-Jun overexpression induced neuronal death, whereas c-Jun suppression or dominant-negative c-Jun expression resulted in the blockade of neuronal death (see references in Herdegen et al., 1997). In line with these observations, Raivich et al. show decreased developmental cell death in the facial motor neuron pools of the *c-jun*$^{\Delta n}$ mice.

Interestingly, neuronal death is also sometimes induced by axotomy. Raivich and colleagues showed that c-Jun also plays a role in mediating axotomy-induced cell death. Neuronal loss was substantially reduced in *c-jun*$^{\Delta n}$ mice following nerve transection compared to the control mice. One tempting explanation for this seemingly counterintuitive effect is that the genetic program induced by c-Jun to promote axon growth overlaps with the genetic program mediated by c-Jun in nonneuronal cells that promotes cell proliferation (Eferl and Wagner, 2003). Attempts to reenter the cell cycle in postmitotic neurons usually lead to apoptosis (Becker and Bonni, 2004). The idea that the axon growth and cell cycle programs overlap is in line with recent findings that suppressing cell cycle repressors in neurons enhances axon growth (Konishi et al., 2004).

Although Raivich et al. provide compelling evidence for a vital role of c-Jun in the adult animal during regeneration, their study does not provide the definitive answer about the specific role of c-Jun in neurons. Previous work has shown that c-Jun is also upregulated in nonneuronal cells after axotomy (see references in Herdegen and Leah, 1998). Nestin-Cre-mediated recombination also mutates the *c-jun* gene in glial cells, which may well contribute to the regeneration phenotype seen in *c-jun*$^{\Delta n}$ mice. Furthermore, the data from Raivich et al. do not rule out the possibility that the lack of c-Jun during development may also contribute to the regeneration defect in the adult animals even though no apparent developmental abnormality is observed in the mutant mouse nervous system. Therefore, a definitive answer for the role of neuronal c-Jun in regeneration can only be obtained with a neuron-specific c-Jun knockout in adult animals. This can readily be achieved via crossing the *c-jun*$^{f/f}$ mice with a neuron-specific and inducible Cre line or by local introduction of a neuron-specific Cre-expressing vector.

Finally, in *c-jun*$^{\Delta n}$ mice, facial nerve regeneration still occurs to some degree, suggesting that multiple redundant pathways exist to promote regeneration. Indeed, another related transcription factor, ATF3, is also upregulated in response to axotomy with a similar expression pattern to that of c-Jun. Dimers of ATF3 and c-Jun or other transcription factors can recognize the cAMP response element (CRE) binding motif (see Hai and Hartman, 2001, for review). Coordination of CRE- and AP-1-regulated genes may be required to control robust axon regeneration. It would be interesting to see whether mutating both of these transcription factors causes an even more profound failure of axon regeneration than was observed in the current study.

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Myosin III Illuminates the Mechanism of Arrestin Translocation

Recent studies have revealed that light adaptation of both vertebrate and invertebrate photoreceptors is accompanied by massive translocations of major signaling proteins in and out of the cellular compartments where visual signal transduction takes place. In this issue of *Neuron*, Lee and Montell report a breakthrough in understanding the mechanism of arrestin translocation in *Drosophila*. They show that arrestin is carried into the light-sensitive microvilli by phosphoinositide-enriched vesicles driven by a myosin motor.

An exciting and active area in photoreceptor biology is the translocation of signaling proteins in and out of the cellular compartment where visual signal transduction takes place. This phenomenon, documented in a broad range of animal species from flies to mammals, is thought to be a major mechanism of light adaptation. Translocation allows the cell to rapidly adjust the protein composi-