physicochemical characteristics, it seems likely that freely circulating small oligomers may be better seeds; however, larger polymers may be more stable against biological clearance.

(4) What are the molecular bases for the selective cellular accumulation of NFTs? Even though spreading of tau pathology may provide a feasible explanation for the mechanism by which deposition of tau aggregates progresses in the brain of AD patients, this phenomenon does not explain why only some of the interconnected neurons develop NFTs. The reason behind the selective accumulation of different types of misfolded aggregates in distinct brain regions is a major unknown in the field. Possible explanations for this intriguing phenomenon could be the involvement of cellular receptors, the differential functioning of clearance mechanisms, or the distinct level of expression of the proteins involved in misfolding.

The finding that tau pathology spreads in the brain by a prion-like mechanism not only helps us understand the process involved in disease pathogenesis and provides a feasible explanation for the stereotypical progression of these lesions in AD brain but may also lead to the identification of new targets for therapeutic intervention. Indeed, preventing the initial formation of seeds or the subsequent spreading of tau aggregates may represent interesting strategies for a much-needed treatment for AD and related tauopathies.

REFERENCES

Aguzzi, A., and Rajendran, L. (2009). Neuron *64*, 783–790.

Braak, H., and Braak, E. (1991). Acta Neuropathol. (Berl) *82*, 239–259.

Brundin, P., Melki, R., and Kopito, R. (2010). Nat. Rev. Mol. Cell Biol. *11*, 301–307.

Chiti, F., and Dobson, C.M. (2006). Annu. Rev. Biochem. *75*, 333–366.

Clavaguera, F., Bolmont, T., Crowther, R.A., Abramowski, D., Frank, S., Probst, A., Fraser, G., Stalder, A.K., Beibel, M., Staufenbiel, M., et al. (2009). Nat. Cell Biol. *11*, 909–913.

de Calignon, A., Polydoro, M., Suárez-Calvet, M., William, C., Adamowicz, D.H., Kopeikina, K.J., Pitstick, R., Sahara, N., Ashe, K.H., Carlson, G.A., et al. (2012). Neuron *73*, this issue, 685–697.

Frost, B., Jacks, R.L., and Diamond, M.I. (2009). J. Biol. Chem. *284*, 12845–12852.

Guo, J.L., and Lee, V.M. (2011). J. Biol. Chem. *286*, 15317–15331.

Johan, K., Westermark, G., Engström, U., Gustavsson, A., Hultman, P., and Westermark, P. (1998). Proc. Natl. Acad. Sci. USA *95*, 2558–2563.

Morales, R., Green, K.M., and Soto, C. (2009). CNS Neurol. Disord. Drug Targets *8*, 363–371.

Moreno-Gonzalez, I., and Soto, C. (2011). Semin. Cell Dev. Biol. *22*, 482–487.

Nonaka, T., Watanabe, S.T., Iwatsubo, T., and Hasegawa, M. (2010). J. Biol. Chem. *285*, 34885–34898.

Santacruz, K., Lewis, J., Spires, T., Paulson, J., Kotilinek, L., Ingelsson, M., Guimaraes, A., DeTure, M., Ramsden, M., McGowan, E., et al. (2005). Science *309*, 476–481.

Soto, C. (2011). Trends Biochem. Sci. *36*, 151–158.

Will, R.G. (2003). Br. Med. Bull. *66*, 255–265.

Bers-ERK Schwann Cells Coordinate Nerve Regeneration

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In this issue of *Neuron*, Napoli et al. (2012) demonstrate that elevated ERK/MAPK signaling in Schwann cells is a crucial trigger for Schwann cell dedifferentiation in vivo. Moreover, the authors show that dedifferentiated Schwann cells have the potential to coordinate much of the peripheral nerve response to injury.

A remarkable feature of the peripheral nerve is the ability to regenerate after injury. Regeneration is associated with an extraordinary series of changes in Schwann cells (reviewed in Chen et al., 2007). After injury, Schwann cells dedifferentiate into a progenitor-like state, proliferate, and repopulate the damaged nerve. In the nerve segment distal to the site of injury, columns of dedifferentiated Schwann cells form the Bands of Bungner and provide an important substrate for regenerating axons. Once axons have regenerated, Schwann cells then redifferentiate and remyelinate. Numerous axonal-, Schwann cell-, and immune-derived mediators are thought to be required for the regenerative response. Given the complex morphological changes and the number of mediators potentially involved, it would seem unlikely that the Schwann cell's multifaceted response to injury could be regulated by a single pathway.

Indeed, within hours of nerve injury, increased activity in multiple pathways including ERK/MAPK, JNK/c-Jun, Notch, and JAK-STAT can be detected in Schwann cells (Sheu et al., 2000; Woodhoo et al., 2009). In vivo studies have

clearly shown that loss of Notch hinders Schwann cell dedifferentiation after injury, whereas virally mediated activation of Notch in intact nerves drives Schwann cell dedifferentiation (Woodhoo et al., 2009). Further, Schwann cell dedifferentiation is inhibited in c-Jun mutant mice, fitting with the overall role of JNK signaling in response to stress and its role in mediating Wallerian degeneration (Parkinson et al., 2008). A key effect of Notch and c-Jun is to inhibit the effects of promyelinating transcription factors, such as Egr2 (reviewed in Pereira et al., 2012).

Despite the importance of JNK/c-Jun and Notch, the elevation and extent of ERK/MAPK activation is apparently more pronounced than that of JNK after nerve transection (Sheu et al., 2000). Indeed after peripheral nerve injury, phosphorylated ERK/MAPK levels in the distal nerve increase >3-fold and are maintained at heightened levels in the Bands of Bungner for up to a month. In previous work, Lloyd and colleagues examined the role of ERK/MAPK activation in vitro by transfecting DRG neuron/Schwann cell cocultures with a tamoxifen (TMX)-responsive, constitutively active Raf construct (Raf-ER) (Harrisingh et al., 2004). TMX administration to these cultures resulted in increased ERK/MAPK phosphorylation, myelin breakdown, and Schwann cell dedifferentiation in vitro (Harrisingh et al., 2004). However, another group has shown that Schwann cell monocultures do not require ERK/MAPK for many aspects of dedifferentiation induced by the withdrawal of cAMP (Monje et al., 2010). An assessment of the importance of ERK/MAPK for Schwann cell dedifferentiation in vivo is clearly important and might resolve the disparate conclusions arising from in vitro analyses.

In this issue of *Neuron*, Napoli et al. (2012) have elegantly tested the function of Raf/MEK/ERK signaling in Schwann cell dedifferentiation in vivo. The authors generated a novel transgenic mouse model that allows for Schwann cell-specific, reversible activation of ERK/MAPK by placing Raf-ER under the control of a modified myelinating Schwann cell-specific promoter, P0 (P0-Raf-ER mice). Injection of TMX into P0-Raf-ER mice induced a robust increase in phosphorylated-ERK/MAPK levels in Schwann cells within 24 hr, comparable to that seen in the distal segment after nerve injury. With a protocol of five consecutive daily injections of TMX, increased ERK/MAPK activity was maintained for a total period of 2 weeks.

Strikingly, 3 days of elevated ERK/MAPK activation clearly stimulated Schwann cell dedifferentiation in vivo (Figure 1). The authors show a rapid decrease in the expression of myelin genes, *P0*, *MBP*, and *periaxin*, and an increase in the expression of Schwann cell progenitor genes, *Krox24*, *p75*, and *cyclinD1*. Further, a significant increase in the number of proliferating p75-expressing Schwann cell progenitors in the nerve was observed. By day 10, overt demyelination in the nerve and motor/proprioceptive deficits on behavioral testing were apparent. Importantly, obvious axonal damage was not observed at any time point analyzed. Thus, activation of a single pathway, RAF/MEK/ERK, is sufficient for the induction of Schwann cell dedifferentiation in vivo, even in a nerve that lacks damaged axons. The result is all the more remarkable in that there was no requirement for direct activation of the JNK and Notch pathways previously implicated as required for the dedifferentiation response.

Importantly, remyelination and motor recovery became apparent in P0-Raf-ER mice a few weeks after ERK/MAPK activity returned to basal levels. A prolonged regimen of TMX injections led to a corresponding delay in motor recovery. These data show that the dedifferentiated state can be maintained as long as ERK/MAPK levels remain high. Further, remyelination may depend upon a subsequent decrease in ERK/MAPK activity.

The authors then asked whether ERK/MAPK signaling was required for the Schwann cell dedifferentiation that normally occurs in injured sciatic nerves. Administration of a pharmacological MEK1/2 inhibitor, PD0325901, immediately before nerve injury strongly inhibited the proliferation changes associated with Schwann cell dedifferentiation. The gene expression changes associated with dedifferentiation were inhibited by PD0325901, but only partially. Due to the side effects of the pharmacological approach, the period of analysis was restricted to 2–3 days after injury, and the dose of inhibitor did not completely block ERK/MAPK activation. This result is consistent with the group's previous in vitro report (Harrisingh et al., 2004), fits with predictions from the P0-Raf-ER model, and supports the view that injury-induced ERK/MAPK signaling is required for Schwann cell dedifferentiation in vivo. However, given the issues with the pharmacological experiments, testing the requirement for ERK/MAPK in Schwann cell dedifferentiation using a conditional knockout approach should be an important future goal.

The P0-Raf-ER model provided a unique opportunity for the authors to test whether dedifferentiated Schwann cells are sufficient to activate other cellular responses to nerve injury. The recruitment of immune cells is particularly important for clearing axon and myelin debris and promoting subsequent revascularization in injured nerves (reviewed in Benowitz and Popovich, 2011). However, it is not clear whether debris, axons, or dedifferentiated Schwann cells provide the cues to initiate the inflammatory response. Napoli et al. therefore examined the inflammatory reaction in the sciatic nerve of P0-Raf-ER mice. Remarkably, a clear infiltration of T cells, macrophages, neutrophils, and mast cells was observed within 3 to 5 days of TMX injection (Figure 1). Moreover, in injured nerves, PD0325901 administration blocked the recruitment of immune cells. Fibroblasts did not appear to undergo any of the changes typically associated with nerve injury. The fibroblast response may require overt tissue damage and presumably depends upon cues that are not Schwann cell derived. Conditioned media from Raf-ER-expressing Schwann cells was also able to recruit immune cells, but not fibroblasts, in vitro. These data demonstrate that dedifferentiated Schwann cells are capable of initiating a complete immune reaction in a normal peripheral nerve.

What are the Schwann cell-derived inflammatory molecules that are increased following dedifferentiation? To identify candidates, a previously reported microarray analysis of cultured Raf-ER-expressing Schwann cells was reanalyzed (Parrinello et al., 2008). A number of relevant secreted cues were regulated, including *c-kit*, *MCP-1*, *IL11*, *Cxcl10*,

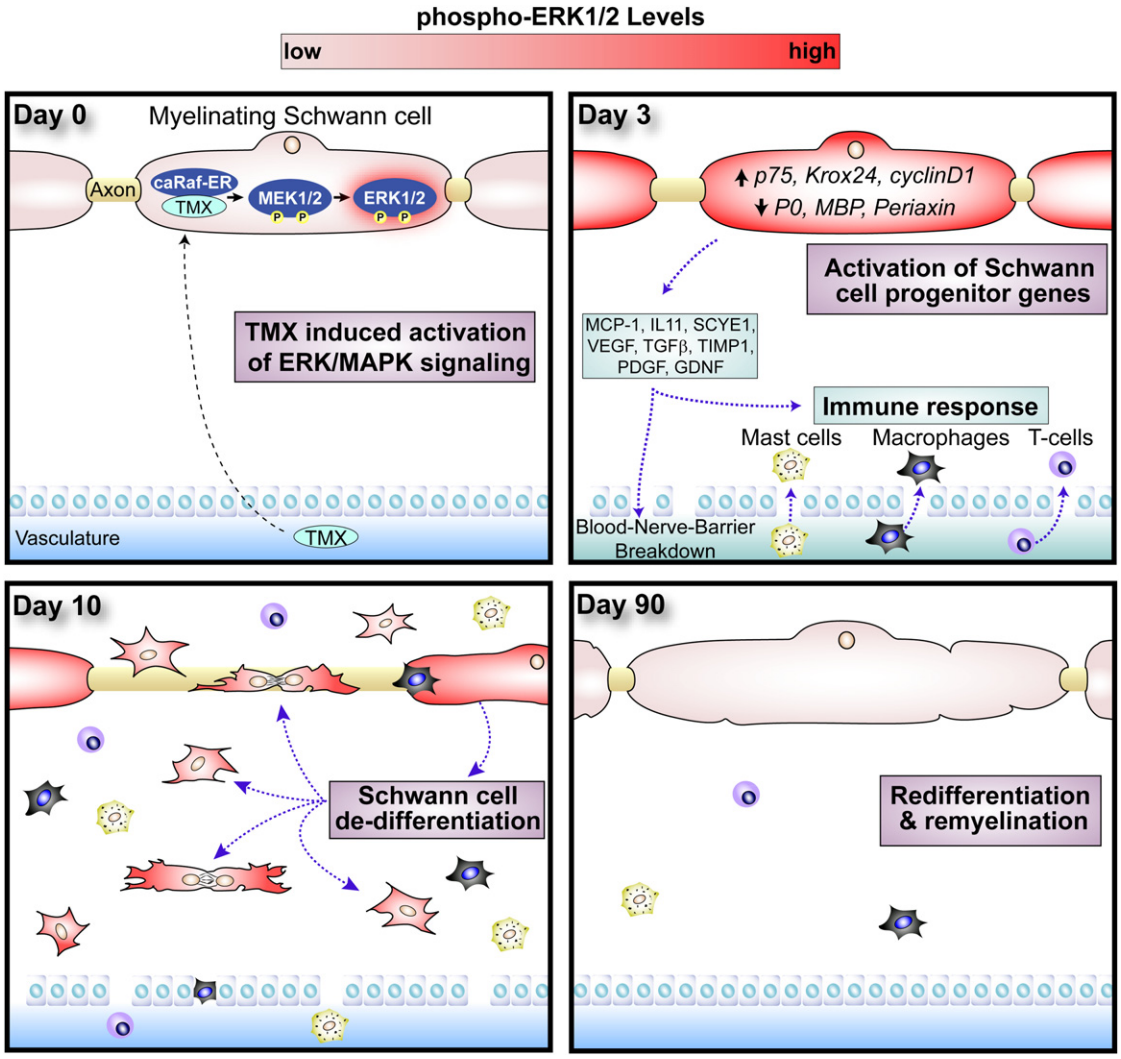

Figure 1. Elevated ERK/MAPK Activation in Myelinating Schwann Cells Is Sufficient to Induce Schwann Cell Dedifferentiation and the Recruitment of Immune Cells to the Peripheral Nerve

On day 0, P0-Raf-ER mice began a regimen of five daily consecutive injections of tamoxifen (TMX) to trigger elevations in phosphorylated ERK/MAPK levels. TMX reversibly activates a constitutively active Raf-ER fusion protein expressed only in myelinating Schwann cells. On day 3, ERK/MAPK activation resulted in changes in gene expression, indicative of Schwann cell dedifferentiation into a progenitor-like state. Further, blood-nerve-barrier breakdown and the recruitment of immune cell types was observed, possibly due to the upregulation of candidate secreted factors by dedifferentiating Schwann cells. At day 10, overt demyelination is apparent. Dedifferentiated Schwann cells and immune cells are present throughout the nerve. Remarkably, no axonal damage is observed. By day 90, after phosphorylated ERK/MAPK levels returned to basal levels, the blood-nerve barrier was restored, and dedifferentiated Schwann cells redifferentiate and remyelinate. However, myelination is more heterogeneous in recovered nerves.

Scye1, *TGFβ*, *GDNF*, *VEGF*, *FGF2*, *Jagged1*, and *Areg*. The upregulation of some candidates was confirmed in vivo by performing qRT-PCR on sciatic nerves samples from P0-Raf-ER mice. Further, an increase in the levels of MCP-1, VEGF, TIMP-1, and PDGF was detected in conditioned media from Raf-ER-expressing Schwann cell cultures. It will be interesting in the future to test the precise role of these candidate molecules in the early stages of the injury response.

It is important to place these results in the context of other studies on regulation of Schwann properties by ERK/MAPK signaling. Interestingly, conditional deletion of ERK/MAPK or Shp2, an upstream ERK/MAPK activator, in embryonic Schwann cell progenitors prevents Schwann cell differentiation and myelination in vivo (Grossmann et al., 2009; Newbern et al., 2011). Thus, there is a requirement for ERK/MAPK signaling both for differentiation of Schwann cell precursors and dedifferentiation of mature Schwann cells. What explains this seemingly paradoxical requirement for ERK/MAPK in Schwann cell differentiation during development and dedifferentiation following injury? The authors suggest that distinct levels of ERK/MAPK activity define the state of Schwann cell differentiation; basal levels are necessary for differentiation of precursors while high ERK/MAPK activity drives dedifferentiation and proliferation.

This quantitative model is reminiscent of the concentration-dependent effects of neuregulin-1 on Schwann cells, in which low levels drive myelination and high levels drive dedifferentiation (Syed et al., 2010).

Another possibility is that ERK/MAPK may interact with other pathways that regulate Schwann cell fate changes. In vitro experiments have shown that cAMP/PKA signaling modulates the Schwann cell response to NRG1 (Arthur-Farraj et al., 2011; Monje et al., 2010). In an extension of this model, heightened cAMP/PKA signaling in developing nerves directs ERK/MAPK signaling toward differentiation. In injured adult nerves, cAMP levels are diminished, which links ERK/MAPK to dedifferentiation. Determining how these signaling pathways control changes in the transcriptional network that regulates Schwann cell behavior will be challenging. For example, the prodifferentiation factor, Egr2, and the dedifferentiation factor, c-Jun, are both activated by ERK/MAPK signaling (Newbern et al., 2011; Syed et al., 2010). Aside from the control of transcriptional mediators, defining how ERK/MAPK might impact epigenetic modifications and the expression of microRNAs important for myelination will be vital as well (reviewed in Pereira et al., 2012).

Schwann cell dedifferentiation is critical to the injury response. However, inappropriate activation of this process may also contribute to pathological states, such as peripheral nerve tumors. Mutations in neurofibromin-1, a Ras-GAP, typically lead to overactive ERK/MAPK signaling and neurofibromatosis type 1 (NF1). A typical feature of NF1 is the formation of peripheral nerve tumors that appear to be composed of progenitor-like Schwann cells. The findings of Napoli et al. provide further support for the idea that heightened ERK/MAPK signaling maintains these precursors in a relatively undifferentiated state and increases susceptibility to oncogenesis (Parrinello et al., 2008). Inhibition of ERK/MAPK signaling or inhibition of factors derived from dedifferentiated Schwann cells may provide a relevant therapeutic strategy for preventing protumorigenic changes in NF1.

In contrast to the robust peripheral nerve regeneration that occurs in rodents, the distances involved after nerve injury in humans often lead to limited recovery. This regeneration failure may be due, in part, to extensive Schwann cell atrophy that has been observed in experimental animals when axon regeneration is delayed. Indeed, regenerating axons are unable to innervate distal nerve stumps that have been denervated for over a month (Gordon et al., 2011). Thus, it is intriguing to consider whether reversibly activating ERK/MAPK in Schwann cells distal to the site of injury via administration of growth factors or other mechanisms would prolong the maintenance of an environment amenable to regrowth. Indeed, the method described here for inducing ERK/MAPK activation in vivo provides a tool for tackling this interesting problem.

REFERENCES

Arthur-Farraj, P., Wanek, K., Hantke, J., Davis, C.M., Jayakar, A., Parkinson, D.B., Mirsky, R., and Jessen, K.R. (2011). Glia *59*, 720–733.

Benowitz, L.I., and Popovich, P.G. (2011). Curr. Opin. Neurol. *24*, 577–583.

Chen, Z.L., Yu, W.M., and Strickland, S. (2007). Annu. Rev. Neurosci. *30*, 209–233.

Gordon, T., Tyreman, N., and Raji, M.A. (2011). J. Neurosci. *31*, 5325–5334.

Grossmann, K.S., Wende, H., Paul, F.E., Cheret, C., Garratt, A.N., Zurborg, S., Feinberg, K., Besser, D., Schulz, H., Peles, E., et al. (2009). Proc. Natl. Acad. Sci. USA *106*, 16704–16709.

Harrisingh, M.C., Perez-Nadales, E., Parkinson, D.B., Malcolm, D.S., Mudge, A.W., and Lloyd, A.C. (2004). EMBO J. *23*, 3061–3071.

Monje, P.V., Soto, J., Bacallao, K., and Wood, P.M. (2010). J. Biol. Chem. *285*, 31024–31036.

Napoli, I., Noon, L.A., Ribeiro, S., Kerai, A.P., Parrinello, S., Rosenberg, L.H., Collins, M.J., Harrisingh, M.C., White, I.J., Woodhoo, A., and Lloyd, A.C. (2012). Neuron *73*, this issue, 729–742.

Newbern, J.M., Li, X., Shoemaker, S.E., Zhou, J., Zhong, J., Wu, Y., Bonder, D., Hollenback, S., Coppola, G., Geschwind, D.H., et al. (2011). Neuron *69*, 91–105.

Parkinson, D.B., Bhaskaran, A., Arthur-Farraj, P., Noon, L.A., Woodhoo, A., Lloyd, A.C., Feltri, M.L., Wrabetz, L., Behrens, A., Mirsky, R., and Jessen, K.R. (2008). J. Cell Biol. *181*, 625–637.

Parrinello, S., Noon, L.A., Harrisingh, M.C., Digby, P.W., Rosenberg, L.H., Cremona, C.A., Echave, P., Flanagan, A.M., Parada, L.F., and Lloyd, A.C. (2008). Genes Dev. *22*, 3335–3348.

Pereira, J.A., Lebrun-Julien, F., and Suter, U. (2012). Trends Neurosci. *35*, 123–134. Published online December 21, 2011.

Sheu, J.Y., Kulhanek, D.J., and Eckenstein, F.P. (2000). Exp. Neurol. *166*, 392–402.

Syed, N., Reddy, K., Yang, D.P., Taveggia, C., Salzer, J.L., Maurel, P., and Kim, H.A. (2010). J. Neurosci. *30*, 6122–6131.

Woodhoo, A., Alonso, M.B., Droggiti, A., Turmaine, M., D'Antonio, M., Parkinson, D.B., Wilton, D.K., Al-Shawi, R., Simons, P., Shen, J., et al. (2009). Nat. Neurosci. *12*, 839–847.