

In order to fully understand the role of soluble GFR $\alpha$ s, it will be necessary to investigate how the release of these receptors is regulated *in vivo*. It is currently unknown whether these receptors are constitutively cleaved from the membrane or whether tight regulation of phospholipases releases these factors at appropriate stages of development or in response to injury. Furthermore, experiments utilizing conditional mutants of GFR $\alpha$ s are also warranted in order to determine the cell type-specific requirements for GFR $\alpha$  receptors during development and nerve regeneration.

The potent ability of GDNF to stimulate survival and neurite outgrowth in a wide variety of neurons has made it an attractive candidate for therapeutic approaches to treat nervous system damage. However, one of the pitfalls of present GDNF treatment strategies is an inability to restrict exogenous GDNF to the damaged tissue. The consequences are clearly demonstrated in the case of a Parkinson's disease patient treated by intracerebroventricular injections of GDNF. The patient demonstrated severe side effects, including nausea, loss of appetite, hallucinations, depression, and inappropriate sexual conduct (Kordower et al., 1999). The ability of sGFR $\alpha$ 1 to guide the activity of GDNF, potentiate axonal growth responses, and confer responsiveness to non-GFR $\alpha$ 1-expressing neurons demonstrates its promise as an effective adjuvant in treating nervous system damage. It is also possible that GDNF signaling may be further potentiated by creating a "hyper-activated" chimeric ligand containing GDNF linked to GFR $\alpha$ 1 by a flexible chain of amino acids. A similar approach has been used to generate hyper-IL-6 molecules that are greater than 100 times more active than IL-6 and sIL-6R $\alpha$  supplied in combination (Marz et al., 1999).

Further investigation may also reveal that the presence of receptors able to function as guidance molecules may be even more widespread than the example given here. For example, the Nogo receptor is a GPI-linked coreceptor and therefore has the potential to act *in trans* on cells expressing the as yet unidentified signaling receptor. For instance, the recently described ligands for the Nogo receptor, Nogo-A, myelin-associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp), are well known inhibitory signals for neurons (Woolf and Bloechlinger, 2002). As it has already been shown that certain "ligands" can also transduce signals through "receptors" to the cytosol of adjacent cells, it should not be surprising that "receptors" can also function outside the cell by regulating the presentation of guidance cues. It seems that the dividing line between "ligand" and "receptor" is becoming more blurred the more that we learn about signaling events between cells.

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## **Spinsters, Synaptic Defects, and Amaurotic Idiocy**

In this issue of *Neuron*, Sweeney and Davis present a beautiful characterization of *Drosophila* mutants in a gene named *spinster*. The results indicate a function of the endocytic pathway in regulating transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling at the *Drosophila* motor synapse. This study provides important new information at an intersection of several disciplines, including membrane traffic, lipid organization, synaptic signaling, and neurodegenerative lysosomal storage disease.

During the three instars of larval life, the *Drosophila* embryonic motor synapse undergoes a more than 50-fold expansion and this expansion is influenced by a number of physiological factors. These likely include homeostatic feedback elicited by demands from growing muscle as well as positive plasticity responses of motor neurons to neural activity (Davis and Goodman, 1998). Genetic screens, enabled by the visualization of fluorescently marked motor terminals in undissected larvae, have identified several *Drosophila* mutants with heritable defects in motor neuron arborization. Molecular analysis of functions disrupted in these mutants has begun to provide important new information on regulatory mechanisms that control synaptic growth.

A mutant with highly expanded synaptic arbors was previously shown to be affected in a presynaptic ubiquitin ligase, Highwire (Hiw), whose function may be antagonized by a ubiquitin hydrolase called "Fat Facets" that regulates membrane traffic in the *Drosophila* eye (DiAntonio et al., 2001). A different mutant with shrunken synaptic arbors carries loss-of-function alleles in the type-2 TGF- $\beta$  receptor Wishful thinking (Wit) required exclu-

sively in the motor neuron for normal synaptic growth (Aberle et al., 2002; Marques et al., 2002).

In this issue of *Neuron*, Sweeney and Davis describe their analysis of yet another *hiw*-like mutant with synaptic arbors almost twice the size of those found in wild-type animals (Sweeney and Davis, 2002). This mutation is allelic to *spinster* (*spin*), originally isolated in 1997 for its vigorous rejection of male courtship and subsequently analyzed at a molecular level (Nakano et al., 2001). Nakano and colleagues observed neural degeneration in *spin* mutants and, more remarkably, the accumulation of autofluorescent lipofuscin-like ("aging") pigment in mutant neurons. These two effects of *spin*, altered neuronal degeneration and accumulation of intracellular aging pigment, are characteristic of a group of heritable human diseases called "neuronal ceroid lipofuscinoses," now called "NCLs" but once included in a wider class of lysosomal storage diseases under the term "amaurotic idiocy" (Hofmann, 2002).

Lysosomes, acidic membrane-bound organelles that hydrolyze carbohydrates, proteins, lipids, and nucleic acids, receive extracellular material and plasma membrane protein predominantly through the endocytic pathway. Lysosome-targeted material, internalized through plasma membrane-derived vesicles, passes through a series of compartments termed "endosomes," whose origin and diversity is as of yet poorly characterized. In probing the interface between a potential *Drosophila* model for NCL and motor synapse growth, Sweeney and Davis took on the considerable challenge of identifying and analyzing endosomes and lysosomes at *Drosophila* motor synapses. They further examine the localization of Spinster relative to these markers and the consequences of *spin* mutations on these endosomal compartments.

Spinster-labeled organelles are present in somatic regions of motor neurons, at presynaptic nerve endings, and throughout postsynaptic muscle. Careful colocalization studies establish that Spinster is a component of late endosomes (poorly distinguished from lysosomes at this level of analysis). In heterologous expression studies, Spinster expressed in mammalian cells is targeted to LAMP-1-positive late endosomes/lysosomes. In *Drosophila* synapses and in cell bodies, three different markers for endosomal compartments show partial colocalization with Spinster-positive puncta. In *spin* mutants, compartments labeled by these endosomal markers, by anti-Spinster antisera or a marker of low pH organelles, are profoundly increased in size and number. Such expansion and enlargement of endosomal compartments is also observed in human NCL patients. By these analyses, Sweeney and Davis associate an increase in the number and size of acidic, endosomal compartments at the synapse with the existence of grossly expanded synaptic arbors. Spinster expression in both nerve and muscle is required for normal synaptic growth, as evidenced by analyzing the ability of tissue-restricted transgene expression to cure the expanded-arbor phenotype of *spinster* mutants.

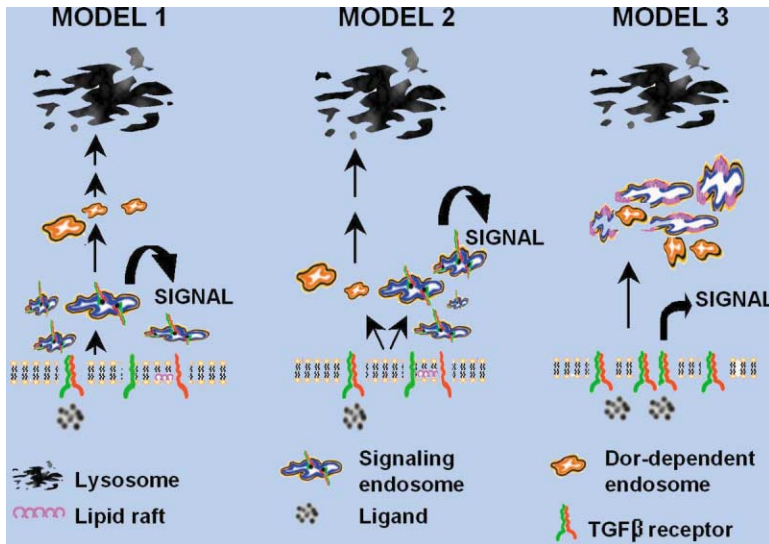
How does an altered endosomal system in *spinster* lead to increased synaptic growth? Recent results show that endocytosis and lysosomal delivery of activated growth factor receptors may be widely utilized for signal downregulation. This suggests that a normally transient

growth factor signal for synaptic growth could be substantially prolonged by blocking lysosomal delivery (Di Fiore and DeCamilli, 2001). What might this growth factor be? A reasonable candidate is TGF- $\beta$  (Aberle et al., 2002; Marques et al., 2002). While TGF- $\beta$  signaling through Wit is necessary for normal synaptic growth, its capacity to induce the entire growth response, though suggested by experiments at the *Aplysia* sensorimotor synapse (Zhang et al., 1997), has not been previously documented at the *Drosophila* NMJ. The sufficiency of TGF- $\beta$  signaling to induce growth is demonstrated by consequences of a loss-of-function allele of a gene named "*daughters against dpp*" (*dad*) that antagonizes TGF- $\beta$  signaling. Mutant *dad* animals, with enhanced endogenous TGF- $\beta$  signaling, show enormously enhanced synaptic growth.

If enhanced TGF- $\beta$  signaling were the sole cause of the expanded synaptic arbors observed at *spin* mutant NMJs, then genetic reduction in TGF- $\beta$  signaling components might be expected to suppress this phenotype of *spin*. This prediction is dramatically borne out by analyzing heterozygous mutations in three different components of TGF- $\beta$  signaling: the type II receptor Wit and the type I receptors Saxophone (Sax) and Thickveins (Tkv). Thus, while *wit/+*, *sax/+*, or *tkv/+* animals show normal synaptic arbor size, the resulting reduction in TGF- $\beta$  signaling is sufficient to cause a substantial reduction in the arbor size of *spin* mutants. Also significant, reduced arbor sizes observed in *wit/wit* mutants are unchanged in *spin/spin; wit/wit* double mutants, indicating that *spin* affects synaptic growth by a pathway completely dependent on TGF- $\beta$  signaling.

Like most unexpected findings, this novel and remarkably specific association between membrane traffic and TGF- $\beta$  signaling raises several important questions regarding the role and mechanism of Spinster and TGF- $\beta$  functions, some of which are considered by Sweeney and Davis in the discussion of their results. These remain unanswered due to our currently sketchy understanding of TGF- $\beta$  signaling at the synapse, incomplete definition of the exact role of Spin in lysosomal delivery, and relatively early knowledge of how endocytic pathways modulate signaling in vivo.

One pressing question concerns the time and location of TGF- $\beta$  signaling. Which cells secrete TGF- $\beta$  and where? Probably muscles, at or near synaptic sites, but this remains to be established. Is TGF- $\beta$  secreted in a continuous dribble during development, at a single early time point during first-instar larvae, or in periodic bursts when muscles have grown too large to be supported without compensatory synaptic growth? Differential tissue requirements revealed for Wit (presynaptic) and Spinster (on both sides of the synapse) in the regulation of synaptic growth point either to unanticipated complexities in TGF- $\beta$  signaling or yet unresolved functions for Spinster. Another important outstanding issue concerns how TGF- $\beta$  signaling relates to and is integrated with cAMP and MAPK signaling that positively regulates structural plasticity of motor neurons. Details of these various *trans*-synaptic signaling events and their relationships should shortly be unraveled, not only by ongoing analyses of various TGF- $\beta$ /BMP homologs and their receptors in *Drosophila*, but also by detailed genetic



Three Models for *spinster*'s Effect on TGF- $\beta$  Signaling

Model 1 (left panel) proposes that signaling endosomes with activated TGF- $\beta$  receptors accumulate in *spinster* mutants. In model 1, these endosomes are part of a general endocytic pathway and occur before a Deep orange-dependent step in lysosome delivery. In model 2 (center), the signaling endosomes are diagrammed as part of a specialized endocytic pathway. In model 3 (right panel), *spinster* mutations are postulated to alter phospholipid composition on cell membranes and thereby cause aberrant activation of TGF- $\beta$  receptors on plasma membrane.

epistasis studies with known components of cAMP and MAPK signaling.

A second category of questions concerns the mechanism by which *spinster* mutations enhance TGF- $\beta$  signaling. A particularly perverse hypothesis is that the primary effect of *spin* mutations is to alter synaptic transmission in a way that triggers TGF- $\beta$  signaling through a compensatory, homeostatic mechanism; thus, *spin* could act to induce new TGF- $\beta$  rather than modulate its endogenous signaling. Though admittedly unlikely, this remains a formal possibility, especially because the exact effect of *spin* on lysosome-directed traffic has yet to be determined. However, based on the reasonable assumption that *spin* alters some step(s) in this pathway, we discuss three models for how TGF- $\beta$  signaling may be enhanced (see Figure).

One explanation (model 1) for this effect of *spin* is slow lysosomal delivery and stabilization of a class of endosomes recently shown to contain activated, signaling TGF- $\beta$  receptors (Hayes et al., 2002). The simplicity of this explanation is certainly attractive, and, if *hiw* phenotypes are also shown to result from altered TGF- $\beta$  signaling, it may tie together the similar effects of *hiw* and *spin* mutants. However, at its simplest level, it makes the prediction that other mutations in the endocytic pathway, like *deep orange*, which has been previously characterized as a positive regulator of endocytic trafficking, should have similar effects. Why then do lethal allelic combinations of *deep orange* (*dor*) have not only smaller but also opposite effects on synaptic growth than completely viable *spin*/+ heterozygotes (Narayanan et al., 2000)? One possibility is that *dor* and *spin* block endocytosis at different steps and that activated TGF- $\beta$  (or other signaling) receptors may only access relevant substrates at specific stages of the endocytic pathway. However, alternative explanations also merit consideration.

Model 2 suggests that activated TGF- $\beta$  receptors reach lysosomes through a class of specialized *spin*-regulated endosomes that are distinct from those affected in *dor* mutants. While specialized endosomes may certainly exist, *spin* phenotypes appear more con-

sistent with a general membrane traffic function for *Spinster*. First, *spin* mutations cause large increases and substantial redistribution of Dor-positive puncta, indicating functions for both proteins in the same pathway. Second, effects of *spin* on endosomal structures are not particularly unique but are rather typical of a large family of lysosomal storage diseases (Hofmann, 2002).

We offer an alternative explanation for the effect of *spin* on TGF- $\beta$  signaling as model 3. Altered late endosome/lysosome function in *spin* may result in substantial changes in the composition of presynaptic plasma membrane. Several sphingolipid storage diseases, for instance, substantially alter the intracellular distribution of cholesterol; this could have potentially wide-ranging effects on signaling (Prior et al., 2001). If local lipid microdomains (rafts) are important for maintaining TGF- $\beta$  receptors in inactive (perhaps monomeric) states, then altering plasma membrane lipids could result in substantial enhancement of both ligand-dependent and perhaps -independent TGF- $\beta$  signaling. This model represents one example of a class of explanations for how defective endosomal organelles could result in altered cell signaling. In this view, phenotypes of *spin* may not inform us about mechanisms normally modulated to influence plasticity but rather of underlying stable cellular mechanisms required for appropriate function of the primary regulators of synaptic change.

Finally, how does analysis of *spin* phenotypes contribute to understanding the etiology of and potentially alleviating human ceroid lipofuscinoses? NCLs are remarkably well studied, and of at least eight different NCL-susceptibility genes mapped, six have been molecularly cloned. Three encode specific lysosomal hydrolases; the other three are lysosomal membrane proteins whose functions, like *Spinster*'s, have yet to be identified (Hofmann, 2002). At the most obvious level, Sweeney and Davis's experiments suggest that altered TGF- $\beta$  signaling may contribute to neuropathologies of some NCL diseases. If this were true, then pharmacological inhibitors of TGF- $\beta$  should be considered candidate therapeutic agents. At another level, the identification of genes that when mutated can suppress organismal and cellular phenotypes

of *spin* may serve to suggest targets for novel drugs potentially useful for NCL therapy. Whether or not *spin* analyses prove valuable for treatment of NCLs, the investigation of the synaptic defects in *spin* and of their origins has opened the door to a wide range of interesting and important research.

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## mRNA at Synapses, Synaptic Plasticity, and Memory Consolidation

Miller et al. (this issue of *Neuron*) report that deletion of the 3'UTR of  $\alpha$ -CaMKII mRNA prevents dendritic delivery of the mRNA in transgenic mice and thus local synthesis of  $\alpha$ -CaMKII protein in dendrites. 3'UTR mutant mice exhibit decreases in  $\alpha$ -CaMKII protein in postsynaptic densities, and deficits in late phase LTP and in memory consolidation.

It has been 20 years since the report describing a selective localization of polyribosomes beneath postsynaptic sites on dendrites, in which it was hypothesized that synapse-associated polyribosome complexes could be the site of synthesis of proteins that were critical for synaptic plasticity and memory consolidation (Steward and Levy, 1982). Much has been learned about the mechanisms underlying mRNA targeting to dendrites, and the role of dendritic protein synthesis in synaptic

plasticity (Steward and Schuman, 2001), but evidence linking local synthesis in dendrites, synaptic plasticity, and memory in intact animals has been lacking. Now, using gene targeting techniques to eliminate dendritic localization of the mRNA for the  $\alpha$  subunit of calcium/calmodulin kinase II ( $\alpha$ -CaMKII), Miller et al. (2002) have provided the strongest evidence yet that local synthesis in dendrites is critical for late phase LTP and memory consolidation.

There is abundant evidence that  $\alpha$ -CaMKII plays a critical role in the signal transduction processes that induce LTP (Lisman et al., 2002). In addition, the mRNA for  $\alpha$ -CaMKII is one of the few mRNAs that is present throughout dendrites, raising the possibility that  $\alpha$ -CaMKII protein that is locally synthesized may be incorporated into synapses that are being modified, altering their signaling capabilities. To test this hypothesis, it is critical to block local synthesis of  $\alpha$ -CaMKII in dendrites while preserving overall expression of the protein. Miller et al. (2002) accomplish this by creating a mutant mouse in which most of the 3'UTR of  $\alpha$ -CaMKII mRNA, which is necessary for dendritic targeting of the message (Mayford et al., 1996), was replaced by the 3'UTR of bovine growth hormone.

### Dendritic Localization of $\alpha$ -CaMKII mRNA Depends on the 3'UTR

In situ hybridization analyses of brains from 3'UTR mutant mice revealed that  $\alpha$ -CaMKII mRNA lacking the 3'UTR targeting sequences remained tightly localized in neuronal somata, in contrast to wild-type  $\alpha$ -CaMKII mRNA that is localized throughout dendrites. These results elegantly confirm that targeting elements within the 3'UTR are necessary and sufficient for dendritic localization of  $\alpha$ -CaMKII mRNA, and also provide new insights into the nature of the targeting element (see Discussion in Miller et al., 2002).

### Dendritic Localization of $\alpha$ -CaMKII mRNA: Role in Maintaining $\alpha$ -CaMKII Protein Levels in Postsynaptic Densities?

Dendrites and especially postsynaptic densities (PSDs) contain large amounts of  $\alpha$ -CaMKII protein, which could be synthesized on site from the mRNA that is present in dendrites or could be synthesized in the neuronal cell body and then delivered throughout dendrites. Miller et al. (2002) found that the overall levels of  $\alpha$ -CaMKII protein were reduced to about 45% of control levels in 3'UTR mutant mice, and the level of  $\alpha$ -CaMKII protein in postsynaptic densities was reduced to an even greater extent (to 17% of control), suggesting that local synthesis is critical for maintaining the levels of  $\alpha$ -CaMKII protein in PSDs. It cannot be excluded, however, that translational control is disrupted because the 3'UTR of  $\alpha$ -CaMKII contains elements that regulate translation, including two cytoplasmic polyadenylation elements (CPEs, see Wells et al., 2001), which are missing in the 3'UTR mutant animals. The absence of CPEs might eliminate the translational repression that comes from short poly-A tails, but at the same time eliminates an element that is responsible for increasing translation in response to synaptic activity. Whatever the mechanism for the reduction in  $\alpha$ -CaMKII protein levels, the substantial reduction in  $\alpha$ -CaMKII in the PSD represents an important