The signal transduction pathway used by BMP and other TGF-β superfamily members is evolutionarily ancient (Raftery and Sutherland, 1999). Homologous molecules and mechanisms are used in both *Drosophila* and mammals. In general, a secreted BMP ligand complexes with both type I and type II receptors at the responding cell's membrane. The receptors are serine-threonine kinases that mediate a cascade of phosphorylation events. Upon activation, the BMP type II receptor phosphorylates the BMP type I receptor, which in turn phosphorylates a receptor-regulated Smad transcription factor (R-Smad). The phosphorylated R-Smad then complexes with a common nonphosphorylated Smad (Co-Smad), to control gene expression in the nucleus. All of these molecular players are found in *Drosophila*. There are two BMP type II receptors, *punt* and the newly characterized *wishful thinking*, as well as three type I receptors, *baboon*, *saxophone*, and *thickvein*. R-Smad and common Smad proteins are encoded by the *mothers against decapentaplegic* and the *medea* genes (Newfeld et al., 1999). Consistent with Wit's involvement in the BMP receptor-mediated signaling cascade, Marqués et al. (2002) showed that the phosphorylation of the *Drosophila* Smad in the CNS is dependent on Wit and that Wit is likely the principal type II receptor of neurons.

Two models for Wit's role in NMJ development can be envisioned. First, Wit might receive a retrograde signal from muscles that controls motoneuron arbor growth. The muscle would secrete candidate BMP ligands, perhaps in an activity-dependent fashion. According to the genome project, there are seven candidate BMP genes, so the identification of Wit's partner should be forthcoming. However, this model has problems. Surprisingly, Wit expression is lowest (as far as antibody staining is concerned) at the synapse itself and is highest in the ganglionic neuropil. This raises questions about whether Wit responds directly to a retrograde signal from muscles. When the identity and source of the ligand is settled, we should be able to better interpret the receptor localization data.

Alternatively, Wit may act as a general regulator of neuronal growth. Thus, the reduced NMJs in mutants would reflect a general failure by motoneurons to grow properly during larval development. One would thus predict that all parts of the neuron would be affected. While the morphology of individual motoneurons remains to be analyzed, Marqués et al. (2002) found that the general organization of the ganglionic neuropil is largely normal in mutants, consistent with a more specific role of the receptor in regulating the NMJ.

We also need to know which genes are downstream to Wit, and their roles in synaptic maturation and neuronal growth. Fortunately, we are not without candidates. Among the genes with dramatic affects on NMJ morphology are *highwire* (*hiw*), which in mutants has elevated numbers of boutons (Wan et al., 2000), and *futsch,* a microtubule-associated protein with reduced bouton numbers in mutants (Hummel et al., 2000; Roos et al., 2000). Other candidates for regulating NMJ development are likely to emerge from the Goodman lab's morphological screen.

Finally, it is possible that the muscle is not the source of the BMP ligand, but rather influences signaling through negative regulation of BMPs (which could come from other sources, or even from the motoneuron, acting in an autocrine fashion). For example, Short gastrulation (Sog), the fly homolog of Chordin, binds to and inactivates the BMP2/4 ligand Decapentaplegic, to define boundaries of the *Drosophila* neurectoderm, in a mechanism reminiscent of vertebrate neural induction (Holley et al., 1995). Even the negative regulators may be regulated: *Drosophila* possess a BMP1 homolog, Tolloid, which is a metalloprotease that cleaves Sog (Marques et al., 1997). It would be informative to test whether these proteins are secreted by muscles or neurons.

In conclusion, with these two papers our understanding of synaptic maturation and neuronal growth has entered a new phase. While these are still early days, with much that remains to be characterized, for the first time in *Drosophila* we have a handle on the molecular mechanisms that tie a neuron's size to its actions.

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Sodium Channels: Grit, Determination, and Persistence

Persistent sodium channel activity modulates neuronal gain in a neurotransmitter-dependent fashion. Previous studies have suggested that persistent and spike-related sodium channel activities are mediated

by separate species. In this issue of *Neuron*, Taddese and Bean (2002) show that a single channel population is sufficient to explain both gating behaviors. A simple allosteric model is provided that can explain the results.

Sodium channels subserve one of the most basic functions in the brain. That is, they are responsible for generation of the digital signals (action potentials) that are unerringly propagated along axonal transmission lines to ultimately trigger intercellular communication at synapses. To fulfill this function, sodium channel gating must be fast and reliable. Channels must be able to activate (open) quickly to promote the avalanche reaction of spike initiation, and then they must rapidly inactivate (close) to allow for termination of the spike. Further, for repetitive spiking to occur, the process of channel inactivation must be readily reversed during the brief intervals between spikes. While these rapid gating functions of activation, inactivation, and deinactivation have been well understood at the macroscopic level since the studies of Hodgkin and Huxley in squid giant axon, in recent years there has been increasing evidence for a second, slower form of sodium channel gating, whose function is especially prominent at near-threshold membrane potentials. This so-called slowly inactivating, or persistent (reviewed in Crill, 1996), channel activity is not as much relevant to the generation of individual spikes, as it is to more exotic firing pattern such as intrinsically driven spontaneous firing or plateau generator potentials that drive high-frequency firing. Varying contributions of persistent sodium current have been observed in different CNS regions, for example, in neurons of hippocampus (Hotson et al., 1979), cerebellum (Llinas and Sugimori, 1980), neostriatum (Bennett et al., 2000), and thalamus (Jahnsen and Llinás, 1984).

Given the widespread expression of persistent sodium channels in the brain and the expected functional consequences, such as boosting of excitatory synaptic inputs, acceleration of firing rates, and promotion of oscillatory neural activities (Crill, 1996), it is an important yet unresolved issue whether persistent versus transient activity arise from distinct voltage-gated sodium channel populations, or rather from different gating properties of a common sodium channel species. Taddese and Bean (2002 [in this issue of *Neuron*]) test the latter possibility in tuberomammillary neurons of the hypothalamus. These neurons exhibit spontaneous firing both in vivo and in vitro. In a technical tour-de-force, the neurons were isolated in a manner that preserved both their membrane integrity and their basic electrical features, most notably their characteristic spontaneous firing. In this isolated state, the neurons were electrically compact and therefore amenable to careful voltage-clamp analysis. The experiments used elegant action potential waveform voltage-clamp to show that nearly all of the membrane current flowing during interspike intervals was delivered by tetrodotoxin (TTX)-sensitive sodium current, and thus in these cells spontaneous firing is largely dependent on persistent sodium channel activity. Ramp and voltage-step analyses were then used to demonstrate that the extent and rate of channel block and unblock by low (5 nM) and high (1 μM) concentrations of TTX were equivalent for transient (action potential related) and persistent currents. Similarly, depolarizing conditioning pulses that triggered slow inactivation of the transient current also caused comparable inactivation of the persistent current. These demanding experiments required nearly perfect voltage-clamp conditions and long-term neuronal stability during bath applications of TTX, as was necessary to extract the sodium channel contribution to the spontaneous firing response.

The authors were then able to derive a simple, yet elegant, model based on that of Kuo and Bean (1994) that can explain the results. The result is an allosteric model whereby the movement of channels through successively activated states causes conformational changes that in turn promote inactivation. A corollary is that binding of inactivation particles stabilizes gating charges in the "activated" state. The model has only two voltage-dependent steps, activation and deactivation, with inactivation and deinactivation (binding and unbinding of the inactivation particle) deriving their voltage-dependence indirectly—binding is promoted and unbinding is slowed to progressive degrees as the channel traverses more activated conformations. Thus, depolarization promotes transitions through successive activation states, each with higher affinity for the inactivation particle. In highly activated states (as with four of four putative charge sensors translocated), the channels are stabilized in an equilibrium with a small but non-zero proportion with unbound inactivation particles, and thus available for conduction. In tuberomammillary neurons, Taddese and Bean estimate the equilibrium ratio to be 1:200, i.e., that the persistent current will be about 0.5% of the peak transient current. The allosteric model is able to reproduce nearly all sodium channel behavior as observed during conventional voltage-clamp, including slow and variable inactivation during voltage steps, and a persistent current activation curve that is similar, but slightly depolarized, to that for steady-state inactivation.

Previous studies have suggested that slowly gating sodium channels may be the target of certain antiepileptic drugs such as phenytoin and carbamazepine and that the blocking effect on persistent sodium channel activity may be more prominent than that on transient sodium current (Quandt, 1988; Segal and Douglas, 1997). Functionally, this would reduce the ability of the persistent sodium current to promote the high-frequency spike firing that occurs during seizures. This study is important because it demonstrates that, at least in one case, a single population of channels underlies both persistent and the transient sodium current. It remains to be seen whether models analogous to the one presented here can explain both transient and persistent sodium channel activity in other cell types, and whether such models are compatible with a specific effect of antiepileptic drugs on persistent channel activity. Persistent activation of sodium channels can be specifically up- and downregulated by neurotransmitters (Mittmann and Alzheimer, 1998; Pierson et al., 2001) and by conditions such as hypoxic stress (Hammarström and Gage, 1998). Further studies will be necessary to test whether this specificity arises through separate sodium channel populations, or through modifications of specific steps in the gating scheme proposed by Taddese and Bean.

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