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Science Signaling **1** (18), pe20. [DOI: 10.1126/stke.118pe20]

NEUROSCIENCE

Synaptic Patterning by Morphogen Signaling

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Published 6 May 2008

Gradients of secreted small morphogenic molecules control cell proliferation and patterning throughout animal development. Recent years have seen the discovery of surprising roles for morphogens in later developmental processes, including axon pathfinding and synaptogenesis. The latest addition is a role for the TGF-β superfamily morphogen Activin in synaptic patterning of the *Drosophila* visual system. In contrast to classical instructive and long-range morphogen gradients, Activin acts as a permissive and local motility restriction signal around several hundred individual photoreceptor axon terminals. Activin must therefore act in concert with other instructively attracting and repelling signals as part of a larger genetic program for brain wiring.

Transforming growth factor β (TGF-β), bone morphogenic proteins (BMPs), and Activins define a family of morphogens, as do the Hedgehog and Wingless/Wnt families of secreted signaling molecules. Morphogens of the TGF-β superfamily direct a plethora of cellular processes, including differentiation, proliferation, and apoptosis. During development, TGF-β signaling plays important roles in processes ranging from cell fate determination and tissue patterning during early animal development (*1*, *2*) to synaptogenesis (*3*). Recent findings in *Drosophila* now implicate Activin signaling in the patterning of synaptic connectivity downstream of axon pathfinding in the visual system (*4*).

The establishment of synaptic connectivity, which occurs late in development compared with cellular differentiation and proliferation, encompasses outgrowth of axons from differentiated neurons, long-range axon pathfinding, and recognition of synaptic partners. Several classes of guidance molecules—including both secreted molecules like Netrins and Slits and transmembrane guidance receptors like Ephrins and Cadherins—are known to control these processes (*5*, *6*). Only in recent years has it become apparent that morphogens of the Hedgehog, Wingless/Wnt, and TGF-β/BMP families can also act as guidance cues in various nervous systems (*7*, *8*). These findings indicate that morphogens that are required for the specification of neuronal cell types early in development may later be reused during the establishment of synaptic connectivity. This raises the question of how developmental programs ensure precise control of very dissimilar processes using the same signals. Are the downstream signaling networks divergent? Or is the separation of the signals in space and time sufficient to confer specificity for distinct developmental processes? The characterization of different events that are controlled by the same signaling molecules provides an important opportunity to answer such questions and uncover underlying principles of the signaling networks.

Drosophila has a long history as both the model organism in which many morphogens and components of their signaling networks were originally discovered (*9*, *10*) and as a model system in which to study synaptic specificity in visual system development (*11*–*13*). *Drosophila* also provides the opportunity to genetically separate distinct functions of the same molecules at different times and places with relative ease. This is critical to the analysis of morphogen-dependent processes occurring at different developmental stages, because severe differentiation and patterning defects caused by early loss of morphogen function would mask any later roles these morphogens might have after cellular differentiation. Recent work by Ting *et al*. (*4*) makes full use of the fly's genetic advantages to uncover and characterize a surprising new role of Activin signaling for a specific task during the establishment of synaptic connections.

After differentiation, *Drosophila* photoreceptors from the ~800 "single eyes" that constitute one compound eye grow axons that terminate in distinct areas of the brain. Of the eight different photoreceptor cell types found in each single eye, subtype R7 projects deepest into the brain to form a precise retinotopic map: Neighboring points in visual space are seen by neighboring single eyes and represented by neighboring synaptic terminals in the brain. It has become clear in recent years that this precise pattern of connectivity, like patterning during embryogenesis, is the product of a genetic program (*14*). Numerous guidance cues that direct axon pathfinding and the target recognition process have been identified (*13*, *15*). Recently, Ting *et al*. uncovered a role for Activin signaling in synaptic patterning (*4*). Normally, during the development of the retinotopic map and after axon pathfinding, R7 terminals segregate into nonoverlapping neighboring terminal "boutons" in a process the authors refer to as "tiling." In the present study, they found tiling defects in flies with mutations in either of two genes, *baboon* (*babo*), which encodes an Activin receptor, and *importin-α3,* which encodes a nuclear import protein.

Baboon is a transmembrane serine/threonine kinase and is the type I Activin receptor required for cell proliferation during larval and pupal development in *Drosophila* (*16*). Upon Activin binding, Babo phosphorylates the transcriptional effector Smad2, which regulates a transcriptional response in the nucleus (*2*). Ting *et al*. identified the Activin receptor Babo as well as a Smad2-interacting importin as playing a role in synaptic patterning in a genetic screen based solely on a cell-specific phenotype. Their cell-specific analyses may reconcile contradicting cell culture reports on the requirement of importins (*17*–*22*); nuclear Smad2 accumulation requires importin-α3 in R7 photoreceptor cells but is importin-α3-independent in the neighboring pigment cells (*4*). Hence, divergent findings in cell culture may reflect divergent signaling networks employed in vivo in a cell-specific manner.

Although importin-dependent nucleocytoplasmic shuttling of Smad proteins may not always be part of the Activin signaling network, the classical mechanism of TGF-β signaling by means of receptor-mediated phosphorylation of Smad proteins seems to be widely used. In flies, Activin signaling

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that results in Smad2-controlled transcription is required for cell proliferation and specification (*16*, *23*), neuronal remodeling in the brain (*24*), embryonic motoneuron pathfinding (*25*, *26*), and patterning of synaptic connectivity downstream of axon pathfinding in the visual system (*4*). The transcriptional targets, however, are generally unknown. These findings suggest that a reusable "signaling cassette" downstream of morphogen action is developmentally exploited for very different processes. Spatiotemporal separation of distinct uses of morphogen signals may thus be sufficient to confer specificity for different developmental processes without substantially altering the molecular composition of the signaling cassette.

What are the special features of morphogen signaling that allow regulation of such diverse developmental processes? Morphogens activate target cells in a concentration-dependent manner, typically along a gradient with cells responding in specialized ways to distinct concentration thresholds. However, when transcriptional regulation is involved, cellular responses to morphogen signals are slow. This constraint on cellular response time is exacerbated in axon pathfinding and synaptic patterning by the added requirement of long-distance axonal trafficking. In contrast, some classical guidance receptors can elicit rapid responses by translating binary attractive or repulsive guidance cues directly into growth cone movement through action on the cytoskeleton (*15*, *27*). It should be noted, however, that the action of classical secreted guidance cues like Netrin can also involve gradients (*28*), and it is unclear how frequently transcriptional regulation is involved.

What, then, is the purpose of a signaling mechanism that reads concentration gradients and takes at least several hours to translate this information into cellular output? Recent work on axon pathfinding in the fly embryo found a permissive, rather than an instructive role for Smad2-dependent Activin signaling (*25*, *26*). A function that promotes outgrowth along a concentration gradient and enables or modulates growth cone responses to other instructive cues is consistent with slow morphogen signaling. During the R7 columnar restriction or "tiling" process described by Ting *et al*., Activin is most likely secreted and sensed by every single one of the ~800 R7 terminals. Thus, local concentration gradients are created around individual terminals through their own activity, making Activin an unlikely repellent signal. Indeed, the authors show that individual *babo* mutant terminals are still partly repelled by their neighbors (Fig. 1). On the other hand, *babo* mutant terminals do extend into neighboring columns, which is inconsistent with a simple role of Activin as an attractant. Instead, the local concentration of Activin normally produced by each R7 terminal might itself serve as a columnar restriction signal, possibly by simply reducing motility and filopodial outgrowth (Fig. 1). As in the case of the role of Activin signaling in axon pathfinding (*25*, *26*), this suggests a permissive role for Activin signaling in concert with other actively attracting or repelling instructive signals. Indeed, the fact that *babo* mutant terminals are still repelled by their neighbors demonstrates the existence of at least one such signal. Furthermore, a classical paper by Ashley and Katz (1994) showed that R7 terminals only invade empty (R7-less) neighboring columns under "competitive pressure" induced by the generation of too many R7 axons per column (*29*). The molecular substrate for this behavior cannot be Activin if it acts as neither an attractant nor a repellent. Taken together, these data suggest that Activin signaling constitutes a novel component of the synapse specification program. It most likely does not instruct the axon where to grow or with whom to synapse; rather, Activin provides a permissive, local columnar restriction signal after target recognition and before synaptogenesis. This "local signaling" model predicts the stabilization of R7 terminals irrespective of whether they have been tiled correctly; in other words, an R7 terminal will also be subject to its own Activin secretion and signaling in a wrong column. Similarly, the formation of a precise number of synapses is independent of partner accuracy in the fly visual system (*14*). Both cases may represent genetically encoded developmental subprograms that are executed regardless of the accuracy of preceding developmental steps. The concatenation of simple, genetically encoded developmental steps may yield a key to understand the emergence of seemingly complicated synaptic connectivity patterns.

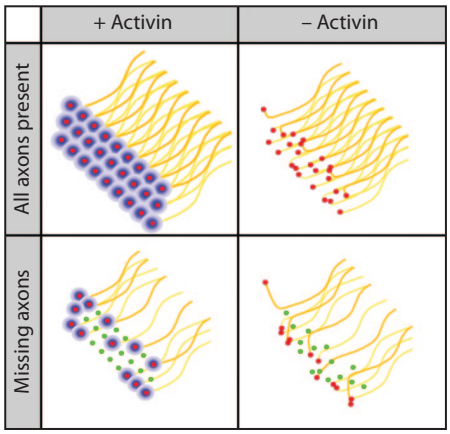

Fig. 1. The role of Activin signaling in columnar restriction and tiling. (**Top left**) R7 axon terminals form a precisely arranged array of terminals in a specialized brain region (red dots). Activin is secreted by each terminal (blue) and restricts filopodial outgrowth and motility in the target field. In the absence of Activin signaling (**top right**), axon terminals exhibit increased filopodial outgrowth and disarray. In contrast, when individual R7 axons are removed (**bottom left**), the remaining R7s still recognize their correct target areas (unoccupied R7 terminal areas are indicated by green dots) (*29*). Thus, the loss of Activin from absent neighboring axons does not interfere with columnar restriction and tiling. However, absence of Activin signaling in a terminal field that also lacks axons (**bottom right**) results in increased filopodial outgrowth and disarray. These data indicate a permissive role of Activin in columnar restriction by reducing motility that acts in concert with other instructively attracting or repelling signals.

A molecular description of Activin signaling in synaptic patterning demands the inclusion of much intracellular machinery to account for signal transduction between the synapse and the far-away nucleus: The Babo receptor needs to associate with Smad2 to phosphorylate it, possibly after endocytosis in an endosomal compartment. Thereafter, phosphorylated Smad2 needs to be retrogradely transported along the axon by means of a Dynein and Dynactin-dependent mechanism and imported into the nucleus. Finally, back in the nerve terminal, the output signals of unknown transcriptional targets need to exert their actions. Indeed, patterning defects of R7 terminals closely resembling the mutant phenotypes of *babo* and *importin-α3* have been described for flies carrying mutations in *synaptobrevin*, which encodes an exocytic vesicle fusion protein (*30*), and *sec15*, which encodes a vesicle cargo targeting protein (*31*). How intracellular signaling and trafficking is spatiotemporally organized during the establishment of synaptic connectivity is fundamentally unknown. Similarly, although we

have some understanding of the input pathways of morphogen signaling, much less is known about the output. The identification of transcriptional targets should yield new insights into the mechanisms regulating cytoskeletal and membranous alterations underlying growth cone movements. Last, the discovery of patterning mechanisms beyond attractive and repulsive guidance cues opens the door to an extended conceptual understanding of how a seemingly complicated brain structure can be the product of a genetic program.

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10.1126/stke.118pe20

Citation: W. R. Williamson, P. R. Hiesinger, Synaptic patterning by morphogen signaling. *Sci. Signal.* **1**, pe20 (2008).