Photoreceptor axons play hide and seek

Javier Morante & Claude Desplan

The outer photoreceptors of *Drosophila* project to their targets in an extremely intricate pattern. Prakash *et al.* in this issue show that homophilic adhesion via N-cadherins is an essential part of the axon guidance mechanism in this system. The new results suggest that axons respond to the level of N-cadherins, and not to any combinatorial cadherin code, as had been postulated previously.

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Drosophila photoreceptor cells face one of the toughest challenges in axon navigation. Each compound eye has 800 functional units called ommatidia, which contain two cell classes: six outer photoreceptor cells (R1–6) detect motion, and two inner photoreceptor cells (R7 and R8) see color and detect polarized light. Because the outer photoreceptor cells are located away from the center of the lens, each one looks in a different direction in space. Still, the outputs of several photoreceptor cells need to be summed to increase light detection and reduce noise[1]. To do this, flies have developed a unique optical system coupled to precise axonal projections known as neural superposition[2].

Owing to the curvature of the eye that matches the diffraction of the lens, six outer photoreceptor cells from six neighboring ommatidia (R1 from one ommatidium, R2 from another, and so on) see the same point in space and project to the same laminar cartridge[3] (**Fig. 1**). The laminar cartridges are formed by six photoreceptor axon terminals that contact a fascicle of axons from a column of five lamina monopolar cells (L_1–L_5). In this issue, Prakash and colleagues[4] report that these photoreceptor axons find their targets through homotypic recognition between the photoreceptor axons and the monopolar cells.

This amazing specificity in the axon projection supposes the involvement of complex axonal cues to allow recognition of the correct target cartridge[3,5]. The bundle of eight axons coming from one ommatidium first reaches the lamina and induces the terminal development of a single lamina cartridge. Although R7 and R8 go through this cartridge to reach their target, the medulla, none of the outer photoreceptor cells, R1–6, contacts the cartridge they have induced. Rather, they defasciculate to reach six different neighboring targets (**Fig. 1**), which is the part of the process that seems to depend on N-cadherins.

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Cadherins form a vast superfamily[6] defined by a unique, tandemly repeated extracellular calcium-binding domain, called the cadherin motif or EC domain. Eighty cadherins have been identified in mammals, and *Drosophila* has 17 members of the family. N-cadherins are classical cadherins. Many roles have been ascribed to these proteins during nervous system development, where they are often found at synapses[7]. This suggested that the main role for classical cadherins in synaptic recognition is to mediate homotypic interactions between pre- and postsynaptic cells. The large number of isoforms has led to the suggestion that a 'cadherin code' could contribute to the specificity of axon targeting. However, the complexity of classical cadherins in mammals (20 different genes and many splice forms) has made it difficult to test this hypothesis. Fruit flies have only three classical cadherins[8]—E-cadherin, N-cadherin and N-cadherin2—with N-cadherin undergoing complex alternative splicing to generate 12 isoforms[9].

Both N-cadherin and E-cadherin are expressed in photoreceptor cells and in their target cells in the lamina[4,10]. Zipursky and colleagues had previously shown that removal of *CadN*, the gene encoding N-cadherin, specifically in photoreceptor cells still allowed R1–6 to reach the lamina correctly, but prevented their extension out of the ommatidial bundle to reach their targets[10]. Now, Prakash and colleagues have extended this observation by showing that N-cadherin proteins are required in the photoreceptor cells for them to recognize their final target, but not their initial waystation. This phenotype could be rescued by providing any of the N-cadherin isoforms in mutant photoreceptor cells (but not by providing E-cadherin), suggesting that they are functionally interchangeable, and that their diversity is not relevant in R1–6 target choice[4]. Thus it seems that an 'N-cadherin code' is not the driving force behind axon targeting.

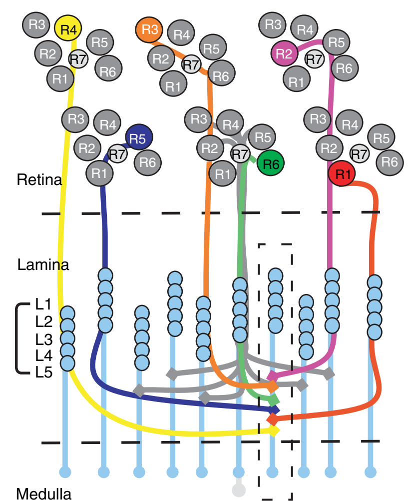

Figure 1 The six outer photoreceptor cells (R1–6) from one ommatidium project to six different cartridges in the lamina (gray). However, the six outer photoreceptor cells from six neighboring ommatidia, which point to the same direction in space (color), project to one lamina cartridge (box). The complex array of these projections is called 'neuronal superposition'.

Because cadherins mediate homotypic interactions, the authors also tested the role of N-cadherins in the target cells, the lamina neurons forming the cartridges. Removing N-cadherins in patches of lamina neurons (but not in single neurons) led to severe disruption of photoreceptor axon projections to the cartridges, which either lacked all contacts to the photoreceptor neurons or had abnormal num-

bers of photoreceptor projections. The authors could also follow what happened to individual photoreceptor neurons when their target lacked N-cadherins: they specifically labeled wild-type R4s[11] and then followed single R4 projections to a mutant cartridge. The R4 projection was abnormal, and the severity of the phenotype correlated with the number of mutant target neurons in an individual group of L_1–L_5 lamina monopolar cells. The authors could not identify any specific lamina neuron that was required for extension outward from a cartridge, thus suggesting that all neurons in a given cartridge contribute together to the extension phenotype[4].

One mechanism proposed to explain these observations is that photoreceptor growth cones sense N-cadherin levels in the lamina. Photoreceptor axons prefer the cartridge with the highest levels of N-cadherins (that is, with the most wild-type and fewest mutant lamina neurons), even if it is not the correct one. The authors therefore conclude that N-cadherin is required in photoreceptor cells for homotypic recognition of N-cadherin in their target cartridge. But then what would prevent a cartridge from receiving extra photoreceptor projections? Perhaps in cartridges that have already received photoreceptor innervation, the N-cadherin in the monopolar neurons is 'neutralized', leading an approaching photoreceptor axon to search for other targets and to project to the nearest cartridge still exhibiting 'free' N-cadherin. Because the homotypic interaction is stronger between a given photoreceptor and its closest cartridge, and because the 'neutralization' limits the number of contacts, cartridges only receive the six correct inputs.

Why do the six photoreceptor neurons from an ommatidium avoid the very cartridge they have induced and choose the cartridge closest to their topographic position? One possibility is that homotypic interaction between fasciculated photoreceptor cells also 'neutralizes' N-cadherins in the photoreceptor cells. Only after defasciculation has pushed growth cones away from the cartridge they induced can photoreceptor cells look for another homotypic adhesion in the next cartridge. Although this model where cadherin is presented or hidden to prevent excess connections is attractive, it does not solve all problems: for instance, equatorial cartridges receive more than six photoreceptor axons (up to eight) because the mirror-image symmetry orientation of ommatidia on either side of the equator leads to more photoreceptor cells pointing in the same direction. These cartridges seem to have the same organization as more peripheral cartridges and still receive excess photoreceptor cells. Possibly the choice of cartridge involves a competition between different cartridges rather than a mechanism based on counting of the absolute number of axons reaching a cartridge.

The lack of full penetrance in most of these experiments suggests the involvement of additional partners in the recognition of lamina neurons by photoreceptor cells. Indeed, Prakash and colleagues have previously reported that the protocadherin Flamingo[5] and the receptor tyrosine phosphatase dLAR[12] are involved in proper targeting of R1–6 to the lamina. However, they have not yet tested the requirement for these molecules in the target tissue. The particular contribution of these molecules in the whole process is unknown so far, but future experiments will help to understand the code that directs the formation of the crystalline array of neuronal superposition in the insect eye.

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Illuminating the calcium sensor for exocytosis in a flash

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A new paper quantifies the dependence of synaptic transmission on transient local changes in internal calcium, examining the equilibrium dynamics of the calcium sensor for exocytosis and its contribution to short-term changes in synaptic strength.

Calcium is known to be essential for synaptic vesicle release, but the quantitative relationship between exocytosis and local, presynaptic calcium has been determined in only a few central neurons[1]. For technical reasons, neurotransmitter release in these studies was triggered by a rapid but relatively sustained change in global internal calcium. Despite yielding valuable insights into the calcium-binding properties of the secretory machinery, these studies did not directly address whether the time window of neurotransmitter release is controlled by the release machinery, local calcium dynamics or both.

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In this issue, Bollmann and Sakmann evaluate the calcium concentration at the secretory machinery in response to a brief calcium transient, and show that the time course of the calcium rise is an important determinant of synaptic strength[2]. They tailored the composition of the presynaptic internal recording solution with an ingenious mixture of caged calcium and calcium buffers to produce a rapid and global calcium transient upon laser photolysis that was similar to the transient produced by an action potential. By triggering exocytosis in this way, the authors avoided the spatial inhomogeneities that are produced by local calcium entry through voltage-gated channels, and they simplified, at least in principle, their ability to estimate the calcium concentration near the release sites.

The authors further recognized that even with a low-affinity fluorescent calcium indicator dye, they could not accurately capture the calcium transient within the first few hundred microseconds after photolysis because multiple