recognized roles in regulation of carcinogenesis function as a double-edged sword, and AIM2 is no exception. Data presented here by Man et al. (2015) suggest that AIM2 is necessary to inhibit cellular, particularly intestinal stem cell, proliferation in response to carcinogens. Yet, overexpression of AIM2 can lead to increased cellular adhesion and invasiveness, which may promote metastasis (Patsos et al., 2010). Therefore, any modulation of *Aim2* expression must be tightly regulated.

Collectively, the intriguing new insights offered by Man et al. (2015) group AIM2 with a growing class of colorectal-cancer-associated immune sensors (Janowski et al., 2013). Based on their findings, interrogating how AIM2 acts in concert with other innate sensors such as NLRP3, NLRC4, NLRP6, and NLRP12 to control colorectal cancer may be the next step forward toward modulation of the innate immune system for therapeutic benefit. Nevertheless, in humans, the underlying heterogeneity and inherent nature of cancer as a multifactorial condition in which genetics and environment impinge upon each other to manifest a disease that is essentially "unique" from individual to individual poses a major challenge for cancer research. Cancer is an emergent property of the dysregulation of multiple epigenetic, transcriptional, molecular, and cellular circuits rather than the result of a single genetic event. Examining these multiple scales may enable a holistic understanding of the underlying factors and/or mechanisms that promote cancer. The road is long, but hopefully through relentless research efforts, literal meaning may be imparted to John Diamond's words—reducing cancer to a word that is no longer perceived as a "sentence."

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Brain Wiring in the Fourth Dimension

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In this issue of *Cell*, Langen et al. use time-lapse multiphoton microscopy to show how *Drosophila* photoreceptor growth cones find their targets. Based on the observed dynamics, they develop a simple developmental algorithm recapitulating the highly complex connectivity pattern of these neurons, suggesting a basic framework for establishing wiring specificity.

Large-scale efforts to precisely reconstruct the connectomes of different visual systems are uncovering a remarkable level of complexity. How this elaborate and precise wiring is established is a critical question, since the sheer number of specific connections presents a major wiring challenge. Design principles common between vertebrate and insect visual systems suggest that basic mechanisms for establishing wiring specificity may be shared between such distantly related species (Sanes and Zipursky, 2010). Using high-resolution time-lapse imaging and mathematical modeling of fly visual system neurons, Langen et al. (2015) (this issue of *Cell*) define a set of simple rules that are sufficient for wiring specificity of these neurons. Hence, a complex interplay of many specific guidance signals may not always be needed to establish precise connectivity.

The *Drosophila* visual system manifests a complex connectivity pattern of photoreceptor axons in the optic lobe and has long served as a model for how individual neurons find their appropriate synaptic partners (Hadjieconomou et al., 2011). The six outer photoreceptor neurons (R1–6) in each ommatidial unit

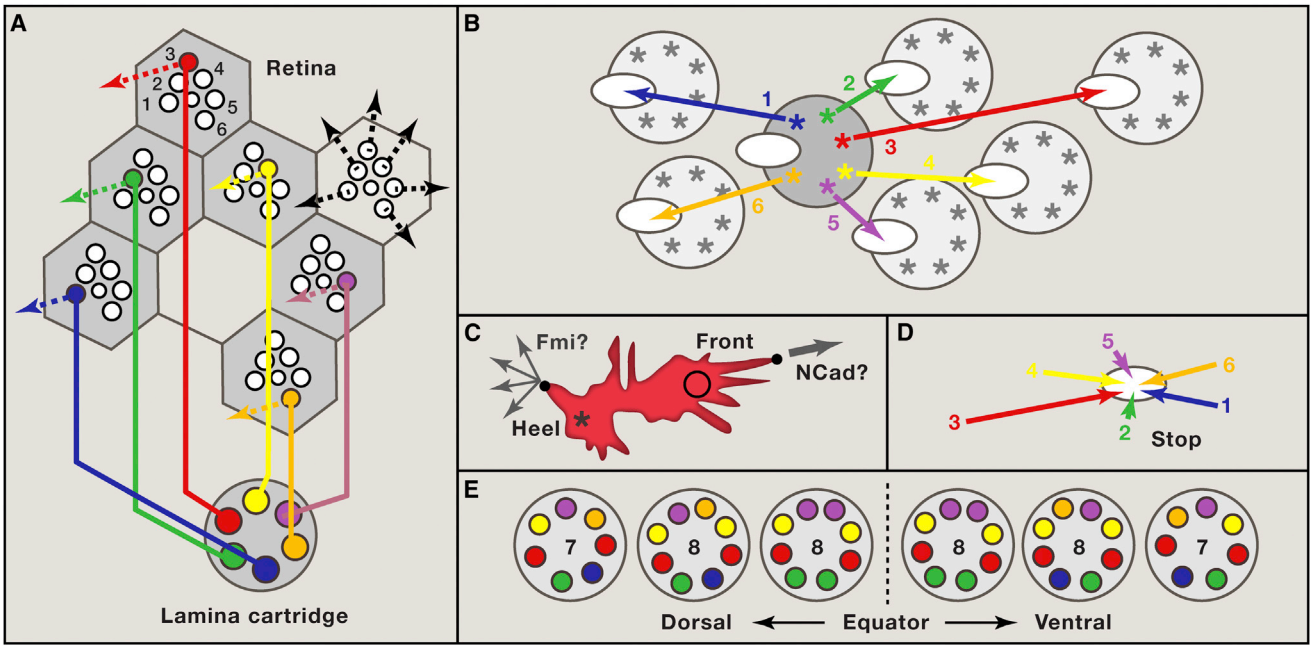

Figure 1. Intravital Imaging of *Drosophila* Neural Superposition

(A) Neural superposition: six outer photoreceptors from six neighboring ommatidia collect visual information from the same point in space (colored dashed arrows) and converge onto the same lamina cartridge. Note that the six outer photoreceptors within the same ommatidium sample different points in space (top right, black dashed arrows).

(B) The combined heels of photoreceptor growth cones (asterisks) form a scaffold defining the repetitive target areas in the lamina (white oval shapes). Different growth cones from one ommatidium must travel shorter (R2, R5) or longer distances (R3) to reach their appropriate target.

(C) Bipolar structure of photoreceptor growth cones: The "heel" structure remains anchored at the primary destination site in the lamina (asterisk), whereas the "front" migrates toward the destination column (open circle).

(D) Six photoreceptor growth cones converge to target the same lamina column.

(E) At the equator, six rows of lamina cartridges receive seven or eight photoreceptors instead of six, which is correctly predicted by the algorithm developed in this study.

of the fly compound eye sample six different points in visual space. However, the curvature of the eye is such that each of six adjacent ommatidia contains a photoreceptor that points in the same direction as its counterpart in the neighboring five ommatidia. In a process known as neural superposition, these six photoreceptors converge onto a single lamina cartridge in the optic lobe, thereby maximizing sensitivity and resolution of the visual response (Figure 1A). Thus, R1–6 axons from the same ommatidium must defasciculate, head in different directions, and travel different distances to reach six separate lamina cartridges. This process sometimes includes growth cones migrating past potential target areas to connect to more distant cartridges. Although a model has been proposed in which cell adhesion molecules ensure correct targeting by polarizing growth cones and regulating differential adhesion between R1–6 and the target cells (Clandinin and Zipursky, 2000; Schwabe et al., 2013, 2014), how such accuracy is achieved has remained mysterious. Notably, neither environmental input nor spontaneous activity plays a role in establishing the precision of this "hard-wired" connectivity (Hiesinger et al., 2006).

As target selection is a highly dynamic process, the authors reasoned that growth cone dynamics, which cannot be observed in fixed tissues, could help explain the process. Using multi-photon microscopy in intact fly pupae to image sparse GFP-labeled photoreceptor cells, they were able to follow each photoreceptor subtype over time. Surprisingly, four of the six photoreceptor subtypes manifest a bipolar growth cone structure in vivo: a stationary "heel" structure that remains anchored at the fascicle's initial entry point into the lamina and a "front" part that exhibits highly directed filopodia oriented toward the final destination cartridge (Figure 1B,C).

Anatomical mapping in combination with rigorous, large-scale quantification of filopodia dynamics, both in the heel region and at the growth cone front, led the authors to formulate a "developmental algorithm" consisting of three simple rules that specify target selection: (1) prospective target areas are defined by the sum of all growth cone heels, which form a scaffold within the lamina (scaffolding rule; Figure 1C); (2) growth cone fronts travel with remarkable constancy, with angle, speed, and developmental time window being photoreceptor subtype specific (extension rule); and (3) the most crucial question is how growth cones stop at the correct target, despite overlapping with multiple wrong targets during their extension (the stop rule; Figure 1D).

In order to understand the logic behind this final step, the authors used parameters determined by intravital imaging

to develop a mathematical model that predicts both the qualitative and quantitative outcome of photoreceptor targeting events. This model could explain why growth cones with a fairly large sensing radius overlapping with incorrect targets consistently ignore these nearby areas in order to reach their more distantly located correct target—their model recapitulates this process perfectly once one requirement is added to the model, and neurons are more likely to stop when multiple growth cones overlap onto the same target. Hence, the accurate, photoreceptor-subtype-specific timing of growth cone extension may serve to ensure that all terminals arrive simultaneously at their destination cartridge. The model predicts that correct wiring could be established even in the absence of cues from the target cells themselves, a conclusion that is at odds with previous reports that showed that a cell adhesion molecule (N-Cadherin) is required in target neurons for photoreceptor axons to stop at their proper cartridges (Clandinin and Zipursky, 2000; Prakash et al., 2005).

Computational models are most powerful when they can correctly predict how an in vivo system will react to perturbations, like mutations. However, the authors did not have to use genetics—instead, they focused on the equator of the eye, where the neural superposition pattern is naturally different. Ommatidia from the dorsal and ventral halves of the eye are mirror images of each other, meeting at the line of symmetry, the equator. As a consequence, because the angular heading of photoreceptor growth cones follows the ommatidial symmetry, more than six photoreceptors converge onto individual cartridges in the equatorial region of the lamina (four central rows have eight inputs, whereas cartridges from the next row on either side receive input from seven photoreceptors; Figure 1E). The authors show that their model correctly recapitulates the equatorial connectivity scheme, which serves as an impressive validation of their computational algorithm. Fascinatingly, their model also recapitulates the higher error rate observed specifically in the more complex equatorial columns but almost never outside this part of the eye (Horridge and Meinertzhagen, 1970; Meinertzhagen, 1972).

Taken together, this work provides an impressive example illustrating the importance of live imaging of dynamic processes. It raises the question whether certain aspects of growth cone morphology and dynamics have been missed in previous studies using fixed tissue. Nevertheless, pressing questions still remain—we need a better understanding of how the outgrowth angle, speed, and time window of each photoreceptor subtype are defined. Previous work on the role of cell adhesion molecules in both polarizing growth cones and regulating differential adhesion to targets are consistent with this study (Schwabe et al., 2013, 2014), yet the molecular mechanisms remain incompletely understood. Perturbing the expression of the atypical Cadherin Flamingo (most likely required at the growth cone "heel") or N-Cadherin (at the growth cone front, as well as in the target cells) in combination with live imaging could provide further validation of the developmental algorithm. This work has general implications toward understanding how complex wiring diagrams form in the absence of specific attractive guidance signals. Instead, a strict geometrical grid, a tight temporal control over growth cone extension, and combinatorial "stop" rules can be sufficient to define complex neural circuitry.

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