

Some of the light passing through a phase-shifted part of the mask destructively interferes with light traveling through adjoining unmodified parts of the mask. Despite important advances in shorter-wavelength exposure systems, sophisticated mask technologies, and more sensitive photoresists (6), there is considerable interest in alternative lithography approaches that can achieve nanoscale resolution with longer-wavelength optical sources, which have much lower maintenance and cost.

The main concept behind the “subdiffraction” resolution approaches reported in this issue is the use of dual exposures to create a spatial exposure pattern. These beams can have a donutlike pattern—the beam that activates the patterning chemistry is surrounded by a ring of intensity of another beam that suppresses the activation while maintaining a valley (or node) at the center of the activating beam. The product of the activation peak and “deactivation” donut pattern gives a spatial dosing pattern that is substantially finer than the far-field diffraction pattern of a tightly focused optical beam.

Subdiffraction resolution is achieved in optical microscopy by using a pair of beams as described above and taking advantage of stimulated emission depletion of chromophores (7). One beam is used for excitation of fluorescence with a peaked spatial distribution, and one with a donutlike pattern is used for rapid de-excitation via stimulated emission. The net

spatial distribution of the excitation provides subdiffraction (nanoscale) imaging resolution.

The studies reported in this issue adapt these ideas for subdiffraction lithography by using photoinhibition or photoinduced absorption, rather than stimulated emission, to narrow the exposure profiles. Scott *et al.* report an optical lithography method based on the use of two wavelengths: One, at 473 nm, excites a photoinitiator that activates free-radical polymerization and gelation of a dimethacrylate monomer and renders the exposed area insoluble (see the figure, panel A). The second beam, with a wavelength of 365 nm, excites a photoinhibitor, which then scavenges free radicals and stops the reaction. Li *et al.* used a different initiator molecule that allowed both beams to share the same wavelength (800 nm). Activation is achieved with an intense initial beam (200 fs) that proceeds through a two-photon process, while long-duration 50-ps or continuous-wave light deactivates the reaction through a one-photon process (see the figure, panel B). This approach created features 1/20 the size of the wavelength of light along the beam direction through beam shaping.

Andrew *et al.* used exposure of a photochromic film at two wavelengths (see the figure, panel C). Ultraviolet light at 325 nm caused the film to become more transparent at that wavelength, whereas red light at 633 nm caused it to become strongly absorbing at the

UV wavelength. By setting up a simple grating interference pattern with peaks of the 325-nm light occurring in the valleys of the 633-nm light and controlling the relative intensities of the beams, nanoscale regions are obtained at the nodes of the red light where the UV light is transmitted. Features as small as 40 nm in width were created in an underlying photoresist layer.

How and when these lithographic schemes will enter into chip fabrication is hard to predict. However, these methods should already offer alternatives to the methods now in use, such as electron-beam lithography and micro-contact printing, for creating nanoscale features in a lab setting. It may well be possible to harness these ideas to create lithographic schemes that will shrink the feature sizes obtainable with shorter-wavelength ultraviolet sources.

References

1. K. Suzuki, B. W. Smith, Eds. (CRC Press, Boca Raton, FL, ed. 2, 2007).
2. T. F. Scott *et al.*, *Science* **324**, 913 (2009); published online 9 April 2009 (10.1126/science.1167610).
3. L. Li *et al.*, *Science* **324**, 910 (2009); published online 9 April 2009 (10.1126/science.1168996).
4. T. L. Andrew *et al.*, *Science* **324**, 917 (2009); published online 9 April 2009 (10.1126/science.1167704).
5. M. Totzeck, W. Ulrich, A. Goehnermeier, W. Kaiser, *Nature Photonics* **1**, 629 (2007).
6. D. Bratton, D. Yang, J. Dai, C. K. Ober, *Polym. Adv. Technol.* **17**, 94 (2006).
7. S. W. Hell, *Science* **316**, 1153 (2007).

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NEUROSCIENCE

Crossing the Line

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A key process in animal evolution was the development of a nerve-rich, bilaterally symmetric longitudinal structure, the central nervous system. Without such a symmetric body axis, Earth might still be populated by just sea anemones, sponges, and similar organisms (1). The switch from radial to bilateral symmetry created a distinct left- and right-hand side to the animal and its nervous system. The dividing line, or axis of symmetry, is known as the midline. One of the earliest decisions a developing neuron must make is whether to extend its long cellular process (the axon) across the midline. On page 944 of this issue, Yang *et al.* (2) uncover an unexpected level of complexity in how this initial decision is made.

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Commissural axons connect the two sides of the nervous system and are attracted to grow toward the midline by netrin proteins. In the fruit fly *Drosophila melanogaster*, Netrins are secreted by specialized glial cells at the midline and are detected by Frazzled/DCC (Fra) receptors on commissural axons (3) (see the figure). As they cross the midline, axons increase expression of Robo-family receptors. These receptors detect Slit, a protein that repels axons. Slit is also secreted by midline glial cells, thereby causing axons to continue growing to the other side of the nervous system (4).

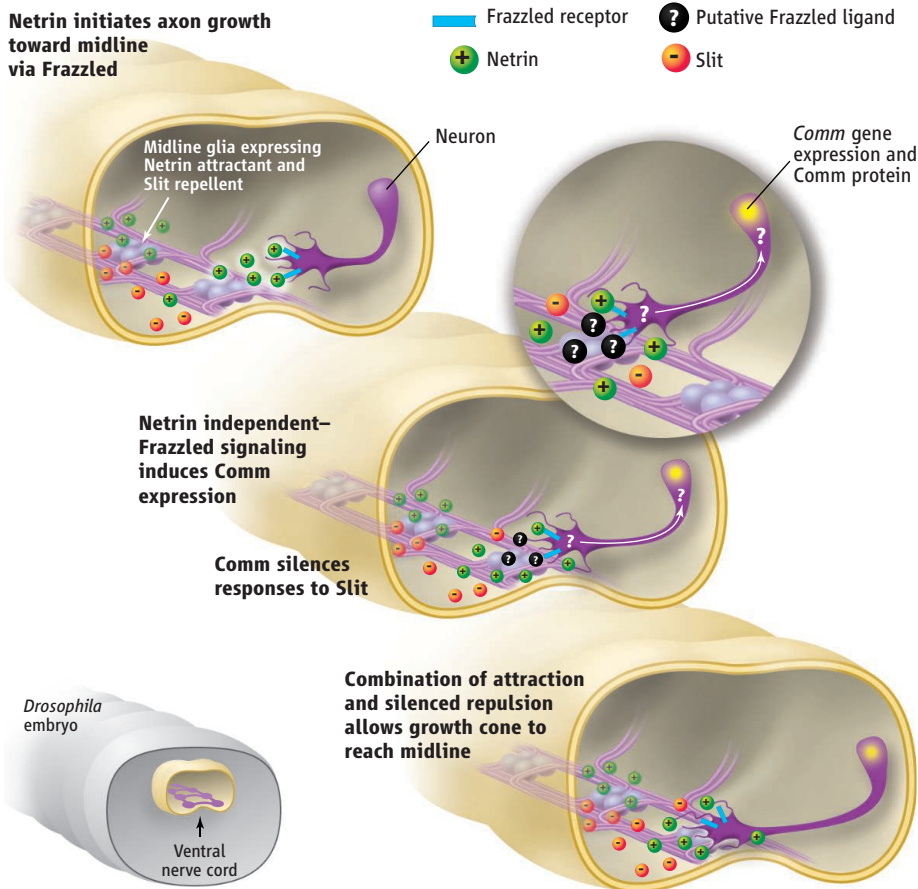
Mutations in the gene *commis sureless* (*comm*) that disrupt expression of the encoded protein Comm abolish all left-right connections in the ventral nerve cord, the fly homolog of the spinal cord (5). Comm promotes midline crossing by silencing axon responses to the midline repellent Slit (6, 7). In the absence

An axon guidance receptor generates a transcriptional response independent of its known ligand.

of Comm, Robo receptors are expressed at the cell surface of commissural axons prematurely, thus allowing Slit-Robo interactions and preventing midline crossing. It appears that commissural neurons begin to express Comm as they approach the midline, and then decrease Comm expression after crossing to allow continued axon growth (7).

A Fra receptor that lacks its cytoplasmic domain (Fra Δ C) also inhibits midline crossing, producing a phenotype far more severe than the *fra* mutant (which lacks Fra receptors), and identical to the phenotype of the *comm* mutant (8). Genetic evidence suggested that Fra Δ C and Comm could act in the same intracellular signaling pathway. This prompted Yang *et al.* to determine that expression of *comm* mRNA in individual neurons is reduced when *fra* is absent. In a complementary experiment, overexpression of *fra* induced *comm* mRNA

Netrin initiates axon growth toward midline via Frazzled



Midline crossing. A schematic of a *Drosophila* ventral nerve cord, in which longitudinal tracts of nerves (axons) are connected in a repeated fashion by midline-crossing (commissural) axons. A commissural axon is shown at successive stages of development. Molecular events in the actively moving tip of the axon are depicted with Fra binding Netrin and a putative unidentified ligand.

expression. Furthermore, neuronal expression of *comm* partially rescued *fra* mutants, showing that Fra induces *comm* expression. Remarkably, however, *comm* expression does not require Fra's ligand Netrin.

What is the signaling pathway that connects Fra to Comm? If the absence of Fra was simply preventing growing axons from contacting the midline and detecting a signal that induces *comm* expression, then expression of *comm* should also be reduced in *netrin* mutants, but Yang *et al.* did not observe this. Furthermore, many axons still cross the midline in *fra* mutants, so they must have been exposed to a candidate midline signal, yet the axons show reduced *comm* expression. The presence of reduced *comm* expression in *fra* mutants indicates that it is not necessary to induce *comm* expression to the level seen in wild-type fly commissural axons to allow midline crossing, and that an additional mechanism also induces *comm* expression.

Why is the FraΔC phenotype so much stronger than that of the *fra* mutant? The likely explanation is that FraΔC interferes with the functions of other proteins. One candidate is

the Down syndrome cell adhesion molecule (Dscam), another receptor expressed by commissural axons. Dscam forms a complex at the cell surface with a protein called Deleted in Colorectal Cancer (9). Dscam responds to an unidentified midline cue as well as to Netrins (10), but Dscam alone cannot account for the severity of the FraΔC phenotype, so other components remain to be discovered.

Although we know some of the transcription factors that specify the complement of receptors expressed in the growing axon tip (growth cone) (11), the study by Yang *et al.* may be the first to show that neuronal gene expression is altered by an external signal encountered during navigation of the growth cone toward the midline. The nature of this external signal is unknown; however, the strong correlation between proximity to the midline and ability to induce *comm* expression suggests a membrane-anchored or short-range midline cue as the most likely explanation.

Identification of a Netrin-independent function for Fra opens up possibilities for identifying novel axon guidance mechanisms. Fra is large enough to accommodate a binding site

for an additional ligand, and the number of external signals that control axon guidance is relatively limited. The Netrin-independent intracellular signaling mechanism does not require any of the previously identified cytoplasmic motifs (sites of interaction with other proteins) in Fra, suggesting a yet unknown signaling pathway. The Fra homolog Neogenin can be cleaved by γ -secretase to produce an intracellular fragment that can regulate gene transcription (12), and such a mechanism might be evolutionarily conserved.

In vertebrates, initial midline crossing appears to be achieved by a different mechanism. A splice variant of Robo3/Rig-1 inhibits Slit repulsion in axons as they approach and cross the midline (13). However, concentrations of Robo1 and Robo2 increase after axons cross the midline (14), suggesting that a mechanism similar to that of Comm could be operating. No *comm* homolog has been found in vertebrates, but even within insects, *comm* sequences are highly divergent.

The next questions include determining the nature of Netrin independent–Fra activation, how this activation transduces a signal to the nucleus, and what signal turns *comm* expression off after midline crossing. Amazingly, Robo proteins engineered to be insensitive to regulation by Comm do not prevent midline crossing (7), suggesting that Comm targets other components required for responding to Slit. In *netrin* mutants, many axons still cross the midline, indicating the existence of a yet unknown midline attractant. This is likely to be a diffusible cue, because in the absence of Netrins, growth cones still orient toward the midline even when at a distance (15). Clearly, the midline still guards many secrets as to how multiple overlapping systems ensure that the decision to cross the midline is made with a high degree of accuracy.

References

1. A. Ghysen, *Int. J. Dev. Biol.* **36**, 47 (1992).
2. L. Yang, D. S. Garbe, G. J. Bashaw, *Science* **324**, 944 (2009); published online 26 March 2009 (10.1126/science.1171320).
3. S. W. Moore *et al.*, *Adv. Exp. Med. Biol.* **621**, 17 (2007).
4. B. J. Dickson, G. F. Gilestro, *Annu. Rev. Cell Dev. Biol.* **22**, 651 (2006).
5. G. Tear *et al.*, *Neuron* **16**, 501 (1996).
6. K. Keleman *et al.*, *Cell* **110**, 415 (2002).
7. G. F. Gilestro, *PLoS One* **3**, e3798 (2008).
8. D. S. Garbe *et al.*, *Development* **134**, 4325 (2007).
9. A. Ly *et al.*, *Cell* **133**, 1241 (2008).
10. G. L. Andrews *et al.*, *Development* **135**, 3839 (2008).
11. S. J. Butler, G. Tear, *Development* **134**, 439 (2007).
12. D. Goldschneider *et al.*, *Mol. Cell. Biol.* **28**, 4068 (2008).
13. Z. Chen *et al.*, *Neuron* **58**, 325 (2008).
14. C. Sabatier *et al.*, *Cell* **117**, 157 (2004).
15. M. Brankatsch, B. J. Dickson, *Nat. Neurosci.* **9**, 188 (2006).

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