In Brief

Multiple Actin Bundlers

Forked and Fascin Act Sequentially

Bundles of parallel actin filaments can impart strength and permanence to structures such as microvilli, stereocilia of the inner ear, and bristles. Large actin bundles can form in *Drosophila* bristles in less than an hour. But fascin, one of the bundling proteins present in the bristle, yields only a small bundle over several days in vitro. One reason for the difference appears to be the presence of multiple bundling proteins in the bristle.

Tilney et al. (page 121) examine various *Drosophila* mutants by electron microscopy to determine the role of the two bundling proteins, forked and fascin, in bristle development. The initial bundling events require forked. Fascin is then needed for ordering the large bundles into a hexagonal array, an arrangement that allows maximal cross-linking of the helical filaments and results in straight and rigid bristles.

The system is sensitive to the level of forked. Too much forked results in gaps, suggesting that the process of grouping smaller bundles together, or polymerization in the gaps, is somehow perturbed. Halving the dosage of forked gives bundles that are the right size but disordered, with little fascin, as if forked was needed for fascin to gain access to the bundle. Fascin may zipper up a filament once it gets started since the few filaments that do have fascin have it all along their length.

Tilney et al. suggest that the building of a hexagonal array may require a trial-and-error process of binding, release, and rebinding of a weak but ultimately rigid link (fascin), even as the bundle is kept intact by a tight-binding but flexible link (forked). The next test will be to reproduce this behavior in vitro.

Small Espin as a Late Modification

In the brush border of the intestinal epithelium, villin and fimbrin are comparable to *Drosophila* forked and fascin. Villin appears first, then fimbrin. Bartles et al. (page 107) add a third bundling protein to this story. Small espin is a shorter version of espin, possibly a splice variant, with the forked homology domain intact. Its in vitro actin-bundling activity (and therefore two actin-binding regions) can be localized to just 116 amino acids near the COOH terminus. Actin binding is tight (the K_d is 50 nM) and calcium insensitive. The calcium-sensitive villin and fimbrin are well on their way to building the actin bundles by the time that small espin accumulates, but the latecomer may add strength and make the bundles insensitive to some later calcium-mediated event.

Less Order and More Movement in the Nucleus

Transcription throughout Chromosome Territories

Individual chromosomes are restricted to nonoverlapping regions of the nucleus called territories. If transcription oc-curred only on the outside surface of these territories, RNA transcripts could be funneled efficiently to nuclear pores, via interchromosomal channels. Support for this hypothesis has, however, rested on the measurement of the position of just a handful of genes.

On page 5, Abranches et al. find that a chromosome or chromosome arm from rye shows no such distribution of transcription sites, at least when it is in a wheat cell. The wheat and rye chromosomes are strongly oriented across the nucleus, with centromeres and telomeres at opposite ends of the cell. This "Rabl" orientation is established by the movement of chromosomes in mitosis and is maintained through multiple cell cycles.

"If the territory hypothesis of transcription is correct, it ought to be blindingly obvious in a nucleus where chromosomes are so highly organized," says senior author Peter Shaw. But staining for incorporated bromouridine triphosphate reveals hundreds of spots scattered throughout the nucleus. There is no clustering on the outside of a chromosome territory, as defined by a rye genomic DNA probe.

The existing physical map of wheat suggests that the telomeric portions of the chromosomes are gene rich, so there should be more transcription foci at the telomeric end of the nucleus. Abranches et al. find no evidence for such polarization. "We are not saying that the physical map is wrong," says Shaw, "but that if it is right, these genes are moving to be transcribed."

Large-Scale Chromosome Movements in Interphase

In *Drosophila* larval neurons, Csink and Henikoff see a more mobile set of chromosomes (page 13). For example, the Rabl configuration in these cells breaks down within 2 h of mitosis.

Another chromosome movement involves the *brown* gene of *Drosophila*. This gene can be silenced by association of a nearby block of heterochromatic DNA with centromeric heterochromatin. Loss of this contact had been presumed to occur because of mitosis, but Csink and Henikoff find that the two regions dissociate early in S phase.

Reestablishment of the contact occurs between 5 and 18 h after mitosis. The delay may reflect the slow reaccumulation of sticky factors, such as heterochromatin protein 1 (HP1), as most of the HP1 is lost from heterochromatin during mitosis. Thus, certain heterochromatic silencing phenomena may be restricted to a window in late G1 phase.

Interphase chromosomes can diffuse, but this diffusion was thought to be confined to a small subset of the nucleus. How regions of *Drosophila* chromosome 2 range across the larger distances involved in *brown* silencing is unknown.

Bax Insertion into Mitochondria

Bax is a good candidate for a mitochondrial initiator of apoptosis. It can form a channel in a lipid bilayer, it causes

release of cytochrome c from mitochondria (which helps induce activation of the caspase destruction machinery), and it induces apoptosis when overexpressed.

But Bax only associates stably with mitochondria after cells have received an apoptotic signal. Goping et al. (page 207) find that two Bax constructs—one lacking the first 19 amino acids, and a second in which the transmembrane domain of Bax is replaced with that of the antiapoptotic Bcl2—can insert into mitochondria in the absence of this apoptotic signal. Insertion of full-length Bax is stimulated by caspase activity. The caspase does not cleave Bax itself; a hypothetical regulator that targets the NH_2 terminus of Bax is a more likely substrate.

Cleavage of Bid, another apoptosis inducer, has recently been shown to trigger its targeting to mitochondria and subsequent release of cytochrome c (Li, H., H. Zhu, C.-j. Xu, and J. Yuan. 1998. *Cell*. 94:491–501; Luo, X., I. Budihardjo, H. Zou, C. Slaughter, and X. Wang. 1998. *Cell*. 94:481–490). Bid is cleaved by caspase 8, which is directly activated as part of a complex with the death receptor Fas. Bax may retain its status as an apoptosis initiator if there is a similar mechanism upstream of it. Alternatively, Bax may be devoted to amplification of cytochrome c release that was initially triggered by other means.

PtdIns(3,5)P$_2$ Gets a Kinase and a Function

Phosphatidylinositol (PtdIns) phosphates have profound effects on signaling and trafficking, so last year's discovery of a new variant, PtdIns(3,5)P$_2$, was significant. Now Gary et al. (page 65) identify Fab1p as the yeast kinase that generates this phospholipid from PtdIns(3)P, based on the lack of PtdIns(3,5)P$_2$ in *fab1* deletion and temperature-sensitive mutants.

Without Fab1p, the yeast vacuole enlarges to take up the majority of the cell volume. Anterograde trafficking to the vacuole is intact. This confirms that the precursor PtdIns(3)P, not the product PtdIns(3,5)P$_2$, is used as a signal for Golgi to vacuole transport. PtdIns(3,5)P$_2$ may induce membrane invagination and destruction in the vacuole or direct a recycling pathway from the vacuole to the endosome or Golgi.

On a first attempt, neither Fab1p nor yeast extracts show in vitro PtdIns(3)P 5-kinase activity, perhaps because Fab1p, or an unstable activator, or both are labile. Candidate activators include the vacuolar proteins Vac7p and Vac14p since their loss results in phenotypes similar to that of a *fab1* deletion.

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