We can deplete it, we have the technology

More can be more, based on two comprehensive RNAi studies of motors and actin-regulating proteins in fly S2 cells—a particularly robust system for RNAi techniques and a convenient cell type for cytological studies.

The first of the two papers, by Goshima and Vale (page 1003), examines the role of every fly kinesin during mitosis—the first such study done in higher eukaryotic cells. The group inhibited 25 kinesins plus dynein, individually and in combination, and examined the lines by live cell imaging. Loss of eight individual motors affected cell division, and three kinesins were absolutely required: BimC/Eg5 (which forms the bipolar spindle), Kip3 (which keeps spindle microtubules from overgrowing), and MKLP1 (which forms and maintains the central spindle necessary for cytokinesis).

The work shows that cells have a backup plan in case spindle formation fails. RNAi of several kinesins caused monopolar spindles to form, but many of these cells reverted to bipolar spindles. A small percentage of wild-type cells also formed and then rescued monopolar spindles. In both cases, acentrosomal poles were formed (as during meiosis and in dividing plant cells), probably through BimC/Eg5-mediated microtubule bundling near the chromosomes.

Double and triple mutants—easily created using RNAi—revealed that chromosome alignment on the metaphase plate is directed by three kinesins (CENP-E, Kid, and chromokinesin) that have partially overlapping functions. Chromosome alignment was completely disrupted only upon triple RNAi of all three motors.

Redundancies were also easily identified using RNAi in the second study, by Rogers et al. (page 1079), which examined the contribution of 90 actin-regulating proteins during lamella protrusion. The findings reveal that either of two independent pathways can activate SCAR, an Arp2/3-activating protein needed for lamella formation. The group also found that SCAR is degraded in the absence of the inhibitors (kette, Sra-1, and Abi), possibly to prevent uncontrolled actin polymerization. In total, seven phenotypic classes were seen from RNAi of 20 of the proteins tested in this study. Live cell imaging should reveal further insight into the function of these proteins.

When nuclear proteins go mitotic

Two articles in this issue indicate that proteins hiding in or around the nucleus in interphase have critical functions during mitosis.

The first of the mystery proteins is the nucleoporin Nup358, a component of the filaments on the cytoplasmic face of the nuclear envelope. On page 991, Salina et al. show that loss of Nup358 does not block nuclear import but does impair mitosis. During division, cells lacking Nup358 formed abnormal spindles and stalled in metaphase.

Several nuclear pore complex proteins associate with kinetochores in dividing cells, including Nup358. Indeed, the division problems stemmed from abnormal kinetochores. Without Nup358, kinetochore morphology was perturbed, and kinetochore proteins such as dynein, CENP-E, and Mad2 were mislocalized. That the mitotic checkpoint was activated in these cells bolsters the recent argument that checkpoint proteins can signal from the cytoplasm.

The authors postulate that loss of Nup358, a RanGAP-binding protein, could increase local RanGTP levels at chromosomes. Ran is essential for microtubule capture by kinetochores. Thus, the kinetochore errors may be indirect effects of changes in the levels of Ran.

A second nuclear protein with a mitotic function is identified on page 1017 by Raemaekers et al. This novel protein, NuSAP, is found in the nucleoli in interphase cells but is needed for spindle formation and chromosome segregation. The spindle defects in cells lacking NuSAP may stem from a loss of stable microtubule bundles at the central spindle, where the protein sat during mitosis.

Storing mitotic proteins in the nucleus might be a simple way for the cell to separate interphase from mitosis. Through nuclear envelope breakdown, proteins crucial for spindle and kinetochore formation are released, thus committing the cell to division. Conversely, nuclear sequestration could prevent untimely interphase activities, such as the bundling of cytoplasmic microtubules that occurs when NuSAP is overexpressed.
The glue that builds bristles

An actin cable is the sum of its parts, according to Guild et al. (page 1069), who find that short bundles of actin filaments are grafted together to form extra long actin cables in fly bristle cells. Bristle cells, which can reach lengths of up to 400 μm, are initially supported by an assembly of multiple short stretches of polarized actin bundles. By looking closely at these bundles as they break, the authors see that individual modules of short bundles are assembled by a grafting-like mechanism. In bent cables, the modules separated slightly to reveal the tapered ends of overlapping bundles, suggesting that the modules are not connected by simple end joining.

The group found that two initially unconnected bundles are joined as one extends over the end of the adjacent bundle. Overlapping bundles are then grafted together by fascin and forked, cross-linking proteins that also bundle individual actin filaments. The grafts are hidden by the addition of more filaments to the original bundles, so that the entire cable appears to be one continuous, smooth entity.

The total length of short cellular extensions, such as microvilli in intestinal cells or stereocilia in hair cells, may be determined by the length of a single actin cable. But overlap and grafting probably create a long yet flexible cable that can either curve, as do bristle cells, or contract by sliding, as do actin cables in fly nurse cells.

More order in DNA

On page 981, Shopland et al. identify a new level of nuclear organization that positions highly transcribed DNA and chromosome segments near proteins needed for mRNA maturation.

Splicing factors and other proteins that process newly made transcripts accumulate in nuclear speckles called SC-35 domains. Shopland et al. find that these domains associate with R-bands, which are cytologically visible, gene-rich, and highly transcribed chromosome regions. Transcripts from both linked and unlinked genes were found within a given SC-35 domain. This shared usage indicates that the domains are not simply spots of RNA metabolic factors at an especially active locus, but are rather organized domains that may promote efficient transcript processing. Clustering of genes with numerous individual components of the large transcription and mRNA processing complexes probably hastens complex assembly and thus increases the efficiency of mRNA maturation.

Certain hyperactive genes may be targeted to or nucleate SC-35 domain formation and then promote the association of the neighboring chromosomal region with the domain. The highly transcribed COL1A1 gene was consistently associated with a domain, although sequences within the same R-band also contacted the domain ~80% of the time. The organization of DNA to optimize the association of active gene regions with SC-35 domains may be the best explanation yet for the evolutionary origins of R-bands versus gene-poor G-bands.

Finding the axon

If you’ve seen one microtubule, you’ve seen them all. Not true, according to Nakata and Hirokawa, who demonstrate on page 1045 that a unique set of microtubules is crucial for protein targeting in neurons.

In neurons, vesicle trafficking must be unusually precise to move proteins efficiently to the axonal plasma membrane, because the entrance to the axon is so narrow. This precision is now shown to be directed by a set of densely packed microtubules extending from the cell body into the proximal portion of the axon. These microtubules preferentially bind KIF5, the motor that carries axon-bound post-Golgi vesicles.

The unique chemical nature of the microtubules that attracts KIF5 is not yet clear. In addition to an unusually high turnover rate (the inhibition of which redistributed axon-directed transport), the population had higher levels of the tip-binding protein EB1. Although it is possible that EB1 somehow directs KIF5 binding, the authors believe this is not the case. They suggest instead that posttranslational modification of tubulin or cross-linking proteins may be involved. Although the KIF5-binding microtubule population was noted only in neurons, other cell types (e.g., motile fibroblasts or activated T cells) may use different microtubule/kinesin pairs to regulate polarized vesicle trafficking.