Aurora B puts chromosomes in their place

Small molecule inhibitors of Aurora B activity, characterized by Hauf et al. (page 281) and Ditchfield et al. (page 267), reveal that the mammalian kinase and its budding yeast counterpart, Ipl1, have similar functions. Without Aurora B, mistakes in kinetochore–chromosome interactions go uncorrected.

Early evidence of a function for the Aurora family in correcting syntelic attachments, those in which both chromatids are attached to the same spindle pole, was provided by the ipl1 mutant. But visualizing spindle–kinetochore attachments in yeast is difficult. The two articles in this issue examine attachments directly, by inhibiting Aurora B in mammalian cells.

The groups used different compounds, but in both cases the Aurora B inhibitors left chromosomes misaligned and compromised the spindle checkpoint, thus causing division failure and endoreduplication. Hauf et al. saw that syntelic attachments were more common in inhibitor-treated cells. They hypothesize that Aurora B senses the lack of tension between syntelic sister chromatids and destabilizes either one or both so that correct attachments can be established. If the checkpoint is activated by unattached kinetochores, its override by Aurora B inhibition may be an indirect result of stable syntelic attachments. Indeed, drugs that destabilize microtubules restored checkpoint function in the presence of the inhibitors, at least in the short term.

Aurora B may also have a more direct effect on the spindle checkpoint through BubR1 or other kinetochore proteins. Low tension between sister chromatids normally leads to recruitment of BubR1 to kinetochores. But BubR1 was absent from kinetochores in the presence of either inhibitor. Ditchfield et al. show that RNA interference of BubR1 caused a chromosome alignment defect resembling that seen in cells treated with their Aurora B inhibitor. It is possible that BubR1 not only monitors kinetochore–microtubule interactions but also regulates them in response to changes in Aurora B activity.

To grow or to shrink…

Looks can be deceiving. According to two articles in this issue, proteins that look like microtubule stabilizing proteins at times do just the opposite, revealing activities that can both build and destroy microtubules.

Originally described as a Xenopus microtubule stabilizing protein, XMAP215 is a defining member of a large family of microtubule-associated proteins. Depletion of XMAP215 or its homologues leads to decreased spindle microtubule length in several systems, including fly, yeast, and worm. On page 349, however, Shirasu-Hiza et al. find that XMAP215 also promotes depolymerization of microtubules stabilized with a nonhydrolyzable GTP analogue (GMPCPP). This destabilizing activity, like its stabilizing activity, is specific to microtubule plus ends. The new work recalls a 10-year-old report demonstrating that XMAP215 has both activities in vitro.

Sirasu-Hiza et al. used EM analysis to reveal a structure that supports a peel–like mechanism of XMAP215, similar to that of Kif11 kinesin. Previously, the plus ends of microtubules stabilized by GMPCPP have been thought to resemble a “GTP cap,” a structure postulated to exist at the ends of growing microtubules. Here, the authors suggest instead that GMPCPP-stabilized structures may mimic a “paused state”—a hypothetical third state in microtubule dynamics, intermediate between the growing and shrinking states. They propose that XMAP215 destabilizes this paused state and increases either polymerization or depolymerization rates depending on cellular conditions, thus explaining its dual activities.

On page 359, van Breugel et al. find another XMAP215 family member with destabilizing activity—the budding yeast homologue Stu2p. In vitro, Stu2p depolymerized microtubules by binding directly to plus ends, probably hindering tubulin dimer addition and thus increasing catastrophe rates. In contrast to the short spindle microtubules seen previously in stu2p mutants, cytoplasmic microtubules of stu2p interphase cells are longer than those in the wild type. Thus, for both yeast and frog proteins, cellular context, such as cell cycle status or protein localization, may determine their effects on microtubules. It remains to be seen whether destabilizing activity has been overlooked in other family members.
H. pylori mobilizes cells

H. pylori infects the gastric track of more than half of the human population and is associated with an increased occurrence of invasive gastric cancers. On page 249, Churin et al. explain how this widespread bug turns tumors metastatic by corrupting a growth factor receptor.

H. pylori mobilizes infected epithelial cells on its course to pathogenicity. The authors now show that mobilization results from activation of the hepatocyte growth factor (HGF) receptor c-Met. During development and differentiation, HGF-induced activation of c-Met initiates cell migration events. Inhibition of c-Met expression by siRNA blocks H. pylori–induced motility. H. pylori corrupts c-Met signaling, however, by injecting host cells with the protein CagA. Binding of CagA to c-Met resulted in modulation of receptor activity and recruitment of PLCγ, a mediator of cell polarity necessary for motility. CagA–PLCγ interactions mobilized infected cells through an ERK-dependent MAP kinase pathway, as inhibitors of MAP kinases or PLCγ blocked motility.

Unlike the gastric tumor cell line, a polarized canine kidney cell line does scatter in response to HGF. H. pylori also induced c-Met–mediated motility in these cells, but did so through a PI3K-dependent pathway rather than through PLCγ, indicating that the bug uses alternative routes to motility depending on the cell type. The authors hope to look at animal models next to determine whether inappropriate activation of c-Met and the resulting mobilization of gastric cells is responsible for increased incidences of metastatic cancers in H. pylori infections.

Choosing replication origins

After mitosis, cells prepare for the next round of DNA replication by assembling complexes of proteins on chromatin that will carry out the task of copying the genome. Pre-replication complexes (preRCs) assemble during telophase, but studies of the dihydrofolate reductase locus in mammalian cells have indicated that, during G1, only a subset of preRCs are designated for use as origins of replication. On page 257, Li et al. demonstrate that the same is true for origins throughout the genome.

Li et al. compared origins used by mammalian cells in vivo with those chosen in isolated mammalian nuclei undergoing premature replication in frog cytoplasmic extracts. Comparison of the two sets of origins revealed that, soon after mitosis, few of the sites used matched. About two hours after mitosis, the sites used in vitro were clustered into domains surrounding in vivo sites, but the actual sites fired still did not necessarily correspond. This domain selection was previously suggested by examinations of the timing of replication of whole chromosomal domains and is known as the timing decision point.

Five hours after mitosis, the sites chosen in vitro were more likely to be the same sites used in vivo. This site selection is known as the origin decision point. Thus, as for the DHFR locus, the general choice of origins used is not random, but why origin selection should be regulated is unclear. Perhaps origins are placed near genes that encode replication proteins so that they are activated early by passage of the replication fork. Alternatively, origins may be regulated to prevent collision of RNA and DNA polymerases. If the latter is true, origin choice may change during differentiation to accommodate transcriptional differences.

Say NO to insulin

Insulin stimulates its own secretion from pancreatic β cells through rapid nitrosylation of glucokinase (GK), according to results by Rizzo and Piston on page 243.

When glucose levels are low, an inhibited form of GK associates with insulin-containing granules in pancreatic β cells. Rizzo and Piston have determined that this localization is mediated through interaction with neuronal nitric oxide synthase (nNOS). Insulin treatment disrupted this association through nitrosylation of a GK cysteine residue. Activation of nNOS and the resulting nitrosylation of GK may be mediated through a rise in intracellular calcium, a known response of PLCγ and the accompanying conformational change—both of which required NO production—activated GK.

GK induces secretion of the granules, thus promoting local increases in insulin levels. A recently developed drug for the treatment of type II diabetes is a GK activator. The new results suggest that the drug may activate GK by preventing granule binding. Piston plans to test this possibility in the near future.

NO regulates association of GK (yellow) with insulin secretory granules (blue).