In This Issue

Shockingly localized

Upon activation by stress, heat shock factor 1 (HSF1) accumulates at a specific heterochromatic locus on human chromosome 9, according to Jolly et al. (page 775).

The final target of HSF1 is DNA, in the form of the heat shock element (HSE), and this binding event is known to activate transcription of adjacent heat shock protein (hsp) genes. But heat shock also causes HSF1 redistribution into nuclear loci known as HSF1 granules. Jolly and colleagues have now shown that the granules are located at chromosomal DNA sites that are distinct from RNA polymerase II transcription sites, meaning they are unlikely to be related to transcriptional activation. The HSF1 granules were found on the 9q11 region, which is primarily composed of heterochromatic satellite III repeats.

What could transcriptional activators be doing at heterochromatic regions following heat stress? Jolly hypothesizes that heterochromatic localization may provide a buffer to avoid over-activation of HSF1, which can be toxic to the cell. Alternatively, she suggests, the transcription factor may have a secondary role as a protective agent for the locus, preventing damage to a DNA region known to be prone to chromosomal rearrangements.

Actin to completion

The actin cytoskeleton is alone on the cytokinesis stage no longer. The final step of cytokinesis cannot be completed without interactions between actin and a microtubule-interacting protein, CHO1, according to a new study by Kuriyama et al. on page 783.

Organization of microtubules into the central spindle is achieved in part by the MKLP1 microtubule-binding motor protein. Kuriyama has determined that alternative splicing of MKLP1 generates CHO1, which has an additional 100 amino acids in the COOH-terminal tail. The group shows that MKLP1 and CHO1 are coexpressed within mammalian cells, both tightly associated with microtubules.

Although CHO1 associates with microtubules, Kuriyama et al. show that it is not required for chromosome separation. The unique CHO1 region imparted an unexpected actin-binding activity both in vitro and in vivo. Inhibition of this actin binding did not impair early steps in cytokinesis, but did prevent the final stage. Although the daughter cells initially appeared to separate completely, within hours the two had merged.

The bud is all in the timing

A new study by Schenkman et al. on page 829 reveals that the subcellular localization of yeast budding proteins can be determined by the time the genes are expressed.

The genes BUD8 and BUD9 are required for establishment of the bipolar budding pattern in normal diploid cells, in which both poles are specified as potential bud sites. Although the two proteins are similar in structure, they show distinct subcellular localizations, consistent with functions as markers of the distal and proximal poles of daughter cells.

Using promoter swap experiments, Schenkman et al. now show that the timing of gene expression determines the localization of Bud8p and Bud9p. The localization of the proteins is probably determined by the direction of the general secretory vesicle traffic at the stage of the cell cycle when the protein is delivered. Although new Bud8p is delivered shortly before or concurrent with bud emergence, and thus will be found at the distal end of the bud, new Bud9p is deposited to the bud side of the neck very late in the cell cycle, and is thus found at the proximal pole of new daughter cells.

The group’s analysis also determined that the peak in transcription of the BUD8 and BUD9 mRNAs precedes the delivery of the corresponding proteins to the cell surface by about an hour. This could be accomplished by a delay in either the translation of the protein or the trafficking of the protein to the cell surface.
Enabling actin

Maybe there can be too much of a good thing. A new report from Woodring et al. (page 879) provides the basis for a self-limiting interaction between F-actin and c-Abl. Although the authors find that c-Abl is an important activator of actin polymerization, the resultant F-actin can shut down c-Abl, thus completing a feedback loop that limits actin polymerization.

The rapid assembly and disassembly of actin fibers is required for controlling cell migration, changing cell morphology, and extending the dendrites and axons of neurons. Some extracellular signals that cause alterations in the F-actin cytoskeleton, such as integrin clustering, have also been shown to activate c-Abl, a nonreceptor tyrosine kinase with an F-actin binding site. In vitro, binding to F-actin inhibits the kinase activity of c-Abl.

Woodring et al. now demonstrate that c-Abl promotes actin polymerization. c-Abl stimulates the formation of F-actin microspikes in both spreading fibroblasts and neurites of embryonic cortical neurons. Whereas inhibition of c-Abl kinase activity blocked the formation of F-actin microspikes, expression of an activated form led to more microspikes, independent of cell surface signals. The stimulation occurred even under conditions in which Rho-family GTPases were inhibited, indicating that either c-Abl acts downstream of Rho or a GTPase-independent actin polymerization pathway exists. Downstream, c-Abl previously has been shown to associate with WAVE1, an activator of the Arp2/3 complex, which is known to initiate actin polymerization.

The inhibition of c-Abl could provide a reciprocal regulation to limit the lifespan of F-actin protrusions. The inhibitory effect of c-Abl on c-Abl may be suppressed in attached cells through the binding of another molecule to c-Abl or the redistribution of c-Abl and F-actin upon attachment, thus promoting actin-dependent spreading of the attached cell.

A kinase kick for endocytosis

Two new studies indicate that cycles of phosphorylation and dephosphorylation may be required during clathrin-mediated endocytosis. A newly identified kinase is responsible for the required phosphorylation.

Clathrin and the adaptor protein 2 (AP2) complex are the major coat proteins of clathrin-coated vesicles (CCVs). Among components of the AP2 complex are α and β2 adaptins, which mediate AP2 membrane targeting and interactions with clathrin, and the µ2 subunit, which may recognize the endocytic cargo.

Phosphorylation and dephosphorylation have been implicated in endocytosis and at least two kinase activities copurify with CCVs. The identity of the kinase and its substrate had not been determined, however. Now, Conner and Schmid (page 921) and Ricotta et al. (page 791) identify one of the kinase activities and demonstrate its effect on cargo binding.

Conner and Schmid show that the serine/threonine kinase AAK1 directly interacts with α-adaptin in vitro and in vivo. AAK1 cofractionates with AP2 complexes and with clathrin, and localizes to regions active in endocytosis. Ricotta et al. demonstrate that AAK1 corresponds to the endogenous kinase that phosphorylates the µ2 subunit. The phosphorylation of µ2 enhances binding of AP2 to sorting signals in vitro by 25-fold.

Previous in vivo experiments indicated that phosphorylation of AP2 was required for clathrin-mediated endocytosis, even though this inhibits AP2 binding to clathrin. Conner and Schmid confirm that maintenance of the µ2 subunit in its phosphorylated form inhibits endocytosis, suggesting that a protein phosphatase activity is also required to dephosphorylate µ2. Possibly, cycles of phosphorylation and dephosphorylation facilitate concentration of the cargo at the plasma membrane through multiple rounds of AP2-mediated recruitment. Perhaps only when clustered cargo inhibits continued cycling can AP2 bind to clathrin and drive vesicle formation and cargo uptake.