In Brief

Cholesterol in Receptor Complex Assembly

Beginning on page 673, Green et al. describe the elucidation of a role for cholesterol in the recruitment of integrin-associated protein (IAP) to a receptor signaling complex. Though previous work had shown that cholesterol is required for signaling in several cell types, the new study is the first demonstration of a specific role for cholesterol in receptor complex assembly and the regulation of integrin function.

IAP seems to regulate some of the functions of αvβ3 integrin receptors and has been shown to form a signaling complex with αvβ3 and trimeric G proteins. The authors show that depletion of cholesterol from the cell membrane prevents the formation of this complex and blocks downstream signaling. Depleting cholesterol from IAP also causes a change in the antigenicity of the protein, and expression of chimeric forms of IAP demonstrates that the multiple membrane-spanning domain is required for both cholesterol binding and complex assembly. "We postulate that cholesterol is directly involved in creating the transmembrane complex that signals," says Eric Brown, senior author on the study. In this model, cholesterol binding to the multiple membrane-spanning domain of IAP produces a protein conformation that can interact with αvβ3, and this entity in turn associates with the trimeric G protein to form the signaling complex.

Transcription and Processing Sites within the Nucleus

Two New Nucleolar Localization Sequences

In contrast to nuclear targeting of proteins, for which consensus sequences and defined pathways have been established, protein localization to the nucleolus remains poorly understood. By studying GFP fusions of the protein subunits of RNase P, Jarrous et al. (page 559) have discovered two amino acid sequences capable of directing proteins to the nucleolus.

To date, three of the subunits of RNase P, a tRNA processing enzyme, have been shown to localize to the nucleolus. Two of these proteins, Rpp38 and Rpp29, carry nucleolar localization sequences, which were mapped using deletion constructs. The third protein, Rpp14, appears to be localized by a piggyback mechanism. No consensus sequence has been established for nucleolar targeting, and most previously discovered nucleolar localization sequences involve multiple domains of a protein. In contrast, the new sequences are functional as single domains and can be transferred to other proteins. The sequences share no homology with each other or with other known nucleolar localization domains.

Sidney Altman, senior author on the paper, explains that Rpp38 and Rpp29 are, "not only components of RNase P, but also components of [rRNA processing enzyme] RNase MRP. It could be that these individual sequences have something to do with the roles they play in each of those two enzymes."

Matrix-associated Transcription Sites in Three-dimensional Networks

Although the nucleolus provides a clearly defined site for RNA polymerase I–mediated transcription, defining the sites of RNA polymerase II–mediated transcription in the nucleoplasm has remained a daunting task. Wei et al. (page 543) describe the three-dimensional mapping of transcription sites in permeabilized cells. The work reveals network-like arrays of transcription sites associated with the nuclear matrix, many of which overlap RNA-processing nuclear speckles.

Using computer analysis of images from laser scanning confocal microscopy, Wei et al. constructed a three-dimensional map of ~2,000 transcription sites in BrUTP-labeled, permeabilized mammalian cells. Over 90% of the sites were extranucleolar, and these were organized into network-like arrays. The network is maintained in the extracted nuclear matrix, and almost half of the transcription sites are associated with splicing factor-rich nuclear speckles. Ronald Berezney, senior author on the paper, asserts that the findings are "bound to create a lot of interest because most researchers in the area are under the impression that the nuclear speckles are not active sites of transcription and perhaps not RNA splicing as well." In a related paper in this issue (Ma et al., page 531), researchers in the same laboratory report that chromosome territories in the interphase nucleus are nuclear-matrix associated.

Coordination of Mitosis

Control of Mitotic Initiation

By following the behavior of chimeric proteins with time-lapse fluorescence microscopy, Karlsson et al. (page 573) have found that the phosphatases Cdc25B and Cdc25C have distinct activities in initiating mitosis in human cells. The nondestructive assay allowed the team to follow proteins in individual cells through an entire cell cycle, and proved the utility of an approach that should be widely applicable to cell cycle studies.

At various points in the cell cycle, the researchers microinjected synchronized HeLa cells with vectors expressing GFP fusions of Cdc25B or Cdc25C, closely related cell cycle regulatory proteins, and observed the cells' behavior and protein localization with time-lapse microscopy. Cdc25C is capable of inducing premature mitosis in G2-phase cells, but only when cyclin B1 is simultaneously overexpressed. In contrast, Cdc25B overexpression can induce premature mitosis from S and G2 phases by itself. When coexpressed with cyclin B1, Cdc25B can also induce premature mitosis in G1-phase cells, demonstrating that cell division and DNA replication can be uncoupled in human cells. "Our observation that Cdc25B but not Cdc25C can cause mitosis without an intervening phase of DNA

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replication may suggest that Cdc25B could also have an important role in meiosis," says Jonathon Pines, senior author on the paper. The researchers are now using the same technique to study other proteins involved in the initiation of mitosis.

Role of Dynein in Mitotic Chromosome Segregation

Taking advantage of the genetic manipulability of *Drosophila*, Robinson et al. (page 597) show that dynein is required for proper centrosome attachment to the nuclear envelope and mitotic spindle. Though previous work has suggested that dynein is involved in mitosis, the new study is the first detailed analysis of dynein's mitotic function in a living multicellular organism.

Because dynein is essential for viability in metazoans, analysis of the protein's role in mitosis has been difficult. To generate embryos lacking functional dynein, Robinson et al. bred flies carrying recessive lethal alleles of the dynein heavy chain gene. Females of this strain lay eggs that lack functional dynein, allowing the scientists to study the protein's role in living cells during early embryonic mitotic divisions. Consistent with earlier studies, the team found defects in centrosome migration along the nuclear envelope and in centrosome attachment to spindle poles in the mutant embryos. The analysis also uncovered a previously unknown role for dynein in maintaining the attachment of centrosomes to the nuclear envelope during prophase. In the few mutant larvae that survive early development, similar defects appear in developing neuroblasts, indicating that dynein is also required for later cell divisions.

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