In This Issue

Utrophin gets a new look

Utrophin, a member of the spectrin superfamily of actin-binding proteins ubiquitously expressed in human cells, helps link the actin cytoskeleton to the extracellular matrix. Galkin et al. report on page 231 that a newly developed image analysis method identifies two different modes of utrophin binding to F-actin. The findings contradict earlier studies on utrophin–actin interactions and provide a clearer explanation of how relatively few proteins may generate the diverse interactions and structures seen in the actin cytoskeleton.

The amino-terminal domain of utrophin contains a tandem pair of calponin homology (CH) domains, which are important for binding to actin. Unfortunately, disorder in binding and the variable twist of F-actin obscure the three-dimensional structure of utrophin–actin complexes seen under the electron microscope, making detailed studies problematic. Galkin et al. tackled this problem with their recently developed image analysis algorithm, which can separate classes of polymorphic structures that are indistinguishable by traditional techniques. First reported last year, the new method is rapidly gaining acceptance. It is now being used to study several other actin-binding proteins, as well as protein–DNA interactions during recombination.

Previous work identified only one type of utrophin binding to actin, but the new study shows that the utrophin actin-binding domain binds to F-actin in two distinct modes with different stoichiometries. The CH domains appear to bind in an extended conformation in both modes, as earlier X-ray crystallography studies had predicted. The two binding modes, and their fit within structural models, suggest that utrophin can establish different interactions with actin on multiple surfaces of actin subunits. Previous work has shown that other actin-binding proteins may exhibit similar diversity in their interactions, suggesting that multiple binding modes are a general theme allowing a small number of actin-binding proteins to create a diverse array of structures.

Consequences of a traffic jam

Identifying the genetic basis of a disease is only a small step toward understanding its pathogenesis, as Simons et al. clearly demonstrate on page 327. The dysmyelinating condition Pelizaeus-Merzbacher disease (PMD) is caused by duplication or overexpression of the myelin proteolipid protein (PLP) gene, but it was not clear if excess PLP caused dysmyelination directly or indirectly. Simons et al. characterized the changes in intracellular trafficking caused by PLP overexpression in three systems. Their findings help define a pathway that may transport myelin rafts in oligodendrocytes, and suggest that PLP overexpression causes PMD by a multistep, indirect mechanism.

The authors examined BHK cells, oligodendrocytes, and transgenic mice in which PLP was overexpressed. In these systems, PLP is not incorporated into lipid rafts, but is routed to endosomes/lysosomes. Cholesterol then accumulates in these compartments, which normally maintain low cholesterol levels. Two fluorescently labeled sphingolipid analogues and GPI-YFP are also missorted to late endosomes/lysosomes in PLP-overexpressing cells.

Based on these and earlier results, the authors propose that PLP overexpression saturates the myelin raft transport pathway, causing the surplus PLP to be routed to the degradative compartment. This rerouting also causes the misdirection of cholesterol, and eventually other raft components, to the late endosome/lysosome.

Thus PLP overexpression may trigger a chain of events that could trap myelin lipids, impair normal trafficking of late endosomes/lysosomes, and interfere with myelination-regulating signaling molecules that localize to lipid rafts. These defects may trigger the premature oligodendrocyte death seen in mouse models of PMD. The authors are now trying to identify the mechanism by which cell death is induced in PLP-overexpressing cells.
Knowing when to let go

During meiosis I, some mechanism must allow homologous chromosomes to separate while keeping sister chromatids paired until meiosis II. How does a cell make this distinction? On page 219, Rogers et al. propose that in *C. elegans* the aurora-B kinase AIR-2 is largely responsible for ensuring that cohesion between chromosomes breaks down at the proper place and time. The authors also identified additional components in what is likely to be a conserved pathway controlling chromosome cohesion.

When AIR-2 activity is inhibited by RNAi, meiotic cells in the worm do not separate homologous chromosomes or sister chromatids. In metaphase I in normal meiotic cells, AIR-2 localizes distal to chiasmata, corresponding to the last points of contact between homologous chromosomes. In metaphase II, AIR-2 localizes to the last points of contact between sister chromatids. AIR-2 phosphorylates the cohesin protein REC-8 at a specific site in vitro, and inhibition of the CeGLC-7α or -β phosphatases causes AIR-2 to localize nonspecifically along chromosomes.

The authors suggest that CeGLC-7α/β phosphatases restrict AIR-2 localization temporally and spatially on meiotic chromosomes. AIR-2 phosphorylates REC-8 in its vicinity, causing the cohesin to be degraded and releasing chromosomal cohesion only in the appropriate location.

Adapting to the pit

Cell surface receptors that are internalized generally interact with the endocytic machinery through adaptor proteins. On page 315, Howard et al. describe the first example of a protein involved in recognizing endocytic targeting signals in yeast. The work links together several earlier observations about yeast actin dynamics and endocytosis, and suggests that an analogous system may exist in mammalian cells.

The authors found that a sequence containing the amino acid motif NPFX<sub>12</sub>D, previously characterized as an endocytic targeting signal in yeast, is sufficient to direct the uptake of a truncated cell surface receptor. A two-hybrid screen for NPFX<sub>12</sub>D-binding proteins yielded Sla1p, which is known to interact with the endocytic machinery and regulate actin dynamics. Disrupting Sla1p expression inhibited NPFX<sub>12</sub>D-mediated endocytosis.

Combined with previous findings, the results imply that Sla1p is part of a complex that links cargo bearing the NPFX<sub>12</sub>D motif to the actin and clathrin-based endocytic machinery. By analogy, a similar complex in mammalian cells might provide a Sla1p-like adaptor function in endocytosis. Searches of the yeast genome database suggest that NPFX<sub>12</sub>D directs endocytosis of a subset of cell surface proteins, and may also mediate other types of protein sorting.

For healthy eyes and bones: got Lrp?

The Wnt family of secreted proteins controls several crucial developmental processes, some of which are apparently mediated by Wnt coreceptors from the LDL receptor-related protein (Lrp) family. Now, on page 303, Kato et al. report that the targeted disruption of Lrp5 in mice causes a phenotype virtually identical to that seen in humans with osteoporosis-pseudoglioma syndrome. In addition to identifying a long-sought genetic component for bone mass determination, the work identifies Lrp5 as a critical component for controlling both osteogenesis and eye vascularization during late stages of development.

Mice lacking functional Lrp5 exhibit lower rates of bone formation than wild-type mice, and fail to achieve normal bone mass early in life. This happens despite normal expression of Cbfa1, a protein thought to be the principal controller of osteogenesis, suggesting that Lrp5 functions in an independent osteogenesis pathway. In addition, Lrp5 knockout mice retain part of the embryonic eye vascularization network, which regresses postnatally in wild-type mice by macrophage-mediated apoptosis. Since normal ocular macrophages are present in the mutant mice, Lrp5 appears to be specifically required for macrophage-mediated apoptosis. The authors are now trying to determine whether other aspects of bone biology are governed by the Lrp5-mediated osteogenesis pathway, and which Wnt proteins signal through this pathway.