In This Issue

Clash of the titin

The sarcomere is more than a complicated piece of structural machinery. According to new results by McElhinny et al. (page 125), a building block of striated muscle cells called MURF-1 may also indirectly regulate gene expression.

MURF-1 localizes to sarcomeres thanks to its interaction with titin, a major structural component of the muscle sarcomere, and the largest vertebrate protein identified to date. Titin is anchored at the sarcomere Z-lines, as are thin (actin) filaments. From there it stretches across entire half sarcomeres to overlap at the mid-line (M-line), where it helps anchor thick (myosin) filaments in a central location.

Now McElhinny et al. show that different domains of titin perform independent functions. Disturbance of the titin–MURF-1 interaction leads to a complete disruption of M-line and thick filament structure. But the remaining titin regions can still stabilize sarcomeric thin filaments and Z-lines.

Then there is the nuclear connection. The authors demonstrate nuclear localization of MURF-1, and interaction with a glucocorticoid-responsive transcriptional activator. Thus, titin may recognize structural alterations in the sarcomere and signal to the nucleus by releasing MURF-1 for translocation to the nucleus and transcriptional activation.

A possible mechanism could involve titin phosphorylation of MURF-1, as titin’s kinase domain lies adjacent to its MURF-1–binding domain.

Seeing through the cilium

To understand vision in vertebrates, one might look to worms and algae, according to recent results. On page 103, Pazour et al. show that a conserved protein transport mechanism in the flagellae of algae and worms is also necessary for development and maintenance of mammalian photoreceptors.

Assembly and maintenance of motor and sensory cells in worms and algae requires the transport of proteins via intraflagellar transport (IFT). The outer segment (OS) of a vertebrate photoreceptor, itself a modified cilium, is formed by transporting membrane and opsin through a connecting cilium that provides a link between the photoreceptor inner (metabolic) and outer (photoreceptive) segments. Photopigments and phototransduction proteins must pass through this connecting cilium to replace components of the OS that have been degraded.

Now, it appears that the mechanism used to transport these photoreceptor molecules is conserved throughout eukaryotes. Pazour et al. identified mouse and human homologues of several proteins that form a complex required for IFT in algae. The vertebrate proteins form a complex of similar density to that of the algal complex and localize to the connecting cilia of photoreceptors.

Defects in one of these proteins, IFT88, leads to abnormal development of the mouse photoreceptor OS. Retinal degeneration occurs via apoptotic death of photoreceptor cells, presumably because necessary OS components are not transported. Mutations in the human IFT genes are candidates for causing degenerative vision disorders such as retinitis pigmentosa.

Peroxide perturbations

Injury triggers invasion of white blood cells into tissues. According to new results from Ji et al. (page 173), PECAM-1, an adhesion molecule on the endothelial cells (ECs) that line blood vessels, does double duty in this invasion process. Direct ligation of PECAM-1 is known to allow neutrophils to crawl between ECs.

But, independent of this function in white cell invasion, PECAM-1 is now shown to activate an EC current that may prime cells by loosening their connections.

The messenger that triggers this current is the reactive oxygen species hydrogen peroxide (H$_2$O$_2$). Neutrophil-produced H$_2$O$_2$ signals increase the EC permeability by inducing cell depolarization and an increase in cytosolic Ca$^{2+}$ concentration, but the mechanism behind these changes remained unknown.

Now, Ji et al. demonstrate that PECAM-1 is required to activate a nonselective cation channel in response to oxidants. The cytoplasmic domain of PECAM-1 is sufficient for activation, suggesting that peroxide-induced Ca$^{2+}$ influx produced during transmigration does not require PECAM-1 binding between neutrophils and ECs. Phosphorylation of tyrosine residues in this domain is required for the H$_2$O$_2$-induced current, and probably allows interaction with other linker or channel proteins.

Although it is not known which channel is activated, inhibitor studies indicate that the calcium is coming from outside the cell, and not by activation of internal stores. The group plans to investigate the function of the PECAM-1–regulated channel from examining pathological responses in PECAM-1 knock-out mice.
A protein known to tether vesicles during intra-Golgi trafficking also ensures their correct docking, according to new results from Shorter et al. (page 45). This provides the first link between the long-range process of tethering and the short-range process of docking.

Vesicle transfer consists of four successive reactions: vesicle formation, tethering, docking, and fusion. Tethering of COP1 vesicles to the Golgi requires the coiled-coil protein p115, which links the Golgins GM130 (on the Golgi) and Giantin (on the vesicle). Subsequent docking of vesicles is a SNARE-dependent event, requiring the correct assembly of cognate SNAREs comprising one vesicle SNARE (v-SNARE) and three target SNAREs (t-SNAREs). The resultant SNAREpin appears to force fusion of the two membranes.

Now, it seems that, in addition to tethering the Golgins, p115 also facilitates vesicle docking. Shorter et al. showed that p115, via one of its coiled-coil domains, binds to specific Golgi SNARE proteins that form SNAREpins containing the t-SNARE syntaxin-5. The binding is reflected in a functional assay with either isolated Golgi membranes or Golgi detergent extracts, where p115 stimulates the assembly of cognate SNARE complexes.

Once established, maintenance of the SNAREpin structure does not require p115, and the protein is not consumed during the process, suggesting that p115 is a catalyst of SNAREpin formation. Thus, p115 is able to couple tethering and docking physically by first linking donor and acceptor membranes through associations with Golgins and then catalyzing SNAREpin assembly through direct interaction with the appropriate SNARE proteins.

The world of topoisomerase II (topo II) is changing. Until now, topo II has been thought to be a major structural protein. But on page 31, Christensen et al. show that this is an unlikely possibility. Then, in another shot to the field, they demonstrate that the two isoforms of mammalian topo II (IIα and IIβ), argued to be functionally redundant, probably make different contributions during mitosis.

Topo II passes DNA strands through each other during DNA replication and separation of sister chromatids during mitosis. It has also been considered the major nonhistone component of the mitotic chromosome scaffold. In vivo evidence to support or refute the latter role has been lacking, in part because it has been tricky to localize mammalian topo II. Although mobile proteins can still sustain a dynamic scaffold, the extent of topo II turnover (50% of all topo II molecules replaced every 1.8 and 3.0 s, respectively) suggests that this is unlikely.

The results also argue against redundancy. Although both isoforms accumulate in nucleoli during interphase, only topo IIα strongly associates with chromosomes during mitosis. Thus, topo IIβ may not be required for mitotic cell division.