Mutant Huntingtin Aggregation Does Not Require tTG

Chun et al. (page 25) provide convincing evidence that tissue trans-glutaminase (tTG) does not contribute to the formation of aggregates of huntingtin, a pathological hallmark of Huntington's disease. Previous in vitro studies suggested that mutant huntingtin is a substrate of tTG, leading some researchers to suggest that tTG could be a therapeutic target for treating the disease, which is caused by the expansion of a poly-glutamine domain in the NH2-terminal region of huntingtin.

To test this hypothesis in vivo, Chun et al. transfected cultured human neuroblastoma cells with wild-type or mutant NH2-terminal huntingtin constructs. Huntingtin aggregates formed in cells expressing the mutant form of the protein, but tTG was completely excluded from the aggregates. The abundance and localization of the aggregates remained unaffected when tTG expression levels were significantly increased when tTG expression was increased. Using fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP) analyses, Chun et al. observed rapid exchange between the nucleolus and the nucleoplasm of factors involved in rRNA transcription, pre-rRNA processing, and ribosome assembly. Ribosomal subunit proteins, however, are exchanged much more slowly. The rapidly exchanged nucleolar factors appear to cycle between the two compartments repeatedly, though it remains to be determined whether they do so individually or in complexes. As components from different steps of the ribosome biogenesis pathway display different mobilities, Chen and Huang suggest that their exchange rates may correspond to the length of time required for each step.

Although ribosome biogenesis has been studied extensively, the spatial and temporal control of this process in living cells has remained poorly understood. Using fluorescence recovery after photobleaching (FRAP) and fluorescence loss in photobleaching (FLIP) analyses of GFP fusion proteins, Chen and Huang observed rapid exchange between the nucleolus and the nucleoplasm of factors involved in rRNA transcription, pre-rRNA processing, and ribosome assembly. Ribosomal subunit proteins, however, are exchanged much more slowly. The rapidly exchanged nucleolar factors appear to cycle between the two compartments repeatedly, though it remains to be determined whether they do so individually or in complexes. As components from different steps of the ribosome biogenesis pathway display different mobilities, Chen and Huang suggest that their exchange rates may correspond to the length of time required for each step.

Cycling of Ribosome Biogenesis Components

Beginning on page 169, Chen and Huang describe the movements of nuclear components involved in ribosome biogenesis. They find that some nucleolar components are rapidly exchanged between the nucleolus and the nucleoplasm, and that proteins involved in different steps of ribosome biogenesis exhibit different mobilities, suggesting that the functional roles of the proteins, rather than specific targeting signals, cause them to associate with the nucleolus.

Twist and Tilt in Actin Depolymerization

Using a novel image analysis method, Galkin et al. (page 75) demonstrate that subunits in F-actin are capable of binding two ADF molecules, and that the depolymerization activity of ADF/cofilin may be due to the ability to induce cooperative changes in the tilt and twist of actin subunits. Rapid F-actin turnover requires proteins of the highly conserved ADF/cofilin family. These proteins can change the mean twist of actin filaments by ~5° per subunit, but other workers have claimed that this change in twist can be uncoupled from subunit dissociation.

In the new work, Galkin et al. analyzed tens of thousands of segments within filaments. They found that segments of pure actin can adopt the ADF/cofilin-like state of twist, even in the absence of other proteins, and, conversely, that the ADF–actin complex can adopt a state of twist close to the normal actin state. Therefore, ADF appears to stabilize a preexisting F-actin conformation. Galkin et al. also discovered that two molecules of ADF bind per actin subunit, and that some actin subunits are tilted significantly from their normal positions under conditions that destabilize filaments. The cooperative twist and tilt resulting from the binding of two molecules of ADF per actin subunit may lead to breakage of longitudinal contacts within the actin filament, and thus to F-actin depolymerization.

Secretory Granule Motion Near the Plasma Membrane

Johns et al. (page 177) describe the poorly understood dynamics of secretory granule motion near the plasma membrane, by developing an automated system to track labeled granules using total internal reflection fluorescence microscopy (TIRFM).

The use of TIRFM, which selectively illuminates features within ~300 nm of the plasma membrane at cell–substrate contact regions, allowed Johns et al. to analyze granule motion in the z-direction (normal to the substrate). Granules were selec-
tively labeled with a GFP-tagged protein that is targeted to the secretory pathway. Software developed for these studies locates and tracks granules, allowing the analysis of hundreds of granules in living cells and reducing the potential for experimental bias.

Statistical analysis of the data shows that granules become more limited in their motion as they approach the membrane, that motion is strongly restricted over distances of tens of nanometers, and that moving granules tend to reverse direction within 0.5 s. These results suggest that granule movement is limited by tethers or a heterogeneous matrix near the membrane. In addition, the transient expression of the light chains of tetanus toxin and botulinum toxin A does not overcome the restrictions on granule movement, demonstrating that the SNARE proteins, SNAP-25 and VAMP, are not required for this process.

**Role of the Centrosome**

Khodjakov and Rieder (page 237) used laser microsurgery to destroy one or both centrosomes in dividing vertebrate cells, and found that the primary role of the centrosome is not spindle formation, but ensuring cytokinesis and subsequent cell cycle progression. Previously, Khodjakov and Rieder demonstrated that cells could form bipolar mitotic spindles in the absence of centrosomes, but it remained unclear whether those cells could complete cell division. In the new work, Khodjakov and Rieder found that “acentrosomal” cells can undergo a normal anaphase to produce two acentrosomal daughter cells. Therefore, centrosomes are not required for progression through mitosis. However, cytokinesis fails more frequently in acentrosomal cells than in normal cells, apparently because the spindle is unable to reposition itself properly, and therefore is not aligned with the long axis or in the geometric center of the cell.

To examine the role of centrosomes in subsequent cell cycle progression, Khodjakov and Rieder destroyed one of the two centrosomes during metaphase, leading to the creation of one acentrosomal and one normal daughter cell after cell division. Whereas the normal cells subsequently carried out DNA synthesis or divided, the acentrosomal cells invariably arrested before S phase, indicating that centrosomes are required for cell cycle progression. Interestingly, the acentrosomal cells do not regenerate their centrosomes, suggesting that centrosome regeneration may require a template that is destroyed by the laser ablation process.

Alan W. Dove, 350 E. Willow Grove Ave. #406, Philadelphia, PA 19118. E-mail: alanwdove@earthlink.net