In the first in vivo characterization of nuclear pore complex (NPC) dynamics in mammalian cells, Daigle et al. (page 71; see also the Comment on page 17) have found that NPCs are remarkably stable complexes that appear to be anchored to a protein network in the nuclear envelope. The work also demonstrates the feasibility of tracking single protein complexes in living cells.

By fusing GFP tags to POM121, lamin B1, or Nup153, the authors were able to track the movement and turnover of NPCs directly. Instead of diffusing freely within the plane of the nuclear envelope during interphase, NPCs remained largely immobile, only moving slowly in unison in large arrays. Individual subunits of the NPC vary considerably in their turnover rates, with POM121 turning over less than once per cell cycle, whereas Nup153 turns over three orders of magnitude faster. The slow turnover of POM121 indicates that the NPC remains stable throughout interphase. This stability, the authors suggest, allows NPCs to form part of a crosslinked protein network in the nuclear envelope composed of lamins, inner nuclear membrane proteins, and peripheral heterochromatin.

Transcription in tight quarters

Müller et al. (page 33) have developed a live-cell system that allows analysis of chromatin condensation during transcription from a natural promoter. Using a tandem array of mouse mammary tumor virus (MMTV) promoters driving ras reporter genes, the authors demonstrate that normal transcription can occur in chromatin that is much more condensed than the transcribed chromatin of previous model systems such as lampbrush chromosomes or polytene puffs. Furthermore, instead of the looping out of DNA observed in these systems, the authors observe a linear unraveling that produces domains of variable packing density.

In response to steroid hormone treatment, the MMTV promoter is first activated by the glucocorticoid receptor, and then down-regulated over a six-hour period. Expression of GFP-tagged glucocorticoid receptor allows visualization of the MMTV array in live cells. Müller et al. found that chromatin decondensation and recondensation paralleled the transcriptional activity of the promoter, and that both the establishment and maintenance of the decondensed state required transcription by DNA polymerase II activity.

Transcription in this system can occur at high chromatin packing densities, a phenomenon previously seen only with artificial promoters that have high densities of transcription-factor binding sites. It remains unclear how the transcriptional machinery interacts with the densely packed chromatin, but these questions should be easier to address now that a live-cell system is available.

RhoA brings up the rear

In fibroblasts, the small GTPase RhoA is required for the formation of integrin-containing adhesions. Surprisingly, Worthylake et al. (page 147) have found that RhoA serves a different purpose in monocytes, apparently operating as a negative regulator of integrin adhesions at the rear of migrating cells. The authors used a tissue culture system that mimics the transendothelial migration of monocytes from blood vessels into adjacent tissues, a model which is relevant to immune system function and tumor metastasis.

When RhoA activity is inhibited in monocytes in this system, the cells attach to the endothelium, crawl forward, and begin to invade between endothelial cells. But the inhibited cells are unable to retract their tails to complete the migration. The RhoA effector p160ROCK is necessary and sufficient to allow tail retraction, and signaling through p160ROCK negatively regulates integrin adhesions. In monocytes, RhoA signaling therefore appears to couple rear retraction with forward movement in order to allow transendothelial migration. If this model is correct, RhoA activity might be localized to the rear of the migrating monocytes, a prediction the authors hope to test in the near future.
Cataracts and NrCAM

The Ig superfamily member NrCAM is believed to help orchestrate neuronal development, so Moré et al. (page 187) were surprised at the phenotype of their recently generated NrCAM /− /− mice; the animals appear to have nearly normal nervous systems, but develop cataracts.

The mice are slightly smaller than their heterozygous littermates and show a slight motor defect, but are viable and fertile, and lack any histological abnormalities in any neural tissues. Their commissural axons cross the spinal cord midline normally, an unexpected result because previous work had suggested a requirement for NrCAM in directing commissural axon growth.

While the absence of NrCAM does not appear to cause serious problems in neuronal tissue, NrCAM /− /− mice develop cataracts because of a failure in establishing contact between lens fiber cells, and thus presumably a failure in forming normal water channels. Flow through these channels appears to be important for lens clarity, and defects in communication between lens fiber cells in the eye leads to the breakdown of the cells. Mice lacking ankyrin-B display a similar disorganization in the lens fiber, providing genetic evidence that NrCAM function requires a link to the cytoskeleton via ankyrin. The new work is also the first demonstration of NrCAM expression in the lens.

Cataracts are the most common cause of visual impairment in humans. If mutations in human NrCAM or ankyrin-B are also involved in the formation of cataracts, then drugs or gene therapy strategies targeting these proteins might be medically important.

Clumping lysosomes

Cataracts form in mice lacking NrCAM.

apo et al. (page 109) find evidence that human Vam6 protein is a mammalian tethering or docking factor with the intrinsic ability to promote lysosome fusion in vivo. The protein is related to a member of a vacuole-fusion complex in budding yeast, but has a unique citron homology domain that is required for lysosome clustering and fusion. Although Rab7 can induce lysosome fusion in yeast, dominant-negative Rab7 does not block hVam6p activity, suggesting that the human protein operates either downstream of, or in parallel to Rab7.

How Toxoplasma hooks up

The intracellular parasite Toxoplasma gondii hides out in a specialized compartment surrounded by the parasitophorous vacuole membrane (PVM). The PVM associates with host cell organelles, possibly as a way of scavenging lipids from the host cell. On page 95, Sinai and Joiner describe a protein that mediates this association. The new work illuminates a poorly understood aspect of parasite biology, and may be relevant for understanding organelle interactions in eukaryotic cells.

Sinai and Joiner focused on the T. gondii ROP2 protein, as this protein appears on the PVM around the time that the PVM associates with mitochondria, and it contains a domain with characteristics of a mitochondrial targeting signal.

In vitro, they find that the N-terminal domain of ROP2 partially translocates into the mitochondrial outer membrane, and appears to stably integrate there. The same domain also contains a motif that promotes a high-affinity interaction with the endoplasmic reticulum (ER). In vivo, an expressed N-terminal domain of ROP2 targets both organelle systems.

The results suggest that one organelle might use a protein such as ROP2 to “capture” other organelles. Because PVM-organelle interactions are morphologically similar to normal interactions between organelles, such as ER-mitochondria interactions, the authors propose that this type of tethering mechanism may be a general phenomenon.