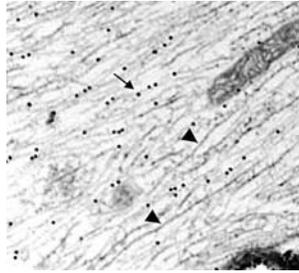


## A motor for neurofilaments

Neurofilaments modulate the shape and size of neurons. On page 279, Rao et al. describe several lines of evidence demonstrating that neurofilaments interact with the motor protein myosin Va, and that this interaction is critical for proper neurofilament organization. Besides illuminating a poorly understood aspect of neuronal biology, the findings may also help explain the pathogenesis of a debilitating human genetic disease.



**Myosin Va (gold particles) binds neurofilaments.**

In both humans and mice, mutations in the myosin Va gene cause severe defects in skin pigmentation and fatal convulsive disorders. Combining biochemical and genetic approaches, the authors found that the neurofilament light chain is a major ligand of myosin Va in mouse nervous

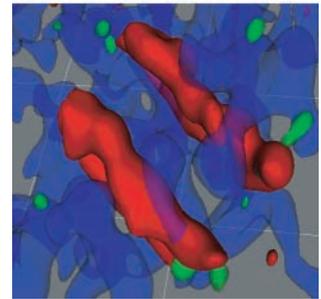
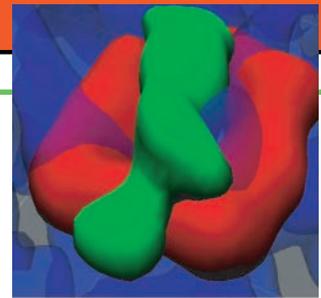
tissue. In mice lacking functional myosin Va, neurofilaments in axons are more densely packed than they are in wild-type mice.

The results suggest that proper neurofilament spacing, and hence normal neuronal shape and function, depends on the dynamic cross-linking of neurofilaments and microfilaments by myosin Va. Myosin Va might also provide the motive force to move membrane-associated enzymes and receptors along neurofilaments, and to carry out the short-range rearrangements of neurofilaments within the axon that occur during early postnatal development. ■

## Initiate then elongate

Studies on yeast have provided significant insights into eukaryotic DNA replication, but it has been difficult to examine the process in more complex organisms. On page 225, Claycomb et al. have taken advantage of a peculiar aspect of insect biology to develop a new model for studying metazoan DNA replication, and have already made one surprising finding with the system.

During a specific period of oocyte development in *Drosophila*, the ovarian follicle cells surrounding the oocyte begin to amplify several defined clusters of genes. Using real-time PCR and a combination of microscopy techniques, the authors discovered that this amplification occurs stepwise. First, initiation takes place at the loci that will be amplified, and then, in a separate developmental stage, the replication forks elongate. The natural separation of initiation and elongation provides an ideal model for studying both processes. The system has already helped uncover one apparent difference between yeast and fly systems. The DUP/Cdt1 protein, which is thought to act only during initiation in yeast, travels with the elongating replication forks in *Drosophila*. Claycomb et al. are now hoping to determine how this and other metazoan replication factors are regulated in vivo. ■

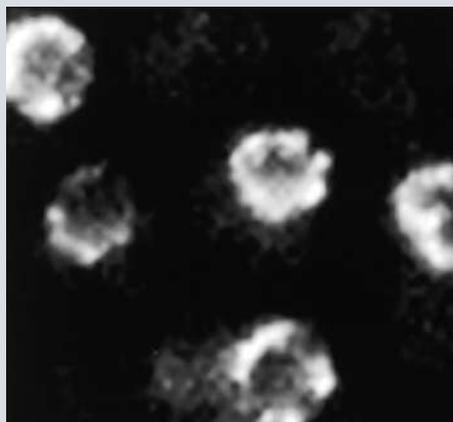


**DUP/Cdt1 (red) functions near replication origins (green, top), but also during elongation (bottom).**

## Building the pore

In examining how the nuclear pore complex (NPC) is assembled, Lusk et al., reporting on page 267, have identified a series of interactions that could help explain both the assembly and the operation of these important structures.

NPCs are built with nucleoporins and then used to traffic karyopherins, proteins that escort molecular cargoes through the nuclear pore. Most nucleoporins bind to multiple karyopherins, but the yeast nucleoporin Nup53p is an exception, interacting only with the karyopherin Kap121p. Taking advantage of this, the authors determined that Kap121p is required for the proper targeting and assembly of Nup53p into the nuclear pore. The data suggest that in addition to binding Nup53p at the pore during translocation,



**A transporter is required for localization of a nucleoporin (white).**

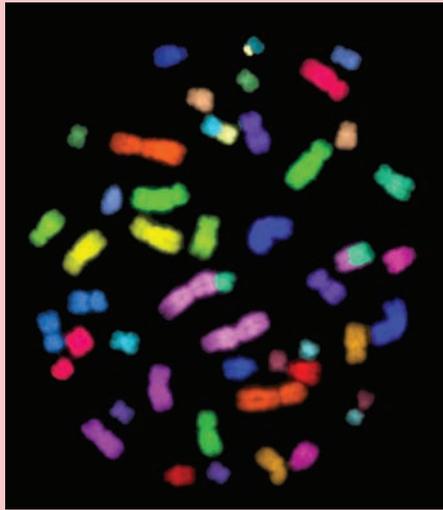
Kap121p can bind to Nup53p that is in the cytoplasm to transport it to the nuclear membrane. Since similar interactions between other karyopherins and nucleoporins could also occur, this may be a general mechanism of nuclear pore assembly.

The authors propose that, once at the pore, Nup53p gets pulled away from its Kap121p escort by competitive binding to the nucleoporin Nup170p. In addition to explaining one aspect of assembly, the model suggests a mechanism for releasing karyopherins from nucleoporins. Competition for nucleoporin binding between a karyopherin and another nucleoporin could allow the now freed karyopherin and its cargo to progress to another nucleoporin on their way into or out of the nucleus. ■

## Chromosomal cliques

Are chromosomes distributed randomly within the cell nucleus during interphase, or do they preferentially cluster into specific groups? On page 237, Cornforth et al. address this question, which has been debated vigorously for nearly a century, and conclude that the distribution of chromosomes within the nucleus of human cells is mostly, but not entirely, random. The work provides the most comprehensive analysis to date of chromosome–chromosome spatial associations in human interphase nuclei, and helps to explain why earlier studies sometimes reached conflicting conclusions.

Previous chromosome painting studies using fluorescence in-situ hybridization (FISH) demonstrated that each chromosome generally occupies its own space during interphase, but this did not



Most translocations, and thus most chromosome positions, are random.

clarify whether or not particular pairs of chromosomes tended to remain close together. Studies that examined just a few chromosomes at a time sometimes gave seemingly conflicting answers. In the new work, the authors exposed human cells to ionizing radiation to produce chromosome breaks, and used 24-color whole-chromosome painting to examine all possible interchanges between heterologous chromosomes after the breaks rejoined. The frequency of interchanges between two chromosomes should indicate whether or not they were in close proximity.

The large number of chromosomal interchanges in the system strengthened the statistical analysis, enabling the authors to identify very small deviations

from spatial randomness. Among all 22 autosomes, most of the deviation from randomness is explained by a single cluster of five chromosomes, with the other chromosomes distributed randomly in the interphase nucleus. The results raise the possibility that this five-chromosome cluster, which has been observed previously, might be functionally significant.

In addition to this cluster, the data show some evidence of other spatial associations that have been suggested by earlier work, but that fail to reach statistical significance in the new work. One possibility is that specific parts of chromosomes might interact without requiring the entire chromosomes to be in close proximity. ■

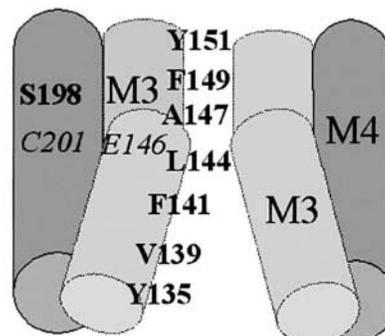
## Gazing into the gap

Gap junctions are a ubiquitous feature of multicellular life, allowing cells to pass particular classes of biomolecules directly to their neighbors. It is clear that these channels are not just nonspecific pores, but it has been difficult to determine what establishes their specificity. Skerrett et al., whose work appears on page 349, developed a cell perfusion system that allowed them to identify the residues lining an intact gap junction channel. The work helps answer lingering questions about earlier structural models, and identifies some unusual features of gap junctions.

Previous studies determined the basic structure of a gap junction, in which one membrane-spanning  $\alpha$ -helix

from each subunit of the channel is exposed to the interior of the pore, but this does not reveal what types of residues line the pore or how specificity is determined. The authors generated a panel of mutated connexin proteins, in each case replacing a single amino acid with a cysteine. These altered connexin subunits were expressed in and formed gap junctions between pairs of *Xenopus* oocytes, and their reactivity with a thiol blocking agent in a novel cell perfusion system pinpointed the residues lining the pore.

The results identify a single  $\alpha$ -helix of the connexin as the main pore-lining segment of



Mostly hydrophobic residues from a single  $\alpha$ -helix line the gap junction pore.

the gap junction channel, and suggest that a single face of that helix is exposed to the pore. Surprisingly, the apparent

pore-lining residues are almost all hydrophobic, an arrangement that is unique among ion channels studied to date.

Skerrett et al. suggest that, unlike classical ion channels, gap junction channels may interact with passing molecules primarily through hydrophobic and weak forces. The new techniques developed for the study also provide a platform for uncovering other structural details about gap junctions. Recently, for example, the authors have used this strategy to map the location of a gate within the pore. ■