An interaction that gets things started

The nuclear envelope clearly helps regulate eukaryotic DNA replication, but many of the molecular pieces of this process are still unknown. On page 177, Martins et al. describe a novel, direct interaction between the nuclear envelope and the genome, mediated by the inner nuclear membrane protein LAP2b and the nuclear matrix-associated protein HA95, that is required for the initiation of replication.

Having cloned HA95 two years ago, the authors now find that it interacts with LAP2b through two distinct domains. Abolishing the HA95–LAP2b interaction leads to the proteasome-mediated breakdown of prereplication complex component Cdc6, and a block in replication initiation. The HA95–LAP2b interaction is not required for DNA replication elongation or nuclear envelope reassembly after mitosis.

The results add to the growing list of functions for HA95, which is also required for normal nuclear envelope breakdown and chromatin condensation, and is believed to facilitate the export of unspliced viral RNA from the nucleus. In replication initiation, HA95 probably works by protecting the prereplication complex from degradation. Martins et al. propose that the global distribution of HA95 in the nucleus and its ability to bind multiple ligands may explain why it appears in several critical regulatory pathways.

Rejection and killing, without MPR

Whether destroying a tumor or rejecting a transplanted organ, cytotoxic lymphocytes rely on proteases called granzymes to kill target cells, so the finding two years ago that the action of a granzyme requires the mannose-6-phosphate receptor (MPR) on a target cell raised hopes for developing new classes of anticancer and antirejection drugs. Now, on page 223, Trapani et al. demonstrate that this optimism was premature, since endocytosis through MPR is actually not required for granzyme-mediated cell killing in a variety of systems.

Seeking to extend previous work (Motyka et al., 2000. Cell. 103:491–500), the authors first used cells defective for dynamin-mediated endocytosis or lacking MPR to study the uptake and effect of granzyme B, the major cell-killing granzyme. In cells unable to endocytose MPR, granzyme B uptake still occurs, albeit at a reduced rate, and cell killing is only slightly decreased. In mouse models, alloreactive tumor cells grafted under the renal capsule are rejected whether or not the cells can internalize MPR. The data suggest that when MPR-mediated uptake is blocked, granzyme B can still kill by entering cells through fluid phase endocytosis.

Taking close-ups of a rafting trip

By combining high-resolution electron microscopy and sophisticated statistical analysis, Prior et al. (page 165) have developed a powerful new technique for studying lipid microdomains, and used it to examine the localization of Ras proteins in the plasma membrane. Since low imaging resolution is a major obstacle to studying microdomains, the authors used electron microscopy to look at sheets of plasma membrane ripped from intact cells. Specific proteins were labeled with gold particles, and each labeled spot on the membrane was numbered and related to every other spot to detect nonrandom distribution patterns. Though the initial focus was on Ras, the technique should be applicable to virtually any membrane-associated protein.

H-ras and K-ras appear to localize to distinct microdomains on the inner leaflet of the plasma membrane, which may help explain how these highly homologous proteins send different signals. To localize correctly, K-ras requires its farnesyl moiety, and H-ras requires the galectin-1 protein. Inner leaflet lipid raft domains, with an average estimated 40-nm diameter, were only loosely associated with the lipid rafts on the outer leaflet of the membrane, suggesting that the strength of the linkage between inner and outer leaflet rafts could be a novel control point for signal transduction.

Different microdomains contain K-ras and H-ras.

Cells lacking MPR still take up granzyme B.