Tumor cells get primitive

When metastatic tumor cells migrate through connective tissue, they use proteases to chew a path for themselves. This finding led to the development of protease inhibitors as potential cancer therapies, but some animal and human clinical tests of these drugs have been disappointing. Wolf et al., reporting on page 267, may have found one reason why: when proteolysis is inhibited, tumor cells resort to an amoeboid type of movement that allows them to squeeze through cracks in the matrix. The results provide substantial new insight into cell migration in multicellular organisms.

Using both in vitro and in vivo cell migration systems, the authors investigated the effects of inhibiting proteases that degrade the extracellular matrix. Normally, transformed cells migrate through three-dimensional collagen matrices or the mouse dermis as individual, spindle-shaped cells, using several proteolytic enzymes and leaving trails through the matrix. When the proteases are shut down, cells undergo a striking change in their appearance, reminiscent of a transition toward amoeboid movement. The cells then continue to migrate through the matrix without breaking it down and without leaving trails.

The change in migration strategy, which Wolf et al. call the mesenchymal–amoeboid transition, suggests that the cells of multicellular organisms retain a more primitive migration system that is normally masked. This type of migration, similar to the movement of the soil amoeba *Dictyostelium discoideum*, could serve as a “salvage” pathway, allowing tumor cells to take a step backward in evolutionary time to continue migrating in the presence of protease inhibitors.

Amoeboid movement may also have more positive functions in multicellular organisms; in separate work, T lymphocytes have been shown to migrate through collagen matrices without using proteases. The authors are now trying to identify the regulatory pathways responsible for controlling the mesenchymal–amoeboid transition. Targeting this process while simultaneously inhibiting proteases might provide a salvage pathway for new tumor therapies as well.

Venus dye trap catches executioners

Fluorescence resonance energy transfer (FRET) between coupled fluorescent proteins is a promising tool for studying caspase activation during apoptosis. Unfortunately, current FRET systems are acutely sensitive to changes in pH and chloride ion concentration, and since these fluctuate considerably during apoptosis the results of FRET experiments are often difficult to interpret. Takemoto et al., whose report appears on page 235, developed a new FRET system that is resistant to acidification and chloride, and used it to obtain a detailed view of caspase-3 and caspase-9 activation in apoptosis. The new technique should also enable studies of caspase activity in vivo that were previously impossible.

To make a caspase-sensitive FRET system, two fluorescent proteins, a donor and an acceptor, are linked with a cleavage site that can be cut by a particular caspase. Caspase activity is then detected as a change in fluorescence. The authors used a new variant of enhanced yellow fluorescent protein called Venus as the acceptor, and linked it to a donor protein through either a caspase-3 or caspase-9 cleavage sequence. Both systems are resistant to acidification and high chloride ion concentrations.

The new systems show that, when apoptosis is induced, caspase-3 is activated rapidly in the cytosol and nucleus, reaching full activation before apoptotic morphological changes begin. This suggests that activated caspase-3 may enter the nucleus to induce the early structural changes. Caspase-9 is initially activated at the same time as caspase-3, but does not become fully activated until the morphological changes are well underway.

The authors have now developed a line of transgenic flies expressing the caspase-3 indicator system, and hope to have a transgenic mouse system soon. The ability to monitor caspase activity in whole tissues should make it possible to study the early steps of this important process, before the apoptotic cells are engulfed by their neighbors.
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.

Cells lacking MPR still take up granzyme B.

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, allogeneic 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.

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.

Different microdomains contain K-ras and H-ras.

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.

Disrupting the chromatin–nuclear envelope link (bottom) prevents DNA replication initiation.