Enter the virus

Single-molecule imaging has enabled Christoph Bräuchle, Michael Hallek (Universität München, München, Germany), and colleagues to track the infection pathway of adeno-associated virus (AAV) as it enters the cell. The AAV particles are tagged with a single molecule of fluorophore and are then individually tracked in real-time at a resolution of 40 nm and 10 msec.

The AAV first bobbles along the surface of the cell, often making several contacts before it undergoes a rapid endocytosis event. Most of the motion from there to the nucleus is diffusional, possibly due to the small size and therefore high diffusibility of the virus. There is, however, some directed movement that is microtubule-dependent.

In the nuclear area, the proportion of microtubule-dependent, directed movement actually increases. Microtubules are not known to exist in interphase nuclei, so the authors suggest that the virus is travelling along invaginations in the nuclear envelope that have been observed by others. These invaginations may provide rapid access to the nuclear volume for both cellular materials and virus particles. Indeed, the speed of infection (with nuclear entry as an endpoint) is fast. Previous estimates of a two-hour infection were limited by the need to detect multiple virus particles. The new methods yield a figure of just 15 min.

Bräuchle says the method can be used to track any virus, and might help gene therapists understand where their virus of choice is getting held up during the infection process. Meanwhile, he has made virus labeled on both capsid and DNA, so that he can track the exact location and kinetics of viral disassembly.

Reference: Seisenberger, G., et al. 2001. Science. 294:1929–1932.

Repair long and prosper

Death can be a good thing when it comes to sunburn—the death of UVB-damaged cells reduces the chances of cancerous growths arising. But now, Thomas Schwarz (University of Münster, Münster, Germany) and colleagues have found a way that the body can reduce the evil effects of UVB without invoking death. A cytokine, IL-12, induces nucleotide excision repair (NER), and thus decreases DNA damage and apoptosis.

Schwarz started out by looking for factors that enhanced apoptosis after UV treatment. Instead, he found the reverse effect with IL-12. A series of follow-up studies failed to demonstrate an underlying mechanism. “At the very end, more in desperation, we checked DNA damage,” he says. The finding of reduced DNA damage was a surprise, as cytokines have not previously been implicated in inducing NER. IL-12’s protective effect seems to be dependent on NER induction, as the improvement was lost in a mouse that had a mutation in the NER system.

Could IL-12 eliminate flaking skin from summer holidays? “It’s tempting to speculate on the prophylactic uses,” says Schwarz. “But I don’t claim that applying IL-12 will be the protective strategy of tomorrow,” especially as such a treatment could increase the chances of autoimmunity. Schwarz suggests, however, that a better understanding of this pathway may help improve sunscreens.

Reference: Schwarz, A., et al. 2001. Nat. Cell Biol. 10.1038/ncb717.

Dead cells get the squeeze

Macrophages can clean up the detritus of dying cells. But that, say Jody Rosenblatt, Martin Raff, and Louise Cramer (University College London, London, UK), is not the whole story. They have found that cells undergoing apoptosis in an epithelial sheet signal to their neighbors to begin squeezing, in a process that maintains the barrier function of the epithelium while extruding the dying cell. Macrophages may come along only after the dying cell is liberated from its neighbors.

Rosenblatt noticed the squeezing when she was looking at wound healing in epithelial sheets. The fate of apoptosing cells “looked very similar,” she says. In retrospect, she noticed that the literature included accounts of cell sloughing in the gut, and possible cell extrusion in developing fly embryos. “But at the most it would be one sentence in the middle of a discussion,” she says.

Rosenblatt set out to characterize the process more fully. She found that actomyosin rings form in both the dying and surrounding cells, although only the acto-myosin contraction in the neighboring cells is necessary for extrusion. The neighboring cells are connected via cell–cell junctions so the whole contracting apparatus acts like a purse string.

Apoptotic cells added to a cell monolayer signal early (before any caspase-dependent events) to induce the acto-myosin contraction. Rosenblatt plans to use this in vitro system and the genetics of processes such as dorsal closure to search for the inducing signal.

Reference: Rosenblatt, J., et al. 2001. Curr. Biol. 11:1847–1857.
Making room for new memories

Joe Tsien (Princeton University, Princeton, NJ) and colleagues have suggested that the creation of new neurons in the hippocampus may allow old memories to be wiped clean, thus making way for the new.

New neurons were thought to form new memories, because widespread adult neurogenesis correlates with song learning in songbirds. But adult primate neurogenesis involves far fewer cells. The new neurons last only a few weeks, during which a memory is transferred from short-term storage in the hippocampus to long-term storage in the cortex.

Tsien found he had a tool for testing the possible link between memory and neurogenesis when he made mice that lacked Presenilin-1 (PS1) in their forebrains. PS1 helps cleave amyloid precursor protein (APP) and Notch, and alterations in PS1 activity are associated with Alzheimer’s disease (AD). Mutant mice lacking forebrain PS1 were normal in appearance, behavior, learning, and nerve conduction.

When Tsien put the mice in an enriched environment (with new toys every day), the normal increase in neurogenesis was reduced in mutants relative to that seen in wild-type mice. But the enrichment still allowed both wild-type and mutant mice to better remember a task that was taught after enrichment. Thus enrichment with lowered neurogenesis still increases subsequent memory abilities, suggesting that the level of neurogenesis is not correlated with levels of learning.

Next, Tsien changed the order of activities: learning came first, then enrichment, and then testing. The enrichment procedure still increased memory retention. But enrichment with lowered neurogenesis (in the mutant mouse) increased memory retention to an even greater extent.

The mutant mouse may still show improvement after enrichment because, based on Tsien’s previous experiments, the relevant changes occur in the cortex. But enrichment also brings new memories into the hippocampus. In the wild-type mouse these new memories may interfere with the old, test-associated memory, and thus reduce the success in the later test. Tsien believes that the interference arises from hippocampal neurogenesis, which helps obliterate the old memories by making random connections to old memory neurons.

With less neurogenesis the mutant mouse can retain a memory more successfully in the short term. But this might not work forever. “These animals spend their entire lives in cages,” says Tsien. “The system really never has a chance to process a lot of memories. In a more natural situation you might show a problem.” Tsien plans to look for such a problem by challenging the mice with multiple, overlapping tasks.

If proven, the new theory would have widespread implications. Those searching for memory drugs would know that the hippocampus, where new memories are first processed, has a far more limited storage capacity than the cortex. “Eventually the hippocampus may run the risk of overloading with memories,” says Tsien. The opposite problem of premature memory obliteration may arise if excessive neurogenesis occurs as a result of either AD or the addition of a neural stem cell transplant.

Reference: Feng, R., et al. 2001. Neuron. 32: 911–926.

Romeo and Leishmania

By faking its own death, Leishmania can gain access to macrophages. Once in the macrophage, say Marcello André Barcinski (Universidade de São Paulo and Instituto Nacional de Câncer, Rio de Janeiro, Brazil) and colleagues, the fake death signal then suppresses the host’s ability to kill the invading parasite.

Leishmania makes the fake death signal by exposing phosphatidylserine (PS) on its surface. Exposed PS is also seen on apoptotic cells, where it induces engulfment by macrophages, and reduces inflammation by prompting production of TGF-β. The same two activities are seen with Leishmania, although the parasite also induces both an increase in IL-10 production (which distracts the immune system by shifting it away from cell-mediated immunity) and a reduction in NO production. The latter change favors parasite survival, as NO is one of the main mechanisms by which macrophages kill Leishmania.

Reference: de Freitas Balanco, J.M., et al. 2001. Carr. Biol. 11:1870–1873.