

Research Roundup

How HIV-1 lost control

A minor change during evolution converted the relatively benign monkey SIV viruses into deadly HIV-1, according to Michael Schindler, Frank Kirchhoff (University of Ulm, Germany), and colleagues. They report that HIV-1, unlike most SIV strains, has lost the ability to protect its T cell hosts, and thus its human host, from death.

The critical difference is in a virus protein called Nef, which increases virus infectivity and replication. But even more important than what HIV-1 Nef does may be what it does not do.

The German group tested 30 different *nef* alleles in an HIV-1 vector. Most SIV Nef proteins down-regulated expression of the T cell receptor protein CD3 and thus blocked activation of infected T cells. Nef from HIV-1 and some closely related SIV relatives had neither of these effects.

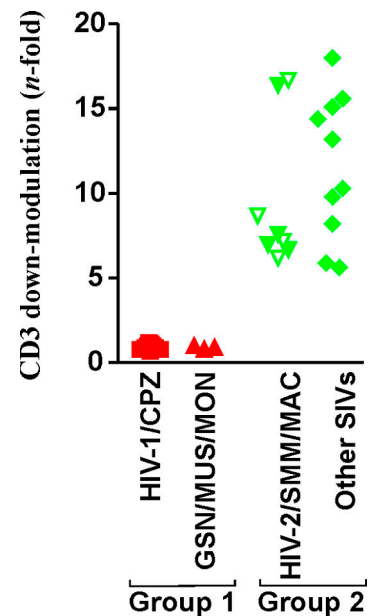
The T cell activation matters because of activation-induced cell death (AICD). A normal immune reaction starts with T cell proliferation. Once an invader is cleared, the reaction is self-limited by AICD—the suicide of most of the recently

proliferating T cells. During HIV-1 infection, however, the virus is not cleared and still remains visible to the immune system. Repeated cycles of activation lead to excessive proliferation and excessive AICD. The vast turnover of T cells eventually exhausts the immune system.

This AICD was high for HIV-1 and the close SIV relatives. But for SIV strains that down-regulated CD3, there was no activation and thus little cell death. This presumably provides a longer-lived cell within which SIV takes shelter.

Kirchhoff plans two further tests of the hypothesis. SIV engineered to express Nef that doesn't down-regulate CD3 should be more pathogenic in monkeys. And less pathogenic HIV-2 strains may express Nef proteins that down-regulate CD3 less.

A more long-term goal will be to reduce AICD, probably by reducing the activation step. This has been tried in patients with full-blown AIDS, "but this is too late," says Kirchhoff. "You need to start this early when the immune system is still functional." Kirchhoff confirms that using immune suppression to fight an immunosuppressive dis-



Less deadly ancestors of HIV-1 down-regulate CD3 so their host cells are spared.

ease will be difficult: "It won't be easy to find the right balance. The [simian] virus can; the question is whether humans can mimic this situation." JCB

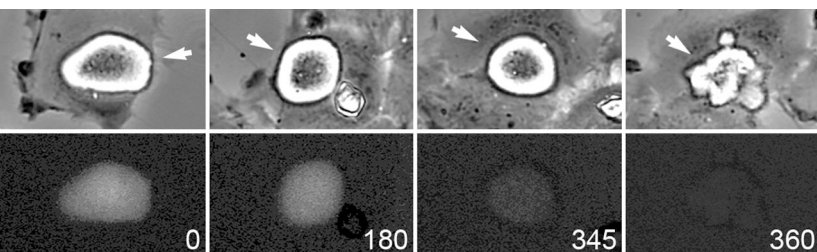
Reference: Schindler, M., et al. 2006. *Cell*. 125:1055–1067.

Slow escape from mitosis

Cells with a defective spindle get delayed—stuck in a mitotic checkpoint—but eventually escape mitosis. In vertebrate cells, say Daniela Brito and Conly Rieder (Wadsworth Center, New York State Department of Health, Albany, NY), that escape is a gradual limp rather than a sudden exit.

The temporary stalling of the cell cycle in a checkpoint gives the cell time to repair damage before continuing. Ted Weinert and Lee Hartwell gave this phenomenon its name, but "they never viewed a checkpoint as leading to a permanent arrest," says Rieder.

Indeed, even in response to a problem that cannot be fixed,



Even when mitotic cells lack a spindle, cyclin (bottom panels) slowly fades away.

such as high levels of the anti-microtubule drug nocodazole, many cells do leak through the mitotic arrest. In yeast and perhaps flies, the escape occurs via phosphorylation of Cdk1 or induction of a Cdk1 inhibitor. This adaptation pathway turns off the Cdk1/cyclin B complex, whose activity defines mitosis.

A similar abrupt change was expected in vertebrate cells, via Cdk1 phosphorylation, induction of a Cdk1 inhibitor, or rapid degradation of cyclin B. "But it turns out it doesn't do any of these," says Rieder. Instead, "human cells slowly degrade cyclin B over the course of time."

This slow, proteasome-dependent degradation of cyclin B was required for nocodazole-treated cells to escape from mitosis. The spindle assembly checkpoint remained intact and active throughout. Thus Brito and Rieder believe that vertebrate cells do not turn on a specific adaptation pathway. Instead there is a constant low level of cyclin B degradation that eventually reduces cyclin B levels below that needed to maintain a mitotic state. In plants the resultant exit may allow a change in ploidy, although its utility in mammals is less clear. JCB

Reference: Brito, D.A., and C.L. Rieder. 2006. *Curr. Biol.* 16:1194–1200.

Flexible tumor metabolism

Tumor cells can survive the hypoxia in the center of tumor masses by using glycolysis, rather than oxidative phosphorylation, to generate energy. Most tumor cells also stick to glycolysis when normally oxygenated. Valeria Fantin, Julie St-Pierre, and Philip Leder (Harvard Medical School, Boston, MA) now report that some tumor cells, despite their preference for glycolysis, nevertheless retain the ability to use oxidative phosphorylation.

The Boston team reached this conclusion by inhibiting lactate dehydrogenase A (LDH-A). This enzyme converts NADH and pyruvate, the products of glycolysis, into lactate and NAD⁺. The lactate is exported and the NAD⁺ used to keep glycolysis going.

Therefore, when LDH-A is shut off and oxygen is limited, NAD⁺ runs low and glycolysis alone cannot continue. When oxygen is around, however, the pyruvate can be converted to acetyl-CoA to feed the Krebs cycle, which in turn feeds oxidative phosphorylation to regenerate NAD⁺.

It was unclear whether tumor cells were capable of switching back to oxidative phosphorylation. It now seems that some are: some cells inhibited for LDH-A got their mitochondria going and grew almost as well as wild-type cells if oxygen levels were normal. The inhibited cells did poorly in low-oxygen and in tumor models, however.

A preference for glycolysis in normoxic conditions, even by cells perfectly capable of oxidative phosphorylation, is somewhat of a surprise given the greater efficiency (per glucose molecule) of oxidative phosphorylation. But in fact oxidative phosphorylation is slower than glycolysis. This difference may be critical for fast-growing tumor cells.

The bias toward glycolysis is probably driven by HIF-1 α , which is induced by both hypoxia and oncogenes, and by oncogenes that increase glucose uptake. Normal cells rely far less on glycolysis and LDH-A, however, so anticancer therapies targeting LDH-A should be nontoxic. **JCB**

Reference: Fantin, V., et al. 2006. *Cancer Cell*. 9:425–434.

Seeing tube formation

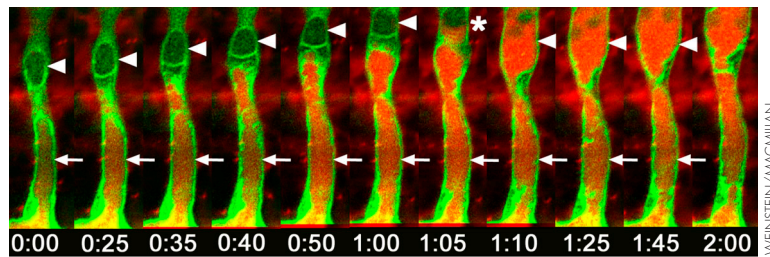
Makoto Kamei, Brant Weinstein (NIH, Bethesda, MD), and colleagues have visualized in vivo tubule formation. As in previous in vitro experiments, large vacuoles fuse to form a tubule lumen that passes through an individual cell. Many such cells adhere in a line to form a blood vessel.

The researchers took advantage of the transparency of zebrafish to look at vessels emerging from the dorsal aorta. Initially, they saw highly dynamic vacuoles that appeared and disappeared. The vacuoles then fused together and enlarged to take up most of the cell volume, thus forming the lumen.

Quantum dots injected into the circulation got into the lumen formed by each cell in turn. Preliminary evidence suggests that vacuoles form independently in each cell, but enlargement may be a progressive process: the cells nearest to established vessels may help out their more distal neighbors.

The tubulating vacuoles formed in vitro are pinocytic in origin. Given the visual similarities, the same may be true in vivo. Weinstein suggests that these vacuoles may mature by establishing their identity as apical membranes, as the inside of a tubule is ultimately defined as apical. He now plans to track apical markers in transgenic zebrafish. **JCB**

Reference: Kamei, M., et al. 2006. *Nature*. doi:10.1038/nature04923.

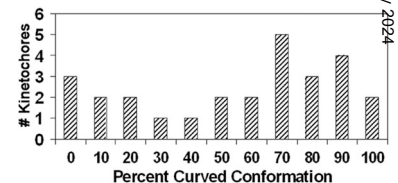
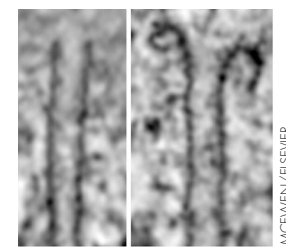


Red dye enters first one and then two cells of a zebrafish vessel sprout.

Mixed kinetochore fiber

The action of a single kinetochore fiber appears unified—it is either pulling or pushing its attached chromosome at any given time. But underlying this unity is diversity, say Kristin VandenBeldt, Bruce McEwen (Wadsworth Center, New York State Department of Health, Albany, NY), and colleagues. They find that the kinetochore microtubules (kMTs) in a single fiber are a mixture of depolymerizing and polymerizing microtubules.

The concept of unified kinetochore action came from light microscopy. But when the New York team used electron microscopy



Polymerizing (left) and depolymerizing (right) microtubules are found in different mixtures at kinetochores (bottom).

they saw that the 10–30 kMTs in one fiber had a mixture of morphologies. The kMTs with straight ends at the kinetochore are believed to be polymerizing, whereas those with curved ends are the unravelling depolymerizing kMTs. Surprisingly, two-thirds of kMTs were in the depolymerizing state during metaphase, suggesting that the kinetochore restrains kMTs that are in the depolymerizing state and retards their shrinkage. This restraint should produce the tension that is central to spindle dynamics.

Some kinetochores had fewer depolymerizing kMTs and some had more, but the differences were on a continuum. Thus the kinetochore appears to change direction based on a balance of pulling and pushing forces rather than a discrete, all-or-none switch. How the polymerization bias and kMT detachment are controlled remains largely mysterious. **JCB**

Reference: VandenBeldt, K.J., et al. 2006. *Curr. Biol.* 16:1217–1223.