When is a virus an exosome?

A bold new theory suggests that retroviruses have hijacked an intercellular communication system for both their biogenesis and spread. The concept, outlined by Stephen Gould, Amy Booth, and James Hildreth (Johns Hopkins University, Baltimore, MD) has implications for HIV treatment and immunization strategies, and may explain why tissue rejection occurs in humans.

Hildreth was looking at human proteins that HIV acquires during its biogenesis, and noticed that lysosomal proteins were in the mix. This ties in with recent findings in this and other journals that HIV is packaged in late endosomes (for review see Amara and Littman, 2003).

In uninfected cells, this endosomal compartment imagines to form small, internal vesicles. The bag of vesicles, or multivesicular body, can fuse with the plasma membrane to disgorge these vesicles, named exosomes, which then travel to other cells to transmit messages. In the immune system, exosomes transfer peptide-laden MHC proteins to noninfected cells, and also act as miniature versions of antigen-presenting cells.

Hildreth now proposes that “the virus is fully an exosome in every sense of the word.” Others have found that HIV particles contain MHC, but by the exosome hypothesis they may also contain proteins that exosomes use to fuse with target cells and to avoid attack by complement. As Gould points out, an exosome makes a perfect vector for HIV, because an exosome “is not just proteins in a vesicle, it’s something that is meant to traffic.”

The idea may explain how HIV both infects cells that lack receptors for its surface gp120 protein, and avoids robust, virus-directed immune responses. “Even if one completely blocks the gp120-related pathway of entry, HIV will have this second, albeit less efficient, means of getting into cells,” says Hildreth.

To block all entry, suggests Hildreth, perhaps the MHC should be the target. Alloimmunization—immunization with a wide range of MHC and other protein variants (e.g., by injecting killed leukocytes) might allow a newly infected individual to mount a quick attack on the incoming HIV, which is packed with foreign MHC. Gould even suggests, “this is why we have tissue rejection responses—[they evolved] to protect us from retroviruses.” He points out that alloimmunity predates and thus could not have arisen from adaptive immunity.

The more extreme idea of xenoinmunization does work in monkeys, which can reject SIV grown in human cells. And for Thomas Lehner (Guy’s Hospital, London, UK), who has been pushing the idea for several years, alloimmunization “is far better than anything we have at the moment.” But it has languished since the monkey experiment, perhaps based on fears that it would prevent later transplants, cause rejection during pregnancies, and fail to catch a handful of HIV particles before they replicate and thus incorporate self-MHC.

Mark Feinberg (Emory University, Atlanta, GA) warns, “for a protective vaccine, the regulatory environment is exceedingly conservative, because you are dealing with healthy people.” Even if concerns such as transplantation are of little importance in the developing world, a vaccine developed in the industrialized world will have to follow the exacting standards of bodies such as the US Food and Drug Administration (FDA). Unfortunately, says Feinberg, “the FDA goes off in one direction, and the epidemic goes off in another.”


Centromere errors get chewed out

Microtubule (MT) ends that invade into the inner centromere may get chewed up, say Ryoma Ohi, Timothy Mitchison (Harvard Medical School, Boston, MA), and colleagues. The process should help prevent MTs emanating from a single pole from attaching to both sister kinetochores.

Ohi proposes that the chewing is performed by the KIN kinasin MCAK. He found a new MT-binding protein, ICIS, that binds to MCAK, relies on it for localization at the inner centromere, and further activates MCAK’s MT-depolymerizing activity in vitro.

Although ICIS depletion causes widespread MT polymerization, probably because of co-depletion of MCAK, it almost certainly has a more specific function at the inner centromere. Just away from the inner centromere—specifically near the kinetochore—a high density of MTs is desirable if the cell is to ensure kinetochore capture. “But it’s also dangerous,” says Ohi. Slightly errant MTs can skirt around the closest kinetochore, cross the inner centromere, and attach to the more distant kinetochore. Ohi predicts that ICIS-stimulated MCAK intercepts these MTs before they can reach their incorrect target. He now proposes to test whether interference with the MCAK/ICIS system causes attachment errors, as his model predicts.

Survival is sweet

Nutrition and cell death are integrated in a single mitochondrial complex, according to Nika Danial, Stanley Korsmeyer (Harvard Medical School, Boston, MA), and colleagues.

The complex was discovered after gel filtration of the pro-apoptotic BAD protein, and contains BAD, a phosphatase, kinase, and kinase-anchoring protein directed at BAD, and glucokinase. In cells lacking BAD, the complex falls apart, respiration is compromised, and blood glucose regulation is abnormal. A nonphosphorylatable BAD leaves the complex intact but otherwise nonresponsive to glucose.

The results are consistent with a simple model: glucose induces phosphorylation of BAD, thus ensuring both cell survival and activation of glucokinase. The active glucokinase clears blood glucose and feeds the mitochondrial with necessary intermediates.

The presumption was always that the cell’s two major survival pathways—glycolysis and apoptosis—were independent. But the complex hints at integration. Korsmeyer speculates that early cells may have relied on this direct integration with sugar levels, as the cells probably lacked fancy growth factors for modulating cell survival. “We could be looking at a primordial role between nutrition and cell death,” he says. “The growth factors may have come later.” This hypothesis is consistent with the ideas of Craig Thompson (University of Pennsylvania, Philadelphia, PA), who has suggested that growth factors act not directly on cell survival but via modulation of glucose levels.


A daughter’s size is not critical

The critical size model of cell division is so well established for budding yeast that, as Warren Heideman says, “it’s on the wall of the Guinness Brewery.” But now Tracy Laabs, Heideman (University of Wisconsin, Madison, WI), and colleagues have found that one of the pillars of the model has an alternative explanation.

The model states that cells only divide once they reach a critical size, which is why smaller daughters delay their division until they reach sizes comparable to those of their mothers. The Madison team found instead that daughters delay thanks to a daughter-localized G1 cyclin inhibitor called Ace2.

After elimination of Ace2, mothers and daughters divided at the same time, so daughters divided at a smaller size than usual. An Ace2 mutant that was no longer localized to daughters also showed simultaneous division because both mothers and daughters were delayed.

Ace2 works at least in part by controlling levels of the G1 cyclin Cln3. In theory Ace2 could be resetting the critical size in daughters. But, says Heideman, “if you stick with critical size you have so many modifications that you are left with something very cumbersome.”

He believes the cell couples growth rate and cell division rate without sensing size. “The critical size model was easily accepted by our minds because it was so elegant,” he says, “but it may be hard [for the cell] to engineer.”


Pulling to the protease

Before destroying proteins, proteases such as bacterial ClpX and the related eukaryotic proteasome must denature them. Jon Kenniston, Robert Sauer (MIT, Cambridge, MA), and colleagues now show that ClpX denatures by repeatedly applying a uniform unfolding force. “The way the enzyme works is to keep trying,” says Sauer.

This strategy works because proteins fluctuate around an average structure over time. Even a very stable protein will, very infrequently, be surprised in a relatively susceptible state. When this happens, the standard pulling force by ClpX is enough to unravel the protein. The enzyme then quickly threads the denatured protein through a narrow hole, toward the protease active site, before the substrate can refold.

The MIT group discovered the repetitive pulling phenomenon by studying stability variants of the muscle protein titin. This allowed “us to deconvolute how much ATP hydrolysis is used for unfolding versus translocating,” says Sauer.

During denaturation of different variants, the ATP hydrolysis rate was constant, but the amount of time taken to denature varied widely. Thus, although unstable variants were destroyed by a handful of ATP cycles, the destruction of a wild-type titin domain took more ATP molecules than were used in the protein’s biosynthesis.

When it fails, the ClpX unfoldase probably lets the pulling process cycle back to a ground state. This slipping may be inevitable given the protein’s construction. “The enzymes aren’t very stable proteins by themselves, so we don’t think they have any way to store energy,” says Sauer. For that reason, he says, “there’s no way of making unfolding a cumulative process.”