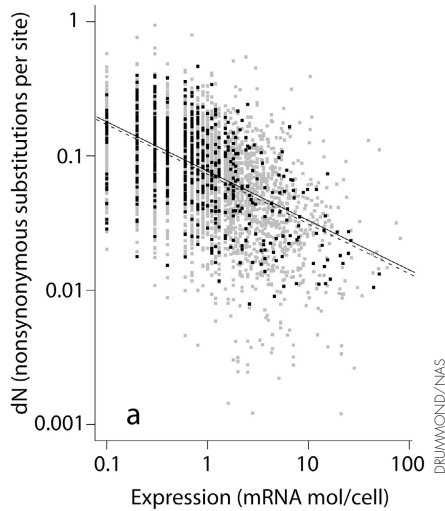


Research Roundup



Pressure not to misfold means that higher translation correlates with slower evolution.

Highly goes slowly

Rates of protein evolution vary widely. Very little of this variation is explained by dispensability (essential proteins evolving slowly), but up to a third is explained by expression levels. For an entirely mysterious reason, highly expressed proteins evolve slowly.

Now, Allan Drummond and colleagues (Caltech, Pasadena, CA; and Keck Graduate Institute, Claremont, CA) argue that this correlation is based on the severe selective pressure on highly expressed proteins to avoid misfolding even when they are mistranslated. More abundant proteins are a greater misfolding threat because even a low misfolding rate would result in many misfolded, potentially

toxic proteins—what Drummond calls “glue-covered monkey wrenches.” Genes encoding abundant proteins therefore get stuck, say the authors, in the few sequences that are “translationally robust”: they usually fold correctly even when there are translational errors.

The study started not with experiments but theory. “The ‘aha!’ moment was lying in bed,” says Drummond. For the theory to work, however, the threat of errors would have to be large enough. Drummond checked his references for translation error rates. “They were absurdly high,” he says.

Ribosomes make ~5 errors per 10,000 codons translated. “When you convert that into how many proteins are translated,” says Drummond, “it becomes much more interesting to look at the cost.” With this error rate,

~19% of average-length yeast proteins should have a missense error; conservatively, 5% of the proteins might misfold. For the abundant PMA1 transporter that raises the scary prospect of ~63,000 misfolded molecules per cell.

The team found that misfolding matters not because it takes proteins out of circulation, but because it creates a potentially damaging molecule. What mattered most for evolution rate was not absolute abundance (the more abundant, the more activity that can potentially be lost by misfolding) but translation frequency (the more translation events, the more opportunities for creating troublesome and long-lived misfolded proteins).

The theory also makes sense of proteins such as the plant enzyme Rubisco, perhaps the most abundant protein on Earth. It is highly conserved but the result of this slow evolution is not functional fragility but, the authors hypothesize, translational robustness—surprisingly few inactivating mutations have been found in its gene. **JCB**

Reference: Drummond, D.A., et al. 2005. *Proc. Natl. Acad. Sci. USA*. doi:10.1073/pnas.0504070102.

Fate trafficking

Asymmetric cell fates can be defined by creating an endocytic compartment in one incipient daughter but not the other, say Gregory Emery, Juergen Knoblich (IMB, Vienna, Austria), and colleagues. Only the daughter with a functioning recycling endosome can direct Delta back to the plasma membrane so that it can activate Notch on the other daughter.

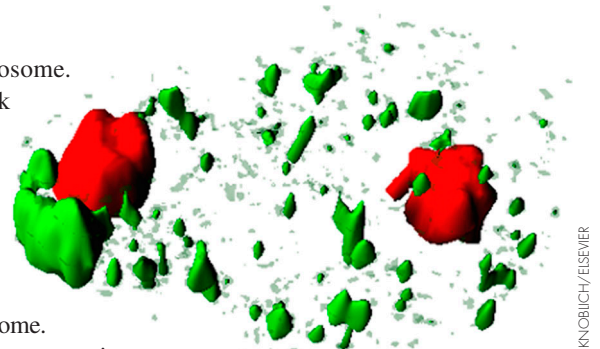
Knoblich studies fly sensory organ precursor (SOP) cells, which divide to form two cells—pIIa and pIIb—with different fates. Those fates are decided largely by Notch and its binding partner Delta. In pIIb, the asymmetry determinants Numb and Neuralized turn off Notch and increase the endocytosis that somehow activates Delta activity.

The Austrian team found that only in pIIb did Delta travel to a compartment

resembling a recycling endosome. From here it could be sent back to the plasma membrane to activate Notch on pIIa. Some Delta endocytosis also happens in pIIa but, with no recycling endosome available, this Delta is sent to its destruction in a lysosome.

The recycling endosome protein Rab11 was present in both of the daughter cells, but only pIIb had centrosome-localized recycling endosomes labeled with Rab11 and the Rab11-binding protein Nuf. Overriding both the Numb and Rab11 pathways, but not either one by itself, resulted in a complete loss of asymmetry.

The work has uncovered not only a new pathway for asymmetric cell division but a more dramatic mode of regulation



Rab11 (green) accumulates at the recycling endosome only in the pIIb cell (left).

by the endocytic system. Endocytosis of select cargos has been known for some time to regulate signal transduction. “What is new,” says Knoblich, “is that for the first time a cell can regulate the endocytic pathway itself—the cell shuts off the recycling endosome. That is unprecedented.” **JCB**

Reference: Emery, G., et al. 2005. *Cell*. 122:763–773.

Antiviral walls

The innate immune system has the tricky task of foiling all invaders rather than targeting a specific few. Eugenia Leikina, Leonid Chernomordik (NICHHD, Bethesda, MD), and colleagues report that defensin antimicrobial peptides use a unique nonspecific method: they cross-link surface glycoproteins and thus freeze them in place. The resulting web of proteins obstructs the fusion of viral and cell membranes.

Defensins caught Chernomordik's eye because he works on membrane fusion and the defensins have broad specificity. He thought they might interfere with membrane properties, but was disappointed when they accelerated rather than inhibited fusion between protein-free liposomes.

With virus-infected cells, however, things got more interesting. Defensins did not block virus binding or endocytic uptake but did block membrane fusion. Membrane glycoproteins no longer diffused over the membrane surface.

The lack of diffusion may be the key. When a virus binds to a cell there is still a gap of ~10 nm between the membranes, which can be closed only when proteins in a tiny patch happen, via random diffusion, to get out of the way. With defensins bound, suggests Chernomordik, "the proteins would not move away."

"This might be the first natural antiviral agent targeting fusion," says Chernomordik. "The fusion stage targeted is elusive, because if proteins are mobile it is not rate limiting." He also notes a tantalizing analogy to prokaryotes, which use covalently cross-linked proteoglycans as a barrier, forcing bacteriophages to use injection rather than fusion mechanisms. "For a short time," he says, "our cells become prokaryotic by erecting these walls." **JCB**
Reference: Leikina, E., et al. 2005. *Nat. Immunol.* doi:10.1038/ni1248.

Repairing for death

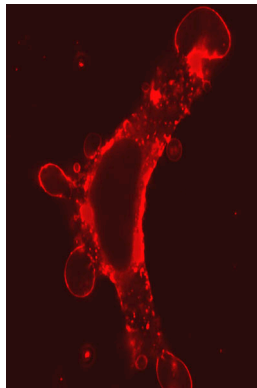
The targets of cytotoxic T lymphocytes (CTLs) determine their own mode of death, say Dennis Keefe, Judy Lieberman, and colleagues (Harvard Medical School, Boston, MA). The dying cells patch themselves up so that rapid and messy necrosis is avoided in favor of a more lengthy and controlled apoptosis.

The CTLs deliver their insult in the form of perforin, a pore-forming protein that helps get proteases called granzymes into the target cell. The Boston group showed that perforin addition to a target cell induced a Ca^{2+} transient and a massive plasma membrane repair response. Similar Ca^{2+} -induced responses, in which lysosomal and other membranes are recruited to patch up plasma membrane holes, are known to be induced by mechanical damage to cell membranes.

Blocking the rise in Ca^{2+} and thus the repair response resulted in far more necrosis. This is presumably a consequence of breaching the plasma membrane barrier. Necrosis is induced before there is time to induce the slower process of apoptosis, which tends to sequester intracellular proteins and thus avoid unwanted autoimmune responses.

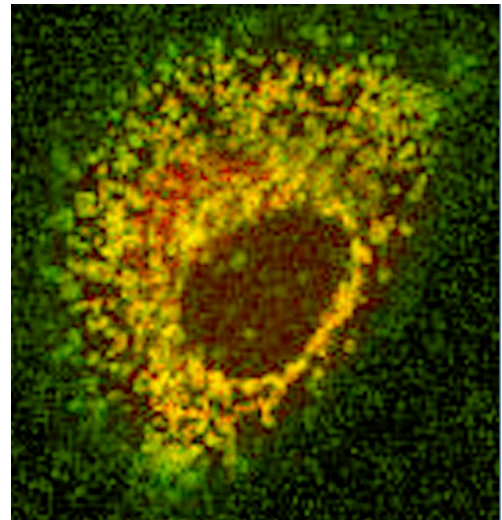
If the plasma membrane is being repaired, perforin is probably acting within endosomes to release granzymes into the cytoplasm. The Boston group gathered more evidence for this theory, but they still want to find out how perforin induces higher levels of endocytosis, and what changes within the endosome turn on perforin's pore-forming activity. **JCB**

Reference: Keefe, D., et al. 2005. *Immunity*. 23:249–262.



Perforin induces membrane repair that saves cells from necrosis.

LIBERMAN/ELSEVIER



MAVS (green) works from mitochondria (red) to direct innate immune responses.

CHEN/ELSEVIER

Mito immunity

Mitochondria may serve as a surveillance site where innate immune responses and apoptosis are coordinated, say Rashu Seth, Zhijian Chen, and colleagues (UTSW, Dallas, Texas). They base this idea on their discovery of an antiviral protein that hangs out on mitochondria.

The protein was discovered by three groups and named MAVS, VISA, and IPS-1. Overexpression of MAVS (named for its mitochondrial antiviral signaling) induces interferon expression and thus increased antiviral defenses. It operates downstream of RIG-I, which detects viral double-stranded RNA, but upstream of the NF- κ B and IRF3 families of transcription factors. RNAi of MAVS abolishes viral activation of NF- κ B and IRF3 and thus knocks out the antiviral response.

MAVS function requires its transmembrane domain, which resembles that of the anti-apoptosis protein Bcl-2. Both proteins are found on mitochondria. This puts MAVS closer to some viruses, which replicate using membranes such as the ER and perhaps mitochondria. It may also allow the coordination of decisions by the innate immune system and the apoptosis machinery; some members of the latter have already been implicated in innate immunity. Chen suggests that innate immunity may be just one more service that mitochondria provide for the cell. **JCB**

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