

Research Roundup

Garbage in, garbage out

Incessant wear and tear on plasma membranes is a fact of cell life, so it's not surprising that cells know how to fix the resulting damage quickly—often within a few seconds. Norma Andrews and her colleagues (Yale University

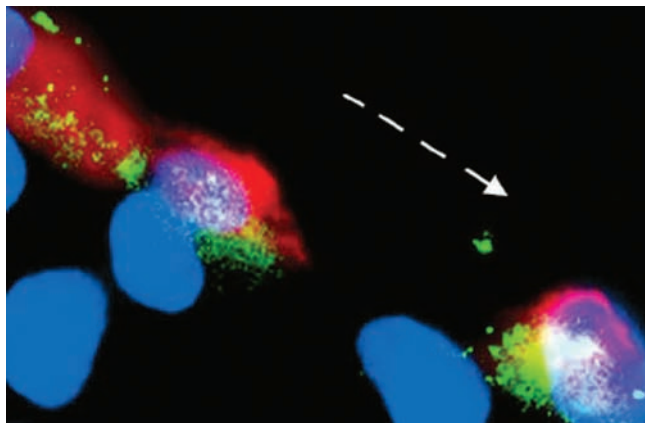
School of Medicine, New Haven, CT) have just revealed how plasma membrane repair works. In the process they have demonstrated a new function for the cell's decomposition specialists, the lysosomes.

Others have observed that membrane repair and calcium-regulated exocytosis often occur at the same time, with unidentified vesicles flocking to the site of injury. Andrews and her colleagues decided to test whether those vesicles were lysosomes, and if they were responsible for membrane repair. Via several methods—for example, using monoclonal antibodies against a major lysosomal glycoprotein—they conclude that

blocking lysosomes' ability to fuse with the plasma membrane in a calcium-dependent manner also interferes with plasma membrane repair.

Andrews says she is now pondering how to investigate an intriguing idea: that lysosomes evolved not just to break down molecules inside cells, but to protect cells from invading microbes by pelting them with the resulting garbage. "We don't have any data indicating that, but it's an interesting speculation," she says. "Paul McNeil suggested that plasma membrane repair may be a primitive form of secretion. We can imagine a scenario in which the involvement of lysosomes provided an additional evolutionary advantage. If the lysosomal contents were dumped on top of a microbe that was secreting membrane-damaging molecules close to a cell, this might create conditions outside the cell that would contain—or maybe even kill—the microbe." ■

Reference: Reddy, A., et al. 2001. *Cell*. 106: 157–169.



Andrews/Elsevier

A scrape (arrow) lets in a red dye and brings lysosomes (green) to the surface.

Running interference

RNA interference (RNAi) is an ancient mechanism for executing the epigenetic phenomenon called gene silencing. It has quickly become a standard way to figure out what a gene is doing, even though not much is known about how it works.

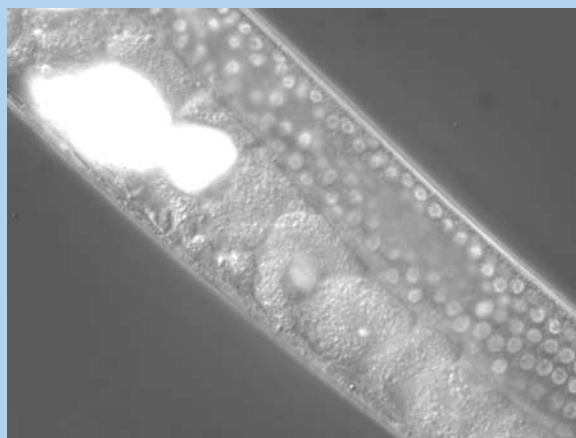
When Thomas Tuschl and Phillip Zamore reported last year that, *in vitro*, RNAi involves cleavage into short pieces of both a double-stranded RNA and subsequently the rest of the target mRNA, Brenda Bass immediately had a hunch that the responsible enzyme was one she had encountered a decade ago. She and her colleague Scott Knight (University of Utah, Salt Lake City, UT) have now verified that hunch. They have shown not only that the enzyme (known appropriately as Dicer) is required for RNAi, but that it is essential for normal development.

With the help of the *C. elegans* Gene Knockout Consortium, Knight and Bass isolated worm knockouts that contained a null mutation in the Dicer gene. The

knockouts had germline defects that rendered them sterile. So Knight mated knockout heterozygotes that also contained transgenes encoding both GFP and a heat-shock inducible RNA hairpin that matched the GFP sequence. Heat shock caused GFP to disappear in wild-type and heterozygote progeny because GFP RNA was knocked out by RNAi. In the mutant homozygote progeny, however, GFP stayed on, suggesting that RNAi needs Dicer in order to work.

Under some conditions, however, RNAi worked just fine in the mutant homozygotes. Bass says one possibility is that Dicer from the heterozygote mother sometimes sticks around, but she's doubtful that this is the whole story. Her latest hunch: "I think this means there are other pathways by which RNA interference can occur." ■

Reference: Knight, S.W., and B.L. Bass. 2001. *Science*. 10.1126/science.1062039 <http://www.sciencemag.org/cgi/content/abstract/1062039>



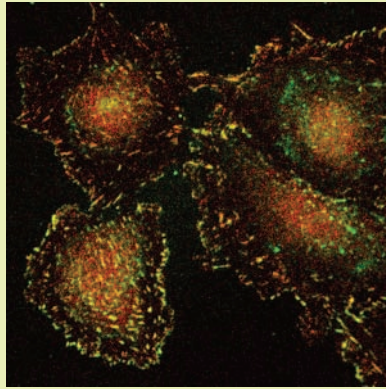
Bass/AAAS

Worms lacking an RNAi enzyme have enlarged oocyte nuclei.

Cell cycling with Rac

When endothelial cells adhere to the extracellular matrix protein fibronectin, they can proliferate in response to growth factors. Adhesion to laminin, however, brings their cell cycles to a dead stop. Why? Given that the response depends on the composition of the extracellular matrix, Filippo Giancotti and his colleagues at the Memorial Sloan-Kettering Cancer Center (New York, NY) proposed in 1996 that progression through the cell cycle depends on signaling from a cell's particular repertoire of adhesion receptors called integrins.

Integrins do not act alone, however. "Our major hypothesis in the past few years has been that the integrins that are able to promote proliferation do so by cooperating with growth factor receptors," says Giancotti. That control was thought to be exerted via the MAP kinase ERK, at the level of cyclin D1 transcription. The new paper, however, reports that ERK activation occurs when cells are plated on either fibronectin or laminin, whereas it is Rac activation that is specific to the proliferation-permissive fibronectin. Furthermore, activated Rac promotes translation, not



Giancotti/Elsevier

Signaling from focal adhesions gets cells through G1.

transcription, of Cyclin D1, initiating progression through G1 to the S phase of the cell cycle.

The specific signaling functions of integrins that promote either proliferation or growth arrest are important in many cell types. "The general issue is the cell's ability to interpret positional information," says Giancotti. In angiogenesis, for example, fibronectin may promote endothelial cell proliferation during the invasive phase, then laminin may aid differentiation by inducing cell cycle exit. ■

Reference: Mettouchi, A., et al. 2001. *Mol. Cell.* 8:115–127.

Shedding light on protein folding

Folding of a bacterial protein is directly related to signal transduction, and temporary partial unfolding can be a mechanism of signaling, according to researchers at the University of Chicago.

Wouter Hoff and his colleagues study photoactive yellow protein (PYP), a circadian photoreceptor from purple bacteria. They found that when PYP absorbs light it switches from the off (trans chromophore) to the on (cis chromophore) conformation, where it is partially unfolded. "The result we obtained," he says, "is that the switch in this case is based on temporary unfolding of the protein." Hoff suggests that a similar switch might be operating in other signal transduction systems.

The findings have some immediate

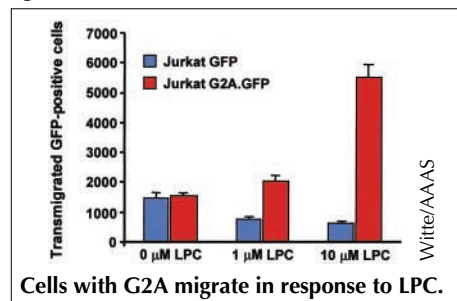
applications. Investigators have had difficulty determining whether a particular intermediate for folding is a productive on-pathway intermediate that will result in a proper conformation, or whether something has gone wrong during folding. With PYP, folding by rapid mixing in denaturants and refolding after signaling both pass through the same state, suggesting that this is an on-pathway intermediate.

PYP could also improve time resolution for protein-folding studies. Rapid mixing experiments are usually limited to 1 ms resolution, but the light-triggered nature of PYP refolding should cut that down to nanoseconds. ■

Reference: Lee, B-C., et al. 2001. *Proc. Natl. Acad. Sci. USA.* 98:9062–9067.

The (formerly) missing link

Approximately 10% of G protein-coupled receptors are orphans whose ligands await identification. Until now, the lymphocyte-expressed receptor G2A has been one of these orphans. But Yan Xu (Cleveland Clinic Foundation, Cleveland, OH) and Owen Witte (University of California at Los Angeles, CA) and colleagues have just reported that a small lipid, the inflammatory mediator lysophosphatidylcholine (LPC), is a high-affinity G2A ligand.



The finding links together two previously unrelated lines of research on atherosclerosis and inflammatory autoimmune disease, and it begins to suggest mechanisms involved in those disorders. Researchers have known that LPC is important in both atherosclerosis (as a component of oxidized low density lipoprotein [LDL]) and autoimmune diseases such as systemic lupus erythematosus. Witte's lab reported in May that mice lacking the G2A gene develop a progressive wasting disease resembling lupus (*Immunity*. 14:561). The new paper suggests that G2A may detect LPC levels at sites of inflammation and may limit expansion of tissue-infiltrating cells, thus slowing progression to autoimmune disease.

"What exactly the cellular function is and how it's regulated is not directly shown yet," Xu notes. She says that another lysophospholipid, sphingosylphosphorylcholine (SPC), is also a G2A ligand, and it too is potentially involved in a number of diseases. The two labs are continuing their collaboration to reveal the physiological or pathological function of both the receptor and the ligands. ■

Reference: Kabarowski, J.H.S., et al. 2001. *Science.* 293:702–705.