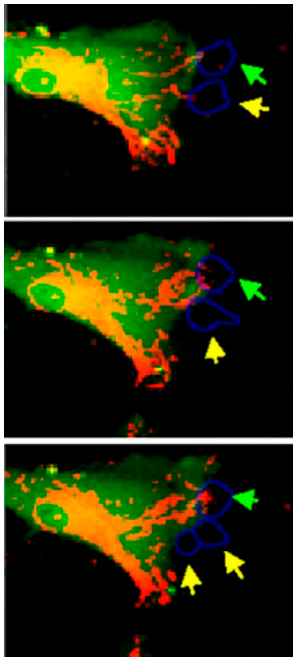
Research Roundup



PROCKOP/NAS

MSC mitochondria (red) approach damaged cells (blue), whose division (bottom) indicates aerobic rescue.

Spreading mitochondria

Mitochondria are passed from cell to cell, as shown by Jeffrey Spees, Scott Olson, Mandolin Whitney, and Darwin Prockop (Tulane University, New Orleans, LA).

Prockop's group stumbled across this remarkable ability while studying bone marrow stem cells (MSCs), which were particularly social in culture, often touching each other and then backing off. Small numbers of MSCs can repair some damaged heart cells, whose main defect is usually a loss of mitochondrial activity. The authors thus wondered whether the contact behavior allowed MSCs to transfer mitochondria. "It was a way out-there hypothesis," says Prockop, "that we wouldn't have mentioned to anyone had the experiment not worked out."

To test their "out-there" idea, the group damaged the mitochondrial genome (mtDNA) of a transformed epithelial cell line using ethidium bromide and then looked for rescue by MSCs. Indeed, rescued clones appeared that contained MSC-derived mitochondrial proteins. Fibroblasts were also able to rescue the damaged cells. It is unclear whether healthy cells take in mitochondria as well.

After ruling out cell fusion as an explanation, the group hoped to show the transfer directly. But a dearth of fluorescent mtDNA tags has made the transfer hard to follow. Cultured cells did not take in isolated, cell-free mitochondria, so donation appears to be an active process.

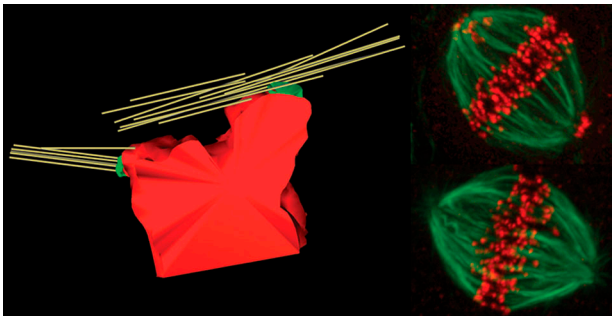
Perhaps the organelles are passed through the recently recognized structures called nanotubes. Or they might be sent out by exocytosis. The group saw that MSCs leave behind mitochondria-filled vesicles that end up attached to other cells, but they have not seen these vesicle internalized as yet. For now, given the therapeutic implications for mitochondrial diseases, the group hopes to prove that transfer occurs in vivo. JCB

Reference: Spees, J.L., et al. 2006. *Proc. Natl. Acad. Sci. USA.* doi:10.1073/pnas.0510511103.

Monooriented in the middle

Chromosomes find an ingenious route to the metaphase plate to improve their chances of biorientation, based on new results from Tarun Kapoor (Rockefeller University, New York, NY), Alexey Khodjakov (Wadsworth Center, Albany, NY), and colleagues.

For their even distribution into daughter cells, chromosomes must first be attached to the opposite spindle poles. For the most part, a chromosome is first monooriented—that is, it is hitched by microtubule bundles (K-fibers), connecting its kinetochore to a single pole. That chromosome is then pulled toward its attached pole, where microtubules from the opposite pole are rare. Eventually, however, the chromosome leaves this pole and congresses to the metaphase plate.



HERGERT AND LAMPSON/AAAS

Lateral interactions with K-fibers (left) allow monooriented chromosomes to get to the metaphase plate, but they accumulate at poles in cells lacking CENP-E (right top).

This congression is generally assumed to be the result of biorientation, but the new results indicate that monooriented chromosomes can also make their way to the metaphase plate. EM images showed that kinetochores of congressing monooriented chromosomes interacted laterally with K-fibers of already bioriented chromosomes.

Monooriented chromosomes, says Khodjakov, "can borrow the K-fibers of other chromosomes and use them as rails to go to the middle of the spindle. At the middle, the chances of acquiring microtubules from the other pole are much higher."

This opportunistic behavior suggests that spindle formation is cooperative—the first chromosome to biorient makes the process easier for its followers. "Late in the process," says Khodjakov, "there are plenty of rails" for the few remaining monooriented chromosomes.

The only known microtubule motor at kinetochores with the appropriate directionality to walk to the metaphase plate is CENP-E. Upon depleting mitotic cells of this protein, the authors found that several monooriented chromosomes lingered near a pole rather than congressing to the center. The ability to pull monooriented chromosomes along neighboring tracks is the first true motor function suggested for CENP-E, which is better known for recruiting spindle checkpoint proteins to the kinetochore. JCB

Reference: Kapoor, T.M., et al. 2006. *Science.* 311:388–391.

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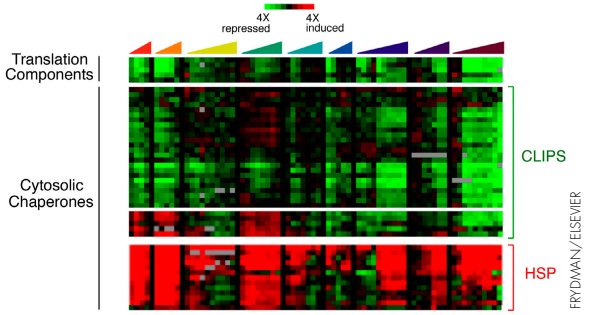
Specialized chaperones

According to Véronique Albanèse, Judith Frydman, and colleagues (Stanford University, Stanford, CA), yeast evolved two distinct chaperone networks—one to fold newly synthesized proteins, and another to deal with stress-induced misfolding. The dedication is a departure from the prokaryotic chaperone system.

Bacteria have only one ribosome-bound chaperone and use primarily the same two chaperones to fold proteins after translation and then again after stress. But bioinformatic analyses by the authors suggested that yeast are different; whereas stresses such as heat and oxidation induced one set of cytosolic chaperones, they repressed another. The proteins from the repressed set were associated with ribosomes, and mutant lines lacking in this set were hypersensitive to translation inhibitors.

The authors propose that eukaryotes have a set of chaperones dedicated to nascent polypeptide folding during translation. This task splitting might have allowed for a better optimization of chaperone duties. "Eukaryotic cells have much larger, multidomain proteins than bacteria," says Frydman. "Maybe this [advancement] was helped by the evolution of a chaperone machinery dedicated to ribosomes."

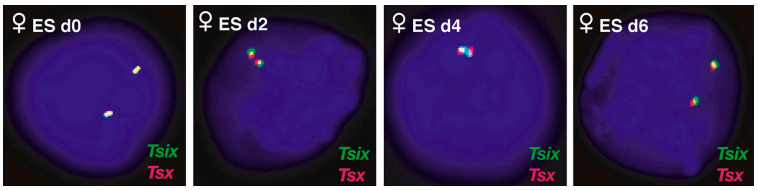


Translation-associated chaperones (CLIPS) are repressed (green) by stress conditions that induce (red) others (HSP-Chaperones).

Some overlap in duties might occur, as the slow growth of a mutant line lacking the major translation-linked chaperone, SSB, was partially rescued by high levels of stress-induced chaperones. Evidence suggests, however, that the replacements are more likely to be helping by cleaning up the mess of unfolded proteins rather than by contributing to cotranslational folding. JCB

Reference: Albanèse, V., et al. 2006. *Cell.* 124:75–88.

XX meetings



X chromosomes (red and green) meet transiently (left to right) before one is inactivated.

An interphase meeting between X chromosomes, revealed by Na Xu, Chia-Lun Tsai, and Jeannie Lee (Harvard Medical School, Boston, MA), ensures that one and only one is silenced.

Silencing of one of the two X chromosomes in a female somatic cell brings the gene dosage level down to that of male cells. Inactivation is controlled by several noncoding RNAs transcribed from, and acting in cis upon, the X inactivation center (XIC). But the field has been perplexed as to how one chromosome knows what the other is doing to keep inactivation mutually exclusive.

The new results suggest that a prior meeting between X chromosomes sets the decision. Although mammalian chromosomes normally only pair during meiosis, the authors saw transient contact between X chromosomes just before the inactivation of one.

Pairing required only the gene sequences of two of the silencing RNAs. Addition of either of these sequences to an autosome drew X chromosomes away from each other and into autosomal pairings. Deletion of the sequences from the X chromosome also interfered with pairing and resulted in none, one, or both X chromosomes being inactivated.

The big next step for the field will be to identify the molecules behind this choice. Thinking on a larger scale, Lee imagines that other epigenetic events might also be preceded by transient chromosomal pairings. In support of this idea, close proximity in late S phase of the two copies of an imprinted locus has been reported. JCB

Reference: Xu, N., et al. 2006. *Science.* doi:10.1126/science.1122984.

APC's order of business

The anaphase-promoting complex (APC) has a hectic schedule. From mitosis through the G1/S transition, the APC is busily targeting cell cycle regulators for degradation. Yet it must keep an ordered degradation schedule for proper cell cycle progression. Now, results from Michael Rape, Sashank Reddy, and Marc Kirschner (Harvard Medical School, Boston, MA) suggest that the APC lets its substrates determine their own death order.

The degradation order of cell cycle regulators correlates with the kinetics of their ubiquitination by the APC. In vitro, securin—one of the first of the APC's substrates to be degraded—rapidly obtained full-length polyubiquitin chains, which are required for proteasome recognition. The late-degraded substrates took much longer for multiubiquitination.

The kinetics reflects differences in substrate processivity—that is, how many ubiquitins are added in a single APC binding event. The most processive substrate outcompeted the rest in vitro for polyubiquitination and thus degradation.

"Why do we need so many ubiquitins in the first place?" asks Kirschner. "Isn't it overboard?" Not according to the group's new results. "We argue that by having multiple steps, chances for things to come off, you could accentuate very small differences in kinetic processes."

Differences are probably further compounded by deubiquitinating enzymes. Nonprocessive substrates easily lost ubiquitin moieties upon dissociation from the APC. JCB

Reference: Rape, M., et al. 2006. *Cell.* 124:89–103.