Research Roundup

The physics of chaperones

A simple physical property based on entropy gives Hsp70 a pulling force, say Paolo De Los Rios (EPFL, Lausanne, Switzerland), Pierre Goloubinoff (University of Lausanne, Switzerland), and colleagues. The new theory resolves two deadlocked models.

Hsp70s have two major activities: they help unfold stable protein aggregates, and they pull proteins through membrane channels. In the Brownian ratchet model for protein import, Hsp70 grabs part of a protein once it is spontaneously unfolded by random thermal fluctuations, and passively prevents it from sliding backward. By contrast, the power stroke model has the Hsp70 using a channel protein as the fulcrum for a lever arm movement that yanks the protein inwards.

The Lausanne group suggested that an emerging protein with a bulky Hsp70 attached would keep bumping against the nearby membrane. But if the whole complex moved further away from the membrane, this bumping no longer happened. The resulting increase in mobility and thus entropy meant a favorable change in free energy—a change that powers Hsp70’s pulling force away from the membrane. Similar physics would draw Hsp70-bound protein segments away from the bulk of an aggregate and thus tease apart the tangles.

“We made a chimeric theory,” says Goloubinoff. “Yes, there is active pulling, but there is no lever.” The Brownian ratchet remains partly intact also, as the unfavorable change of free energy forbids Hsp70 from being pushed back toward the membrane.

“The physics behind it is very simple,” says statistical physicist De Los Rios. “It’s been known that you can generate forces on polymers when you manipulate the number of conformations that the polymer can exist in.” With the Hsp70 case, he worked out how much work this change could do and found it was compatible with the forces required for protein unfolding and accelerated import.

In theory, this leap in thinking could have been made at any time in the last 20 years. In fact, says De Los Rios, “the first thing I said is, this is a nice application, but I asked who must I cite. But I couldn’t find anything.” Goloubinoff suggests a precedent. “When I was doing my Ph.D. we had all the tools of molecular biology,” he says. “But there was only one person who thought of PCR.”

Reference: De Los Rios, P., et al. 2006. Proc. Natl. Acad. Sci. USA. doi:10.1073/pnas.0510496103.

Outdoor decision-making

Cells often weigh conflicting signals. They do so in part, say Kevin Janes, Peter Sorger (MIT, Cambridge, MA), and colleagues, by generating extracellular signaling circuits that allow for group consensus.

The MIT group measured 19 signaling markers over 13 time points after cells were stimulated with different combinations of proapoptotic TNF and mitogenic insulin and EGF. The data were plotted and regressed in 19-dimensional space. “You take this space and try to reduce the number of dimensions while retaining important information,” says Sorger. The resulting two-dimensional graph showed that a few markers were solely related to signaling by one pathway, but most were clustered somewhere in between.

This crosstalk resulted in alternating pro- and antiapoptotic signals. First, TNF receptor activation induces shedding of TGFα, which turns on the EGF receptor. The two active receptors (TNFR and EGFR) combine to stimulate secretion of IL-1α; later the TNFR acts alone to promote secretion of an antagonist of the IL-1α receptor.

“Much to our astonishment, a large part of the crosstalk was extracellular,” says Sorger. “And it is so time variant. You can pulse for 10 minutes, then 36 hours later it is still changing.” Exactly why the cell flip-flops through pro- and antiapoptotic pathways is not clear, but based on the pathways being extracellular, “we presume that this is involved in a kind of communal decision making,” says Sorger.

“Computation was necessary to discover connections that you can’t get from simple inspection,” says Sorger. These are pathways that have been well-studied by more traditional means, so “it might have been that we just rediscovered the obvious.” But, he suggests, “what [the signaling community has] now is a bewildering set of molecular data without any clear understanding of pathways.” Self-consistent datasets and computation help make sense of large amounts of information. Now the group is investigating why the same signals affect different tissues and cancer cells in different ways.

Reference: Janes, K.A., et al. 2006. Cell. 124:1225–1239.
Immune to weight loss

Leptin always seemed a bit too good to be true. Why would our chronically undernourished ancestors have evolved a hormone for weight loss? Now, Ke Chen, Allan Zhao (University of Pittsburgh, PA), and colleagues explain why leptin doesn’t work as the weight loss wonder drug that initially seemed a possibility.

Leptin was discovered as a hormone produced by fat cells. When reinstated in leptin-deficient mice and humans it drastically reduced their obesity. But individuals who were obese for other reasons had paradoxically high leptin levels, and adding more leptin did not induce significant weight loss.

The Pittsburgh group looked to leptin-binding proteins for an answer. The first that they found was C-reactive protein (CRP), which is famous as a marker for immune reactions but whose exact function remains mysterious. CRP bound to leptin, and inhibited leptin signaling in tissue culture. In mice lacking their own leptin, CRP reduced or eliminated the effects of added leptin on food intake, body weight, blood glucose, and lipid metabolism.

The CRP connection is not completely without precedent, as others have noted inflammation (and elevated CRP) as a complication of obesity. The adipogenic and inflammatory systems also share many of the same mediators, so there is plenty of potential for crosstalk. It is not clear whether this is an accident of evolution or has a particular function.

Zhao’s one clue is that low levels of leptin turn on CRP expression. He suspects CRP helped our ancestors to accumulate some fat in the few periods of plenty interspersed amongst the more common lean times. “If leptin worked unimpeded you would have a very difficult time accumulating fat,” he says.

Now that energy rich food is always available, however, the system has become pathological. Zhao’s next step is to find out whether those who are morbidly obese have a different set point for CRP expression or accumulation. JCB

Reference: Chen, K., et al. 2006. Nat. Med. 12:425–432.

Mixed-up DNA

Interphase human chromosomes show significant intermingling, say Miguel Branco and Ana Pombo (MRC Clinical Sciences Centre, London, UK). The idea is a counter to the most extreme view of chromosome territories.

Territories were so named based on microscopists’ early observations: that individual chromosomes did not spread out over an entire nucleus. DNA labeling also indicated that nuclei had gaps between DNA, although it was not clear if these were between DNA of the same or different chromosomes. Still, this was enough to spark talk of an interchromatin domain separating territories. “In this model, it will always be right because two pieces of chromosome will never be in exactly the same place,” says Pombo. “The field couldn’t move forward.”

She and Branco responded by labeling two different chromosomes in ultrathin slices and following up with EM for even higher resolution. Based on extrapolation, an average of 46% of each chromosome territory intermingles with other chromosomes. Furthermore, extents of intermingling for different chromosomes correlated with translocation frequencies.

The nucleus is not a complete tangled mess, however. “Chromosomes as polymers will tend to expand,” says Pombo. “But what stops them is interaction with each other and with the nuclear membrane.” She is now seeing if transcription factories help keep particular chromosomes fixed to themselves or each other. JCB

Reference: Branco, M.R., and A. Pombo. 2006. PloS. 4:e138.

Stressful betrayal

During heat shock recovery, a helper becomes a destroyer, say Shu-Bing Qian, Cam Patterson, and colleagues (University of North Carolina, Chapel Hill, NC).

The traitor protein is called CHIP. After the group discovered CHIP, their interest was piqued when they realized it was both an Hsp70-binding protein and a ubiquitin ligase. The finding suggested that “folding versus degradation is a regulated decision, not a stochastic decision, and CHIP ties together these two processes,” says Patterson.

They now find that CHIP helps to mark unfolded proteins that are bound to Hsp70 by adding the ubiquitin destruction signal. But once those marked proteins are destroyed, the Hsp70 itself becomes CHIP’s next target. The effect could be reconstituted in vitro with only unfolded protein, Hsp70, and CHIP.

The resultant return to baseline Hsp70 levels is important to restore cellular normality. Hsp70 helps halt the cell cycle and alter signaling pathways so that cells can recover from stress. Once unfolded proteins are cleared, says Patterson, “the cell has to get out of this suspended animation phase.” JCB

Reference: Qian, S.-B., et al. 2006. Nature. 440:551–555.