Research Roundup

Proteins on the edge of stability

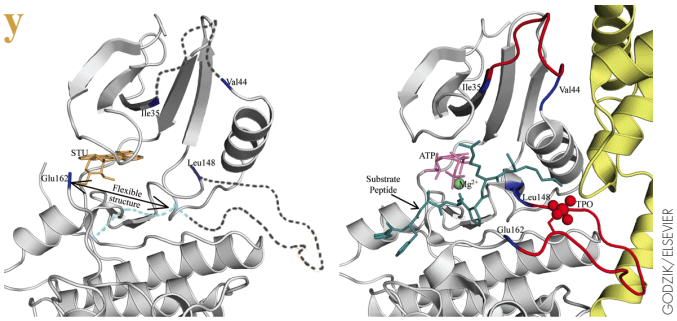
Proteins harbor regions with split personalities, as suggested by findings from Ying Zhang, Boguslaw Stec, and Adam Godzik (Burnham Institute, La Jolla, CA). Straddling the edge of order and disorder, these dual personality (DP) fragments might be common regulation sites.

Ordered parts of protein structures can be revealed by crystallography, while disorder is usually hidden. Occasionally, solving a structure under a new condition reveals previously unseen protein parts. Scientists usually consider the exposure an improvement, especially if a new structure is obtained at a better resolution, and might even discard previous "inferior" models.

"I believe that's wrong," says Godzik. "Different conditions have changed the protein; these are independent experiments." In the new work, his group compared independently solved—previously thought of as redundant—crystal structures from the Protein DataBank. "As far as I know, the idea is new," says Godzik. "And once you have the epiphany, you can see so many new things."

What the authors saw were peek-a-boo fragments that only appeared structured under certain conditions. Such DP fragments were common; 50% of the examined proteins had at least one DP fragment, based on the authors' conservative definition.

Many proteins are also fully disordered and can become ordered upon major events, such as the binding of a protein partner. But the DP regions—which often coincided with regulatory regions—need a smaller push. "They are sitting on the edge of stability," says Godzik. "Phosphorylation, small molecule binding, or even a local environmental change could all give them structure."

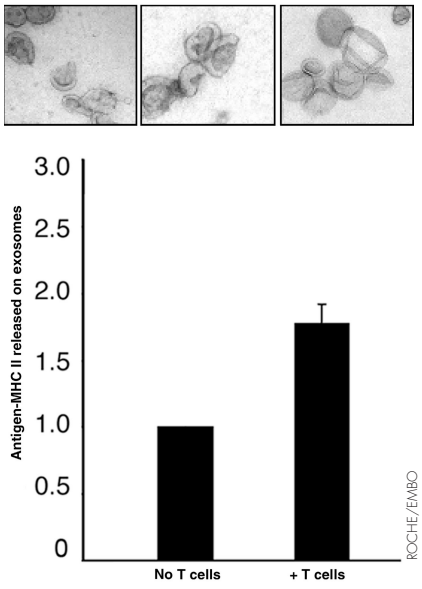


DP fragments (red) of CDK2 cannot be seen in its inhibited form (left) but come into view (right) when cyclin (yellow) activates the kinase.

The induced structure might have big effects on protein activity. Godzik uses protease-mediated activation as an example. "Things that are floppy are easier to cut," he says. "So make the substrate stiffer by phosphorylating it, and you can block that [and prevent its activation]."

DP fragments had their own unique amino acid characteristics that set them apart from intrinsically disordered and fully ordered proteins. They favored a few specific types of residues and were more likely to pair a hydrophobic residue next to a charged one. This schizophrenic trait might help them easily cross the stability border. JCB

Reference: Zhang, Y., et al. 2007. *Structure*. 15:1141–1147.



Exosomes (top) containing antigen and MHC are shot out of B cells that meet partner T cells.

MHC and antigen in exosomes

T cells tell B cells to spit out exosomes loaded with T cell stimulants, report Aura Muntasell, Adam Berger, and Paul Roche (NIH, Bethesda, MD).

Exosomes are the membrane pockets that lie within larger vesicles called multivesicular bodies (MVBs). In B cells and other antigen-presenting cells, MVBs and exosomes house antigens in complex with the MHC class II molecules that present them to T cells. When an MVB fuses with the plasma membrane, MHC-antigen from the MVB membrane is displayed on the B cell surface, while the exosome allotment of MHC-antigen is sent out into the extracellular environment.

In the new study, the authors found that a B cell shot out twice as many exosomes when it met a T cell that recognized its antigen. In turn, the exosomes stimulated the T cells to divide rapidly and make cytokines that further activate B cells.

The resulting T cell progeny also need to see antigen to be activated. Roche wondered, "How are those daughters getting stimulated from one lone B cell?" He imagines that the exosome secretion speeds the process.

"We think of it like a shotgun blast," says Roche. "The T cell tells the B cell to shoot out its exosomes, and they help activate the new T cells." Testing the theory will be difficult, however. "No one knows what exosomes do in real life; we have not been able to stop a cell from secreting them without killing it."

The signal to release exosomes seems to stem from the MHC molecule, probably upon binding to the T cell receptor. Artificially cross-linking MHC molecules also stimulated the release. Downstream tyrosine kinases were also required, but more signaling details are so far unavailable.

Exosomes acquired their MHC-antigen pairs via recycling from the cell surface. Perhaps sending out the exosomes saves recycled MHC-antigen from its alternative fate—lysosomal degradation. After all, once an enemy is found, MHC weapons are in high demand. JCB

Reference: Muntasell, A., et al. 2007. *EMBO J.* doi:10.1038/sj.emboj.7601842.

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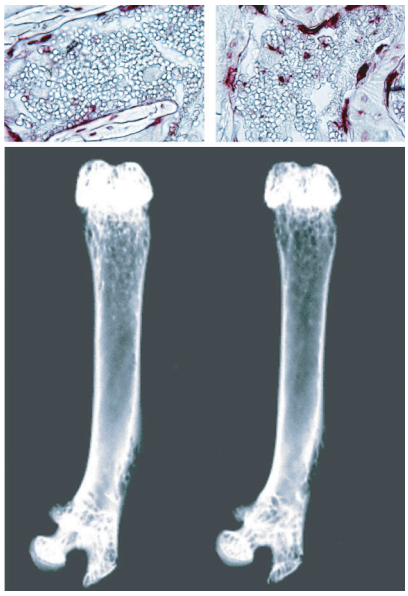

KATO/ELSEVIER

The survival of osteoclasts (top, purple) in mice lacking estrogen receptors (right) leads to loss of bone mass (bottom).

Apoptosis prevents osteoporosis

Grandmothers everywhere know well that estrogen deficits lead to osteoporosis. Now, the molecular basis for this debilitating bone loss is finally identified. Estrogen is needed to kill off bone-destroying osteoclasts, show Takashi Nakamura, Shigeaki Kato (University of Tokyo, Japan), and colleagues.

The root cause of osteoporosis has been difficult to pin down, in part because bones are not frail in female mice lacking estrogen receptors. These mice make extra androgen, which builds bone in male mice and might compensate for bone loss in the mutant females.

To avoid the androgen rise, Kato's group knocked out estrogen receptors only in mature osteoclasts, which accumulate in osteoporotic bones. These female mutants developed rickety bones due to losses within the central bone shafts.

The authors then isolated osteoclasts to determine why they are so abundant in diseased bone. Microarray analyses revealed that estrogen induced apoptotic proteins, including Fas ligand, that were not induced in the estrogen-blind osteoclasts.

Men who have estrogen receptor mutations develop osteoporosis. But male mice were not affected by the loss of estrogen receptors in osteoclasts. Perhaps the androgen-headed pathway is more dominant in mice than in humans.

Currently, potential drugs to treat osteoporosis are screened through mice whose ovaries have been removed. Screens for the induction of Fas ligand in cultures of estrogen-blind osteoclasts should be much simpler. JCB

Reference: Nakamura, T., et al. 2007. *Cell.* 130:811–823.

Nervous blood cells

Blood cells feel nervous impulses, reveal findings from Asaf Spiegel, Tsvee Lapidot (Weizmann Institute, Rehovot, Israel), and colleagues. Human blood stem cells express receptors for stress-induced neurotransmitters.

Protective white blood cells swarm into the circulation when the body is in alarm mode. Infection, injury, and even anxiety can cause blood stem cells to proliferate and escape from their birth sites in bone marrow, which is heavily innervated. Lapidot's group now shows that this response stems from receptors on young blood cells that sense stress-induced neurotransmitters such as epinephrine.

Receptor expression was increased by cytokines such as granulocyte colony-stimulating factor (G-CSF), which mobilizes blood progenitor cells. G-CSF is secreted during inflammation and activates early responding immune cells.

The bound receptors activated a known proliferation pathway headed by Wnt and β-catenin. As a result, blood progenitor cells proliferated, polarized, and mobilized in the presence of the neurotransmitters.

Lapidot believes that blood cells aren't picky about their neurotransmitters. "We chose to look at dopamine, epinephrine, and neuroepinephrine," he says. "But we think others are also involved in stem cell regulation." His team would now like to determine whether unchecked activation of neurotransmitter pathways also helps blood cancers such as leukemia thrive. JCB

Reference: Spiegel, A., et al. 2007. *Nat. Immunol.* doi:10.1038/ni1509.

Special skin cells get pigments

Skin coloration comes from melanocytes, which donate pigment to epithelial cells. Now, findings from Lorin Weiner, Rong Han, Janice Brissette (Massachusetts General Hospital, Charlestown, MA), and colleagues reveal that color is given out selectively to the dedicated skin cells that request it.

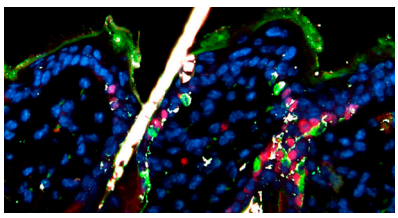
Since melanocytes are the givers of melanin, they've been the focus of studies on coloration. "But we are now showing that epithelial cells tell the melanocytes what to do," says Brissette. "It's analogous to a child's coloring book. Pigment recipients collectively form the outline of the picture, where the pigments should be placed. And the melanocytes color in the picture."

Brissette's group is interested in a transcription factor called Foxn1 and its role in the skin. For functional studies, they created mice whose skin precursor cells made extra Foxn1. The mice developed dark skin that had extra melanocytes. As the mice matured, the cells making Foxn1 became sparser. Melanocytes persisted mainly near the remaining Foxn1-expressing cells, suggesting that those cells might help melanocytes survive or proliferate.

One survival factor that melanocytes don't make themselves but do need in culture is Fgf2. The authors found that Foxn1 activated Fgf2 expression, resulting in its secretion from epithelial cells. Blocking Fgf2 activity killed off the extra melanocytes in the transgenic mice.

Humans, who have darker skin than do mice, expressed Foxn1 more widely in epithelial cells. But whether Foxn1 defects cause human pigmentation diseases is not yet known. JCB

Reference: Weiner, L., et al. 2007. *Cell.* 130:932–942.


BRISSETTE/ELSEVIER

Melanocytes (green) accumulate around extra Foxn1 (red) in mouse skin.