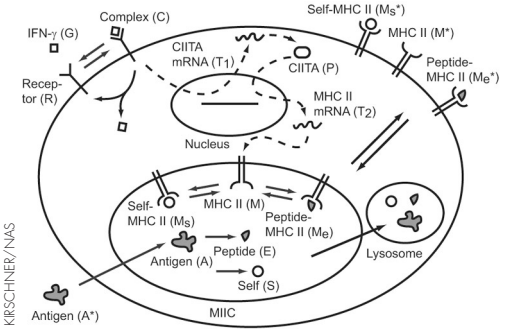
Research Roundup



KIRSCHNER/NAS

There are multiple TB resistance pathways in the cell.

TB bug inhibits any which way

Each new study of *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (TB), seems to come up with a new resistance mechanism, and fails to see evidence for the mechanisms claimed by others. Now Stewart Chang, Jennifer Linderman, and Denise Kirschner (University of Michigan, Ann Arbor, MI) use a mathematical model to conclude that multiple mechanisms operate. Different mechanisms are best suited to protection under different conditions, and some are masked by experimental protocols used in previous studies.

Mtb prevents macrophages from doing their job primarily by inhibiting antigen presentation by MHC class II. The Michigan group included four parts of this process (identified by others) that could be inhibited: MHC transcription; MHC protein maturation; antigen processing; and peptide loading onto MHC. IFN-γ and antigen got things going, and surface expression of peptide-loaded MHC was the readout.

Two inhibition classes served distinct time frames: effects were immediate for inhibition of antigen processing or peptide loading but delayed for inhibition of MHC transcription or maturation. The long pulses of IFN-γ used by two previous groups resulted in inhibition mediated primarily by effects on either maturation or transcription, respectively, with some part of the effect unexplained.

To suggest candidates for these unexplained effects, Kirschner and colleagues identified a number of processes whose inhibition in silico has major effects on antigen presentation efficiency. In vitro time series will allow many of the model's predictions to be tested. JCB

Reference: Chang, S.T., et al. 2005. *Proc. Natl. Acad. Sci. USA*. doi:10.1073/pnas0500362102

Split motifs

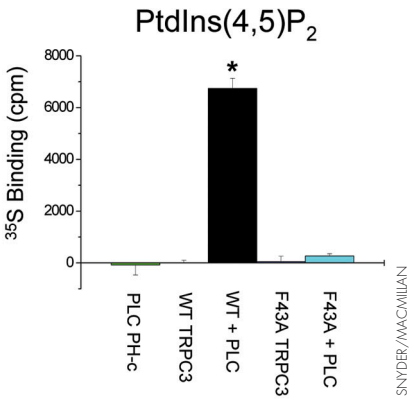
Many more motifs may be lurking in proteins than previously expected. Randen Patterson (Pennsylvania State University, State College, PA), Damian van Rossum, Solomon Snyder (Johns Hopkins University, Baltimore, MD), and colleagues report that functional protein–lipid interaction motifs can be formed when partial motifs from two proteins unite.

The group's initial example of a split domain comes in the context of a Ca^{2+} entry system. In this system, neurotransmitters bind receptors that trigger production of inositol 1,4,5-trisphosphate (IP3), which in turn prompts release of intracellular Ca^{2+} stores and entry of extracellular Ca^{2+} through TRPC3 channels. Snyder and colleagues previously established that phospholipase Cγ1 (PLC-γ1) was needed for this latter TRPC3 action, independent of PLC-γ1's enzymatic activity in generating IP3.

They now report that the binding of TRPC3 and PLC-γ1 to each other brings together two halves of a split PH domain, a motif associated with the binding of lipids and other proteins. Mutation of the partial PH domains changes the lipid-binding profile of the TRPC3–PLC-γ1 complex, and reduces the amount of TRPC3 at the plasma membrane 24 h after stimulation.

The phenomenon of split domains may be widespread. A modified search algorithm spotted not only the half-domains in TRPC3 and PLC-γ1 but also split domains in additional proteins. "It has opened up a whole new world," says Snyder. "It will amplify perhaps many-fold the number of protein recognition motifs." This profusion of motifs, he says, allows the cell to deliver on a promise: "Everything is hand delivered." JCB

Reference: van Rossum, D.B., et al. 2005. *Nature*. 434:99–104.



SNYDER/MACMILLAN

Only TRPC3 (WT) combined with PLC-γ1 generates a PH domain that binds lipids.

PML goes to the centrosome

The promyelocytic leukemia gene (PML) has "too many things it is involved in," says Kun-Sang Chang (University of Texas MD Anderson Cancer Center, Houston, TX). But now Chang, Zhi-Xiang Xu, and colleagues have added another function: the PML3 isoform prevents centrosome reduplication.

PML has at least 7 isoforms but most studies have used only one (PML4). Chang developed isoform-specific antibodies and saw that PML3 antibodies gave staining that coincided with that of centrosome proteins. Knock-down by siRNA of PML3, but not of other PML isoforms, resulted in centrosome amplification. And only PML3 interacted with and, when overexpressed, reduced the phosphorylation of Aurora A kinase.

It is known that in cells with activated Aurora A kinase there is a failure to inhibit Cdk2/cyclin E, leading to reduplication of centrosomes. Cells lacking PML had higher levels of Cdk2 kinase activity, which could explain the centrosome reduplication. What is signaling to PML from upstream is still mysterious, but the new findings certainly provide one possible explanation for why PML is lost in so many tumor types. JCB

Reference: Xu, Z.-X., et al. 2005. *Mol. Cell.* 17:721–732.

Text by William A. Wells
wellsw@rockefeller.edu

Ready, set, wait

Stem cell differentiation is all about cautious preparation, say Deborah Lang, Jonathan Epstein (University of Pennsylvania, Philadelphia, PA), and colleagues. They find that a transcription factor called Pax3 sends conflicting messages: it gets stem cells ready for the differentiated state, by building up proteins used in the differentiation program, but inhibits the action of those differentiation proteins and thus holds the cells back. Only a separate signal can relieve this repression by Pax3 and unleash the full differentiation program.

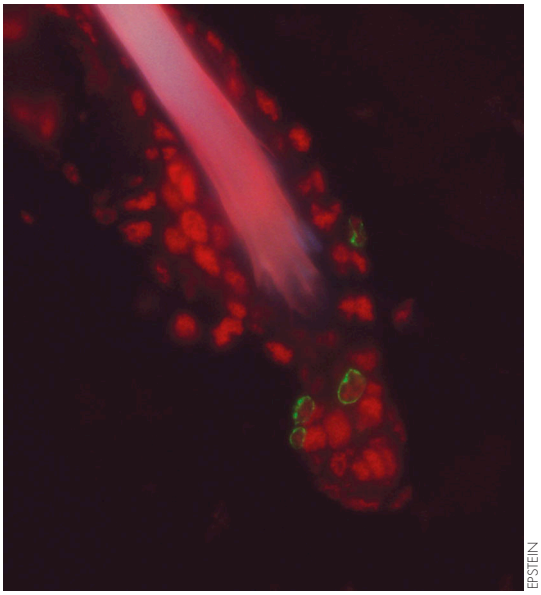
"The general concept that there are classes of transcription factors that yield paradoxical results—both activating and inactivating pathways—has been around," says Epstein. "What's an addition here is a molecular mechanism."

Epstein's group came across the phenomenon while studying neural crest cells. Pax3 was required in mice for the eventual induction of melanocyte differentiation markers such as dopachrome tautomerase (Dct), but in cell culture experiments Pax3 was found to repress Dct expression.

The paradox could be understood based on a series of relationships. Epstein found that Pax3 helped turn on Mitf, and Mitf was needed to turn on Dct. But if Pax3 was still around, it could prevent Mitf from binding and turning on the Dct enhancer. Only when an activated Wnt signal displaced Pax3 was Mitf able to do its job.

Consistent with this model, only areas positive for Wnt signals were expressing both Pax3 and Dct. In the embryo, such Wnt-expressing areas are thought to be terminal migratory locations for neural crest cells. In the adult, skin damage may generate Wnt signals that prompt the necessary melanocyte differentiation.

The group suggests that Mitf acts like a "biological capacitor." It builds up, but is repressed until needed. Epstein is now looking for a similar organization in other stem cell systems. "It's a tight little circuit," he says, "and I bet it will come up again and again." JCB



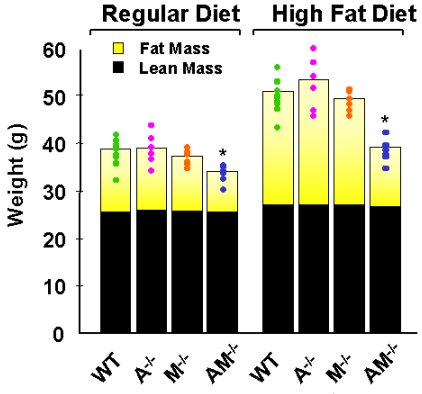
EPSTEIN

With Pax3, cells are prepared for but inhibited from becoming differentiated (green).

Reference: Lang, D., et al. 2005. *Nature.* 433:884–887.

Mice with no fat carriers are skinny

The two related fatty acid binding proteins (FABPs) called aP2 and mal1 did not seem destined for fame. "These are seemingly very dull molecules," says Gökhan Hotamisligil (Harvard School of Public Health, Boston, MA). "They are like little clams attaching to fatty acids." Yet somehow, he says, these "idle chaperone proteins," which shuttle fatty acids around inside the cell, turn out to "determine lipid metabolism."



HOTAMISLIGIL/ELSEVIER

Mice lacking both fat carriers ($AM^{-/-}$) don't get fat on a high-fat diet.

They may do so by bringing fatty acid species to regulatory proteins or enzymes.

Mice lacking one or the other FABP had been generated before by Hotamisligil and shown mild phenotypes. Now the Boston group, including Kazuhisa Maeda and Haiming Cao, characterizes mice lacking both proteins. The mice have less fat than normal, and after a high fat diet their body composition, blood glucose, blood insulin, and insulin resistance do not worsen but are all indistinguishable from those of wildtype mice on a normal diet.

The proximal cause of these changes may be an unusual lipid profile. The double mutants have more shorter chain fatty acids in adipocytes and muscle cells, and more longer chain fatty acids in liver. The results, both in the mutants and in vitro after similar manipulations of lipid content, include increased activating phosphorylations of both AMP-activated kinase (AMP-K) and members of the insulin receptor pathway in muscle. AMP-K can induce greater fatty acid oxidation and energy expenditure, as is seen in the mutant mice. And an activated insulin receptor pathway would protect animals from insulin resistance.

FABP acts predominantly in adipose tissue, so the effects on other tissues must be mediated by other messengers. Hotamisligil is pursuing these messengers using cocultures. His "larger aspiration," meanwhile, is to "tie FABP to a specific biological pathway."

This may involve tracking down lipid mediators, which is notoriously difficult, even with the protein now in hand. The altered profile of fatty acids is also not yielding answers easily. The pattern seen in mutant adipocytes and muscle "has not been noted as a signature pattern in any other state" such as fasting or feeding, says Hotamisligil, so the significance is not immediately clear. He hopes the answer will come from large-scale "lipomics" efforts to identify lipid signatures at specific sites and under specific dietary conditions. JCB

Reference: Maeda, K., et al. 2005. *Cell Metab.* 1:107–119.