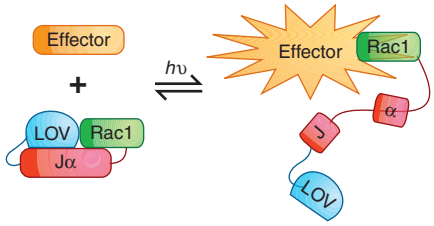
CELL BIOLOGY

'Blue' lighting cell signaling research

By fusing a light-sensitive domain of an oat plant protein to Rac1, researchers created a genetically encoded protein fusion that can be reversibly activated with blue light and control cell movement—an attractive alternative to current caging tools.

Protein caging tools are a staple in the arsenal of cell biologists. Though researchers are constantly developing new means to study rapid, spatiotemporally controlled processes, the drawbacks of current tools include irreversible activation and/or cell damage when the caged molecules are introduced into cells or uncaged with UV light.

In their quest to hone in on cell signals more precisely, Klaus Hahn, with postdoc Yi Wu, at the University of North Carolina, Chapel Hill, and their colleagues took a different caging approach, creating genetically encoded photoactivatable GTPases. As the 'cage' they used the photoreactive light oxygen voltage (LOV) domain of the *Avena sativa* (common oat) phototropin 1 protein (Wu *et al.*, 2009). "It's like a yo-yo," explains Hahn: "it's a globular protein on a string, which is an alpha helix that changes length depending on the irradiation."



Exposure of PA-Rac1 to blue light results in unwinding of the Jα helix, which frees the previously caged Rac1 GTPase to interact with effector proteins. Adapted from *Nature*.

In the dark, this LOV domain interacts with a C-terminal alpha helix (Jα) and, when fused to a target protein, sterically blocks effector protein binding. When it is exposed to light—458- or 473-nm blue light—photon absorption results in a conformational change and subsequent dissociation and unwinding of the Jα helix, which releases the steric block and allows the fused protein to interact with downstream signaling molecules. As controls, a light-insensitive mutant as well as a constantly 'lit' mutant of the LOV domain can be used.

Using this approach, the researchers tagged Rac1, a GTPase that regulates actin cytoskeletal dynamics. After optimizing the fusion, they could turn on this photoactivatable Rac1 (PA-Rac1) by irradiation with blue light—reversibly and repeatedly—generating cell protrusions and ruffling in the activated area of the cell. Combining this tool with a Rho protein biosensor, they found that in mouse fibroblasts Rac1 inhibits RhoA, another protein involved in cytoskeleton dynamics. "We saw that Rho was specifically blocked in particular regions of the cell, so this now allows us to look at pathway interactions spatially in different spots and see that they are in fact different in different spots," says Hahn.

The group also tagged a similar GTPase, Cdc42, with LOV-Jα. The strategy used for PA-Rac1 resulted in a Cdc42 fusion with residual activity, but structural modeling of the interface pointed to a mutation that would

PROTEIN BIOCHEMISTRY

A LEADER ANYONE CAN FOLLOW

The development of leader sequences that stimulate mRNA translation in a species-independent manner could offer new possibilities for eukaryotic protein production and proteomic research.

Proteins are stubborn things, and achieving high yields of properly folded and modified polypeptides can become a truly Herculean task, with cell-based production often hampered by issues of efficiency and toxicity as well as the difficulty of collecting target protein.

Existing cell-free lysate-based systems offer a promising alternative but not a perfect solution. "You can do cell-free preparation in *Escherichia coli* on the multiliter scale," says Kirill Alexandrov of the University of Queensland, Australia. "But if you go to the eukaryotic world, which you have to do because *E. coli* doesn't properly fold complex eukaryotic proteins, then things become much more difficult." Preferred systems like rabbit reticulocyte lysate and wheat germ extract fall short when it comes to yield and scalability, and Alexandrov was keen to develop a more efficient and universal approach to eukaryotic protein production.

The capacity to specifically and efficiently engage lysate translational machinery with exogenous mRNAs is vital for successful cell-free protein production, and Alexandrov's team achieved a breakthrough in this regard by reexamining the leader sequences on their starting transcripts. Previous evidence has suggested that relatively unstructured poly(A)-rich sequences stimulate translational complex assembly, and by designing a customized 5′ untranslated region based on this concept, they considerably boosted the efficiency of translation for linked transcripts in virtually any cell-free system. "This translation is truly species-independent and worked in all organisms that we tested, including both eukaryotes and prokaryotes," says Alexandrov.

His team has worked closely with one particularly intriguing organism, the trypanosome *Leishmania tarentolae*, with characteristics that make it a promising candidate for cell-free extracts. "It's essentially a transitional organism between prokaryotes and eukaryotes," he says, "because they have prokaryotic gene expression [machinery] but eukaryotic protein folding and modification machinery." Another potent advantage of this organism is the existence of a ubiquitous 35-nucleotide leader sequence on all endogenous mRNAs; as a result, translation of these could be readily blocked with a *Leishmania*-specific antisense nucleotide, eliminating the need for RNase digestion as in other extract-based methods.

This combination of *L. tarentolae* extract and their species-independent translational sequence proved potent, resulting in markedly higher yield of protein than competing eukaryotic extracts—up to 300 micrograms of protein per milliliter in 90

stabilize the LOV-Cdc42 interaction with additional hydrogen bonds in the caged, 'dark' state, allowing the researchers to make a PA-Cdc42. This suggests that on the basis of a structural understanding of the steric block for the protein family, the interface can be optimized for each individual protein to create other caged GTPases.

Hahn hopes that the LOV domain has an ideal structure to become a general caging tool, but he notes that the tagging approach will need to be worked out for each individual protein family. "The GTPases have a similar enough structure that we could come up with a general solution," he says. "But if you now try to do something with a different shape, you are going to have to ask how you put the yo-yo on a string,… but I hope that there will be broadly applicable solutions for each family." His group is now working with other protein families to see how general the approach can be.

And as more such caged proteins are made, tools will be needed to keep track of them. In a companion paper (Machacek *et al.*, 2009), Hahn's group, along with Gaudenz Danuser's team at Scripps, used a computational multiplexing approach to delineate the spatiotemporal relationships between activities of the GTPases Rac1, RhoA and Cdc42 during cell protrusion.

As for PA-Rac1, because the protein controls cell motility, according to Hahn others are using it to make cells move around in animals. So more work is yet to come on many fronts.

Irene Kaganman

RESEARCH PAPERS

Machacek, M. *et al.* Coordination of Rho GTPase activities during cell protrusion. *Nature* **461**, 99–103 (2009).

Wu, Y.I. *et al.* A genetically encoded photoactivatable Rac controls the motility of living cells. *Nature* **461**, 104–108 (2009).

minutes—with a diverse array of protein-coding sequences, although the efficiency of folding varied depending on the protein in question. As a further demonstration of the utility of their method, the researchers monitored diffusion and interaction behavior of individual proteins directly in the cell-free translation mixture using fluorescence correlation spectroscopy.

Based on these initial experiments, Alexandrov is encouraged that his method offers a strong combination of efficiency and the capacity to ratchet up eukaryotic protein production to the liter scale at a fraction of the cost of existing commercial systems—a serious boon to scientists looking to generate crystals or produce antibodies. In contrast, there are also important advantages in 'going small', and the capacity for highly multiplexed protein production using the species-independent translational sequence leader, either with *L. tarentolae* extract or any other extract system, could enable powerful, high-throughput interaction analysis studies or even new approaches for tackling proteomic research. "You can find an organism, make an extract and back-translate its genome," says Alexandrov. "Craig Venter estimates a total pool of 20 million genes on the planet, and most of them are in microorganisms on which we have no handle—and so this could be a way of quickly generating expressed proteomes."

Michael Eisenstein

RESEARCH PAPERS

Mureev, S. *et al.* Species-independent translational leaders facilitate cell-free expression. *Nat. Biotechnol.* **27**, 747–752 (2009).

NEWS IN BRIEF

STEM CELLS

Mice from iPSCs

Two groups show that mouse induced pluripotent stem cells (iPSCs) can generate fertile adult mice in tetraploid complementation assays, in which all tissues are derived from the pluripotent cells. Failure to achieve this so far has raised questions about the pluripotency of iPSCs, but these results show that full pluripotency via reprogramming is possible. The mice will be useful models to study the function of cells and tissues that are entirely iPSC-derived.

Boland, M.J. *et al. Nature* **461**, 91–94 (2009).
Zhao, X.Y. *et al. Nature* **461**, 86–90 (2009).

PROTEOMICS

Mammalian tethered catalysis

The tethered catalysis method, originally developed in yeast, is used to identify post-translational modification–specific binding proteins (PTMBPs). A substrate peptide is tethered to an enzyme that post-translationally modifies the peptide, which then serves as bait for PTMBPs. The PTMBPs can be identified by affinity purification and mass spectrometry. Spektor and Rice now report an expansion of the tethered catalysis approach for application in mammalian cells.

Spektor, T.M. & Rice, J.C. *Proc. Natl. Acad. Sci. USA* **106**, 14808–14813 (2009).

CHEMICAL TOOLS

Cleavable linkers for affinity chromatography

Biotin-streptavidin–based affinity chromatography is widely used for identifying protein-protein and protein-ligand interactions. However, releasing the biotinylated target protein from the streptavidin support is a harsh and inefficient process. Park *et al.* now report an acylhydrazone-based linker that can be incorporated into a biotin tag, and which is cleaved under mildly acidic conditions. This allows for a clean release of the target protein without requiring denaturation, facilitating downstream analysis.

Park, K.D. *et al. Chem. Biol.* **16**, 763–772 (2009).

GENOMICS

Predicting copy-number variation

Though duplicated regions of the human genome are the culprits of various conditions ranging from color-blindness to lupus, these regions have been very difficult to study. Alkan *et al.* describe an algorithm called mrFAST, which can predict absolute copy-number variation in duplicated genome segments from next-generation sequencing data. By examining three human genomes, Alkan *et al.* estimated that 73–87 genes vary in copy number between individuals.

Alkan, C. *et al. Nat. Genet.* advance online publication (30 August 2009).

DRUG DISCOVERY

Mapping drug space

Drugs that target pathogenic bacteria or cancer cells can potentially also interfere with human enzyme function. Thus, an understanding of how drugs can affect metabolism is very important. Adams *et al.* present an online resource of interactive maps of potential drug action in metabolism, which compares the chemical structural similarities of drugs and human metabolites. The resource contains information on 246 drug classes and 385 organisms; such maps could be used to design more effective drugs.

Adams, J.C. *et al. PLoS Comput. Biol.* **5**, e1000474.