IMAGING AND VISUALIZATION

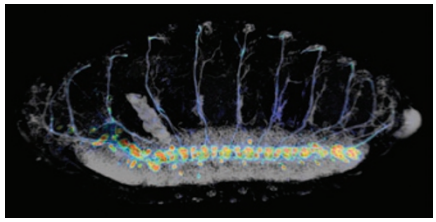
Actions speak louder

A fluorescence resonance energy transfer (FRET)-based biosensor helps scientists monitor the activation of an essential signaling protein over the course of embryogenesis in *Drosophila melanogaster*.

Woody Allen once noted that "eighty percent of success is just showing up"—but a new *in vivo* imaging study demonstrates that just showing up is not nearly enough for some proteins to get their jobs done right.

It has been known for some time that Cdc42, a widely conserved eukaryotic GTPase involved in diverse signaling pathways, has an important role in embryonic development. At the same time, experimental evidence also suggests that this protein spends a considerable amount of time just sitting around in its inactive, GDP-bound form rather than its active, GTP-bound form.

This caught the attention of University of Miami researchers Akira Chiba and Daichi Kamiyama, who were developing probes to study proteins whose activities cannot be explained by expression data alone. "Knowing exactly when those GTPases are activated must be very important," says Chiba, "and we were interested in seeing this kind of activity within a living, undissected insect embryo."



At hour 15 of *Drosophila* embryonic development, the A-probe reveals localized activation of Cdc42, with red and orange spots indicating the highest FRET signal. Image courtesy of D. Kamiyama and A. Chiba.

Kamiyama and Chiba designed specialized activation bioprobes, or A-probes, in which Cdc42 is physically linked to the Cdc42-binding domain (CBD) polypeptide. These two components are linked to chromophores suitable for FRET. As the CBD interacts with Cdc42 only in the latter's activated state, the A-probe can provide a rapid visual readout of Cdc42 activation both in whole *Drosophila* embryos and in individual cells.

To their surprise, the researchers observed consistently low levels of Cdc42 activation throughout the embryo until hour 15 of embryogenesis—nearly two-thirds of the way into the developmental process—even though the protein itself is ubiquitously expressed throughout embryogenesis. Indeed, embryos lacking functional Cdc42 altogether matured normally until the latest stages of development.

The central nervous system is among the tissues in which Cdc42 activation is greatly increased at hour 15, and

VIROLOGY

NEW SHAPES OF PRIONS

Modification of a system for rapid amplification of misfolded prion proteins allows *de novo* generation of these infectious molecules and provides a glimpse of the diverse range of possible misfolded prion strains.

Of the three origins of prion diseases—familial, infectious and what is called 'spontaneous'—the latter is most common in humans. In this case, a normal prion protein spontaneously 'flips' to a misfolded form that kills neurons and then autocatalytically replicates, causing neurodegenerative disease.

Though many studies have shown that misfolded prion protein (PrP^{Sc}) induces misfolding of the normal cellular prion protein (PrP^{C}), the idea that PrP^{Sc} replicates in the absence of nucleic acids, contrary to the dogma of molecular biology, is known as the 'prion hypothesis'. As a step toward convincing the remaining critics of this hypothesis, Claudio Soto at the University of Texas Medical School in Houston wanted to reproduce this spontaneous prion conversion process *in vitro*.

His group had previously described protein misfolding cyclical amplification (PMCA), a technique in which PrP^{Sc} induces the misfolding of normal PrP^{C} in a test tube in a process catalyzed by ultrasonic waves (Saborio *et al.*, 2001). "So something that *in vivo* takes many years—or in humans can take decades—to happen, in a test tube we can do it in a matter of hours," says Soto, further reasoning that "if the normal protein becomes transformed into the misfolded form *in vivo* spontaneously at a very low frequency, this process of PMCA is very powerful and should detect spontaneous formation of prions."

To this end, Soto's group modified the PMCA assay, essentially increasing the number of sonication cycles from 144 to 240 to detect these low-frequency events (Barria *et al.*, 2009). Starting with brain homogenate from disease-free hamsters, mice and humans in the extended PMCA assay, they observed PrP^{Sc} formation in 2 and 1 out of 10 hamster and mouse samples, respectively—but not in any of the human samples. Notably, even after modifying the conditions, the group saw no spontaneous conversion in the human samples or in samples from transgenic mice expressing human PrP^{C}, suggesting that the human version of the protein misfolds at a lower rate.

Interestingly, the extended PMCA yielded novel forms of misfolded prions that caused new disease phenotypes. When injected into wild-type hamsters, the PMCA-generated hampster PrP^{Sc} caused disease that was distinct from known hamster prion diseases, suggesting that their work yielded a

the researchers took a closer look at this protein's activation profile during the development of individual anterior corner cell (aCC) motoneurons. As the researchers noted in the whole embryos, FRET signals from the A-probe were highly restricted in aCC cells. "When they're born and migrate and start generating axons, Cdc42 is silent," says Chiba. "Only towards the end of axonogenesis and the beginning of dendrogenesis does this molecule become activated for the first time." Notably, Cdc42 activity was also spatially restricted to the axon segment of the developing aCC neuron from which the dendrites emerge.

Overexpression of a constitutively active form of Cdc42 in aCC cells led to a variety of developmental defects, whereas overexpression of the wild-type protein had no apparent effect at all—providing additional confirmation of the primacy of specific activation over mere presence as a determinant of Cdc42 activity.

Chiba and Kamiyama believe such constructs could provide a generalizable strategy for tracking the activation of other signaling proteins *in vivo* and are currently developing FRET- and non-FRET-based activation bioprobes for other proteins involved in nervous system development. "If you do *in situ* hybridization in a tissue, you can see what genes are expressed," says Chiba. "But this kind of FRET-based bioimaging allows us to study the behavior of signaling proteins and monitor them at the level of protein-protein interactions."

Michael Eisenstein

RESEARCH PAPERS

Kamiyama, D. & Chiba, A. Endogenous activation patterns of Cdc42 GTPase within *Drosophila* embryos. *Science* **324**, 1338–1340 (2009).

new prion. Thus, Soto proposes that "the universe of possible prions is not restricted to what we know in nature…, and the sequence of the protein can accommodate many more prions that we [know of] today, and some of these could be potentially more virulent or more transmissible," analogous to today's situation with the influenza virus. But unlike with the flu, for which the new culprit mutant can be identified using existing technologies, protein-to-protein transmission complicates the study of prion disease. PMCA, however, is a powerful tool for this task: the original PMCA conditions can be used as a diagnostic assay to detect preformed prions in samples, and the extended PMCA is a model for studying the sporadic origin of prions.

The question of the molecular basis for this phenomenon still remains: "How can one single protein without changes in the amino acid sequence encode all the diversity that you have in prions? What are the differences between them, and how they produce the diseases? We are now studying this with natural prions and *de novo*–produced prions," says Soto.

So new insights into these confounding diseases are on the way, and PMCA is an adaptable tool for the task.

Irene Kaganman

RESEARCH PAPERS

Barria, M.A. *et al. De novo* generation of infectious prions *in vitro* produces a new disease phenotype. *PLoS Pathog.* **5**, e1000421 (2009).

Saborio, G.P. *et al*. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. *Nature* **411**, 810–813 (2001).

NEWS IN BRIEF

PROTEOMICS

Defining RNA-binding protein preferences

RNA-binding preferences for only a few RNA-binding proteins (RBPs) have been determined, in part because existing methods are costly and laborious. Ray *et al.* describe a rapid approach, called RNAcompete, and use it to define RNA-binding specificities for nine RBPs. In this method, a diverse RNA pool is first generated; RNAs that bind to an RBP of interest are pulled down, fluorescently labeled and then analyzed via a microarray.

Ray, D. *et al. Nat. Biotechnol.* **27**, 667–670 (2009).

CHEMICAL BIOLOGY

Quantum dot biosynthesis

Quantum dots are widely used in imaging applications. The synthetic methods used to make CdSe quantum dots, however, are anything but environmentally friendly. Seeking a more benign synthetic approach, Cui *et al.* now report a method to biosynthesize quantum dots in yeast cells. They do this by carefully controlling the timing and duration of the incubation of the yeast with Na_2SeO_3 and $CdCl_2$ to generate CdSe quantum dots of various sizes with various fluorescence emission wavelengths.

Cui, R. *et al. Adv. Funct. Mater.* advance online publication (12 June 2009).

GENOMICS

Designing GWASs

Genome-wide association studies (GWASs) are very powerful methods for finding genetic variants that indicate risk for disease. Designing good GWASs, however, takes a lot of careful planning and usually a big budget. Spencer *et al.* describe a simulation method to assess the statistical power of different genotyping chips. Contrary to popular belief, they show that the chip with the highest coverage is not necessarily the best tool for the job.

Spencer, C.C.A. *et al. PLoS Genet.* **5**, e1000477 (2009).

BIOSENSORS

Detecting ozone

Although the stratospheric ozone layer crucially protects life on Earth from harmful ultraviolet rays, ground-level ozone is toxic. Garner *et al.* describe a small molecule–based, fluorescent turn-on probe for ozone, which can be used both as an atmospheric and a cell-based ozone sensor. This probe is highly selective for ozone and is not sensitive to the presence of other reactive oxygen species; it is a promising tool for better understanding the role of ozone in tissue damage.

Garner, A.L. *et al. Nat. Chem.* **1**, 316–321 (2009).

PROTEIN BIOCHEMISTRY

Enzymes for glycosphingolipid synthesis

Hancock *et al.* describe the generation of 'designer' enzymes for glycosphingolipid synthesis, using rational mutagenesis–based directed evolution and an enzyme-linked immunoassay (ELISA)–based screen to select glycosynthase mutants with improved catalytic activity. These enzymes could potentially be used to synthesize large quantities of pure glycosphingolipids for therapeutic applications.

Hancock, S.M *et al. Nat. Chem. Biol.* **5**, 508–514 (2009).