SYNTHETIC BIOLOGY

Tethered together

Researchers describe a genetic approach to identify the native components responsible for forming molecular transport junctions between the mitochondria and the endoplasmic reticulum.

Eukaryotic cells evolved to have specialized compartments—organelles—which perform different functions, thus optimizing the specificity and efficiency of biochemical reactions. Concomitant to this compartmentalization, routes for communication and molecular transport between organelles also had to evolve. As one example, lipids that are synthesized in the endoplasmic reticulum (ER) are exported via vesicles that ship the lipids to other organelles. However, no mitochondrial ports are found on these vesicular shipping routes. It is not well understood how such necessary components are transported from the ER to the mitochondria.

Previous research has suggested that junctions for molecular transport form between the ER and the mitochondria. Several proteins have been implicated in forming these junctions, but they have not been confirmed. "It has been known for a long time that there were connections, but the nature of them was very mysterious," says Benoît Kornmann, a postdoc in Peter Walter's lab at the University of California, San Francisco. Kornmann and his colleagues recently described a powerful approach that attempts to understand the character of these junctions, by first pinning down which proteins are involved.

They designed a genetic screen to identify proteins involved in connecting the mitochondria and ER in yeast, by constructing a synthetic mitochondria-ER tether, called ChiMERA (construct helping in mitochondria-ER association) intended to rescue the function of mutants with mutations in the native tethering components. ChiMERA consists of an N-terminal mitochondrial signal sequence and a transmembrane domain from the protein Tom70, connected via a central GFP domain to a C-terminal ER tail anchor from the protein Ubc6.

They then screened for yeast mutants that did not grow without a plasmid expressing the synthetic tether. They discovered only two mutants, both of which contained mutations in Mdm12, a peripheral outer mitochondrial membrane protein. Mdm12 is known to form a complex with Mmm1, Mdm10 and Mdm34; this complex has actually been quite well studied and was thought to be important for regulating mitochondrial morphology. "But nothing was known about it connecting the ER to the mitochondria," says Kornmann. The researchers found that by expressing ChiMERA, they could rescue the growth of yeast strains with these genes deleted, thus suggesting that the core function of the complex, which they renamed ERMES for ER-mitochondria encounter structure, is to connect the mitochondria and the ER. They also discovered that ERMES was necessary for phospholipid exchange between the ER and mitochondria.

This unique approach could be used to discover other native organelle tethers, something that Kornmann plans to pursue in the future. "For example, it is known that the ER and the plasma membrane are also found in proximity and probably owing to tethering,"

NEUROSCIENCE

LOCATION, LOCATION, LOCATION

A strategy for selectively disabling activated neurons helps researchers to characterize brain circuitry controlling addiction-related behaviors.

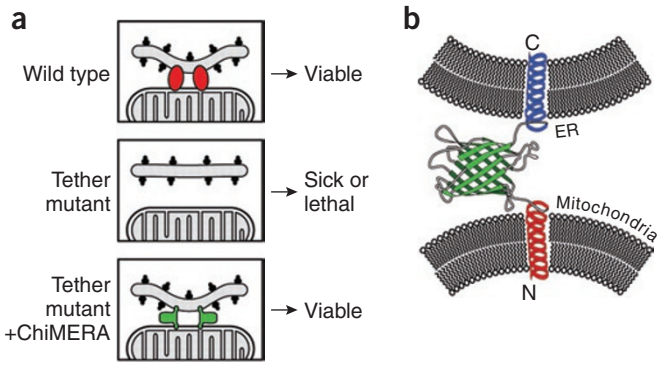
Even though decades of research have shown that the familiarity of a physical location where addicts use cocaine or heroin can markedly affect their drug response—or even their vulnerability to an overdose—the mechanisms of this process remain poorly understood. According to Bruce Hope, a researcher at the US National Institute on Drug Abuse, this is due in part to belated recognition by molecular and cellular biologists of the effects of external conditions on animals used in drug studies.

"We didn't appreciate environment," he says. "We'd always assumed that the mechanisms we saw in the home cage are going to be the same ones operating in a novel environment, and it turns out not to be so." In fact, several studies from recent years have revealed neuronal subpopulations that are specifically activated only when drugs are consumed in an environment previously associated with such behavior.

Hope was interested in studying the neurons involved in these circuits, but found himself limited by existing neurobiological techniques designed to examine brain tissue as homogenous cell clusters rather than sparsely distributed, specialized cellular circuits. To counter this limitation, his team devised a new approach for disabling only those cells involved in environment-specific response to cocaine intake.

They noted that rats injected with repeated doses of cocaine in a particular environment (for example, a square, flat-floored chamber) exhibit increased locomotor activity—behavior that is enhanced by additional treatments in the same environment, but not when the rat is treated with cocaine in an unfamiliar environment (for example, a round chamber with woodchips). Upon examining *Fos* (*c-fos*) expression in these rats—a general indicator of neuronal activity—they found that ~2–3% of the neurons in the nucleus accumbens, a brain region involved in 'reward-seeking' behavior, are active in these environmentally sensitized rats.

To selectively target these cells, the researchers used transgenic rats in which the *c-fos* promoter was linked to the gene encoding β-galactosidase. By treating these rats with Daun02, a compound that is converted by this enzyme into an action potential–blocking drug, they specifically inhibited neurons engaged in active signaling. When they infused these transgenic rats with Daun02 after initial cocaine treatment, the rats lost their sensitized response, with subsequent cocaine doses inducing movement equivalent to that observed in rats receiving their first dose, regardless of whether it was



A synthetic ER-mitochondrial tether. (**a**) Mitochondria and ER are tethered by a native complex in yeast cells (top). Mutations to this complex cause slow growth or cell death (middle). A synthetic mitochondria-ER tether, ChiMERA, rescues cell growth (bottom). (**b**) The ChiMERA protein consists of an ER-directed domain (blue), a central GFP domain (green) and a mitochondria-directed domain (red). Reprinted with permission from The American Association for the Advancement of Science.

he says. Perhaps most interestingly, at least from a methods standpoint, the approach is also likely to have general value, as Kornmann explains: "If one is able to replace cellular function by an artificial protein—not necessarily tethering—any kind of function in principle should be amenable to doing this kind of method."

Allison Doerr

RESEARCH PAPERS
Kornmann, B. *et al.* An ER-mitochondria tethering complex revealed by a synthetic biology screen. *Science* **325**, 477–481 (2009).

given in familiar or unfamiliar surroundings.

Notably, this treatment did not appear to exert any nonspecific neurological effects, nor did it affect the rats' capacity to respond to cocaine or engage in normal locomotor activity. "It's a unique set of neurons that were only activated by the repeated presence of both the drug and the environment together," says Hope. "This means that it could be a mechanism for how the learned association is produced between those two factors."

His team is now investigating characteristics of the neurons affected in these experiments, but their initial findings suggest that Daun02 inactivation could offer a general strategy for precisely manipulating neural circuitry underlying other complex behavioral processes. Accordingly, Hope is now collaborating with National Institute on Drug Abuse colleague Yavin Shaham to apply their method to 'cue-induced reinstatement', the process by which certain environmental cues can restore seeking behavior in animals previously weaned off of drugs—in this case, heroin. "The parallel might be exposing an addict to drug paraphernalia, and all of a sudden they crave heroin," says Hope. "This is what that's supposed to model."

Michael Eisenstein

RESEARCH PAPERS
Koya, E. *et al.* Targeted disruption of cocaine-activated nucleus accumbens neurons prevents context-specific sensitization. *Nat. Neurosci.* **12**, 1069–1073 (2009).

NEWS IN BRIEF

SYNTHETIC BIOLOGY

Engineering *Escherichia coli* with new functions

Wang *et al.* present multiplex automated genome engineering (MAGE), a method to rapidly generate combinatorial genetic modifications by repeatedly introducing synthetic DNA to cells. The approach is based on the concept that for a pool of degenerate oligonucleotides, those with greater homology to the chromosomal target will be incorporated with higher frequency via allelic replacement. They applied MAGE to program *E. coli* to overproduce the antioxidant lycopene by optimizing the biosynthetic pathway.

Wang, H.H. *et al. Nature* **460**, 894–898 (2009).

CHEMICAL BIOLOGY

Multiplexed kinase activity profiling

Protein kinases have differential levels of activity under different biological conditions. Yu *et al.* describe an approach to profile the activation state of kinases in a cell lysate by monitoring the phosphorylation of 90 synthetic known peptide substrates via mass spectrometry, using heavy isotope–labeled versions of the peptides as internal quantitative standards. They applied the method to profile kinase activity during the cell cycle, in breast cancer cell lines and after applying kinase inhibitors.

Yu, Y. *et al. Proc. Natl. Acad. Sci. USA* **106**, 11606–11611 (2009).

NANOTECHNOLOGY

Fluorescent-plasmonic nanoparticles

Jin and Gao describe an approach to make multifunctional nanoparticles that are both fluorescent and plasmonic. This presented a challenge because gold, the plasmonic material, can quench fluorescence. Jin and Gao overcame this by precisely controlling the spacing between a quantum dot core and an ultrathin gold shell via layer-by-layer assembly.

Jin, Y. & Gao, X. *Nat. Nanotechnol.* advance online publication (26 July 2009).

PROTEOMICS

The Dub interactome

Although the ubiquitin conjugation machinery is fairly well understood, the functions of deubiquitinating enzymes (Dubs), which catalyze the removal of ubiquitin from proteins, have not been vastly studied. Sowa *et al.* profiled the human Dub protein family using an affinity purification scheme followed by mass spectrometry to analyze Dub protein interaction partners. They also introduce CompPASS, a software platform for identifying high-confidence interactions.

Sowa, M.E. *et al. Cell* **138**, 389–403 (2009).

BIOPHYSICS

Transcription initiation on a single-molecule scale

Single-molecule fluorescence resonance energy transfer can be used to monitor molecular motions over time, but multiplexing is limited. Sorokina *et al.* describe a single-molecule method that requires only a single fluorophore, using time correlated single-photon counting for monitoring fluorescence lifetime trajectories of an immobilized molecule. They used this approach to follow the complex process of transcription initiation by T7 RNA polymerase.

Sorokina, M. *et al. J. Am. Chem. Soc.* **131**, 9630–9631 (2009).