CHEMICAL TOOLS

Pony express to mitochondria

Synthetic peptides that can enter the cell and localize to mitochondria are promising tools for delivering cargo to the organelle.

To deliver plasmids into bacteria, one can just add DNA to the medium and wait a bit. Eukaryotic cells, however, have less promiscuous membranes, so researchers have devised several ways to deliver molecules into cells. One of them uses cell-penetrating peptides, cationic molecules that can cross the plasma membrane and bring fused cargo with them into the cell.

Shana Kelley from the University of Toronto would like to take this cargo a step further—into various organelles—and along the way learn how chemical structure influences where molecules end up in the cell. With mitochondria as their first target, she and her colleagues designed cationic peptides that were also lipophilic enough to pass through the very hydrophobic mitochondrial inner membrane.

"Cyclohexylalanine looked to be one of the most hydrophobic things you could incorporate into a peptide easily, and so we thought this would be a good amino acid to try," says Kelley. Along with these hydrophobic residues, her group's 4–8-amino-acid synthetic peptides contained arginines and lysines to provide the positive charge necessary to harness the mitochondrial membrane potential for transport.

When incubated with live human cells, these peptides, fused to a fluorophore for detection, crossed the plasma membrane and specifically localized to mitochondria: the fluorescence signal strongly correlated with that of a mitochondrial marker. Interestingly, peptides containing the amino acid phenylalanine, which is less hydrophobic than cyclohexylalanine, were found in the mitochondria as well as in the nucleus. In contrast, Tat, a control cell-penetrating peptide, local-

ized to the nucleus and was excluded from mitochondria. Based on these results, the researchers then designed other peptides to more precisely define the chemical rules for mitochondrial localization.

In addition to having possible applications for drug delivery, these mitochondria-penetrating peptides will be useful cell biology tools. Kelley also hopes they can be used to deliver nucleic acids, to manipulate the mitochondrial genome, for example. Toward these goals, her group is now testing their mitochondria-penetrating peptides to deliver molecules ranging from proteins to small-molecule drugs. She concludes: "It's going to be challenging, but I think we now know what the rules for organellar entry are."

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RESEARCH PAPERS

Horton, K.L. *et al.* Mitochondria-penetrating peptides. *Chem. Biol.* **15**, 375–382 (2008).