GENE TRANSFER

Supercharging through the cell membrane

Researchers show that superpositively charged GFP enters mammalian cells with ease and can be used as a nucleic acid delivery vehicle.

It's a bird, it's a plane, it's … supercharged GFP!

Supercharged GFP may not be faster than a speeding bullet, but it is quite an interesting molecule. As part of a project to understand how to make designed proteins less prone to aggregation, David Liu's group at Harvard University discovered that mutants containing large numbers of charged, surface-exposed residues were more aggregation-resistant than their neutral counterparts. They made supercharged variants of GFP as well as glutathione *S*-transferase and streptavidin, and found that the supercharged versions were not only virtually immune to aggregation, but they largely maintained their fold and biological function (Lawrence *et al.*, 2007). "Then we began to wonder if some of these supercharged proteins could have other interesting and potentially useful features by virtue of their extremely unusual net charge," says Liu.

Liu and his colleagues now report that one of the interesting things that supercharged GFP in particular can do is potently enter mammalian cells (McNaughton *et al.*, 2009). They observed that GFP with a theoretical net charge of +36 (+36 GFP) penetrated several different mammalian cell lines at very low (nanomolar) concentrations. Cationic peptides and proteins have been observed to penetrate cells, and both natural and synthetic cell-penetrating peptides are widely used as delivery vehicles. However, whereas a covalent link between the cell-penetrating peptide and its cargo is typically required, Liu and his colleagues found that +36 GFP can simply form a complex with negatively charged nucleic acids and carry them into the cell.

The researchers harnessed +36 GFP to deliver *GAPDH*-targeting short interfering RNA (siRNA) into HeLa cells, inner medullary collecting duct cells, 3T3-L preadipocytes, rat pheochromocytoma PC12 cells and Jurkat T cells—the latter four of which are resistant to siRNA delivery using the common lipid-based transfection reagent, Lipofectamine 2000. They observed suppression of *GAPDH* expression in all of the cell lines except in Jurkat T cells. The +36 GFP–based siRNA delivery was also much more effective than several tested cell-penetrating peptides. The researchers also delivered plasmid DNA complexed with a +36 GFP variant and observed plasmid-driven β-galactosidase expression in four of the five tested cell lines.

Liu and his colleagues have worked out that +36 GFP enters the cell via an endocytotic pathway, but they are not yet quite sure why it is such a potent delivery agent. "We know that charge is important, because we see a beautiful relationship between charge and efficiency of delivery, but we are still in the process of probing, for example, how the distribution of that charge around the surface of the protein might affect delivery," says Liu. They are also currently investigating how to improve the escape of nucleic acids delivered via supercharged proteins from endosomes, as well as exploring whether supercharged proteins can be harnessed to deliver molecules other than nucleic acids into cells and whether *in vivo* delivery applications are possible.

Though these results are preliminary, supercharged proteins are an intriguing new class of intracellular delivery tools. Existing delivery methods do not work for all applications or all cell lines, and safe and effective approaches are still needed for *in vivo* applications. "There's a long list of challenges that have to be met before any macromolecule delivery approach can really have an impact in the life sciences both inside and hopefully outside of the research lab," notes Liu. "It's too early to know if supercharged proteins will provide a general solution to a major delivery problem, but we're excited to explore that possibility."

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RESEARCH PAPERS

Lawrence, M.S. *et al.* Supercharging proteins can impart unusual resilience. *J. Am. Chem. Soc.* **129**, 10110–10112 (2007).

McNaughton, B.R. *et al.* Mammalian cell penetration, siRNA transfection, and DNA transfection by supercharged proteins. *Proc. Natl. Acad. Sci. USA* **106**, 6111–6116 (2009).