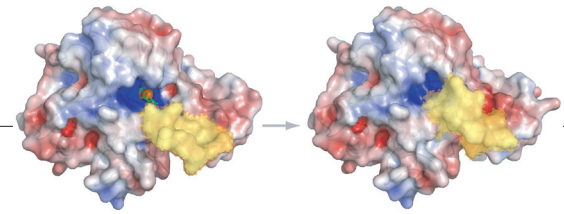
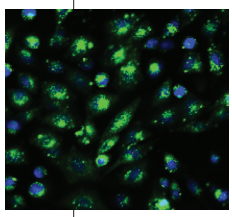
ACS chemical biology

In this ISSUE



Positively Permeable

Most molecules are unable to permeate mammalian cell membranes. Positively charged peptides are an exception, and appending polyarginine or other positively charged entities to proteins can facilitate their entry into cells, but not without altering the size or homogeneity of the protein. Fuchs and Raines (p 167) cleverly graft arginine residues onto the surface of enhanced GFP (eGFP) to create a GFP variant capable of permeating the cell membrane.



eGFP was chosen as a model protein for the grafting experiment because of its well-characterized structure and handy fluorescent properties. Using site-directed mutagenesis, the authors strategically replaced five acidic residues with arginines, and the result was a highly positively charged patch on the eGFP surface. This modified protein, termed cell-permeable GFP (cpGFP), was as stable and as fluorescent as eGFP. However, fluorescence microscopy showed that cells exposed to cpGFP, but not eGFP, became fluorescent. The fluorescence was dose-dependent and was detected primarily in vesicles. This innovative approach to solving the impermeability problem should have a positive impact in this challenging area.

Dipping into the Daptomycins

The pool of drugs that are effective against bacterial infections is becoming shallower because of the emergence of antibiotic-resistant strains of bacteria. Researchers are continually searching for new antibiotics that can defeat these "superbugs", and in their quest some have begun wading into the pool of lipopeptide antibiotics. This group includes daptomycin, which was recently approved for the treatment of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. Strieker *et al.* (p 187 and Point of View on p 152) report the biochemical and structural characterization of AsnO, an enzyme involved in the biosynthesis of a related lipopeptide, calcium-dependent antibiotic (CDA).

CDA is an 11-residue acidic lipopeptide lactone that contains a β-hydroxylated asparagine (hAsn). The fogginess surrounding how hAsn is incorporated into CDA cleared when the authors determined that AsnO hydroxylates free L-asparagine to generate hAsn, which is subsequently incorporated into the growing peptide chain. Generation of the crystal structures of AsnO alone and in complex with hAsn revealed the basis of the stereoselectivity of the enzyme along with the presence of a lid-like structure that likely guides the enzyme's substrate specificity. This insight could facilitate the creation of additional β-hydroxylated amino acids, expanding the diversity of lipopeptide structures.

Pathway to Power

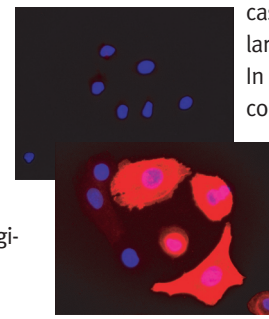
Mitochondria have been referred to as the "powerhouses" of the cell because of their role in ATP production, and they have captured additional attention for their role in apoptosis. The intermembrane space (IMS) of mitochondria houses specific proteins, including the apoptosis regulator Smac, but the events that transpire within this region are not well understood. Although peptide targeting sequences for various other cellular organelles are known, the targeting sequence for the IMS has not been identified. Ozawa *et al.* (p 176) now characterize the IMS targeting signal.

Clever assembly of a Smac-containing reporter construct that incorporates protein splicing and fluorescence elements enabled identification of proteins located in the IMS. Random mutagenesis experiments revealed that residues 10–57 comprise the minimal sequence needed to target Smac to the IMS and that the N-terminal four residues are especially critical. Fusion of this IMS-targeting sequence to other proteins also resulted in delivery of these proteins to the IMS. This study indeed provides a powerful molecular tool for exploring the physiological processes that occur in the powerhouse of the cell.

Getting the Skinny on Skin Cells

The skin provides a protective barrier against pathogens, helps regulate body temperature, and synthesizes vitamins D and B. Unfortunately, diseases such as psoriasis and skin cancer are the result of the misregulation of the proliferation and differentiation of skin cells. Hong *et al.* (p 171) perform a cell-based screen to identify small molecules that induce terminal differentiation of human skin cells.

Differentiation of primary normal human epidermal keratinocytes results in a variety of cellular events, including increased expression of the protein involucrin. A collection of ~13,000 small molecules were screened for their ability to induce expression of an involucrin-specific luciferase reporter. Several 7-aryl-substituted 2-(3,4,5-trimethoxyphenylamino)-pyrrolo[2,3-*d*]pyrimidines were identified, and the most potent was used to explore the mechanism by which these compounds induce skin cell differentiation. Gene expression analysis revealed that this compound affects the cell-cycle-related p38 mitogen-activated protein kinase signaling pathway and integrin-mediated signaling. Using affinity chromatography, the authors identified casein kinase 2 (CK2) as one molecular target of these pyrrolopyrimidines. In addition, a closely related derivative competitively inhibited CK2. These molecules may help researchers "get the skinny" on skin cell differentiation by providing molecular tools for exploring this process and pointing to jumping-off points for potential therapeutic agents.



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