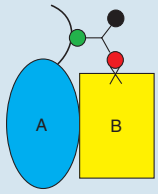
Protein-protein interactions in a FlAsH



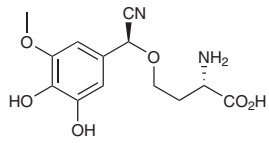
Because many biological processes are mediated by protein-protein interactions (PPIs), the development of methods for identifying physiologically relevant PPIs remains an important aim. Chemical approaches for validating PPIs have focused on covalent cross-linking, but such 'label transfer' chemistry, in which a tag is transferred from one protein to partner proteins, has been limited by the need to chemically modify one protein with a cross-linker. Now Liu *et al.* report an *in vitro* PPI labeling method that avoids these difficulties. The authors synthesized a FlAsH probe that contains a biarsenical fluorophore, an orthoquinone cross-linker and a biotin tag. They validated the reagent on the known interaction of a Gal80-binding peptide (G80BP) with Gal80 protein. G80BP was engineered to carry a FlAsH receptor peptide (FRP) that was designed to capture the FlAsH probe in the G80BP–Gal80 complex and bring the orthoquinone moiety in proximity to Gal80. Cross-linking to Gal80 was then initiated by activation of the orthoquinone by sodium periodate treatment, with cleavage of the biarsenical-FRP interaction completing the biotin label transfer to Gal80. The authors adapted the approach to identify physiological partners of the viral protein VP16. By inserting an FRP into the acidic activation domain of VP16, the authors demonstrated specific biotinylation of two components of the proteasome that are thought to be important for regulating expression of viral genes. Combined with other analytic techniques, the new label transfer method offers a useful approach for the identification of protein interaction partners. (*J. Am. Chem. Soc.*, published online 26 September 2007, doi:10.1021/ja072904r) *TLS*

NAD$^+$ meets stress in mitochondria

NAD$^+$ acts as a cosubstrate for various enzymes, including poly(ADP-ribose) polymerase-1 (PARP1) and the sirtuin family of deacetylases and mono-ADP-ribosyltransferases. It is generally recognized that genotoxic stress causes cell death by depleting nuclear and cytosolic NAD$^+$ levels. In yeast, the *PNC1* gene product responds to nutrient restriction and stress by catalyzing the first step in NAD$^+$ biosynthesis, thereby positively regulating the life-span-extending properties of the yeast sirtuin Sir2. In mammals, Nampt is thought to be the functional equivalent of Pnc1 and is thought to depend on the mammalian Sir2 homolog, SIRT1. Now, Yang *et al.* show that like *PNC1*, expression of Nampt is induced by cell stress and nutrient restriction. Also, overexpression of Nampt protected cells from treatment with the DNA alkylating agent MMS, which causes cell death through hyperactivation of PARP1 and subsequent depletion of NAD$^+$. Surprisingly, Nampt protection against MMS treatment did not affect MMS-mediated NAD$^+$ depletion, as total NAD$^+$ levels were similar. Also, protection was not dependent on SIRT1 but did require the mitochondrial sirtuins SIRT3 and SIRT4. This led the authors to suspect that the mitochondrial pools of NAD$^+$ might provide a clue. Indeed, using a new method to measure mitochondrial NAD$^+$, they found that mitochondrial NAD$^+$ levels are upregulated by nutrient restriction and remain intact in mitochondria from MMS-treated cells overexpressing Nampt. Probing further, the authors propose that an NAD$^+$ salvage pathway exists whereby Nampt generates NAD$^+$ in response to nutrient restriction and cell stress and also increases the activity of sirtuins, thereby affording protection from cell death—all from within mitochondria. (*Cell* **130**, 1095–1107, 2007) *MB*

Written by Mirella Bucci, Catherine Goodman, Joanne Kotz & Terry L. Sheppard

Primed to poison



Plants, bacteria and fungi rely on a sophisticated set of chemical defense agents to discourage feeding by herbivores and other attacks. While these molecules must be extremely unpleasant to the predator, they must also be innocuous to the host, and so they are frequently stored as inactive counterparts to be converted and released upon external stress. One important agent in this arsenal is cyanide, which is stored in the form of cyanogenic glucosides or glycine. Kindler and Spiteller have now identified a new oxidative pathway for HCN release in their discovery of a novel endogenous cyanohydrin. The authors were intrigued by the release of hydrocyanic acid from injured fruiting bodies of the fungus *Aleurodiscus amorphus*; extraction of intact and injured fruiting bodies revealed a significant amount of two different compounds, termed aleurodisconitrile and aleurodiscoester. Because of the ether linkage in aleurodisconitrile, it is not possible for the nitrile to be released enzymatically by known mechanisms. Instead, the authors found that the three substituents on the aromatic ring are optimized for release of HCN upon oxidative stress. Examination of the reaction using model systems allowed the capture of two potential intermediates in the pathway: a quinonemethide, which would result from the initial oxidation of aleurodisconitrile, and a tetrahedral intermediate mimic, which suggests that water is sufficient to convert the quinonemethide to the final ester. These steps outline a new mechanism for self-preservation. (*Angew. Chem. Int. Ed.*, published online 20 September 2007, doi:10.1002/anie.200702481) *CG*

Fine-tuning protein expression

In directed evolution experiments, assays in which the reaction product is required for cellular growth are often used to select for enzymatic activity. However, once a threshold of activity has been reached that permits wild type-like cell growth, there is no further selective pressure to increase enzymatic activity. Thus, the dynamic range of directed evolution experiments can be very limited. Neuenschwander *et al.* now report a system for precisely modulating protein expression levels in the cell to enable the selection of protein variants with high enzymatic activity. By placing the target gene under the control of a tetracycline-inducible promoter, cell growth with a weakly active variant of chorismate mutase (an enzyme that is essential for the biosynthesis of tyrosine and phenylalanine) was distinguishable from growth of cells expressing a moderately or highly active variant. However, discriminating between moderately active and highly active enzymes required the combination of the tunable promoter with an SsrA degradation signal, which targets the tagged protein for protease-mediated degradation. Using this combination for regulating protein levels, the authors were able to increase the activity of a weakly active chorismate mutase 80-fold—to an activity level comparable to that of the wild-type enzyme and significantly higher than had been achieved by other directed evolution strategies. This method for precisely adjusting cellular protein levels is likely to be useful in a wide range of cell-based selection assays. (*Nat. Biotechnol.*, published online 16 September 2007, doi:10.1038/nbt1341) *JK*