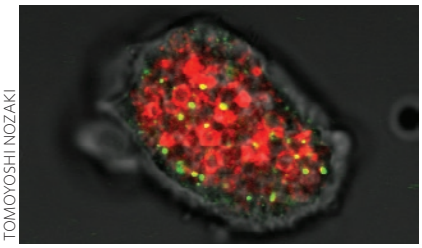
METABOLISM

A location for sulfation

Proc. Natl. Acad. Sci. USA, published online 7 December 2009, doi:10.1073/pnas.0907106106



TOMOYOSHI NOZAKI

Mitosomes are reduced, mitochondrion-related organelles found in protists, or simple eukaryotes, that can perform divergent functions. For example, the mitosomes of *Entamoeba histolytica* and *Mastigamoeba balamuthi* have a nitrogen fixation system, but the main purpose of these organelles, and indeed most of the contents, are unknown. To identify specific proteins in and ascribe function to the *E. histolytica* mitosome, Mi-ichi *et al.* first created a two-tier purification method to identify 95 putative proteins cofractionating with the known mitosomal marker chaperonin 60 (Cpn60); 22 of 25 proteins tested were also observed to colocalize with Cpn60 in cells, providing support for the proteomics results. Three enzymes involved in sulfate activation—capable of catalyzing the transformation of ATP into 3′-phosphoadenosine-5′-phosphosulfate (PAPS)—were detected at high levels, along with a sodium/sulfate symporter, suggesting that these sulfate reactions likely occur in the mitosome. Incubation of the parasite with radioactive sulfate resulted in labeling of several lipids with unknown structures. These results suggest a model in which mitosomal PAPS, a sulfate donor, is used to synthesize modified lipids by sulfotransferases. However, these latter enzymes are predominantly in the cytoplasm, and the only obvious PAPS transporter in the genome does not appear to be localized to the mitosome. These intriguing results suggest that the remaining proteins in the mitosome—many of which are annotated as hypothetical proteins—may have important roles in membrane transport or lipid biosynthesis. *CG*

SIGNALING

PTTH is connected to Torso

Science, **3260**, 1403-1405 (2009)

MICHAEL O'CONNOR

Metamorphosis from immature larvae into adult *Drosophila* fruit flies is initiated by the neuropeptide hormone PTTH, though the receptor involved is not known. Rewitz *et al.* suspected a receptor tyrosine kinase (RTK), since PTTH is structurally similar to some growth factor RTK ligands. As well, the MAPK pathway has been implicated in signaling downstream of PTTH. The authors measured the expression of all *Drosophila* RTKs in the prothoracic gland (PG), the endocrine organ that receives the signal from PTTH to trigger production and release of the molting hormone 20E. They found that the RTK Torso had the proper spatial and temporal expression to be a good candidate for the PTTH receptor. RNAi against *torso* delays the onset of pupariation and causes an increase in pupal size, much like the loss of PTTH does, and these phenotypes were reversed by feeding the larvae with 20E. As well, a constitutively active *torso* allele causes precocious pupariation, similar to the known effect of PTTH overexpression. The authors used RNAi-mediated downregulation of MAPK components and a cell culture-based signaling assay to further support the conclusion that Torso mediates PTTH signaling in the PG, and that the MAPK pathway lies downstream. This study has implications in understanding body size determination as well as the timing of fundamental developmental transitions, such as occurs during puberty in mammals. *MB*

TRANSLATION

Channeling order

Proc. Natl. Acad. Sci. USA, published online 17 December 2009, doi:10.1073/pnas.0903750106

Though *in vitro* studies on isolated polypeptides have uncovered the chemical principles that govern protein folding, it remains unclear whether these principles apply to co-translational folding that occurs as nascent polypeptide chains (NCs) emerge from the ribosome exit channel *in vivo*. Cabrita *et al.* now report a method for isolating stalled ribosome–nascent chain (RNC) complexes from *Escherichia coli* and analyzing the folded states of these ribosome-bound proteins by multidimensional NMR spectroscopy. By stimulating *E. coli* growth to high cell densities and then inducing expression of a model protein construct in the presence of ^{15}N-ammonium chloride and ^{13}C-glucose, the authors were able to produce RNCs with the NCs labeled with NMR-active nuclei in a background of unlabeled ribosomes. The NMR spectra of the RNC consisting of full-length polypeptides were remarkably similar to those of the *in vitro* folded protein. However, analysis of the RNC derived from a shorter segment of the model protein, in which the domains were only partially extruded from the ribosome channel, showed more significant line broadening and fewer crosspeaks, indicative of a dynamic set of partially folded structures. In addition to providing high-resolution snapshots of co-translational folding at the ribosome, the new method offers the potential for analyzing the role that the ribosome channel and other cellular factors play in protein folding within the cell. *TLS*

METALLOPROTEINS

Design-NOR enzyme

Nature **462**, 1079–1082 (2009)

Nitric oxide reductase (NOR), an enzyme that reduces NO to N_2O in the nitrogen cycle of anaerobic bacteria, contains both a heme and non-heme metal site. Although synthetic models and spectroscopic studies have been reported, progress in understanding the NOR reaction mechanism has been hindered by the lack of a three-dimensional structure. To provide a model for structure-function studies of NOR, Yeung *et al.* turned to myoglobin, an easily crystallizable protein containing a heme binding site, as a scaffold for designing NOR activity. Starting from a histidine near the heme site, the authors used computational modeling along with mutagenesis to introduce two additional histidines and a glutamate in the orientation expected in the non-heme binding site of NOR. Spectroscopic and crystallographic studies confirmed that these ligands formed a functional Fe(II) binding site with the expected geometry less than 5 Å away from the heme site. Similarly to the native enzyme, addition of Fe(II) to the Fe(III) heme form of this protein resulted in spin coupling between the two metal sites and an increase of more than 100 mV in the heme reduction potential. Providing the final confirmation of NOR activity, addition of NO to the designed protein resulted in the formation of N_2O. Rational design of enzymes, particularly metalloenzymes, presents a significant challenge. These results suggest an increased tractability of metalloenzyme design and provide a new model for investigating NOR. *JK*

PROTEIN FOLDING

Thermodynamic compromise

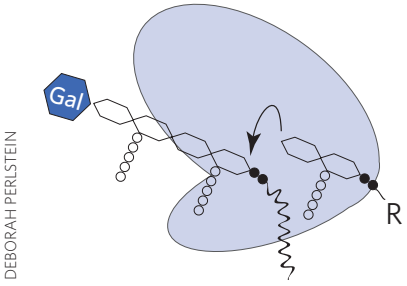
Mol. Cell **36**, 861–871 (2009)

A full understanding of the factors that govern protein stability *in vivo* is incomplete since studies of the kinetics and thermodynamics of protein folding have almost exclusively come from *in vitro* studies of dilute, pure proteins. Foit *et al.* have now developed a system to evolve protein stability *in vivo* by fusing a model protein between the two halves of the antibiotic resistance enzyme β-lactamase. Among known variants of the Im7 test protein that alter its stability *in vitro*, there was a strong correlation between thermodynamic stability and the minimal inhibitory concentration when expressed *in vivo*. By selecting for enhanced antibiotic resistance in a library of mutagenized Im7, the authors identified 13 residues whose mutation caused increased stability when tested *in vitro* (shown as spheres on the figure). Increased stability for Im7 variants correlated with decreased sensitivity towards protease digestion, an increase in steady state expression levels and increased kinetic stability, a measure related to protein unfolding rates. Functionally, a majority of the stabilized Im7 mutations (shown as blue spheres) map to the interface of Im7 binding with its cognate toxin, colicin E7. Some mutations reduced binding, but none abolished it. These results highlight a balance between functional, kinetic and thermodynamic properties and help to explain why protein evolution does not necessarily favor the most stable fold. *MB*

GLYCOBIOLOGY

Probing polymerization

J. Am. Chem. Soc., published online 17 December 2009, doi:10.1021/ja909325m



DEBORAH PERLSTEIN

Peptidoglycan glycosyltransferases create the bacterial cell wall in a processive reaction using glycolipids in the membrane. In particular, these enzymes catalyze the transfer of a disaccharide unit from a glycolipid in the donor site to a growing polymer chain in the acceptor site, followed by translocation of the chain to the donor site and a return to the beginning of the reaction. Previous work using chemically synthesized substrate analogs had shown that variations in the conformation, saturation and length of the lipid chain prevent homopolymerization, indicating that one or both of the donor and acceptor sites have strict substrate specificity. To investigate the unique properties of the two sites, Perlstein *et al.* used a second modified substrate, terminating in a galactose residue, which could serve as a glycosyl donor but not an acceptor. By testing the original analogs in the presence of the galactose construct, the authors were able to identify whether the acceptor site could accommodate the lipid modifications. Surprisingly, all of the analogs tested were converted to product, demonstrating that the donor site is responsible for the strict specificity observed. Though it remains to be seen how exactly the lipid tail impacts enzyme function—affecting initial binding versus translocation, for example—these results demonstrate an elegant strategy for studying a repetitive reaction and provide new insights into peptidoglycan biosynthesis. *CG*

EPIGENETICS

Methylation coordination

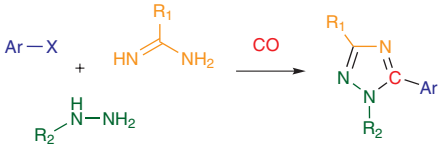
Nat. Struct. Mol. Biol., published online 20 December 2009, doi:10.1038/nsmb.1753

The reversible methylation of lysine residues within histones is a central regulatory mechanism for eukaryotic gene expression. Because histones often contain activating marks, such as Lys4-trimethylated histone 3 (H3K4me3), along with repressive marks such as H3K9me2 or H3K27me2, it remains unclear how histone-modifying enzymes manage these conflicting signals. Horton *et al.* now suggest that certain histone lysine demethylases target different dimethylated lysine residues by virtue of their structural architectures. PHF8 and KIAA1718 are two related demethylases that contain an N-terminal plant homeodomain (PHD) that binds to H3K4me3 sites and a jumonji demethylase domain that removes downstream dimethyllysine marks. H3K9me2 is a poor substrate for PHF8, but the addition of an upstream H3K4me3 modification enhances the ability of PHF8 to remove K3K9me2 marks by 12-fold. In contrast, KIAA1718 is active on H3K9me2 alone, but addition of a H3K4me3 mark abrogates demethylase activity. Structural analyses of PHF8 and KIAA1718 showed that although their PHD and jumonji domains adopt virtually identical structures, the relative three-dimensional relationship of these two domains was strikingly different: PHF8 bends to bring the PHD and jumonji sites in proximity, while KIAA1718 adopts a more extended conformation that enables it to target the more distant H3K27me2 modification. In addition to providing new insights into the 'histone code', the study opens the possibility that histone-modifying enzymes could be rationally engineered. *TLS*

CHEMICAL SYNTHESIS

Triazole in four parts

Angew. Chem. Int. Ed., published online 8 December 2009, doi:10.1002/anie.200905897



Triazoles, which are five-membered aromatic heterocycles containing two carbon and three nitrogen atoms, are commonly found in bioactive molecules. Access to highly substituted 1,2,4-triazoles from aryl halides typically requires complex multistep synthesis involving transition metal–catalyzed C-C coupling that proceeds via unstable metallo-containing intermediates. Staben and Blaquiere now report an alternative synthetic approach to trisubstituted 1,2,4-triazoles via Pd-catalyzed C-C-N coupling to form carbonyl precursors that cyclize to yield the triazole products. The modular synthetic approach involves the initial carbonylative coupling of amidines to aryl or heteroaryl halides, followed by *in situ* reaction with monosubstituted hydrazines. This four-component, one-pot reaction proceeded in good yield with a wide range of commercially available reactants. A variety of aryl and heteroaryl halides as well as alkyl, aryl and heteroaryl amidines and hydrazines were well tolerated. The pharmaceutical relevance of this synthetic approach was demonstrated by the synthesis of deferasirox, a metal chelator that is approved for the treatment of iron-overload disease. This method provides a new, facile approach for accessing a broad range of analogs of this important scaffold. *JK*

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