BluB's clues

Although vitamin B_{12} was identified almost 100 years ago, the mechanism by which the pendant piece, 5,6-dimethylbenzamidazole (DMB), is constructed has remained a mystery. The source of this substructure had been identified as flavin, but the complexity of the necessary flavin-DMB transformation suggests that several biosynthetic enzymes are involved. The recent discovery of BluB as a protein involved in vitamin B_{12} synthesis provided a starting point for identification of these enzymes. Surprisingly, characterization of BluB by Taga *et al.* now demonstrates that this single protein makes and breaks four bonds to deliver the DMB core. Although NAD(P)H serves as a cofactor to reduce the flavin in related flavin oxidoreductases, biochemical assays and a crystal structure demonstrated that the reduced flavin is the direct substrate in BluB. Additional biochemical evidence indicates that molecular oxygen drives the conversion of flavin to DMB. The capture of a step along the reaction coordinate in a second crystal structure suggests the specific role of O_2 in cleavage of the ribityl side chain and identifies functionally significant residues. In particular, O_2 is found in a putative peroxyanion hole, where it is likely to stabilize a tetrahedral intermediate, a function reminiscent of monooxygenases. The specific details of this intriguing structural conversion remain to be seen, but the discovery of this hybrid enzyme is an important step toward explaining DMB's mysterious origins. (*Nature* **446**, 449–453, 2007) *CG*

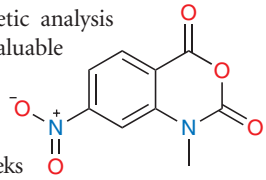
Divide to the rhythm

Thomas Dickmeis

Circadian oscillations of endogenous clocks regulate behavioral and physiological responses such as feeding and the timing of cell division in most plants and animals. The mammalian circadian system consists of a central "systemic" pacemaker in neurons of the suprachiasmatic nucleus of the hypothalamus, which synchronizes cellular circadian oscillators of the self-sustained and cell-autonomous peripheral clocks. Although evidence exists that both the systemic and cell-autonomous mechanisms control the cell division cycle, the neurochemical pathways used by the suprachiasmatic nucleus to transmit timing information to the peripheral clocks and ultimately to clock outputs are unknown. Dickmeis *et al.* used zebrafish mutants to begin to answer this question. By studying cell cycle progression in blind zebrafish mutants, they showed that ocular photoreception is not required to establish cell cycle rhythms during early larval development. *rx3*, one of the blind mutants, did have defects in cell division, but this was unrelated to the blindness and not caused by global deregulation of the circadian clock. The nighttime synthesis of the hormone melatonin was normal in the *rx3* mutants, but concentrations of corticotrope cell lineage markers and the corticotrope target cortisol were lower than in controls, which suggests that cortisol is a systemic signal required for circadian cell cycle rhythmicity. Indeed, cortisol could rescue the defective cell cycle rhythms of the *rx3* mutant. Both the mechanism for how cortisol affects cell cycle rhythmicity and the relative importance of the systemic and peripheral mechanisms are still unclear, but cortisol's role downstream of clocks in regulating output is now clearer. (*PLoS Biol.* **5**, e78, 2007) *MB*

Research Highlights written by Mirella Bucci, Catherine Goodman and Terry L. Sheppard

RNA structure rapidly takes SHAPE



The traditional approaches of phylogenetic analysis and biochemical probing have been invaluable for understanding RNA folding, but more precise tools for profiling RNA structure in the absence of three-dimensional structural information are required. Mortimer and Weeks now report an enhancement of their "SHAPE" RNA probing technology that allows for more rapid and accurate prediction of RNA secondary and tertiary structure. In their original SHAPE approach, the authors probed the structure of a folded RNA with the acylating agent NMIA, which reacted preferentially with more mobile 2′-hydroxyls found in unpaired or unstructured regions of RNA. In the current study, Mortimer and Weeks prepared 1-methyl-7-nitroisatoic anhydride (1M7), an NMIA derivative that is enhanced with an electron-withdrawing nitro group in its aromatic side chain. As expected by its stereoelectronic properties, 1M7 proved more reactive than NMIA to hydrolysis and acylation of RNA 2′-hydroxyls. The speed and precision of 1M7 SHAPE analysis was shown using an RNA element with a known structure—the specificity domain of bacterial RNase P. Because 1M7 reactivity is unperturbed by ionic strength, the reagent could also be applied to the magnesium-dependent folding of the RNase P element. The use of 1M7 SHAPE data, when applied as a constraint in modeling experiments, greatly enhanced the accuracy of RNA folding algorithms. The study reflects a useful application of physical organic chemistry principles to enhance our understanding of RNA structure. (*J. Am. Chem. Soc.*, published online 17 March 2007, doi:10.1021/ja0704028) *TLS*

Sugar manipulation

Structural genomics efforts intend to solve the crystal structures of every cloned protein. So far, the structures of only 2.5% of the expressed proteins have been solved, and the structures of glycoproteins are particularly scarce. This is a result of several complications. The levels of glycoprotein expression achievable in the eukaryotic systems required for proteins with post-translational modifications are low, as is the extent of solubility (and therefore the ability to form crystals) and the success of crystallization itself. N-linked glycoproteins present an additional challenge because N-glycans are typically found in fully folded regions of the polypeptide and cannot be easily removed after expression. Because these challenges have been insurmountable, it is desirable to develop strategies to obtain minimally glycosylated proteins. To do this, Chang *et al.* designed an expression system in which they manipulated the activity of glycosidases and glycosyltransferases to generate proteins that were minimally N-glycosylated but could still fold properly. The authors tested their inhibition strategy in the easily transfected human embryonic kidney cell line because these cells can allow for transient, high-level expression. One of the earliest stages of N-linked processing is carried out by α-glucosidase. Generally, inhibiting this enzyme is not effective because in most cell lines, a "shunt pathway" exists that allows for downstream processing. Kifunensine and swainsonine are alkaloid inhibitors of α-mannosidases I and II and work downstream of the shunt pathway. When cells were treated with kifunensine and swainsonine, the purified proteins (which were in high yield) could be treated with endoglycosidase H to reveal a minimally glycosylated structure. Because the glycoproteins are transiently expressed, this strategy eliminates the clone selection required for stable transfectants, thus making it desirable for high-throughput structural studies. (*Structure* **15**, 267–273, 2007) *MB*