Heme watches the clock

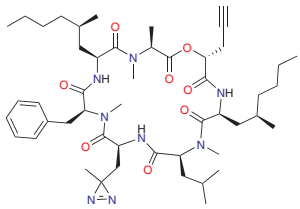
Although components of the mammalian circadian clock have been characterized, the physiological mechanisms of periodicity in organisms have been more difficult to elucidate. A new study shows that the REV-ERB class of nuclear hormone receptors may sense the concentration of heme, a cofactor important in energy metabolism, and couple it to changes in expression of genes associated with circadian rhythms. Previous studies had established that REV-ERBα and REV-ERBβ are related 'orphan' nuclear receptors that act as transcriptional repressors. The current study by Raghuram *et al.* demonstrates that heme is the physiological ligand of these proteins. The authors showed that the receptors copurify with heme and validated, using thermodynamic measurements, that heme binds to REV-ERB proteins in a 1:1 stoichiometry with micromolar affinity, which is mediated in part by a conserved histidine in the ligand binding domain. Heme binding is required for the repressor activity of REV-ERB and enhances REV-ERB thermal stability. In cells, inhibition of heme biosynthesis led to enhanced expression of genes, including *BMAL1*, a known REV-ERB target gene and component of the circadian oscillator, whereas elevated heme levels led to transcriptional repression. The authors further provide evidence that heme binding regulates the ability of REV-ERB proteins to associate with NCoR, a co-repressor required for downregulation of genes such as *BMAL1*. The study adds heme to the growing list of orphan receptor ligands, but it also raises the possibility that targeted regulation of REV-ERB may offer a strategy for circadian clock modulation. (*Nat. Struct. Mol. Biol.*, published online 25 November 2007, doi:10.1038/nsmb1344) *TLS*

Pulling apart chaperone contributions

Protein folding is assisted *in vivo* by a diverse group of chaperones that typically bind to hydrophobic peptide sequences to prevent aggregation of misfolded or unfolded proteins. Though many protein-chaperone partners have been elucidated, the specific mechanism of how and when chaperones function is less clear. In particular, maltose binding protein (MBP) is a substrate for the chaperone SecB, which not only protects MBP from aggregation but also promotes MBP's travel across the cell membrane. Now Bechtluft *et al.* have investigated the SecB-MBP interaction in more detail. The authors tethered MBP between two spheres and monitored the protein's behavior as the spheres were stretched apart and released using optical tweezers. The protein displayed the same behavior in subsequent unfolding curves, which indicates that MBP successfully refolded in between experiments. With the addition of SecB, however, the protein did not resume the native state, but instead maintained a molten globule-like state. Further insight into SecB's role came from the comparison of an unfolding intermediate observed in the force measurements with molecular dynamics simulations. The calculations showed that unfolding of six segments, including five C-terminal helices, leads to a stable core structure, which is in good agreement with the experimental unfolding curves. Surprisingly, SecB had no impact on this intermediate in unfolding experiments, leading to a model in which SecB interacts specifically to prevent formation of the 'core' structure, and also providing new testable hypotheses as to where and when SecB intervenes. (*Science* **318**, 1458–1461, 2007) *CG*

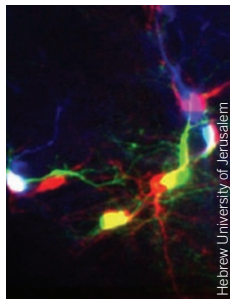
Written by Mirella Bucci, Catherine Goodman, Joanne Kotz & Terry L. Sheppard

Target hunting with photoleucine



Proteins destined for the secretory pathway are translocated into the endoplasmic reticulum (ER) through a channel formed by the Sec61 complex. HUN-7293 and related cyclodepsipeptides have previously been shown to block an early step in the translocation of selected proteins into the ER. However, the molecular target of these inhibitors is unknown. Photoaffinity labeling is an important approach for identifying small-molecule targets, but large photoreactive groups can perturb the biological activity of small molecules. The amino acid isosteres photoleucine and photomethionine had previously been inserted into proteins to probe protein-protein interactions, but they had not been incorporated into small molecules as an affinity label. To investigate the target of HUN-7293, MacKinnon *et al.* first optimized the synthesis of the photocrosslinker Boc-(*S*)-photoleucine from a low-yield six-step procedure to an efficient two-step synthesis via ozonolysis of a commercially available starting material followed by diazirine formation. The authors then synthesized a photoaffinity probe based on HUN-7293 in which a leucine was replaced with photoleucine. Incubation of the probe with crude ER fractions resulted in the specific labeling of Sec61α, a subunit of the Sec61 complex that is believed to form the translocation channel. Identification of the major target of the cyclodepsipeptides forms an important foundation for investigating the mechanism by which these compounds selectively inhibit translocation into the ER. (*J. Am. Chem. Soc.* **129**, 14560–14561, 2007) *JK*

Turning up the visual volume



The neurotransmitter acetylcholine (ACh) is involved in various higher brain functions, including learning and memory as well as reward and addiction. ACh also has a role in visual processing, but this is poorly understood. Neurons that use ACh—the so-called cholinergic neurons—are found in several regions of the brain including the neocortex. In the well-studied visual system of the macaque monkey, most visual information reaches the cortex via V1, the largest visual cortical area. To begin to understand ACh's role in vision, Disney *et al.* determined the subcellular distribution of both nicotinic ACh receptors (nAChRs) and muscarinic ACh receptors (mAChRs) in the various macaque V1 layers. nAChRs were found at thalamic excitatory synapses while mAChRs were not. This nAChR localization revealed that ACh modulates thalamic input to cortical excitatory neurons (thalamocortical synapses) via nAChRs in the 4c layer, the main thalamic recipient zone in V1. This is supported by the fact that nicotine, which acts on nAChRs, elevates thalamocortical transmission in layer 4c. This application of nicotine transiently increases the spiking response to a given stimulus contrast and represents a visual gain that allows the individual cortical neurons of 4c to detect stimuli that, without ACh's enhancement, are below the detection threshold for these neurons. This effect of nicotine closely mimics the effect of attention on early visual processing, which suggests that the nicotinic pathway is a key modulator of visual attention. (*Neuron* 56, 701–713, 2007) *MB*