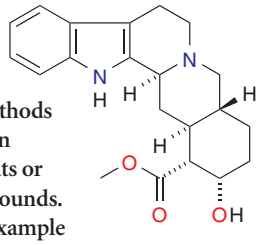
CoACTING HUNGER SIGNALS

The hypothalamus sits at the crossroads of nutritional signal input and feeding behavior output. This part of the brain can take signals from hormones such as leptin and translate them into signals to decrease food intake. Similarly, direct metabolic signals, such as the levels of freely circulating fatty acids, are interpreted by the hypothalamus to modulate feeding behavior. To test whether this input pathway plays a part in energy homeostasis in rats, He, Rossetti and colleagues induced the degradation of the fatty acid nutrient sensors malonyl-coenzyme A (malonyl-CoA) and long-chain fatty acyl-CoAs (LCFA-CoAs) in the mediobasal hypothalamus (MBH). This caused a 35% increase in glucose production in the rats. Also, increasing their lipid availability caused insulin resistance, the first step towards a diabetic phenotype. Thus, the levels of malonyl-CoA and LCFA-CoAs are critical for liver glucose homeostasis and for the hypothalamic sensing of circulating lipids. The malonyl-CoA- and LCFA-CoA-depleted rats also progressively gained weight and became obese, most likely owing to an increased appetite. Leptin, agouti-related protein (AgRP) and neuropeptide Y (NPY) levels increased during depletion of malonyl-CoA and LCFA-CoAs. However, under these conditions, the leptin did not act as an anorexic compound, as it normally does. Curiously, AgRP and NPY stimulate appetite, which may help to explain the hyperphagia the authors observed. Much work remains to be done to untangle the network of chemical signals that the hypothalamus receives and transmits, but it is clear that the malonyl-CoA and LCFA-CoA signals have a central role. (*Nat. Neurosci.* **9**, 227–233, 2006). *MB*

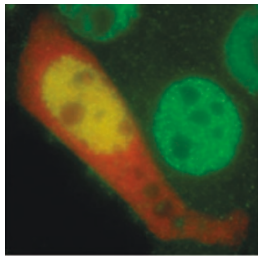
Tuning expression at the source



Control of protein expression allows insight into protein function as well as assignment of relationships within cellular pathways and disease states. Traditional methods for directing protein synthesis have relied on control at the genetic level, such as knockouts or the use of DNA-binding (polyamide) compounds. Manipulation of the mRNA sequence, for example with RNAi or antiswitches, offers some advantages over DNA-based techniques, including temporal control over the protein of interest. These methods, however, require the addition of an exogenous RNA strand. Tibodeau, Fox and coworkers now demonstrate that direct control of mRNA expression is possible. Their approach uses a known stem-loop structure (an iron-responsive element, or IRE) within ferritin mRNA as a binding site target for small-molecule screening. RNA footprinting experiments identified yohimbine as a ligand for the IRE; introduction of this molecule resulted in the upregulation of ferritin by >40% in cell-free extracts. The observed increase in expression can be explained in part by inhibition of a regulatory protein, but it also indicates that the ligand must enhance the translation of free mRNA. This report describes the first example of the selection of a small molecule based on a known RNA structure and is a key step forward in the consideration of RNA sequences as drug targets. One key undertaking of this general strategy will be to identify RNA structures that are stable and unique as suitable candidates for design. (*Proc. Natl. Acad. Sci. USA* **103**, 253–257, 2006) *CG*

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

An unexpected transfer



Mary G. Goll and Alison Getz

Genomic DNA methylation is essential for modulating eukaryotic gene expression. Methylation is mediated by DNA methyltransferase enzymes, which add methyl groups to cytosine bases in DNA using *S*-adenosylmethionine (AdoMet) as a cofactor. Recent studies have identified signature DNA binding and catalytic motifs within Dnmt1- and Dnmt3-family DNA methyltransferases. The related Dnmt2-family proteins are similar to these canonical enzymes, but they possess an altered DNA binding domain and, surprisingly, their absence in cells has no effect on genomic methylation levels. These observations were recently explained by Bestor and colleagues, who showed that human Dnmt2 (hDNMT2) is a transfer RNA (tRNA) methyltransferase rather than a DNA methyltransferase. The authors searched for a non-DNA substrate after they found hDNMT2 in the cytoplasm rather than in the nucleus. To determine the identity of the methyl acceptor, nucleic acid extracts were incubated with purified hDNMT2 and radiolabeled AdoMet. A small (~80-nucleotide) RNA component from Dnmt2-deficient cells was labeled by hDNMT2. The authors showed that 5-methylcytosine was produced, and that the cytosine substrate was situated in the anticodon loop of $tRNA^{Asp}$. Selective Dnmt2 methylation of cytosine 38 requires other modified nucleotides in the anticodon loop, which may explain why Dnmt2 is unable to methylate other cytosines in the same tRNA. In addition to clarifying the role of the Dnmt2 enzyme family, the work also raises the interesting possibility that RNA methyltransferases may represent an evolutionary precursor to eukaryotic enzymes that methylate genomic DNA. (*Science* **311**, 395–398, 2006) *TLS*

How tightly coupled?

Proton-coupled electron transfer (PCET), which involves the simultaneous transfer of an electron and a proton, is still not well understood. An example of putative PCET occurs in class I ribonucleotide reductase (RNR), which catalyzes the reduction of nucleoside diphosphates (NDPs) to deoxynucleoside diphosphates (dNDPs). During the catalytic cycle of RNR, a stable tyrosyl radical triggers nucleotide reduction by generating a transient thiyl radical, across a distance of more than 35 Å. An electron transfer pathway has been proposed that involves radical hopping across aromatic amino acids between the cysteine and the tyrosine residues. To investigate PCET in RNR, Stubbe, Nocera and colleagues synthesized six fluorotyrosines in which one to four of the aromatic protons were replaced with fluorines. These fluorine substitutions allowed the authors to tune the tyrosine acidity and reduction potential across 5 pK_a units and 320 mV, respectively. The authors used protein-ligation methods to specifically replace Tyr356, one of the residues suggested to be in the RNR electron transfer pathway, with the fluorotyrosine analogs. They found that the reduction potential of Tyr356 significantly influenced enzyme activity, supporting the idea that Tyr356 has a redox role. Although there is a hydrogen-bonding network along the proposed electron transfer path, the surprising observation that RNR activity is insensitive to Tyr356 protonation state suggests that a proton is not transferred along the reaction pathway, but is probably lost to solvent. These fluorotyrosines can now be used to further investigate PCET in this and other enzyme systems. (*J. Am. Chem. Soc.*, published online 11 January 2006, doi:10.1021/ja055927j and doi:10.1021/ja055926r) *JK*