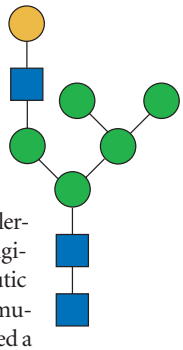
MIGRATE ThiS

Thiamine diphosphate (ThDP), the active metabolite of vitamin B1, is an enzymatic cofactor involved in carbon-transfer chemistry in the cell (see p. 324). Because of the unusual heterocyclic structure of ThDP, its biosynthesis has fascinated chemists for decades. In *Bacillus subtilis*, the carbon skeleton of the thiazole moiety of ThDP is derived from 1-deoxy-D-xylulose-5-phosphate (DXP). Sulfur is incorporated early in the pathway through the action of several enzymes, including ThiG and ThiS. ThiS is a carrier protein charged with a thiocarboxylate group (ThiS-COS$^-$), and ThiG binds DXP by formation of an imine at C2, which facilitates the attack of ThiS-COS$^-$ at C3. However, the regiochemistry of the chemical steps leading to stable sulfur incorporation has remained unclear. Begley and colleagues now resolve this question using a mechanistic probe of the sulfurization reaction. The authors synthesized 1,4-dideoxy-D-xylulose-5-phosphate (DDXP), a non-natural substrate for ThiG and ThiS, and showed that DDXP forms an imine and is converted to a ketone tautomer. This result demonstrates that DDXP serves as an alternative substrate for ThiG. However, DDXP, which lacks a critical hydroxyl group at C4, is not a substrate for ThiS-COS$^-$ and blocks stable incorporation of sulfur. The authors concluded that ThiS undergoes an S/O-acyl shift from C3 to the C4 hydroxyl group of DXP to complete the incorporation of sulfur in thiazole biosynthesis. (*Angew. Chem. Int. Ed.*, published online 20 April 2006, doi:10.1002/anie.20050461404) *TLS*

Plantibodies rerouted



The complex *N*-glycans found on mature proteins are a result of successive reactions with glycosyltransferases and glycosidases as the protein passes from the endoplasmic reticulum through the Golgi stacks. Plant *N*-glycans are unique in that they contain β-1,2-xylose and α-1,3-fucose residues linked to the trimannosyl chitobiose core. These atypical residues may be responsible for the allergenicity of certain plant proteins and of proteins engineered for production in plants, such as therapeutic antibodies. To generate proteins lacking these immunogenic sugars, Bakker, Bosch and colleagues created a transgenic tobacco plant that expresses human β-1,4-galactosyltransferase I bearing the localization signal for β-1,2-xylosyltransferase (xylGalT), thereby modifying the order of glycosylation events. The complex patterns of nearly 40 *N*-glycans attached to the tobacco-cell proteins included high-mannose oligosaccharides as well as galactosylated hybrid oligosaccharides that were not present in plants not expressing the rerouted xylGalT. Also, the levels of xylose and fucose were drastically diminished. These uniquely modified proteins could not bind to fucose- and xylose-specific immunoglobulin E from allergic patients, suggesting that the immune system would be unable to detect them. The authors went on to use the new xylGalT-expressing plants to exogenously express a monoclonal antibody. Like the endogenous glycoproteins, the exogenously expressed antibody had greatly reduced fucosylation and xylosylation. These results suggest a use for xylGalT in producing therapeutic antibodies that can escape detection by the patients' immune system. (*Proc. Natl. Acad. Sci. USA*, published online 4 May 2006, doi:10.1073/pnas.0600879103) *MB*

Research Highlights written by Mirella Bucci, Kaspar Mossman and Terry L. Sheppard

CLOCK wears a new HAT

Diverse organisms undergo periodic physiological and behavioral changes that are controlled by a circadian clock. A cell's innate timekeeping mechanism is maintained by cyclic production of transcriptional repressors and activators that regulate the expression of downstream circadian genes. The CLOCK transcription factor serves as a central control element in circadian oscillators. CLOCK forms a heterodimer with BMAL1 and activates transcription of circadian genes, such as *period* and *cryptochrome*. Periodic changes in chromatin remodeling by histone modification reactions have previously been associated with CLOCK function. However, Doi, Hirayama and Sossone-Corsi now establish a molecular link between chromatin remodeling and circadian regulation by demonstrating that CLOCK itself possesses histone acetyltransferase (HAT) activity. The authors showed that CLOCK catalyzes the acetylation of specific lysine residues in histones H3 and H4. The HAT activity of CLOCK is enhanced in the presence of BMAL1 and requires acetyl-CoA. CLOCK mutations that disrupt the proposed acetyl-CoA–binding site result in reduced HAT activity. Mutant CLOCK could not substitute for wild-type CLOCK to promote cyclic expression of downstream circadian genes in mouse embryonic fibroblast cells. The expression of active CLOCK results in a parallel increase in acetylation of H3 and H4 histones in these cells. Taken together, these results suggest that CLOCK may regulate circadian oscillations through its intrinsic HAT activity; they also raise the intriguing possibility that CLOCK–transcription factor interactions may provide a mechanism to integrate multiple inputs for control of circadian rhythms. (*Cell* **125**, 497–508, 2006) *TLS*

Virtual search finds a winner

The βγ subunits of G proteins (Gβγ) regulate numerous signaling events that are essential for cellular functions such as chemotaxis and nociception. Thus, selective blockage of multiple downstream targets remains a challenging problem. Chemical inhibition of signaling pathways can be difficult because protein-protein interactions, such as that between Gβγ and phospholipase C (PLC), are typically mediated by flat surfaces and are generally not well suited for small-molecule binding. Bonacci, Smrcka and colleagues have used virtual screening to identify small molecules that modulate G protein signaling and enhance the pain-killing effect of morphine in mice. A 'hotspot' had previously been identified on $G\beta_1\gamma_2$, which suggested that it would be possible to identify inhibitors that could block the ability of Gβγ to bind and activate downstream targets. The authors computationally docked 1,990 small molecules into this hotspot and screened the top 85 for their ability to bind to $G\beta_1\gamma_2$ *in vitro*. One compound, M119, inhibited $G\beta_1\gamma_2$ binding to PLCβ2 and PLCβ3; another compound, M201, which binds a different hotspot subsurface, enhanced the interaction between Gβγ and PLCβ3. These effects of M119 and M201 *in vitro* were reflected in their differential ability to modulate Gβγ activation of PLCβ2 in leukocytes. Because PLC-deficient mice are particularly sensitive to morphine, the researchers hypothesized that M119 might boost the opiate's potency in wild-type mice. Indeed, they observed an 11-fold increase in the analgesic effect in mice that were injected with both M119 and morphine. The study suggests that Gβγ may offer a versatile target for selective manipulation of downstream signaling pathways initiated by G protein–coupled receptor (GPCR) signaling. (*Science* 312, 443–446, 2006) ***KM***