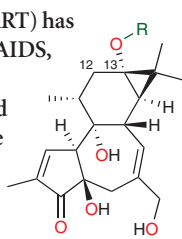


ACPs add up

Polyunsaturated fatty acids (PUFAs) are important in human nutrition, and understanding their biosynthetic pathways could lead to the controlled and sustainable availability of specific PUFAs. Unlike other biosynthetic systems that use one acyl carrier protein (ACP) per iterative cycle, the PUFA biosynthesis machinery contains multiple copies of these domains in tandem. Jiang *et al.* investigated the purpose of this apparent redundancy using the *Shewanella japonica pfa* system, which contains six tandem ACP domains. The authors first confirmed that the individual domains can be charged with a phosphopantetheinyl arm at the standard active site cysteine, either by a promiscuous PPTase *in vitro* or by the PPTase PfaE *in vivo*, which strongly supports the functional relevance of the ACPs. When studied as part of the full biosynthetic cluster, cysteine-to-alanine mutations of all six domains interrupted PUFA production, further demonstrating that ACP function is required for product formation. Finally, creation of 15 additional constructs containing varying numbers of cysteine mutations allowed the authors to conclude that the number of functional ACP domains, independent of position, determines the overall yield of the reaction. This study not only establishes the role of these intriguing tandem ACP domains but also raises new questions, including why ACP redundancy is needed in PUFA production, how each ACP domain interacts with the rest of the biosynthetic machinery, and whether tandem ACP domains can be exploited to improve PUFA yields. (*J. Am. Chem. Soc.*, published online 29 April 2008, doi:10.1021/ja801911t) CG

Prostratin in a snap

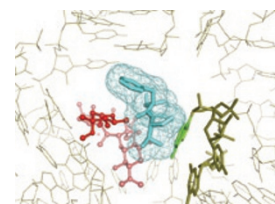
Though highly active antiretroviral therapy (HAART) has demonstrated effectiveness for treatment of HIV/AIDS, it fails to eradicate pools of latent HIV in many patients. The development of adjuvants that would make these viral reservoirs accessible may enhance therapeutic efficacy. The natural product prostratin is an adjuvant candidate due to its ability to activate latent HIV-1 pools and block viral infectivity. However, because prostratin is currently isolated from plant sources in limited quantities, new synthetic approaches are needed to support future mechanistic studies and therapeutic development. Wender *et al.* now report an efficient and scalable synthesis of prostratin and related analogs. The authors started from phorbol, a diterpene natural product that resembles prostratin but contains a C12 hydroxyl group. They surmised that removal of the C12 hydroxyl by standard methods would prove challenging and devised an alternative route to prostratin from phorbol in a 16% overall yield in five steps. Acid hydrolysis of phorbol eliminated the C12 hydroxyl group and produced a ketone by ring opening of the cyclopropane. The authors were able to restore the cyclopropane and C13 oxygen groups by a four-step sequence involving hydrazone formation, heterocyclization, pyrazoline oxidation and nitrogen photoextrusion. The approach was also successful for DPP, a prostratin analog, and was extended to a new class of C13 ether analogs. This synthetic strategy, starting from a readily available plant metabolite, provides a more rapid method to produce prostratin analogs with enhanced potential for application as HAART adjuvants. (*Science* 320, 649–652, 2008) TLS



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

Reasons to stall

Most nascent peptides transit through the ribosome tunnel smoothly, but some are stalled due to specific interactions with tunnel elements. Ribosome stalling is often related to regulation of gene expression, as is the case with the translation of the *ermC* methyltransferase cassette. Here, the macro-lide antibiotic erythromycin binds in the ribosome tunnel near the peptidyl transferase center (PTC), thereby stalling the ribosome and inducing a conformational change in the *ermCL* mRNA that activates the gene's expression. To understand this process in more detail, Vazquez-Laslop *et al.* established an *Escherichia coli* cell-free system that recapitulates erythromycin-induced stalling. To determine the position of the stalled ribosome on the mRNA, the authors used a "toe-printing" technique that maps the 3' boundaries of mRNA-associated complexes. They found erythromycin-dependent formation of a stable stalled complex on the leader mRNA and placed the Ile9 codon in the P site and the Ser10 codon at the A site of the ribosome. Selective amino acid labeling showed that Ile9 is important for stalling of the ribosome and is therefore the last amino acid incorporated into the nascent peptide before translation arrest; further experiments suggested that the stalled ribosome is unable to catalyze peptide bond formation and identified key roles for specific attributes of the nascent peptide, the ribosome and the antibiotic responsible for ribosome stalling. When compared to other ribosome stalling systems, these insights into the structure of the stalled complex and the mechanism of erythromycin-dependent stalling highlight the generality of ribosome stalling mechanisms in terms of critical interactions in the ribosome tunnel and intrinsic properties of the ribosome that promote stalling. (*Mol. Cell* 30, 190–202, 2008) MB



Chromatin remodeling in PHO₅

The cellular response to inorganic phosphate (Pi) in yeast is controlled by the transcription factor Pho4. At high Pi levels, Pho4 is phosphorylated at four sites and is inactive, whereas at low Pi levels Pho4 is unphosphorylated and fully active. At intermediate levels of Pi the situation is more complex: Pho4 is phosphorylated at one position and induces low levels of expression of some target genes and high levels of expression of others, raising the question of how gene transcription levels are controlled. The promoter region of one target gene, *PHO5*, contains a low-affinity Pho4 site in a nucleosome-free region and a high-affinity Pho4 site that is occluded by a nucleosome. To investigate the interplay between nucleosome positioning and gene expression, Lam *et al.* altered the accessibility of the two Pho4 sites by moving them into and out of nucleosomal regions and monitored the effects on transcription of a reporter gene. The authors found that the affinity of the accessible Pho4 site quantitatively determines the kinetics and level of gene expression at a given concentration of Pi. However, once nucleosome-occluded sites are exposed upon chromatin remodeling, the maximum level of gene expression was controlled by the number, affinity and placement of all Pho4 sites. Based on these results, the authors propose a model in which Pho4 binding is in equilibrium with dynamic chromatin remodeling such that transcription factor binding sites in nucleosome-free regions determine the threshold of gene activation and all dynamically accessible Pho4 binding sites determine the ultimate level of gene expression. (*Nature*, published online 16 April 2008, doi:10.1038/nature06867) JK