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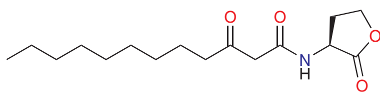
Making sense of RNA activation

Small RNAs are central players in numerous pathways that regulate gene expression. Recently, antigene RNAs (agRNAs), short duplex RNAs complementary to the DNA at gene promoters, have been shown to repress and in some cases activate expression of the targeted gene by an unknown mechanism. Now Schwartz *et al.* provide evidence that agRNAs modulate gene expression by binding to transcripts localized at the promoter site. In their study of agRNA-mediated gene activation of the progesterone receptor (PR), Schwartz *et al.* showed that antisense transcripts that overlap with the PR promoter domain are produced in multiple cell types. Targeted 'gapmer'-mediated knockdown demonstrated that a particular antisense transcript (AT2) was required for activation of PR expression by agRNAs. Affinity chromatography demonstrated that biotinylated PR agRNAs bind AT2 within cells, and RNA immunoprecipitation showed that this interaction attracts Argonaute protein. The resulting tripartite complex promotes localization of proteins that are typically associated with DNA, including hnRNP-k, HP1γ and RNA polymerase, to the RNA complex at the promoter site. Though many questions remain, the current study formulates the compelling model that agRNAs engage transcripts at promoter sites and induce protein remodeling that leads to activation or repression of gene expression. Given the large number of small RNAs in the genome, the study also raises the possibility that endogenous agRNAs may be discovered. (*Nat. Struct. Mol. Biol.*, published online 6 July 2008, doi:10.1038/nsmb1444) *TLS*

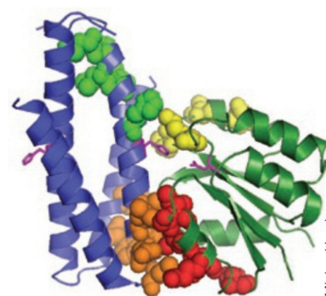
C12 κaps immunity



NFκB coordinates the immune system's response to bacterial infection. During infection, lipopolysaccharide (LPS) induces several macrophage responses, including degradation and resynthesis of inhibitor of NFκB (IκB), phosphorylation of RelA (subunit of NFκB), activation of the IκB kinase complex (IKK) and expression of NFκB-dependent pro-inflammatory genes. Subsequently, the immune system relies on tumor necrosis factor α (TNF) to sustain the NFκB-mediated response. Despite the multilayered response of the immune system, some pathogens establish persistent infections. Kravchenko *et al.* demonstrate that *N*-(3-oxo-dodecanoyl)homoserine lactone (C12), a small molecule produced by *Pseudomonas aeruginosa*, impairs the NFκB-mediated immune response. The authors found that macrophages exposed to *P. aeruginosa* exhibit altered temporal patterns of IκB degradation and resynthesis compared to those challenged with other pathogens. C12 was detected exclusively in the *P. aeruoginosa* cultures, and *P. aeruginosa* deficient for the production of C12 did not elicit the altered IκB response. C12 application also interfered with each of the aforementioned LPS-dependent macrophage responses in culture and interfered with NFκB-dependent gene expression in mouse models. Furthermore, C12 diminished the response of macrophages to other pathogens. Finally, the authors demonstrated that C12 inhibits TNF-dependent NFκB activation. Though the exact mechanism of action remains unknown, these observations provide an important link between a bacterial small molecule and host physiology. (*Science*, published online 19 June 2008, doi:10.1126/science.1156499) *AD*

Written by Amy Donner, Catherine Goodman, Joanne Kotz & Terry L. Sheppard

Signaling specificity swap


Michael Laub

Paralogous families of enzymes and domains, such as MAP kinases and SH2 domains, regulate many critical signaling pathways. Although the details of some individual protein-protein interactions are known, the molecular determinants that maintain the specificity of endogenous partners and prevent promiscuous pathway crosstalk are not always understood. To investigate signaling specificity, Skerker *et al.* turned to the pathways of bacteria. In these two-component signaling systems, an extracellular signal triggers autophosphorylation of a histidine kinase (HK), which then catalyzes the transfer of the phosphate group to a cognate response regulator (RR) that controls the downstream physiological response. By computationally searching for residues that covary between endogenous HK-RR pairs, the authors identified two regions of the HK that seemed likely to be important in determining specificity. Most of the covarying residues occurred in the histidine phosphotransfer domain, and swapping this domain was enough to completely switch HK specificity. Further, transferring a short segment of this domain that contained a cluster of seven of the residues with the highest covariance was nearly sufficient to completely swap HK-RR pairs. In fact, mutating the seven residues identified in the computational analysis plus a small adjacent loop reengineered HK substrate specificity, and introduction of these chimeras into *Escherichia coli* rewired the *in vivo* signaling pathways. The ability to rationally control bacterial two-component signaling may now make it possible to investigate the evolutionary forces that shape pathway specificity. (*Cell* **133**, 1043–1054, 2008) *JK*

Manning all sites

Golgi α-mannosidase II (GMII) is a key enzyme in N-linked glycosylation, removing two mannoses from an *N*-acetylglucosamine (Gn)/mannose (Man) octasaccharide to generate $GnMan_3Gn_2$, which is then transferred to acceptor proteins. Though it was known that the enzyme cleaved both mannoses in the same active site, the specific details of this reaction sequence were unclear. Now, a cocrystal structure of the substrate and a catalytically inactive enzyme mutant by Shah *et al.* has identified three distinct binding sites on the protein, which provide important clues about this process. One sugar, an α-1,6-linked mannose (or M5), resides in the catalytic site, well positioned for the first cleavage to occur. M4, an α-1,3-linked mannose, is situated in a 'holding' site that is tightly packed and unable to accommodate the longer M5 chain; this arrangement forces M5 into the catalytic site ahead of M4, thereby defining the order of mannose processing in the enzyme. Finally, G3, a required Gn, is found in an 'anchor' site, where it forms a hydrogen bond with His273. Interestingly, though a second mannosidase structure with a substrate lacking *N*-acetylglucosamine residues demonstrates that G3 is not absolutely necessary for successful binding of M4 and M5, previous biochemical data indicate that G3 is required for efficient mannose cleavage, which suggests that allosteric changes in conformation or proton state mediated by His273 may play an important role in facilitating catalysis. This multivalent binding provides new insights into glycan processing by these tailoring enzymes. (*Proc. Natl. Acad. Sci. USA*, published online 30 June 2008, doi:10.1073/pnas.0802206105) *CG*