COMMENTARY

Linking glucose metabolism to the stringent response through the PTS

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pppGpp and ppGpp (here abbreviated as ppGpp) are signaling molecules synthesized throughout the bacterial domain of life, serving as second messengers that respond to nutritional deprivation, a phenomenon called the stringent response. ppGpp accumulation causes the coordinated inhibition of macromolecule synthesis resulting in growth arrest, as well as the activation of a number of stress responses to alleviate problems resulting from the nutritional deprivation. In *Escherichia coli*, there are two enzymes responsible for synthesizing ppGpp: RelA and SpoT. However, SpoT's primary activity is ppGpp hydrolysis to prevent uncontrolled ppGpp production, which is lethal. In PNAS, Lee et al. (1) report a link between SpoT activity and the function of the phosphoenolpyruvate-dependent sugar transferase system (PTS), thereby connecting sugar metabolism with the stringent response.

Rsd Has Another Activity

Surprisingly, the connection between these two major cellular pathways is conferred by regulator of SigmaD (Rsd), a well-characterized antisigma factor. Rsd binds to the cell's major sigma factor, σ^{70}, reducing its interaction with core RNA polymerase (RNAP) (2–5). Thus, Rsd facilitates transcription from promoters recognized by alternative RNAP holoenzymes instead of promoters recognized by the major RNAP holoenzyme, Eσ^{70}. However, Lee et al. (1) show that Rsd's regulatory role in ppGpp metabolism is unlinked to its role as an antisigma.

In most proteobacterial species like *E. coli*, ppGpp binds directly to two sites on RNAP, increasing or decreasing transcription initiation depending on the kinetic properties of the individual promoter (6, 7). ppGpp regulates the expression of hundreds of genes, reprogramming a major fraction of the cell's transcriptome. Although much is known about how ppGpp binds to RNAP and changes its conformation to increase or decrease the activities of specific promoters, much less is understood about how RelA and SpoT sense nutritional signals and set the level of ppGpp in response to certain nutrient conditions and not others.

RelA and SpoT belong to the widely conserved RelSpo homolog (RSH) protein family. Proteins in this family have two domains. The N-terminal domain contains the enzymatic activities (ppGpp hydrolase and/or synthetase), and the C-terminal domain autoregulates these activities. Recent cryoelectron microscopy structures of RelA bound to the ribosome demonstrate how RelA responds to the absence of amino acid(s) (8–10). The C-terminal domain of RelA interacts with the 3′-end of an uncharged tRNA in the ribosomal A site, freeing the N-terminal domain of RelA to synthesize ppGpp, whereas charging the 3′-end of the tRNA with an amino acid interferes with RelA binding and prevents activation of ppGpp synthesis.

As with RelA and ppGpp synthesis, the C-terminal domain of SpoT autoregulates its ppGpp hydrolase activity, maintaining it at a basal level (11, 12). However, how does SpoT regulate ppGpp levels in response to a variety of nutritional cues other than amino acids (which are sensed by RelA)? This is still mysterious. Lee et al. (1) find that binding of the Rsd protein to the C-terminal domain of SpoT activates SpoT hydrolase activity, both in vivo and in vitro. By increasing SpoT hydrolase activity, Rsd thereby decreases ppGpp levels.

Rsd–HPr Interactions Regulate ppGpp Levels

The Seok laboratory showed previously that HPr, a conserved component of the PTS whose phosphorylation status depends on the availability of PTS sugars like glucose, antagonizes the antisigma activity of Rsd (13). Wild-type Rsd or a mutant Rsd without its antisigma activity bind similarly to SpoT. Rsd binding to Hpr prevents Rsd from binding to SpoT. Thus, the competition between SpoT and HPr for binding to Rsd determines the extent to which SpoT hydrolase activity is stimulated, controlling ppGpp levels in response to the nature of the carbon source.

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Rsd can thus bind to three different proteins: SpoT, dephosphorylated HPr, and σ^{70}. When glucose is present, then HPr is mostly dephosphorylated and it sequesters Rsd. In this condition, there is no need to stimulate SpoT hydrolase activity since ppGpp does not accumulate. Presumably, in this condition, Rsd is also unable to bind to σ^{70} and prevent it from inhibiting housekeeping promoter activity. However, when ppGpp accumulates, such as during a diauxic shift, then activation of SpoT ppGpp hydrolase activity is required, in order for the cell to resume growth in the new carbon source. In this situation, Lee et al. (1) demonstrate that an *rsd* mutant has a clear and convincing phenotype.

Using truncated proteins, Lee et al. show that Rsd binds the TGS subdomain within the C-terminal domain of SpoT. Because RelA and SpoT evolved from a common ancestor, and because the TGS domain is highly conserved, one might expect that Rsd would also bind to the RelA TGS, regulating RelA activity. However, this was not the case (1).

Using very similar approaches, it was shown previously that SpoT also binds to acyl carrier protein (ACP), the central cofactor of lipid synthesis (12). As with the Rsd–SpoT interaction, the presence of the TGS domain is necessary for ACP binding, and ACP does not bind to the RelA TGS. Because ACP carries precursors and intermediates in the fatty acid biosynthesis pathways, it was proposed that the ACP–SpoT interaction might modulate SpoT activity depending on the status of cellular fatty acid metabolism. Accumulation of ppGpp also has a direct role in the control of promoters of fatty acid synthesis genes, reducing their expression if fatty acids are ample (14). Thus, there seems to be a striking parallel between the mechanism of regulation of SpoT by carbon source through Rsd and the regulation of SpoT by fatty acids through ACP, although the molecular details of these mechanisms remain unclear.

Other connections between the stringent response and PTS systems have been found in proteobacteria. The SpoT enzymes from *Ralstonia eutropha* and *Caulobacter crescentus* bind to enzyme IIA^{Ntr} of the nitrogen-related PEP-dependent phosphotransferase system (15, 16). Although Lee et al. and others (1, 15) have shown that this particular interaction is not present in *E. coli*, clearly evolution has selected for similar metabolic connections to control ppGpp levels in bacteria, even though the molecular mechanisms differ.

ObgE, a GTPase that inhibits 50S ribosomal subunit assembly, has also been proposed to interact with and control SpoT activity in *Vibrio cholerae* (17). However, whether the ObgE–SpoT interaction affects ppGpp levels or not is unclear (18). Interestingly, ppGpp has also been reported to regulate ribosome assembly by binding to ObgE directly in *E. coli* (19) and to regulate expression of the operon encoding ObgE (20).

In summary, although the interactions of different proteins with SpoT may have different effects on ppGpp production, the emerging picture is that SpoT serves as a hub for different metabolic signals to impact ppGpp levels (21, 22). Stay tuned for more surprises about how diverse partners interact with RSH proteins and control their enzymatic activities.

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