Regulatory Features Deduced from the Structure of the Catalytic Domain of a Streptococcal Enzyme that Synthesizes and Degrades (p)ppGpp

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Work in many laboratories over three decades has led to understanding that eubacterial (p)ppGpp nucleotides can act as generalized signals of nutritional stress. These nucleotides differ from GDP and GTP only by the presence of a pyrophosphate on the ribose 3' hydroxyl and have been called alarmones.

It is now known that starvation for either amino acids, phosphate, nitrogen or energy will quickly elevate (p)ppGpp levels. Restoring nutrient sufficiency restores low basal levels. Intracellular levels of (p)ppGpp can now be manipulated at will. Exploiting these tools allows regulatory roles are assigned to (p)ppGpp. Complete elimination abolishes regulation during starvation. Artificially elevating (p)ppGpp mimics many regulatory effects usually accompanying starvation. Responses to (p)ppGpp are now thought to be superimposed on effects of more specific repressors and inducers. These responses include limiting expression of genes coding for protein synthesis components, inducing expression of adaptive genes to insure survival (as by inducing RpoS) and participation in other stress response networks. An emerging literature links (p)ppGpp with a variety of phenomena in microbiology. These include pathogenicity, microfilm formation, production of toxins and antibiotics, quorum sensing and persistence of the carrier state in *Mycobacteria tuberculosis*.

Effects of (p)ppGpp at the level of gene expression predominately operate at the level of transcription initiation consistent with (p)ppGpp binding sites found on RNA polymerase. The complete absence of (p)ppGpp results in several phenotypes that allow selection of suppressor mutants, including multiple amino acid requirements and killing during prolonged stationary phase exposure. These suppressors map exclusively in three RNA polymerase subunits: *rpoB, rpoC* and *rpoD*. These missense suppressors are located on surfaces involved in DNA contact and several decrease open complex stability. Although a consensus has now been reached that (p)ppGpp mediates growth rate control of stable RNA accumulation, different mechanisms for (p)ppGpp effects on transcription have been proposed and are currently hotly contested.

In any case, the balance of rates of (p)ppGpp synthesis and degradation determines signal strength. For example, activation of (p)ppGpp synthesis can be triggered when the supply of any aminoacyl tRNA fails to keep up with the demands of protein synthesis leading to preferential binding of uncharged tRNA to empty ribosomal acceptor (A) sites with "hungry" mRNA codons. This activates the (p)ppGpp synthesis by RelA, an *E. coli* enzyme bound to ribosomes. Degradation of (p)ppGpp is

controlled by sensing starvation for nutrients other than amino acids. The degrading enzyme in *E. coli* is a (p)ppGpp-specific 3'-pyrophosphoryl hydrolase called SpoT in most enteric bacteria. Whereas enteric bacteria contain separate genes encoding homologous proteins specialized for either synthesis (RelA) or degradation (SpoT), most other bacteria contain a single bifunctional Rel Spo homolog protein (Rsh).

The Rsh protein from Streptococcus has its two catalytic domains located within the N-terminal half of the protein (NTD). It was found these could exist in two reciprocal activity states: hydrolase off - synthetase on versus hydrolase on - synthetase off. Switching states depends in part on the C/terminal half-protein (CTD). These effects are quantitatively consistent with findings of others that the full length Rsh protein from *M. tuberculosis* switches states on even *E. coli* ribosomes when uncharged tRNA binds. Genetic mapping of hydrolase and synthetase mutants revealed nonoverlapping domains but unusual missense mutants of one activity can be reactivated by reassembly with the missing domain. These allele-specific suppressors suggest that certain residues within one domain can regulate the activity of a neighboring domain. We conclude that reciprocal regulation of opposing catalytic activities is an intrinsic "hard wired" feature of these proteins that can be changed by NTD-CTD interactions or by signals operating on the CTD perhaps through ribosome interactions. Although genetic studies established functional domains, little was known of structures.

Dr. Tanis Hogg in the laboratory of Dr. Rolf Hilgenfeld (Jena, Germany) have now determined the structure of the NTD region at a resolution of 2.1 Angstroms. The hydrolase is similar to cyclic phosphodiesterases with a characteristic metal-dependent phosphohydrolase fold. The synthetase catalytic site is 30 Angstroms away from the hydrolase site and consists of a 5-stranded mixed beta sheet sandwiched by 5 alpha helices palm domain. The structure is similar to the nucleotidyl transferases superfamily with human DNA polymerase beta, polyA polymerases and terminal deoxynucleotidyl transferases. This structural relatedness is not evident from the linear amino acid sequence. The structures actually analyzed consist of a dimer, with each monomer in a different conformation and each deduced to represent different reciprocal activity states. Coordination of opposing activities seems to occur without covalent modification.

These structures provide insights into a portable regulatory system for mediating transitions between states of rapid growth and the near stasis of stress. Similar genes have been found widely distributed in the plant kingdom, presumably by lateral transmission.