Scratching the surface of Metabolic complexity through the glimpse of CodY

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Central to life are the metabolic processes from which all energy is derived. Given the unique and diverse array of habitats in which organisms live, it follows metabolism too is diverse. Therefore, although glucose is usually considered the preferred carbon source, in times or environments where it is not abundant, alternative energy sources may be utilized. Because the citric acid cycle has major biosynthetic and energetic function, its components are found ubiquitously. Microorganisms employ a variety of organic acids as carbon sources and electron donors. Complex proteins and aromatic compounds are digested and then converted to products such as succinate, acetyl-CoA and pyruvate. Fatty acids undergo beta oxidation which too releases acetyl-CoA and then is able to be translated into energy. Ironically, through the citric acid cycle, acetyl-CoA may be used for fatty acid synthesis. Key intermediates of the citric acid cycle namely α-ketoglutarate and oxalacetate are precursors of amino acids or possible to be converted into a glucose precursor. Given such drastic and opposing outcomes possible it is not surprising this cycle is highly regulated. Overexpression of a particular product is a costly use of cellular resources including ATP and may impose a metabolic drain [1].

The enormous complexity involved in regulating these processes occurs with concerted action between regulatory genes, proteins and sensory processes. Signaling is non-linear, genes' regulatory proteins have many protein targets, may compete with each other or even act upon their own genetic elements. In gram-positive bacteria with low G + C content, CodY is one of the global regulators ensuring cellular metabolism remains energetically favorable. Many of CodY functions remain unknown and vary from organism to organism but direct or indirect effects have been numbered in the hundreds. Activity varies in response to metabolites like GTP and the branched-chain amino acids (BCAAs) isoleucine, leucine and valine (IVL) [2]. More specifically *in vitro* CodY is more active in nutrient rich media, behaves poorly when lacking BCAAs in opposition to the observed higher binding affinity in the presence of both GTP and BCAAs [3]. It seems CodY senses intracellular status through metabolite binding via a two-component system. Once binding ILV or GTP CodY is activated as a DNA-binding protein commonly in an overlapping arrangement and binds to the palindromic 15nt AATTTTCWGAAAATT [4]. The resulting cascade most frequently represses genetic elements but may activate transcription of nitrogen related genes to trigger various metabolic effects [1,4]. Given higher repressive and derepressive activity, it is

unsurprising IDAP-Seq identified an overwhelming 81% of CodY binding within gene's coding sequences rather than upstream of promoter regions. This patterned observation in *Bacillus subtilis* is suggested to repress gene activation in a "road block" manner where the binding of CodY is likely to result in early termination of the transcript. However, in *Listeria monocytogenes* CodY binds upstream of the *mfd* coding sequence possibly blocking continued transcription from the upstream gene. What is interesting is that despite the differently proposed methods of action CodY is highly similar in both B. subtilis and L. monocytogenes. It is likely the similar effector response is related to the completely identical helix turn helix motif and 79.5% sequencing identity [3]. It must be noted however activity *in vivo* may vary significantly and although dissimilar in sequence and structure the gram-negative leucine-responsive protein provides many of the same functional roles [5].

Functional role similarity between species is a direct result of the biosynthetic and bioenergetic capacity of the citric acid cycle. ILV are some of the most abundant amino acids found in proteins thus essential to maintaining appropriate structural balance. As such global regulation of these processes is regulated as much by availability of a particular substance as by the accumulation of the synthesis end product [5]. For example, leucine and valine increase the CodY binding affinity to its DNA-binding sites but high levels of accumulated BCAAs have negative feedback on expression. Metabolic shifts can identify cellular status. In regards to CodY high GTP and BCAAs levels suggests late exponential or stationary growth phases because it is inactivated with limited nutrient availability [1, 2]. In deed most of the CodY regulon is repressed in times of rapid growth. Derepression thus is regulated by both metabolite quantity and the presence of low and high affinity cis acting elements [2].

The need to acquire nutrients especially in times of cellular stress may trigger a variety of responses. Actions perceived as virulence factors may be merely an attempt for survival [5]. CodY activity levels such as repression during rapid growth suppresses sporulation. Its activity balances the production of degrative enzymes [2]. In *Staphylococcus aureus* the sae two-component system is responsible for *arg* controlling quorum sensing and biofilm formation. However, the sae system is also under the control of global regulator CodY [4]. In non-emetic *Bacillus cerus* CodY not only controls biofilm production but also the production of enterotoxin. Cody is essential for activating full virulence of *Bacillus anthracis* [2]. Truly, metabolism is at the heart of virulent activity.

CodY is only one global regulator at the metabolic intersection of cellular control and sustained life. The CodY negatively controlled operon products are additionally involved in nitrogen uptake and utilization processes. Synergistic advantageous activity occurs when examined in combination to catabolite control protein A (CcpA), also a global metabolism regulator. CodY binds with varying affinity frequently in competition with CcpA [1]. When decreasing CodY and increasing CcpA proteins levels there is reduced carbon metabolism and increase reactions within nitrogen metabolism. It has been shown while CodY-regulated genes may act with sensitivity CcpA regulon genes remain more stable during the changes in metabolite levels [1]. Ultimately, central metabolic need augments control over all necessary cellular action. The basal need to eat, grow and reproduce has dictated the evolution of all processes from the production of toxins to sporulation and systematic control of the resources within the cell. The representations here of CodY are intended not to be exhaustive but a simplistic look at an extensive system science has only begun to understand.

References

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