Linking nutritional status to gene activation and development

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One of the most daunting challenges in biology is elucidating the mechanisms by which cells sense and respond to changes in their food supply. The reason for the difficulty in addressing this problem even in primitive cells such as bacteria is that nutrient limitation can perturb the complex web of metabolic interactions that govern the physiological state of the cell. Thus, when a cell is deprived of nutrients, how are we to pinpoint the precise cue among the myriad alterations in metabolic intermediates that is responsible for the ensuing adaptive response? And how are we to tie this cue to the molecular mechanisms that execute the resulting changes in gene expression? In some cases, such as the response to growth-limiting levels of an amino acid or a particular carbon source, the specific nutrient is the signal to which the cell responds, and the mechanisms by which the cell perceives this signal and adapts to it are well understood. In other cases, however, in which the general nutritional status of the cell has been perturbed, the challenge of linking nutrient availability to alterations in gene expression has met with success in only a few instances. Here, after a brief review of a few classic examples of nutrient-sensing mechanisms in bacteria, we focus on a wonderfully simple solution, as reported in this issue by Ratnayake-Lecamwasam et al. [2001], to the long-standing problem of how a bacterium responds to nutritional signals that trigger the elaborate adaptive response of spore formation and entry into stationary phase.

A classic example of a transcriptional response to a change in the level of a specific nutrient is the trp operon of Escherichia coli. The operon is regulated in part by a repressor whose capacity to bind to the trp operator is determined by direct interaction with tryptophan [Rose et al. 1973]. The trp operon also responds to tryptophan levels through a mechanism called attenuation that acts at the level of transcription termination (Oxender et al. 1973). The 5′ region of the trp mRNA contains a short open reading frame for a tryptophan-containing leader peptide. When tryptophan, and hence charged tRNA\textsuperscript{trp}, levels are high, the ribosome translates the leader peptide-coding sequence and allows the formation of a hairpin that terminates transcription. In contrast, when tryptophan levels are low, the ribosome stalls in the short open reading frame, thereby preventing the formation of the transcription termination hairpin. In this example, the response to the levels of a specific nutrient in the medium is determined by the intracellular concentration of the amino acid, which is monitored by the Trp repressor, and the intracellular concentration of charged tRNA\textsuperscript{trp}, which is sensed by the ribosome as it translates a Trp codon-containing leader sequence.

As a final example of a transcriptional response to a change in the level of a nutrient is the phenomenon of “catabolite repression” in E. coli. In the presence of a readily metabolized carbon source such as glucose, transcription of genes encoding proteins responsible for the catabolism of other carbon sources is inhibited. It is caused in part by a drop in the intracellular concentration of cAMP, the response to which is mediated by the transcription factor cAMP receptor protein [Busby and Kolb 1996]. The level of glucose in the medium is linked to the level of cAMP in the cell by the phosphotransferase system, a sugar transport system in which the uptake from the medium is driven by phosphorylation of the sugar [Safer 1998]. In the case of glucose, a phosphoryl group is transferred to the sugar from the phosphorylated form of the phosphotransferase protein IIA\textsuperscript{phc}. This generates unphosphorylated IIA\textsuperscript{ph}, which, among other effects, acts allosterically to inhibit the cAMP-generating enzyme, adenylate cyclase. Thus, in this instance a nutrient-specific transport system senses the presence or absence of a sugar, transducing the signal into a second messenger that interacts directly with a transcriptional regulatory protein.

As a final example of a transcriptional response to a specific nutrient, we consider the case of ammonium, the favored source of nitrogen for enteric bacteria such as E. coli and Salmonella, which convert ammonium to glutamine via the enzyme glutamine synthetase. These bacteria sense low ammonium levels by monitoring the pool of intracellular glutamine [Ikeda et al. 1996] and respond in part by increasing the transcription of the gene for glutamine synthetase [Magasanik 1999]. The molecule that senses glutamine directly is uridylyl transferase/uridyl removing enzyme (UTase/UR) [Ninfa and
Atkinson 2000). At low glutamine levels, UTase/UR uridylates a protein called PII, thereby generating PII-UMP, which is inactive. Conversely, when glutamine levels are high, UTase/UR deuridylates PII-UMP, generating the active form of the protein. PII, in turn, sets in motion a cascade of events that culminates in determining the phosphorylation state of the transcription factor NtrC (or NR), which directs transcription of the gene for glutamine synthetase. Thus, the level of ammonium is sensed by monitoring the intracellular concentration of an amino acid that is derived from it.

In the examples considered so far, the cell senses a specific nutrient directly (e.g., an amino acid or a sugar) or a molecule derived from the nutrient (e.g., a charged tRNA or a nitrogen-rich amino acid) (Table 1). But how do cells sense a general alteration in their nutritional status? One such classic example is growth rate-dependent control in which the rate of ribosome biosynthesis is dependent on the doubling time of the cells, increasing roughly with the square of the growth rate (Bremer and Dennis 1999). In growth rate-dependent control, ribosomal protein synthesis is regulated to match the level of ribosomal RNA (rRNA) synthesis. Thus, the critical regulatory step in ribosome synthesis is the rate of transcription of rRNA genes. It turns out that despite their high strength (accounting for perhaps 50% of total transcription at high growth rates), the promoters for rRNA genes form unusually unstable open complexes that are particularly sensitive to the concentration of the initiating nucleotide, either ATP or GTP (Gaal et al. 1997). Thus, the rate of rRNA synthesis is determined by the cellular concentration of ATP or GTP (depending on the particular RNA promoter), which is in turn a reflection of the general nutritional state of the cell. Growth rate-dependent control is therefore a model of simplicity: Regulation is achieved without the need for any dedicated regulatory protein!

No prokaryote shows more elaborate responses to conditions of nutrient limitation than Bacillus subtilis. This gram-positive bacterium displays a wide range of adaptations to nutrient limitation, including the secretion of enzymes capable of degrading complex carbon sources, the production and secretion of antibiotics to ward off competing bacteria, the import and utilization of secondary metabolites, entry into the state of genetic competence, and the elaboration of systems for motility and chemotaxis. As a more extreme response to nutrient limitation, the bacterium undergoes a profound physiological and morphological transformation that culminates in the formation of a dormant cell type, the endospore.

What is the specific nature of the physiological signal or signals that triggers these adaptive responses, and how precisely does the cell respond to this signal(s)? An important clue came from the work of the late microbiologist Ernst Freese and his colleagues almost a quarter of a century ago. Careful physiological experiments led them to conclude that the expression of genes involved in adaptation to nutrient limitation and sporulation was correlated closely with, and indeed probably caused by, a transient but significant (70%–80%) decrease in the cellular pools of GDP or GTP (and not other purine or pyrimidine nucleotides) (Lopez et al. 1979). They found that leaky purine auxotrophs, in particular leaky guanine auxotrophs, were able to sporulate in the presence of excess ammonia, glucose, and phosphate when the purine required for the normal growth of such mutants was removed from the medium (Freese et al. 1979). They also showed that sporulation could be induced under conditions of nutrient excess by treating cells with the drug decoyinine, an inhibitor of GMP synthetase (Mitani et al. 1977).

Finally, Freese and colleagues investigated the possibility that the inducing signal was pppGpp or ppGpp, which are synthesized by the RelA protein from GTP and GDP under conditions of amino acid starvation by an idling reaction of the ribosome. These highly phosphorylated guanine nucleotides (also known as “magic spots”) are physiological signals probably acting directly on RNA polymerase to elicit the so-called stringent response in which rRNA synthesis is reduced under conditions of amino acid deprivation (Cashel et al. 1999). It was known that a mutation in the gene for RelA (relA) inhibits entry into sporulation. In an elegant analysis, Freese and colleagues showed that the inhibition was indirect by showing that treatment of relA mutant cells with decoyinine restored their capacity to sporulate (Ochi et al. 1981). Rather than pppGpp and ppGpp themselves being a signal for sporulation, their synthesis drains the pools of GTP and GDP. Thus, by preventing pppGpp and ppGpp synthesis, the relA mutation causes GDP and GTP levels to remain high under conditions of amino acid starvation.

If a decrease in GDP or GTP levels is the physiological signal for stationary phase and sporulation gene expression, then how are guanine nucleotide levels monitored and how is this signal transduced to activate the genes involved in adaptation to nutrient limitation? A possible clue, at least for the sporulation response, was the discovery of an elaborate phosphorelay that governs entry into the developmental pathway for spore formation. This relay, which consists of multiple kinases, phosphatases, and phosphotransferases, determines the phosphorylation state of the transcription factor Spo0A, the master regulator for entry into sporulation (Burbulys et al. 1991; Perego et al. 1994). Elegant experiments by J.

### Table 1. Nutrient-sensing mechanisms

| Specific nutrient limitation | Signal | Sensor |
|-----------------------------|--------|--------|
| Tryptophan                  | tryptophan, TrpR | Trp-tRNA<sup>tr</sup> ribosome |
| Carbon source               | glucose | IIA<sup>lec</sup> |
| Nitrogen                    | glutamine | UTase/UR |
| General nutrient limitation | GTP, ATP | RNA polymerase |
| Amino acid starvation       | pppGpp, RNA polymerase | CoDY |
| Stationary phase            | GTP | CoDY |
Hoch, M. Perego, and A. Grossman have shown that the phosphorelay integrates various physiological and environmental signals into the decision to sporulate (Burkholder and Grossman 2000). Many in the field had fully anticipated that a link between Freese’s guanine nucleotides and the phosphorelay would eventually emerge, bridging the gap between nutrient limitation and entry into sporulation. Frustratingly, no such link has been discovered.

Enter A.L. Sonenshein and his colleagues, who have been painstakingly investigating the mechanisms that control gene expression induced by nutrient limitation as cells enter stationary phase. This work has led to the discovery of the repressor CodY, which mediates the inhibitory effects of glucose and amino acids on stationary phase gene expression (Slack et al. 1995). The list of genes under the negative control of CodY includes numerous genes that are normally switched on during stationary phase, as well as certain genes, including evidently the gene for Spo0A, that are needed for entry into sporulation. Ratnayake-Lecamwasam et al. (2001) now report that CodY, which is conserved broadly among low G + C species of gram-positive bacteria, is a GTP-sensing transcriptional regulator. Noticing that the amino acid sequence of CodY displays a predicted guanine nucleotide-binding pocket, they show that CodY binds GTP. GTP does not seem to have an important effect on specific binding of CodY to DNA, but the capacity of CodY to block transcription is strongly dependent on the guanine nucleotide. Importantly, this repression requires physiological (millimolar) concentrations of GTP, corresponding to the levels of GTP observed in rapidly growing cells. Thus, CodY fulfills all the requirements to act as a global regulator of postexponential phase gene expression by sensing cellular levels of GTP directly (Fig. 1).

One strong prediction of the hypothesis is that a codY mutant should be able to sporulate even in the presence of excess nutrients. Indeed, Ratnayake-Lecamwasam et al. (2001) find that a codY mutant sporulates at an efficiency of ~90% under nutritional conditions that prevent sporulation, as compared with an efficiency of <0.2% for the wild type, matching or exceeding the sporulation efficiency observed following treatment of wild-type bacteria with decoyinine. In light of these new findings, the picture that emerges is that entry into sporulation is governed by two pathways: the CodY pathway, which monitors the nutritional status of the cell, and the phosphorelay, which monitors nonnutritional signals, such as cell population density and chromosome replication state (Fig. 2). The phosphorelay may also respond to nutritional signals, but clear evidence for such a role is lacking, and the findings of Ratnayake-Lecamwasam et al. (2001) indicate that CodY is the principal device for sensing and responding to nutrient limitation.

The CodY system therefore joins the short list of known molecular mechanisms that sense the nutritional status of the cell and transduce this physiological information into an adaptive transcriptional response. Intriguingly, all three of the systems we have considered here operate by sensing and responding to purine nucleotides (Table 1). It will be interesting to see whether responses to changes in cellular pools of purine nucleotides and other related, small molecules prove to be a pervasive feature of nutrient-sensing mechanisms in a wide range of organisms. In this regard, we draw attention to recent work attributing the effect of caloric restriction on lifespan extension in yeast and nematodes to a putative sensor of cellular NAD levels (Lin et al. 2000; Tissenbaum and Guarente 2001).

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