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Molecular Mechanism of Carbon and Nitrogen Regulation in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa PAO1 utilizes a variety of compounds as carbon and nitrogen sources. In this bacterium, the CbrA-CbrB and NtrB-NtrC two-component regulatory systems regulate the expression of several catabolic pathways for amino acids and polyamines in response to carbon and nitrogen availability. The CbrB response regulator appears to control the relevant catabolic pathways by different mechanisms. It elicits the polyamine-dependent expression of a putative regulatory protein of the *spuABCDEFGH* operon for putrescine transaminase and the polyamine transport system. CbrB activates the arginine (or ornithine)-dependent expression of the *aot-argR* operon encoding ArgR regulatory protein of the AraC/XylS family and the arginine/ornithine transport system via an unknown mechanism. Expressed ArgR induces the *aruCFGDBE* operon encoding the arginine succinyltransferase pathway (the major arginine/ornithine catabolic pathway in this bacterium), whereas CbrB, as well as NtrC, appears to directly activate expression of the *hut* (histidine utilization) operon. When histidine is the sole source of carbon and nitrogen, CbrB mainly activates the transcription from the *huU* promoter of this operon. However, when histidine is the nitrogen source along with succinate as the carbon source, CbrB is no longer active. Instead, NtrC actively expresses the operon. CbrB or NtrC activates transcription at the same two sites at appropriate distances from the consensus sequences for the σ^{54}-recognition and binding regions. Two types of suppressor mutants have been isolated from a *cbrAB* null mutant that restored the ability to utilize histidine as the sole carbon and nitrogen source; one has a transition of G at 407 to A (causing a change of Arg136 to His) in *cbrB*, the other is a C to T transition at 251 (Ala84 to Val) in *ntrC*. In contrast to the wild type strain in which *hut* expression is repressed by succinate (a catabolite repressor of *P. aeruginosa*), mutant cells harboring either suppressor gene on a plasmid express the *hut* operon independently of the co-existing carbon and nitrogen sources. Thus, the CbrA-CbrB system together with that of NtrB-NtrC controls catabolic pathways in response to carbon and nitrogen status in growth medium. This novel function of the two-component regulatory system in carbon regulation presents a novel view of the catabolite controls exerted by metabolically versatile pseudomonads.

Introduction

The γ-proteobacteria pseudomonads can persist in water and in soil where nutrients are limited as well as in plants and animals that are rich in nutrients and some species cause plant and animal diseases. The metabolic versatility of pseudomonads that accounts for their omnipresence has two facets. They can utilize a wide range of organic compounds including phenolic compounds and xenobiotics. They can also utilize amino acids, polyamines and other C-N compounds as both carbon and nitrogen sources, in contrast to *Escherichia coli* and *Bacillus subtilis,* which can only use these compounds as a nitrogen source. Pseudomonads should have a regulatory mechanism that modulates catabolic pathways in response to both carbon and nitrogen sources. Since *E. coli* and *B. subtilis* control amino acid metabolism by nitrogen

regulators, these organisms might lack such a mechanism. Two-component regulatory systems, consisting of a sensor/histidine-kinase (also called a transmitter) and a cognate response regulator, are the paradigm of bacterial adaptation to changes in environmental status such as oxygen tension, osmolarity, and cell population, as well as nutrient availability (1, 2). Environmental signals can trigger autophosphorylation of the transmitter in the histidine kinase domain through the sensor domain. Subsequent transfer of phosphosphate to the receiver domain of the response regulator activates the regulator protein. The active form of the response regulator then induces expression of the genes necessary for adaptation to changed environmental status. In *E. coli* the NtrB-NtrC two-component regulatory system mediates nitrogen-responsive regulation of *glnA* (glutamine synthetase) and other nitrogen-assimilation genes (1). Nitrogen signals (ammonia) control phosphorylation levels of the response regulator NtrC (also called NRI, the *glnL* product) through modulation of the phosphatase activity of NtrB (also called NRII, *glnG*) via helper PII (*glnB*). NtrC is a representative of the NtrC family regulators that activate transcription by the σ^{54}-RNA polymerase holoenzyme (1). We found that the CbrA-CbrB two-component regulatory proteins are essential for the utilization of arginine/ornithine, histidine, some other amino acids, and polyamines (3). This system appears to control the expression of amino acid and polyamine catabolic pathways in response to carbon status, whereas the NtrB-NtrC pair mediates nitrogen-responsive regulation as in *E. coli*. In this symposium, I describe the regulation of arginine and polyamine metabolism by CbrA-CbrB and present the current view of regulation of the histidine utilization (*hut*) operon. When a preferable carbon source is present, CbrB is not activated, but NtrC directs *hut* operon expression to utilize the amino acid as the nitrogen source. If histidine is supplied as the carbon source, CbrB essentially actives the *hut* operon and the Ntr system does not function because histidine degradation produces excess ammonia. This view supports the notion that the CbrA-CbrB system is involved in catabolite control of histidine metabolism and perhaps that of other amino acids and polyamines.

CbrA-CbrB Two-Component System

The cbrAB genes were initially identified as being essential for the utilization of arginine or ornithine as carbon sources (3). The membrane sensor domain of CbrA with 12 possible transmembrane helices at the amino-terminal half (1 - 490 residues) has about 20% identity with sodium/solute symporters, and a histidine kinase domain at the carboxyl-terminal region (730 - 983 residues) shares 34% identity with *E. coli* NtrB. The CbrB response regulator resembles (45% identity) NtrC. Mutants of *cbrA* or *cbrB* are also defective in the utilization of proline, alanine, histidine, polyamines (putrescine, spermidine, and spermine), gluconate and citrate (3).

Regulation of Arginine, Proline and Polyamine Metabolism by CbrA-CbrB

The *aruCFGDBC* operon encodes the arginine succinyltransferase (AST) pathway (Fig. 1). Expression of this operon is activated by ArgR protein of the AraC/XylS family, which is encoded by the upstream *aot-argR* operon together with the AotJQMP arginine/ornithine transport system (4-7). The expression of the *aot-agR* operon requires arginine (or ornithine), ArgR, and the CbrA-CbrB functions. Proline catabolism proceeds via bifunctional proline dehydrogenase and Δ^1-pyrroline 5-carboxylate dehydrogenase encoded by the *putA* gene (8). The *putA* gene constitutes an operon with *putP* (proline permease) and the upstream *pruR* gene positively controls expression of the *putAP* operon. In *cbrA* or *cbrB* mutant, the *putAP* operon is normally expressed in response to exogenous proline. Why proline utilization by these mutants is defective remains unknown, but it is almost completely restored by adding 1 mM succinate, an amount that is not

sufficient for growth (3). Perhaps the CbrA-CbrB pair also controls a specific central pathway that is dependent on the carbon source. In the *cbrA* or *cbrB* mutant, the *spuABCDEFGH* operon that encodes putrescine transaminase (SpuC) and spermidine/spermine transport proteins (SpuDEFGH) is not expressed even in the presence of polyamine, which accounts for the inability of the mutants to grow on polyamine (9). SpuC is essential for putrescine metabolism, whereas the SpuDEFGH transporters are necessary for spermidine and spermine uptake. The wild type strain when grown on polyamine produces a DNA-binding protein that interacts with the promoter regions of the operon, but mutants do not (9). This suggests that CbrA-CbrB are involved in the synthesis of a protein that regulates the operon and which is inducible by the substrate.

Fig. 1. Structure and regulation of arginine catabolic operons. Upstream *aot-argR* operon encodes an arginine/ornithine transport system, AotJ (periplasmic arginine-ornithine binding protein), AotQ (membrane transport protein), AotM (membrane transport protein), AotP (ATP-binding cassette transport protein), and ArgR regulatory protein (7). In the presence of arginine (or ornithine) the *aotJ* promoter is expressed through the functions of CbrA and CbrB. Expressed ArgR then activates transcription of the *aruCFGDBE* operon that encodes the arginine succinyltransferase pathway (4).

Fig. 2. Carbon- and nitrogen-controls of the *hutU* promoter by two-component regulatory systems. When histidine is absent, HutC repressor prevents expression from the *hutU* promoter for the *hutUHIFG* operon for histidine utilization by binding to the operator site between the -12 and -24 regions of the two σ^{54}-promoters. Histidine abolishes repressor function. Under carbon limited (N>C) conditions, CbrB is activated by phosphorylation via CbrA and activates transcription of the *hut* operon by σ^{54}-RNA polymerase holoenzyme. In contrast, when nitrogen is limited (N<C) and where CbrB is not phosphorylated, NtrC becomes phosphorylated and activates the transcription from the same promoters by σ^{54}-RNA polymerase.

Regulation of the Histidine Utilization Operon by CbrA-CbrB and NtrB-NtrC

The histidine utilization operon (*hutUHIFG*) is negatively controlled by the upstream *hutC* gene, and urocanate, a metabolite of histidine, induces operon expression by antagonizing the repressor function. Whereas a mutant of *cbrB* cannot utilize histidine as a carbon source, the *ntrC* mutant cannot use it as nitrogen source. Enzyme and fusion assays confirmed that ammonia represses *hut* operon expression in the *cbrB* mutant and that succinate (a catabolite repressor for *P. aeruginosa*) prevents its expression in *ntrC* mutant. In other words, CbrB and NtrC activate the operon in the absence of a carbon or nitrogen source, respectively. Two transcription start sites determined by primer extension experiments, are identical in *cbrB* and *ntrC* mutants. Promoter sequences similar to those for the σ^{54}-RNA polymerase holoenzyme could be found at -12 and -24 bp upstream of the start points (Fig. 2). Moreover, two regions resembling the NtrC-binding sequences were also located at the -160 and -180 regions. These structural features of the *hutU* promoter agree with the notion that the response regulators directly activate transcription of the promoter in response to carbon or nitrogen status.

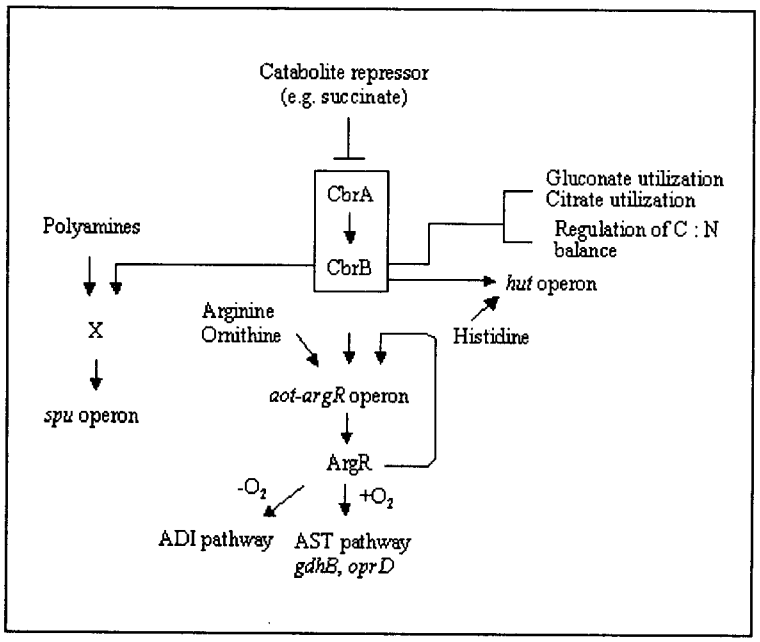

Fig. 3. Schematic regulatory circuit controlled by the CbrA-CbrB pair. Carbon-limitation triggers phosphorylation of CbrA and subsequent phosphotransfer to CbrB. Phosphorylated CbrB, an active transcription factor, expresses several catabolic pathways depending on available substrates. Arginine (or ornithine) induces the *aot-argR* operon to express ArgR regulatory protein (Fig. 1). Under aerobic conditions, ArgR elicits the AST pathway that leads arginine and ornithine to glutamate. It also stimulates the expression of the *oprD* and *gdhB* genes that specify an outer membrane porin protein and catabolic (NAD^+-dependent) glutamate dehydrogenase, respectively; OprD porin facilitates diffusion of arginine/ornithine into the periplasmic space, whereas GdhB channels the pathway product glutamate into TCA cycle via 2-ketoglutarate (10, 11). Under anaerobic conditions, ArgR stimulates the *acrDABC* operon for the ADI pathway that generates ATP from arginine (12). Polyamines (putrescine, spermidine, and spermine) produce a putative transcription activator of the *spuABCDEFGH* operon encoding putrescine aminotransferase and spermidine/spermine transport system (9). Histidine induces the *hut* operon for histidine utilization (Fig. 2). CbrA-CbrB pair also controls the utilization of gluconase and citrate by yet unknown ways and contribute to the maintenance of cellular ratio of carbon and nitrogen (3).

Possible Involvement of the CbrA-CbrB Pair in Catabolite Repression

The finding that CbrB is not activated in the presence of succinate, a catabolite repressor, suggested that CbrA-CbrB modulates catabolite control of the *hut* operon. To confirm this hypothesis we isolated suppressor mutants from a *cbrAB* null mutant. We also introduced a mutation into *cbrB* by error-prone PCR and screened *cbrB* mutants that can complement the defective histidine utilization of the *cbrAB* mutant. Nucleotide sequencing identified a base-substitution of C to T at 251 (resulting in an amino acid change of Ala84 to Val) in a mutant *ntrC* and a base-change G to A at 407 (Arg136 to His) in a mutant *cbrB*. The growth of a *cbrAB* null mutant harboring these mutant suppressor genes on a plasmid was restored on arginine as well as on histidine. When wild type *P. aeruginosa* is cultured in histidine medium supplemented with succinate, the catabolite repressor diminishes *hut* operon expression about fourfold. In contrast, *hut* operon expression is marginally repressed by the catabolite repressor. Thus, structurally related two-component pairs (CbrA-CbrB and NtrB-NtrC) in *P. aeruginosa* regulate catabolite and nitrogen control, respectively, in response to carbon and nitrogen status. The impaired growth of the *cbrA* or *cbrB* mutant on proline suggests that this two-component system also plays an important role in the maintenance of a healthy C:N balance (Fig. 3).

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