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Molecular Regulation of Pyrimidine Nucleotide Synthesis in Bacterial Genomes

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Summary

Regulation of pyrimidine nucleotide synthesis has been studied extensively in enteric bacteria and *Bacillus* species. Varieties of control modes have been proposed for regulation of pyrimidine nucleotide biosynthetic *(pyr)* genes. In *Bacillus caldolyticus* and *B. subtilis*, it has been proved that pyrimidine *de novo* biosynthetic operon is controlled by a regulatory protein PyrR-mediated attenuation. Another Gram-positive bacteria including *Enterococcus faecalis, Lactobacillus plantarum,* and *Lactococcus lactis* have been found to constitute a *pyr* gene cluster containing the *pyrR* gene. In addition, it has been proposed that the structure of the 5' leader region of the Gram-negative extreme thermophile Thermus strain ZO5 *pyr* operon provides a novel mechanism of PyrR-dependent coupled transcription-translation attenuation.

Bacterial genome sequencing projects have identified the PyrR homologues in *Haemophilus influenzae, Synechocystis* sp., *Mycobacterium tuberculosis, Streptococcus pneumoniae, S. pyogenes,* and *Clostridium acetobutylicum,* which are currently investigating for their physiological functions.

Introduction

Nucleotides are components of DNA and RNA which are indispensable macromolecules in all living cells. Most procaryotes can produce their nucleotides both by *de novo* biosynthesis and by recycling the performed nucleosides and nucleobases. Pyrimidine nucleotide synthesis has initially been studied in *Escherichia coli* and *Salmonella typhimurium* (7).

Expression of all genes involving pyrimidine nucleotide synthesis is under negative control by pyrimidine compounds, but their maximal regulatory response is quite different. Regulatory pattern of pyrimidine effectors on the six *pyr* genes involving UMP biosynthesis is diverse. Complicated data from different group's studies suggest that the pyrimidine synthesis genes are regulated in a highly complex manner. Therefore several specific control modes have been proposed for regulation of each gene: *pyrB* and *pyrE* genes are controlled by transcriptional attenuation; *pyrC* and *pyrD* genes by dual transcriptional initiation; and *carAB, pyrF, codBA, upp,* and *udk* genes by abortive transcription.

The aims of this research was to accomplish a structural and functional analysis of all the *pyr* genes from various sources of bacteria. In addition, the studies concerning the pyrimidine regulation and its possible mechanism are also included.

Materials and Methods

Bacterial strains and plasmids

B. caldolyticus and other *Bacillus* strains were obtained from the DSMZ (Braunschweig, Germany)

and BGSC (Ohio, USA), respectively. *E. coli pyr* mutants were obtained from J. Neuhard. The pBEM215 plasmid containing the *E. faecalis pyr* gene cluster was kindly provided by B.E. Murray. The pPSH12 plasmid containing the *H. influenzae pyrR* orthologous gene and *Synechocystis* sp. strain PCC6803 were provided by R.L. Switzer.

DNA and RNA techniques

PCR, DNA, and RNA techniques were applied according to standard (9) and suppliers' protocols. DNA was sequenced by the dideoxy chain-termination method. Gel mobility shift analysis for RNA binding was performed using a Bio-Rad PROTEAN II_{Xi} electrophoresis apparatus with the core cooled to 2?.

Enzyme assays

Crude cellular extracts prepared by sonic disruption were used for enzyme assays. Uracil phosphoribosyltransferase (UPRTase) was assayed by measuring the PRPP-dependent conversion of [^{14}C]uracil to [^{14}C]UMP. Aspartate transcarbamylase activity was determined by the method of Prescott and Jones (8). Protein content was determined by using the Coomassie protein assay reagent (Pierce, Rockford, Ill.) with bovine serum albumin as the standard.

Computer analysis

RNA secondary structure was predicted by the FOLD program. Computer analyses of nucleotide and amino acid sequences were carried out with DNA Strider and DNA Star programs.

Results and Discussion

In both *B. caldolyticus* and *B. subtilis*, it turned out to be that PyrR encoded by *pyrR* gene is a unique bifunctional protein whose primary function is the regulation of *pyr* gene expression, but which also catalyzes the UPRTase reaction (1,2). A control model for transcriptional termination-antitermination in the pyrimidine regulation of the *pyr* operon expression was first proposed in *B. caldolyticus* by Ghim and Neuhard (1). They found an approximately 60-bp conserved sequence for the putative binding site of the PyrR protein and two mutually exclusive stem-loop structures called antiterminator (AT) and terminator (T) in each of the three non-coding regions of the *pyrB* upstream on the *B. caldolyticus pyr* transcripts. Under high UTP conditions, the PyrR regulatory protein would be in its active conformation, the terminator structure will form and transcription will be terminated. Under low UTP conditions, the RNA binding PyrR protein is inactive, the antiterminator structure will form and lead to transcriptional readthrough.

Simultaneously and independently, Turner *et al.* (11) discovered that PyrR has both regultory and UPRTase activity in *B. subtilis*. Detailed genetic and biochemical studies of *B. subtilis* PyrR have clarified the transcriptional attenuation mechanism by which it regulates *pyr* transcription (4, 5). Actually, the *pyr* operon in *B. subtilis* is regulated by an autogenous transcriptional attenuation mechanism in which the first gene of the operon, *pyrR*, encodes a regulatory protein that causes transcriptional termination by binding in a uridine nucleotide-dependent manner to three specific sites in the *pyr* mRNA (10). These sites are located in the 5' untranslated leader of the operon, between the first (*pyrR*) and second (*pyrP*) cistrons, and between the second and third (*pyrB*) cistrons of the operon. The

same arrangement of genes and regulatory sites is found in the *B. caldolyticus pyr* operon.

Several lines of evidence demonstrated that the *E. faecalis pyr* operon is repressed by uracil via transcriptional attenuation at the single 5' leader termination site and that attenuation is mediated by the PyrR protein (3). A similar attenuation mechanism appears to regulate *pyr* genes in *L. lactis* and in *L. plantarum*, but different organization of the *pyr* genes and attenuation sites are found in these cases (6). Similarly, all of necessary attenuation elements including overlapping AT and T structures and a consensus PyrR binding sequence in the 5' region of *pyr* transcript exist immediately upstream the *pyrRP* operon of *S. pyogenes* and *pyrRP* and *pyrBIFDIIDI* operons of *Clostridium acetobutylicum*. It could be concluded that the mechanism of transcriptional attenuation by PyrR in all of these species described above is fundamentally the same as is known to occur in *Bacillus* species.

There have also been alternative mechanisms for PyrR-mediated regulation of *pyr* gene expression. Van de Casteele *et al.* (12) have proposed that it is very likely that in *Thermus* PyrR regulates transcriptional attenuation by acting as a translational repressor in a coupled transcription-translation system, rather than acting to disrupt an AT structure. The *pyr* operon in *M. tuberculosis* showed no A or AT structure but a putative PyrR binding site overlapped with the ribosome binding site of *pyrR* gene, which might role as a simple translational repressor. Other PyrR homologues were found in *H. influenzae* and in *Synechocystis* after whole genome sequencing but none of the known features required for PyrR-dependent regulation of *pyr* genes has been identified. Clearly, much more needs to be investigated about PyrR homologues and their functions in various species of bacteria.

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