S1-4

Regulation of Purine Nucleotide Synthesis.

Howard Zalkin,

Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907.

This talk at the International Meeting 2000 of The Microbiological Society of Korea will cover a few of the highlights of our research on the regulation of purine nucleotide synthesis. Although it is likely that the biosynthetic pathway for synthesis of purine nucleotides is regulated in all organisms, it is only in *Escherichia coli* that the regulatory mechanisms have been determined at atomic resolution and in *Bacillus subtilis*

they are partially worked out. In these bacteria changes in gene expression and enzyme activity together adjust the rate of biosynthesis to meet cellular requirements. Here, I will focus on the transcriptional regulation of gene expression. The synergistic end product inhibition of glutamine PRPP amidotransferase, the key regulatory enzyme of the pathway has been described (1, 2).

The genes for the 15 steps required for synthesis of AMP and GMP are organized in nine coregulated operons in *E. coli* (3). Addition of purines to the growth medium results in a parallel repression of each of the operons. Transcriptional regulation involves a repressor protein that binds to a conserved operator sequence located within the promoter of each of the operons. A transcription start site, promoter and operator were first identified in *purF* (4,5). The 16 bp 5'-ACGCAAAC·GTTTTCTT *purF* operator sequence was subsequently proved to be the repressor binding site by DNase I footprinting (6). It was identified in the promoter region of all of the other coregulated *pur* genes (3).

The cloning of *purR* was made possible by the isolation of stable, non repressible *pur* regulon mutants (5). Earlier, genetically uncharacterized regulatory mutants had been obtained (7,8) but the regulatory phenotype was uncharacterized due to high rates of spontaneous mutations and the lack of sensitive assays for enzymes of the pathway. The *E. coli purR* gene was cloned by Ronda Rolfes using a mini-Mu phage library for functional complementation of a *purR* mutant containing a *purF-lacZ* reporter gene that she had constructed (6). Cloning was difficult due to the fact that multiple copies of *purR* strongly inhibit the growth of cells.

The nucleotide sequence of *purR* defined several key properties of the repressor protein. The PurR subunit is a two domain protein having a 50-amino acid N-terminal DNA binding domain connected by a hinge sequence to a 289-amino acid domain that functions in corepressor binding and dimerization. The DNA binding domain has a typical helix-turn-helix (H-T-H) DNA binding motif but it is the hinge region that is the key to DNA binding. Before addressing the role of the hinge region, I should describe a few other properties of the repressor. First, PurR exhibits 26% sequence identity with LacI, the *lac* operon repressor. Thus, PurR is one of the Lac family of bacterial transcription factors, which includes more than 25 proteins. In addition, the corepressor binding domain (CBD) is homologous with a family of periplasmic binding proteins for ribose, arabinose and galactose. Thus, we visualize that PurR evolved by the fusion of genes for a periplasmic binding protein and a DNA binding domain. A second important feature of PurR is the corepressor. A cellular "excess purines" signal is required for high affinity DNA binding. Once PurR was over produced and purified, hypoxanthine and guanine were identified as corepressors (9). Affinity for operator DNA was increased about 40-fold by corepressor (10). Structural work on PurR in Dick Brennan's laboratory at Oregon Health Sciences University helped put the finishing touches on the *E. coli* PurR story. In 1994 Schumacher *et al.* reported the structure for the PurR-hypoxanthine-*purF* operator ternary

complex (11) and in 1995 the structure for a corepressor-free CBD dimer appeared (12). This was the first high resolution structure for a LacI family repressor bound to DNA and together with the structure for the aporepressor provided new insights into how this family of repressors bind to DNA. PurR is a dimer (13). The N-terminal DNA binding domain contains four helices. Helices 1 and 2, the H-T-H DNA binding motif, bind in the major groove of each half operator. The key to DNA binding, however, is helix 4, the "hinge helix". Leu54 in the hinge helix of each subunit intercalates the central C-G step in the operator minor groove and kinks the DNA helix resulting in a 45° bend away from the protein, enabling the H-T-H interactions with the major groove. What is the role for corepressor? The structures have established that PurR exists in corepressor-free "open" and corepressor-bound "closed" conformations. When a corepressor binds to the CBD, the N-subdomains of each subunit rotate by about 20° causing Lys60 of each subunit, the N-terminal CBD residue, to move 3.5 Å closer to each other. This change is propagated to hinge residues 48 to 56, resulting in their interaction by van der Waals contacts. In the presence of a specific DNA site, local hinge folding into helices occurs. The specific interaction of the hinge helices with the operator DNA minor groove kinks the DNA and permits the subsequent interactions with the major groove of each half operator. Thus, binding of a corepressor to a site 40 Å from the DNA-binding domain modulates DNA-binding affinity by an allosteric mechanism through interactions made by the hinge helix. Mutations have been constructed which prevent the structural changes needed to transmit the corepressor binding signal to the hinge helix. Interestingly, the important role of the hinge region (14) as well as allosteric properties (13) were discovered by Kang Yell Choi prior to the structure determination.

Due to limitations of space the transcriptional regulation of purine biosynthesis in *B. subtilis* by a purine repressor that is unrelated to *E. coli* PurR is not covered.

References

1. Chen, S. Tomchick, D.R., Wolle, D., Hu, P., Smith, J.L., Switzer, R.L., and Zalkin, H. 1997. Mechanism of the synergistic end-product regulation of *Bacillus subtilis* glutamine phosphoribosylpyrophosphate amidotransferase by nucleotides. *Biochemistry* **36**, 10718-10726.

2. Zhou, G., Smith, J.L., and Zalkin, H. 1994. Binding of purine nucleotides to two regulatory sites results in synergistic feedback inhibition of glutamine 5-phosphoribosylpyrophosphate amidotransferase. *J. Biol. Chem.* **269**, 67894-6789.

3. Zalkin, H., and Nygaard, P. 1996. Biosynthesis of purine nucleotides. In *Escherichia coli and Salmonella, cellular and molecular biology* (Neidhardt, F.C., Curtiss, R., III, Ingraham,J.L., Lin, E.C.C., Low, K.B., Magasanik, B., Reznikoff, W.S., Riley, M., Schaechter, M., and Umbarger, H.E., Eds.) pp 561-579, American Society for Microbiology, Washington, DC.

4. Makaroff, C.A., and Zalkin, H. 1985. Regulation of *Escherichia coli purF*. Analysis of the control region of a *pur* regulon gene. *J. Biol.. Chem.* **260**, 10378-10387.

5. Rolfes, R.J., and Zalkin, H. 1988. Regulation of *Escherichia coli purF*. Mutations that define the promoter, operator and purine repressor gene. *J. Biol. Chem.* **263**, 19649-19652.

6. Rolfes, R.J., and Zalkin, H. 1988. *Escherichia coli* gene *purR* encoding a repressor protein for purine nucleotide synthesis. Cloning, nucleotide synthesis, and interaction with the *purF* operator. *J. Biol. Chem.* **263**, 19653-19661.

7. Gots, J.S., Benson, C.E., Jochimsen, B., and Kodure, R. 1977. Microbial models and regulatory elements in the control of purine metabolism. *Ciba Found. Symp.* **48**, 23-41.

8. Levine, R.A., and Taylor, M.W. 1981. Selection for purine regulatory mutants in an *E. coli* hypoxanthine phosphoribosyltranserase-guanine phosphoribosyltransferase double mutant. *Mol. Gen. Genet.* **181**, 313-318.

9. Rolfes, R.J., and Zalkin, H. 1990. Purification of the *Escherichia coli* purine regulon repressor and identification of corepressors. *J. Bacteriol.* **172**, 5637-5642.

10. Lu, F., Brennan, R.G., and Zalkin, H. 1988. *Escherichia coli* purine reprssor. Key residues for the allosteric transition between active and inactive conformations and for interdomain signaling. *Biochemisty* **37**, 15680-15690.

11. Schumacher, M.A., Choi, K.Y., Zalkin, H., and Brennan, R.G. 1994. Crystal structure of LacI member, PurR, bound to DNA: Minor groove binding by helices. *Science* **266**, 763-770.

12. Schumacher, M.A., Choi, K.Y., Lu, F., Zalkin, H., and Brennan, R.G. 1995. Mechanism of corepressor-mediated specific DNA binding by the purine repressor. *Cell* **83**, 147-155.

13. Choi, K.Y., and Zalkin, H. 1992. Structural characterization and corepressor binding of the *E. coli* purine repressor. *J. Bacteriol.* **174**, 6207-6214.

14. Choi, K.Y., and Zalkin, H. 1994. Role of the purine repressor hinge sequence in repressor function. *J. Bacteriol.* **176**, 1767-1772.