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Studies on Novel Regulatory Mechanism For Nucleotide Biosynthesis Through the Amino Acid Signal in Bacteria

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Research aims

Amino acids and nucleotides are important constituents of every organism, and intracellular levels of these compounds are strictly regulated in cells at various levels. Thus, to optimally produce these molecules by fermentation, it is very important to understand the mechanisms of homeostasis of each biomolecule. In general, when concentration of a specific amino acid dropped below the usual level, relevant uncharged tRNA increases and binds to ribosome, which results in the activation of stringent response provoked by alarmone nucleotide, (p)ppGpp. (p)ppGpp inhibits de novo ribosome biosynthesis and peptide elongation to suppress the amino acid consumption, and on the other hand, activate transcription of amino acid biosynthetic genes to increase the amino acid biosynthesis. These effects indicate that (p)ppGpp plays a central role for the amino acid homeostasis. Moreover, (p)ppGpp inhibits initiation and elongation of DNA replication, which suppress the consumption of nucleotides, while the effects on the nucleotide biosynthesis have not been well evaluated yet.

Recently, using luciferase genes of *Vibrio fischeri* as the reporter, we constructed a real-time monitoring system of intracellular redox level in *Escherichia coli* (1). In a series of studies, we found the induction of stringent response, which was mediated by addition of a certain amino acid such as serine or isoleucine, results in significant decrease of the intracellular redox pool. Since this decrease was not observed in ppGpp^0 strain lacking the (p)ppGpp synthetic activity, it was likely mediated by (p)ppGpp (data not shown). Another finding was that the decrease of redox pool was disappeared or alleviated by addition of one-carbon-metabolites (OCM; glycine, methionine, thymidine, adenosine and panthotenate (2)) into the medium. OCM are metabolites that are synthesized by the help of folate, and thus, (p)ppGpp was suggested to inhibit folate metabolism which results in the inhibition of nucleotide biosynthesis and the decrease of redox pool. This could be a critical regulatory point to couple amino acid metabolism and nucleotide metabolisms, and this study was performed to make clear the underlying molecular mechanism.

Methods

In this study, a *relA*$^+$ derivative of *E. coli* MC4100 strain, CK8, was routinely used. Luciferase reporter plasmid, pLXUV5, was previously constructed by inserting the constitutive *lac*UV5 promoter to drive the *luxCDABE* operon of pUCD615 (1, 3). Bacterial strains harboring pLXUV5 were cultivated in MOPS medium, and the luciferase activity (RLU) was monitored by luminometer (Berthold Lumat LB9507) in 100 µl for 10 seconds. 0.4% glucose or galactose was added to the medium as the carbon source. Amino acids were added at 50 µg/ml. A *crr*::*kan* allele was obtained from KO collection at National Institute of Genetics, Japan (4), and strains were constructed by P1 transduction. Measurements of intracellular metabolites were performed in cooperation with Keio University.

Results

1. CK8 strain harboring pLXUV5 was cultivated in MOPS medium containing glucose as the carbon source, and isoleucine was added to the medium during the logarithmic growth phase. Since excess isoleucine represses the acetohydroxy acid synthase (AHAS) that catalyzes the first common step of isoleucine/valine biosynthesis, this isoleucine addition was expected to induce starvation of valine and thus weak stringent response. Examining the intracellular metabolite concentrations revealed significant decreases of glycolytic intermediates, such as glucose-6-phosphate and glyceraldehyde-3-phosphate. Consistently, similar decreases of glycolytic intermediates were observed in case of stringent response caused by sudden removal of amino acids from the rich cultivating medium (Dr. N. Saito, personal communication). Thus, (p)ppGpp was suggested

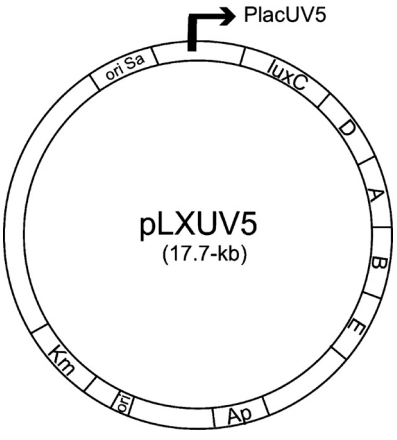

Fig. 1. Structure of pLXUV5 (PlacUV5::luxCDABE)

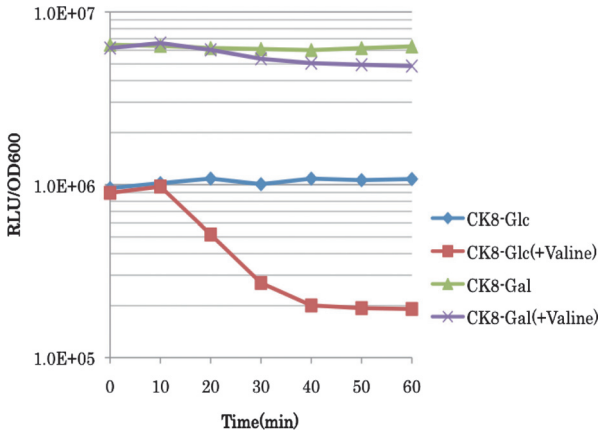

Fig. 2. Effect of carbon source on the valine-induced redox decline. Valine (50 µg/ml was added to the medium at time 0, and luciferase activity was monitored.

to inhibit glycolytic flux and generation of reducing equivalents, and induce decrease of the intracellular redox pools.

2. CK8 (pLXUV5) was cultivated in MOPS medium in the presence of either glucose or galactose as the sole carbon source, and the luciferase activity was monitored after addition of valine into the medium. While the valine addition greatly decreased the luciferase activity in case of glucose culture, the same valine addition made almost no effect on the luciferase activity in case of galactose cultivation (Fig. 2). Other carbon sources such as maltose and glycerol resulted in similar results with the galactose case. Thus, it was suggested that the glucose culture was the special case.

3. Given that the effect of stringent response was specifically observed in case of glucose culture, involvement of PTS (PhosphoTransferase System) for glucose transport was considered. Thus, we examined the effect of a null mutation of the *crr* gene that encodes IIA^{Glc} for the phosphotransfer reaction to glucose. Other than the PTS function, it is known that IIA^{Glc} activates adenylate cyclase in its phosphorylated form and repress non-PTS lactose transporter in its non-phosphorylated form. We constructed the *crr* mutant of CK8, and examined for the valine effect. As the results, we found that weak decline of luciferase activity was observed similarly in both carbon sources. This result was explained if IIA^{Glc} regulates PTS activity cooperating with (p)ppGpp either directly or indirectly.

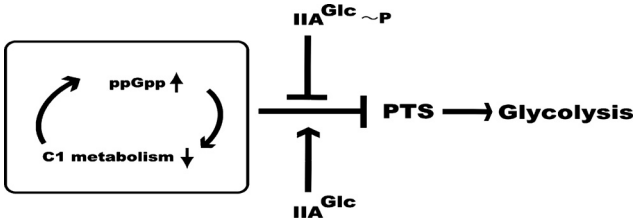

Fig. 3. Model for Redox Pool Control

Conclusion

To explain the obtained results, we constructed a working model as in Fig. 3. Amino acid starvation response affects sugar catabolic flux through (p)ppGpp, and thus results in the decline of cell redox pools. In this pathway, IIA^{Glc} of glucose PTS system has an important role. With respect to the relationship with one carbon metabolism, (p)ppGpp is likely has an inhibitory effect on the folate metabolism and subsequent PTS inhibition, while the direct target has not been identified. In case of folate starvation induced by trimethoprim, starvation of glycine and methionine is induced and thus stringent response and decline of redox pools are also induced (5). In conclusion, this study has revealed close relationship among amino acid metabolism, one carbon metabolism and sugar metabolism.

Acknowledgment

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