Identification and Classification of Two-component Systems That Affect \textit{rpoS} Expression in \textit{Escherichia coli}

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The \textit{rpoS}-encoded \(\sigma^5\) subunit of RNA polymerase regulates the expression of stationary phase and stress response genes in \textit{Escherichia coli}. Recent study of our DNA microarray analysis suggested that the \textit{rpoS} expression is affected by multiple two-component systems. In this study, we identified two-component-system mutants in which the \textit{rpoS} expression increased. The regulatory manner of the systems on \textit{rpoS} expression is suggested.

Key words: RpoS; \textit{Escherichia coli}; two-component system; His-Asp phosphorelay; sigma factor

In \textit{Escherichia coli}, a general stress response is dependent on the \(\sigma^5\) (RpoS) subunit of RNA polymerase.\(^{11}\) The expression of the \textit{rpoS} gene is stimulated by a variety of stimuli, suggesting the existence of several sensory systems that are integrated in the \textit{rpoS} regulation. It is known that the regulation is conducted in a complex manner at the levels of transcription, translation, and protein stability.\(^{11}\) Several factors involved in these regulations were also known.\(^{11}\) However, the whole framework of \textit{rpoS} regulation remains to be discovered.

In \textit{E. coli}, the two-component system (TCS) is one of the most characterized and universal systems that work in adapting to a variety of stresses.\(^{2,3}\) The typical TCS are composed by a histidine kinase (HK) that works as a stress sensor and a response regulator (RR) that works as an effector (in many cases, as a transcriptional factor). \textit{E. coli} has 29 HKs, 32 RRs, and a sole HPt protein which works as an inter-mediator of phosphate transfer between HK and RR.\(^{2}\) One of the RR, RssB, is known to be involved in the stability control of \(\sigma^5\), however the cognate HK that transfers phosphate to RssB has not been identified to date.\(^{4,6}\) Because the recent our microarray analysis using TCS mutants suggested that the \textit{rpoS} expression is induced in many TCS mutants, first in this study we analyzed the expression of \textit{rpoS} in all of TCS mutants by using an \textit{rpoS}-lacZ protein fusion reporter gene.\(^{7}\) The \textit{rpoS}-lacZ protein fusion (PF977) constructed in strain CU263 is known to be a reliable indicator of the \textit{rpoS} expression because it contains both a turnover control element for \(\sigma^5\) protein and a \(\mathit{cis}\)-acting element required for translational control in addition to the \textit{rpoS} promoter region (Fig. 1A).\(^{8,9}\) Thirty-six TCS deletion mutants that cover all of the TCSs found in \textit{E. coli} were constructed on CU263 and their \(\beta\)-galactosidase activities were examined at logarithmic growth phase.\(^{3}\) From this analysis, we identified nine TCS mutants, \textit{cusRS}, \textit{rstAB}, \textit{arcA}, \textit{narP}, \textit{cheABYZ}, \textit{arcB}, \textit{uvrY}, \textit{rssB}, and \textit{barA} mutants, in which the \textit{rpoS} expression increased significantly (Fig. 1B). The previously identified function of these TCS systems is as follows; \textit{cusRS} (copper response), \textit{ArcAB} (respiratory control), \textit{NarP} (nitrate regulation), \textit{CheABYZ} (chemotaxis), \textit{UvrY} (hydrogen peroxide sensitivity), \textit{RssB} (\(\sigma^5\) degradation), and \textit{BarA} (\textit{rpoS} transcription).\(^{7}\) The function of \textit{RstAB} has not yet been identified. In other mutants we did not find significant alterations of \(\beta\)-galactosidase activity compared with wild type (data not shown). Next, to confirm directly the increased expression of \textit{rpoS}, the amount of \(\sigma^5\) in these mutants was analyzed by Western blotting by using \(\sigma^5\) antiserum. As shown in Fig. 1C, the amount of \(\sigma^5\) correlated well with the \(\beta\)-galactosidase activity. Under the experimental conditions we used, \(\sigma^5\) protein was hardly detectable in wild type cells, as previously shown.\(^{7,9}\) These results clearly indicated that \textit{rpoS} expression was increased in these TCS mutants.

As mentioned first, the expression of \textit{rpoS} is under the control of many factors. So, the effect of each TCS deletion on \textit{rpoS} expression could be caused by the quantitative change of such regulatory factors. Then, in this study, we addressed our attention to two factors, CRP and H-NS, both of which were known to control the \textit{rpoS} expression negatively.\(^{10-12}\)

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Abbreviations: HK, histidine kinase; RR, response regulator; TCS, two-component system

The amount of both proteins was examined by Western blotting analysis. As shown in Fig. 1C, the amount of both CRP and H-NS proteins in the selected TCS mutants was nearly the same as that of wild type. From these results, we concluded that the up-regulation of *rpoS* in the selected TCS mutants was not derived from the quantitative control of CRP and H-NS by the TCS mutations. Besides CRP and H-NS, many factors such as small RNAs encoded by *dsrA*, *rprA*, and *oxyS* genes, an RNA binding protein Hfq, and a histone-like protein, HU, are known to be involved in the complex regulation of *rpoS*. 1,13 So, it is needed to investigate whether these factors are participating in the up-regulation of *rpoS* in each TCS mutant. These studies are waiting in the future.

Next, to clarify the regulatory steps at which these TCS mutants affect, two additional *rpoS-lacZ* fusion construct were used as reporter genes (Fig. 1A).

CU264 carries the *rpoS-lacZ* protein fusion (PF212) which contains a cis-acting element required for translational control in addition to the *rpoS* promoter region. Because the *rpoS-lacZ* protein fusion (PF212) does not contain the turnover control element that is necessary for the $\sigma^S$ degradation, the $\beta$-galactosidase activity is assumed to reflect the sum of transcriptional and translational efficiency of the *rpoS* gene. 8,9) GY29 carries the *rpoS-lacZ* operon fusion (OF) and was used to evaluate the transcriptional efficiency. 12) The deletion constructs of each TCS were transferred into these strains and $\beta$-galactosidase activities expressed at the logarithmic growth phase were measured. As shown in Fig. 2A, in which we used CU264 as a reporter strain, we have no more observed the higher activity of $\beta$-galactosidase in *cusRS*, *narP*, *cheABYZ*, *uvrY*, and *rssB* mutants, compared with wild type cells. These results suggested that the up regulation of *rpoS* in *cusRS*, *narP*, *cheABYZ*, *uvrY*, and *rssB* mutants shown in Fig. 1 resulted from the increased stability of the $\sigma^S$ protein itself. Indeed, the *rssB* mutant that was known to be involved in the stability control of $\sigma^S$ was classified in this group, supporting the accuracy of our classification. 4,5) The data using *rpoS-lacZ* operon fusion as a reporter gene (Fig. 2B) showed that in *arcA*, *arcB*, and *barA* mutants, higher expres-

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**Fig. 1.** Expression of *rpoS* in TCS Mutants.

A. A set of *rpoS-lacZ* fusion genes used in this study is schematically shown. GY29, CU264, and CU263 are derivatives of MC4100 and carry $\lambda$ *rpoS-lacZ* OF, $\lambda$ *rpoS-lacZ* PF212, and $\lambda$ *rpoS-lacZ* PF977, respectively. 8,12) B. The indicated TCS mutants were constructed on strain CU263 that carries the *rpoS-lacZ* protein fusion (PF977) and then grown at 37°C in Luria broth. At the logarithmic growth phase, the $\beta$-galactosidase activity was measured. C. The indicated protein content in each TCS mutants was analyzed by Western blotting using specific antiseraums.

**Fig. 2.** Effects of the TCS Mutations on a Set of *rpoS-lacZ* Fusion Genes.

TCS mutants constructed on either CU264 that carries the *rpoS-lacZ* protein fusion (PF212) (panel A) or GY29 that carries the *rpoS-lacZ* operon fusion (OF) (panel B) were grown at 37°C in Luria broth. At the logarithmic growth phase, the $\beta$-galactosidase activity was measured.
Although our data suggested that the rpoS mutants increased the expression of the rpoS gene. Because all of these TCS deletion mutants affect the regulatory steps at which these TCSs, for example, CheA or CusS, increase the expression of rpoS. The deletion of rstAB resulted in increased translation of rpoS. And the deletion of cusRS, narP, cheABYZ, uvrY, and rssB TCSs resulted in increased stability of σ^S protein.

All these results together, we would like to propose the regulatory steps at which these TCS deletion mutants affect rpoS expression. As shown in Fig. 3, both mutants of arcA and arcB, which composed a pair of TCSs, increase the transcription of rpoS. Although our data suggested that the barA mutant increases the transcription of rpoS, Mukhopadhyay et al. reported that BarA is required for the transcriptional induction of rpoS. This functional inconsistency may have been caused by the difference of the barA mutants used or growth conditions used. So, it is necessary to interpret this result carefully. The rstAB mutant activates translation of rpoS and the cusRS, narP, cheABYZ, uvrY, and rssB mutants increase the σ^S protein stability. At present it is not clear why these unrelated TCSs affected σ^S protein stability similarly. There may be functional interactions among these TCSs, for example, CheA or CusS may be a kinase for an orphan response regulator, RssB or UvrY. Clarification of these possibilities is waiting in the future. Because all of these TCS deletion mutants increased the rpoS expression, these TCSs seemed to function as negative factors for rpoS expression in wild-type cells at the logarithmic growth phase. At present, we do not know whether these TCSs have their negative effect directly on rpoS. Rather, their effect might be indirect, for example, defects of proper response caused by TCS deletion resulted in the stress signal that induces the rpoS expression. Anyway, the finding of TCS deletion mutants that affect rpoS expression, and their regulatory mode on rpoS regulation, should give a clue to resolve the complex regulatory mechanism of rpoS expression.

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