氏　　名	寺社下　美　樹
学位（専攻分野）	博士(理学)
学位記番号	総研大乙第64号
学位授与の日付	平成１１年3月２４日
学位授与の要件	学位規則第４条第２項該当
学位論文題目	Control of the Synthesis and Functions of RNA Polymerase Sigma Subunits in Escherichia coli
論文審査委員	主　査　教　授　嶋本 伸雄 教　授　荒木　弘之 助教授　西村 昭子 助教授　和田　明（大阪医科大学）

論文内容の要旨

Selection of a σ subunit of RNA polymerase is a powerful mechanism for switching transcription of a group of genes. For understanding the regulation of σ subunit utilization in *Escherichia coli*, I determined the intracellular levels of four members among 8 known σ subunits of *E. coli* strains, W3110 and MC4100, at various growth phases by a quantitative Western immunoblot analysis. The level of σ^{70} is rather constant throughout the growth, and those of σ^{54} and σ^{28} are maintained at 10 and 50% the level of σ^{70} in both strains growing at exponential and stationary phases, respectively. At the exponential growth phase σ^{38} is undetectable but increases to up to 30% of σ^{70} at the stationary phase, supporting the concept that σ^{38} plays a key role in transcription of the stationary phase-expressed genes. Stress-coupled change in the intracellular level was observed for two σ subunits: an increase in σ^{38} level and a decrease in σ^{28} level upon exposure to heat shock at the exponential phase; and the increase in σ^{38} level under a high osmolality condition at both the exponential and the stationary phases. I also found that the composition of σ subunits is different between bacterial strains, even under the same strain name. For instance, the compositions of $\sigma^{28}$and σ^{38} differ among three W3110 strains.

The growth transition from exponential to stationary phase is accompanied by the replacement of RNA polymerase-associated σ^{70} subunit with σ^{38}. She found that the GST-fused σ^{70} in stationary-phase cell extracts exists as a complex with a new protein, and it was designated Rsd (Regulator of Sigma D). A gene located at 90 min on the *E. coli* chromosome encodes Rsd, and is transcribed from upstream and downstream promoters with σ^{38} and σ^{70} holoenzymes, respectively. Over-expressed and purified Rsd protein can form a complex with σ^{70} *in vitro* but not with any other σ subunits; σ^{54}, σ^{38}, σ^{32}, σ^{28} and σ^{24}. A binding assay with proteolytic fragments of σ^{70} suggested that Rsd interacts with the conserved region 4, the promoter -35 recognition domain, and its downstream of σ^{70}. The isolated Rsd inhibited transcription *in vitro* to various extents depending on the promoters used. The intracellular level of Rsd starts to increase during the transition from growing to stationary phase.

Experiments using strains with disrupted *rsd* gene and those with overproduced Rsd indicated that Rsd is a negative factor for transcription directed by σ^{70} and a positive factor for that by σ^{38}. The results indicate that Rsd is a stationary-phase *E. coli* protein regulating the activity of the σ^{70} function, providing an evidence for the existence of the control of the activities as well as the levels of the σ subunits of RNA polymerase, contributing to a group switching of genes of*E. coli*. Taking all the observations together, she discussed possible models for the switching mechanism.

論文の審査結果の要旨

Selection of a σ subunit of RNA polymerase is a powerful mechanism for switching transcription of a group of genes. For understanding the regulation of σ subunit utilization in *Escherichia coli*, Ms. M. Jishage determined the intracellular levels of four members among 8 known σ subunits of *E. coli* strains, W3110 and MC4100, at various growth phases by a quantitative Western immunoblot analysis. The level of σ^{70} is rather constant throughout the growth, and those of σ^{54} and σ^{28} are maintained at 10 and 50% the level of σ^{70} in both strains growing at exponential and stationary phases, respectively. At the exponential growth phase σ^{38} is undetectable but increases to up to 30% of σ^{70} at the stationary phase, supporting the concept that σ^{38} plays a key role in transcription of the stationary phase-expressed genes. Stress-coupled change in the intracellular level was observed for two σ subunits: an increase in σ^{38} level and a decrease in σ^{28} level upon exposure to heat shock at the exponential phase; and the increase in σ^{38} level under a high osmolality condition at both the exponential and the stationary phases. She also found that the composition of σ subunits is different between bacterial strains, even under the same strain name. For instance, the compositions of $\sigma^{28}$and σ^{38} differ among three W3110 strains.

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The committee concluded that this dissertation fulfills the requirements for the degree of PhD (Science) for Miki Jishage.