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学位論文題目	Physiological and Morphological Changes during the Transition of *Escherichia coli* from Exponential Growth to Stationary Phase
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Upon depletion of essential nutrients from the culture medium, the growth rate of bacteria slows down and eventually reaches zero. At this point the culture enters into the stationary phase, which has been operationally defined as the absence of increase in cell number. The transition from rapid growth, where cells grow with a generation time less than 20 min, into stationary phase is accompanied by adaptation of bacterial cells to the new state. In this period of the transition of *Escherichia coli* culture, a number of morphological and physiological changes take place, including a decrease of cell volume, an alteration of cell shape, a modulation of the nucleoid, an alteration in the components of cell wall, and an accumulation of several storage materials, and alteration of the transcription and the translation machineries. These changes are accompanied by changes in gene expression in such a way that the growth-coupled genes are mostly switched off while the stationary phase-specific genes are up-regulated. More than 100 stationary phase-specific genes have been identified, and these genes appear to be expressed sequentially in a definite order. These findings altogether mean that there is temporal alteration in the *E. coli* phenotype even after the cessation of cell growth.

The morphological and physiological differentiation of *E. coli* during the growth transition was studied in terms of the global regulation of gene expression. Based on the transcriptome analysis by using a DNA microarray assay I identified more than 70 genes that were induced and other 70 repressed in the stationary-phase. Among the induced genes, those whose expression depends on the sigma S (RpoS) were identified by comparison of the transcriptome between the wild-type and a *rpoS* disruptant. To observe the changes in the promoter activities associated with these stationary-phase genes, a novel vector was constructed. It allows expression of two fluorescent proteins in different way: the green fluorescent protein under the control of a test promoter and the other, dsRed protein, under the control of reference promoter. Using this double-reporter vector, the levels and growth phase-dependent variations were determined by FACS for several representative promoters from the exponential phase- and stationary phase-specific genes. This analysis of the promoter activities indicated that the population heterogeneity of *E. coli* culture increases in the stationary phase.

Attempts were then made to fractionate stationary-phase cultures into homogenous populations. Cultures of *E. coli* were separated into more than 15 cell populations, each forming a discrete band after centrifugation with Percoll gradient. The separation resulted from the difference in buoyant density but not the size difference. The cell density increased upon transition from exponential growth to stationary phase. Exponential-phase cultures formed at least 5 discrete bands with lower densities, whereas stationary-phase cultures formed more than 10 bands with higher densities. These findings altogether

suggest that the growth phase-coupled transition of *E. coli* phenotype is discontinuous. Two molecular markers characterizing each cell population were identified: the functioning promoter species, as identified by measuring the expression of green fluorescent protein under the control of test promoters; and the expressed protein species, as monitored by quantitative immunoblotting. The analysis of chemical composition revealed that significant increase was observed only for polysaccharides. In concert with this finding, glycogen granules were found to accumulate in the stationary-phase cells as revealed by thin section microscopy. This finding suggests that at least one component, which contributes the increase in cell density is polysaccharides.

As an initial attempt to identify the gene or genes involved in each step of cell density increase, a random screening was performed, by analyzing the Percoll centrifugation pattern of a set of *E. coli* mutants, each with deletion of a large segment of the genome. Among of a total of mutant strains tested, the density increase stopped for mutants at specific steps, forming discrete intermediate bands along Percoll gradient. One or more genes within the deleted genome segments must be involved in the density shift during the growth transition of *E. coli* into stationary phase. In parallel, I also tested some of the known stationary-phase genes in the cell density shift. As an initial attempt, the role of RNA polymerase sigma S.(RpoS) in the cell density shift was examined. The *rpoS* disruptant formed apparently a single low density band even in the stationary phase, and the growth phase-coupled density increase was small. Even after prolonged culture, no further increase in the cell density was observed for this *rpoS* disruptant. Thus I concluded that the growth phase-coupled increase in *E. coli* cell density ceased at an early stage for the *rpoS* disruptant. These findings of mutant studies indicate that a specific gene or a set of genes are involved in each step of the cell density increase during the transition period from exponential growth to stationary phase, and the RpoS sigma factor is one such factor that is needed at an early step of the cell density increase.

論文の審査結果の要旨

バクテリアは培養液中の栄養源の枯渇により、増殖を止め定常期に入る。大腸菌では、２０分程度で分裂し増殖する対数増殖期から定常期へ移行し、さらに何日もかけながら、様々な形態や遺伝子発現の異なる生理的状態を取ることが知られている。しかし、その変化の詳細はもとより、対数増殖期から定常期への変化と、定常期内での変化は、遺伝子発現や生理状態、形態の変化のシグナルとシグナル伝達もほとんど理解されておらず、未開拓な分野である。この問題に挑戦するために牧野嶋君は、転写を中心とした状態の変化と、電子顕微鏡による形態の変化、様々な物質の蓄積状態を調べると共に、定常期の細胞が、どの程度均一な集団からなっているのかを調べた。それらの状態の記述的なデーターとして、GFP と dsRED の発現を利用した多数のプロモーター活性の比較、FACS による細胞での発現の不均一性の検証等、かなりの量の観察をおこなった。

牧野嶋君のこの一連の研究における最大の貢献は、定常期の細胞は、１５以上の不連続な比重の値を持つ異なる集団から構成され、その構成も経時的に変化することを明らかにしたことである。特に定常期の細胞が不連続な比重を持つことは、大きな驚きをもって、バクテリオロジーでは国際的にも注目された。

次に牧野嶋君は、比重が不連続になる機構を解明すべく、様々な実験をおこなった結果、DNA や RNA、鞭毛の数などには、比重は依存しなかったが、電子顕微鏡観察でのグリコーゲン顆粒と推定されるものが関与する可能性が示された。また、破壊すると細胞の比重の分布パターンが変化する幾つかの遺伝子を同定した。代表的なものは、定常期への移行の時に発現する転写開始因子、rpoS である。また研究の副産物として、rpoS 欠損株では、定常期にほとんど鞭毛が無くなることを見出し、鞭毛の遺伝子発現と rpoS との関連を初めて明らかにした。

これらの成果は、膨大な実験をこなした上での新規の発見を含んでいるので、学位論文としての必要条件を満たすとの結論に到達した。