Loss of Flagellation in dnaA Mutants of Escherichia coli

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A dnaA46 mutant of Escherichia coli showed loss of motility at 37°C, a permissive temperature for cell growth of this mutant. Other dnaA mutations near the middle of the gene also caused an immotile phenotype. The amount of flagellin was much less in the dnaA46 mutant than in the wild-type control, as was the promoter activity. DnaA protein may play an important role in expression of the fliC gene.

Bacteria swim by the propeller function of flagella, and this motility is important for the cell to acquire nutrients (1, 2, 8). The formation of flagella is strictly regulated and is coupled with stages of cell proliferation. For example, flagellum formation initiates at a late period of exponential growth (17) and ceases when cells are exposed to various stresses which inhibit growth (11). Flagellin, a subunit of flagella, suppresses its own synthesis under conditions which are adverse. Therefore, cell proliferation and flagellation formation may be coregulated.

Nishimura and Hirota reported that some temperature-sensitive mutants of cell growth and cell division showed a defective phenotype of motility (13). Repression of flagellin synthesis as a result of mutations in heat shock protein genes, the dnaK and dnaJ genes, which are essential for chromosomal DNA replication, has also been reported (16). Therefore, mechanisms which regulate chromosomal DNA synthesis may effect the synthesis of flagellin. DnaA protein is the initiator of chromosomal DNA synthesis in Escherichia coli (5, 7). The abundance of this protein in cells (800 to 1,200 molecules per cell) (15) and the presence of DnaA boxes to which DnaA protein specifically binds in the upstream regions of various genes, including the dnaA gene itself (3, 4, 10, 19), suggest that DnaA protein acts as a transcription factor of various genes. On the basis of the thesis that DnaA protein may be involved in the cooperative regulation of cell growth and flagellation synthesis, we did a series of experiments, the descriptions of which are given here.

dnaA mutants and their isogenic parent, AQ5425 (metE46 trp-3 his-4 thi-1 galK2 lacY1 or lacZ4 met-l ara-9 tsx-3 ton-l rpsL8 or rpsL9 supE44 maa::TnlO λ-) (6), were kindly provided by T. Kogoma (University of New Mexico) via T. Katayama. pHB10 DNA with the wild-type dnaA gene (14) was kindly provided by Y. Sakakihara. pflIC-lacZ DNA and its vector were kindly provided by K. Kutsukake. pflIC-lacZ DNA was constructed by fusion of FZY1 (9) and the promoter region of the fliC gene of Salmonella typhimurium (10a). Formation of swarm rings was observed in semisolid agar containing 1% tryptone, 0.5% NaCl, 50 μg of thymine per ml, and 0.3% agar (2). Each strain was cultured to the stationary phase in Luria-Bertani (LB) medium supplemented with 50 μg of thymine per ml. At the time of full growth, the suspension (2 μl) was placed on the swarm plate, and the preparation was incubated at 37°C for 6 h.

We compared the motility phenotype of the AQ5480 strain, with the dnaA46 mutation, with that of AQ5425, the parent strain with the wild-type dnaA gene, by measuring diameters of swarm rings in soft agar plates. As shown in Fig. 1, the swarm ring formed with the dnaA46 mutant was much smaller than that formed with the wild-type strain.

To confirm that the loss of motility in the AQ5480 strain was caused by the dnaA4 mutation, we transformed the strain with plasmid pHBI0 DNA having the wild-type dnaA gene and examined the motility of the transformants. The transformants were highly motile in the swarm assay (Fig. 1), and recovery of motility was nil when the cells were transformed with the vector, pBR322 (data not shown). Transformation of the wild-type strain with pHBI0 did not affect motility (data not shown).

We then examined whether the motility phenotype of the dnaA46 mutation would be cotransducible with the temperature-sensitive phenotype in P1 transduction experiments. In the AQ5480 cells, Tnl0 with the tetracycline resistance marker is located at a position close to the dnaA gene. P1 phages were grown in AQ5480 cells, whose growth was restricted at 42°C, and transduced into W3110 cells, and the transductants were selected on tetracycline plates; the temperature sensitivity and motility phenotypes were then examined. Of 100 independent transductants, 21 were high temperature resistant and motile, and all of the remaining 79 were temperature sensitive and immotile. There was no transductant with either high-temperature-resistant and immotile or temperature-sensitive and motile phenotypes. Thus, the temperature-sensitive and immotile phenotypes of AQ5480 cells were cotransducible.

At 28°C, strain AQ5480 had much the same motility as wild-type cells in the swarm assay (data not shown). Since growth of the AQ5480 strain is temperature sensitive, there remained the possibility that the loss of motility was the result of inhibition of cell proliferation at 37°C. We then compared the growth of AQ5480 at 37°C with that of AQ5425. The time required for generation of these strains in liquid medium, determined either by measuring the number of viable cells which formed colonies on agar plates (Fig. 2) or by measuring culture absorbance, was approximately 60 min, and the size of colonies on agar plates of these strains grown at 37°C was indistinguishable. We isolated more than 10 independent suppressor mutants from the AQ5480 strain, with high-temperature-resistant (42°C) and immotile phenotypes. We carried out a transductional backcross of the dnaA region in the four independent revertants to W3110 cells having the wild-type dnaA gene. P1 phage was proliferated in the revertants and transduced into W3110 cells. Fifty independent transductants were isolated on tetracycline plates, and their temperature sensitivity and motility were examined. In all cases, 12 to

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15 transductants showed temperature-resistant and motile phenotypes, and other transductants showed temperature-sensitive and immotile phenotypes. These results suggest that all of these four revertants having high-temperature-resistant and immotile phenotypes carry an intergenic suppressor mutation of the \textit{dnaA} gene. We also isolated motile revertants from the AQ5480 strains and examined their temperature-sensitive growth phenotype. Of 33 independent revertants, 8 were temperature sensitive and 25 were temperature resistant. From these results, we concluded that the immotile phenotype had no accompanying temperature-sensitive phenotype.

\textit{dnaA} mutants have been isolated, and their mutated sequence positions were determined. To observe the relationship between the location of mutations in the \textit{dnaA} gene and the extent of the loss of motility phenotype, we examined the motility phenotypes of various \textit{dnaA} mutants at 37°C. All of these mutants grew normally at 37°C but not at 42°C. As shown in Fig. 3, some \textit{dnaA} mutations, including \textit{dnaA446} and \textit{dnaA4604}, showed a clear phenotype of the loss of motility, whereas other \textit{dnaA} mutations, including \textit{dnaA203}, \textit{dnaA204}, and \textit{dnaA508} were motile. This means that the N- and C-terminal regions of DnaA protein are not likely to be required for the motile phenotype.

We then asked whether the immotility phenotype of AQ5480 is due to the lack of formation of flagella. Flagella can be released from cells by vigorous vortexing of the bacterial cell suspension (18). We analyzed flagellum fractions of the AQ5480 (\textit{dnaA46}) and the AQ5425 (wild-type) cells grown at various temperatures. As shown in Fig. 4, the amount of flagellin, a subunit of flagella, in the AQ5480 cells growing at 37°C was much less than that in the AQ5425 cells. In the AQ5425 cells, the amount of flagellin was constant at temperatures between 28 and 37°C, and at temperatures below 31°C, a significant amount of flagellin was observed in the AQ5480 cells.

The synthesis of flagellin is regulated at the level of transcription (8). We examined the promoter activity of the \textit{fliC} gene in AQ5480 cells by using the transcriptional fusion plasmid \textit{pflC-lacZ} containing the promoter of the \textit{fliC} gene fused with the \textit{lacZ} gene. Cells transformed with the fusion plasmid were grown at various temperatures, and \beta-galactosidase activities in the cell extracts were determined. As shown in Fig. 5, the promoter activity of the \textit{fliC} gene in the AQ5480 cells decreased concomitant with temperature shift-up and yet was constant in the AQ5425 cells, despite a change in temperature. Extracts prepared from cells transformed with the vector showed a low level of enzyme activity, thereby indicating that \beta-galactosidase was synthesized, dependent on transcription from the \textit{fliC} promoter of the fusion plasmid. These results suggest that the inhibition of flagellum synthesis in the \textit{dnaA46}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Swarm plate assay. Full-growth suspensions of AQ5480 (\textit{dnaA46}), AQ5425 (\textit{dnaA}\textsuperscript{+}), and AQ5480 transformed with pHBl0 (\textit{dnaA46/dnaA}\textsuperscript{+}) were placed on a soft agar plate, incubated at 37°C, and then photographed.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure2.png}
\caption{Growth of AQ5480 (\textit{dnaA46}) and AQ5425 (\textit{dnaA}\textsuperscript{+}) cells at 37°C. Cells were grown in LB medium supplemented with 50 \mu g of thymine per ml at 37°C for the indicated time period. Appropriate dilutions of the culture were plated to monitor growth.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Swarming abilities of various \textit{dnaA} mutants. Diameters of swarm rings on the soft agar plates at 37°C after 6 h were measured. The diameter of the motility ring of the wild-type cells was 60 mm. The mutation positions of each \textit{dnaA} mutant are from reference 6.}
\end{figure}
The possibility that the lacZ expression is due to the inhibition of transcription of the fliC gene. The possibility that the lacZ expression is regulated by some sort of posttranscriptional control would need to be ruled out.

In this article, we suggest that DnaA protein plays an important role in expression of the fliC gene. With regard to the manner in which DnaA protein participates in transcription of the fliC gene, two possibilities are being entertained: one is that DnaA protein acts as a transcription factor of the fliC gene or other regulatory genes of flagellation, and the other is that adverse effects on DNA synthesis caused by dnaA mutations reduce expression of the fliC gene, even though the period of generation of cell growth is not prolonged.

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