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Cell Cycle Regulation of Flagellar Genes

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The expression of the flagellar master operon, *flhDC*, peaked in the middle of three consecutive cell cycles. The level of expression was lowest at the time of cell division. The expression of the second-level operon, *flhB*, peaked at cell division. The swimming speed of individual cells was also highest at the time of cell division.

The expression of flagellar genes in response to environmental stress takes place on multiple transcriptional levels (7). At the top of this hierarchy is the flagellar master operon, *flhDC*, which acts as a transcriptional activator (2) for the downstream flagellar genes. The expression of *flhDC* is tightly regulated by biochemical and molecular signals (11, 14, 15, 17).

Connections between FlhD and other stress response systems have been demonstrated recently (3–5). The question of whether FlhD is a global regulatory protein involved in many stationary-phase processes in the cell has been raised (12).

One function of FlhD, other than the regulation of flagella, is the regulation of the cell division rate (12). Cells with mutations in FlhD divided at a faster rate than wild-type cells in stationary phase. The regulation of the cell division rate by FlhD involves the acid response gene *cadA* (13). Earlier, Nishimura and Hirota had found that the expression of flagella by cell division mutants and DNA replication mutants decreased when the nonpermissive temperature was reached (10). It was proposed that the expression of flagella is regulated by the bacterial cell cycle. The possibility of an involvement of FlhDC in this regulation was discussed.

It has now been demonstrated that the expression of flagellar genes is indeed regulated throughout the cell cycle. By using fusions to the reporter gene *lacZ*, it has been shown that the level of expression of the master operon, *flhDC*, is highest in the middle of the cell cycle. The expression of the second-level operon, *flhB*, increases toward the end of the cell cycle and is highest at the time of cell division. The swimming speed of individual cells is also highest at the time of division.

Growth phase regulation of *flhDC* expression. Amsler et al. (1) determined that the expression of the second-level operon, *flhB*, peaked in late exponential phase. The swimming speed of individual cells was highest in post-exponential phase.

The growth phase dependence of *flhDC* expression was determined. An *flhDC*::*lacZ* construct (15) was kindly provided by C. Park (Korea Advanced Institute of Science and Technology, Taejon, South Korea) and introduced, via P1 transduction (16), into the parent strain YK410 [F^- *araD139* Δ*lac*(*U169*) *strA thi pyrC46 nalA thyA his* (8)]. Bacteria of the resulting strain, BP64, were grown at 34°C in tryptone broth (TB; 1% tryptone, 1% NaCl) after inoculation from an overnight culture. The expression of *flhDC* corresponded to the activity of β-galactosidase (9).

Figure 1 shows the growth phase regulation of *flhDC* expression. During the first 30 min of growth, the total activity was so low that a statement about the expression level cannot be made. The level of FlhDC expression was highest during the exponential phase between an optical density at 600 nm (OD_{600}) of 0.04 to 0.1 and a cell density of 3.5×10^7 to 7×10^7 cells/ml. Maximum expression was first reached at mid-exponential phase. After 1.5 h of growth, the expression of *flhDC* decreased steadily. A second increase in expression was observed after 3 h of growth during the transition to stationary phase.

According to Amsler et al. (1), the expression of *flhB* was also growth phase dependent. Expression from a *flhB*::*lacZ* fusion was at its peak in late exponential phase. This corresponds to an OD_{600} of 0.2 to 0.3 and a cell density of 10^8 cells/ml under our growth conditions.

Expression of the master operon, *flhDC*, peaks in the middle of the cell cycle. Bacteria were synchronized with a modification of the membrane filtration technique, or "baby machine" (6), as described previously (12). Cells were grown in TB for 3 h. Cells were then loaded onto a 0.22-μm-pore-size nitrocellulose filter (Millipore Corp., Bedford, Mass.) coated with poly(D-lysine). The filter was inverted, and TB was pumped through it at a speed of 1 ml/min. Cells were checked microscopically for homogeneity of size. Freshly divided baby cells eluted after approximately 20 min and were collected on ice. These cells were used to start a synchronously growing culture.

Figure 2A shows the cell cycle dependence of *flhDC* expression. The total activity of β-galactosidase increased stepwise. During three consecutive cell cycles (cycles two to four), the

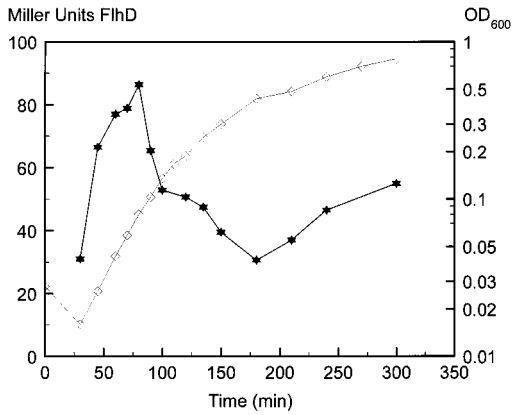

FIG. 1. Expression of *flhDC* in an asynchronously growing culture. Cells were grown in TB over a time period of 5 h at 34°C. The open diamonds represent the OD_{600} (right *y* axis), and the stars represent the expression of *flhDC* as the activity of β-galactosidase (in Miller units; left *y* axis). The experiment was done twice, and the means for the populations were determined.

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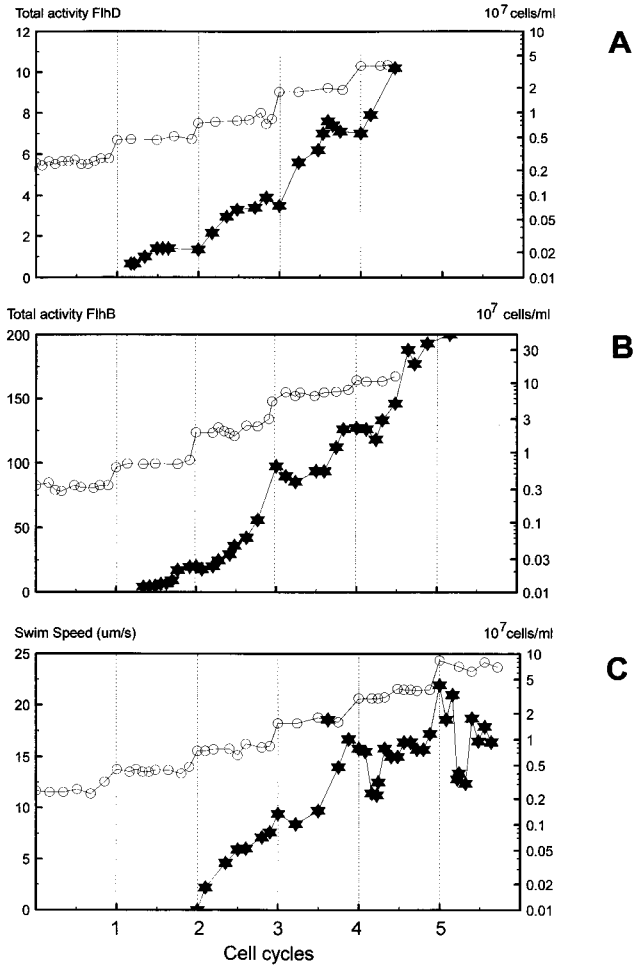

FIG. 2. Expression of *flhDC* (A) and *flhB* (B) and swimming speed (C) of cells in a synchronously growing culture. Freshly divided baby cells were grown in TB over a time period of 3 h at 34°C. The open circles represent the number of cells per milliliter (right *y* axis), and the stars represent the expression of *flhDC* (A) or *flhB* (B) in units of total activity of β-galactosidase or the swimming speed (C) in units of micrometers per second (left *y* axis). Vertical lines represent the cell divisions. The experiment was done two to three times, and the means for the populations were determined. Out-of-range data were included but not connected to the other points.

expression from the *flhDC* promoter increased during the first half of each cycle, reached a plateau at midcycle, and stayed at a relatively steady level until cell division. Alternatively, the specific activity of β-galactosidase (in Miller units) was plotted (data not shown). The specific activity increased during the first half of cycles two to four, as demonstrated for the total activity. Toward the end of each cycle, the specific activity decreased due to an increase in the OD.

Expression of the second-level operon, *flhB*, peaks at the time of cell division. A *lacZ* gene, fused transcriptionally to the second-level operon, *flhB*, was obtained from strain MC453 (1) and transduced into strain YK410.

Figure 2B shows the cell cycle dependence of *flhB* expression. FlhB expression increased stepwise during cycles two to five. During these four consecutive cell cycles, the expression increased during the second half of each cycle and reached a maximum level at or just prior to cell division. This is, on average, half a cell cycle later than the expression maximum of *flhDC*. The total activity of β-galactosidase stayed relatively steady over the course of cell division.

Swimming speed. The swimming speed of cells was determined according to the protocol of Amsler et al. (1). Samples were videotaped and processed with a VP110 video processor (Motion Analysis Corp.). Computer motion analysis programs were developed with CellTrak software (Motion Analysis Corp.).

The samples from the two previous experiments were videotaped immediately after collection and analyzed (Fig. 2C). With respect to growth phase, the swimming speed of the cells peaked at an OD_{600} of 0.25 to 0.3 and a cell density of 10^8 cells/ml. The highest cell speed was observed at the time of division; it dropped immediately after the division.

Expression of *flhD* and *flhB* and the swimming speed of individual cells are regulated in a timely order. The data presented in this paper indicate the consecutive increase in the level of master operon expression, second-level gene expression, and, finally, swimming speed. This consecutive order can be seen in both the growth phase dependence and the cell cycle dependence of *flhD* expression, *flhB* expression, and swimming speed.

With respect to growth phase regulation, *flhD* expression, *flhB* expression, and swimming speed of individual cells reach their maxima at OD_{600}s of 0.08, 0.2, and >0.3, respectively. This is consistent with the data of Amsler et al. (1), who observed the highest level of expression of *flhB* in late exponential phase and the highest swimming speed in post-exponential phase.

With respect to cell cycle regulation, *flhD* expression peaks in the middle of a cycle and *flhB* expression peaks half a cycle later. The swimming speed was highest at the time of cell division. Since the peak in *flhB* expression at the second cell division did not lead to an increase in swimming speed at the same time, it could as well be that the formation of flagella takes one full cycle.

Among the stresses that regulate the expression of *flhDC* are catabolite repression (17), heat shock (14), and the concentration of acetyl phosphate (11), mediated by phosphorylation of OmpR (15). Any one of these could be involved in the cell cycle regulation of *flhDC*.

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