Temporary Expression of Flagellar Phase-1 in Phase-2 Clones of Diphasic Salmonella

By T. IINO AND TOMOKO OGUCHI
Laboratory of Genetics, Faculty of Science, University of Tokyo, Hongo, Tokyo, Japan
AND T. HIRANO
Tokyo Metropolitan Isotope Research Center, Setagaya, Tokyo, Japan

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SUMMARY

Diphasic Salmonella strains, Salmonella typhimurium TM2, S. abony sw803 and their derivatives differing in flagellar shape and antigen type, were found to produce copolymer segments of phase-1 and phase-2 flagellins among flagella in phase 2, except for a strain which is non-flagellate in phase 1. The copolymer segments were not detected in phase-1 clones of any of the strains. The wave-forms of the copolymers are homologous with those of the copolymer filaments obtained by in vitro reconstitution of the corresponding phase-1 and phase-2 flagellins. Thus, in the mutant producing normal flagella in phase 1 and straight ones in phase 2, copolymer segments with curly or small waves appear among the straight filaments.

Formation of the copolymers was attributed to temporary derepression of the structural gene for phase-1 flagellin, H1, in phase 2. Copolymerization occurred in a fraction of the phase-2 cell population at late exponential and early stationary phase in nutrient broth cultures. When a phase-2 cell was temporarily derepressed, the copolymers formed almost simultaneously in every growing flagellar filament of the cell. Their formation continued for a short period until the supply of phase-1 flagellin was exhausted after re-establishment of repression. This period was estimated to be 7.7 min on average, fluctuating between 4 and 13 min in a cell population of a straight flagellar mutant whose generation time was 55 min in late exponential phase.

INTRODUCTION

A diphasic Salmonella strain possesses two non-allelic structural genes for flagellin, the component protein of flagellar filaments. They were termed H1 and H2. These two genes are activated alternately in a bacterial clone and their alternating expression results in the characteristic phenomenon of phase variation (Lederberg & Iino, 1956). The cells producing flagella composed of H1 or H2 product are said to be in phase 1 or phase 2, respectively. In an ordinary diphasic Salmonella, the alternation between the two phases occurs with a probability of $10^{-3}$ per cell division (Stocker, 1949). Genetical (Pearce & Stocker, 1967; Fujita, Yamaguchi & Iino, 1973) and biochemical (Suzuki & Iino, 1973) studies showed that H2 constitutes an operon together with rhl which produces a repressor of transcription of H1. When the H2-operon is active, phase-2 flagellin is produced and simultaneously H1 is repressed by the product of rhl. When the H2-operon becomes inactive, mRNA for phase-1 flagellin, and consequently phase-1 flagellin itself, start to be synthesized. The present
Table I. Bacteria

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phase 1</th>
<th>Phase 2</th>
<th>Characteristics and derivation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM2</td>
<td>i-N</td>
<td>1,2-N</td>
<td>Wild-type <em>Salmonella typhimurium</em></td>
<td>Lederberg &amp; Iino (1956)</td>
</tr>
<tr>
<td>sw803</td>
<td>b-N</td>
<td>e,n,x-N</td>
<td>Wild-type <em>Salmonella abortus</em></td>
<td>Lederberg &amp; Iino (1956)</td>
</tr>
<tr>
<td>SJ770</td>
<td>i-N</td>
<td>1,2-S</td>
<td>Straight flagella mutant derived from TM2</td>
<td>Iino &amp; Mitani (1967)</td>
</tr>
<tr>
<td>SJ814</td>
<td>NF</td>
<td>1,2-S</td>
<td>O-H variant derived from SJ770</td>
<td>Asakura &amp; Iino (1972)</td>
</tr>
<tr>
<td>SJ4067</td>
<td>b-N</td>
<td>1,2-S</td>
<td>Recombinant of SJ814 with <em>H1</em>-b transduced from sw803</td>
<td></td>
</tr>
<tr>
<td>SJ4142</td>
<td>a-N</td>
<td>1,2-S</td>
<td>Recombinant of SJ814 with <em>H1</em>-a transduced from a phase-1 clone of <em>Salmonella abortus-equus</em> SJ241</td>
<td>Iino (1962)</td>
</tr>
<tr>
<td>SW577</td>
<td>i-C</td>
<td>1,2-N</td>
<td>Phase-1 curly flagella mutant of <em>Salmonella typhimurium</em></td>
<td></td>
</tr>
<tr>
<td>SJ4158</td>
<td>i-C</td>
<td>1,2-S</td>
<td>Recombinant of SJ814 with <em>H1</em>-i transduced from a derivative of sw577</td>
<td></td>
</tr>
<tr>
<td>SJ4159</td>
<td>i-C</td>
<td>1,2-N</td>
<td>Recombinant of SJ4158 with <em>H2</em>-1,2 transduced from TM2</td>
<td></td>
</tr>
</tbody>
</table>

N, Normal; S, straight; C, curly; NF, non-flagellate.

paper reports that under certain growth conditions the repression of *H1* in phase-2 clones is not complete and consequently copolymer filaments of phase-1 and phase-2 flagellins are temporarily produced during the growth of phase-2 flagella.

METHODS

*Bacteria.* Salmonella strains used in the present experiment are listed in Table 1. The recombinants, SJ4067, SJ4142, SJ4158 and SJ4159, were obtained by phage P22-mediated transduction following the procedure of Lederberg & Iino (1956).

*Cultivation.* Nutrient broth comprised 1% (w/v) peptone, 1% (w/v) meat extract and 0.25% (w/v) NaCl. Nutrient agar was prepared by the addition of 1.2% (w/v) agar to the nutrient broth. To obtain phase-specific bacterial samples, cultures were initiated in each experiment from colonies grown on a nutrient agar plate. Their predominant flagellar phases were identified by a slide agglutination test with the phase-specific anti-flagellar sera (Iino & Enomoto, 1966). They were then transferred to nutrient broth and incubated at 37°C without shaking. Cell numbers were counted with a bacterial counting chamber (Elma Co., Tokyo).

*Electron microscopy.* The bacterial specimens were fixed with formalin to a final concentration of 5% (v/v), kept for at least 1 h at room temperature, then harvested by centrifugation and suspended in de-ionized water. The final concentration of bacteria was adjusted to approximately 10^6/ml. Droplets of each suspension were placed on to a Formvar-coated copper grid, and after 2 min the excess solution was removed by touching the edge of the grid with a piece of filter paper. When the antibody labelling method (Elek, Smith & Highman, 1964; Asakura, Eguchi & Iino, 1968) was applied, droplets of specific antiserum of agglutination titre 1000 were then placed on to the grid. After 2 min, its wet surface was brought into contact for a few seconds with the surface of a large volume of de-ionized water and the antiserum was washed off. Excess water was removed, and droplets of 1% (w/v) phosphotungstic acid solution pH 6.8 were placed on to the grid. The excess solution was removed after 2 to 3 min and the sample quickly dried in a vacuum. The specimens were
examined in a JEM-100U electron microscope. Electron micrographs were taken at initial magnifications of \( \times 5000 \) or \( \times 10000 \). As a standard for magnification, polystyrene latex particles (diam \( 0.7900 \pm 0.0044 \) \( \mu \)m; Dow Chemical Co.) were photographed simultaneously.

**RESULTS**

Detection of copolymer segments composed of phase-1 and phase-2 flagellins in phase-2 flagella of a straight flagellar mutant

Salmonella strain \( \text{SJ}4067 \) produces b-normal and 1,2-straight type flagella in phase 1 and phase 2, respectively (Table 1). When its phase-2 cells were in early stationary phase in nutrient broth, their flagella were examined by electron microscopy. Unexpectedly, short segments, up to 3.2 \( \mu \)m in length, were found to be wavy in 10 to 20 \% of the straight flagella. These wavy segments must have been copolymers of both b-type and 1,2-type flagellins, since when the flagella were treated with either anti-b or anti-1,2 serum, their straight portions were stained only by the latter while the wavy segments were stained homogeneously by either (Fig. 1). In form they were either curly or in small waves (compare Fig. 1a with Fig. 1c and \( d \)), belonging to a group of small wave-forms manifested by \textit{in vitro} copolymers of normal and straight flagellins (Asakura & Iino, 1972).

The possibility that these copolymers were formed in a transient stage of ordinary phase variation from phase 2 to phase 1 is excluded for the following reasons. (i) Under our culture conditions, the cells carrying b-type normal flagella, i.e. in phase 1, were detected in less than 0.5 \% of the cells in the population, as expected from the frequency of phase variation (Stocker, 1949), while the maximum frequency of those carrying the copolymers was as high as 20 \%. (ii) The copolymer segments were detected more frequently at an intermediate portion of a phase-2 flagellar filament than at the tip. Because a flagellar filament grows only at its tip (Asakura \textit{et al.} 1968; Iino, 1969; Emerson, Tokuyasu & Simon, 1970), a copolymer segment at the middle of a filament means that it was formed over a short period of time during the growth of the phase-2 filament and thereafter the filament grew again only with phase-2 flagellin.

In a diphasic Salmonella clone, mRNA for phase-1 flagellin synthesis is produced only when repression of \( \text{Hr} \) is removed (Suzuki & Iino, 1973). Therefore, the copolymer segments observed here must have been produced by temporary derepression of \( \text{Hr}-b \) in a phase-2 clone.

When phase-1 clones of \( \text{SJ}4067 \) were examined under the same experimental conditions as described for the phase-2 clones, none of the b-normal filaments among 300 examined carried a segment characteristic of the copolymer.

**Growth-phase dependence of copolymer formation**

The copolymer segments were looked for in phase-2 clones of \( \text{SJ}4067 \) at various growth phases after the cells were inoculated into nutrient broth. When bacteria in early to middle exponential phase were examined, the copolymer filaments were not detected among 300 flagella. Among bacteria entering late exponential phase, 2 to 3 \% of straight flagella carried them. Thereafter, their frequency increased up to 20 \% until the culture reached stationary phase.

Confirmation of this growth-phase dependence of the copolymer formation was obtained by further, reproducible, experiments with cultures grown from various initial inoculum sizes at temperatures between 25 and 37 \(^{\circ} \)C.
Fig. 1. Flagella of Salmonella strain SJ4067: (a) normal in phase 1; (b) straight in phase 2; (c) and (d) straight with segments of short wave copolymers in phase 2. (a), (b) and (c) were stained with b-antibody specific for phase 1, and (d) with 1,2-antibody specific for phase 2.
Fig. 2. Phase-2 cells of *S. typhimurium* carrying the copolymer segments on their flagella: (a) SW577; (b) TM2. Both were stained with i-antibody specific for phase 1.

Copolymer formation in other *Salmonella* strains

Copolymer formation during late exponential phase was surveyed in the strains listed in Table 1.

Strains SJ770 and SJ4142 produced 1,2-straight flagella in phase 2 and normal flagella in phase 1, but their phase-1 flagella are different from those of SJ4067 in their antigenicity. On both of these strains, the short waved copolymer segments stainable by both phase 1- and phase 2-specific antisera were detected among the straight flagella of their phase-2 cells.

Strain SJ814 carries the same *H2* gene as the above three strains but is non-flagellate in phase 1 because it carries *ahr*- the inert operator of *H1* (Iino, 1961; Suzuki & Iino, 1973). The copolymer segments were not detected on phase-2 cells of this strain among 500 flagella examined.

Strains SW577, SJ4158 and SJ4159 produce i-curl flagella in phase 1 and either 1,2-normal or 1,2-straight flagella in phase 2. The copolymer segments in phase-2 flagella were detected in all three strains as curly or short waves (Fig. 2a) homologous with those obtained by *in vitro* copolymerization of the corresponding flagellins (Asakura & Iino, 1972).

The wild-type *Salmonella* strains, TM2 and SW803, have normal flagella in both phases. Therefore, it is impossible to identify the copolymer segments morphologically. However, when their flagella were treated with antiserum specific for the corresponding phase-1 flagella, segments stained by the antibody were detected (Fig. 2b). Their frequencies were similar to those of the copolymer segments in phase-2 cells of SJ4067; 500 flagellar filaments treated with antiserum specific for the corresponding phase-2 flagella were stained throughout.

Thus the copolymer segments of phase-1 and phase-2 flagellins were produced in phase-2 cells of all diphasic *Salmonella* strains examined, except one which is non-flagellate in...
Table 2. Distribution of the cells of S14067 carrying various numbers of copolymer flagella

Total number of cells observed, 244. Average ratio of straight filaments to those carrying copolymer, 3.8. For an example of how to interpret the Table, see the footnote.

<table>
<thead>
<tr>
<th>No. of flagellar filaments on a cell which were:</th>
<th>Straight</th>
<th>Carrying copolymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 1 2 3 4 5 6 7 8 9 10</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>41 6 3 2 1 1 1 3 2 3 0 0</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>39 1 0 0 2 0 0 0 0 0 1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>36 3 0 1 0 2 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>29 0 4* 1 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>23 0 1 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>14 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>12 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>5 0 0 0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3 0 0</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1 0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*As an example, '4' here means that there were four cells (out of the 244 observed) which had 3 straight flagella and 2 copolymer-carrying flagella.

phase-1 because of ahf-). In every strain, they were detected in between 2 and 20% of the flagella of the phase-2 cells examined. None of the copolymers was detected among 200 to 300 flagella on phase-1 cells of these strains. Because of the ease of identification of the copolymer segments, phase-2 cells of S14067 treated with anti-b serum were used exclusively for further detailed observations.

Distribution of the copolymer segments among the cells and flagella

Distribution of the copolymer segments among phase-2 bacteria of S14067 indicated that they did not appear randomly among the flagella but appeared on the whole cell as a unit: if they appeared on one flagellum of a cell, then they appeared on most or all flagella of that cell (Table 2). A cell carrying the copolymers will hereafter be termed a 'derepressed cell'.

A copolymer segment was detected most frequently between two straight portions of a filament (I-type; Fig. 3a) and less frequently at the tip (T-type; Fig. 3b). Occasionally it appeared at its base (B-type; Fig. 3a) or throughout the whole region of a short filament (W-type; Fig. 3b). As a flagellar filament grows at its tip (Asakura et al. 1968; Iino, 1969; Emerson et al. 1970), both T- and W-type filaments are interpreted as undergoing copolymer formation at the time of fixing and I- and B-type filaments as having undergone copolymer formation followed by re-establishment of phase-1 flagellin synthesis.

Combination of these types in a derepressed cell was found to be non-random and most of the cells could be classified into two groups (Table 3; Fig. 3). The cells belonging to the predominant group, R ('re-repressed'), carried only filaments in which copolymer formation had finished (i.e. either of I-type or of both I- and B-types). Out of 52 cells belonging to group R found among the 75 derepressed cells observed, 48 were composed of I-type filaments only, and each of the other four cells carried one B-type filament in addition to the I-type. The cells of the second group, D ('derepressed'), on the other hand, carried only filaments in which copolymer formation was still occurring (i.e. either of T-type or of both T- and W-types), irrespective of the variation in length of the filaments on a cell. Eighteen
Temporary derepression in Salmonella

Fig. 3. Derepressed cells of s14067: (a) an R-group cell with I- and B-type flagella; (b) a D-group cell with T- and W-type flagella. Both stained with b-antibody specific for phase 1.

cells belonged to this group, of which 13 carried only T-type and five both T- and W-type filaments. There was thus a clear-cut separation of the cells carrying copolymer into the two groups D and R, since 93 % of the derepressed cells (70 out of 75) definitely belonged to one or other of these groups.

From this we inferred that the copolymers are formed almost simultaneously at the growing tips of the flagellar filaments of a derepressed cell. Consequently, assuming that the longer filaments generally initiated their growth earlier than the shorter ones on the same cell, the straight portion proximal to the cell must be longer in them. This prediction was
Table 3. Distribution of the derepressed cells of S14067 carrying flagella in the course of or having finished copolymer formation

For examples of how to interpret the Table, see the footnotes.

No. of flagella on a cell:

<table>
<thead>
<tr>
<th>In the course of copolymer formation (T- and W-types)</th>
<th>Having completed copolymer formation (I- and B-types)</th>
<th>Total no. of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 (R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D, Cells belonging to group D; R, cells belonging to group R.
* That is, the number of cells which had 1 flagella in the course of copolymer formation and 3 flagella which had finished copolymer formation, was one.
† That is, a total of ten cells was observed which had 1 flagella in the course of copolymer formation.
‡ That is, there were eighteen cells which possessed 2 flagella which had completed copolymer formation.

studied for each of the derepressed cells. On an R-group cell, B-type filaments were shorter than all I-type filaments and the position of the copolymer on a longer I-type filament was more distal from the cell than on a shorter I-type. Similar relationships were observed for W-type filaments and T-type filaments of various lengths on a D-group cell. Typical results are shown in Fig. 4.

The difference between the overall length and the length of the proximal straight portion...
Temporary derepression in Salmonella

![Graph showing correlation between the length of the proximal straight portion (μm) and the length of the copolymer portion (μm). Each closed circle denotes one flagellar filament. I-type filaments on a single cell are connected by a solid line. Ten representative cells differing in the distribution of copolymer length in their I-type filaments were chosen from three independent experiments. O, Average lengths of flagellar filaments of S. typhimurium TM2 grown in 1 h at late exponential phase after the filaments reached the lengths indicated by the abscissa (Iino, 1974).

of either I- or T-type filaments (i.e. the amount of growth after initiation of copolymer formation) was generally smaller, the longer the filament (Fig. 4). Because the growth rate of a flagellar filament generally decreases with increase in length (Iino, 1974), this result was expected when copolymer formation was initiated simultaneously on filaments of different lengths.

In addition to the I-type filaments, it was found that 44% of the R-group cells had some entirely straight filaments (between 1 and 6 per cell), which were shorter than the I-type filaments on the same cell (Fig. 4) and were inferred to have initiated their growth after the period of copolymer formation. Entirely straight filaments were generally not present in D-group cells.

Nine of the 75 derepressed cells did not fit the above pattern; five possessed one T-type filament together with the I-type (Table 3), while four belonged to group D but possessed one or two entirely straight filaments. Most of these exceptional filaments were longer than the other filaments on the same cell; it is therefore possible that they did not grow detectably after derepression or re-establishment of repression, because the growth rate of a flagellar filament declines exponentially with increase in length (Iino, 1974). The explanation for the few exceptional shorter filaments is either that they had been broken during the process of specimen preparation for electron microscopy or that they accidentally stopped their growth before reaching the full length as has been observed for in vitro flagellar growth (Hotani & Asakura, 1974).

**Duration of copolymer formation in a derepressed cell**

The lengths of the copolymer segments varied from 0.2 to 3.2 μm among different flagella on the derepressed cells. A correlation was observed between the length of the proximal straight portion (p) (i.e. length of the filament when copolymer formation was initiated) and that of the copolymer segment (m) of each I-type filament: m was smaller in a filament with a larger p.

The pattern of the decrease of m with increase of p was noticed to be homologous with
that of the decrease of growth rate of a flagellar filament with increase in its length (Iino, 1974) (Fig. 5). The homology between them suggested that the diversity of \( m \) reflects a difference in growth rate among filaments of different lengths, and that the time taken for the copolymer segment to grow is similar among the filaments of a derepressed cell.

When flagellate Salmonella cells grow in nutrient broth, the growth rate of each of their flagella decreases according to the following equation:

\[
V = V_0 e^{-KL},
\]

where \( V \) denotes the growth rate of the filament at length \( L \), \( V_0 \) the initial rate at \( L = 0 \), and \( K \) the constant characterizing degree of decrease in growth rate per unit length (Iino, 1974). Integration of eqn (1) gives

\[
\tau = (e^{KL_2} - e^{KL_1})/KV_0,
\]

where \( \tau \) denotes the time taken for the flagellar filament to increase in length from \( L_1 \) to \( L_2 \). When \( p \) and \( p+m \) of an I-type filament are equated to \( L_1 \) and \( L_2 \), respectively, \( \tau \) corresponds to the time taken for the copolymer to grow on the filament.

The lengths of flagellar filaments were measured on a culture of \( s4067 \) whose cell generation time was 55 min in late-exponential phase. From their distribution the average growth curve of the flagellar filaments was constructed, and \( V_0 \) and \( K \) under these growth conditions were estimated to be 0.13 and 0.15 \( \mu m/min \), respectively, according to the procedures described by Iino (1974). On the assumption that the growth rate of the copolymer is not significantly different from that of the phase-2 homopolymer, the \( \tau \) values for \( V_0 = 0.13 \) and \( K = 0.15 \) were calculated from eqn (2) on I-type filaments of seven R-group cells detected in the bacterial sample. The calculated values for flagella on a single cell were distributed in a narrow range with a maximal standard deviation of 2-2 min and the average (between 5 and 10 min) differing among different cells (Fig. 6). Their overall distributions were within the range 4 to 13 min, with an average of 7.7 min.

**DISCUSSION**

The temporary formation of copolymers of phase-1 and phase-2 flagellins occurs commonly among phase-2 clones of diphasic Salmonella. This phenomenon may previously have been
overlooked because of the wave-forms of homopolymers and the copolymers in wild-type bacteria are similar, and because it occurs in a limited stage of growth phase. Observations on the phase-2 straight flagellar mutant facilitated detection of the phenomenon, because the wave-forms of copolymers are distinct from both phase-1 and phase-2 flagella.

Applying the information obtained from the experiments on *in vitro* flagellin synthesis (Suzuki & Iino, 1973), the temporary copolymer formation in phase-2 cells was attributed to temporary derepression of *Ht* at the transcriptional level. The appearance of copolymer segments in all filaments on a cell simultaneously, and the absence of copolymer formation in cells carrying *ahi*− (which corresponds to an inert allele of *Ht*-operator, *ahi*) conform with this explanation.

In inducible enzyme systems, it is well known that enzyme is often synthesized in small amounts by the non-induced cell population (Miller, 1970). The basic mechanism of the temporary derepression described in the present report may essentially be the same as the leaky repression in non-induced cell populations. The present system, however, has the advantages that the derepression is easily identified on individual cells and the effect is measured on a localized structure of a cell at a defined period in the growth cycle.

Studying the composition of flagella of F'-merozygotes of *Escherichia coli*, Silverman & Simon (1974) showed that the flagellins produced by an endogenotic structural gene for flagellin and by its exogenotic allele are together incorporated into a flagellar filament and form a homogeneous copolymer. We found this to be true even when the two structural genes for flagellin were non-allelic, i.e. when one was *Ht* and the other *H2*. Detection of the I- and T-type filaments showed that a flagellum-forming apparatus can accept newly synthesized flagellin of phase 1 even after it has already started filament formation in phase 2. Thus, the flagellum-forming apparatus of each flagellum was found to be phase non-specific.

When a cell was derepressed, the copolymers were formed at the tip of every growing phase-2 flagellum of the cell. Moreover, the lengths of the copolymer segments were not the same among the different flagella on the cell, but paralleled the growth rate of each flagellar filament at the time of derepression. Copolymer formation continues for about the same length of time for every flagellum of a derepressed cell. This suggests that the pool of phase-1 flagellin produced in a derepressed cell is equally available to every growing flagellum on the cell.

The duration of detectable copolymer formation in a cell is of the same order as the half-life of flagellin mRNA (about 7 min according to Suzuki, Enomoto & Hirota, 1974). The resemblance of the derepressed period to the half-life of flagellin mRNA suggests that the derepressed state of the *Ht*-operon lasts for only a very short period which corresponds to the initiation of one or a few rounds of mRNA synthesis. Whether the temporary derepression of *Ht* is caused by a turning off of the *H2*-operon or by under production of the phase-1 repressor at the translational level is not known; it may even occur through ‘leaky’ repression by the phase-1 repressor.

**REFERENCES**


