JOURNAL OF BACTERIOLOGY, Oct. 1977, p. 254-261
Vol. 132, No. 1

Printed in U.S.A.

Ribonucleic Acid Polymerase Mutant of *Escherichia coli* Defective in Flagella Formation

T. YAMAMORI,[1] K. ITO,[1] T. YURA,[1]* T. SUZUKI,[2] AND T. IINO[2]

Institute for Virus Research, Kyoto University, Kyoto, Japan,[1] and Laboratory of Genetics, Faculty of Science, University of Tokyo, Tokyo, Japan[2]

Received for publication 20 June 1977

Escherichia coli K-12 mutants that are resistant to bacteriophage χ, defective in motility, and unable to grow at high temperature (42°C) were isolated from among those selected for rifampin resistance at low temperature (30°C) after mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Genetic analysis of one such mutant indicated the presence of two mutations that probably affect the β subunit of ribonucleic acid (RNA) polymerase: one (*rif*) causing rifampin resistance and the other (Ts-74) conferring resistance to phage χ (and loss of motility) and temperature sensitivity for growth. Observations with an electron microscope revealed that the number of flagella per mutant cell was significantly reduced, suggesting that the Ts-74 mutation somehow affected flagella formation at the permissive temperature. When a mutant culture was transferred from 30 to 42°C, deoxyribonucleic acid synthesis accelerated normally, but RNA or protein synthesis was enhanced relatively little. The rate of synthesis of β and β' subunits of RNA polymerase was low even at 30°C and was further reduced at 42°C, in contrast to the parental wild-type strain. Expression of the lactose and other sugar fermentation operons, as well as lysogenization with phage λ, occurred normally at 30°C, suggesting that the mutation does not cause general shut-off of gene expression regulated by cyclic adenosine 3',5'-monophosphate.

The deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase of *Escherichia coli* is a complex enzyme consisting of at least five subunits (i.e., 2α, β, β', and σ) (3). It plays a major role in genetic transcription and is thought to be involved in the synthesis of most, if not all, cellular RNA species. It is not unexpected, therefore, that certain mutations affecting the structure of this enzyme bring about a variety of phenotypic alterations directly or indirectly. For example, some rifampin resistance mutations presumably affecting the β subunit of RNA polymerase in *E. coli* or other bacteria have been reported to affect sporulation (30), nutritional requirements (20), the capacity to support phage growth (e.g., 5), or the ability to be lysogenized with a temperate phage (8). Analysis of these pleiotropic mutants should provide an interesting approach not only to the regulatory mechanism involved in each system, but also to the general problem of the role of RNA polymerase in transcriptional control.

Bacterial flagella have been studied extensively in past years (10), but relatively little is known about the molecular mechanism controlling their formation. Thus, it was thought interesting to examine the role of RNA polymerase in flagella formation by looking for mutants defective in flagella formation as the result of a mutational alteration of RNA polymerase. Indeed, such mutants were found among temperature-sensitive strains carrying a mutation at the *rpoB* region. In this paper, we report on some properties of one such mutant that exhibits a reduced capacity to form flagella at low temperature and fails to grow at high temperature.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains used in this study are listed in Table 1. Bacteriophage χ is a flagellotropic phage that grows only on motile cells of *E. coli* and *Salmonella* (18, 26). P1*vir* (11) was used for transductional mapping of genes. The specific transducing phage λd*rif*$^+$ (referred to here as λd*rpoB*$^+$) used in some genetic analysis was described previously (12). Wild-type λ phage was used for determining lysogenization frequency.

Media. Peptone-glucose medium contained 10 g of polypeptone (Wako Chemicals Co.), 5 g of NaCl, and 1 g of glucose per liter (pH 7.4). Minimal medium was medium E (32) supplemented with 0.5% glucose and each required amino acid at 20 μg/ml. L broth contained 10 g of tryptone, 5 g of yeast extract (Difco

TABLE 1. *E. coli K-12 strains*[a]

Strain	Sex	Genetic characters	Derivation/reference
KY1394	F⁻	*his ilv trp*(Am) *tyr*(Am) *lac*(Am) *tonA*(Am) *tsx*(Am) *bfe sup-126*	KN238; ref. 21
B74	F⁻	*rif* Ts-74; other markers same as in KY1394	KY1394; this paper
KY1324	F⁻	*his metB ppc argE15 purD thi str supE*	N. Glansdorff
KY1400	F⁻	*argH his ilv trp*(Am) *tyr*(Am) *lac*(Am) *tonA*(Am) *tsx*(Am) *bfe sup-126*	KN255; ref. 22
KL16-99	Hfr	(*O-lysA-thyA*---) *recA*	B. Low
W3110	F⁻	Prototroph	
KY3601	F⁻	*argH*	W3110; this paper
AB1884	F⁻	*thr-1 leu-6 thi-1 argE his-4 proA2 uvrC34 lacY1 galK2 mtl-1 xyl-5 ara-14 str-31 tsx-33 supE44*	Ref. 9

[a] Gene symbols are those described in reference 1. (Am) denotes an amber mutation.

Laboratories, Detroit, Mich.), 10 g of NaCl, and 2 g of glucose per liter (pH 7.2). For testing motility, peptone medium containing 5 g of polypeptone, 3 g of beef extract, 2.5 g of agar, and 80 g of gelatin per liter was used.

Chemicals. Recrystallized products of acrylamide and *N,N'*-methylene-bis-acrylamide were obtained from Wako Chemicals Co., and *N,N'*-diallyltartardiamide was from Eastman Kodak Co., Rochester, N.Y. Recrystallized sodium dodecyl sulfate and Brij-58 were products of Wako Chemicals Co. and Atlas Chemicals, respectively. [^{3}H]thymidine (23 Ci/mmol), [^{3}H]uridine (43 Ci/mmol), and L-[^{14}C]leucine (348 Ci/mol) were obtained from the Radiochemical Centre, Amersham, England. L-^{3}H-amino acids were a synthetic mixture of 15 L-^{3}H-amino acids (NET-250) obtained from New England Nuclear Corp., Boston, Mass.

Isolation of rifampin-resistant, temperature-sensitive mutants. The parental wild-type strain (KY1394) was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and rifampin-resistant mutant colonies were obtained at 30°C on peptone-glucose agar containing 50 μg of rifampin per ml. Temperature-sensitive mutants were then screened by replica-plating these colonies onto peptone-glucose agar at 42°C. About 1% of the colonies tested were found to be temperature sensitive; most of them were subsequently shown to carry a second mutation, in addition to *rif*, at or near the *rpoB* locus. These mutants, which are resistant to rifampin at 30°C and are unable to grow at 42°C, were used as materials for the further screening of mutants defective in flagella formation, because temperature-sensitive mutants were thought to be more amenable to further analysis (see Results).

Electron microscopy. Cells were taken from at least two colonies on peptone agar plates that had been incubated for 18 h at 30°C, suspended in phosphotungstic acid (pH 6.8), and spread on mesh covered with Formvar film. After the mixture stood for 3 min, excess water was removed with filter paper and dried in a vacuum. A JEM 7A (100 kV) electron microscope was used at an original magnification of ×3,000.

P1 phage transduction. All transduction experiments were carried out with P1*vir* essentially as described by Ikeda and Tomizawa (11).

Macromolecular synthesis. Cells were grown to mid-log phase in medium E supplemented with 0.2% glucose and 0.2% Casamino Acids (Difco), diluted 20-fold with prewarmed medium, and labeled with [^{3}H]thymidine (or [^{3}H]uridine) and L-[^{14}C]leucine. Deoxyadenosine (250 μg/ml) was added to the medium when [^{3}H]thymidine was used. After growth for one to two generations, the cultures were shifted to 42°C. Samples (0.05 ml) were taken at appropriate intervals onto a filter paper and washed three times with cold 5% trichloroacetic acid and twice with ethanol before being dried. Radioactivity was determined in a toluene-based scintillator with a Nuclear-Chicago liquid scintillation counter.

β-Galactosidase synthesis. Experiments on induced β-galactosidase synthesis were carried out by the procedures of Miller (19).

RESULTS

Isolation of the mutant. A number of temperature-sensitive mutants that can grow at 30°C but not at 42°C were found among those selected for rifampin resistance at 30°C. When these mutants were examined for sensitivity to χ phage at the permissive temperature, some (10 to 20%) were found to be resistant by streak tests. The latter mutants were shown to be nonmotile when tested on semisolid agar medium (Fig. 1). To determine whether these mutant properties were due to a single mutation or to multiple mutations, temperature-independent (Ts^+) revertants were isolated spontaneously from each mutant and examined for possible simultaneous changes in other properties. Two of 10 mutants tested produced Ts^+ revertants that regained χ sensitivity (and motility) while retaining rifampin resistance (Table 2). These results suggest that, at least in these two instances, a temperature-sensitive mutation is responsible for both χ phage resistance (defect in motility) and temperature sensitivity for growth, whereas a separate mutation (*rif*) is responsible for rifampin resistance. One such mutant, designated B74, was used for further study.

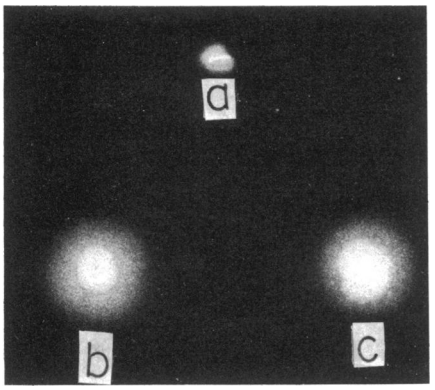

FIG. 1. *Motility on semisolid agar medium. A petri dish containing semisolid peptone agar medium was inoculated with an overnight culture of each strain and was incubated at 30°C for 24 h. (a) Mutant (B74), (b) wild type (KY1394), (c) Ts⁺ revertant.*

TABLE 2. *Temperature-independent revertants derived from temperature-sensitive rifampin-resistant mutants*[a]

Parental temperature-sensitive mutant no.	Temperature-independent revertants obtained[b]	
	χ^s Rif^r	χ^r Rif^r
1 (B74)	29	4
2	2	8
3	0	10
4	0	10
5	0	10
6	0	10
7	0	10
8	0	10
9	0	10
10	0	10

[a] Spontaneous temperature-independent revertants were obtained from each temperature-sensitive mutant by plating cells on peptone-glucose agar at 42°C. A number of independently occurring revertants were examined by streak tests for their sensitivities to χ phage and rifampin at 30°C. s, sensitivity; r, resistance; χ, χ phage; Rif, rifampin.

[b] No χ^s Rif^s or χ^r Rif^s revertants of any mutant were obtained.

Electron microscopic observation. Cells of the mutant grown at 30°C were examined under an electron microscope to determine whether the observed defect in motility was due to functional deficiency or lack (or decrease in number) of flagella. Figure 2 shows histograms of the number of flagella per parental, mutant, and revertant cell. Most mutant cells (about 99.5%) lacked flagella, whereas at least one-third of the parental cells had one or more. The mutant cells tended to elongate to some extent, and filamentous cells exceeding the maximum length (4 μm) of parental cells were observed at an appreciable frequency. The temperature-independent revertants produced flagella (Fig. 2), as expected from their motility and sensitivity to χ phage. These and other results presented below clearly demonstrate that mutation Ts-74 somehow affects flagella formation at low temperature and, at the same time, renders the cell unable to grow at high temperature.

Genetic mapping of the mutation. Preliminary experiments indicated that the mutation responsible for the temperature sensitivity of strain B74 was located near the *rpoB* gene, since introduction of an F′ plasmid (KLF10) or a prophage, λd*rpoB*⁺, carrying this region into the mutant conferred on cells the ability to grow at high temperature. Further mapping experiments were carried out by transduction with phage P1. Cells of strain KY1400 (Arg) were infected with P1 phage grown on the mutant (B74), and Arg⁺ transductants were selected at 30°C and examined for unselected markers. As seen in Table 3, the mutation (Ts-74) causing temperature sensitivity is separate from, although closely linked to, that responsible for rifampin resistance (*rif*), the probable order being *arg-rif*-Ts-74. The χ phage resistance and motility could not be separated from temperature sensitivity in this and other similar experiments. These results, taken together with those from the reversion studies (Table 2), strongly indicate that temperature sensitivity and the defect in flagella formation and motility are due to a single mutation (Ts-74) closely linked to the *rif* mutation. In one experiment (III) shown in Table 3, transductants forming smaller colonies were selectively picked and examined to facilitate possible detection of Rif^s Ts transductants (0–1 class). However, none of the transductants obtained (0/363) belonged to

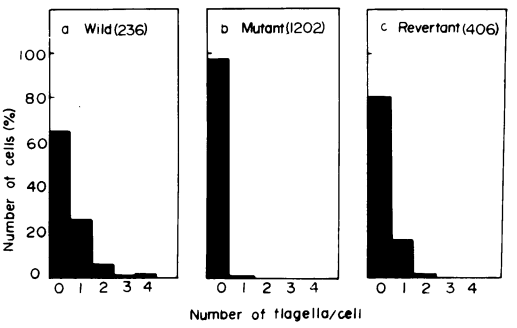

FIG. 2. *Histogram showing the frequency distribution of cells with varying numbers of flagella as determined by electron microscopy. The ordinate represents the percentage of each fraction among total population of cells. The figures in parentheses indicate the number of cells examined. (a) Wild type (KY1394), (b) mutant (B74), (c) Ts⁺ revertant.*

TABLE 3. *Transductional mapping of Ts-74*[a]

Expt.	Selection	Unselected marker			No. of transductants obtained
		Rif	Ts	χ	
I	Arg+	1	1	1	18
		1	0	0	4
		0	1	1	0
		0	0	0	28
II	Arg+	1	1		60
		1	0		2
		0	1		0
		0	0		60
III	Arg+	1	1		303
		1	0		0
		0	1		0
		0	0		60

[a] Donor (B74): Arg+ Rif[r] Ts χ[r]. Recipient (KY1400): Arg Rif[s] Ts+ χ[s]. Cells of strain KY1400 were infected with phage P1*vir* grown on strain B74 and were plated on medium E containing all amino acids except arginine. After incubation at 30°C for 4 to 5 days, transductant colonies were picked, purified on peptone-glucose agar, and scored for unselected markers. Rifampin sensitivity was examined on peptone agar containing 50 μg of the drug per ml at 30°C. Temperature sensitivity was tested by growth on peptone agar at 42°C. Rif, Rifampin sensitivity; Ts, temperature sensitivity for growth; χ, sensitivity to χ phage. 1, Marker from the donor; 0, marker from the recipient. In experiment III, transductants with relatively small colony size were selectively picked (see text).

this class. This provides further support of the order suggested above.

Identification of the gene affected by the Ts-74 mutation. The structural genes for the β and β′ subunits of RNA polymerase (*rpoB* and *rpoC*, respectively) are clustered, probably forming an operon (4), and are mapped in the order *argH-bfe-rpoB-rpoC-thi*. Rifampin resistance mutations affect *rpoB*, resulting in the production of structurally altered β polypeptides (7, 13). The results of mapping experiments therefore suggest that the Ts-74 mutation affects either *rpoB* or *rpoC*. To determine which of the RNA polymerase genes was being affected, a *recA* derivative of the mutant was cross-streaked at 42°C against λd*rpoB*+-6 phage carrying the intact β gene (*rpoB*+) and a portion of the β′ gene (*rpoC*+) (T. Osawa, Y. Nakamura, T. Ikeuchi, and T. Yura, Jpn. J. Genet. 50:489, 1975). Growth occurred only at the intersection, and these cells were purified at 42°C and analyzed for their properties. Most of them turned out to be lysogenic for both λd*rpoB*+-6 and λ+ phage that were present in the lysate used (12); they were immune to λ, sensitive to rifampin (*rif*+ is dominant to *rif*), and able to grow at high temperature on peptone agar. Furthermore, when these lysogens were "cured" by heteroimmune curing, most of the λ-sensitive clones obtained were shown to be temperature sensitive and rifampin resistant. These results indicate that both the Ts-74 and *rif* mutations can be complemented with λd*rpoB*+-6 carrying intact *rpoB*+ without *rpoC*+. Thus, the Ts-74 mutation most probably affects *rpoB* rather than *rpoC*.

Flagella formation in Ts-74 transductants derived from strain W3110. The results of the genetic analysis presented above indicate that the Ts-74 mutation is responsible for both the inability to grow at high temperature and the defect in flagella formation at low temperature. However, since the parental strain used (KY1394) produces only a few flagella per cell, the effect of the Ts-74 mutation in another strain with a greater capability to form flagella was examined. Thus, a chromosomal segment around Ts-74 was transduced into an Arg derivative (KY3601) of strain W3110 by selecting for Arg+ clones, and the resulting transductants were examined, under an electron microscope, for the ability to form flagella. Transductants carrying Ts-74 produced, on the average, four flagella per cell, with relatively little variation (Fig. 3). On the other hand, Ts+ transductants and parental strain KY3601 produced more than 11 flagella per cell, with much broader

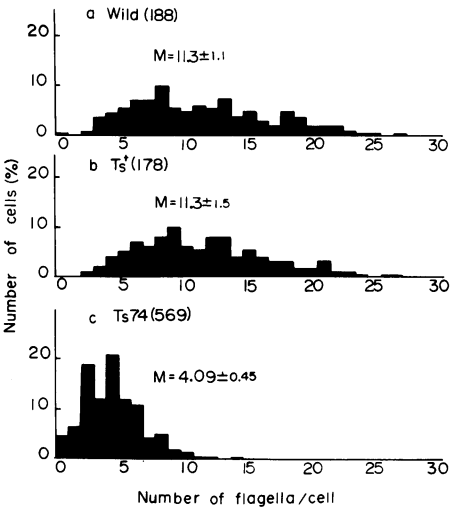

FIG. 3. *Histogram showing the frequency distribution of cells with varying flagella number for transductants derived from strain W3110. Flagella numbers were determined and the results are presented as for Fig. 2. M, Mean with standard error. (a) Wild type (KY3601), (b) arg+ Ts+ transductant, (c) arg+ Ts-74 transductant.*

distribution. These results clearly show that the Ts-74 mutation, when present in the genetic background of strain W3110, causes a significant decrease in the number of flagella formed per cell but does not inhibit flagella formation completely. This indicates that the mutation quantitatively affects flagella formation in *E. coli*.

Macromolecular synthesis in the mutant. When a mutant culture grown in minimal-Casamino Acids medium at 30°C was shifted to 42°C, growth as judged by optical density accelerated slightly but then gradually decelerated until it ceased after 8 to 10 h. A double-labeling experiment was then carried out with [^{3}H]thymidine and [^{14}C]leucine to compare DNA and protein synthesis. The temperature shift-up resulted in normal acceleration of DNA synthesis in the mutant but significantly less acceleration of protein synthesis as compared with that of the wild-type control (Fig. 4). Similar experiments with [^{3}H]uridine and [^{14}C]leucine showed that RNA and protein synthesis in the mutant can be accelerated only slightly after temperature shift-up, in contrast to the marked acceleration in the parental strain (data not shown). Thus, the mutation appears to reduce the synthesis of at least certain classes of RNA and protein at the restrictive temperature.

Synthesis of RNA polymerase β and β' subunits. To examine possible gross alterations in protein synthesis in the mutant, cells were exposed to high temperature (42°C) for 1 h and pulse-labeled with ^{3}H-amino acids, and crude extracts were analyzed by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. Profiles of ^{3}H-labeled proteins obtained for the mutant and the parent are similar (Fig. 5), although radioactivities associated with some

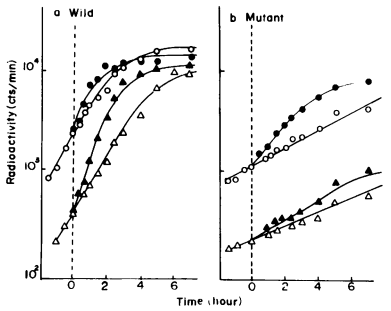

FIG. 4. *Effect of temperature shift-up on DNA and protein synthesis. Cells were grown in medium E-Casamino Acids as described in the text and diluted into prewarmed medium containing [^{3}H]thymidine (10 μCi/8 μg per ml), L-[^{14}C]leucine (0.5 μCi/20 μg per ml), and deoxyadenosine (250 μg/ml). After growth for one to two generations, cultures were divided into two portions (zero time) and shaken further at 30°C (○, △) and 42°C (●, ▲). Samples were taken at the times indicated, and acid-insoluble ^{3}H (○, ●) and ^{14}C (△, ▲) radioactivities were determined as described in the text. A pair of transductants derived from strain KY3601 to which arg$^+$ Ts-74 or arg$^+$ Ts$^+$ had been transduced by Phage P1 (grown on B74) was used. (a) Wild type (Ts$^+$); (b) mutant (Ts-74).*

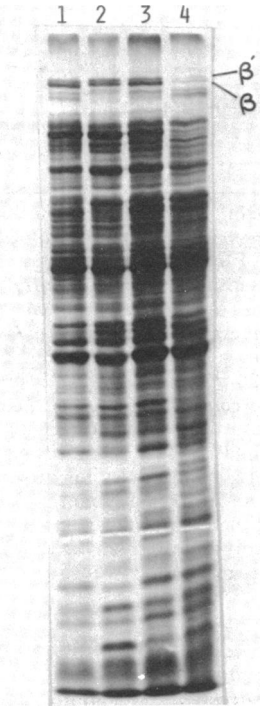

FIG. 5. *Sodium dodecyl sulfate-gel electrophoresis of ^{3}H-labeled proteins. Cells were grown in medium E with appropriate supplements, pulse-labeled for 2 min with a ^{3}H-amino acid mixture (10 μCi/ml) at 30°C or after shift to 42°C, and chased with 0.2% Casamino Acids for 3 min. Cells were lysed by treatment with lysozyme-ethylenediaminetetraacetate and Brij-58, followed by sonic oscillation as described elsewhere (21). Crude extracts were analyzed by sodium dodecyl sulfate-gel electrophoresis on slab gels (15 by 14 by 0.1 cm; 5% stacking gels and 10% separation gels), using the discontinuous buffer system described by Laemmli (17). Migration is from top to bottom as shown here. After running for 6 h at 20 mA, gels were stained with Coomassie brilliant blue. For detection of tritium-labeled proteins, fluorographs (2) were taken by exposing Fuji X-ray films to scintillator-treated gel slabs for 4 days. Total acid-insoluble radioactivities applied to the gels were 1.16 (1), 1.50 (2), 1.52 (3), and 1.37 (4) (×10^5 cpm). (1) Wild type (KY1394) at 30°C, (2) wild type (KY1394) after 1 h at 42°C, (3) mutant (B74) at 30°C, (4) mutant (B74) after 1 h at 42°C.*

protein bands may be quantitatively altered in the mutant. Synthesis of β and β′ polypeptides of RNA polymerase that can be identified unequivocally in these profiles appeared to decrease appreciably in the mutant at 42°C.

To determine the rates of synthesis of β and β′ proteins quantitatively, crude extracts were first treated with anti-RNA polymerase serum, and the precipitates formed were analyzed by sodium dodecyl sulfate-gel electrophoresis. The results (Table 4) clearly demonstrate that the level of β and β′ synthesis in the mutant was lower even at 30°C compared with that of the wild type and was further reduced upon shift to 42°C. Similar reductions in β and β′ synthesis at 42°C have been observed with transductants carrying Ts-74 but not with Ts$^+$ transductants or revertants (data not shown), suggesting that the Ts-74 mutation is responsible for the reduced synthesis of β and β′ proteins.

Expression of genes regulated by cyclic adenosine 3′,5′-monophosphate. Flagella formation in *E. coli* has been reported to be regulated by cyclic adenosine 3′,5′-monophosphate (29, 33). Thus, we examined possible effects of the Ts-74 mutation on the expression of other cyclic adenosine 3′,5′-monophosphate-dependent genes at the permissive temperature. First, induction of β-galactosidase was found to proceed normally, as judged by differential synthesis rates, although the growth rate was much lower in the mutant (Fig. 6). Second, no appreciable difference was found between the mutant and wild type in the lysogenization frequency with phage λ at 30°C. Third, there were no great differences between the two strains in the capacity to utilize galactose or arabinose as well as lactose. These results suggest that the observed phenotype of the present mutant at the permissive temperature is not due to general depression of the cyclic adenosine 3′,5′-monophosphate-controlled genes.

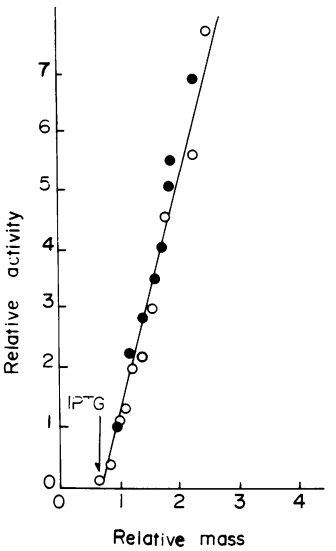

FIG. 6. *Induction of β-galactosidase synthesis. Cells were grown in medium E supplemented with 0.5% glycerol and required amino acids at 30°C to the log phase, and isopropyl-thio-β-D-galactoside was added to induce β-galactosidase synthesis. After incubation for 60 min, samples were withdrawn at appropriate intervals, treated with toluene, and assayed for enzyme activity. Enzyme activities thus obtained were normalized to that found at the time of temperature shift. Optical density was determined at 600 nm with a Zeiss spectrophotometer (PMQ III) and was also normalized to the value obtained at the time of shift. The same pair of transductants carrying Ts$^+$ or Ts-74 used in the experiment of Fig. 4 was employed. Symbols: ○, wild type (Ts$^+$); ●, mutant (Ts-74).*

TABLE 4. *Synthesis of RNA polymerase β and β′ subunits*[a]

Strain	Expt	Synthesis of: β			Synthesis of: β′		
		30°C (A)	42°C (B)	B/A	30°C (A)	42°C (B)	B/A
Wild type (KY1394)	I	0.71	0.85	1.2	0.67	0.93	1.4
	II	0.64	0.70	1.1	0.65	0.65	1.0
Mutant (B74)	I	0.44	0.30	0.68	0.49	0.30	0.62
	II	0.35	0.22	0.63	0.32	0.24	0.75

[a] Cells were grown and labeled with a ^{3}H-amino acid mixture as described in the legend to Fig. 5. The crude extracts were treated with anti-RNA polymerase serum, and the precipitates formed were analyzed by sodium dodecyl sulfate-gel electrophoresis as described elsewhere (14, 21, 22), except that exponentially growing wild-type cells (KY1394) labeled with [^{14}C]leucine at 37°C were added to each sample of ^{3}H-labeled cells as an internal reference before preparing crude extracts. The values presented here represent ^{3}H/^{14}C ratio for each polymerase subunit divided by that for the bulk protein, thus giving differential synthesis rates relative to those for wild-type cells actively growing at 37°C.

DISCUSSION

The structural genes for RNA polymerase β and β' subunits are probably located adjacent to each other, forming a single operon on the *E. coli* chromosome (4, 16). The present data indicate that the Ts-74 mutation lies at the site very close to, but distinct from, the rifampin resistance (*rif*) mutation; it is presumably located between the site of the *rif* mutation and *purD*, but not on the β' subunit gene (*rpoC*). From this and other evidence, it was surmised that the Ts-74 mutation affected the β subunit gene (*rpoB*). Consistent with this conclusion is the finding that a temperature shift-up of a mutant culture from 30 to 42°C normally accelerates DNA synthesis but enhances RNA or protein synthesis only slightly. Furthermore, synthesis of β and β' polymerase subunits in the mutant was markedly reduced particularly at high temperature. Some mutations, probably affecting the β subunit gene, have been reported to cause reduced synthesis of both polypeptides (15, 23). On the other hand, the present data do not exclude the possibility that Ts-74 affected a gene other than *rpoB* or *rpoC*.

The cellular capacity for flagella formation is evidently reduced by the Ts-74 mutation at the permissive temperature (30°C), although the extent of the mutational effect differs depending on the genetic background of the strain into which the mutation has been introduced. It seems remarkable that the Ts-74 mutation exerts a significant effect on the flagella content in both strains KY1394 and W3110, which exhibit such diverse capacities with respect to flagella formation. Although little is known at present about factors determining the number of flagella per cell, several mechanisms can be suggested to explain the reduced flagella formation in the Ts-74 mutant.

It has been reported that flagella formation in *E. coli* is regulated by cyclic adenosine 3',5'-monophosphate and its receptor protein (29, 33). However, since the Ts-74 mutation does not seem to have a general effect on the synthesis of the catabolite-sensitive operons, the observed effect on flagella formation would not be attributable to the reduced interaction between RNA polymerase and cyclic adenosine 3',5'-monophosphate receptor protein. It has also been suggested that the expression of the *fla* gene cluster is controlled by at least one specific regulator gene, *flaI* (28, 29). Thus, it is possible that the present mutant produces an altered RNA polymerase that is defective in interacting with the *flaI* gene product, resulting in a decreased level of expression of the genes required for flagella formation. Alternatively, the mutant polymerase may be unable to recognize effectively the promoter of these genes, which might have a special nucleotide sequence.

On the other hand, evidence suggests that flagella may be formed at a specific stage in the cell cycle of *E. coli* (H. Kondoh and H. Ozeki, Jpn. J. Genet. 48:426, 1973; A. Nishimura, H. Suzuki, and Y. Hirota, Jpn. J. Genet. 50:484, 1975). This finding is of particular interest in view of the observations (6, 24, 25, 27) that some proteins that constitute the bacterial cell surface are synthesized or assembled at a specific phase in the cell cycle. Thus, a common regulatory mechanism somehow coupled with cell division might be operative in the synthesis of a number of cell surface proteins, including flagella. The altered RNA polymerase produced by the Ts-74 mutant might prevent normal functioning of such a regulatory mechanism. The observation that the mutant cells tend to elongate to some extent at the permissive temperature is consistent with this view. Finally, it is possible that reduced flagella formation in the Ts-74 mutant is a secondary consequence of a mutation that primarily affects transcription of the genes involved in cell surface architecture, which in turn is essential for normal biogenesis of flagella.

Whatever the mechanisms underlying the observations reported here, the present experiments seem to emphasize the role of RNA polymerase in transcriptional control of flagella formation in bacteria. This also agrees with the expectation that the expression of the *fla* genes is regulated at the level of specific messenger RNA synthesis (31). Further work both with RNA polymerase mutants and with other mutants defective in flagella and membrane biogenesis may provide an interesting approach to the general control mechanisms for the synthesis and assembly of bacterial surface structures, including flagella.

ACKNOWLEDGMENTS

We are grateful to H. Kondoh for valuable discussions and a supply of bacteriophage χ used in this study. Helpful discussions and advice of the members of the Institute for Virus Research are also gratefully acknowledged.

LITERATURE CITED

1. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. 40:116-167.
2. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acid in polyacrylamide gels. Eur. J. Biochem. 46:83-88.
3. **Burgess, R.** 1969. Separation and characterization of the subunits of ribonucleic acid polymerase. J. Biol. Chem. 244:6168-6176.
4. **Errington, L., R. E. Glass, R. S. Hayward, and J. G.**

Scaife. 1974. Structure and orientation of an RNA polymerase operon in *Escherichia coli*. Nature (London) 249:519-522.
5. Georgopoulos, C. P. 1971. Bacterial mutants in which the gene N function of bacteriophage lambda is blocked have an altered RNA polymerase. Proc. Natl. Acad. Sci. U.S.A. 68:2977-2981.
6. Gudas, L. J., R. James, and A. B. Pardee. 1976. Evidence for the involvement of an outer membrane protein in DNA initiation. J. Biol. Chem. 251:3470-3479.
7. Heil, A., and W. Zillig. 1970. Reconstitution of bacterial DNA-dependent RNA polymerase from isolated subunits as a tool for the elucidation of the role of the subunits in transcription. FEBS Lett. 11:165-171.
8. Hong, J., G. R. Smith, and B. N. Ames. 1971. Adenosine 3',5'-cyclic monophosphate concentration in the bacterial host regulates the viral decision between lysogeny and lysis. Proc. Natl. Acad. Sci. U.S.A. 68:2258-2262.
9. Howard-Flanders, P., R. P. Boyce, and L. Theriot. 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics 53:1119-1136.
10. Iino, T. 1969. Genetics and chemistry of bacterial flagella. Bacteriol. Rev. 33:454-475.
11. Ikeda, H., and J. Tomizawa. 1965. Transducing fragments in generalized transduction by phage P1. I. Molecular origin of the fragments. J. Mol. Biol. 14:85-109.
12. Ikeuchi, T., T. Yura, and H. Yamagishi. 1975. Genetic and physical studies of lambda transducing bacteriophage carrying the beta subunit gene of the *Escherichia coli* ribonucleic acid polymerase. J. Bacteriol. 22:1247-1256.
13. Iwakura, Y., A. Ishihama, and T. Yura. 1973. RNA polymerase mutants of *Escherichia coli*. II. Streptolydigin resistance and its relation to rifampicin resistance. Mol. Gen. Genet. 121:181-196.
14. Iwakura, Y., K. Ito, and A. Ishihama. 1974. Biosynthesis of RNA polymerase in *Escherichia coli*. I. Control of RNA polymerase content at various growth rates. Mol. Gen. Genet. 133:1-23.
15. Kirschbaum, J. B., I. U. Glaeys, S. Nasi, B. Molholt, and J. H. Miller. 1975. Temperature-sensitive RNA polymerase mutants with altered subunit synthesis and degradation. Proc. Natl. Acad. Sci. U.S.A. 72:2375-2379.
16. Kirschbaum, J. B., and J. Scaife. 1974. Evidence for a λ transducing phage carrying the genes for the β and β' subunits of *Escherichia coli* RNA polymerase. Mol. Gen. Genet. 132:193-201.
17. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (London) 227:680-685.
18. Meynell, E. W. 1961. A phage, χ, which attacks motile bacteria. J. Gen. Microbiol. 25:253-290.
19. Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. Morishita, T., and T. Yura. 1976. Altered nutritional requirements associated with mutations affecting the structure of ribonucleic acid polymerase in *Lactobacillus casei*. J. Bacteriol. 125:416-422.
21. Nakamura, Y., and T. Yura. 1975. Evidence for a positive regulation of RNA polymerase synthesis in *Escherichia coli*. J. Mol. Biol. 97:621-642.
22. Nakamura, Y., and T. Yura. 1975. Hyperproduction of the sigma subunit of RNA polymerase in a mutant of *Escherichia coli*. Mol. Gen. Genet. 141:97-111.
23. Oeschger, M. P., and K. B. Berlyn. 1975. Regulation of RNA polymerase synthesis in *Escherichia coli*; a mutant unable to synthesize the enzyme at 43°. Proc. Natl. Acad. Sci. U.S.A. 72:911-915.
24. Ohki, M. 1972. Correlation between metabolism of phosphatidylglycerol and membrane synthesis in *Escherichia coli*. J. Mol. Biol. 68:249-264.
25. Ryter, A., H. Shuman, and M. Shwartz. 1975. Integration of the receptor for bacteriophage lambda in the outer membrane of *Escherichia coli*: coupling with cell division. J. Bacteriol. 122:295-301.
26. Schade, S. Z., and J. Adler. 1967. Purification and chemistry of bacteriophage χ. J. Virol. 1:591-598.
27. Shen, B. H. P., and W. Boos. 1973. Regulation of the β-methylgalactoside transport system and the galactose-binding protein by the cell cycle of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 70:1481-1485.
28. Silverman, M., P. Matsumura, R. Draper, S. Edwards, and M. I. Simon. 1976. Expression of flagellar genes carried by bacteriophage lambda. Nature (London) 261:248-250.
29. Silverman, M., and M. Simon. 1974. Characterization of *Escherichia coli* flagellar mutants that are insensitive to catabolite repression. J. Bacteriol. 120:1196-1203.
30. Sonenshein, A. L., and R. Losick. 1970. RNA polymerase mutants blocked in sporulation. Nature (London) 227:906-909.
31. Suzuki, H., and T. Iino. 1975. Absence of messenger ribonucleic acid specific for flagellin in non-flagellate mutants of *Salmonella*. J. Mol. Biol. 95:549-556.
32. Vogel, H. J., and D. M. Bonner. 1956. Acetylornithinase of *Escherichia coli*; partial purification and some properties. J. Biol. Chem. 218:97-106.
33. Yokota, T., and J. Gots. 1970. Requirement of adenosine 3',5'-cyclic phosphate for flagella formation in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. 103:513-516.