Molec. gen. Genet. 165, 7–14 (1978)

MGG

Biosynthesis of RNA Polymerase in *Escherichia coli*

VII*. Regulation of $\beta\beta'$ Operon on Lambda Transducing Phage in Assembly-Defective Mutants

Makoto Taketo, Ryuji Fukuda, and Akira Ishihama
Department of Biochemistry, Institute for Virus Research, Kyoto University, Sakyo-ku, Kyoto 606, Japan

Summary. Effect of temperature-sensitive, assembly-defective mutations in *Escherichia coli* RNA polymerase β (*rpoB*) or β' subunit gene (*rpoC*) was investigated on the expression of wild-type $rpoB^+C^+$ operon, which was introduced by infection of a lambda transducing phage $\lambda drif^+$ ($rpoB^+$)-6 after UV-irradiation of the mutant cells. In *rpoB2·rpoB7* strain which accumulates assembly-intermediates, free α, $\alpha_2\beta$ complex and premature core, the expression of $rpoB^+C^+$ operon measured by the rate of β subunit synthesis was considerably inhibited whereas that of EF(translation elongation factor)-Tu, ribosomal proteins L1 and L7/L12, and some λ-coded proteins remained unaffected. On the other hand, the expression was enhanced specifically for only $rpoB^+C^+$ operon in either *rpoC*4 or *rpoC*1 mutants, which are defective in the association of $\alpha_2\beta$ complex and β' subunit or the activation of premature core enzyme, respectively. Upon preincubation of the mutant cells at 42° C prior to phage infection, during which assembly intermediates degraded rapidly, the rate of β subunit synthesis relative to other phage-corded proteins increased remarkably in *rpoB2·rpoB7* mutant as well as in *rpoC*4 and *rpoC*1 mutants. These observations strongly suggested the autogenous regulation for at least $\beta\beta'$ ($rpoB^+C^+$) operon by some trans-active diffusible protein complexes built of RNA polymerase subunits. Nature of the regulatory molecules is discussed.

Introduction

In exponentially growing *Escherichia coli* cells, the intracellular concentration of the DNA-dependent RNA polymerase [EC 2.7.7.6] as well as ribosomes is maintained at certain levels characteristic of the rate of cell growth (Iwakura and Ishihama, 1975; Ishihama et al., 1976). Several lines of evidence indicated that biosynthesis of such constitutive components essential for cell growth as RNA polymerase and ribosomes is regulated both positively and negatively by complex mechanisms, in which various kind of cell components participate (for example see review, von Myenburg, 1976). One of the control mechanisms involved is the autogenous regulation, that is, the expression of the $\beta\beta'$ (*rpoBC*) operon including the structural genes for RNA polymerase β and β' subunits is regulated directly by RNA polymerase proteins (for example see review, Scaife, 1976). Supporting the hypothesis is the influence of mutations in the structural genes for RNA polymerase subunits on their own synthesis (Kirschbaum et al., 1975; Taketo et al., 1976). The transient increase in RNA polymerase subunit synthesis caused by the addition of rifampicin but not streptolydigin, both of which are potent inhibitors of RNA polymerase, can also be explained by the autogenous regulation (Hayward et al., 1976; Nakamura and Yura, 1976).

On the other hand, the expression of α subunit gene, *rpoA*, which belongs to the same transcriptional unit as those for ribosomal proteins S11, S4 and L17 (Jaskunas et al., 1975) is subject to the stringent control mediated by the magic spot, ppGpp, either in vivo (Maher and Dennis, 1977) or in vitro (Lindahl et al., 1976).

Among multiple regulation loops which operate in the biosynthesis of RNA polymerase subunits, the autogenous regulation of at least *rpoBC* operon is specific for RNA polymerase and thus considered to be the smallest loop. Analysis of the influence of RNA polymerase proteins on the expression of *rpoBC* genes is one of the short cuts to reveal the molecular mechanism of this autoregulation. For the purpose, the *E. coli* mutants defective in the subunit

* Paper VI in this series is Saitoh and Ishihama (1977)

For offprints contact: Dr. Akira Ishihama

0026-8925/78/0165/0007/$01.60

assembly would be useful because such mutants accumulate large amounts of intermediate subassemblies or premature core enzyme (Taketo and Ishihama, 1976, 1977). In fact, the three temperature-sensitive assembly-defective mutants identified so far in our laboratory exhibited dramatic changes in the rate of RNA polymerase synthesis (Taketo et al., 1976), implying that the expression of at least *rpoBC* operon is regulated directly by intracellular concentrations of RNA polymerase itself and/or intermediate subassemblies such as free α subunit, $\alpha_2\beta$ complex and premature core enzyme.

In the present report, the effect of such protein complexes accumulated in the assembly-defective mutants was examined on the expression of a wild-type *rpoBC* operon introduced into these cells by infection of a lambda transducing phage $\lambda drif^+$-6 after UV-irradiation of host cells. Part of this study was published previously (Taketo et al., 1977).

Materials and Methods

Phage and Bacterial Strains. The bacterial strains used in this study were all derivatives of *E. coli* K12 and are listed in Table 1. The lambda transducing phage $\lambda drif^+$-6 which contains the *bfe-rpoB* region of *E. coli* chromosome in the left arm of the phage DNA was prepared from the lysogen KY3372 as described by Ikeuchi et al. (1975). The lysogen was cultured in L-broth (Lennox, 1955) and the phage $\lambda drif^+$-6 was titrated on W3350 grown on peptone-glucose (PG) agar plate, which contained (per liter) 10 g of polypeptone (Difco Laboratories), 5 g of NaCl and 0.5% agar. Purified phage preparations were stored in CsCl solution at 4° C, and was dialysed against λ buffer (phage adsorption buffer supplemented with 10^{-2} M $MgSO_4$) before use.

Transduction with P1*vir* was carried out by the method of Lennox (1955). For preparation of the P1*vir* lysate, L broth was employed and the phage P1 was titrated on L agar plate (Lennox, 1955). Phage adsorption buffer was 0.01 M Tris·HCl (pH 7.4) containing 0.15 g of $MgSO_4 \cdot 7H_2O$, 0.08 g of $CaCl_2$ and 0.01 g of gelatin per litter.

M9-medium (Clowes and Hayes, 1968) with 0.2% maltose and 50 µg/ml of tryptophan was used for $\lambda drif^+$-6 infection experiments.

Infection of $\lambda drif^+$-6 onto UV-Irradiated Cells. N3-1 transductants were grown in 10 ml of M9-maltose (0.2%)-tryptophan (50 µg/ml) medium at 34° C, or at 42° C for 15 or 90 min following shift from 34° C, to a cell density of about 40 turbidity unit (measured with Klett-Summerson photometer), which corresponds to approximately 3.5×10^8 cells/ml. Cells in 7 ml cultures were collected by centrifugation and resuspended into 3 ml of the same medium at 0° C. The cell suspensions in sterilized petri-dishes were irradiated with UV-light (15 watt germicidal lamp (Toshiba Electric Co., Japan)) at a distance of 25 cm with swirling at 0° C for 3 min. After adding $MgSO_4$ to the final concentration of 10 mM, 2.5 ml aliquots of UV-irradiated cell suspensions were transfered into brown tubes and infected with $\lambda drif^+$-6 phage at a multiplicity of infection of about 1. The phage adsorption was performed at 34° C for 20 min. The infected cells were diluted into 8 ml of M9 medium prewarmed at 34° C and, after shaking for 15 min at 34° C, labelled with 0.5 µCi of [^{14}C]protein hydrolysate (specific activity, 56 mCi/mAtom carbon; the Radiochemical Centre, England) for 10 min. For termination of the labelling, the cultures were chilled in ice and added with NaN_3 and chloramphenicol to the final concentrations of 10 mM and 100 µg/ml, respectively.

Table 1. Bacterial strains used and their genetic characters

Strain	Sex	Genetic characters		Derivation (Reference)
		rpoBG genes	other chromosomal markers	
X240	F^-	*rpoB*$^+$ *rpoC*$^+$	*thi lac his metB strA* λ^R	J.B. Kirschbaum (Kirschbaum et al., 1975)
X240 A2R7	F^-	*rpoB2·rpoB7 rpoC*$^+$	same as in X240	same as X240
X240 Ts4	F^-	*rpoB*$^+$ *rpoC4*	same as in X240	same as X240
T16	F^-	*rpoB*$^+$ *rpoC1*	*thi str*	R.B. Khesin (Ilyina et al., 1971)
N3-1	F^-	*rpoB*$^+$ *rpoC*$^+$	*trp gal str uvrB*	H. Ogawa via N. Ryo
N3-1A	F^-	*rpoB*$^+$ *rpoC*$^+$	*argECBH*; other makers same as in N3-1	Arg$^-$ derivative of N3-1
N3-1AR	F^-	*rpoB rpoC*$^+$	same as in N3-1A	Rif-R derivative of N3-1A
KY3409	F^-	*rpoB*$^+$ *rpoC*$^+$	*argG metB1 his-1 leu-6 recA mtl-2 xyl-7 malA gal-6 lacy-1 str-104 tonA2 tsx-1 sup44* $\lambda^R\lambda^-$/KLF10 *metB*$^+$	T. Yura
N3-1 Xt	F^-	*rpoB*$^+$ *rpoC*$^+$	same as in N3-1	N3-1AR transduced with P1 grown in X240
N3-1 ARt	F^-	*rpoB2·rpoB7 rpoC*$^+$	same as in N3-1	N3-1A transduced with P1 grown in X240 A2R7
N3-1 4t	F^-	*rpoB*$^+$ *rpoC4*	same as in N3-1	N3-1AR transduced with P1 grown in X240 Ts4
N3-1 Tt	F^-	*rpoB*$^+$ *rpoC1*	same as in N3-1	N3-1AR transduced with P1 grown in T16
KY3372	F^-	*rpoB195 rpoC*$^+$	*thi thr leu metB rha str tsx* λ^R (λcI^+) ($\lambda cI857drif^+$-6)	T. Yura (Ikeuchi et al., 1975)
W3350	F^-	*rpoB*$^+$ *rpoC*$^+$	*gal-1 gal-2 lac*	T. Yura

Gene symbols are according to Bachmann et al. (1976). Nomenclature for *rpo* genes mutations are those employed by Hayward and Scaife (1976)

Determination of Synthesis Rates for RNA Polymerase Subunits and EF-Tu. The relative synthesis rates for RNA polymerase subunits was determined as described previously (Taketo et al., 1976): Labelled cells were collected by centrifugation and lysed in Tris-sucrose-lysozyme-EDTA solution; crude extracts obtained were treated with anti-RNA polymerase holoenzyme antiserum and precipitates formed were fractionated into each subunit by SDS-polyacrylamide gel electrophoresis. The electrophoresis was carried out essentially according to the method of Laemmli (1970) except that the gradient of acrylamide concentration was from 10 to 16% as employed by O'Farrell (1975). Radioactive polypeptides were identified by taking autoradiograms of gel slabs treated for fluoroautoradiography by the method of Laskey and Mills (1975), and quantitated by weighing the paper weight for each peak of traces scanned with Joyce-Loebl MKIII densitometer.

The rate of EF-Tu synthesis was determined according to the method of Miyajima and Kaziro (1977) with use of [^{14}C]labelled Tu and anti-Tu. Unlabelled and [^{14}C]labelled EF-Tu, and anti-EF-Tu antiserum were kindly donated by Drs. A. Miyajima and Y. Kaziro.

Ribosomal proteins were identified by the electrophoretic profile on two-dimensional polyacrylamide gels. Unlabelled and [^{35}S]labelled L7/L12 was purified as a authentic marker from 50S ribosome particle of *E. coli* W3350 by the method of Hamel et al. (1972).

Results

Preparation and Properties of uvrB Transductants with rpoB or rpoC Mutations

Identification and quantitation of polypeptides coded for by a phage has been successfully achieved by infecting the phage onto UV-sensitive (*uvr*) cells which were previously irradiated with UV to minimize host protein synthesis (Watson et al., 1975; Jaskunas et al., 1975). This system was employed to analyze the effect of RNA polymerase proteins on the expression of $\beta\beta'$ (*rpoBC*) operon on the transducing phage $\lambda drif^+$-6. For the purpose, *E. coli* strains which are UV-sensitive and defective in the assembly of RNA polymerase were constructed by transferring *rpoB* or *rpoC* mutations into *uvr* strains by phage P1-mediated transduction. Since it is known that phage-coded protein synthesis in UV-irradiated cells is greatly influenced by various genetic and physiological backgrounds of host cells beside the nature of *uvr* genes, several *uvrA* and *uvrB* strains were tested for protein synthesis after infection with $\lambda drif^+$-6; among them, the strain N3-1 carrying *uvrB* was found to exhibit the highest activity of phage protein synthesis after UV irradiation, and used for transduction.

Inititally, Arg$^-$ derivative (N3-1A) of the strain N3-1 was isolated by penicillin screening and checked for the mutation to be located in *argECBH* cluster by cross-streaking with KY3409 which carries an episome KLF10 that contains intact *argECBH* region of the *E. coli* chromosome. Transduction of *rpoB* or *rpoC* locus was checked by the sensitivity of cell growth to temperature and rifampicin-resistant double mutations of β-subunit gene in the assembly-defective mutant X240A2R7, phage P1 grown in this strain was infected to N3-1A. From Arg$^+$ transductants, temperature-sensitive and rifampicin-resistant clones were selected, and one of the clones, N3-1 ARt (designated as ARt in this report), was used for $\lambda drif^+$-6 infection experiments described in this report. On the other hand, for transduction of *rpoC4* and *rpoC1*, both temperature-sensitive mutations of the β'-subunit gene in the respective assembly-defective strains Ts4 and T16, P1 phage was first grown on these strains and the progeny phage was infected to N3-1AR, which is a rifampicin-resistant derivative of N3-1A, Arg$^+$ transductants sensitive to both 42° C and 50 μg/ml rifampicin were established and the strains, N3-1 4t and N3-1 Tt (designated as 4t and Tt), of the respective transductants were used for the phage infection experiments. The original strain T16 carrying *rpoC1* is temperature-sensitive only when grown on minimal plates (Ikeuchi and Yura, personal communication; Taketo, unpublished observation) and this character was also observed in the *uvrB* transductant N3-1 Tt. The control strain N3-1 Xt (*rpoB*$^+$ *C*$^+$) was constructed by selecting temperature-resistant, rifampicin-sensitive clones from P1-infected Arg$^+$ descendants of N3-1AR.

The original assembly-defective strains harboring mutations *rpoB2*·*rpoB7*, *rpoC4* or *rpoC1* synthesize RNA polymerase subunits at irregular rates after shift to the nonpermissive temperature of 42° C; such irregular profile of polymerase synthesis is also observed at the permissive temperature (30° C) for the cell growth, though to a lesser extent (Taketo et al., 1976). These properties were transferred thoroughly to the transductants, because relative rates of synthesis for RNA polymerase subunits were as high as those in the original mutant strains, *i.e.* at the permissive temperature (30° C), N3-1 ARt (*rpoB2*·*rpoB7*) showed almost equal rate to that in the wild-type control N3-1 Xt, whereas N3-1 4t (*rpoC4*) and N3-1 Tt (*rpoC1*) overproduced about 3 and 2 folds, respectively, compared to N3-1 Xt (Fig. 1). These results indicated that the *rpoB* and *rpoC* genes transferred into the *uvrB* host were expressed as in the original mutant strains.

On the basis of the observation that the overproduction and rapid degradation of RNA polymerase proteins in the original mutants were closely coupled with the defects in the subunit assembly or activation of premature core enzyme (Taketo and Ishihama, 1976; 1977), we proposed that some protein complexes containing β and/or β' subunits themselves might regulate the expression of both $\beta\beta'$ and α operons (Taketo et al., 1976). The autogenous nature of

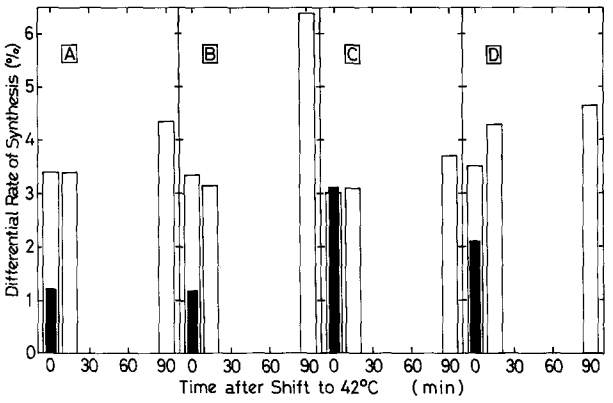

Fig. 1 A–D. Differential rates of synthesis for RNA polymerase and elongation factor Tu in *rpoB* or *rpoC* transductants. Cells of transductants, Xt(**A**), ARt(**B**), 4t(**C**) and Tt(**D**), were labelled at 30° C or at 15 or 90 min after shifting up to 42° C, and differential rates for RNA polymerase and EF-Tu were determined as described in Materials and Methods. Open bar, EF-Tu; closed bar, RNA polymerase (sum of core enzyme subunits)

this regulation was further established since the rates for the synthesis of EF-Tu were essentially the same among the three transductants and the wild-type control strain either at 30° C or after incubating at 42° C for 15 and 90 min, except that N3-1 ARt (*rpoB2·rpoB7*) produced approximately twice the amount of EF-Tu at 90 min after the temperature shift.

Preliminary experiments suggested that the amounts of the ribosomal protein L7/L12, whose structural gene is located between those for EF-Tu (*tufB*) and $\beta\beta'$ (*rpoBC*) operon (Lindahl et al., 1976) was also unaffected in the *rpoB* and *rpoC* transductants (data not shown).

Identification and Quantitation of λdrif⁺-6 Coded Proteins

A lambda transducing phage *λdrif*⁺-6 was constructed by induction of a lysogen which has integrated prophage *λcI857* into the *bfe* locus located near *rif* (*rpoB*), the structural gene for the β subunit of RNA polymerase (Ikeuchi et al., 1975). Recently, several groups reported that another *rif*-transducing lambda phage *λdrif*^{d}18 isolated by Kirschbaum and Konrad (1973) contained the *E. coli* genes coding for not only complete $\beta\beta'$ subunits of RNA polymerase, but also elongation factor Tu (*tufB*), ribosomal proteins L1, L10, L11 and L7/L12 (*rplA, J, K* and *L*), ribosomal RNAs (*rrnB*) and some tRNAs (*glyT, tyrU, thrT* and *gluT*) (Lindahl et al., 1976; Kirschbaum et al., 1976). Because the selction procedure for *λdrif*⁺-6 was essentially similar to that employed for the isolation of *λdrif*^{d}18, it was expected that the phage *λdrif*⁺-6 also carried the genes for these components in addition to the complete *rpoB* gene.

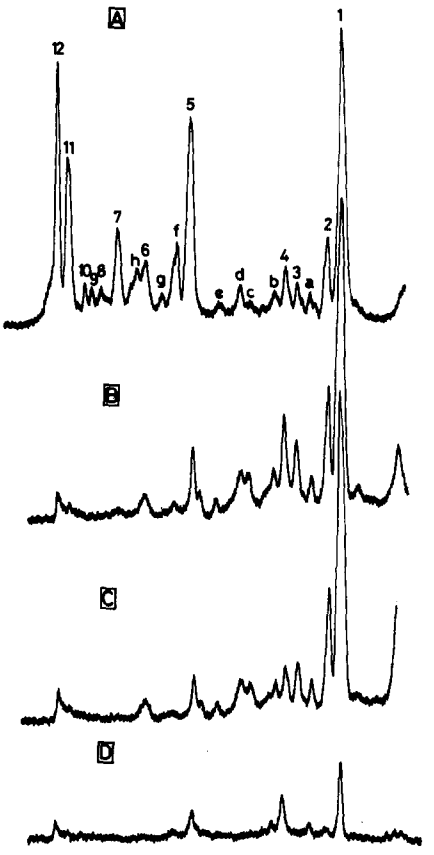

Fig. 2 A–D. Electrophoresis of *λdrif*⁺-6-coded proteins. UV-irradiated *uvrB* cells were infected with *λdrif*⁺-6 and labelled with [^{14}C]amino acids. Cell lysates was prepated as described in Materials and Methods and directly subjected to electrophoresis on polyacrylamide slab gel (**A**). Portions of the cell lysate were treated with anti-RNA polymerase holoenzyme (**B**), anti-β subunit (**C**), anti-β' subunit (**D**) sera, and precipitates formed were analyzed as above. Fluorograms of the gel slabs were traced with micordensitometer. Electrophoresis was from right to left

In order to verify this expectation, the phage was infected onto UV-irradiated *uvrB* cells and the phage coded proteins were labelled with [^{14}C]amino acids; crude cell extracts were electrophoresed on polyacrylamide slab-gel in the presence of SDS, and gel slabs were exposed to X-ray films in the presence of scintillator. Figure 2A shows one of the typical fluorograms, in which bands no. 1–12 were reproducibly observed with use of independent preparations of the phage and the UV-irradiated cells whereas bands a-h were found in some experiments and in various proportions. Among the reproducibly observed products, bands no. 1, 5 and 12, the major three polypeptides, were identified as RNA polymerase β subunit, elongation factor Tu, and ribosomal protein L7/L12, respectively, because they comigrated on electrophoresis with purified authentic samples and cross-reacted with specific antisera against each component. Bands no. 7 and 11 were identified as ribosomal protein L1 and a mixture of L10 and L11, respectively. Since

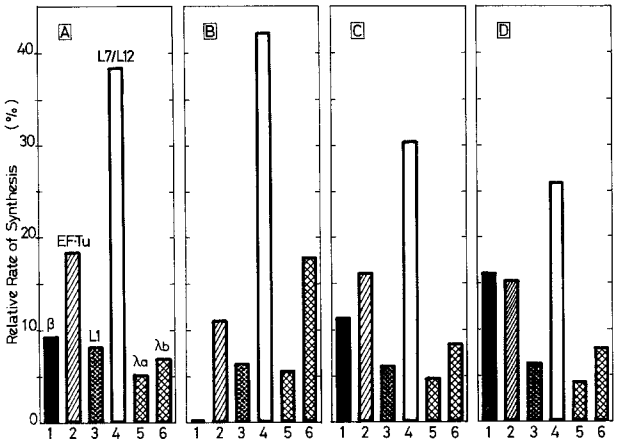

Fig. 3A–D. Relative synthesis rates of some $\lambda drif^+$-6-coded proteins in *rpoB* or *rpoC* transductants. Phage $\lambda drif^+$-6 was infected at 34° C to UV-irradiated cells of transductants, Xt(**A**), ARt(**B**), 4t(**C**), and Tt(**D**), and labelled for 10 min with [^{14}C]amino acids. Cell lysates were directly subjected to electrophoresis on polyacrylamide gel. The amounts of each protein synthesized were determined as described in Materials and Methods, and are expressed as percentage to the sum of the labelled proteins except λa and λb. The amounts of λa and λb are expressed as percentage to the total proteins including λa and λb. *1*, β subunit; *2*, EF-Tu; *3*, ribosomal protein L1; *4*, robosomal protein L7/L12; *5*, λa; *6*, λb

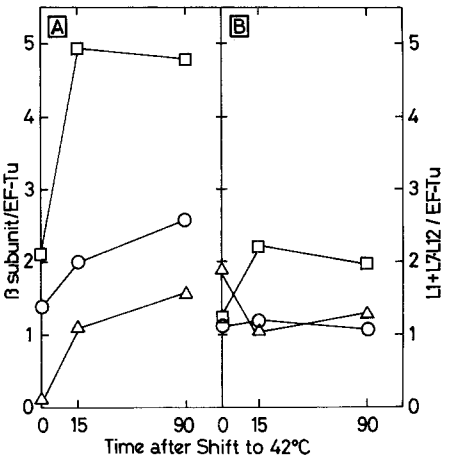

Fig. 4A and B. Relative synthesis rates of RNA polymerase β subunit and ribosomal proteins L1 plus L7/L12 to EF-Tu in *rpoB* or *rpoC* transductants preincubated at 42° C. Phage $\lambda drif^+$-6 was infected at 34° C or after temperature shift to 42° C to UV-irradiated cells of transductants, ARt (△——△), 4t (○——○) and Tt (□——□), and labelled for 10 min with [^{14}C]amino acids. Cell lysates were directly subjected to electrophoresis on polyacrylamide gel, and the amounts of each protein synthesized were determined as described in Materials and Methods. The ratios of RNA polymerase β subunit (**A**) and ribosomal proteins L1 plus L7/L12 (**B**) to EF-Tu in the *rpoB* or *rpoC* transductants were compared to those in $rpoB^+C^+$ transductant, Xt

bands no. 8 and 10 were not observed when the phage was infected onto a λ^+-lysogen, these polypeptides were identified as gene products of phage lambda and tentatively called as λa and λb, respectively.

When the cell lysate was treated with anti-RNA polymerase holoenzyme serum, bands no. 2, 3, 4 and 6 polypeptides were quantitatively recovered in the precipitates, as well as β subunit (Fig. 2A), implying that these polypeptides are fragments of β and/or β′ subunit. In addition, most of the alphabetized bands were observed in the antibody precipitates. However, the synthesis of β′ subunit has never been found in $\lambda drif^+$-6 phage infected UV-irradiated cells. Lack of β′ subunit synthesis was confirmed in an *in vitro* protein synthesizing system directed by $\lambda drif^+$-6 DNA (Fukuda et al., 1978). Since both ββ′ subunits were synthesized in the same system when $\lambda drif^d18$ was used instead of $\lambda drif^+$-6 (Fukuda, unpublished observation), it was concluded that $\lambda drif^+$-6 phage carries only a part, if any, of β′ subunit gene (*rpoC*). The nature of putative ββ′ fragments was then investigated employing anti-subunit antisera. As shown in Figure 2C, the amount of polypeptides, except band no. 4, precipitated by anti-β subunit serum was essentially the same with that recovered in anti-holoenzyme precipitates. In contrast, only band no. 4 polypeptide was quantitatively recovered in anti-β′ subunit precipitates. Taken together these observations indicate that bond no. 4 represents the fragment of β′ subunit encoded by incomplete *rpoC* gene on phage $\lambda drif^+$-6.

Expression of ββ′ Operon on $\lambda drif^+$-6 in the UV-Irradiated rpoB or rpoC Mutants

The regulation of a wild-type ββ′ operon on the phage $\lambda drif^+$-6 in the *rpoB* and *rpoC* mutants was investigated by infecting the phage onto UV-irradiated cells. In order to increase the rate of phage protein synthesis in UV-irradiated cells, the *uvrB* transductants constructed as above were cultured at 34° C, and irradiated immediately or after preincubation at 42° C. After phage infection onto the UV-irradiated cells, the synthesis rate of β subunit as well as EF-Tu, ribosomal proteins L1 and L7/L12, and phage proteins λa and λb, was measured at 34° C, and parts of the results are shown in Figures 3 and 4.

The synthesis of β subunit at 34° C was almost completely repressed in the transductant ARt (*rpoB2*·*rpoB7*), whereas the rates of β subunit synthesis in the transductants 4t (*rpoC4*) and Tt (*rpoC1*) were slightly and remarkably higher than those in the control strain Xt ($rpoB^+ \cdot rpoC^+$), respectively (Fig. 3). On the other hand, EF-Tu, L1, L7/L12, λa and λb were synthesized in these *rpoB* and *rpoC* transductants essentially at the same rates as those in the

control Xt, except that the amounts of EF-Tu in ARt and of L7/L12 in 4t and Tt were slightly less, and the amount of λb in ARt was twice more than those in the control Xt. The dramatic change in the synthesis rate peculiar to β subunit clearly demonstrated that the expression of ββ′ operon on the phage λd*rif*$^+$-6 was regulated specifically by some trans-active protein complexes, accumulated in the assembly-defective transductants.

The expression of *rpoB* gene on the phage was also analyzed in the transductants previously exposed to non-permissive temperature for some time. The cells were first grown at 34° C and then shifted up to 42° C for 15 or 90 min. The rate of λd*rif*$^+$-6-coded protein synthesis was determined as above and parts of the results are shown in Figure 4, in which the ratios of β subunit to EF-Tu and of a combination of ribosomal proteins L1 and L7/L12 to EF-Tu are compared among the transductants. Although the amount of β subunit as well as another proteins synthesized in the transductants was reduced following preincubation of host cells at 42° C, the relative amount of the β subunit synthesized in all the transductants was significantly higher than that in the control Xt. After 90 min exposure to 42° C, transductants Tt, 4t and ARt exhibited approximately 5-, 2.5- and 1.5-fold higher rate of β subunit synthesis than control Xt, respectively (Fig. 4). In contrast, the relative synthesis rate for EF-Tu and the sum of ribosomal proteins L1 and L7/L12 remained virtually unaffected among the transductants including the control Xt and even after shift to 42° C. The marked repression of ββ′ operon expression, observed in the transductant ARt grown at 34° C, was not observed when the culture was incubated at 42° C for either 15 or 90 min but the rate of β subunit synthesis rather exceeded that in the control strain.

Discussion

We have examined the effects of trans-active diffusible substances in the temperature-sensitive, assembly-defective mutants on the expression of *rpoB*$^+$ gene introduced into the cells by infecting the transducing phage λd*rif*$^+$-6. In all the experiments described in this report, the phage was infected at the multiplicity of infection of about one. Thus, the ratio of the putative regulatory molecules to the functional *rpoBC* operon in UV-irradiated phage-infected cells might be close to that in unirradiated uninfected cells.

When the *rpoB*2·*rpoB*7 transductants carrying *uvrB* (ARt) was grown and infected with λd*rif*$^+$-6 phage at 34° C, the synthesis of β subunit was markedly reduced compared to that in phage-infected control strain harboring wild-type *rpoB* gene, whereas that of elongation factor Tu, ribosomal proteins L1 and L7/L12 and λ-coded protein λa remained unaffected. This strong inhibition of β subunit synthesis reminds us the transient decrease in the rate of ββ′ subunit synthesis in the original strains A2R7 (*rpoB*2·*rpoB*7) shortly after the shift of culture temperature from 30 to 42° C (Taketo et al., 1976). Analysis of labelled cell lysates by glycerol gradient centrifugation revealed that the assembly of RNA polymerase is defective in this mutant leading to an abnormal accumulation of $\alpha_2\beta$ complex and $\alpha_2\beta\beta'$ premature core at 30° C, and in addition, of free α subunit at non-permissive temperature of 42° C (Taketo and Ishihama, 1977). These observations suggest that free subunits, $\alpha_2\beta$ complex and/or premature core enzyme accumulated in the mutant cells may have "repressor activity" for *rpoBC* expression. Specific inhibition of the *rpoBC* gene expression, however, was not observed if the transductant ARt was exposed to 42° C for 15 or 90 min, suggesting that the repressor molecule(s) had been inactivated during incubation at the non-permissive temperature. In agreement with this interpretation, β and β′ subunits in the assembly intermediates are degraded quite rapidly at 42° C, though σ and α subunits are rather stable (Taketo et al., 1976; Taketo and Ishihama, 1977). However, all the polymerase subunits are metabolically stable in this mutant at 30° C (Taketo et al., 1976) as in wild-type cells (Iwakura et al., 1975).

As reported previously (Taketo et al., 1976), both β and β′ subunits are overproduced in the mutants Ts4 (*rpoC*4) and T16 (*rpoC*1) grown at 30° C. Upon shift to 42° C, the mutant Ts4 exhibited marked but transient decrease in the rate of ββ′ subunit synthesis whereas the other mutant T16 showed a remarkable burst; after about 30 min or later, however, both mutants overproduced ββ′ subunits at more than twice the rate at 30° C. In agreement with these observations, the expression of *rpoBC* operon on the phage was enhanced slightly and markedly in temperature-sensitive *rpoC* transductants 4t and Tt, respectively; this enhancement was observed not only for cells grown at permissive temperature but also for cells further incubated at 42° C for 15 or 90 min. The transient decrease in ββ′ subunit synthesis in the mutant Ts4 can be explained by the repressor hypothesis of $\alpha_2\beta$ complex which accumulates in a large amount shortly after shift to 42° C, due to the alteration of β′ subunit at the non-permissive temperature (Taketo and Ishihama, 1976). On this occasion, the inhibition must be transient because the $\alpha_2\beta$ complex is rapidly degraded at 42° C (Taketo and Ishihama, 1976; Taketo et al., 1976).

Two alternative explanations can be made for marked overproduction of β subunit in the phage-

infected transductants 4t and Tt, e.g., increase of "stimulator(s)" or decrease of "repressor(s)". In this respect, it is worthwhile to note that about 50% of newly synthesized σ subunit in the strain Ts4 and almost all the σ subunit in the strain T16 was recovered in free form, not associated with core enzyme though almost all portion of newly synthesized σ in wild-type cells is associated with core enzyme (Taketo and Ishihama, 1976). Thus, based on the assumption that free σ subunit and/or active holoenzyme is another "repressor", the overproduction can be best explained by a decrease in the concentration of σ subunit or holoenzyme concentration, resulting in the derepression of *rpoBC* operon.

The repressor hypothesis discussed above prompted us a great importance to make an in vitro experiment which might allow us to identify the regulatory protein(s) with repressor activity. Recently, we have found that both $\alpha_2\beta$ complex and holoenzyme repress the in vitro synthesis of β subunit directed by $\lambda drif^+$-6 DNA template (Fukuda et al., 1978).

Acknowledgements. We are grateful to Drs. T. Saitoh and K. Kawakami for discussions, Drs. J.B. Kirschbaum, R.B. Khesin, N. Ryo and T. Yura for kind donation of bacterial strains and phage lysogens. Our thanks also to Drs. A. Miyajima and Y. Kaziro for generous gift of purified EF-Tu preparations and anti-EF-Tu antiserum. Many fruitful discussions made by Dr. T. Yura and some members of the Department of Genetics of this Institute are especially acknowledged. This work was supported in part by grants, No. 210608, No. 220619 and No. 268084, from the Ministry of Education, Science and Culture, of Japan.

References

Backmann, B.J., Low, K.B., Taylor, A.L.: Recalibrated linkage map of *Eschierichia coli* K-12. Bact. Rev. **40**, 116–167 (1976)

Clowes, R.C., Hayes, W.: In: Experiments in microbial genetics. Oxford and Edinburgh: Blackwell Scientific Publications 1968

Fukuda, R., Taketo, M., Ishihama, A.: Autogenous regulation of RNA polymerase β subunit synthesis in vitro. J. biol. Chem., **253**, 4501–4505 (1978)

Hamel, E., Koka, M., Nakamoti, T.: Requirement of an *Escherichia coli* 50S ribosomal protein component for effective interaction of the ribosome with T and G factors and with guanosine triphosphate. J. biol. Chem. **247**, 6–9 (1972)

Hayward, R.S., Scaife, J.G.: Systematic nomenclature for the RNA polymerase genes of prokaryotes. Nature (Lond.) **260**, 646–648 (1976)

Hayward, R., Tittawella, I., Scaife, J.: Evidence for specific control of RNA polymerase synthesis in *Escherichia coli*. Nature (Lond.) New Biol. **243**, 6–9 (1973)

Ikeuchi, T., Yura, T., Yamagishi, H.: Genetic and physical studies of lambda transducing bacteriophage carrying the beta subunit gene for the *Escherichia coli* ribonucleic acid polymerase. J. Bact. **122**, 1247–1256 (1975)

Ilyna, T., Ovadis, M., Mindlin, S., Gorlenko, Zh., Khesin, R.: Interaction of RNA polymerase mutations in haploid and merodiploid cells of *Escherichia coli* K12. Molec. gen. Genet. **110**, 118–133 (1971)

Ishihama, A., Taketo, M., Saitoh, T., Fukuda, R.: Control of formation of RNA polymerase in *Escherichia coli*. In: RNA Polymerase (Losick, R., Chamberlin, M., eds.), pp. 485–502. New York: Cold Spring Harbor Laboratory 1976

Iwakura, Y., Ito, K., Ishihama, A.: Biosynthesis of RNA polymerase in *Escherichia coli*, I. Control of RNA polymerase content at various growth rates. Molec. gen. Genet. **133**, 1–23 (1974)

Jaskunas, S.R., Burgess, R., Nomura, M.: Identification of a gene for the α subunit of RNA polymerase at the *str-spc* region of the *Escherichia coli* chromosome. Proc. nat. Acad. Sci. (Wash.) **72**, 5036–5040 (1975)

Kirschbaum, J., Claeys, I., Nasi, S., Molholt, B., Miller, J.: Temperature sensitive RNA polymerase mutants with altered subunit synthesis and degradation. Proc. nat. Acad. Sci. (Wash.) **72**, 2375–2379 (1975)

Kirschbaum, J.B., Greenblatt, J., Allet, B., Rochaix, J.-D.: Studies with a $\lambda drif$ transducing phage carrying the genes for the β and β' subunits of *E. coli* RNA polymerase. In: RNA Polymerase (Losick, R., Chamberlin, M., eds.), pp. 503–518. New York: Cold Spring Harbor Laboratory 1976

Kirschbaum, J.B., Konrad, E.B.: Isolation of a specialized lambda transducing bacteriophage carrying the genes for *Escherichia coli* RNA polymerase. J. Bact. 517–526 (1973)

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.) **227**, 680–685 (1970)

Laskey, R.A., Mills, A.D.: Quantiative film detection of ^3H and ^{14}C in polyacrylamide gels by fluorography. Europ. J. Biochem. **56**, 335–341 (1975)

Lennox, E.S.: Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1**, 190–206 (1955)

Lindahl, L., Post, L., Nomura, M.: DNA dependent in vitro synthesis of ribosomal proteins, protein elongation factors, and RNA polymerase subunit α: Inhibition by ppGpp. Cell **9**, 439–448 (1976)

Lindahl, L., Yamamoto, M., Nomura, M., Kirschbaum, J., Allet, B., Rochaix, J.P.: Mapping of a cluster of genes for components of the transcriptional and translational machineries of *Escherichia coli*. J. molec. Biol. **109**, 23–47 (1977)

Maher, D.L., Dennis, P.P.: In vivo transcription of *E. coli* genes coding for rRNA, robosomal proteins and subunits of RNA polymerase: Influence of the stringent control system. Molec. gen. Genet. **155**, 203–211 (1977)

Miyajima, A., Kaziro, Y.: Coordination of levels of elongation factors Tu, Ts, and G, and ribosomal protein S1 in *Escherichia coli*. J. Biochem. (Tokyo), **83**, 453–462 (1978)

Myenburg, K.V., Neidhardt, F.C.: Retrospectives-perspectives. In: Control of ribosome synthesis (Kjeldgaard, N.C., Malløe, O., eds.), pp. 447–463. copenhagen: Munksgaard 1976

Nakamura, Y., Yura, T.: Effects of rifampicin on synthesis and functional activity of DNA-dependent RNA polymerase in *Escherichia coli*. Molec. gen. Genet. **145**, 227–237 (1976)

O'Farrell, P.H.: High resolution two-dimensional electrophoresis of prpteins. J. biol. Chem. **250**, 4007–4021 (1975)

Saitoh, T., Ishihama, A.: Biosynthesis of RNA polymerase in *Escherichia coli*, VI. Distribution of RNA polymerase subunits between nucleoid and cytoplasm. J. molec. Biol. **115**, 403–416 (1977)

Scaife, J.: Bacterial RNA polymerases: The genetics and control of their synthesis. In: RNA Polymerase (Losick, R., Chamberlin, M., eds.), pp. 207–225. New York: Cold Spring Harbor Laboratory 1976

Taketo, M., Ishihama, A.: Biosynthesis of RNA polymerase in *Escherichia coli*, IV. Accumulation of intermediates in mutants

defective in the subunit assembly. J. molec. Biol. **102**, 297–310 (1976)

Taketo, M., Ishihama, A.: Biosynthesis of RNA polymerase in *Escherichia coli*, V. Defects of the subunit assembly in a temperature-sensitive β subunit mutant. J. molec. Biol. **112**, 65–74 (1977)

Taketo, M., Ishihama, A., Kirschbaum, J.B.: Altered synthesis and stability of RNA polymerase subunits in mutants of *Escherichia coli* with mutations in the β or β' subunit genes. Molec. gen. Genet. **147**, 139–143 (1976)

Watson, R.J., Parker, J., Fill, N.P., Flaks, J.G., Friesen, J.D.: New chromosomal location for structural genes of ribosomal proteins. Proc. nat. Acad. Sci. (Wash.) **72**, 2765–2769 (1975)

Wittmann, H.G.: Purification and identification of *Escherichia coli* ribosomal proteins. In: Ribosomes (Nomura, M., Tissieres, A., Lengyl, P., eds.), pp. 93–114. New York: Cold Spring Harbor Laboratory 1974

Communicated by E. Bautz

Received June 16, 1978