Defective Assembly of Ribonucleic Acid Polymerase Subunits in a Temperature-Sensitive α-Subunit Mutant of *Escherichia coli*†

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ABSTRACT: The subunit assembly of RNA polymerase was investigated for the temperature-sensitive *Escherichia coli* strains carrying the mutation *rpoA*101 or *rpoA*112 in the gene encoding its α subunit. In cells carrying *rpoA*112, the sequential assembly of enzyme subunits is blocked at an early step, i.e., either the dimerization of altered α subunit or the subsequent association of altered α dimer with β subunit. As a result, the unassembled free α subunit accumulates and the unassembled β and β′ subunits are degraded rapidly, in particular at a nonpermissive temperature. The assembly defect is accompanied by an overproduction of enzyme subunits apparently due to the decrease in the concentration of repressor holoenzyme involved in the autogenous regulation. These results together with the previous observations [Ishihama, A., Shimamoto, N., Aiba, H., Kawakami, K., Nashimoto, H., Tsugawa, A., & Uchida, H. (1980) *J. Mol. Biol. 137*, 137–150] indicate that the temperature-sensitive growth of *rpoA*112 mutants is attributed to the assembly defect of RNA polymerase as well as to the thermolability of assembled polymerase. In contrast, the altered α subunit in a mutant carrying *rpoA*101 is assembled into the polymerase structure as efficiently as in wild-type cells; nevertheless, both β and β′ subunits are rapidly degraded in this mutant. This indicates that the mutant polymerase is structurally different from the metabolically stable wild-type enzyme. Thus, the *ts* character of *rpoA*101 mutant is explained by the alteration in the structure and function of assembled RNA polymerase.

The DNA-dependent RNA polymerase[1] is the key enzyme responsible for gene transcription in *Escherichia coli* and thus one of the cell components essential for cell growth [see review by Yura & Ishihama (1979)]. The RNA polymerase holoenzyme is composed of at least five subunits, two α, one β, β′, and one σ, and extensive work has been devoted to reveal the role each subunit plays in RNA synthesis [see reviews by Zillig et al. (1976) and Yura & Ishihama (1979)]. For this purpose, a number of conditionally lethal as well as drug-resistant strains carrying mutations in the β- or β′-subunit genes (*rpoB* and *rpoC*) have been isolated and characterized [see reviews by Scaife (1976) and Yura & Ishihama (1979)]. However, no mutation has been found for the α subunit gene (*rpoA*) except the mutation *groP*109 (later renamed as *rpoA*109) which cannot support the growth of phage P2 (Sunshine & Sauer, 1975; Fujiki et al., 1976). Recently, we isolated two temperature-sensitive *E. coli* strains carrying mutations *rpoA*101 or *rpoA*112 in the α-subunit gene and so far have found several unique properties associated with the mutants (Ishihama et al., 1980b). RNA synthesis stops after a considerable lag period upon up-shift of culture temperature, both purified and reconstituted RNA polymerases containing the mutant α subunits exhibit irregular profiles of thermal inactivation, and the mutant RNA polymerases show a low fidelity of transcription in vitro though no evidence has been obtained for the incorrect transcription in vivo.

During the enzyme reconstitution experiments from isolated subunits, it was recognized that the recovery of enzymic activity was remarkably lower for the mutant polymerases than that for the wild-type polymerase. The observation immediately suggested that the assembly of RNA polymerase subunits does not proceed in the mutants as efficiently as it does in wild-type cells, in analogy with the assembly-defective mutants carrying mutations in the β- or β′-subunit genes (Taketo & Ishihama, 1976, 1977; Gross et al., 1977). On this sequence, we have examined the in vivo assembly of the two mutant RNA polymerases and it was indeed found that at least one of the mutants harboring *rpoA*112 had a defect in the assembly at the step of either the dimerization of altered α subunit or the association of altered α dimer with β subunit. In agreement with the autogenous regulation of RNA polymerase synthesis [see reviews by Scaife (1976), Yura & Ishihama (1979), and Ishihama & Fukuda (1980)], the defect in the assembly of RNA polymerase in this α-subunit mutant was accompanied by the overproduction of β and β′ subunits. Some of the excess β and β′ subunits was degraded rapidly into several unique fragments. On the other hand, the temperature-sensitive growth of the mutant carrying *rpoA*101 is due to the alteration in the function and structure of RNA polymerase composed of altered α subunit because the assembly of RNA polymerase proceeds normally in this mutant.

Materials and Methods

Bacterial Strains and Media. The following strains of *E. coli* K12 were provided by Dr. H. Nashimoto, University of Tokyo, and used in the present study: HN198, a *malA* derivative of AB2834 F⁻ *aroE thi su*⁻ (Pittard & Wallace, 1966); HN206, a *his* derivative of HN198; HN315*ts*101, a *rpoA*101 derivative of HN198; HN316*ts*112, a *rpoA*112 derivative of HN206; HN317*ts*112, a *rpoA*112 derivative of HN198. Cultures were grown and labeled in M9-glucose medium (Clowes & Hayes, 1968) supplemented with 20 μg/mL shikimic acid, 20 μg/mL phenylalanine, 10 μg/mL tyrosine, and 10 μg/mL thiamin hydrochloride. In addition, 50 μg/mL histidine was supplemented for the cultures of HN206 and HN316*ts*112.

Chemicals. Recrystallized acrylamide and sodium dodecyl sulphate ($NaDodSO_4$) were purchased from Wako Chemicals Co., Japan, and *N,N′*-diallyltartardiamide was from Eastman Kodak Co. L-[4,5-$^3H_2$]Leucine (370 mCi/mg) and L-[U-

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[1] Abbreviations used: RNA polymerase, ribonucleoside-5′-triphosphate:RNA nucleotidyltransferase (DNA dependent) (EC 2.7.7.6); $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetate.

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^{14}C]leucine (324 mCi/mmol) were purchased from the Radiochemical Centre, England. Pancreatic deoxyribonuclease (DNase I) and ribonuclease (RNase A) were purchased from Worthington Biochemical Co. and Sigma Chemicals, respectively, while lysozyme was from Seikagaku Kogyo, Japan. Brij 58 was a product of Atlas Chemicals.

RNA Polymerase and Antisera. RNA polymerase holoenzyme was purified as described previously (Fukuda et al., 1974). Antisera against RNA polymerase holoenzyme and each subunit were prepared in rabbits essentially according to the procedure of Iwakura et al. (1974).

Determination of Subunit Synthesis Rate. The rate of subunit synthesis was determined as described previously (Iwakura et al., 1974; Taketo et al., 1976). Log phase cultures of the parental and mutant strains were grown at 34 °C to a cell density of 30 turbidity units as measured with a Klett-Summerson photometer. Portions of the cultures were pulse-labeled either at 34 °C or at various times after a shift to 42 °C with [^{3}H]leucine for 2 min followed by a 3-min chase with excess unlabeled leucine, isoleucine, and valine to allow completion of full-size subunit molecules. The labeled cells were harvested by centrifugation, washed with Tris–saline–EDTA, lysed by treatment with lysozyme–EDTA–Brij 58, and disrupted by sonication. The sonicated lysates were digested with a mixture of DNase I and RNase A and subsequently treated with specific rabbit antiserum made against RNA polymerase holoenzyme. The antigen–antibody precipitates formed after 3 days of incubation at 4 °C were collected by centrifugation, washed 3 times with 10 mM Tris-HCl buffer (pH 7.4) containing 0.15 M NaCl and 1 mM EDTA, solubilized in NaDodSO$_4$–sample buffer, and fractionated by electrophoresis on polyacrylamide cylindrical gels according to Shapiro et al. (1967) except that the gels were cross-linked with diallyltartardiamide. In some experiments, the two-step indirect method of Kessler (1975) was employed, in which immune complexes were bound to protein A on the cell wall of formalin-fixed, heat-inactivated *Staphylococcus aureus*. The amount of ^{3}H radioactivity associated with each subunit band on the gels was measured and corrected for the recovery of the ^{35}S-labeled carrier RNA polymerase added prior to the immunoprecipitation. The differential rate of subunit synthesis was estimated by dividing the corrected amount of radioactivity for each subunit by the total amount of acid-insoluble radioactivity in the crude extracts used.

Determination of Subunit Distribution. Crude extracts of labeled cells were fractionated by centrifugation on a 30-mL linear gradient of glycerol from 15 to 35% in 10 mM Tris-HCl (pH 7.8 at 4 °C) containing 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 0.5 mM *p*-methylbenzenesulfonyl fluoride, and 0.2 M KCl. After centrifugation in a Spinco SW25.1 rotor at 24000 rpm for 70 h, gradients were fractionated and aliquots of the fractions were subjected to determination of subunit content.

Identification of Fragments of Subunits. For identification of the fragments of enzyme subunits, the antigen–antibody precipitates prepared as described above were fractionated by NaDodSO$_4$–polyacrylamide slab gel electrophoresis in the phosphate buffer system of Shapiro et al. (1967). Gels were treated for fluorography as described by Lasky & Mills (1975). In some experiments, the electrophoresis system of Laemmli (1970) was also employed.

Results

Assembly of RNA Polymerase in α-Subunit Mutants. The assembly of RNA polymerase subunits was investigated for *E. coli* cells carrying the temperature-sensitive mutation

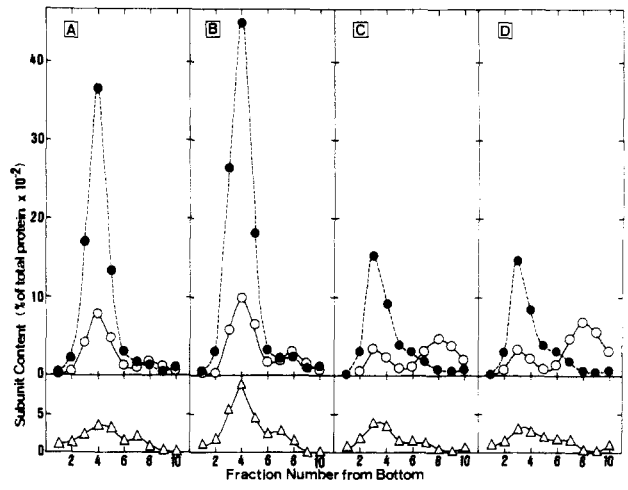

FIGURE 1: Distribution of RNA polymerase subunits in cell lysates fractionated by glycerol gradient centrifugation. 20 mL of each cell culture (A, HN198; B, HN315*ts*101; C and D, HN317*ts*112) at 34 °C was continuously labeled with radioactive leucine (B and D, 0.1 mCi of [^{14}C]leucine; A and C, 0.5 mCi of [^{3}H]leucine) until a turbidity of 30, as measured with a Klett-Summerson photometer. Cells were harvested for analysis of subunit distribution in (C), while in (A), (B), and (D) the culture temperature was shifted thereafter to 42 °C until 40 Klett units. Cell lysates were fractionated by glycerol gradient centrifugation, and the resulting glycerol fractions were analyzed for their content of labeled enzyme subunits as described under Materials and Methods. (●) β plus β' subunits; (○) α subunit; (△) σ subunit.

*rpoA*101 or *rpoA*112 in the α-subunit gene. For this purpose, cells of the two α-subunit mutants, HN315*ts*101 and HN317*ts*112, were continuously labeled with [^{3}H]leucine before or after a shift of the culture temperature from 34 to 42 °C; labeled cell lysates were fractionated by glycerol gradient centrifugation, and the amount of each subunit in the fractions was determined separately by polyacrylamide gel electrophoresis of precipitates formed after the addition of a mixture of specific antisera against holoenzyme and α subunit. The efficiency of immunoprecipitation was routinely measured based on the precipitation of ^{35}S-labeled carrier RNA polymerase added into glycerol fractions prior to the addition of antisera.

Figure 1 shows the results, in which one can identify that the pattern of subunit distribution for the wild-type parent HN198 is essentially similar to those obtained with other wild-type *E. coli* strains: e.g., K12 W3350 (Ito et al., 1975) and K12 X240, the parental strain of assembly-defective β- or β'-subunit mutants (Taketo & Ishihama, 1976, 1977). In these strains, most of the β and β' subunits are assembled into the enzyme structure but a small amount (10–20%) of the α subunit exists as a free unassembled form(s). The existence of free α subunit is consistent with the observation that α subunit is synthesized in excess over β and β' subunits in these strains (Iwakura et al., 1974; Taketo et al., 1976) and at least during the exponentially growing phase (Kawakami et al., 1979). In sharp contrast with these wild-type strains, more than half of the α subunit in a cell lysate from HN317*ts*112 was recovered in slowly sedimenting fractions (Figure 1C) and the amount of this unassembled free α subunit increased furthermore following the temperature up-shift (Figure 1D). For improvement of the accuracy of the determination of unassembled α subunit, mixtures of ^{3}H-labeled cell lysates and ^{35}S-labeled RNA polymerase were treated with low concentrations of NaDodSO$_4$ and the dissociated enzyme subunits were precipitated with a mixture of antisubunit sera. The amount of α subunit obtained by the improved method was essentially identical with the values by the present method

(data not shown). Thus, it was concluded that the altered α subunit in this mutant was inefficiently assembled into the enzyme structure at the nonpermissive temperature and thus the defect in the assembly was a block of the early step reaction(s) in the known sequence [see review by Ishihama (1980)]. The lack of unassembled pairs of the two large subunits, β' and β, is due to rapid degradation of these subunits, resulting in the accumulation of degradation fragments (see Figure 3). Rapid degradation of unassembled β' and β subunits but not of α subunit is a metabolism common to all the assembly-defective mutants (Taketo et al., 1976) as well as those under a block of the subunit assembly during the stationary phase of a wild-type strain (Kawakami et al., 1979). However, the degradation pathway of the two large subunits is different for isolated subunits, assembly intermediates, and native core enzyme (Ishihama et al., unpublished results). Since the degradation fragments in the assembly-defective mutant (see Figure 3) coincided well with those produced from isolated individual subunits, it was further supported that the assembly was blocked at an early step prior to the association of β' and β subunits. The assembly defect in this mutant is consistent with the previous observation that, following the temperature up-shift, RNA synthesis stops only after a considerable lag period (Ishihama et al., 1980b).

When another α-subunit mutant, HN315*ts*101, carrying the mutation *rpoA*101 was examined as described above, the sedimentation profile of enzyme subunits, shown in Figure 1B, was rather similar to those of wild-type *E. coli* cells. It was unexpected that the amounts of assembled RNA polymerase proteins in this mutant were within the same range as those in the wild-type parent, since the rate of cell growth was remarkably different between the two strains, i.e., 70–77 min for each doubling of the parent and 90–118 min for the α-subunit mutant under the experimental conditions. Thus, it appears that the mutant cells contain higher amounts of assembled RNA polymerase than the value expected from the linear relationship between the rate of cell growth and the content of RNA polymerase (Iwakura et al., 1974; Iwakura & Ishihama, 1975; Ishihama et al., 1976b). The exceptionally higher amount of RNA polymerase in HN315*ts*101 is discussed below in connection with the abnormal function and structure of mutant polymerase.

Rate of Subunit Synthesis in α-Subunit Mutants. It has been established that the autogenous regulation operates for the determination of the subunit synthesis rate [see reviews by Scaife (1976), Yura & Ishihama (1979), and Ishihama & Fukuda (1980)], in which both holoenzyme and $\alpha_2\beta$ complex function as the regulatory molecules with repressor activity (Taketo et al., 1978; Fukuda et al., 1978). In fact, the defective assembly of RNA polymerase more or less influenced the rate of subunit synthesis (Taketo et al., 1976). Thus, the two α-subunit mutants were examined for the rate of subunit synthesis.

Figure 2 shows the results, in which the differential synthesis rates were measured at various times before and after a shift of the culture temperature from 34 to 42 °C. In agreement with the previous observation that, shortly after the shift of culture temperature, the transient change occurs in the synthesis rates of some *E. coli* proteins, including RNA polymerase (Leumaux et al., 1978; Yamamori & Yura, 1978), the synthesis rates of all the polymerase subunits change not only for the mutants but also for the wild-type parent within 15 min after the temperature shift. In addition to the general and transient changes, unique changes were observed thereafter in the synthesis rates of enzyme subunits for the two α-subunit

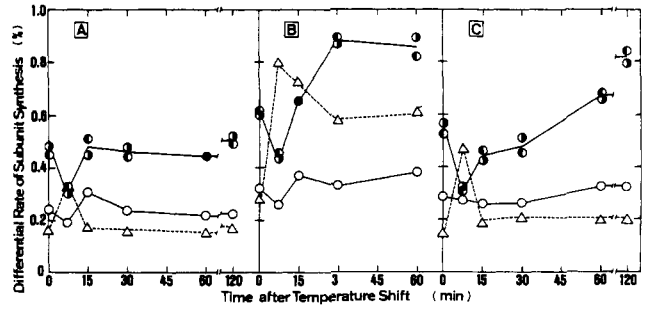

FIGURE 2: Differential synthesis rate of RNA polymerase subunits. Log phase cultures of wild-type parent (A, HN198) and temperature-sensitive α-subunit mutants (B, HN315*ts*101; C, HN317*ts*112) grown at 34 °C were pulse-labeled for 2 min with 1 μCi/mL [^{3}H]-leucine at 34 °C or at various times after a temperature shift to 42 °C followed by a 3-min chase with unlabeled leucine, isoleucine, and valine. The differential rate of subunit synthesis was determined for 10-mL aliquots at the indicated times as described under Materials and Methods. (●) β' subunit; (◑) β subunit; (○) α subunit; (△) σ subunit.

mutants whereas those for the wild-type parent stay at a constant level characteristic of the post-shift rate of cell growth. The assembly-defective α-subunit mutant HN317*ts*112 showed an increased synthesis rate of β and β' subunits but not of α and σ subunits. The kinetics is similar to that observed with the assembly-defective β'-subunit mutant Ts4, in which the association of $\alpha_2\beta$ complex and β' subunit is blocked, resulting in the derepression of β and β' synthesis supposedly due to the decrease in the holoenzyme concentration (Taketo & Ishihama, 1976; Taketo et al., 1976).

As described above, another α-subunit mutant, HN315*ts*101, exhibited a regular profile of subunit distribution and thus an apparently normal assembly of enzyme subunits. However, the synthesis rates of all the enzyme subunits, including that of the σ subunit, were considerably increased in this mutant, i.e., 1.5–2-fold higher than those of wild-type parent cells at a permissive temperature and more than 2-fold higher at a nonpermissive temperature. The gradual decrease in the rate of RNA synthesis in HN315*ts*101 (Ishihama et al., 1980b) can be best explained to be due to some defect in the function of RNA polymerase, in particular at high temperatures. In this context, it is noteworthy that purified RNA polymerase from this strain exhibits significant infidelity in transcription (Ishihama et al., 1980b). The functional defect may induce the overproduction of enzyme subunits for compensation.

Degradation of RNA Polymerase in α-Subunit Mutants. It is well-known that RNA polymerase is metabolically as stable as most of the constitutive proteins in exponentially growing *E. coli* cells (Iwakura et al., 1974) but that most of unassembled β' and β subunits in the assembly-defective mutants as well as in the stationary phase cells of a wild-type strain are rapidly degraded (Taketo et al., 1976; Kawakami et al., 1979). Thus, the metabolic stability of RNA polymerase proteins in the two α-subunit mutants was investigated. For this purpose, cells were pulse-labeled with radioactive leucine at both permissive and nonpermissive temperatures and immediately chased by adding nonradioactive leucine for various periods. Cell lysates were treated with antiholoenzyme serum or a mixture of antisubunit sera; precipitates formed were analyzed by polyacrylamide gel electrophoresis, and the gels were subjected to fluorography for identification of subunits and their degraded fragments. One of the typical fluorograms is shown in Figure 3, in which a number of labeled polypeptides are identified beside enzyme subunits for not only the assembly-defective HN316*ts*112 but also for HN315*ts*101.

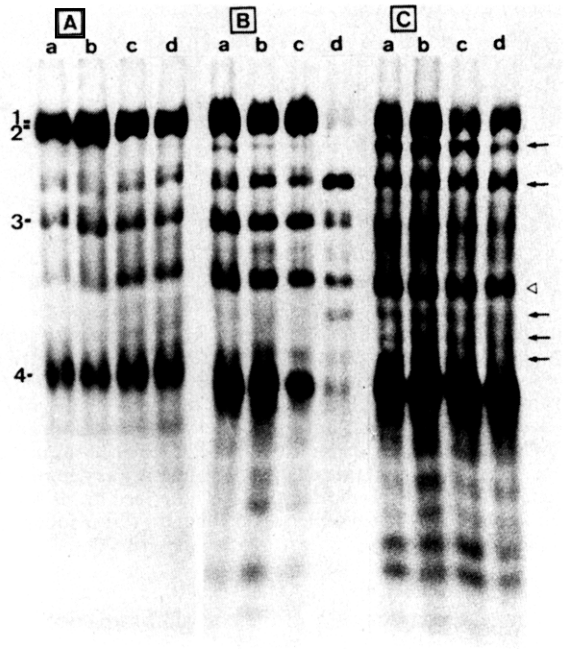

FIGURE 3: Degradation of RNA polymerase subunits. 50 mL of each log phase culture (A, HN198; B, HN315*ts*101; C, HN316*ts*112) at 30 °C was pulse-labeled for 3 min with 0.25 mCi of [^{3}H]leucine and immediately chased by adding excess leucine, isoleucine, and valine at final concentrations of 200, 50, and 50 μg/mL, respectively, for 3 (a), 15 (b), 60 (c), and 120 min (d). Cell lysates were prepared from 10-mL aliquots of the labeled cultures as described under Materials and Methods. Portions of the cell lysates containing 2.61 × 10^6 cpm were mixed with 17.4 μg of purified RNA polymerase holoenzyme and treated with 20 μL each of antiholoenzyme serum. Antigen–antibody precipitates formed after 3 days of incubation at 4 °C were subjected to the polyacrylamide slab gel electrophoresis method of Shapiro et al. (1967). Gels were stained with Commassie brilliant blue R250 and treated for fluorography according to Lasky & Mills (1975). The numbers on the left side indicate the positions of subunits of RNA polymerase holoenzyme: (1) β′ subunit; (2) β subunit; (3) σ subunit; (4) α subunit. The arrows on the right side indicate the major degradation fragments of the enzyme subunits, and the triangle indicates the ATPase coprecipitated with RNA polymerase (Ishihama et al., 1976a).

In the gel pattern obtained for the assembly-defective mutant HN316*ts*112, at least five polypeptides, shown by arrows in Figure 3C, with approximate molecular weights of 135K (K represents 1000), 110K, 60K, 50K, and 45K were identified beside the enzyme subunits. Among these polypeptides, only 110K protein was found in the wild-type parent (Figure 3A) and thus it might represent one of the τ proteins found associated with purified RNA polymerase (Burgess et al., 1969; Kajitani et al., unpublished results). Since all these polypeptides were precipitated with the antisera even when the cell lysates were treated with low concentrations of $NaDodSO_4$ prior to the addition of antisera, it was concluded that they are degraded and/or prematurely terminated fragments of enzyme subunits and not accessory factors associated with RNA polymerase. For identification of the origin of these fragments, the cell lysates were also treated with $NaDodSO_4$ and the antigen–antibody precipitates formed in the $NaDodSO_4$-treated lysates after the addition of either anti-β′- or anti-β-subunit serum were analyzed as described above. The results indicated that all of these fragments but no α and σ subunits were coprecipitated along with β′ or β subunits (data not shown). Moreover, these fragments were not precipitated when the same lysates were treated with either anti-α- or anti-σ-subunit serum. Although the amount of labeled α subunit stayed at a constant level at least within 120 min following the chase, not only the amounts of complete β′ and β subunits but also the amounts of fragments of β′ and β subunits decreased significantly after 60 min of the chase (Figure 3C, c and d).

On the other hand, three major degradation fragments with the antigenicity of the β′ or β subunit were identified for HN315*ts*101. They had approximate molecular weights of 110–115K, 55–60K, and 45K (Figure 3B). However, these fragments did not comigrate, under the slab gel electrophoresis system of Laemmli (1970), with the degradation fragments of similar molecular weights in HN316*ts*112 (data not shown). The difference in the degradation pathway of subunits of RNA polymerase is in good agreement with the above observation that the newly synthesized subunits are differently assembled in the two mutants. In fact, it has been demonstrated that unassembled free subunits, assembly intermediates, and intact polymerase are degraded in different fashions both in vitro and in vivo (Ishihama et al., unpublished results). It is worthwhile to note that enzyme subunits including the α subunit are also degraded in HN315*ts*101 (Figure 3B) although most of the enzyme subunits in this mutant are assembled into the enzyme complex. RNA polymerase in this mutant is therefore not only functionally defective (Ishihama et al., 1980b) but might also be altered in structure, which is recognized as a target for the degradation system of abnormal proteins in *E. coli* cells.

Discussion

A number of peculiar properties have been found for the temperature-sensitive *E. coli* strains carrying the mutation *rpoA*101 or *rpoA*112 in the gene for RNA polymerase α subunit, which include the considerable thermolability or unexpected thermostability of RNA polymerases purified from the mutant HN316*ts*112 or HN315*ts*101, respectively, and the low fidelity of in vitro transcription catalyzed by purified RNA polymerases from the mutant cells (Ishihama et al., 1980b). The observation that RNA synthesis in the mutants stops only after certain lag periods following the temperature up-shift raised the possibilities that the formation of functional RNA polymerase might be repressed in these mutants at nonpermissive temperature and/or that the preexisting polymerases might be converted into inactive forms gradually upon the temperature up-shift. We have examined the assembly of RNA polymerase subunits in these mutants and found that it is defective at least in the mutant HN317*ts*112, in particular at a nonpermissive temperature, at an early step, i.e.,, at either the dimerization of altered α subunit or the subsequent association of altered α dimer with β subunit, of the known sequence of subunit assembly [see review by Ishihama (1980)]. Thus, more than half of the α subunit in this mutant accumulated in an unassembled free form(s). Taken together with the previous observations (Ishihama et al., 1980b), it is natural to explain the temperature-sensitive growth of the strains carrying *rpoA*112 mutation by the defective assembly of enzyme subunits as well as by the thermolability of assembled polymerase.

In agreement with the findings with other assembly-defective β′- or β-subunit mutants (Taketo et al., 1976), the assembly-defective α-subunit mutant HN317*ts*112 overproduced both β′ and β subunits without affecting the rate of α- and σ-subunit synthesis and the excess but unassembled β′ and β subunits were degraded rapidly. The degradation fragments of β′ and β subunits identified in this particular mutant are identical, at least in the size estimated based on electrophoretic mobility, with those formed by limited digestion of isolated β and β′ subunits by proteases (Ishihama et al., unpublished results). The identification of the origin of these fragments

was performed by an immunological method in which cell lysates were treated with low concentrations of $NaDodSO_4$ and the dissociated subunits were specifically precipitated with antisera against individual subunits. The method was successfully employed for the discrimination of enzyme-associated transcription factors from both enzyme subunits and their degradation fragments (Ishihama et al., 1980a). The content of assembled RNA polymerase core enzyme in this mutant at 34 °C was approximately one-third of that in the wild-type parent HN198. The slow rate of cell growth for this mutant, i.e., 120–240 min for one doubling at 34 °C where its parent grows at the generation time of 70–77 min, might be attributed to the decreased content of RNA polymerase.

On the other hand, the assembly of RNA polymerase in the mutant HN315*ts*101 seems to proceed as efficiently as that in its parent HN198. As described previously (Ishihama et al., 1980b), RNA polymerase purified from this mutant exhibited a thermostability higher than that of the wild-type polymerase. The low fidelity of transcription observed in the in vitro system with purified RNA polymerase from HN315*ts*101 has not been shown to be the intrinsic property of a transcriptase in the mutant cells. Taken together, none of the known properties alone appears to be enough to explain the *ts* character of this mutant. Since the rate of RNA polymerase production in this mutant is higher, in particular after the temperature up-shift, than the rate in wild-type *E. coli* and thus higher than the value expected from the rate of cell growth, it is inferred that RNA polymerase synthesis is derepressed in this mutant due to decreased concentrations of the functional repressors, holoenzyme or $\alpha_2\beta$ complex. Thus, it appears that the mutant holoenzyme is altered even in its repressor function. The considerable degradation of RNA polymerase in this mutant also supports the notion that not only the function but also the conformation of RNA polymerase is altered in this mutant, which might be responsible for the *ts* character of the mutant cell. It is noteworthy that not only assembly intermediates but also assembled polymerase is subject to degradation when the polymerase is structurally and functionally different from the complete enzyme in wild-type *E. coli* cells.

Acknowledgments

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