A TEMPERATURE-SENSITIVE MUTANT OF ESCHERICHIA COLI WITH AN ALTERED RNA POLYMERASE β' SUBUNIT

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SUMMARY: A temperature-sensitive RNA polymerase mutant of Escherichia coli K-12 was isolated. Subunit reconstitution experiments showed that the mutation was located in the gene for β' subunit. RNA polymerase purified from the mutant was stable and showed reversible temperature sensitivity in vitro. At high temperature, the initiation of RNA synthesis was impaired and the degree of inhibition was different with templates used. These results suggested that the β' subunit had an essential role for promoter selection.

DNA-dependent RNA polymerase of E. coli consists of four different subunits: α, β, β' and σ (1, 2). The catalytic activity of this enzyme has been extensively studied (3), but the definite information on the role for each subunit in the complex process of transcription is scarce (4). As an approach toward understanding the precise role of each subunit in RNA polymerase during transcription, we undertook isolation of temperature-sensitive (ts) mutants with altered RNA polymerases which expressed their temperature sensitivity in the usual in vitro reaction system.

In this paper we report the isolation and the identification of such a β' subunit mutant. RNA polymerase purified from this mutant, unlike enzymes of previously isolated ts

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Abbreviations: ts, temperature-sensitive; tr, temperature-resistant

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mutants (5, 6), was stable and showed reversible temperature sensitivity *in vitro*. This property allowed us to analyze the role of the β' subunit during transcription *in vitro*.

EXPERIMENTAL

An *E. coli* K-12 strain, PA3092 (F^-, *thr*, *leu*, *trp*, *his*, *thyA*, *argH*, *thi*, *lacY*, *malA*, *mtl*, *xyl*, *mel*, *tonA*, *supE*, *str*) was mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine and 5000 ts mutants of independent origin were isolated as described previously (7, 8). This mutant-collection served as the source of strains with altered RNA polymerases. All cells were grown in L-broth supplemented with 50 μg/ml thymine.

The rates of RNA synthesis in exponentially growing cells at 30°C and after a shift to 43°C for 16 min were measured by incubating 0.5 ml cells for 2 min with [^{3}H]uridine (0.2 μCi, 41.5 Ci/mmol) at 30°C and 43°C and counting acid-insoluble material. About 70 strains which were low in the rate of RNA synthesis at 43°C were selected from 900 ts mutants of the collection. Their RNA polymerases were prepared essentially according to Burgess up to the DEAE-cellulose step (1). After assaying the enzyme activity at 30°C and 43°C, we obtained one strain, JE10092, which appeared to be a ts RNA polymerase mutant.

Enzyme assay was done as described previously (9) with modifications described in the legends. Core enzyme and subunit σ were prepared as described (2, 10). Separation of the core subunits and reconstitution of the enzyme were done essentially according to Lill *et al.* (11).

The method used for transduction experiments was described by Lennox (12). Rifampicin (100 μg/ml) was added to agar-plates when necessary.

RESULTS AND DISCUSSION

Temperature sensitivity of the mutant RNA polymerase

RNA polymerases were partially purified from parent strain PA3092 and ts mutant strain JE10092 and the temperature sensitivity *in vitro* was examined. As shown in Table 1, RNA synthesis by the parent enzyme at 43°C was about 40% of that at 30°C. On the other hand, the JE10092 enzyme was significantly less active at 43°C than at 30°C. Furthermore, the mutant enzyme was several fold less active even at 30°C than the wild-type enzyme. The RNA polymerases isolated from the strains which were made temperature-resistant (tr) by phage P1

Table 1

Enzymic activity of RNA polymerases of PA3092, JE10092 and its transductants

Enzymes	[^{3}H]UMP incorporated (cpm)		
	30°	43°	43°/30°
PA3092	5510	2123	0.39
JE10092	1446	256	0.17
9225 (tr, Rif^s)	5438	1874	0.35
9226 (tr, Rif^r)	2128	1077	0.51
9249 (ts, Rif^r)	953	114	0.12

P1 phage that had been grown on strain E5014 Rif^r (F'128, thi, rel, mal, spc12, supE, Δ(proB-lac), rif) was used to infect recipient strain JE10092 (ts, Rif^s), selecting for tr transductants; 9225 (tr, Rif^s) and 9226 (tr, Rif^r). JE10092 was crossed with Hfr P4X and Lac$^+$ Met$^+$ recombinants were selected at 30°C; 9202 (Hfr P4X, his, ts). Strain 9202 was made Rif^r by the above P1; 9234 (Hfr P4X, his, ts, Rif^r). Transduction was carried out with 9234 as donor and wild-type strain W3110 as recipient, selecting Rif^r transductants; 9249 (ts, Rif^r). Reaction mixtures contained total volume of 0.2 ml: 0.04 M Tris-HCl (pH 7.9 at 25°C), 8 mM $MgCl_2$, 0.1 mM dithiothreitol, 0.1 mM EDTA, 0.2 mM each of ATP, CTP, GTP and [^{3}H]UTP (10 μCi/μmol), 0.2 M KCl, 0.4 mM K_2HPO_4, 100 μg BSA, 5 μg T_4-DNA and 30 μl enzyme preparation (DEAE-cellulose fraction, approximately 1 OD_{280}/ml). Incubations were for 10 min at 30°C and 43°C.

transduction from a tr strain regained original activity at high temperature. On the other hand, the wild-type (W3110) which was made ts by phage P1 transduction from the ts mutant produced a ts RNA polymerase. We, therefore, concluded that the ts cell growth was due to the ts RNA polymerase.

Identification of the defective subunit

Highly purified RNA polymerase was obtained from JE10092 cells by the modified procedure of Burgess (1, 13). The holoenzyme was subjected to phosphocellulose column chromatography to separate σ subunit and core enzyme and the core enzyme

Table 2

Enzymic activity of reconstituted RNA polymerases

Wild subunits	[^{3}H]UMP incorporated (nmol)		
	30°	43°	43°/30°
-	0.30	0.19	0.63
α	0.20	0.13	0.65
β	0.31	0.21	0.68
β'	1.41	1.22	0.87

50 pmol of JE10092 holoenzyme and 5-fold excess of respective PA3092 subunit were combined and incubated for 2 hr at 23°C in the presence of 7 M urea, 0.01 M Tris-HCl (pH 7.9 at 25°C), 0.01 M $MgCl_2$, 0.5 M KCl, 10 mM dithiothreitol, 0.1 mM EDTA and 50% glycerol (total volume 0.1 ml). After denaturation, the mixture was dialyzed for 3 h at 23°C followed by overnight at 4°C against 0.01 M Tris-HCl (pH 7.9 at 25°C), 0.01 M $MgCl_2$, 0.5 M KCl, 1 mM dithiothreitol, 0.1 mM EDTA and 50% glycerol. Enzyme assay was as in the legend to Table 1 except that 5 μg equivalent enzyme and about 10% glycerol were included.

was found to be ts. To identify the defective subunit in the core enzyme, the enzyme reconstitution experiment was carried out by mixing excess amount of a wild-type subunit with the ts holoenzyme. As shown in Table 2, only the ts enzyme mixed with wild-type β'subunit resulted in tr and in great recovery of activity at 30°C and 43°C. Apparent high ratios of activity at 43°C to 30°C in the mutant enzyme in the reconstitution assay was due to the presence of glycerol in assay mixture. Glycerol was found to stabilize the enzyme at high temperature. We concluded that the ts mutation (rpoC92) was located in the gene for β' subunit of RNA polymerase.

Genetic characterization of rpoC92

The transduction experiments were carried out with a rifampicin-resistant strain as donor and JE10092 as recipient,

Table 3

Co-transduction of *rpoC92* with *rif*

Selection	Growth on L (42°C)	+Rif	Number of transductants
Tr		+	99
		-	9
Arg^+	+	+	8
	+	-	24
	-	+	52
	-	-	0

The tr transductants were obtained from a cross between the recipient strain JE10092 (ts, Rif^S) and the donor strain E5014 Rif^r (see Table 1) and their rifampicin sensitivity was examined. The Arg^+ transductants were obtained from a cross between the recipient strain LC161 (F^-, *leu*, *proA*, *argH*, *tsx*, *str*) and the donor strain 9234 (see Table 1). Their temperature sensitivity and rifampicin sensitivity were examined. + growth, - no growth.

and with a JE10092 derivative (*rpoC92*, *rif*) as donor and a strain carrying *argH* as recipient. From these results shown in Table 3, the *rif* and *rpoC92* mutations were co-transduced by phage P1 at a frequency of 92%, and the map order was *argH* - *rif* (β) - *rpoC92* (β') which was consistent with the earlier report (14).

Enzymic characterization of the ts RNA polymerase

Ts enzymes are in general unstable and often difficult to purify. However the RNA polymerase of strain JE10092 was easy to purify by a usual method with appropriate modifications and the enzyme so obtained was as stable as the parent enzyme up to at least 6 months. When a reaction mixture containing JE10092 enzyme was incubated for 10 min at 43°C and then transferred to

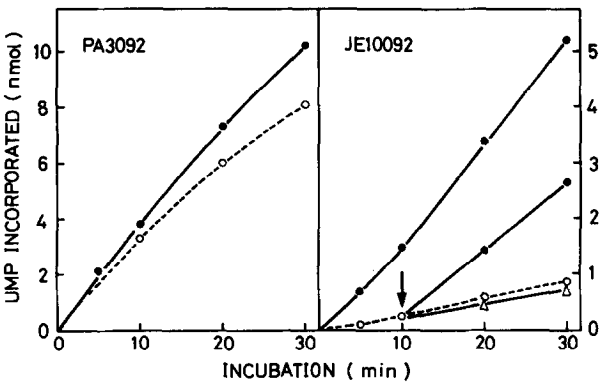

Fig. 1. RNA synthesis with PA3092 and JE10092 RNA polymerases at 30°C and 43°C. Reaction mixtures were as described in the legend to Table 1 except that 25 μg/ml enzyme was added. Reaction mixtures containing JE10092 enzyme were incubated for 10 min at 43°C and transferred to 30°C with or without addition of 5 μg/ml rifampicin (indicated by an arrow). ● 30°C, o 43°C, Δ 30°C with rifampicin.

Table 4

Enzymic activity of PA3092 and JE10092 RNA polymerases with various templates

Templates	[^{3}H]UMP incorporated (43°/30°)	
	PA3092	JE10092
T4-DNA	1.12	0.19
Calf thymus-DNA	0.88	0.31
T7-DNA	1.22	0.34
λ-DNA	0.82	0.48
φX174 RF-DNA	0.90	0.65

Enzyme assay was done as described in the legend to Table 1 except that 5 μg of DNA and 5 μg of enzyme were added.

30°C, the activity was restored (Fig. 1). This indicated that the loss of activity at high temperature was not due to an irreversible inactivation of the mutant enzyme. The RNA polymerase with reversible temperature sensitivity should be

more useful for studying transcription process in vitro than previously reported ts enzymes (5, 6). No recovery was observed when rifampicin was added simultaneously with the temperature shift-down (Fig. 1). This result indicated that high temperature affected the initiation of RNA synthesis.

The β' subunit of RNA polymerase has the greatest affinity for DNA (4). Therefore, we compared the temperature sensitivity of the JE10092 enzyme with various templates. The ratios of activity at 43°C to 30°C differed with templates used (Table 4). This result suggested that the β' subunit played an important role in promotor selection. Gross et al. reached a similar conclusion (6).

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