THE ROLE OF RNA POLYMERASE IN GENETIC TRANSCRIPTION

K. Igarashi and T. Yura
Institute for Virus Research
Kyoto University, Kyoto, Japan

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The DNA-dependent RNA polymerase found in many organisms including bacteria (see Hurwitz and August, 1963) is generally believed to be the genetic transcriptase (Hayashi et al, 1964) in that it catalyzes the transcription of the genetic information from DNA to RNA. However, this is mostly based on the properties of RNA polymerase studied in vitro, and no direct evidence is available for the role of this enzyme in vivo.

We have recently isolated a number of temperature-sensitive mutants of Escherichia coli K12 that produce heat-labile RNA polymerase (Igarashi and Yura, 1968). In this communication, we will report on properties of one of these mutants, KY5544, and will present a proof that RNA polymerase indeed represents the genetic transcriptase in E. coli.

The mutant KY5544 (stv-266) was isolated from nitrosoguanidine-treated culture of strain KY131 (a derivative of PA678) as a mutant that is resistant to an antibiotic streptovaricin (SV, a specific inhibitor of RNA polymerase in bacteria; Mizuno et al, 1968) at 30°C and has simultaneously lost the ability to grow at high temperature (42°C) in polypeptone medium without SV. This mutant, when grown at 30°C, produces RNA polymerase which is heat labile and resistant to SV, as compared to the parental enzyme. As shown in Fig. 1, the mutant enzyme is more labile than the wild-type enzyme when heated in the presence of 0.5M KCl. Under these conditions, the enzyme is in the dissociated form with a sedimentation coefficient of about 15S following centrifugation in a glycerol density gradient. In contrast, the mutant and the wild-type enzymes

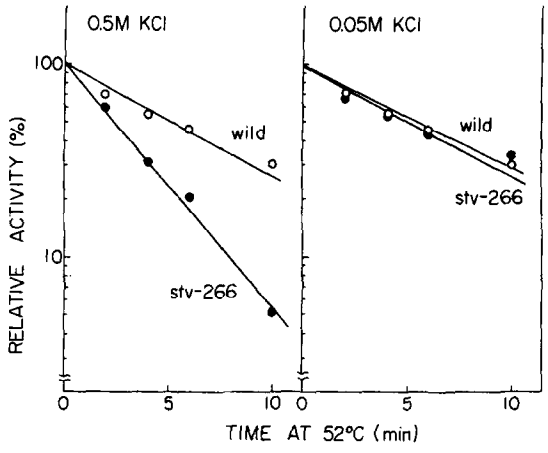

Fig. 1. Thermostability of the mutant and wild-type RNA polymerase. Cells were grown in medium E supplemented with 0.2% Casamino acids and thiamine (2 μg/ml) at 30°C to a late log phase, and RNA polymerase prepared by a modified procedure of Chamberlin and Berg (1962). The partially purified enzyme was dialyzed overnight in the presence of 0.5M or 0.05M KCl. Aliquots of the enzyme were heated at 52°C, chilled in ice, and incubated with an equal volume of other standard components of the reaction mixture (including H^3-UTP and E. coli DNA) for 30 min at 30°C. The radioactivity in the acid-insoluble fraction was determined in a liquid scintillation counter.

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are about equally stable when heated under low KCl concentration (0.05M), where the enzyme takes an associated form with a sedimentation coefficient of about 22S. These results clearly indicate that the mutant produces the structurally altered RNA polymerase and therefore the gene involved in this mutation represents a structural gene for this enzyme. Moreover, the fact that the mutant enzyme is heat labile only at high KCl concentration may suggest the physiological role of the dissociated form of RNA polymerase, at least in some stage of the transcription process.

When the mutant culture that had been grown at 30°C is transferred to 45°C, H^3-uridine incorporation into acid-insoluble fraction ceases after about 20 min, in contrast to the wild-type bacteria (Fig. 2). Similar results were obtained when RNA was either pulse-labeled with H^3-uridine or determined by the orcinol method at various times after the temperature shift. DNA and protei

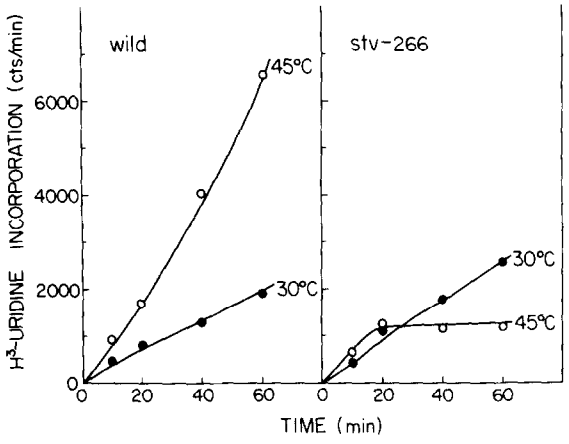

Fig. 2. Incorporation of H^3-uridine with mutant and wild-type bacteria. Cells were grown in the minimal-Casamino acids medium at 30°C and were divided into two at time 0, uridine-5-H^3 (0.2 μc/10 μg/ml) was added, and incubated further at 30°C or 45°C. Aliquots were taken at intervals and the radioactivity in the acid-insoluble fraction was determined.

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syntheses as determined colorimetrically are also markedly reduced shortly after the cessation of RNA synthesis.

To determine the effect of the temperature shift on the synthesis of various RNAs in this mutant, RNA pulse-labeled with H^3-uridine was examined by sucrose density-gradient centrifugation and by DNA-RNA hybridization. As the results in Fig. 3 and Table 1 indicate, the synthesis of ribosomal, transfer, and messenger RNA is all reduced appreciably under these conditions.

The gene determining the SV resistance and the temperature sensitivity in strain KY5544 (stv-266) has been mapped close to argH by P1 phage transduction. Other SV-resistant mutants have previously been mapped in this region (Yura and Igarashi, 1968). When an F14 episome (Pittard et al, 1963) is transferred into strain KY5544, the resulting merodiploid strain exhibits the wild-type phenotype with respect to both temperature sensitivity and SV resistance. This suggests that the F14 episome carries the stv^+ allele and that the mutant allele (stv-266) is recessive to stv^+.

The foregoing experiments clearly show that the RNA polymerase indeed

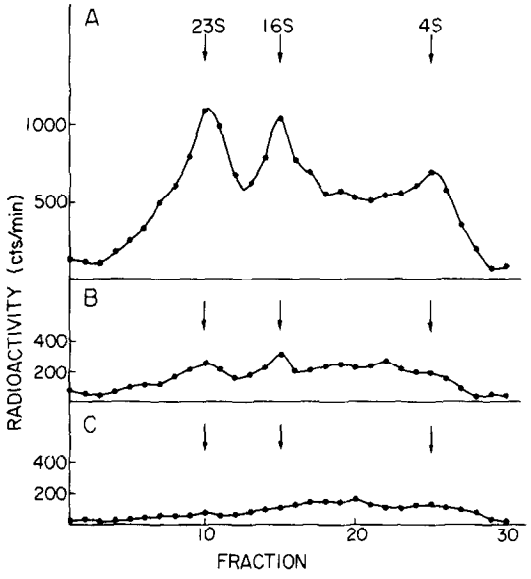

Fig. 3. Sucrose density-gradient analysis of pulse-labeled RNA of the mutant. Cells were grown at 30°C and were transferred, at time 0, to 45°C as in Fig. 2. RNA was pulse-labeled with H^3-uridine (A) from -2 to 0 min at 30°C, (B) from 10 to 12 min at 45°C, and (C) from 20 to 22 min at 45°C. RNA was extracted with phenol and examined by sucrose density-gradient centrifugation as described previously (Yura and Igarashi, 1968). H^3-RNA was centrifuged through a sucrose gradient (5-20%) at 100,000 x g for 6 hr with C^{14}-labeled *E. coli* RNA for density reference.

represents the major, and perhaps the only, enzyme that catalyzes the transcription *in vivo* of all major species of RNA from the corresponding regions on the template DNA. Consistent with this conclusion is the finding that SV which specifically inhibits RNA synthesis in *E. coli* by presumably binding to RNA polymerase (Mizuno *et al*, 1968) does inhibit the synthesis of ribosomal, transfer and messenger RNA in the wild-type *E. coli* (Yura and Igarashi, 1968). Obviously, temperature-sensitive mutants such as the one described here should be useful for further studies on the role of RNA polymerase in bacterial and phage growth as well as for structural and functional analysis of this enzyme.

TABLE 1

DNA-RNA hybridization analysis of pulse-labeled RNA

	H^3-RNA		RNA hybridized	
Temperature	pulse(min)	cts/min	cts/min	per cent
30°C	-2 - 0	109,790	11,009	10.0
45°C	10 - 12	42,600	5,497	12.9
45°C	20 - 22	17,964	2,304	12.8

Pulse-labeled RNA used for sucrose density-gradient analysis (Fig. 3) was hybridized with denatured E. coli DNA by the method of Gillespie and Spiegelman (1965). About 0.5 μg of H^3-RNA and 50 μg of denatured E. coli DNA were used. Values represent the average from two separate experiments.

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