INITIATION BY RNA POLYMERASE ON UV OR X-RAY DAMAGED T7 DNA

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Summary: It is possible to dissociate template activity from initiation specificity of E. coli RNA polymerase. Ultraviolet damage in DNA diminishes the template activity of bihelical T7 DNA but does not result in new nonspecific initiation points. On the other hand, X-ray damage in DNA not only decreases template activity, but also increases nonspecific initiation. These results show that the quality of the template DNA is a critical consideration in studies on the initiation specificity of RNA polymerase in vitro.

Recent experiments on DNA-dependent RNA polymerase from bacteria have illuminated many details of the mechanism of transcription (e. g. see review by Richardson, 1969). One rather generally ignored aspect of this problem is that of initiation specificity. This is understandable since the criteria for 'biologically meaningful' initiation are not usually available. In experiments with DNA template from coliphages T7 and T3, however, one such criterion is obvious. Since all the mRNA made in vivo by these phages is complementary to the r-strand of the DNA, it follows that any in vitro copying of the l-strand must be the result of improper initiation (Summers and Szybalski, 1968). The experiments described in this paper use this asymmetry test to monitor initiation from template which has been damaged specifically by either ultraviolet or X-irradiation. These results confirm the prediction (Summers, 1969a) that initiation specificity is a function of the state of the DNA template.

MATERIALS AND METHODS

T7 DNA was isolated in either of two ways which gave similar results. DNA was extracted by the phenol method of Mandell and Hershey (1960), or by heat release at 65°C (Freifelder, 1965) in the presence of 0.1% sodium

dodecyl sulfate (SDS). The SDS and most of the phage protein were precipitated at $0^{o}C$ with 0.3 M KCl. The DNA was then dialyzed into 0.01 M Tris Cl, pH 7.9.

RNA polymerase was isolated and purified by the method of Burgess (1969) from commercial E. coli B cell paste. The enzyme was purified through the low ionic-strength glycerol gradient sedimentation step. Analysis by SDS-polyacrylamide gel electrophoresis showed that this enzyme was about 70% pure and that the sigma specificity subunit (Burgess, Travers, Dunn and Bautz, 1969) was present.

Standard incubation conditions for the in vitro transcription of T7 DNA were described previously (Summers and Siegel, 1969). The following concentrations were used: .04 M Tris Cl, pH 7.9; 0.2 M KCl; 0.07 mM EDTA; 4.6 mM $MgCl_2$; 2 mM $MnCl_2$; 0.15 mM CTP; 0.15 mM GTP; 0.15 mM UTP; 0.15 mM ^{14}C-ATP; 1 ci/mole; 0.04 M mercaptoethanol; 25 μg/ml DNA. The amount of enzyme added was adjusted to approximate equivalence with the template. Incubations were for 30 minutes at $37^{o}C$. The reaction was terminated by chilling to $0^{o}C$ and the addition of 0.1% SDS containing 1.6% diethyl pyrocarbonate (Solymosy, Fedorcsăk, Gulyăs, Farkas and Ehrenberg, 1968).

RNA extraction from the incubation mixture was done following the method of Summers (1969b). The mixture containing SDS and diethyl pyrocarbonate was precipitated with 2 M NaCl at $0^{o}C$ and the supernatant which contained the RNA was removed and used directly in the hybridization assay.

Hybridization of the RNA to the separated strands of the T7 DNA was done as previously described (Summers and Siegel, 1969). The strands of T7 DNA were preparatively separated as described by Summers and Szybalski (1968). Experiments were carried out so that the hybridization efficiency was about 40% and so that about 1000 cpm of RNA hybridized to the DNA. Controls without DNA were included for each sample to estimate the background of self-annealing and nonspecific retention on the nitrocellulose filters.

Ultraviolet irradiation was from a G15T8 Sylvania Germicidal Lamp. The DNA solution (525 μg/ml, in 0.01 M Tris Cl, pH 7.9) was constantly stirred during exposure. The dose rate was 10 ergs/mm^2/sec.

X-irradiation was from a 250 kvp X-ray machine. The DNA was

irradiated in 0.01 M Tris Cl, pH 7.9 at 525 μg/ml.

RESULTS

Nine replicate determinations of the asymmetry of initiation from the unirradiated T7 DNA sample used for most of the experiments reported here gave an average (± s.d.) of 89 ±2% r-strand specific RNA. The presence of Mn^{++} in the incubation mixture seemed necessary for highly asymmetric synthesis by most preparations of RNA polymerase. However, for reasons not understood, some preparations did not exhibit this requirement. Since Mn^{++} did not inhibit activity significantly, it was included in all incubations in an attempt to insure asymmetric initiation.

Figure 1 shows the effects of ultraviolet irradiation of the template DNA on the template activity and the asymmetry of initiation. At doses that reduce template activity to less than 35% of the control, no change in the initiation specificity could be detected. Inactivation of template activity decreased exponentially after an initial rapid inactivation of about 10% of the control activity.

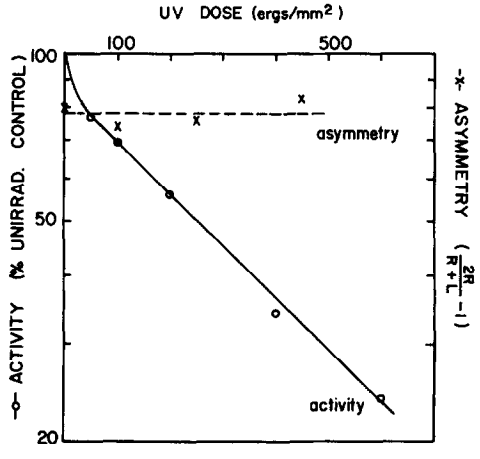

Figure 1. Effect of UV-irradiation on template activity of T7 DNA (left hand axis) and on initiation specificity (right hand axis). The template activity is given as percent of unirradiated control DNA. The initiation specificity is given in terms of the asymmetry of the RNA product. This is measured by the ratio $\frac{2R}{R+L} - 1$, where R is the amount of RNA complementary to the r-strand of T7 DNA, and L is the amount of RNA complementary to the l-strand. This ratio is 1.00 for perfectly asymmetric (r-strand) synthesis and 0 for random (symmetric) synthesis.

Figure 2 shows the effects of X-irradiation of the template DNA. The loss of template activity was accompanied by a concomitant increase in nonspecific initiation as inferred from the increase in symmetry of the RNA product. At low doses of X-rays, there was a small increase in template activity to about 10-15% greater than the control DNA template. As in the case of the ultraviolet-irradiated template, the X-ray inactivation appeared to be exponential after an initial rapid inactivation of about 10-15% of the control activity.

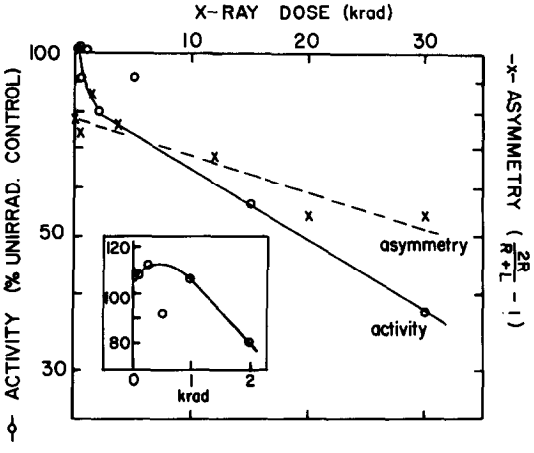

Figure 2. Effect of X-irradiation on template activity and initiation specificity. Data plotted as in Figure 1. Inset shows enlargement of initial part of inactivation curve.

DISCUSSION

The experiments presented here suggest that the deformation of the DNA bihelix by UV-photoproducts, mainly thymine dimers (Wacker, 1963), is not responsible for new initiation sites for synthesis by RNA polymerase. It can not be excluded, however, that the enzyme could be bound and inactivated at such sites in the DNA. These results are compatible with the model in which the UV photoproducts induce premature chain termination. The exponential inactivation is in accord with such a model of fixed initiation sites together with the introduction of random sites of chain termination. This model was previously proposed by Michalke and Bremer (1969) to explain the effect of UV on RNA synthesis *in vivo*.

On the other hand, X-ray damaged DNA contains lesions which serve as new initiation sites for RNA synthesis. DNA irradiated under these con-

ditions contains many single-strand breaks (Summers and Szybalski, 1967), some of which may serve as new initiation sites. At low doses, this might result in an increase in template activity (Figure 2, insert). This view is supported by the recent finding of Vogt (1969) that mild treatment of DNA with endonuclease can increase its template activity. The inactivation that is observed at high doses of X-rays may be the result of several factors. First, since the DNA is fragmented by the accumulation of single-strand breaks and occasional double-strand breaks, premature termination may be the result of loss of linear continuity of the DNA. Second, newly broken ends and internal single-strand breaks may bind polymerase but in most instances not permit synthesis, thereby inactivating the enzyme (Weiss and Wheeler, 1967). Third, some single-strand breaks and damaged bases may hinder the progress of the enzyme and result in premature termination.

A preliminary report (Graziosi, 1967) of similar experiments with phage α suggests that such new initiation points are also produced *in vivo* by X-irradiation, since some RNA from irradiated, infected cells is complementary to the strand not normally transcribed.

These results, together with those of Vogt (1969), show some of the pitfalls inherent in studying RNA synthesis *in vitro*. Enzyme activity, template activity, and initiation specificity are distinct parameters and must be assessed individually. Among several variables, the state of the template DNA is critical for proper initiation specificity.

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