X-Ray Inactivation of the *Escherichia coli* Deoxyribonucleic Acid Dependent Ribonucleic Acid Polymerase in Aqueous Solution. II. Studies on Initiation and Fidelity of Transcription†

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ABSTRACT: The ability of complementary ribosyl pentamers to bypass chain initiation and to stimulate poly(A) synthesis in poly(U)- and poly(dT)-directed reactions using the *Escherichia coli* DNA-dependent RNA polymerase was examined. Utilizing this method of bypassing the rate-limiting step in *in vitro* RNA synthesis, we examined how X-irradiation influences inactivation of enzymatic activity of the RNA polymerase. We previously showed (preceding paper) that X-ray inactivation of RNA polymerase, when assayed for enzymatic activity against homoribopolymers, was directly dependent on substrate concentration. At low substrate levels, where the initiation step is rate limiting, radiosensitivity of the RNA polymerase was most acute. However, the substrate-dependence phenomenon can be eliminated with addition to the reaction mixture of initiator molecules (oligomer primers). Furthermore, response of the irradiated enzyme, when assayed against homodeoxyribopolymers, becomes less radiosensitive in the presence of oligomer primers. We suggest that the initiation process can account for the radiosensitive step in RNA synthesis. The fidelity of RNA synthesis by X-irradiated RNA polymerase was also examined. We found a linear increase in misreading, or insertion of noncomplementary nucleoside triphosphates into the RNA product, for both poly(U), and poly(dT)-directed synthesis of poly(A) as a function of dose. Misreading was three to four times more pronounced in poly(dT)-directed reactions compared to poly(U)-directed reactions.

It has been shown that the initiation or rate-limiting step in RNA synthesis can be bypassed with the use of complementary oligoribonucleotides for a variety of polymer templates (Niyogi and Stevens, 1964, 1965a,b; Downey and So, 1970). The effectiveness of bypassing chain initiation with primer oligoribonucleotides in the RNA polymerization reaction is a function of complex formation between template molecule and oligomer and is dependent on template, oligomer, and substrate concentrations (Straat *et al.*, 1971). It has also been shown that incorporation of [γ-^{32}P]nucleoside triphosphates (a measurement of chain initiation, Maitra and Hurwitz, 1965) is decreased significantly with the addition of complementary oligomers, even in the presence of the RNA polymerase subunit σ, implying that the oligomer is replacing the [γ-^{32}P]nucleoside triphosphate as a chain initiator (Downey and So, 1970; Niyogi, 1972). It is also known that a free 3′-OH group is essential in the oligonucleotide for stimulation (Niyogi and Stevens, 1965b).

With the ability to bypass chain initiation we decided to investigate how the presence of oligomer primers in the reaction mixture might influence the enzymatic activity of X-irradiated, DNA-dependent RNA polymerase isolated from *Escherichia coli*. In the preceding paper (Strniste *et al.*, 1973), we demonstrated how inactivation of binding ability of RNA polymerase to a template molecule with X-irradiation could not account for the radiosensitivity of the enzyme. Furthermore, we showed that the D_{37} value for inactivation of RNA polymerase, when the assays for enzymatic activity were conducted with homoribopolymer templates, was a function of substrate concentration. This latter phenomenon implied that the initiation step in RNA synthesis may (at least in part) contribute to the radioresponse of the enzyme. Therefore, it was argued that elimination of this step in RNA synthesis with oligomer primers should alter the response of activity of RNA polymerase to X-irradiation.

We also examined how radiation could influence fidelity of transcription when X-irradiated RNA polymerase is assayed with poly(U) or poly(dT) templates in the presence of both complementary and noncomplementary nucleoside triphosphates. Our results for *E. coli* RNA polymerase agree favorably with the recent results of Goddard *et al.* (1969) who showed, with X-irradiated *Micrococcus luteus* RNA polymerase and a poly(U) template, an increase in misreading or incorporation of noncomplementary bases in the RNA product with an increase in radiation dose. Our results for this and the preceding paper (Strniste *et al.*, 1973) suggest that radiation might influence the transfer of genetic information by interfering with the mechanisms of RNA transcription. These perturbations potentially could influence the formation of sublethal and lethal types of damage in irradiated cells.

Experimental Procedures

Isolation of Polymerase. The DNA-dependent RNA polymerase was isolated from *E. coli* strains B/r and B_{s-1} using the method of Burgess (1969), as described in the preceding paper (Strniste *et al.*, 1973).

Materials. All chemicals were of analytical grade unless otherwise specified. Radioactive nucleoside triphosphates (^{3}H or ^{14}C) were purchased from Schwarz/Mann, and unlabeled nucleoside triphosphates were purchased from P-L

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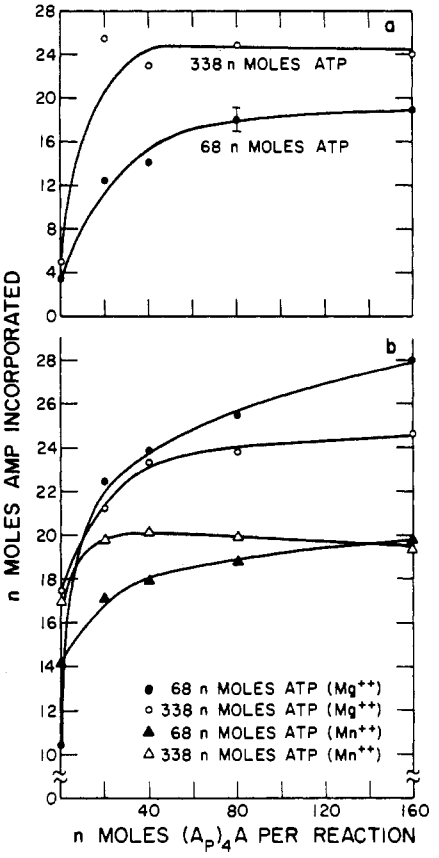

FIGURE 1: Stimulation of poly(U)-directed poly(A) synthesis (a) or poly(dT)-directed poly(A) synthesis (b) with increasing amounts of the complementary oligomer $(Ap)_4A$ added to the reaction. [^{3}H]-ATP (1.1×10^6 cpm/μmol) at either 68 or 338 nmol per reaction, 120 nmol of either poly(U) or poly(dT), and 0.5 μmol of $MnCl_2$ or 2.0 μmol of $MgCl_2$ were mixed with 7.5 μg of *E. coli* B/r RNA polymerase. Poly(U)-directed poly(A) reactions were incubated for 20 min at 37°, and poly(dT)-directed poly(A) reactions were incubated for 10 min at 37°. The reactions were then assayed for CCl_3COOH-insoluble ^{3}H-labeled material.

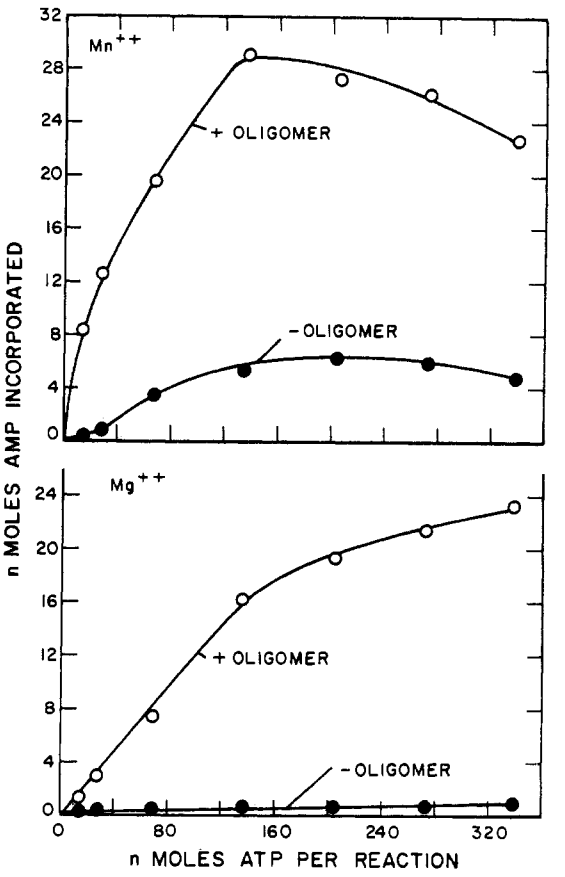

FIGURE 2: Stimulation of poly(U)-directed poly(A) synthesis as a function of amount of substrate (ATP) added to the reaction with or without addition of the oligomer primer $(Ap)_4A$. Reaction conditions were the same as noted in Figure 1 with the exceptions of varying amounts of [^{3}H]ATP added to the reaction and ±80 nmol of $(Ap)_4A$.

Biochemicals, Inc. All labeled and unlabeled nucleoside triphosphates were checked for purity by paper chromatography. Poly(dT) was synthesized as described in the preceding paper (Strniste *et al.*, 1973). The oligoribonucleotide $(Ap)_4A$ was purchased from Miles Laboratories, Inc.

X-Irradiation. Purified samples of RNA polymerase which had been dialyzed extensively against 0.01 M Tris–0.15 M KCl (pH 7.9) were irradiated as previously described (Strniste *et al.*, 1973).

Polymerization Assays. Reactions assayed using the synthetic homopolymers poly(U) and poly(dT) contained per 225-μl reaction (unless stated differently elsewhere): 10 μmol of Hepes[1] buffer (pH 7.9), 0.5 μmol of $MnCl_2$ or 2.0 μmol of $MgCl_2$, 2.5 μmol of 2-ME, 120 nmol[2] of homopolymer, and varying amounts of ATP ([^{3}H]ATP, 1110 cpm/nmol) plus RNA polymerase. Assay mixtures were incubated at 37° for the desired times, spotted on glass fiber disks (Whatman GF-82), and soaked in 5% CCl_3COOH–0.01 M $Na_4P_2O_7$ for 10 min at 0°. The filters were washed with 25 ml of 2% CCl_3COOH–0.01 M $Na_4P_2O_7$ (0°), rinsed with 95% ethanol, and dried. The disks were placed in scintillation vials filled with 15 ml of toluene base scintillation cocktail and counted in a Packard Tri-Carb scintillation spectrometer (Model 3003). Reactions assayed using the polymers poly(U) or poly(dT) and the complementary oligomer $(Ap)_4A$ were conducted as outlined above with the addition of varying amounts of oligomer to the reaction mixture at zero time.

Results

RNA Polymerase Catalyzed Poly(A) Synthesis. INITIATION BYPASS WITH ADDITION OF COMPLEMENTARY OLIGONUCLEOTIDES. It has been demonstrated recently that, in the process of RNA synthesis using a variety of homopolymer templates including poly(U) and poly(dT), there exists a dependence on the σ subunit of the RNA polymerase especially at low substrate concentrations where chain initiation is the rate-limiting step in this process (Niyogi, 1972). However, this σ dependence can be circumvented by addition to the reaction mixture of complementary oligoribonucleotides which presumably bypass this σ-dependent initiation step (Niyogi and Stevens, 1964, 1965a,b; Downey and So, 1970; Niyogi, 1972). In Figure 1a stimulation of enzymatic activity of *E. coli* B/r RNA polymerase using the poly(U) template and varying amounts of the complementary ribopentamer $(Ap)_4A$ is shown at both low and high substrate concentrations. Figure 1b shows the

[1] Abbreviations used are: Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; 2-ME, 2-mercaptoethanol.

[2] Quantities of synthetic homopolymers or complementary oligomers are always expressed in terms of monomer units.

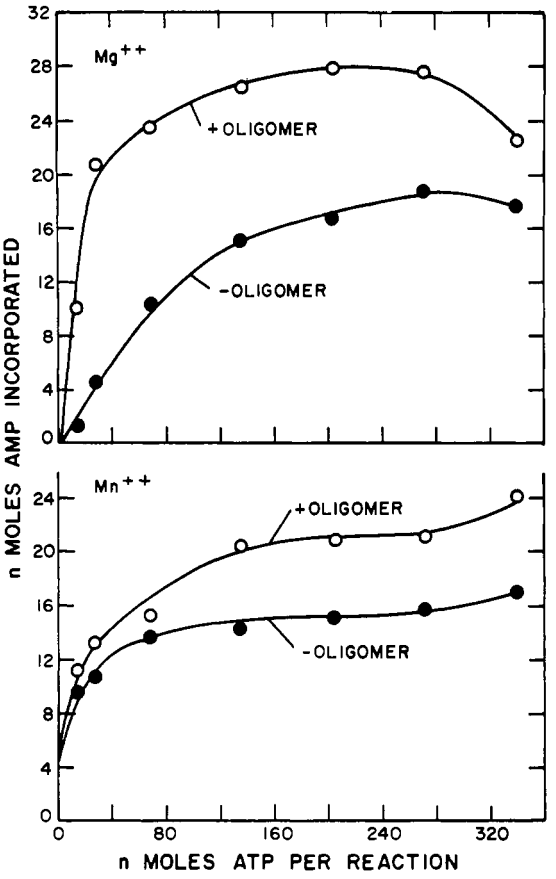

FIGURE 3: Stimulation of poly(dT)-directed poly(A) synthesis as a function of amount of substrate (ATP) added per reaction with or without addition of the oligomer primer $(Ap)_4A$. Reaction conditions were the same as noted in Figure 1 except for the varying amounts of [^{3}H]ATP added and ±80 nmol of $(Ap)_4A$.

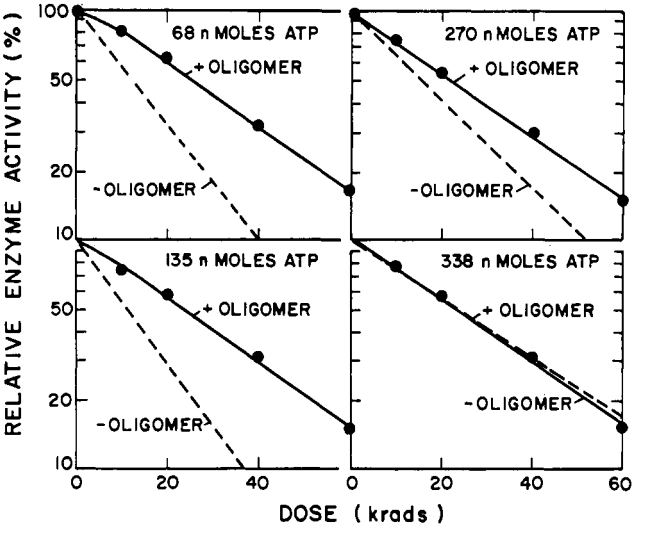

FIGURE 4: X-Ray inactivation of enzymatic activity of the *E. coli* B/r RNA polymerase when assayed with a poly(U) template with or without addition of the oligomer primer $(Ap)_4A$ and as a function of amount of substrate (ATP) added per reaction. Reactions contained 68–338 nmol of [^{3}H]ATP, 0.5 μmol of $MnCl_2$, 120 nmoles of poly(U), and 7.5 μg of enzyme with or without addition of 80 nmol of primer and were incubated at 37° for 20 min.

results of an analogous experiment; in this case the template molecule is poly(dT), and either Mg^{2+} or Mn^{2+} is used as the divalent metal ion cofactor. It is apparent from our data that, at low substrate concentrations, maximum stimulation for both poly(U)- and poly(dT)-directed reactions occurs at an oligomer:polymer ratio (expressed in monomer units) between 1:2 and 1:1. Our results are in agreement with findings for *M. luteus* RNA polymerase stimulation experiments for either ribo- or deoxyribopentamer primers and a poly(U) template (Straat and Ts'o, 1970; Straat *et al.*, 1971).

In Figure 2 we demonstrate how the presence of the oligomer primer $(Ap)_4A$ at 80 nmol (monomer units)/reaction stimulates the synthesis of poly(A) [as a function of amount of substrate (ATP) per reaction] using a poly(U) template with either Mn^{2+} or Mg^{2+}. In accordance with the idea that oligomer primers bypass the initiation or rate-limiting step in RNA synthesis, our data—in agreement with others—show maximum stimulation with oligomer primers at low substrate concentrations where chain initiation is the rate-limiting step (Niyogi and Stevens, 1964, 1965a,b; Steck *et al.*, 1968). Furthermore, it is seen that oligomer-primed, poly(U)-directed synthesis of poly(A) occurs in the presence of Mg^{2+}; without oligomer addition, this reaction is abortive as has been demonstrated previously with *M. luteus* RNA polymerase (Fox *et al.*, 1964; Steck *et al.*, 1968). Figure 3 shows a similar experiment concerning oligomer stimulation of poly(A) synthesis using a poly(dT) template, 80 nmol of $(Ap)_4A$, either Mg^{2+} or Mn^{2+}, and varying amounts of substrate (ATP). Again, stimulation is highest at low substrate levels, especially in reactions conducted in the presence of Mg^{2+}.

FUNCTION OF INITIATION BYPASS. The objective in using complementary oligomer primers in RNA synthesis was to examine how inactivation of RNA polymerase, after exposure to X-irradiation, proceeded when the initiation or rate-limiting step in RNA polymerization was bypassed. In the following experiments we examined the inactivation kinetics of X-irradiated B/r RNA polymerase using the homopolymer templates poly(U) and poly(dT) with addition of the ribopentamer primer $(Ap)_4A$ and as a function of amount of substrate per reaction. The oligomer primer was always used at a fixed amount of 80 nmol/reaction. In Figure 4 inactivation of enzymatic activity of X-irradiated RNA polymerase using a poly(U) template is shown. The dashed line represents inactivation of enzyme under identical assay conditions minus the oligomer primer. It is quite apparent that, for the range of amount of substrate used per reaction shown, inactivation of enzymatic activity of the RNA polymerase is substrate independent when the oligomer primer is added to the reaction mixture; that is, the D_{37} values obtained from inactivation curves are approximately equal (34–36 krads) for substrate amounts per reaction ranging from 68 to 338 nmol. In contrast, as has been shown in the preceding paper, inactivation of RNA polymerase with X-irradiation when assayed against a poly(U) template (minus oligomer primer) was substrate dependent; the D_{37} values from inactivation curves varied from 16 to 36 krads for a substrate range of 68–375 nmol/reaction.

In Figure 5 the analogous experiment shows X-ray inactivation of enzymatic activity of B/r RNA polymerase when assayed with a poly(dT) template with addition of an oligomer primer and varying amounts of substrate. For comparison, inactivation of enzyme under identical conditions except minus the oligomer primer is shown (dashed line). Although the D_{37} values obtained from inactivation curves in Figure 5 with or without oligomer addition show substrate independence, there exists a decrease by a factor greater than two in

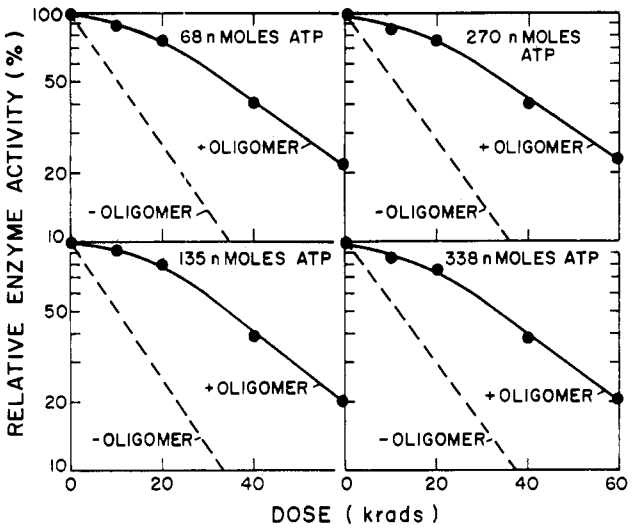

FIGURE 5: X-Ray inactivation of enzymatic activity of the *E. coli* B/r RNA polymerase when assayed with a poly(dT) template with or without addition of the oligomer primer $(Ap)_4A$ and as a function of amount of substrate (ATP) per reaction. Reactions contained 68–338 nmol of [^{3}H]ATP, 2.0 μmol of $MgCl_2$, 120 nmol of poly(dT), and 7.5 μg of enzyme with or without addition of 80 nmol of $(Ap)_4A$ and were incubated 10 min at 37°.

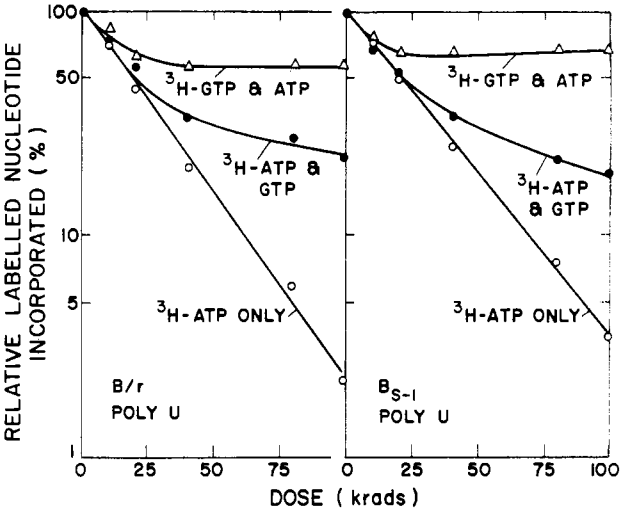

FIGURE 7: Relative incorporation of either ATP or GTP by X-irradiated *E. coli* B/r or B_{s-1} RNA polymerase using a poly(U) template. Each reaction contained either 375 nmol of [^{3}H]ATP (1.1×10^6 cpm/μmol) and 67 nmol of GTP or 375 nmol of ATP and 67 nmol of [^{3}H]GTP (4.0×10^6 cpm/μmol) plus 120 nmol of poly(U), 0.5 μmol of $MnCl_2$, and either 7.5 μg of B/r or B_{s-1} RNA polymerase. Reactions were incubated for 20 min at 37°. For comparison, incorporation of [^{3}H]ATP, where this is the only nucleoside triphosphate in the reaction mixture, by the X-irradiated RNA polymerase is shown. Control values for NTP incorporation by nonirradiated RNA polymerase–poly(U)-directed reactions: ATP incorporation ([^{3}H]ATP only), 4 nmol/20 min; ATP incorporation ([^{3}H]ATP and GTP), 2 nmol/20 min; and GTP incorporation ([^{3}H]GTP and ATP), 0.06 nmol/20 min.

radiosensitivity of enzyme when the D_{37} values from inactivation of enzyme assayed in the presence of oligomer primer are compared to those obtained for inactivation of enzyme in the absence of oligomer primer. The D_{37} values for inactivation of the RNA polymerase when assayed with a poly(dT) template in the absence or presence of oligomer primer equal 16 or 35 krads, respectively.

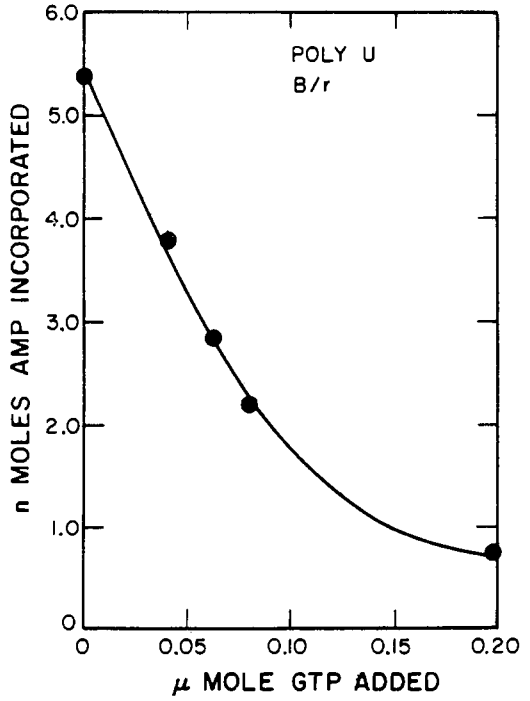

FIGURE 6: Inhibition of poly(U)-directed poly(A) synthesis by addition of a noncomplementary nucleoside triphosphate to the reaction mixture. Reactions contained 375 nmol of [^{3}H]ATP (1.1×10^6 cpm/μmol), 0.5 μmol of $MnCl_2$, 120 nmol of poly(U), 10 μg of B/r RNA polymerase, and increasing amounts of unlabeled GTP. Reactions were incubated at 37° for 20 min and then assayed for ^{3}H-labeled CCl_3COOH-insoluble material.

FIDELITY OF TRANSCRIPTION. We next considered how the fidelity of transcription might be influenced by X-irradiation of RNA polymerase. It has been shown previously that, in *in vitro* RNA synthesis using a variety of homopolymer templates, incorporation of complementary nucleoside triphosphate can be inhibited partially by introduction of noncomplementary nucleoside triphosphates into the reaction mixture (Chamberlin and Berg, 1962; Niyogi and Stevens, 1965a). In Figure 6 the influence of increasing amounts of GTP in the reaction mixture on synthesis of poly(A) using the B/r RNA polymerase and poly(U) template is shown. A similar inhibition is seen also for poly(A) synthesis using a poly(dT) template as a function of increasing amounts of GTP added (data not shown).

In the following experiment, in which we examine the effects of X-irradiation on misreading or insertion of a noncomplementary nucleoside triphosphate into the synthesized RNA by the irradiated RNA polymerase, a mole ratio of ATP:GTP of 5.6:1.0 was used in all reaction mixtures. In control reactions, using a poly(U) template, the inhibitory effect on poly(A) synthesis by the presence of noncomplementary nucleoside triphosphate (GTP) was reduced to about 50% of the value obtained when no GTP was present in the reaction mixture; for poly(dT)-directed poly(A) synthesis, the inhibitory effect reduced synthesis to about 70% of the value obtained when no GTP was present in the reaction. On the other hand, when [^{3}H]GTP incorporation into acid-insoluble material was monitored at a mole ratio of ATP:GTP of 5.6:1.0, incorporation of GTP using a poly(U) template and B/r or B_{s-1} RNA polymerase averaged 2.5–3.5% (moles of GTP:moles of ATP incorporated), whereas, using a poly-

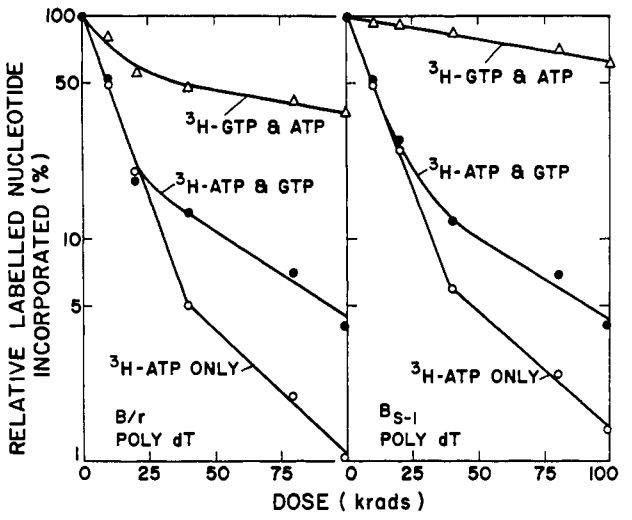

FIGURE 8: Relative incorporation of either ATP or GTP by X-irradiated *E. coli* B/r or B_{s-1} RNA polymerase using a poly(dT) template. Each reaction contained 375 nmol of [^{3}H]ATP (1.1×10^6 cpm/μmol) and 67 nmol of GTP or 375 nmol of ATP and 67 nmol of [^{3}H]GTP (4.0×10^6 cpm/μmol), 120 nmol of poly(dT), 2.0 μmol of $MgCl_2$, and either 7.5 μg of B/r or B_{s-1} RNA polymerase. Reactions were incubated for 10 min at 37°. For comparison, incorporation of [^{3}H]ATP, where this is the only nucleoside triphosphate in the reaction mixture, by the X-irradiated RNA polymerase is shown. Control values for NTP incorporation by nonirradiated RNA polymerase–poly(dT)-directed reactions: ATP incorporation ([^{3}H]ATP only), 18 nmol/10 min; ATP incorporation ([^{3}H]ATP and GTP), 12.6 nmol/10 min; and GTP incorporation ([^{3}H]GTP and ATP), 0.38 nmol/10 min.

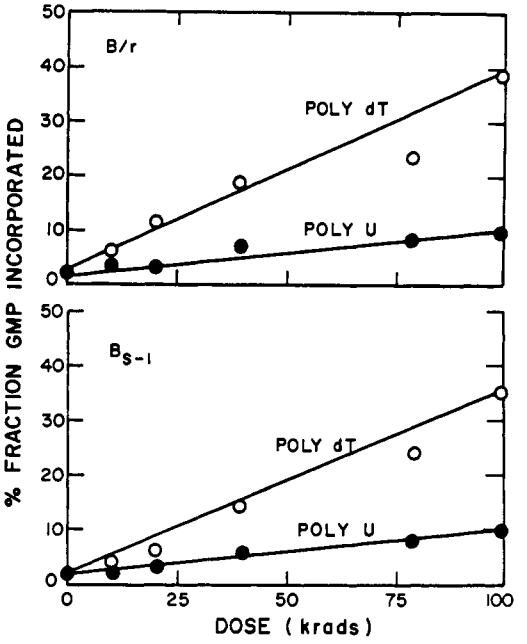

FIGURE 9: Misreading or per cent fraction of GMP incorporated in a poly(U)- or poly(dT)-directed poly(A) synthesis reaction by X-irradiated *E. coli* RNA polymerase as a function of dose. Reactions were conducted as outlined in Figures 7 and 8; the per cent fraction of GMP incorporated at a particular dose by either the B/r or B_{s-1} RNA polymerase was calculated from the amount of GMP incorporated, compared to the amount of AMP incorporated, and multiplied by 100.

(dT) template and B/r or B_{s-1} enzyme, incorporation of GTP was 2.0–4.0% that of ATP incorporated.

Figure 7 shows incorporation of either [^{3}H]ATP or [^{3}H]GTP into acid-insoluble material by X-irradiated B/r or B_{s-1} RNA polymerase using a poly(U) template and a mole ratio of ATP:GTP of 5.6:1.0 in the reaction mixture as a function of dose. For comparison, inactivation curves for both enzymes are shown when assayed only in the presence of [^{3}H]-ATP. A similar experiment is shown in Figure 8; in this case, a poly(dT) template was used.

In order the examine how the fidelity of RNA synthesis is affected by X-irradiation, the per cent fraction of noncomplementary base (GTP) incorporated (moles of GMP incorporated compared to moles of AMP incorporated $\times$ 100) was calculated as a function of dose for both RNA polymerases when assayed with either a poly(U) or poly(dT) template. The results are plotted in Figure 9, where it is seen that misreading or insertion of a noncomplementary base into the synthesized RNA product increases linearly with dose. Furthermore, misreading is a factor of three to four times more prominent when the irradiated RNA polymerase is assayed against a poly(dT) template compared to an assay against a poly(U) template.

Discussion

The process of RNA polymerization is a complex series of reactions involving the interaction between RNA polymerase, a template molecule, nucleoside triphosphates, and a divalent metal ion. This process can be subdivided into at least four discrete steps: binding, initiation, elongation or polymerization, and termination. Prior treatment of RNA polymerase with a variety of chemicals or antibiotics can affect the ability of the enzyme to catalyze RNA synthesis at one or more of these steps in the polymerization process. It was the objective of this and the preceding report to examine how radiation, in particular ionizing radiation, could influence the overall enzymatic activity of *E. coli* RNA polymerase and, furthermore, to determine if a particular step(s) in the polymerization process was the radiosensitive target.

Our data from this report demonstrate that, when the initiation or rate-limiting step in RNA synthesis is bypassed with addition of complementary oligomer primers, enzymatic activity of irradiated RNA polymerase becomes less radiosensitive. Furthermore, in the preceding paper, we have shown that the radiosensitivity of RNA polymerase, when assayed with homoribopolymer templates, is a function of substrate concentration. However, inactivation of the polymerase, when assayed against homoribo- or homodeoxyribopolymers in the presence of oligomer primers, is substrate independent over a wide range of substrate concentrations tested. This result, along with the finding that the binding step is quite radioresistant, suggests strongly that the initiation step in RNA synthesis is a reaction strongly influenced by X-irradiation. However, in the absence of oligomer primers, inactivation of RNA polymerase with X-irradiation is complex, a function of type of template molecule, substrate concentrations, and divalent metal ions used in the assay. For example, at low substrate concentrations in the reaction mixture, where initiation is the rate-limiting step, X-ray inactivation of RNA polymerase when assayed with homoribopolymers is probably a consequence of inactivation of the σ subunit. Niyogi (1972) has shown that poly(U)-directed poly(A) synthesis is σ dependent, especially at low substrate concentrations; therefore, inactivation of this subunit would result in inability of the enzyme to initiate synthesis. However, if this

rate-limiting, σ-dependent step were bypassed, then the radioresponse of the enzyme should become less acute. Our data demonstrate that, with increasing substrate concentrations or addition of oligoriboprimers which bypass the initiation step, there exists a significant decrease in radiosensitivity of RNA polymerase to X-irradiation when assayed against homoribopolymers.

X-Ray inactivation of RNA polymerase, when assayed against homodeoxyribopolymers, is similar at low substrate concentrations. Again, the σ-dependent initiation step appears to be the radiotarget; bypassing this step with oligoriboprimers reduces the radiosensitivity of the enzyme. However, in this case the radiosensitive step is substrate independent, suggesting that the mechanism of inactivation of the ability of the enzyme to initiate is complex and a function of variables in the reaction mixture.

Thus, it is apparent that inactivation of RNA polymerase by X-irradiation is a complex process directly affecting the initiation step. Bypassing this step, inactivation of the enzyme is probably a consequence of inability of the enzyme to elongate and/or terminate. Which step(s) in the polymerization process is influential in dictating the radioresponse of RNA polymerase is dependent on variables in the reaction assay mixture, including type of template molecule, divalent metal ion, and substrate concentrations used.

We have demonstrated also how radiation can influence the fidelity of *in vitro* RNA transcription. When X-irradiated RNA polymerase is assayed against either a poly(U) or poly(dT) template, there exists a linear increase in misreading or insertion of a noncomplementary base into the RNA product as a function of dose. Our data for *E. coli* RNA polymerase agree remarkably well with misreading data obtained for X-irradiated *M. luteus* RNA polymerase and a poly(U) template (Goddard *et al.*, 1969). However, our findings indicate that misreading occurs at a rate three to four times greater in a poly(dT)-directed reaction compared to a poly(U)-directed reaction.

In both this work and the work of Goddard and colleagues (1969) with *M. luteus* RNA polymerase, rather extensive (2–4%) misincorporation of GMP was observed in nonirradiated controls with the single-stranded poly(U) and poly(dT) templates. Such high levels of misincorporation have not been observed in studies with double-stranded repeating polymers (Bujard and Heidelberger, 1966). Incorporation of [^{3}H]GMP into acid-insoluble material was not affected by the inclusion of 0.4 mM potassium phosphate (pH 7.5), in the reaction mixture, indicating that our RNA polymerase preparations were not contaminated with polynucleotide phosphorylase (Burgess, 1969). The effects of irradiation upon misreading by the RNA polymerase are being further investigated.

Treatment of RNA polymerase with *p*-chloromercuribenzoate, which reacts with sulfhydryl groups, abolishes the enzymatic activity of the enzyme yet at low salt concentrations does not interfere with the ability of the enzyme to bind to synthetic polynucleotides (Smith *et al.*, 1971). Treatment of the polymerase with β-napthoquinone-4-sulfonic acid, which reacts with amino groups, inactivates the ability of the enzyme to bind nucleoside triphosphates (Ishihama and Hurwitz, 1969). It is known that there exists a dependence of the σ subunit of RNA polymerase at low substrate levels for maximum stimulation of both poly(U)- and poly(dT)-directed poly(A) synthesis (Niyogi, 1972). Furthermore, Sümegi and colleagues (1971, 1972) have shown that a primary radiation action upon RNA polymerase occurs with sulfhydryl groups; the extreme radiosensitivity of the σ subunit had been associated with inactivation of these very reactive, highly essential sulfhydryl groups present in this subunit. Thus, if the primary radiotargets are the highly reactive sulfhydryl groups in RNA polymerase, then the irradiated enzyme should be most affected in those functions dependent on these groups. One function would be initiation, which is dependent on the highly radiosensitive σ subunit; however, it would appear that the binding reaction, which does not depend directly on sulfhydryl groups, would be less affected. Our data are in agreement with these observations. Of course, it is possible that other essential groups associated with RNA polymerase are also radiotargets. For example, alteration by radiation of the free amino groups could result in basic failure of the enzyme to recognize nucleoside triphosphates; consequently, both the initiation and elongation processes would be affected by this damage.

RNA polymerase directs the intricate process of RNA transcription which involves transfer of genetic information from DNA to RNA. Production of RNAs must be exceedingly precise to ensure production of the multitude of cellular products dependent on RNA synthesis. Perturbations on the activity of RNA polymerase by X-irradiation could influence the radioresponse of viable cells.

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