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## BRIEF COMMUNICATION

### THE EFFECTS OF IONIZING RADIATION ON PROMOTER-DEPENDENT RNA SYNTHESIS

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The template activity of DNA for RNA synthesis *in vitro* markedly decreased in parallel with increasing dose of irradiation (5-8). The radiation-induced loss of template activity was mainly due to the formation of a specific lesion in the DNA which stops the process of RNA synthesis along the strand (5,6). The translational ability of RNA directed by irradiated calf thymus DNA was also reduced (9). In these earlier *in vitro* studies, RNA was synthesized from irradiated calf thymus DNA as a template by *Escherichia coli* RNA polymerase. In both prokaryotic and eukaryotic cells, RNA synthesis is initiated at specific promoter regions on DNA and unique sizes of RNA which correspond to those of the cistrons are synthesized. However, if calf thymus DNA is used as a template, *E. coli* RNA polymerase does not recognize the promoter region of calf thymus DNA and does not synthesize the specific RNA corresponding to the cistrons (2, 3). Thus, in those systems, the effect of radiation on the expression of a specific gene could not be evaluated. Plasmid pGem1<sup>TM</sup> has T7 and SP6 RNA polymerase promoters. A single species RNA which corresponds to the length from the promoter to restriction endonuclease site is synthesized by using restriction endonuclease-digested pGem1<sup>TM</sup> DNA as a template and T7 or SP6 RNA polymerase. In this study using the model system, we investigated the effects of radiation on specific RNA synthesis from particular regions of DNA by using restriction endonuclease-digested plasmid pGem1<sup>TM</sup> and T7 RNA polymerase.

Plasmid pGem1<sup>TM</sup> was obtained from Promega Biotec. Co. Ltd. Restriction en-

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donuclease BglI was purchased from Nippon Gene Co. Ltd., T7 RNA polymerase and RNasin from Boeringer Mannheim Co. Ltd., and  $\alpha$ - $^{32}\text{P}$ -uridine 5'-triphosphate (30 Ci/mmol) from ICN Radiochemicals. Plasmid DNA was prepared by the method of MANIATIS et al. (10). BglI-digested pGem1<sup>TM</sup> DNA was X-irradiated at a concentration of 0.1 mg/ml (in 10 mM Tris-HCl and 1 mM EDTA, pH7.4), with Toshiba KXC-18 generator operated at 170 kVp and 25 mA at a dose rate of 61 Gy/min. DNA solution was irradiated at 15 °C under aerobic condition. The reaction mixture for the RNA synthesis *in vitro* contained the following components in 20  $\mu\text{l}$ : 40 mM Tris-HCl (pH 7.5), 6 mM  $\text{MgCl}_2$ , 2 mM spermidine, 10 mM NaCl, 10 mM dithiothreitol, 20 units of RNasin, 0.5 mM each of ATP, GTP and CTP, 12  $\mu\text{M}$  of UTP, 2  $\mu\text{Ci}$  of  $\alpha$ - $^{32}\text{P}$ -UTP, 1  $\mu\text{g}$  of DNA and 3 units of T7 RNA polymerase. The mixture was incubated for 1 hr at 37 °C. The template activity of irradiated plasmid DNA was determined as described previously(8). The synthesized RNA was denatured, and subjected to the denatured agarose gel electrophoresis (GOLDBERG 1980). Agarose gel was dried on Whatmann 3MM paper. Autoradiography using Fuji RX X-ray film (Fuji photo film Co., Ltd.) was carried out with an intensifying screen at -80 °C. In order to determine the radioactivity of a certain size of RNA in agarose gel, the agarose gel with radioactive spot was cut out, minced, and dissolved in a small volume of distilled water by heating. After an addition of 10 ml of Aquasol-2 (NEN Research Products), the radioactivity was counted by liquid scintillation counter.

The digestion sites of restriction endonuclease, the sites of T7 and SP6 RNA polymerase promoters, and the multiple cloning sites in pGem1<sup>TM</sup> DNA are shown in figure 1. The length from T7 RNA polymerase promoter to BglI site was about 1.2 kilobase pairs(kbp). The radiation effects on the template activity of BglI-digested pGem1<sup>TM</sup> DNA as a template for RNA synthesis were shown in figure 2. The template activity for RNA synthesis was reduced depending on the irradiation doses. However, the degree of reduction of the template activity was found to be much smaller than that from the experiment consisting of calf thymus DNA and *E. coli* RNA polymerase(8). This difference in the radiation sensitivity between those two experiments may be mainly due to the difference in size of template between calf thymus DNA ( $M_n=6 \times 10^6$ ) (1) and digested pGem1<sup>TM</sup> DNA ( $6 \times 10^5$ )(5, 6). The size of RNA synthesized from irradiated pGem1<sup>TM</sup> DNA was analyzed with denatured agarose gel (fig. 3). Figs. 3a and b show 1 hr and 16 hrs exposed autoradiograms. Using unirradiated template, RNA with about 1.2 kb in length was synthesized. This size corresponds to the length from the initiation site of RNA synthesis to the BglI site in pGem1<sup>TM</sup> DNA. The amount of RNA synthesized with original size markedly reduced by the irradiation (figs. 2 and 3). These results indicate that although the template activity was kept sufficiently high after irradiation, intact RNAs were not synthesized. No bands smaller than 1.2 kb were shown in the autoradiogram. This result suggests that the lesions, which inhibit the RNA synthesis, were randomly formed on DNA. If

Fig. 1 The restriction endonuclease map of pGem1<sup>TM</sup> DNA. MCS, Ori and Amp<sup>R</sup> stand for the multiple cloning site, origin of replication of the plasmid DNA and ampicilin resistance gene, respectively.

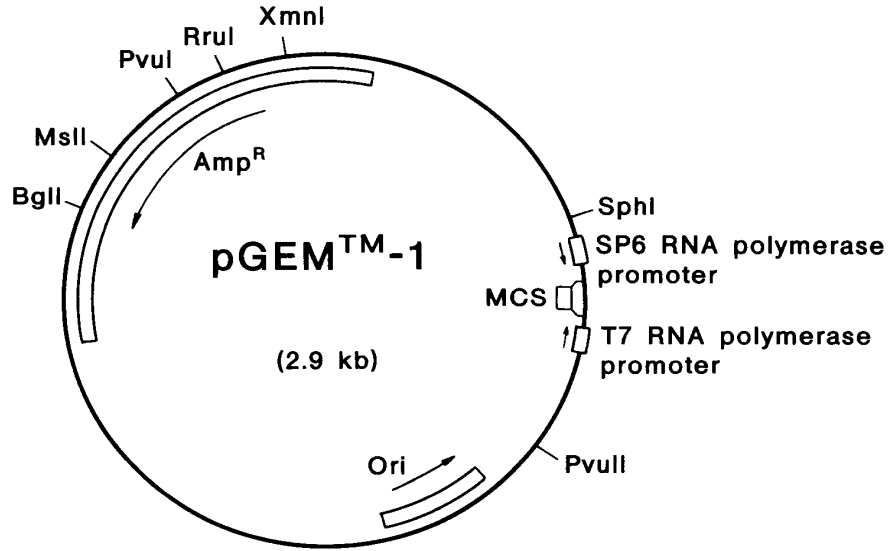


Fig. 2 The dose-effect curves of the template activity for RNA synthesis. UMP incorporation with irradiated DNA is related to the UMP incorporation with unirradiated DNA. —○—; total RNA synthesis. ····×····; the synthesis of 1.2 kb RNA. Each point was average of two separate experiments.

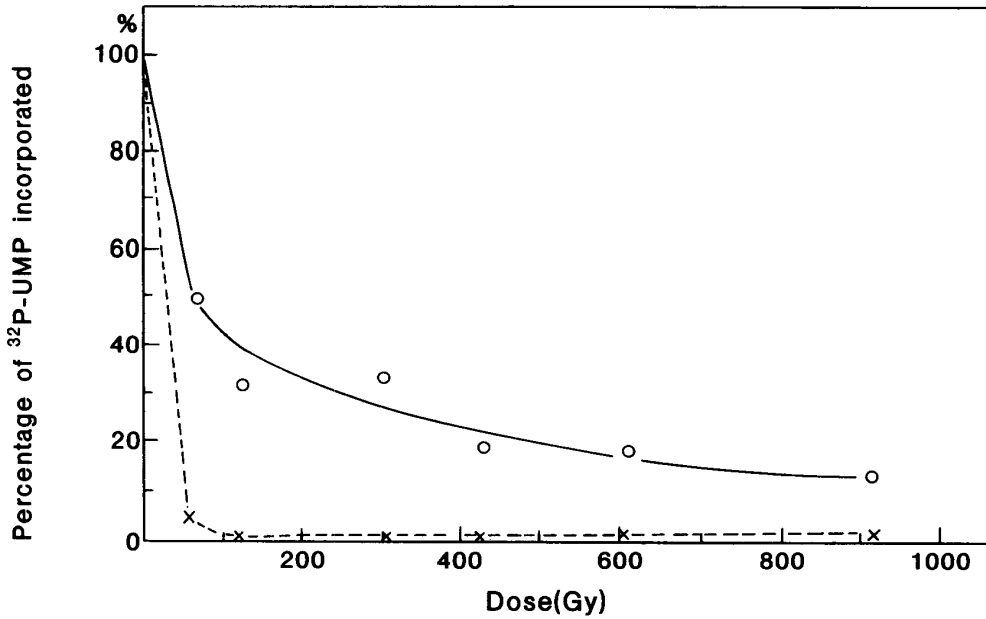
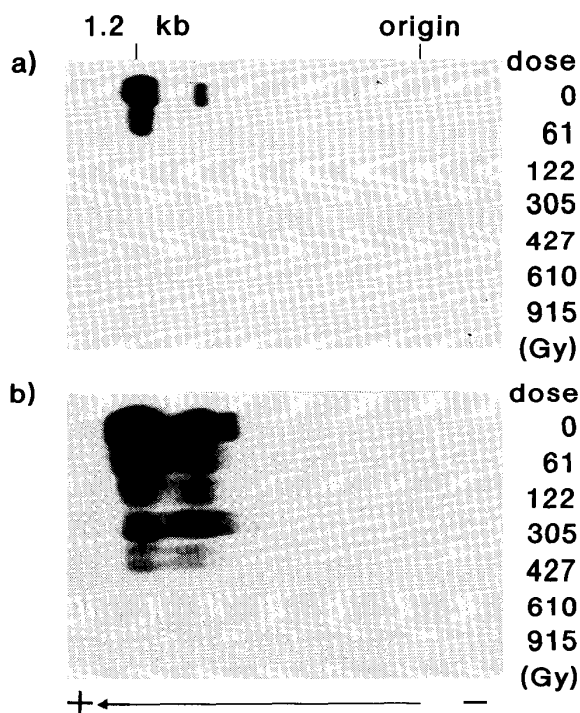


Fig. 3 The effects of radiation on the synthesis of full-length RNA.  
 a) 1 hr exposure autoradiogram  
 b) 16 hrs exposure autoradiogram  
 Arrow indicates the direction of electrophoresis.  
*E. coli* 16S and 23S rRNAs were used as size markers.



the lesions were formed at unique sites, the bands corresponding to the length from the promoter site to the lesion sites must be seen. The faint band with the size larger than 1.2 kbp was shown in figure 3. This band may originate from the RNA synthesized by undigested template or the RNA with secondary structure due to the incomplete denaturation. These results suggested that by using this model system, the effect of radiation on the specific RNA synthesis from DNA *in vitro* can be evaluated more exactly than by using calf thymus DNA and *E. coli* RNA polymerase.

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