The Protein Synthesis Dependent on the RNA Directed by Irradiated Calf Thymus DNA and Deoxyribonucleoprotein

M. HAYASHI and G. YOSHII

Department of Radiation Biology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan
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DNP/RNA synthesis/Protein synthesis

The translational ability of RNA directed by irradiated calf thymus DNA or deoxyribonucleoprotein (DNP) in vitro was investigated.

Both the template activity of calf thymus DNA for RNA synthesis and the incorporation of amino acids into acid insoluble materials in vitro markedly decreased by irradiation with 20 increasing dose.

The activity of calf thymus DNP, however, increased with irradiation at a dose lower than krads. The incorporation of amino acids also increased with the same dose.

It was shown that the translation dependent on the RNA directed by irradiated templates was qualitatively different from that of unirradiated templates.

INTRODUCTION

The template activity of irradiated DNA for RNA synthesis in vitro markedly decreased with increasing dose\(^1\). On the other hand, the template activity of irradiated DNP increased up to a certain level with increasing doses; upon further irradiation, however, it decreased\(^2\). The enhancement of the template activity of irradiated DNP could be attributable to the labilization of the bond between the protein and the DNA in the DNP\(^2\). It has been reported that the chain length of RNA synthesized from irradiated DNA distinctly decreased\(^3\), and that the nucleotide composition of RNA synthesized from irradiated DNA\(^3\) or DNP\(^4\) changed. These changes could affect the translational ability of RNA. No reports on the change of translational ability of RNA transcribed from irradiated templates have yet appeared.

The present paper is an attempt to obtain some informations on the translational ability of RNA transcribed from the irradiated calf thymus DNA or DNP in vitro.

MATERIALS AND METHODS

Reagents

*E. coli* RNA polymerase (EC 2.7.7.6), four nucleoside triphosphates, phosphoenolpyruvate, pyruvate kinase (EC 2.7.1.40) and rifampicin were purchased from Sigma.
Chemical Co. 8-14C-ATP (56 mCi/m mole, L-4,5-3H-leucine (50 Ci/m mole), and 2-3H-glycine (3.4 Ci/m mole) from Radiochemical Centre, Amersham, England.

Preparation of calf thymus DNA and DNP

Calf thymus DNA was prepared by the method of Kay et al. and dissolved in 10 mM NaCl (pH 7.0). Calf thymus DNP was prepared essentially by the method of Zubay and Doty. The protein to DNA ratio in the DNP preparation was about 1.5:1.

Irradiation conditions

Gamma-irradiation of DNA and DNP was performed as described previously.

Incubation conditions for determination of protein synthesis and RNA synthesis in vitro

The E. coli preincubated S-30 extract for cell-free protein synthesis was prepared by the method of Nirenberg from a mutant strain Q13 (RNase I-).

The reaction mixture for determination of protein synthesis in vitro contained the following components in 0.275 ml: 12.5 μmoles of Tris-HCl (pH 8.0); 12.5 μmoles of NH₄Cl; 12.5 μmoles of potassium acetate; 2.5 μmoles of magnesium acetate; 2 μmoles of 2-mercaptoethanol; 27.5 μmoles of each unlabelled 19 amino acids; 2.5 μmoles of phosphoenol pyruvate; 125 μmoles of each CTP, GTP and UTP; 625 μmoles of ATP; 6.25 μg of pyruvate kinase; 2 units of RNA polymerase; 10 μg of DNA or 25 μg of DNP; 0.1 ml of preincubated S-30 extract (4.0 mg protein/ml; 2.1 mg RNA/ml); and 6.25 μCi of labelled amino acid.

RNA polymerase and the template were mixed with four nucleoside triphosphates and incubated for 20 min at 37°C, and 27.5 μg of rifampicin were added to the mixture to stop the RNA synthesis. After the addition of phosphoenol pyruvate, pyruvate kinase, preincubated S-30 extract and labelled amino acid, the reaction mixture was incubated for an additional 30 min at 37°C. After the mixture was chilled to 0°C, 0.5 ml of 5% casamino acid and 5 ml of 7% trichloroacetic acid were added to the mixture, and the mixture was heated for 20 min at 90°C. 250 μg of bovine serum albumin were added to the mixture, and it was centrifuged. The pellets were dissolved in a small volume of 0.2 M NaOH and reprecipitated with 5 ml of 5% trichloroacetic acid. The precipitate was collected on Whatman GF/C glass fibre paper, washed twice with 5 ml of 5% trichloroacetic acid and 5 ml of ethanol, then washed once with 5 ml of ethyl ether, and allowed to dry. After addition of 10 ml of toluene-PPO-POPOP scintillator, the radioactivity was measured with a Horiba LS-500 liquid scintillation counter.

The reaction mixture for determination of RNA synthesis in vitro contained the same components of the reaction mixture for protein synthesis, except that the preincubated S-30 extract, phosphoenol pyruvate, pyruvate kinase and labelled amino acid were omitted, and 0.1 μCi of 14C-ATP was present instead of 625 μmoles of unlabelled ATP. The reaction mixture was incubated for 20 min at 37°C. The radioactivity was measured as described previously.

Counts of control tube having no added RNA polymerase in the reaction mixture were subtracted from the sample radioactivity counts.
Acrylamide gel analysis of proteins synthesized *in vitro*

Proteins were synthesized *in vitro* as described above, precipitated with 5 ml of 7% trichloroacetic acid, and then heated for 20 min at 90°C. The precipitate was collected by centrifugation. The pellets were dissolved in a small volume of 0.2 M NaOH, and the solution was dialyzed overnight against 0.01 M sodium phosphate (pH 7.2) containing 1% 2-mercaptoethanol and 1% SDS. SDS-polyacrylamide gel electrophoresis (10% acrylamide, pH 7.2) was performed according to the method of Weber and Osborn\(^{12}\). The elution of the labelled proteins from gel was carried out essentially by the method of Mahin and Lofberg\(^{13}\). After the addition of 10 ml of aquaso 1-2, the samples were counted in a liquid scintillation counter. 13 marker proteins were used to determine the molecular weight of the proteins synthesized *in vitro*.

RESULTS AND DISCUSSION

*Protein synthesis directed by RNA synthesized *in vitro*

*E. coli* cell-free protein synthesis system of Nirenberg\(^{11}\) was used to investigate the translational ability of RNA synthesized from calf thymus DNA or DNP *in vitro*. In this coupled system, protein synthesis was carried out following the synthesis of RNA (see MATERIALS AND METHODS). Using this system, \(^{3}H\)-labelled amino acid was incorporated into proteins (Table 1). In order to confirm that protein synthesis in this system was dependent on RNA synthesized *in vitro*, protein synthesis was carried out under the condition where RNA synthesis *in vitro* was inhibited. When template or RNA polymerase was omitted from the reaction mixture, or when the action of RNA polymerase was prevented by the addition of rifampicin, only small amounts of \(^{3}H\)-labelled leucine were incorporated into proteins (Table 1). These results indicated that the incorporation of \(^{3}H\)-labelled amino acids into proteins was dependent on RNA synthesized *in vitro*. Moreover, with using SDS-polyacrylamide gel analysis of synthesized protein *in vitro*, it was shown that proteins which incorporated \(^{3}H\)-labelled amino acid have a molecular weight from \(1 \times 10^4\) to \(1 \times 10^5\) (Fig. 1). From these results, it was proved that RNA synthesized from calf thymus DNA or DNP *in vitro* was served as messenger RNA.

### Table 1. Requirement of newly synthesized RNA *in vitro* for protein synthesis *in vitro*

| cell-free protein synthesis system            | incorporation of \(^{3}H\)-leucine (cpm) |
|---------------------------------------------|------------------------------------------|
| complete (DNA as a template)                | 1709                                     |
| (DNP as a template)                         | 380                                      |
| minus template                              | 154                                      |
| minus RNA polymerase                        | 156                                      |
| plus rifampicin*                            | 156                                      |

Protein synthesis was carried out as described in MATERIALS AND METHODS.

* 27.5 μg of rifampicin was added to the reaction mixture prior to RNA synthesis.
Protein synthesis directed by RNA synthesized from irradiated DNA

The template activity of irradiated DNA for RNA synthesis in vitro markedly decreased with increasing dose (Fig. 2). The markedly decrease in the template activity of irradiated DNA agrees with previous reports\(^1\^-3\). The loss of template activity in the irradiated DNA has been attributed to the introduction of breaks in the polynucleotide chains\(^1\).

The incorporation of leucine or glycine on irradiation decreased similarly in template activity with increasing dose (Fig. 2). This result suggests that the decrease in the template activity of irradiated DNA would result in the decrease in the incorporation of amino acids into proteins.

Protein synthesis directed by RNA synthesized from irradiated DNP

With DNP as a template for RNA synthesis, an entirely different result was observed. The template activity of DNP increased with irradiation at a dose lower than 20 krads (Fig. 3). The enhancement of template activity in the irradiated DNP was in agreement with previous reports\(^2\^-5\). The enhancement has been explained by a previous proposal\(^2\^-3\): DNA restricted by the interaction between the DNA and the protein in the native state DNP is uncovered by irradiation and acts as a template for RNA synthesis. However, it has not been proved whether or not the synthesized
Fig. 2. Effects of ionizing radiation on RNA synthesis and protein synthesis directed by irradiated calf thymus DNA. RNA synthesis and protein synthesis were carried out as described in MATERIALS AND METHODS. —○—, the incorporation of AMP; ——●——, leucine; ——×—, glycine. Each point was average of five separate experiments. The errors are not exceeding the size of the symbols.

Fig. 3. Effects of ionizing radiation on RNA synthesis and protein synthesis directed by irradiated calf thymus DNP. RNA synthesis and protein synthesis were carried out as described in MATERIALS AND METHODS. ——O——, the incorporation of AMP; ——●——, leucine; ——×—, glycine. Each point was average of four separate experiments (±S.D.).
RNA, which was occurred through the enhancement of template activity of DNP on radiation, acted as messenger RNA.

The incorporation of leucine or glycine into proteins was well enhanced by irradiation at a dose lower than 20 krads (Fig. 3). These results suggested that the enhancement of RNA synthesis by irradiation would lead to the enhancement of protein synthesis. Therefore, it was likely that RNA occurred through the uncovering of proteins in the irradiated DNP was served as messenger RNA. As shown in Fig. 3, some differences in the level of increase of incorporation between leucine and glycine suggested that the translational ability of RNA directed by irradiated DNP was qualitatively different from that of unirradiated DNP.

In the present paper, it was shown that the decrease in the template activity of DNA by irradiation led to the decrease in the protein synthesis in vitro (Fig. 2), that RNA occurred through the uncovering of proteins in the irradiated DNP was served as messenger RNA and that the translational ability of RNA directed by irradiated DNP was qualitatively different from that of unirradiated DNP (Fig. 3). Moreover, these results suggest that DNP is not affected by irradiation in the same way as DNA alone (Fig. 2, Fig. 3 and ref. 14, 15). If the DNP is damaged by irradiation in vivo, producing changes in the template and in the translational ability of RNA, such changes may lead to the formation of proteins which would not be normally formed.

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