Initiation of DNA Synthesis by the Avian Oncornavirus RNA-Directed DNA Polymerase: Tryptophan tRNA as the Major Species of Primer RNA

WILLIAM R. FOLK AND ANTHONY J. FARAS*

Department of Biological Chemistry, The University of Michigan, Ann Arbor, Michigan 48104, and Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455*

Received for publication 6 October 1975

The major species of primer RNA required for the initiation of DNA synthesis by the Rous sarcoma virus RNA-directed DNA polymerase can be aminoacylated by tryptophan. Furthermore, an intact 3' terminus is required for the primer to function in the initiation of DNA synthesis.

The major species of primer RNA utilized by the avian oncornavirus RNA-directed DNA polymerase for the initiation of DNA synthesis in vitro is a 4S RNA molecule with structural and functional features of tRNA (4-7, 11). Primer RNA appears to be host cell derived, since a structurally similar 4S RNA can be identified in uninfected avian cells (6, 11). Although the Rous sarcoma virus (RSV) primer molecule has been completely sequenced and shown to contain the tryptophan anticodon (8), there nevertheless exist conflicting reports regarding the aminoacylation properties of the primer RNA species (2, 3, 8, 13). In one report, primer RNA could only be aminoacylated with methionine (2), whereas in another report it could only be aminoacylated with tryptophan (8). In an effort to reconcile these differences we have attempted to establish a relationship between the capacity of the primer to be aminoacylated and its ability to function as primer in the initiation of DNA synthesis by the avian oncornavirus RNA-directed DNA polymerase in vitro. In this communication, we present conclusive evidence indicating that the particular species of tRNA capable of reconstituting the bulk of the template activity of the oncornavirus genome can be aminoacylated with tryptophan but not methionine.

Two experimental approaches were employed in these studies, both utilizing preparations of partially purified tryptophan and methionine aminoacyl synthetases to ensure that adequate levels of synthetase could be provided without, at the same time, adding unlabeled amino acids or extraneous RNase activities. In the first approach, highly purified RSV primer RNA was incubated with preparations containing tryptophyl tRNA synthetase (from human placenta) or methionyl tRNA synthetase (from chick liver) and the corresponding radiolabeled amino acid. Under reaction conditions in which both synthetases exhibit maximum aminoacylation activity with total chick RNA, primer RNA can only be aminoacylated with tryptophan (Fig. 1).

The second approach is based on the protection against periodate oxidation of the cis-diol at the 3' terminus of tRNA that is afforded by aminoacylation (10). Since the primer acts by providing a 3' hydroxyl terminus to the RNA-directed DNA polymerase (12), the capacity of the RSV primer molecule to function in the initiation of DNA synthesis in vitro can be related to its capacity to be aminoacylated. In these experiments, primer RNA was incubated with either methionine or tryptophan and the corresponding aminoacyl tRNA synthetase preparations, subjected to periodate oxidation, enzymatically discharged, and then tested for its ability to restore the template activity of the oncornavirus genome from which primer RNA had been previously removed (6). As can be seen in Fig. 2, only tryptophan is capable of protecting the 3' terminus of the RSV primer molecule from periodate oxidation so that it can participate in the initiation of DNA synthesis. Furthermore, the bulk of the primer activity observed with total 4S RNA obtained from either chick 4S or human placenta 4S RNA can also be protected with tryptophan, indicating that these are the major species of cellular tRNA's capable of functioning as primer for the initiation of RNA-directed DNA synthesis in vitro (Table 1).

These studies demonstrate that the primer RNA molecule required for the initiation of
Fig. 1. Aminoacylation of RSV primer RNA. RSV primer RNA or total chick 4S RNA purified as previously described (6) was incubated with partially purified tryptophanyl tRNA synthetase from human placenta, or partially purified methionyl synthetase from chicken liver, and the corresponding amino acid in 0.05 ml of a buffer containing 100 mM Tris-chloride (pH 7.5), 10 mM KCl, 10 mM MgCl₂, 1 mM ATP, 4 mM glutathione, and 0.1 mM ³H-labeled amino acid (400 µCi/µmol). After 30 min at 37°C, 2 ml of cold 2 N HCl containing 0.1% Casamino Acids was added and the precipitate was collected on GF/C glass fiber filters as described by Calendar and Berg (1). The preparation of the partially purified amino acid tRNA synthetases will be described in a later communication, but was essentially an S-100 purified by DEAE-cellulose chromatography as described by Muench and Berg (9), followed by passage through a column of Bio-Gel A 1.5. The peak fractions of each activity were pooled and concentrated by ultrafiltration. With the Bio-Gel column, groups of aminoacyl tRNA synthetases can be separated from each other and from the predominant RNase activity. "Mischarging" of tRNA's by heterologous aminoacyl tRNA synthetases is not likely to be a problem, as the tryptophanyl tRNA from avian and human tissues appears to be similar (8, 11). Symbols: (●) incorporation of [³H]-tryptophan; (○) incorporation of [³H]methionine.

Fig. 2. Protection by tryptophan of primer RNA 3' terminus from periodate oxidation. Purified primer RNA was incubated with either tryptophan or methionine and the appropriate aminoacyl tRNA synthetase preparation, as described in the legend to Fig. 1. Separate incubations were performed with chick 4S RNA substituted for primer RNA and radiolabeled amino acids to ensure that the aminoacyl tRNA synthetases were active. The samples containing primer RNA were subsequently acidified to pH 5.0 by addition of acetic acid, 50 µg of carrier Escherichia coli tRNA was added, and the samples were deproteinized by shaking with water-saturated phenol. The nucleic acids were precipitated from the aqueous supernatant with 2 volumes of ethanol, and dissolved in 0.5 ml of 0.1 M sodium acetate, pH 5.0. NaIO₄ was added to 0.05 M, and the samples were placed in the dark at room temperature for 30 min. The excess IO₄⁻ was eliminated by addition of glycerol to 1%, and the samples were dialyzed overnight against water at 4°C. The aminoacylated tRNA's were discharged enzymatically with the same aminoacyl tRNA synthetase preparations described above, in reaction mixtures in which 2 mM AMP was substituted for the amino acid. After sufficient time to allow discharging (usually 30 min at 37°C), the samples were deproteinized by phenol extraction, dialyzed, and ethanol-precipitated. The primer RNA was dissolved in a small volume of buffer and reannealed to RSV 35S RNA (2 µg) from which all 70S-associated 4S RNA, including primer, had been removed, and template activity of the reconstituted templates-primer complexes was determined by incubation with the purified AMV DNA polymerase as described (6). Data are corrected for low amounts of residual primer activity (<10%) in preparations of RNA that were not incubated with aminoacyl tRNA synthetase, but subjected to all the following steps. Complete reconstitution (100%) is that observed with primer RNA not incubated with aminoacyl tRNA synthetase, nor with NaIO₄. Symbols: (●) primer RNA incubated with tryptophan and tryptophanyl tRNA synthetase preparation; (○) primer RNA incubated with methionine and methionyl tRNA synthetase preparation.
RNA-directed DNA synthesis on the RSV genome is a tryptophanyl tRNA. Furthermore, they indicate that an intact 3' terminus containing an unoxidized ribose is required for the initiation of DNA synthesis by the oncornavirus RNA-directed DNA polymerase in vitro. Our results are consistent with those obtained from nucleotide sequence analysis of the primer RNA species obtained from uninfected chick cells (8) and aminoacylation studies of the avian myeloblastosis virus RNA primer molecule (8). They are also consistent with the recent observations concerning the ability of preparations of tryptophan tRNA from avian cells to reconstitute template activity of the oncornavirus genome (13). We cannot presently explain the basis for the previous observed discrepancies in aminoacylation of the avian oncornavirus primer molecule.

We thank N. Dibble and Barbara Fishel for excellent technical assistance.

This investigation was supported by Public Health Service Research grant no. CA19790-01 from the National Cancer Institute and grant NP-172 from the American Cancer Society.

### LITERATURE CITED


