A reverse transcriptase-like activity of wheat (*Triticum aestivum*) embryo microsomal fraction

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A reverse transcriptase-like activity was isolated from germinating wheat (*Triticum aestivum*) embryos. The activity was found to be associated with a microsomal fraction (70 000 g pellet) of the embryo homogenate. The microsome-associated enzyme prefers homologous polyadenylated RNA to any other polynucleotides as template and requires all four deoxyribonucleoside triphosphates for maximal activity. The reaction product appears in the incubation mixture in the form of an RNA–DNA hybrid, which can be converted into single-stranded DNA by mild alkaline hydrolysis. These observations suggest that normal wheat embryo cells contain an enzyme which, functionally, is similar to retroviral reverse transcriptase.

INTRODUCTION

Viral reverse transcriptase is a well characterized enzyme, known to play a key role in the replication of the viral genome in retrovirus-infected vertebrate cells. The occurrence of a similar enzyme in uninfected eukaryotic cells has been reported, but still remains a matter of controversy (Verma, 1977; Bauer *et al.*, 1978; Nelson *et al.*, 1981; Steinhilber & Cummings, 1986). Nevertheless, the process of reverse transcription is commonly thought to be involved in some intracellular events, and retro-transposon-mediated DNA sequence rearrangements in particular (Baltimore, 1985). According to Bernstein *et al.* (1983), the reverse transcription may be a secondary activity of one of the known cellular DNA polymerases. The most close to meeting such an expectation seems to be the γ -like polymerase of wheat embryos, first described by Castroviejo *et al.* (1979) and shown to be able to mimic the activity of reverse transcriptase *in vitro* (Litvak *et al.*, 1985).

Prompted by these findings, we have undertaken a search for reverse transcriptase activity in germinating wheat embryos. By using a simple isolation procedure, we have obtained a crude DNA polymerase preparation that prefers wheat embryo mRNA to any other polynucleotides as template and gives an RNA–DNA hybrid as the direct reaction product.

MATERIALS AND METHODS

Reagents

Wheat embryo mRNA was a polyadenylated fraction of the cytoplasmic RNA. The preparation was kindly isolated and checked for its ability to support amino acid incorporation *in vitro* by Dr. M. Tomaszewski of this Institute, as described elsewhere (Tomaszewski, 1982). The same procedure resulted also in the separation of a non-polyadenylated RNA fraction which served as rRNA. Brome-grass-mosaic-virus RNA was a gift of Dr. M. Welnicki (this Institute) and was prepared as described by Zagórski (1978). All other reagents, including labelled nucleoside triphosphates (Amersham

International, Amersham, Bucks., U.K.), were of commercial origin and were of analytical grade. Some of them (ribohomopolymers and oligonucleotides) were generously given by Dr. A. Paszkowski (Friedrich Miescher-Institut, Basel, Switzerland).

Plant material

Viable embryos of the wheat *Triticum aestivum* (var. Mironovska 808) were obtained as described by Marciniak *et al.* (1978) and, in 2 g portions, germinated for 3 h at 21 °C. Then the embryo sample was rinsed with water and immediately used for the preparation of microsomal fraction.

Preparation of microsomal fraction

The procedure was similar to that described by Steinhilber & Cummings (1986), except that the post-mitochondrial supernatant, instead of the nuclear/mitochondrial pellet, was used for the further treatments. The overall protocol was as follows. The whole embryo sample (see above) was ground with a prechilled pestle and mortar in ice-cold 50 mM-potassium phosphate, pH 7.6, containing 0.66 M-sucrose, 10 mM-2-mercaptoethanol, 0.5 mM-EDTA and 0.15% (w/v) bovine serum albumin. The homogenate was filtered through Miracloth and the resulting filtrate (approx. 20 ml) was centrifuged at 20 000 g for 20 min at 4 °C. The postmitochondrial supernatant was sonicated (10 × 15 s in a Branson-type sonifier at setting 4), clarified by centrifugation as described above and, finally, centrifuged at 70 000 g for 2 h at 4 °C. The resulting microsomal pellet was suspended in 0.2 ml of 0.1 M-KCl/2.5 mM-MgCl₂/10 mM-Tris/HCl, pH 7.0. The preparation was pipetted off into 10 μ l portions and these were either stored in liquid N₂ or immediately assayed for reverse transcriptase activity.

Enzyme assays

For homopolymer synthesis, 10 μ l (160 μ g of protein) of the microsomal fraction was added to 50 μ l of a mixture (Heine *et al.*, 1980) containing 63 mM-Tris/HCl, pH 7.8, 75 mM-KCl, 0.63 mM-MnCl₂, 2.5 mM-dithio-

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Fig. 1. Time course of [^3H]dTTP incorporation by wheat embryo microsomal fraction

Freshly prepared (●) and heat-denatured (○) samples of the microsomal fraction were incubated at 37 °C for the indicated time periods under the conditions of the homopolymer-synthesis assay (see the Materials and methods section).

threitol, 4 μg of poly(rA), 1 μg of (dT)₁₂₋₁₈ and 6.3 μCi of [*methyl*- ^3H]dTTP (40 Ci/mmol). For heteropolymer synthesis the same amount of the enzyme preparation was added to 50 μl of a mixture containing 100 mM-Tris/HCl, pH 8.3, 50 mM-KCl, 10 mM-MgCl₂, 10 mM-dithiothreitol, 5 μg of wheat embryo mRNA, 1 μg of (dT)₁₂₋₁₈, 10 μCi of [^3H]dCTP (30 Ci/mmol) and 1 mM each of dATP, dGTP and dTTP. Replacements, when made, were equivalent on a molar (substrates) or weight (templates and primers) basis. After incubation at 37 °C for 1 h, the trichloroacetic acid-precipitable radioactivity was measured by a filter-paper-disc method (Mans & Novelli, 1961). Duplicate experiments were performed, and data (except those given in Fig. 1 below) were corrected for the background radioactivity of heat-inactivated enzyme preparations.

Protein was determined by the method of Bradford (1976).

Buoyant-density-gradient centrifugation

The putative reverse transcriptase reaction product of heteropolymer series was characterized by centrifugation in CsCl/ethidium bromide gradients. The reaction product was obtained as described above, except that the trichloroacetic acid precipitation step was replaced by filtration through a small column (0.5 cm $\times$ 10 cm) of Sephadex G-50. The high-*M*_r product thus separated was treated with Pronase (1 mg/ml) at 37 °C for 1 h before centrifugation. Buoyant-density measurements were done by the method of Radloff *et al.* (1967), with the starting concentrations of CsCl and ethidium bromide equal to 1.55 g/ml and 200 $\mu\text{g}/\text{ml}$ respectively. The solution (8 ml) was centrifuged at 41 000 rev./min in a Beckman Ti 75 rotor at 18 °C for 48 h. Fractions (0.5 ml

Table 1. Template/primer requirements for the synthesis of homopolymers by microsomal fraction of wheat embryo homogenate

Samples of the microsomal fraction were incubated at 37 °C for 1 h under the conditions used for the homopolymer-synthesis assay (see the Materials and methods section) with the use of ^3H -labelled substrates, templates and primers as indicated.

^3H -labelled substrate	Template/primer	Nucleotide incorporated (pmol/assay)
dTTP	Poly(rA) + oligo(dT)	2.42
dTTP	Poly(rA)	0.13
dTTP	Oligo(dT)	0.20
dTTP	None	0.11
dTTP	Poly(dA) + oligo(dT)	0.18
dTTP	Poly(rC) · oligo(dG)	0.10
dGTP	Poly(rC) · oligo(dG)	1.25
dGTP	Poly(rA) + oligo(dT)	0.12
rUTP	Poly(rA) + oligo(dT)	0.14

each) were collected from the bottom. Positions of density markers were determined in parallel experiments. A reference sample of the RNA–DNA hybrid was obtained with the use of wheat embryo mRNA and commercial avian-myeloblastosis-virus reverse transcriptase. The direct reaction product was treated with S1 nuclease (2 units/ml, 37 °C, 30 min) before centrifugation. Single-stranded DNA was obtained by alkaline hydrolysis (0.1 M-NaOH/50 mM-EDTA, 60 °C, 1 h) of the hybrid. Pure DNA (*Escherichia coli*) and RNA (wheat germ) were of commercial origin.

RESULTS

The microsomal fraction (70 000 *g* pellet) of wheat embryo homogenate was found to be able to catalyse the incorporation of [^3H]dTTP into an acid-precipitable product. The reaction was linear with the incubation time for nearly 1 h (Fig. 1). As summarized in Table 1, the polymerization of dTTP was strongly dependent on the simultaneous presence of poly(rA) and oligo(dT), which served probably as the template and primer respectively. There was no efficient dTTP incorporation when poly(rA) was replaced by poly(dA). Poly(rC) could support the polymerization of dGTP but not that of dTTP. Poor incorporation of rUTP was observed with any template/primer system, and the highest value found is included in Table 1 (see also Table 2).

To test the ability of the enzyme preparation to catalyse the synthesis of heteropolymers, wheat embryo mRNA was used as a template in further experiments. However, to avoid measuring the copying mRNA polyadenylate tails, [^3H]dTTP was replaced by [^3H]dCTP. In such a system, all four deoxynucleoside triphosphates and oligo(dT) primer were required for maximal enzyme activity (Table 2). A substantial incorporation of [^3H]dCTP could, however, be observed when dATP, dGTP and dTTP were omitted from the assay. Similarly, the omission of mRNA diminished the incorporation considerably, but not completely. The lack of a clear-cut dependence of the reaction rate on these

Table 2. Template/primer requirements for the synthesis of heteropolymers by a microsomal fraction of wheat embryo homogenate

Samples of the microsomal fraction were incubated under conditions used for the heteropolymer-synthesis assay (see the Materials and methods section), with the use of substrates, templates and primers as indicated. '3 dNTP' and '3 rNTP' stand for mixtures of the supplementary deoxy- and ribo-nucleoside triphosphates respectively. Abbreviation: BMV, brome-grass mosaic virus.

Substrate		Template/primer	[³ H]Nucleotide incorporated (pmol/assay)
³ H-labelled	Unlabelled		
dCTP	3 dNTP	mRNA + oligo(dT)	4.02
dCTP	3 dNTP	mRNA	2.19
dCTP	3 dNTP	Oligo(dT)	1.57
dCTP	3 dNTP	None	1.30
dCTP	None	mRNA + oligo(dT)	1.28
dCTP	None	None	0.76
dCTP	3 dNTP	rRNA + oligo(dT)	1.30
dCTP	3 dNTP	BMV RNA + oligo(dT)	1.65
dCTP	3 dNTP	Activated DNA	1.43
dGTP	3 dNTP	mRNA + oligo(dT)	3.91
rUTP	3 rNTP	mRNA + oligo(dT)	0.16

Fig. 2. Distribution in CsCl/ethidium bromide gradients of the putative reverse transcriptase reaction product

The reaction product was obtained and centrifuged as described in the Materials and methods section. Untreated (●) and 0.1 M-NaOH-hydrolysed (○) samples (5300 c.p.m.) were centrifuged in parallel. The arrows indicate the positions of density markers, RNA, single-stranded DNA, RNA-DNA hybrid and double-stranded DNA (in that order, from the bottom to top).

essential components may be due to their endogeneous presence in our crude enzyme preparation. Moreover, the preparation may be contaminated with some interfering enzyme activities, in particular with terminal deoxynucleotidyltransferase, which is known to occur in wheat embryos (Brodiewicz-Proba & Buchowicz,

1980). Nevertheless, the pattern of all responses to changes in supplying templates, primers and substrates strongly suggests that a reverse transcriptase-like activity prevails in our enzyme preparation. Clearly, neither of the templates tested could support the heteropolymer synthesis as efficiently as did mRNA, and the rate of heteropolymer synthesis was considerably higher than that observed for homopolymer synthesis.

In attempts to establish the nature of the heteropolymer formed, the ³H-labelled reaction product was subjected to centrifugation in a CsCl/ethidium bromide density gradient. As Fig. 2 shows, the main radioactive product bands near the density of an RNA-DNA hybrid. A less-prominent peak of radioactivity seems to correspond to double-stranded DNA. The putative hybrid disappears after alkaline hydrolysis and gives rise mainly to single-stranded DNA. A less-dense product of unknown nature also appears. These observations allow us to assume that an RNA-DNA hybrid may be a direct product of the reaction catalysed by the wheat embryo microsomal fraction under our experimental conditions.

DISCUSSION

With respect to template/primer requirements, the microsomal-fraction-associated enzyme of wheat embryos differs essentially from typical eukaryotic DNA polymerases. The ability to use polyribonucleotides as template makes it, at the first approximation, similar to DNA polymerase- γ and, especially, to the plant γ -like DNA polymerase A described by Litvak *et al.* (1985). Unlike the DNA polymerase A, however, the microsomal-fraction-associated enzyme prefers homologous mRNA to any other polynucleotides as template. Thus functionally the enzyme seems to be most similar to viral reverse transcriptases and, in particular, to that occurring in cauliflower-mosaic-virus-infected plants (Volovitch *et al.*, 1984). It should be emphasized, however, that wheat is never infected with this or related viruses. We therefore believe that the wheat harbours a gene related to reverse transcriptase.

The apparent association of the cellular reverse transcriptase-like activity with the microsomal fraction may be not fortuitous. Particle-bound reverse transcriptase has been found in various eukaryotic cells, but is usually classified as the activity of virus-like entities (Bauer *et al.*, 1978; Heine *et al.*, 1980; Garfinkel *et al.*, 1985; Müller *et al.*, 1987; see also Baltimore, 1985, and papers quoted therein). We do not, however, extend this interpretation to wheat. Instead we take a view that is similar to that expressed by Adams (1985), namely that the microsomal fraction usually contains some DNA of unknown origin. It has been postulated that this DNA may originate from reverse transcription (Buchowicz *et al.*, 1978), and the physiological significance of such a reverse transcription has been discussed elsewhere (Buchowicz & Kraszewska, 1984).

Notwithstanding the validity of the interpretation given above, the wheat embryo microsomal fraction remains of interest as a promising source of truly eukaryotic reverse transcriptase.

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