SELECTION OF REPEATED SEQUENCES OF HOMOLOGOUS AND HETEROLOGOUS DNA DURING IN VITRO TRANSCRIPTION BY MAIZE RNA POLYMERASE

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SUMMARY

RNA synthesized in vitro by maize RNA polymerase II arises in part from repeated DNA sequences, since significant hybridization to the parent DNA occurs with low concentrations of RNA and DNA. Over three times as much "repeated sequence" RNA is transcribed from maize as from calf thymus DNA.

INTRODUCTION

Differential selection of DNA sequences for transcription and subsequent translation of the defined messenger population may underlie a variety of phenomena from differentiation to carcinogenesis[1-4]. How transcription is controlled in higher organisms is virtually unknown, except for the recent discovery of multiple RNA polymerases with correlated cellular compartmentalization[5] and the possible involvement of poly(A) in the initiation of synthesis[6], processing or transport of nuclear transcripts[7]. We have studied which DNA sequences are transcribed by the eukaryotic RNA polymerase II of Zea mays seedlings[8]. Our approach has been RNA-DNA hybridization using filter-bound DNAs and RNA synthesized in vitro from maize and calf thymus DNAs, native or heat denatured, alone or mixed.

SOURCE AND PREPARATION OF MATERIALS

Maize DNA was prepared from seedling roots and shoots by the method of Britten, Pavich and Smith[9] modified as described previously[10]. Chinese hamster ovary (CHO) cell DNA was the gift of Dr. M. Schneiderman. T_4 DNA was from Dr. C. Mead. Calf thymus, E. coli and M. luteus DNAs were from Worthington (Freehold, NJ). Maize and calf thymus DNAs were heterogeneous in molecular weight, with mean sizes greater than 20×10^6. Template DNAs were dissolved in 15 mM NaCl, 1.5 mM sodium citrate, pH 7 (0.1 x SSC). Maize RNA polymerase II

TABLE I. ACCUMULATION OF ACID-INSOLUBLE PRODUCT AFTER INCUBATION OF MAIZE AND BOVINE DNAS WITH MAIZE RNA POLYMERASE II.

	Template DNA Type	μg	Labeled product accumulated nmoles
A.	Maize native	318	4.2
	Maize den'd	267	15.1
	Bovine native	563	6.3
	Bovine den'd	444	11.3
B.	Maize native, bovine den'd	41.5 each	1.9
	Bovine native, maize den'd	41.5 each	1.4
	Both native	41.5 each	0.8
	Both denatured	41.5 each	1.9

Reaction mixtures (2 ml) for single templates (Part A) contained 200 μmoles Tris, pH 8, 7.4 μmoles 2-mercaptoethanol, 2.1 μmoles [α-^{32}P]ATP (specific activity 21 Ci/mole; 4 μmoles each CTP, GTP and UTP, 10 μmoles $MnCl_2$, 1 mg bovine serum albumin, 0.09 mg polymerase and an excess of DNA as indicated. For mixed template syntheses (Part B) reactions volumes were 0.5 ml and all reagents were proportionately decreased. Mixtures were incubated at 30°C for 60 min and acid-insoluble radioactivity determined on filter paper disks as described previously[16]. DNA was denatured in a boiling water bath for 10 min and quenched on ice.

was purified through DEAE-cellulose chromatography from shoots of 5-day-old seedlings to a specific activity of 3.2 nmoles AMP incorporated/min/mg protein at 30°C as described by Mans[8].

RESULTS AND DISCUSSION

RNA was transcribed *in vitro* from single or mixed DNA populations of maize and bovine DNAs, either native or heat denatured, with RNA polymerase II of *Zea mays*. Both DNAs served as templates and, at the concentrations used, denatured DNA supported greater product accumulation than native DNA (Table I, Part A)[11].

Single-template RNA. Hybridization is specific for the DNA used as template, the homologous reaction being at least 10-fold greater than hybridi-

TABLE II. HYBRIDIZATION SPECIFICITY OF MAIZE RNA POLYMERASE PRODUCTS SYNTHESIZED WITH SINGLE TEMPLATES[a].

Template	Radioactive product (pmoles AMP) bound to filters DNA on filter					
	Maize	Bovine	CHO cell	E. coli	M. luteus	Th
Maize native	33.3(62)	0.3(59)				0.1(49)
Maize den'd	69.7(40)	0.4(82)	1.2(46	4.4(47)	0.4(28)	0.0(40)
Bovine native	0.1(42)	10.5(50)	0.1(51)	0.0(51)	0.0(24)	0.0(41)
Bovine den'd	0.1(40)	24.0(38)	1.4(73)	0.3(43)	0.9(23)	1.3(44)

[a] Results are reported as pmoles AMP bound per filter; 40 cpm/pmole AMP.

[b] Number in parentheses is µg DNA per filter.

Radioactive products accumulated after 60 min incubation as described in Table I were prepared for hybridization by the addition of an equal volume of phenol saturated with acetate buffer (0.1M NaCl, 0.01M sodium acetate, pH 5). The mixtures were shaken for several minutes at 70°C, the phases separated by centrifugation and the aqueous layers desalted by Sephadex column chromatography. DNAs were loaded onto nitrocellulose membranes (25mm, 0.45 µm) according to Gillespie[15]. Hybridization was done in scintillation vials with 1 ml medium and 2-4 filters including one without DNA. Medium consisted of 7.5M urea, 0.01 M Tris, pH 8, 0.05% sodium dodecyl sulfate, 0.39M Na+ as SSC and 3500-25000 cpm of RNA. After 4 days incubation at 37°C filters were rinsed with 2 x SSC, treated 30 min at 23°C with 20 µg/ml pancreatic ribonuclease A (Worthington, Freehold, NJ; brought to 80°C at pH 5 prior to use), and washed on both sides with 50 ml 2 x SSC. RNase treatment reduced counts on blank filters from twice background to background (~20 cpm) and removed 77% of the counts originally bound to DNA-containing disks. Bound radioactivity was then assayed by counting dried filters in a liquid scintillation spectrometer. During hybridization there was no loss of RNA acid-precipitable counts. After hybridization DNA was hydrolyzed from filters by boiling in 0.3% perchloric acid. The amount of DNA per filter was estimated from the A_{260} released, using a standard curve constructed from filters loaded but not hybridized. DNA retention was 100%.

zations with other than template DNA (Table II). Specificity is maintained whether the template DNA is native or heat-denatured, but denaturation increases the amount of RNA hybridizing to the parent DNA at least twofold (Table II). Since low RNA (<1 µg/ml) and DNA (<83 µg/filter) concentrations were used, the observed hybridization is due to RNA transcribed from repeated DNA sequences[12,13]. Such "repeated sequence" RNA represents a significant fraction of the in vitro product, since up to 30% of the input RNA became bound in RNase-resistant

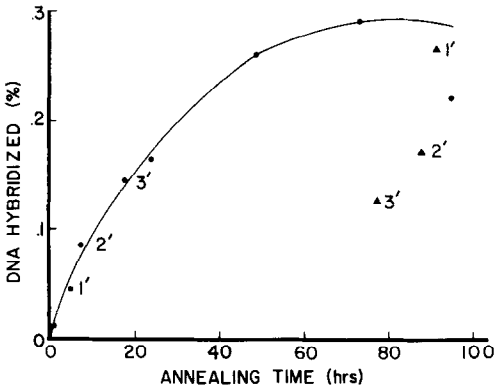

Figure I. Kinetics of RNA-DNA hybridization. Product transcribed from denatured maize DNA as described in Table I was isolated and hybridized to maize DNA as described in Table II. Percent DNA hybridized was calculated by assuming a DNA-like composition of the RNA[16]. Circles = primary hybridizations; triangles = secondary hybridizations in which new filters (1', 2' and 3') were added to vials from which other filters (1, 2 and 3, respectively) had been removed.

hybrids. That RNAs other than those from highly reiterated sequences are transcribed is suggested by the fact that both percent DNA and RNA hybridized decrease as DNA and RNA concentrations increase. Further evidence for product from less repeated DNA sequences is obtained from experiments in which a second (or third) DNA filter was either included in the hybridization vial or added after removal of a primary filter[14]. In the former experiment none of the multifilter vials yielded disks with counts as great as on disks from equivalent vials incubated with only one DNA filter (data not shown). With sequential hybridizations, secondary filters failed to bind as much RNA as equivalent-time primary disks (Fig. 1). These results indicate the presence of at least two different RNA classes differing in the repetition frequency of the parent DNA sequences.

Mixed template RNA. It may be noted that in single-template transcription maize DNA produces threefold more hybridizable RNA than does bovine DNA (Table II). Since the transcripts accumulated *in vitro* with maize RNA polymerase II anneal selectively to the DNA used as template, we investigated the products transcribed in the presence of two potential templates. In essence we asked if the polymerase could selectively transcribe its homologous DNA in

TABLE III. HYBRIDIZATION SPECIFICITY OF MAIZE RNA POLYMERASE PRODUCTS SYNTHESIZED WITH MIXED TEMPLATES[a].

Template Mixture	Input RNA (pmoles AMP)	Radioactive product (pmoles AMP) bound to filters — DNA on filter[b]		Ratio[c]
		Maize	Bovine	
Both native	307	54.0 (106)	8.9 (127)	6.1
Both denatured	413	23.8 (107)	19.8 (79)	1.2
Maize native, bovine den'd	527	8.0 (105)	47.3 (90)	0.17
Maize denatured, bovine native	573	73.3 (120)	4.2 (122)	17

[a] Results are reported as pmoles AMP bound per filter; 44 cpm/pmole AMP.

[b] Number in parentheses is µg DNA per filter.

[c] Ratio of pmoles AMP bound on the maize DNA filter to pmoles bound on the bovine DNA filter.

RNA was synthesized and purified and hybridization performed as described in Tables I and II.

the presence of a heterologous template. Products were accumulated in reaction mixtures containing equal amounts of maize and bovine DNAs with neither, one or both denatured (Table I, Part B). Hybridization of the RNA with both DNAs was observed (Table III). However, (1) maize DNA is a 6-fold better template for making hybridizable RNA than is bovine DNA when both templates are native; (2) when both templates are denatured product RNA hybridizes with about equal efficiency to both DNAs; and (3) with RNA from mixed native and denatured templates, hybridization strongly favors the DNA which was denatured during synthesis of the RNA; however, the dominance of maize DNA in this regard is much greater than that of bovine DNA (Table III, lines 3 and 4).

We conclude that the maize RNA polymerase transcribes more repetitious regions of the homologous maize DNA than of bovine DNA, whether the templates are native or denatured. Furthermore the selection of reiterated maize DNA sequences is enhanced during transcription in the presence of a competing, native transcribable DNA.

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