Theor Appl Genet (1988) 75: 751–753

An analysis of the quantity and diversity of messenger RNAs from pollen and shoots of *Zea mays*

R. P. Willing, D. Bashe and J. P. Mascarenhas

Department of Biological Sciences, State University of New York at Albany, Albany, NY 12222, USA

Received February 17, 1987; Accepted June 25, 1987
Communicated by P. L. Pfahler

Summary. The mRNAs of the mature pollen grain of *Zea mays* and of vegetative shoots were analyzed by comparing the kinetics of hydridization between homologous and heterologous reactions of cDNA to poly-(A)RNA in excess. The total complexity of pollen poly-(A)RNA is 2.4×10^7 nucleotides, whereas in the seedling shoot it is 4.0×10^7 nucleotides. This corresponds to 24,000 different sequences in pollen and about 31,000 diverse mRNAs in shoots. The mRNAs in pollen can be divided into three abundance classes that constitute 35%, 49% and 15% of the total mRNAs having complexities of 2.1×10^5, 6.4×10^6 and 1.8×10^7 nucleotides, respectively. Estimates have been made of the number of copies of each sequence per pollen grain or shoot cell, and also of the pollen mRNA sequences shared with those of the shoot.

Key words: Hybridization kinetics – mRNA copy numbers – Pollen mRNA complexity

Introduction

Mature pollen grains of *Tradescantia paludosa* (Frankis and Mascarenhas 1980), maize (Mascarenhas et al. 1984), and tobacco (Tupy 1982) contain presynthesized messenger RNAs (mRNAs). These presynthesized mRNAs appear to have functions during germination and early pollen tube growth (Mascarenhas 1975). The mRNAs present in the mature pollen grain of *Tradescantia* are the products of about 20,000 genes, compared to approximately 30,000 different genes for vegetative shoots (Willing and Mascarenhas 1984). Since this is the only estimate of the numbers of genes expressed in pollen, it is of interest to determine the complexity and diversity of mRNAs in pollen of maize.

Materials and methods

Plant material

Seedlings of maize (*Zea mays* L.), inbred line W-22 (seed obtained from Illinois Foundation Seeds) were grown in the dark at 25 °C on moist paper towels. Shoots were harvested when the average length was about 1.5 cm and frozen in liquid nitrogen. Pollen was harvested from field-grown plants and both pollen and shoots were stored at −70 °C until used for RNA extraction.

Isolation of poly(A)RNA, determination of poly(A)RNA size and preparation of cDNA

Total RNA and poly(A)RNA were isolated from pollen and shoots as described by Willing and Mascarenhas (1984). The justification for using total cellular poly(A)RNA rather than polysomal poly(A)RNA has been discussed in Willing and Mascarenhas (1984). Poly(A)RNA concentrations were determined by hybridization to ^{3}H-poly(U) as previously described (Willing and Mascarenhas 1984). The size of poly(A)RNAs from shoot and pollen total RNA was determined as described earlier (Willing and Mascarenhas 1984). The number average size of shoot poly(A)RNA was calculated to be 1,300 nucleotides, and that of pollen poly(A)RNA to be 1,020 nucleotides. The average amount of poly(A)RNA per pollen grain was taken to be 13.35×10^{-12} g (Mascarenhas et al. 1984) and 5.6×10^{-14} g per young seedling shoot cell (Table 1). ^{32}P-cDNA was prepared to pollen or shoot poly(A)RNA using ^{32}P-dCTP (New England Nuclear; specific activity 3,000 Ci/mmole) and murine virus reverse transcriptase according to the supplier's protocol (Bethesda Res. Labs.).

Hybridization of RNA to ^{32}P-cDNA and analysis of the data

Hybridization conditions were as described earlier by (Willing and Mascarenhas 1984). In brief, the reaction mixture contained 30 mM PIPES-NaOH, pH 6.7, 1 M NaCl, 1 mM EDTA and 0.5% SDS. For Cot values of 150 or less, the poly(A)RNA concentration was 0.5 μg/μl and the poly(A)RNA to cDNA ratio was about 1,000:1. For Cot values over 150, the poly(A)RNA concentration was 2.0 μg/μl and the poly(A) RNA to cDNA ratio was about 4,000:1. Two μl aliquots of the reaction mixture were sealed in a 5 μl capillary tube,

Table 1. Summary of homologous cDNA-poly(A)RNA hybridizations from pollen and shoots. The homologous hybridization reactions in Fig. 1 were fit by computer and calculations made as described in the text and in Willing and Mascarenhas (1984)

Tissue	Component	F[a]	K_p[b]	Complexity[c] (nucleotides)	No. of diverse mRNAs	No. of copies per sequence per pollen grain or shoot cell
			$m^{-1} \cdot s^{-1}$			
Pollen	1	0.35	6.56	2.1×10^5	245	32,000
	2	0.49	0.22	6.4×10^6	6,260	1,700
	3	0.15	0.08	1.8×10^7	17,250	195
Shoot	1	0.19	19.0	7.4×10^4	57	245
	2	0.54	0.47	3.0×10^6	2,300	17
	3	0.26	0.04	3.7×10^7	29,365	1

[a] Fraction of the total poly(A)RNA, determined by the fraction of reacting cDNA (computer derived) and normalized to 100%
[b] K pure, the pseudo first order rate constant for a given component, derived by computer
[c] No. of nucleotides summed for all diverse sequences of a given class

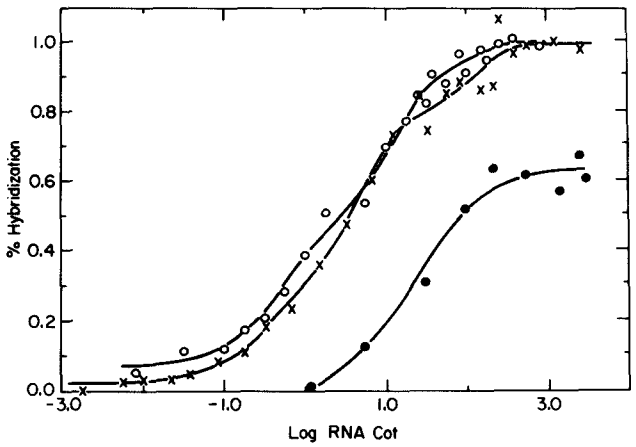

Fig. 1. Hybridization of ^{32}P-cDNAs with various poly(A)RNA fractions. cDNA synthesized using poly(A)RNA from pollen was hybridized to pollen poly(A)RNA (○) or to shoot poly(A)RNA (●); cDNA synthesized to poly(A)RNA from shoots was hybridized to shoot poly(A)RNA (×)

denatured for 3 min at 110 °C, and incubated to different RNA Cot values at 65 °C. RNA Cot values were corrected to the standard 0.18 M salt conditions by multiplying by 2.9 (Van Ness and Hahn 1982). The fraction of ^{32}P-cDNA resistant to S-1 nuclease was determined by the DE-81 filter disc assay (Goldberg et al. 1978; Maxwell et al. 1978). A least squares computer program (Pearson et al. 1977) was used to fit the data to a plot. Complexity of poly(A)RNA was determined by comparison to the hybridization kinetics of a known standard as described previously by Willing and Mascarenhas (1984).

Results

Complementary DNAs synthesized from pollen poly(A)RNA and from shoot poly(A)RNA were each hybridized to homologous poly(A)RNA. The kinetics of these hybridizations are shown in Fig. 1 and summarized in Table 1. Both hybridizations occurred over several log units of Cot, indicating a wide range in abundance of the different mRNA classes (Bishop et al. 1974). The RNAs can thus be divided into a number of abundance classes, and the kinetics of each class can be analyzed by the least squares computer program (Pearson et al. 1977). For both pollen and shoots there was a reduction in the error term when the number of components was increased from two to three (0.026 to 0.023 for pollen and 0.031 to 0.029 for shoots). There was no change in the error terms when four rather than three components were used. Thus three abundance classes best describe the data for both pollen and shoots.

In pollen (Fig. 1 and Table 1) 35% of the mRNAs are abundant and comprise about 240 sequences each present, on an average, in about 32,000 copies per pollen grain. The middle abundance class, which makes up the major fraction of the mRNAs (49%), consists of about 6,000 different sequences each present in about 1,700 copies per pollen grain. The least abundant fraction (15%) is made up of about 17,000 sequences each present in about 200 copies per pollen grain.

In contrast, the most abundant fraction of the young seedling shoot is made up of 19% of the RNAs and consists of about 60 different sequences each present in about 250 copies per cell. The middle abundance class (54%) is made up of 2,300 sequences each present on an average in about 20 copies per cell, and the least abundant class (26%) has about 29,000 different sequences in about one copy per cell.

The total complexity of corn pollen poly(A)RNA is 2.4×10^7 nucleotides corresponding to about 24,000 different sequences, whereas in the seedling shoot it is 4.0×10^7 nucleotides which corresponds to about 31,000 diverse mRNAs. The complexity of pollen mRNA sequences is about 60% of that of shoot mRNAs.

The heterologous hybridization of pollen cDNA to shoot poly(A)RNA (Fig. 1) does not show any hybridization at log Cot values below 0, and appears to reach completion by a log Cot of 3 and with about 65% of the pollen cDNA being hybridized to the shoot poly(A)RNA.

Discussion

The complexity of corn pollen poly(A)RNAs (2.4×10^7 nucleotides) is very similar to that (2.3×10^7 nucleotides) found for *Tradescantia* pollen (Willing and Mascarenhas 1984). The individual sequences in the three poly(A)RNA components in corn pollen are all much more abundant than the corresponding components in shoot cells, a situation similar to that found for *Tradescantia*. In similarity with *Tradescantia*, the complexity of corn pollen RNAs is also about 60% to that of shoot mRNAs (Willing and Mascarenhas 1984). The fact that when pollen cDNA is hybridized to shoot poly(A)-RNA, the reaction reaches completion with about 65% of the cDNA being hybridized to the shoot poly(A)RNA, could indicate that about 65% of the sequences in pollen are similar to those in shoots. The kinetics of the heterologous reaction, the mass ratio of the reacting nucleic acid components, and the complexities of the components in each tissue, however, place constraints on this estimate of shared sequences and could indicate that between 20% and 95% of the pollen mRNA sequences are present in the shoot. Of the abundant pollen mRNA sequences which are present, the majority are either absent or at substantially reduced concentrations in the shoot. This estimate is in agreement with an estimate made by Sari-Gorla et al. (1986) who have studied isozymes in various tissues of corn. They have shown that about 72% of the isozymes are expressed both in pollen and sporophyte tissues. Our results lend further support to recent studies that indicate that the genetic program expressed during pollen development is quite extensive, and that there is a substantial overlap between genes active in gametophytic and sporophytic tissue (Mulcahy 1979; Tanksley et al. 1981; Willing and Mascarenhas 1984; Sari-Gorla et al. 1986).

Acknowledgements. This work was supported by National Science Foundation Grant DCB-8501461.

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