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Messenger RNAs in corn pollen and protein synthesis during germination and pollen tube growth

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Summary. Mature ungerminated pollen grains of *Zea mays* L. contain presynthesized messenger RNAs. This has been demonstrated by the isolation of poly(A)RNA and its translation in the wheat germ and reticulocyte cell free systems into polypeptides many of which are similar to those synthesized in germinating pollen. Each corn pollen grain contains between 352–705 pg of total RNA and 8.9–17.8 pg of poly(A)RNA. During germination of corn pollen at least 260 different polypeptides are synthesized as determined by labeling and 2-dimensional gel electrophoresis. These results are discussed with reference to other plants and the number of different genes expressed during pollen development.

Key words: *Zea mays* L. – Pollen – mRNAs – Protein synthesis – Germination

Introduction

Pollen grains of *Tradescantia paludosa* contain messenger RNAs (mRNAs) that were synthesized during development prior to maturity and that are utilized during pollen germination and early tube growth (Mascarenhas 1966; Frankis and Mascarenhas 1980). These mRNAs code for at least 230 polypeptides (Mascarenhas and Mermelstein 1981). Mature pollen grains of tobacco also contain poly(A)RNA (Tupý 1982). Germinating corn pollen incorporated ^{35}S-methionine into protein but whether this was due to synthesis from performed mRNAs in the grains or from the new synthesis of mRNAs during germination was not distinguished (Porter 1981). Because of the agronomic value of corn and the potential for utilizing the extensive genetic information available for this plant for studies of gene expression and regulation during pollen development we have characterized the mRNAs of the corn pollen grain and determined the types and numbers of proteins synthesized in vitro and in the germinating grain.

Materials and methods

Pollen collection and growth

Pollen for the isolation of RNA was collected from field grown plants of *Zea mays* L., cultivar 'Gold Cup' (Harris Seeds, Rochester, New York), courtesy of Mr. H. LeVie of LeVies' Farm, Voorheesville, New York. Pollen was quick frozen in liquid N_2 and stored at –70 °C. For germination of pollen and labeling of proteins, tassels were cut and the cut ends placed in a flask of tap water in the laboratory on the day prior to collecting the pollen. Pollen after collection was kept in a petri dish in the refrigerator at 4 °C for ca 2 h prior to addition to the growth medium (Pfahler, P., personal communication). Pollen was spread evenly in a 5 cm sterile petri plate over 1.2 ml of Pfahler's (1973) medium containing 25 μg/ml chloramphenicol and 50 μCi of ^{35}S-methionine (New England Nuclear, 1,236 Ci/mmol). The plate was incubated for 2 h at 26 °C in the dark during which time tubes were four to eight times the diameter of the grain. Germination of 50 to 70% was obtained with different batches of pollen. The pollen tubes were removed from the plate and collected in a tube with several rinses of Laemmli (1970) sample buffer without sodium dodecyl sulfate (SDS). SDS was then added to a concentration of 1% and the tube was immediately placed in boiling water for 2.5 min. The suspension was homogenized in a ground glass homogenizer, centrifuged at full speed for 10 min in a Clay Adams Dynac desk top centrifuge and the supernatant stored at –20 °C for further analysis.

Isolation of poly(A)RNA, determination of the poly(A) content of RNA, cell free protein synthesis and analysis of in vitro synthesized proteins

Poly(A)RNA was prepared from total RNA isolated from ungerminated pollen essentially as described previously (Frankis

and Mascarenhas 1980) except that the homogenization buffer was 50 mM Tris-HCl (pH 9.0) containing 0.2 M NaCl, 1 mM EDTA, 0.1% diethyl pyrocarbonate and 1.0% SDS. The poly(A) content of RNA was determined by hybridization with ^{3}H-poly(U) as described previously (Mascarenhas and Mermelstein 1981). The poly(A)RNA was translated in both the wheat germ cell free system (Roberts and Paterson 1973; kindly provided by Dr. Donald Nuss) to which was added 10 μg/ml of placental ribonuclease inhibitor (Bolton Biochemicals, Richmond Heights, Missouri) and the reticulocyte system (Pelham and Jackson 1976) to which was added 10 μg/ml of 2-aminopurine (DeBenedetti and Baglioni 1983) and 10 μg/ml of placental ribonuclease inhibitor. The ^{35}S-methionine labeled polypeptides synthesized both in vivo and in vitro were analyzed on single dimension 12.5–20% gradient acrylamide-SDS gels (Laemmli 1970) or by two dimensional electrophoresis according to O'Farrell (1975). The extracts were cooled in ice to precipitate SDS, the SDS was pelleted and the extracts treated with DNase and RNase. The proteins were then precipitated with five volumes of acetone and the pellets washed with 70% acetone to remove all SDS prior to solubilizing in lysis buffer (O'Farrell 1975) for 2-dimensional analysis. The isoelectric focusing gels contained pH 5–8 ampholytes and the second dimension was in 12.5 to 20% gradient acrylamide gels. The gels were dried, followed by autoradiography with Kodak X-Omat AR film.

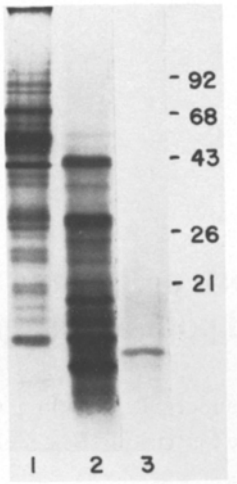

Fig. 1. Autoradiogram of in vivo and in vitro (wheat germ system) synthesized proteins after SDS-polyacrylamide gel electrophoresis. *Lane 1* proteins synthesized in vitro during pollen germination; *Lane 2* in vitro translation products of poly(A)RNA from ungerminated pollen; *Lane 3* endogenous activity of the wheat germ system without added poly(A)RNA. The numbers at the right are molecular weights in kilodaltons of standard proteins

Results

RNA was isolated from corn pollen and from this analysis one gram of pollen was found to contain 1.41 mg of total RNA. Porter (1981) has estimated there are 2,000 pollen grains per mg of corn pollen and if one calculates the number of grains from a weight of 247×10^{-9} g per pollen grain (Miller 1982) one would arrive at a figure of 4,000 grains/mg. Using 2,000 grains/mg the total amount of RNA per corn pollen grain is 705 pg and if one uses 4,000 grains/mg for the calculations each pollen grain contains 352 pg of RNA.

The poly(A) content of corn pollen RNA was determined by ^{3}H-poly(U) hybridization. On an average, 1 mg of total RNA contains 1.69 μg of poly(A) or 25.3 μg of poly(A)RNA, using the value determined for *Tradescantia* pollen mRNA (which is similar to that for tissues from various plants) that 1/15 of the length of the mRNA on an average is poly(A). This works out to either 17.8 or 8.9 pg poly(A)RNA per pollen grain depending on whether one uses 2,000 or 4,000 pollen grains/mg respectively for the calculations.

Poly(A)RNA isolated from ungerminated corn pollen was translated in both the wheat germ and reticulocyte cell free systems. The polypeptides synthesized in vitro and in vivo in germinating pollen were analyzed by one dimensional and 2-dimensional electrophoresis (Figs. 1–3). A large number of radioactive bands (greater than 40) spanning a range of sizes can be resolved in the single dimension gels of in vivo labeled proteins (Figs. 1 and 2), several of which comigrate

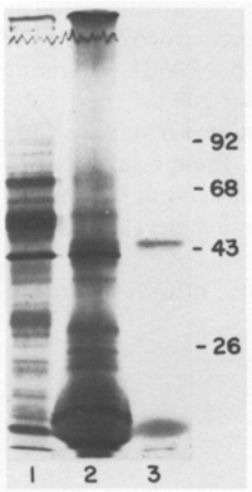

Fig. 2. Autoradiogram of in vivo and in vitro (reticulocyte lysate) synthesized fractions after SDS-polyacrylamide gel electrophoresis. *Lane 1* proteins synthesized in vivo during pollen germination; *Lane 2* in vitro translation products of poly(A)RNA from ungerminated pollen; *Lane 3* endogeneous activity of the reticulocyte cell free system without added poly(A)RNA. The numbers at the right are molecular weights in kilodaltons of standard proteins

with polypeptides synthesized in both the wheat germ (Fig. 1) and reticulocyte (Fig. 2) systems. In the reticulocyte system larger polypeptides are synthesized and the correspondence in migration of in vivo and in vitro synthesized polypeptides is better than with the wheat germ system.

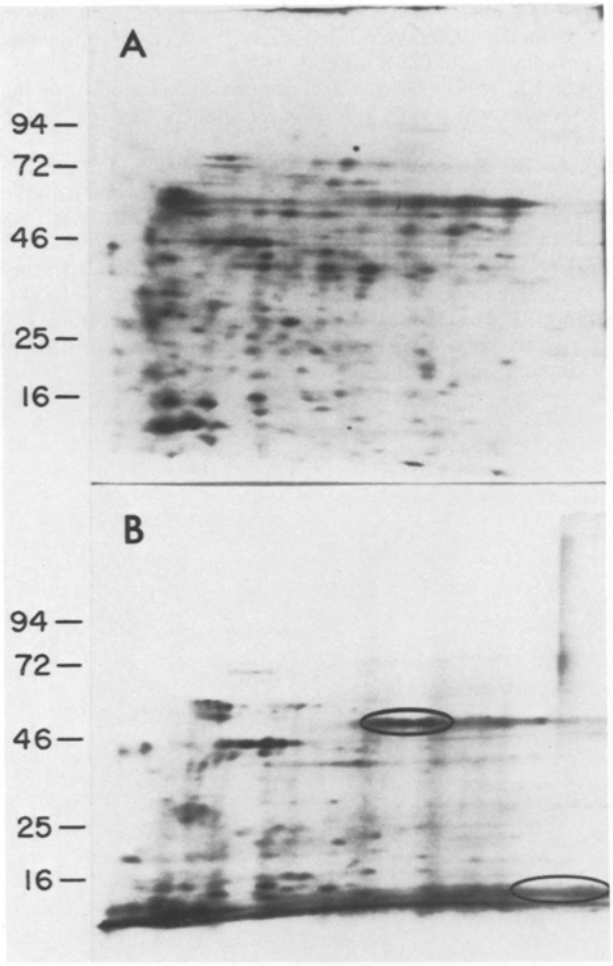

Fig. 3. Analysis of proteins synthesized (**B**) in the reticulocyte cell free system with corn pollen poly(A)RNA and (**A**) by pollen during germination. Autoradiograms of 2-dimensional gels with the acidic end shown to the *left*. The numbers at the *left* are the molecular weights in kilodaltons of standard proteins. *Circled spots* are the endogenous polypeptides synthesized by the reticulocyte system without any added poly(A)RNA

Autoradiograms of polypeptides synthesized by germinating corn pollen when analyzed by 2-dimensional gel electrophoresis (Fig. 3 A) show about 260 spots. The reticulocyte lysate translation products of pollen poly(A)RNA (Fig. 3 B) show a large number of polypeptides many of which appear identical in their size and isoelectric points to the polypeptides synthesized in germinating pollen.

Discussion

One gram of corn pollen contains 1.4 mg of RNA. In comparison, 1 g of *Tradescantia paludosa* pollen contains 17 mg of RNA (calculated from results in Mascarenhas and Mermelstein 1981). Each corn pollen grain, however, contains between 352–705 pg of total RNA and 8.9–17.8 pg of poly(A)RNA compared to 196 pg of total RNA and 5.1 pg of poly(A)RNA per *Tradescantia* pollen grain (Mascarenhas and Mermelstein 1981). This is because of the large size of corn pollen grains and their greater degree of hydration relative to *Tradescantia*. There are 88,100 pollen grains per mg of *T. paludosa* pollen (Mascarenhas and Mermelstein 1981) compared to 2,000–4,000 grains/mg in corn. *Nicotiana tabacum*, the only other pollen studied in this manner contains 230 pg of total RNA and 6.2 pg of poly(A)RNA (Tupý 1982).

The ability of the poly(A)RNA from mature, ungerminated corn pollen, in both the wheat germ and reticulocyte cell free systems, to be translated into polypeptides many of which are similar in their migration after electrophoresis to polypeptides synthesized during pollen germination is conclusive evidence that the mature corn pollen grain has a store of presynthesized mRNAs.

During germination of corn pollen at least 260 different polypeptides are synthesized as visualized after labeling with ^{35}S-methionine and 2-dimensional gel electrophoresis (Fig. 3 A). Approximately the same number of newly synthesized proteins can be detected in germinating *Tradescantia* pollen (Mascarenhas and Mermelstein 1981). The actual number of proteins synthesized is probably much higher and the 260 polypeptides detected are probably only those that are synthesized from mRNAs that are present in many copies per pollen grain. In *T. paludosa* based on the kinetics of hydridization of cDNA (made to pollen poly(A)RNA) to pollen poly(A)RNA in excess it has been estimated that about 20,000 different mRNAs, i.e. different gene products are present in the mature ungerminated pollen grain (Willing and Mascarenhas 1984). One might accordingly expect a similar if not larger number of different transcripts in the corn pollen grain since the corn pollen grain contains more RNA than *Tradescantia* and it grows to a much greater length to reach the embryo sac.

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