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Microspore and Microgametophyte Development in Relation to Biological Activity of Environmental Pollutants

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The pattern of synthesis of nucleic acids and proteins in the development of the microspore and later during pollen germination and tube growth is discussed. In the pollen grain at the time of anthesis all the proteins that are required for germination and early tube growth are either already present, or if new proteins are synthesized, the messenger RNAs for their synthesis already exist in the ungerminated pollen grain. In addition, similar proteins are synthesized on new mRNAs during germination and pollen tube growth as are synthesized on premade mRNAs. The genetic program during at least the latter part of pollen maturation prior to anthesis is thus the same as that during pollen germination and tube growth. Accordingly, one cannot treat mature pollen with mutagens and expect to be able to score the pollen tubes for mutant proteins. Treatment with mutagenic compounds would have to be during pollen maturation in the anther, before the transcription for the proteins required during germination and pollen tube growth has occurred. Available evidence indicates that this is very early in pollen development, possibly soon after meiosis.

The value of pollen tubes to monitor for chemicals that affect the intracellular motility systems of organisms is also discussed.

Pollen can, in theory, be used to assay for effects of chemicals on cellular processes that are general to all plant systems. In addition, there are other processes that can be studied that are unique to pollen and are concerned with the fertilization process.

In this paper certain aspects of the molecular biology of pollen development will be discussed. This information might be of value in devising experimental protocols for the use of pollen as a monitoring system for potentially toxic chemicals released into the environment.

Protein and RNA Synthesis in the Mature Pollen Grains and Pollen Tube

Any rapid pollen assay for mutagenic activity would of necessity require individual pollen grains to be assayed for a specific enzyme activity. Thus, it would be of interest to know which proteins are already present in the pollen grain at anthesis, and when during development the genes for these proteins are transcribed. It would also be necessary to know which proteins, if any, are not present in the mature pollen grain but are newly synthesized during pollen germination, because these would be likely candidates for any rapid assay system. Experiments done in my laboratory that bear on these points will be discussed.

Most of the work has been done with pollen of *Tradescantia paludosa*. Tradescantia pollen is easy to obtain throughout the year, stores well in the freezer for several months, is easy to handle experimentally, grows rapidly, and has other properties, such as synchrony of development, that would make it an ideal pollen for assaying the activity of chemicals.

Proteins and RNA are synthesized during pollen germination and tube growth. Protein synthesis is initiated during the early stages of pollen germination prior to tube outgrowth (*1*, *2*). Cell membrane

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synthesis is one of the major synthetic events in pollen tube growth and in Tradescantia a 50-fold increase in surface area of the pollen tube occurs within a short period of growth (*3*). We have accordingly studied the enzymes for lipid synthesis to determine whether they were newly synthesized during germination or were already present in the pollen grain at anthesis (*4*). In these experiments the incorporation of [^{14}C]-acetate into individual lipids was studied in the absence and presence of inhibitors of protein and RNA synthesis. The incorporation of the precursor into a lipid was used as an indication of the presence of the enzymes necessary for the synthesis of that particular lipid (*4*). The total radioactivity incorporated into both neutral lipids and polar lipids showed no differences whether or not pollen was grown with or without cycloheximide and actinomycin D. These results indicate that all the proteins, i.e., enzymes required for the synthesis of both neutral and polar lipids are already present in the mature ungerminated pollen grain and are functionally stable for the 2.5-hr period which was studied. None of these enzymes appear to be synthesized during pollen tube growth (*4*). The conclusions about the presence of enzymes in the pollen grain made from the study of enzymes of lipid synthesis are generally found to be true for all the enzymes that have been investigated. A large number of enzymes have been reported to be present in pollen grains of various plant species (*3, 5-11*). All the enzymes studied have been found in ungerminated pollen and although increases in the activity of a few enzymes have been reported during pollen tube growth, there is no good evidence for the new synthesis of any enzyme that was not present in the mature pollen grain (*3*).

Experiments studying the effects of blocking RNA and protein synthesis by inhibitors, on pollen germination and early tube growth of several different pollens also indicate that the proteins and/or messenger RNAs (mRNAs) for the proteins necessary for germination and early tube growth are already present in the pollen grain at the time of anthesis (*3*). Direct evidence for the presence of stored mRNAs in the ungerminated pollen grain has now been obtained (*12*).

RNA synthesis occurs during pollen germination (*13*). In Tradescantia, the RNA that is synthesized is not ribosomal (*13*) or transfer RNA (*14*). These two species of RNA are present in the mature pollen grain and were synthesized prior to another dehiscence, and ribosomal and transfer RNA genes are inactive during pollen germination and tube growth (*13, 15*). The RNAs that are synthesized during pollen germination are thus mRNAs or mRNA precursors (*16*). The proteins synthesized from newly made mRNAs during Tradescantia pollen germination are similar to the proteins made on mRNAs that are already present in the mature pollen grain and that were synthesized some time earlier during pollen maturation (*16*). When ^{14}C-labeled proteins synthesized during the first hour of Tradescantia pollen germination and tube growth are analyzed by SDS-polyacrylamide gel electrophoresis, followed by autoradiography, about 20 radioactive bands, most of them comigrating with the Coomassie blue-stained bands, are seen (*16*). Moreover, no new protein bands appear in pollen grown for up to 6 hr. When mRNA synthesis is blocked with actinomycin D, the same pattern of synthesis of proteins is obtained, indicating that the presynthesized stored mRNAs and the newly made mRNAs code for the same proteins (*16*).

Since the resolution of single dimension gels is relatively poor, we have labeled pollen proteins for 1 hr to high radioactivity with ^{35}S-methionine in the presence and absence of actinomycin D and analyzed the proteins by two-dimensional gel electrophoresis after the procedure of O'Farrell (*17*). On autoradiograms of two-dimensional gels about 230 radioactive spots, i.e., polypeptides, can be resolved. The pattern of labeled spots is the same whether or not actinomycin D is present during growth (*18*). These results confirm the results obtained with single-dimensional gels; that there are no qualitative differences in the types of proteins synthesized during pollen germination on previously existing and newly synthesized mRNAs.

The results discussed above indicate that, in the pollen grain at maturity, either all the proteins that are required for germination and early tube growth are already present or if new proteins are synthesized the mRNAs for their synthesis already exist in the mature pollen grain. Moreover, similar proteins are synthesized on new mRNAs during germination as are synthesized on premade mRNAs. In other words, the genetic program during at least the latter part of pollen maturation prior to anthesis and that during pollen germination and tube growth are the same. There is no evidence yet for any protein that does not exist in the mature pollen grain but is synthesized on newly made mRNA during germination.

What are the implications of these observations for the use of pollen to assay for mutagens? It is apparent that one cannot use mature pollen for this purpose because, even if mutations were induced, there would be no rapid procedure to select for possible faulty proteins because either all the proteins required for pollen germination and tube growth are already present, or the gene transcrip-

tion for proteins required during germination has already occurred during pollen maturation. If mature pollen were treated with mutagens, it would only be possible to select for mutations after fertilization in the embryo or after seed germination. The time and effort involved in this sort of a selection procedure would make it unsuitable for routine testing.

To assay pollen directly for mutations, it would be necessary to treat the pollen with mutagens during pollen maturation in the anther before the transcription for the proteins had occurred.

There is at present very little information concerning when, during pollen development, the mRNAs for proteins present in the mature pollen grain and active during germination and tube growth are synthesized. Vithanage and Knox (*19*) have by quantitative cytochemical methods studied the synthesis of two wall-held enzymes during pollen development in the rye grass, *Lolium perenne*. One of the enzymes, an acid phosphatase, which is known to occur in the intine, was synthesized at two periods, the first in the late vacuolate stage of pollen development prior to microspore mitosis, and the second during late maturation of the pollen during the time of generative cell division. The second enzyme studied, a nonspecific esterase, occurs in the exine. The esterase appears to be primarily synthesized in the tapetum soon after meiosis and the formation of tetrads and is probably contributed to the exine after tapetal breakdown (*19*). The acid phosphatase is a wall-held enzyme, and its pattern of synthesis might not be the same for cytoplasmic proteins. Nevertheless, these results suggest that the enzymes present in pollen might be synthesized early in pollen development and the transcription of the genes for the enzymes would occur even earlier.

Accordingly, to check a compound for mutagenic activity, it would have to be applied to the anther early during pollen development possibly even prior to meiosis, i.e., one to two weeks before anthesis, to be able to detect mutations that might result and be expressed in the mature pollen grains.

Intracellular Motility

Pollen has many unique properties that make it a good system to monitor the effects of chemicals other than mutagens, on several important cellular processes. Intracellular motility is one such process. All plant cells exhibit cytoplasmic streaming. Streaming is important for the proper functioning of plant cells and probably plays an important role in intracellular transport. Cytoplasmic streaming can be affected by chemicals. In growing pollen tubes cytoplasmic streaming is extremely active and can be easily visualized under the microscope.

When Tradescantia pollen grains are germinated in the presence of cytochalasin B, both streaming and tube growth are reversibly affected (*20*). Cytochalasin B is a fungal product which interferes with microfilament function by blocking actin polymerization into the growing end of the microfilament (*21*, *22*). The first evidence that flowering plants contained actin was obtained with pollen of Amaryllis (*23*). With low concentrations of cytochalasin B, streaming and growth rates are slowed down but not entirely stopped (*20*). Increasing the cytochalasin B in the growth medium within the range of 0.10 to 0.16 μg/ml, resulted in a continuously decreasing rate of streaming, which was directly related to the reduction in the growth rate of the pollen tube (*20*). The contractile microfilament system thus seems to be responsible for cytoplasmic streaming and effects pollen tube growth by possibly playing a role in the transport of cell wall precursor materials from the sites of their synthesis to the pollen tube tip where growth occurs (*20*).

How tightly are the processes of streaming and pollen tube growth coupled? It is possible to separate the effects on growth and cytoplasmic streaming. The calcium ionophore A23187 at low concentrations can inhibit pollen tube growth completely while only partly affecting cytoplasmic streaming (*24*).

Pollen is probably the best assay system available for monitoring the effects of chemicals on the intracellular motility processes of plants. Using Tradescantia pollen, such assays could be completed within a few minutes. Some developmental work would be needed, however, to work out the easiest method for making quantitative streaming observations.

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REFERENCES

1. Mascarenhas, J. P., and Bell, E. Protein synthesis during germination of pollen: studies on polyribosome formation. Biochim. Biophys. Acta 179: 199 (1969).
2. Linskens, H. F., Schrauwen, U. A. M., and Konings, R. N. H. Cell-free protein synthesis with polysomes from germinating petunia pollen grains. Planta 90: 153 (1970).
3. Mascarenhas, J. P. The biochemistry of angiosperm pollen development. Bot. Rev. 41: 259 (1975).
4. Whipple, A. P., and Mascarenhas, J. P. Lipid synthesis in germinating Tradescantia pollen. Phytochem. 17: 1273 (1978).
5. Makinen, Y., and Macdonald, T., Isoenzyme polymorphism in flowering plants. III. Pollen enzymes and isoenzymes. Physiol. Plant. 21: 477 (1968).
6. Brewbaker, J. L. Pollen enzymes and isoenzymes. In: Pollen: Development and Physiology J. Heslop-Harrison. Ed., Butterworths, London, 1971, pp. 156-170.

7. Dickinson, D. B., and Davies, M. D. Metabolism of germinating lily pollen: pollen enzymes. In: Pollen: Development and Physiology, J. Heslop-Harrison, Ed., Butterworths, London, 1971, pp. 190-193.
8. Dickinson, D. B., Hopper, J. E., and Davies, M. D. A study of pollen enzymes involved in sugar nucleotide formation. In: Biogenesis of Plant Cell Wall Polysaccharides, F. Loewus, Ed., Academic Press, New York, 1973, pp. 29-48.
9. Shaykh, M., Kolattukudy, P. E., and Davis, R. Production of a novel extracellular cutinase by the pollen and the chemical composition and ultrastructure of the stigma cuticle of nasturtium (*Tropaeolum majus*). Plant Physiol. 60: 907 (1977).
10. Kroh, M., and Loewus, M. W. Pectolytic enzyme activity from *Nicotiana tabacum* pollen. Phytochem. 17: 797 (1978).
11. Bryce, W. H., and Nelson, O. E. Starch-synthesizing enzymes in the endosperm and pollen of maize. Plant Physiol. 63: 312 (1979).
12. Frankis, R. C., and Mascarenhas, J. P. Messenger RNA in the ungerminated pollen grain: A direct demonstration of its presence. Ann. Bot. 45: 595 (1980).
13. Mascarenhas, J. P., and Bell, E. RNA synthesis during development of the male gametophyte of Tradescantia. Develop. Biol. 21: 475 (1970).
14. Mascarenhas, J. P., and Goralnick, R. D. Synthesis of small molecular weight RNA in the pollen tube of *Tradescantia paludosa*. Biochim. Biophys. Acta 240: 56 (1971).
15. Peddada, L., and Mascarenhas, J. P. The synthesis of 5S ribosomal RNA during pollen development. Develop. Growth Diff. 17: 1 (1975).
16. Mascarenhas, J. P., Terenna, B., Mascarenhas, A. F., and Rueckert, L. Protein synthesis during germination and pollen tube growth in Tradescantia. In: Fertilization in Higher Plants. H. F. Linskens, Ed., North-Holland Publishing Co., Amsterdam, 1974, pp. 137-143.
17. O'Farrell, P. H. High resolution, two-dimensional electrophoresis of proteins. J. Biol. Chem. 250: 4007 (1975).
18. Mascarenhas, J. P., and Mermelstein, J. Messenger RNAs: their utilization and degradation during pollen germination and tube growth. Acta Soc. Bot. Polon., in press.
19. Vithanage, H. I. M. V., and Knox, R. B. Periodicity of pollen development and quantitative cytochemistry of exine and intine enzymes in the grasses *Lolium perenne* L. and *Phalaris tuberosa* L. Ann. Bot. 45: 131 (1980).
20. Mascarenhas, J. P., and Lafountain, J. Protoplasmic streaming, cytochalasin B, and growth of the pollen tube. Tissue Cell 4: 11 (1972).
21. Lin, D. C., and Lin, S. Actin polymerization induced by a motility-related high-affinity cytochalasin binding complex from human erythrocyte membrane. Proc. Natl. Acad. Sci. (U.S.) 76: 2345 (1979).
22. Grumet, M., Flanagan, M. D., Lin, D. C., and Lin, S. Inhibition of nuclei-induced actin polymerization by cytochalasins. J. Cell Biol. 83: 316a (1979).
23. Condeelis, J. S. The identification of F actin in the pollen tube and protoplast of *Amaryllis belladona*. Exptl. Cell. Res. 88: 435 (1974).
24. Herth, W. Ionophore A23187 stops tip growth, but not cytoplasmic streaming, in pollen tubes of *Lilium longiflorum*. Protoplasma 96: 275 (1978).