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LIPID SYNTHESIS IN GERMINATING *TRADESCANTIA* POLLEN

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Key Words Index—*Tradescantia paludosa*; Commelinaceae; germinating pollen; lipid synthesis; enzymes; protein and RNA inhibitors.

Abstract—Synthesis of neutral and polar lipids in pollen of *Tradescantia paludosa* during germination and tube growth was studied by the incorporation of acetate-[1-^{14}C] into lipids in the presence and absence of inhibitors of RNA and protein synthesis. The proteins required for the synthesis of both neutral lipids and phospholipids are not made *de novo* during germination but are already present in the mature ungerminated pollen grain and they are functionally stable during the first 2 hr of pollen growth.

INTRODUCTION

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]. When protein synthesis is blocked, *Tradescantia* pollen tube growth is stopped within 20 min and at a length of about 70 μm. When RNA synthesis is inhibited the tubes grow for *ca* 30 min to a maximum length of 200–300 μm. Control tubes at 30 min are longer than 300 μm and continue growing for a few hours at a linear rate of growth to a length of several mm [3, 4]. Cell membrane 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]. These observations indicate that initial membrane synthesis occurs on presynthesized enzymes. However, for later growth, i.e. beyond 70 μm, are the presynthesized enzymes still stable and functional, or are new enzymes synthesized for continued pollen tube growth and are these enzymes synthesized on preexisting or newly made mRNAs? The results reported in this study indicate that the enzymes for lipid synthesis are presynthesized during maturation of the pollen grain and are stable and active during almost the entire life of the pollen tube and no new synthesis of these enzymes occurs during pollen tube growth.

RESULTS AND DISCUSSION

In order to determine whether the enzymes required for the synthesis of lipids were already present in the ungerminated pollen grain or were newly synthesized on pre-existing or newly made messenger RNAs, the incorporation of acetate-[^{14}C] into individual lipids was studied in the absence and presence of inhibitors of protein and RNA synthesis. The incorporation of a precursor into a lipid was used as an indication of the presence of the enzymes necessary for the synthesis of that particular lipid. These experiments were carried out by germinating 20 mg of *Tradescantia* pollen in 2 ml of pollen medium. Lipids were labeled during a 12 min pulse of acetate-[1-^{14}C] (final concentration, 1 μCi/ml) at 3 different phases of germination and growth, in the presence and absence of 30 μg/ml actinomycin D (AmD) or 100 μg/ml cycloheximide.

The inhibition of protein synthesis by cycloheximide at 100 μg/ml, as measured by the incorporation of ^{14}C-reconstituted protein hydrolyzate into hot trichloroacetic acid insoluble material, was complete during the first 5 min of incubation of pollen, *ca* 85% during the first 15 min and between 70 and 85% after 30 min. AmD at 30 μg/ml essentially blocks RNA synthesis [5]. The 3 phases of germination and tube growth were—(1) The period of germination and very early tube growth which is not sensitive to AmD or cycloheximide at the morphological level, from 0 to 12 min of placing the pollen in growth medium; (2) The time period in which protein synthesis inhibitors have stopped tube growth and AmD just begins to slow tube growth, from 30 to 42 min; (3) A period in which both RNA and protein synthesis inhibitors have stopped growth but control pollen tubes continue to grow, from 120 to 132 min [4]. The synthesis of neutral lipids was studied during the last 2 periods and polar lipid synthesis during all 3 periods. There were no significant differences in the total counts incorporated into either polar or non-polar lipids (Tables 1 and 2). There are several minor differences in the labeling patterns of neutral and polar lipids which may or may not be of significance, but all lipids which are labeled in control pollen tubes also become labeled in pollen which is germinated in the presence of both protein and RNA synthesis inhibitors. These results show conclusively that the synthesis of individual neutral lipids and polar lipids is insensitive to both cycloheximide and AmD. This would indicate that the proteins required for the synthesis of both neutral lipids and phospholipids are already present in the mature ungerminated pollen and are functionally stable for the 2 hr period which was studied. This would also mean that none of these enzymes are synthesized *de novo* during pollen tube growth on

Table 1 Incorporation of acetate-[1-^{14}C] into neutral lipids during two different 12 min labeling periods of germination and tube growth. Chromatography with solvent A Am-D, 30 μg/ml actinomycin-D, control, no cycloheximide, CHX, 100 μg/ml cycloheximide

Spot No	R_f × 100	Tentative identification	% Total counts incorporated (−) Am-D Control	(−) Am-D CHX	(+) Am-D Control	(+) Am-D CHX
(a) Labeling period 30–42 min						
1	0	polar lipids	61	63	54	51
2	7	diglycerides	7	10	7	9
4 + 5	17, 24		2	1	2	2
6 + 7	33, 37		1	0·4	0·4	0.5
8 + 9	44, 50		11	9	10	9
18 + 19	98, 99	pigment	19	17	25	29
Total cpm			56000	53000	50500	56000
(b) Labeling period 120–132 min						
1			41	42	40	43
2			1	0·4	1	1
4 + 5			4	2	5	1
6 + 7			0·6	0·4	0·6	0·5
8 + 9			11	17	9	17
18 + 19			43	37	45	37
Total cpm			40800	44500	35000	55000

pre-existing mRNA. Although a fairly large number of proteins are synthesized during *Tradescantia* pollen tube growth [6], we have at present no information as to the identity of any of these proteins [3].

It is of interest that total incorporation of acetate-[^{14}C] into lipid material was not appreciably diminished compared to the control at 2 hr in those cultures containing RNA or protein synthesis inhibitors even though all tube growth had stopped in these cultures. In the presence of chloramphenicol or AmD Dexheimer [7, 8] observed an increase in the amount of membranous structures within the germinating pollen of *Lobelia erinus* even though tube growth was blocked. The production of membrane 'whorls' has also been seen in rat liver treated with cycloheximide or emetine [9]. These studies suggest that membrane synthesis may well continue in the presence of inhibitors of RNA and protein synthesis, even in pollen tubes not growing in length, resulting in a build-up of excess membrane.

Table 2. Incorporation of acetate-[1-^{14}C] into polar lipids during three different 12 min labeling periods of germination and tube growth Chromatography with solvent B. (PC = phosphatidyl choline, PI = phosphatidyl inositol, PS = phosphatidyl serine, PE = phosphatidyl ethanolamine)

Spot No	R_f × 100	Tentative identification	% Total Counts Incorporated (−) Am-D Control	(−) Am-D CHX	(+) Am-D Control	(+) Am-D CHX
(a) Labeling period 1–12 min						
1	20	PC	25	35	28	42
2 + 3	33, 40	PI & PS	1	1	2	2
5	58	PE	11	14	13	14
6	64		4	3	3	1
10 + 11 + 12	91, 98, 100	neutral lipids	59	48	54	42
Total cpm			35000	33700	35100	44200
(b) Labeling period 30–42 min						
1			30	33	24	33
2 + 3			2	3	2	3
5			12	14	11	13
6			4	4	4	4
10 + 11 + 12			53	47	58	48
Total cpm			52400	54100	51700	72600
(c) Labeling period 120–132 min						
1			36	31	38	39
2 + 3			11	14	11	12
5 + 6			10	21	10	17
10 + 11 + 12			43	35	42	31
Total cpm			32100	39900	26000	30000

EXPERIMENTAL

Pollen of *Tradescantia paludosa* (L.) was grown as described in ref. [10]. Lipids were extracted by a modification of Folch's technique [11]. Pollen was extracted with 19 vols of $CHCl_3$–MeOH (2:1). The extracted pollen was filtered and washed × 3 with $CHCl_3$–MeOH–H_2O (3:48:47). All operations were carried out on ice or at 4°.

The extracts were dried under N_2, redissolved in a minimal vol of $CHCl_3$–MeOH (2:1), divided into two and each spotted under a stream of N_2 onto a 0.25 mm thick Si gel plate, one of which was chromatographed with Solvent A [petrol–Et_2O–HOAc (90:10:1)] and the other with Solvent B [$CHCl_3$–MeOH–HOAc–H_2O (60:35:4·4)]. The neutral lipids ran at the solvent front in Solvent B and thus their presence did not obscure the results.

TLC chromatograms were scanned for radioactivity and those spots that were radioactive were scraped off and counted in a liquid scintillation spectrometer.

A tentative identification of the different lipid spots was made by comparison with R_f values of standard phospholipids and by comparison with a lipid extract of rat liver as suggested in ref. [12]. General and specific spray reagents were used for the detection and tentative identification of the lipid spots [12, 13].

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