The metabolic incorporation of label from myoinositol-2-³H by the growing young anther of *Lilium longiflorum*

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Abstract

Fine structural autoradiography involving the incorporation of myo-inositol-2-³H (MI-2-³H) into lily anther during development has been used to explore the metabolic processes of microspore development and the interaction between the developing microspore and degenerating tapetal cells.

INTRODUCTION

The ultrastructural ontogeny of the microspore in vascular plants has been studied by many investigators. In a previous paper (Miki-Hiroshige, Nakamura, 1979) we traced the incorporation of myoinositol-2-³H (MI-2-³H), a precursor of polysaccharidic units, into young lily anther with the use of the fine structural autoradiography to assess polysaccharide metabolism during anther development. Label was incorporated into the intine layer of the young microspore at the 28-mm bud-length stage. It was assumed that the labeled substance in the intine was pectin. Takats (1962) and Taylor (1959) also reported that the accumulation of radioactivity was found on the microspore wall.

In this paper, fine structural autoradiography involving the incorporation of MI-2-³H into lily anther during development has been used to explore the metabolic processes of microspore development and the interaction between the developing microspore and degenerating tapetal cells.

MATERIALS AND METHODS

Flower buds of greenhouse-grown *Lilium longiflorum* were used. To label the anthers, the cut surface of the stalk of a detached bud was immersed in 50ml of MI-2-³H (1.7 µg/ml) containing 50 µCi, 24 h. The feeding vial was enclosed by a larger test tube that was kept at 25°C. After labeling, the radioactive medium was replaced by Nitsch's culture medium (1951). Each day for the 6 days subsequent to labeling,

one anther of the 6 available from each bud, was removed and fixed. The stages of bud development used in this study were: 1) anaphase of microspore meiosis, 20-mm bud; 2) post meiosis (tetrad microspore), 25-mm bud; 3) initiation of inner cell wall formation of the microspore, 29-mm bud; 4) the stage just preceding mitosis of the microspore, 50-mm bud.

Fine structural and autoradiographic methods were described in the previous paper (Miki-Hirosige, Nakamura, 1979). The coating emulsion was developed with Konidol X. To identify labeled material by the MI-2- ^3H in ultrathin sections, selected sections were treated with 0.1% pectinase, pH 4.0 or 0.1% cellulase, pH 4.8 (Sigma Chem. Co.) in 0.02 M acetic acid at 25°C for 24 h prior to coating with emulsion. Polysaccharides were detected with silver methenamin. With the latter test 200 meshed nickel grids were used.

RESULTS

1. Regions of lily anther exhibiting label after feeding MI-2- ^3H to buds at the designated stages of microspore development are summarized in Table 1.

Table 1
Tritiated regions in MI-2- ^3H -labeled lily anthers

Stage	Bud length mm	Regions bearing tritium		
		microspore	tapetum	endothecium
1. Anaphase (Fig. 1)	20	cell plate coated vesicles nucleus Golgi body oil droplet	wall residue pro-orbicle	cell wall nucleus amyloplast
2. Young microspore (Fig. 2)	25	exine nucleus	orbicular wall nucleus	cell wall nucleus amyloplast
3. Onset of intine formation (Fig. 3)	29	intine nucleus Golgi body Golgi vesicle oil droplet	orbicle core nucleus	cell wall nucleus amyloplast
4. Mitosis (Fig. 4)	50	intine generative cell wall generative and vegetative (slight) starch oil droplet	orbicular wall oil droplet	cell wall (slight) nucleus

During stage 1, a portion of the polysaccharidic component of the cell plate and coated vesicles was labeled by MI-2- ^3H . These regions also contained electron dense stain when treated with silver methenamin (Fig. 1d). Treatment of sections with cellulase and pectinase failed to remove the ^3H -labeled material and it is assumed that the labeled substance is callose. Labeled substance was found on nuclei, namely on chromatin (Fig. 1b). Presumably, the label from MI-2- ^3H was incorporated into the metabolically active chromatin region during meiosis.

During stage 2, exine layer formation is initiated by the fresh microspore which has just separated from the tetrad microspore. Label appears on the exine layer and the orbicular wall (Fig. 2a). Treatment with cellulase and pectinase fail to remove this labeled material.

Fig. 5. The total amount of released ^3H -water from the labelling lily buds with MI-2- ^3H

After exine formation, intine is built up in the young microspore (stage 3). Treatment with pectinase removes the labeled substances derived from MI-2- ^3H (Fig. 3c, d). Treatment with cellulase is ineffective in this regard (Fig. 3e, f). This result suggests that the intine layer contains pectic substance. Following mitosis of the microspore, new cell wall material which had first accumulated inside of the intine of the pollen wall now is formed around the generative cell (Fig. 4a). The label was found in this new cell wall in intine 3 (Nakamura, 1979, Fig. 4b), and slight in new generative and vegetative nuclei. In each stage, it was clear that MI-2- ^3H derived label incorporated into new structures and the most biosynthetically active regions of pre-existing structures.

2. Exchange of tritium from MI-2- ^3H into water was investigated by culturing two 20-mm buds (0.492 g) in medium containing 45 μCi of MI-2- ^3H . Release of ^3H -water is shown in Fig. 5. Presumably, this

PLATE I

Fig. 1. Stage 1. MI-2- ^3H -labeled microspore mother cell

Developed silver grains which overlay regions bearing ^3H -labeled components are found in a) the cell plate, CP; b) the nucleus, N; and tapetal cell wall, TCW; c) coated vesicles, CV; which are making cell plate. d) This section was treated with silver methenamin to reveal polysaccharide-rich regions such as the cell plate which is formed from coated vesicles. callose wall, CaW.

Fig. 2. Stage 2: MI-2- ^3H -labeled microspore and orbicules, Or.

a) Silver grains appear over exine, E; and orbicular wall. b) Similar material treated with silver methenamin. Polysaccharide is condensed on the surface of the orbicule and exine of the microspore.

^3H -water was produced in the course of conversion of MI-2- ^3H to polysaccharides.

3. To explore possible patterns in the rate of incorporation of MI-2- ^3H into labeled structures, relative amounts of ^3H in labeled regions at each stage were compared over six days using one anther per flower per day. After one day, very little ^3H appeared in the fixed tissue but this increased to reach a maximum at four days and then decreased to a low value on the sixth day.

In this experiment a search was made for evidence of transfer of labeled tapetal components to developing microspore. None was found.

DISCUSSION

Incorporation of label from MI-2- ^3H into structural components of the developing anther falls into four groups: 1) polysaccharidic regions — cell plate (stage 1 and 4), intine (stage 3 and 4), endothelial cell wall and amyloplast (starch grain); 2) oil droplet and orbicule core; 3) sporopollenin-rich regions — exine and orbicular wall; 4) chromatin — nucleus (stage 1). Results suggest that MI-2- ^3H was converted into polysaccharides in group 1, into oil via fatty acid in group 2 and into new DNA via ^3H -hexose in group 4. Marimuthu (1970) found that growing plants of *Tradescantia paludosa* given ^3H -thymidine produced labeled nuclei in the pollen mother cell at pre-meiotic interphase and in young binucleate pollen grains. Takats (1962) reported that ^3H -thymidine was incorporated into "the inner part of the microspore wall" of the microspore DNA synthesis stage. Because it is supposed that the inner part may be the intine 3 was made of the breakdown products from tapetal DNA, this result is similar to that of stage 4. Labeled components of Group 3 were not solubilized by treatment with pectinase and cellulase and

PLATE II

Fig. 3. Stage 3: MI-2-³H-labeled microspore and endothecium

Silver grains appear over regions in microspore corresponding to: a) orbicular core, Oc; and oil droplet, OD; b) intine, I; and Golgi body, G; sections were treated with pectinase prior to addition of emulsion. Silver grains are no longer seen over intine of the microspore (Fig. 3c) or the cell wall of the endothecium (Fig. 3d). Sections were treated with cellulase prior to addition of emulsion. Silver grains appear over of the microspore (Fig. 3e) and cell wall of the endothecium (Fig. 3f).

Fig. 4. Stage 4: MI-2-³H-labeled pollen grain after mitosis

Silver grains appear: a) over the generative cell wall, GCW; generative nucleus, GN; b) the inner layer of intine (intine 3). I1 — intine 1; I2 — intine 2, I3 — intine 3.

were not stained by silver methenamin (Fig. 2b). The precursor of MI-2-³H labeled sporopollenin was not detected. Christensen et al., (1972), Dickinson and Bell (1972), Heslop-Harrison and Dickinson (1969) and Skvarla and Larson (1966) indicate that sporopollenin is synthesized in the tapetum and then condensed upon the orbicular surface and microspore wall. The present study suggests a scheme in which a MI-2-³H-derived ³H-precursor of sporopollenin, synthesized in the tapetum, is deposited on the exine of the microspore and orbicular wall and converted to sporopollenin at the latter sites. Rowley (1963) reported that sporopollenin is not homogeneous during early development of exine but becomes homogeneous in mature pollen. Tsinger and Petrovskaya-Baranova (1961) suggested that the pollen wall is a physiologically active structure playing a very important role in the process of interchange between the pollen grain and its substrate.

About 2.5% of the radio-activity used by the anther was released as ³H-water. Rosenfield and Loewus (1978) found that the germination of lily pollen in MI-2-³H labeled medium released 21% of the label as ³H-water. In our experiment MI-2-³H was incorporated into the most metabolically active regions at each stage of development. It is assumed that ³H-water was released during this metabolism and that the decreased incorporation is reflected by loss of ³H as ³H-water. Our next study will attempt to resolve the process of conversion.

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