ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLVI, nr 2
1977

Radioautographic visualization of incorporation of lipid precursors into anthers of *Muscari comosum* (L.) Mill.

BARBARA GABARA

Laboratory of Plant Cytology and Cytochemistry, Institute of Physiology and Cytology, University of Łódź

(Received: December 7, 1976)

Abstract

Incorporation of the following lipid precursors: DL-mevalonic acid-2 ^{3}H, ^{3}H palmitic acid and acetic acid-^{3}H sodium salt, into the anther cells of *Muscari comosum* (L.) Mill. has been investigated. These lipid precursors have been demonstrated to incorporate into pollen mother cells, pollen grains and the tapetum at the consecutive developmental stages. All used isotopes are incorporated into the cytoplasm of these cells and in the case of pollen grain the radioactivity of pollen grain wall (mainly composed of sporopellenin) is noticed.

The highest radioactivity of pollen grain wall is observed after acetic acid, the lower one after palmitic acid, whereas the lowest uptake of lipid precursor occurs after mevalonic acid. In comparison with tetrad stage the distinct inrease of the cytoplasmic radioactivity of tapetum, which appears to accompany the labelling of pollen grain wall, seems to indicate the participation of tapetum in the formation of exine. A possible role of Ubisch bodies in the formation of pollen grain wall is discussed.

INTRODUCTION

In plants characterized by the secretory type of tapetum the development of pollen grain wall-exine is closely associated with the appearance of Ubisch bodies at the surface of tapetal cells. The walls of the Ubisch bodies coating consist of sporopollenin, the main constituent of the mature pollen grain exine. Ubisch bodies have been considered by some workers (Heslop-Harrison, 1963; Banerjee and Barghoorn, 1973) to be a transport form of sporopollenin produced in the tapetum and utilized in the development of the pollen grain exine.

In spite of many reports concerning the formation of exine (Echlin and Godwin, 1968; Heslop-Harrison, 1968a; Risueño et all., 1969; Dickinson and Bell, 1972; Dunbar, 1973; Dickinson,

1976) the role of Ubisch bodies in the exine development remains still obscure. Moreover, the place of sporopellenin synthesis and polimerization is controversial. At first, the mitochondria were supposed to be the place of sporopollenin synthesis (Heslop-Harrison, 1962). On the contrary, Horner (1968) ascribed that to Golgi vesicles and endoplasmic reticulum and Risueño et all (1969) consider the vesicles associated with endoplasmic reticulum and the surface of lipid droplets to be the place of sporopollenin condensation. Similarly, the observations of *Berberis vulgaris* pollen mother cells indicate that endoplasmic reticulum may be a place of synthesis and polimerization of that exine component (Gabara, 1974, 1976).

On the other hand, some authors consider the electron lucent lamella formed at the surface of plasmalemma to be responsible for the sporopollenin formation (Rowley and Southworth 1967; Dickinson and Heslop-Harrison, 1968).

In the present study an attempt is made to examine the place of synthesis of lipids composing the exine and to relate the findings to the developmental processes which lead to the formation of the pollen grain wall.

MATERIAL AND METHODS

Pollen mother cells in the first and the second meiotic division up to the formation of pollen grains, and also tapetal cells at the appriopriate developmental stages were used to study.

The bud flowers of *Muscari comosum* were incubated in the following isotopes: ^{3}H palmitic acid 9, 10 at the concentration of 50 μC/ml (spec. activity 500 mCi/mM) during 1, 3, 6 and 24 h; DL-mevalonic acid — 2 ^{3}H at the concentration of 50 μC/ml (spec. activity 382 mCi/mM) during 1, 3, 6, 24 h; acetic acid — ^{3}H sodium salt at the concentration 50 μC/ml (spec. activity 500 mCi/mM) during 1, 6 and 24 h. To facilitate the penetration of isotopes, the bud flowers were excised from the plant under the water before they were incubated in the radioactive precursors.

After incubation in an appropriate isotope, whole bud flowers were fixed in 3.6% glutaraldehyde buffered with sodium cacodylate to pH 7.2, during 12 h, at temp. 0-4°.

Fixed individual anthers were dehydrated with alcohol and propylene oxide and embedded in Epon 812. One micron thick sections were covered with Ilford liquid emulsion L4 and exposed 230 days in the dark room at temp. 0-4°. Developed and fixed specimens were stained with Mayer's haematein, Unna solution or with toluidine blue adopted to staining of the thick sections by Fowke and Pickett-Heaps (1969).

The radioactivity of cells was calculated on the basis of number of silver grains per 100 μm^2 of cell wall or cytoplasm including nucleus. The

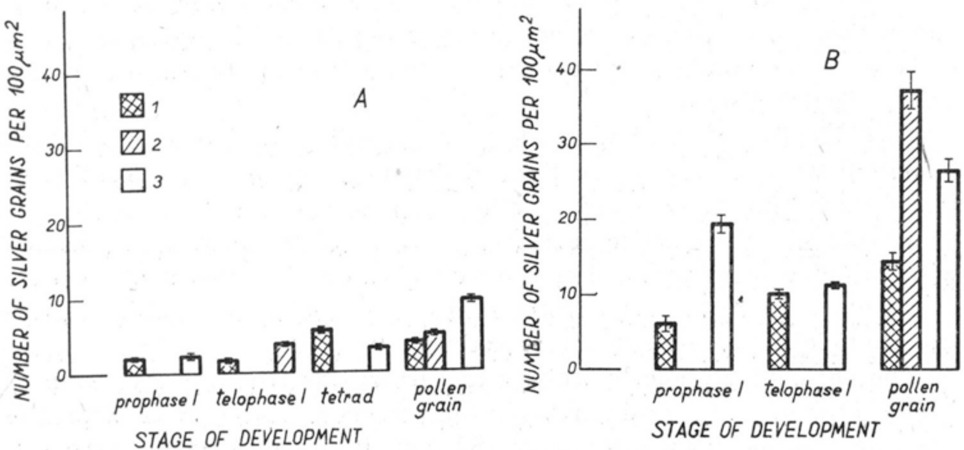
area of cytoplasm and cell wall was measured planimetrically in the micrographs enlarged 4310 ×. Mean value of intensity of the isotope incorporation into each (studied) developmental stage of pollen mother cells and tapetum is based on the silver grains counting and the area measurements of 50 cells. Frequency of mean value was calculated on the basis of the following formula:

$$S.E. = \pm \sqrt{\frac{\sum(\bar{x} - x^2)}{n(n-1)}}$$

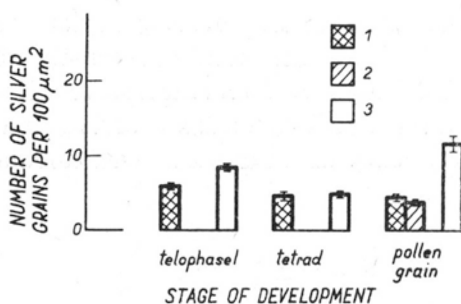
RESULTS AND DISCUSSION

Pollen mother cells, pollen grains and the tapetum of *Muscari comosum* in the consecutive stages of their development incorporate the radioactive lipid precursors only after 6 and 24 h of incubation (Text-Fig. 1-3). After the shorter incubation (1, 3 h) no uptake of these radioisotopes into tapetum and pollen mother cells or pollen grains was revealed after 230 days of exposure. Incorporation of the radioactive lipid precursors into pollen mother cells, as well as the radioactivity of special callose wall (Southworth, 1973b; Gabara, 1976) contradict the conception of Heslop-Harrison's (1966) and Heslop-Harrison and Mackenzie's (1967) concerning the existence of callose barrier preventing the uptake of various substances into pollen mother cells enclosed by callose wall.

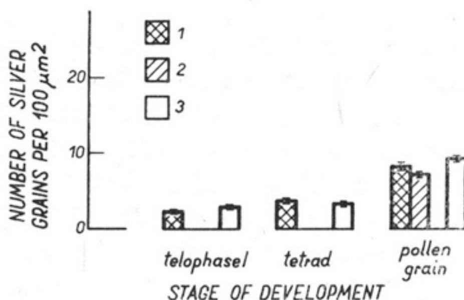
All used isotopes (acetic acid, mevalonic acid and palmitic acid) are incorporated into pollen mother cells, pollen grains and tapetal cells, that



Text-Fig. 1. Incorporation of acetic acid-^{3}H sodium salt into cytoplasm (1) and cell wall (2) of developing pollen and tapetum (3) of *Muscari comosum*. A-6 h incubation, B — 24 h incubation



Text-Fig. 2. Incorporation of DL-mevalonic acid — 2 ^{3}H into cytoplasm (1) and cell wall (2) of developing pollen and tapetum (3) of *Muscari comosum;* 24 h incubation



Text-Fig. 3. Incorporation of ^{3}H palmitic acid into cytoplasm (1) and cell wall (2) of developing pollen and tapetum (3) of *Muscari comosum;* 24 h incubation

is into the cytoplasm and the pollen grain wall-exine as well (Text-Fig. 1—3, Plate I, Fig. 1—3). As can be seen from Text-Fig. 1—3 the highest radioactivity of cell wall occurs in the pollen grain after feeding with acetic acid, the lowest one after mevalonic acid. The shortening of the incubation time in the radioactive acetic acid from 24 to 6 h causes sevenfold decrease of the number of silver grains placed over the cell wall of pollen grains (Text-Fig. 1 A).

Analogically, Southworth (1973a) feeding the cells of *Gerbera jamesonii* with radioactive acetic acid demonstrated the incorporation of that isotope into the developing pollen grain wall at all stages of the cell wall synthesis. Since acetic acid incorporates into the material associated with the exine and sporopollenin, Southworth (1973 a, b) suggests that acetic acid may be used in the synthesis of fatty acids and incorporates into lipids (?) of sporopollenin fraction.

The chromatographic hydrolysis of carotenoids from sporopollenin of *Lilium henryii* pollen grains reveals the presence of fatty acids with palmitic acid as the main component (Shaw, 1971). Moreover, Green et all. (Shaw, 1971) incorporating ^{14}C-acetic acid and ^{14}C-palmitic acid to *Cucurbita pepo* cells and then extracting the carotenoids have demonstrated intensive incorporation of anther carotenoids into sporopollenin. On the

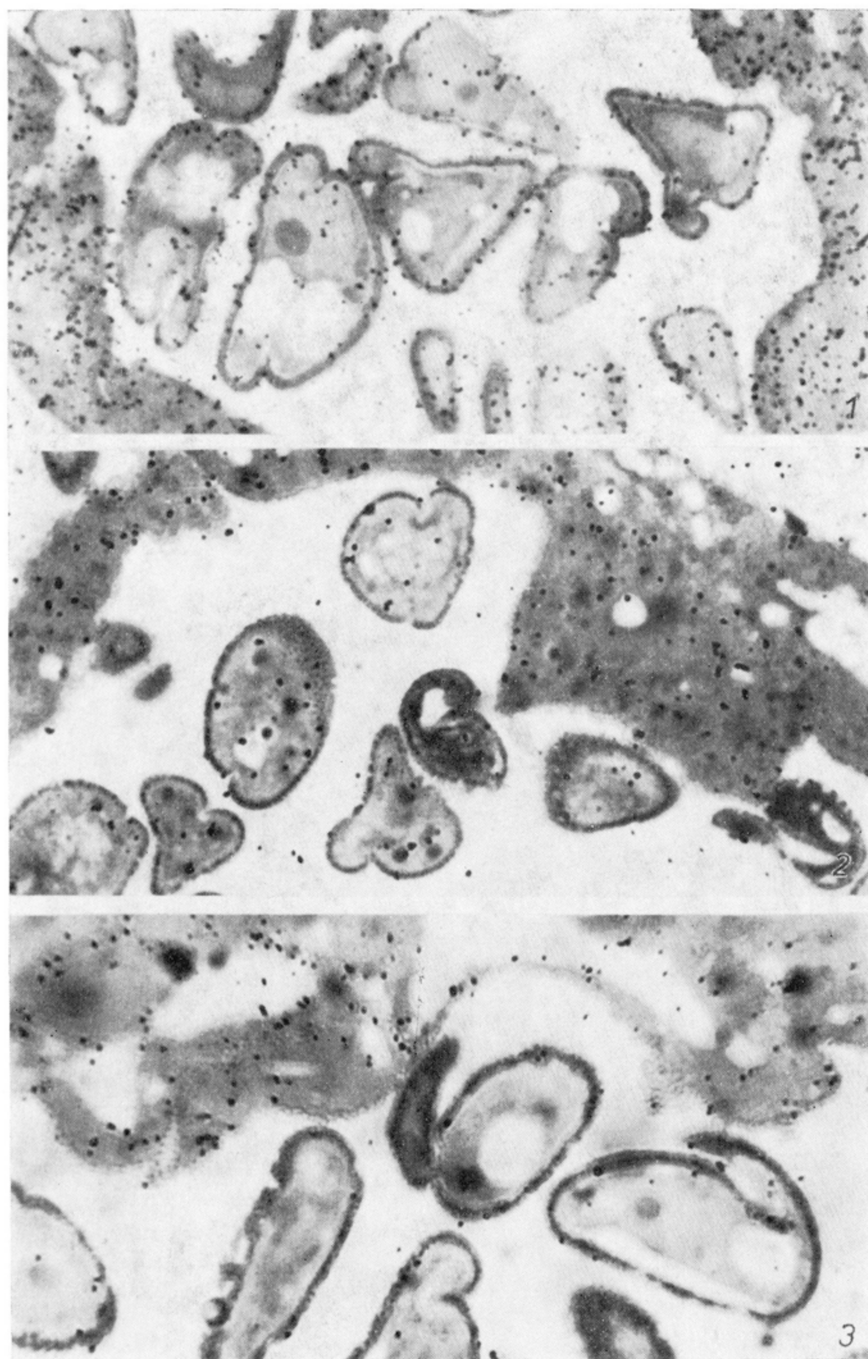


Fig. 1—3. Incorporation of lipid precursors into pollen grains and tapetum of *Muscari comosum* after 24 h incubation: 1) acetic acid-^{3}H sodium salt, 2) ^{3}H palmitic acid, 3) DL-mevalonic acid-2 ^{3}H. × 1500

basis of these data and Brooks and Shaw's results (1968) describing the sporopollenin as an oxydative polimer of carotenoids and their esters one may assume that labelling of the cell wall of *Muscari comosum* pollen grain with ^{3}H-palmitic acid (Text-Fig. 3) is due to that isotope uptake into lipid substances of carotenoids composing the sporopollenin of pollen grains.

On contrast to the cells of *Gerbera* (Southworth, 1973 b) mevalonic acid incorporates into pollen grains wall of *Muscari* during the exine development (Text-Fig. 2, Plate I, Fig. 3) Since Krzeminski and Quackenbush (1960) demonstrated the incorporation of mevalonic acid into carotene, and Treharne et all.. (1966) stated that although mevalonic acid uptakes into terpenoids yet a small amount of it incorporates into β-carotene, so we can consequently assume that mevalonic acid may be used in the carotenoids synthesis like acetic acid in the synthesis of fatty acids (Southworth, 1973 b).

Analogically to the labelling of pollen grain wall the lowest incorporation into pollen grain cytoplasm was visible after incubation with ^{3}H mevalonic acid and the highest one in the case of radioactive acetic acid. The shortening of the incubation to 6 h causes threefold decrease of acetic acid uptake to the pollen grain cytoplasm. It seems probable that such high cytoplasmic labelling (including nucleus) after the incubation with acetic acid is caused by the incorporation of that isotope to the nuclei (probably due to the acetylation of histones — Bouvier and Chevillier, 1976) and to the other cytoplasmic organelles not distinguishable at the level of light microscope. 24 h incubation with radioactive acetic acid seems to be sufficient to incorporate that isotope not only to the lipids but also to the other substances in their metabolic pathways.

In contrast to acetic acid neither mevalonic acid nor palmitic acid were incorporated to the cell nuclei.

Incorporation of palmitic acid, mevalonic acid and also acetic acid into tapetal cells is the highest in the pollen grain stage and, analogically as in the case of pollen grain wall radioactivity, the highest number of traces per 100 μm^2 of cell area is visible in the tapetal cells after 24 h incubation with acetic acid. On the other hand, the shortening of incubation to 6 h causes over twofold decrease of the uptake of that isotope to the tapetum. If the increase of palmitic acid incorporation into the cytoplasm of pollen grains in comparison with telophase I or tetrad could be explained by the increase of the lipid synthesis of sporopollenin composing the cell wall of pollen grain, a low cytoplasmic radioactivity caused by DL--mevalonic acid-2 ^{3}H and keeping on the same level during the pollen grain wall development does not seem to prove the participation of pollen grain protoplast in exine formation.

Contrariwise, twofold increase of the tapetal radioactivity after mevalonic acid allows to assume the participation of tapetum and particularly

of Ubisch bodies in the formation of exine. This suggestion seems to be most probable because mevalonic acid is considered the carotenoid precursor and carotenoids build the sporopollenin- the exine component of pollen grains (Brooks and Shaw, 1968; Shaw, 1971).

No incorporation of three used lipid precursors into the cell wall of tetrad cells was observed and the radioactivity of their cytoplasm was low and almost on the same level as the labelling of tapetum accompanying the tetrad cells. Only after 6 h incubation with acetic acid the radioactivity of tetrad cell cytoplasm was higher than that of the tapetum at this stage. This distinctly high radioactivity of the tetrad cell cytoplasm can indicate the synthesis of lipid material composing probacula but we cannot exclude that the radioactivity is due to the acetic acid incorporation into other metabolic pathways.

The above observations suggesting the participation of Ubisch bodies in the development of exine do not allow to state whether these bodies incorporate completely into the cell wall of pollen grains, or only their outher part is incorporated to the exine and then lipopolisaccharide central core can participate in the formation of the pollenkit substance formed later (Heslop-Harrison, 1968 b; Heslop-Harrison and Dickinson, 1969; Dickinson, 1973).

There is another possibility that Ubisch bodies may play a role in the transport of sporopollenin (condensed on the surface of proUbisch bodies) from the tapetum to cell wall of pollen grain. If Ubisch bodies participate in the development of exine there remains an unresolved problem of the formation of pollen grain wall in the anthers with the amoeboidal tapetum where Ubisch bodies are not formed at all. (Godwin et all., 1967; Mepham and Lane, 1969; Echlin, 1973).

Acknowledgment

The author is indebted to Professor M. J. Olszewska for critical reading of the manuscript.

REFERENCES

Banerjee U. C., Barghoorn E. S., 1973. The tapetal membranes in grasses and Uhisch body control of mature exine pattern. [In:] Pollen, development and physiology ed. J. Heslop-Harrison London, Butterworths.

Bouvier D., Chevaillier Ph., 1976. A correlation between histone acetylation and chromatin changes in spermatids of the locust (Locusta migratoria). Cytobiologie, 12: 287 - 304.

Brooks J., Shaw G., 1968. Chemical structure of the exine of pollen walls and a new function for carotenoids in nature. Nature, 219: 523 - 524.

Dickinson H. G., Heslop-Harrison J., 1968. A common mode of deposition for the sporopollenin of sexine and nexine. Nature, 220: 926 - 927.

Dickinson H. G., Bell P. R., 1972. The role of the tapetum in the formation of sporopollenin-containing structures during microsporogenesis in *Pinus banksiana*. Planta, 107: 205 - 215.

Dickinson H. G., 1973. The role of plastids in the formation of pollen grain coatings. Cytobios, 8: 25 - 40.

Dickinson H. G., 1976. Common factors in exine deposition [In:]. The evolutionary significance of the exine, ed. I. K. Ferguson and J. Muller, Linn. Soc. Symp. Series no 1.

Dunbar A., 1973. Pollen development in the *Eleocharis palustris* group (*Cyperaceae*). I. Ultrastructure and ontogeny. Bot. Notiser. 126: 197 - 254.

Echlin P., Godwin H., 1968. The ultrastructure and ontogeny of pollen in *Helleborus foetidus* L. I. The development of the tapetum and Ubisch bodies. J. Cell Sci., 3: 161 - 174.

Fowke L. C., Pickett-Heaps J. D., 1969. Multivesicular structures and cell wall growth. Can. J. Bot., 12: 1873 - 1877.

Gabara B., 1974. A possible role for the endoplasmic reticulum in exine formation. Grana, 14: 16 - 22.

Gabara B., 1976. Udział organelli komórkowych w tworzeniu ścian podczas mikrosporogenezy. Praca habilitacyjna, UŁ.

Godwin H., Echlin P., Chapman B., 1967. The development of the pollen grain wall in *Ipomoea purpurea* (L.) Roth., Rev. Paleobot. Palynol., 3: 181 - 195.

Heslop-Harrison J., 1962. Origin of exine. Nature, 195: 1069 - 1071.

Heslop-Harrison J., 1963. Ultrastructural aspects of differentiation in sporogenous tissue. Symp. Soc. exp. Biol., 17: 315 - 340.

Heslop-Harrison J., 1966. Cytoplasmic continuities during spore formation in flowering plants. Endeavour, 25: 65 - 72.

Heslop-Harrison J., Mackenzie A., 1967. Autoradiography of soluble (2-^{14}C) thymidine derivatives during meiosis and microsporogenesis in *Lilium* anthers. J. Cell Sci., 2: 387 - 400.

Heslop-Harrison J., 1968 a. Pollen wall development, Science, 161: 230 - 237.

Heslop-Harrison J., 1968 b. Tapetal origin of pollen coat substances in *Lilium*, New Phytol., 67: 779 - 786.

Hesiop-Harrison J., Dickinson H. G., 1969. Time relationship of sporopellenin synthesis associated with tapetum and microspores in *Lilium*. Planta, 84: 199 - 214.

Horner H. T., 1968. Sporopollenin formation and secretion by tapetal cells. Amer. J. Bot. (Abstr.) 55: 709.

Krzeminski L. F., Quackenbush F. W., 1960. Incorporation of ^{14}C-labelled compounds into carotenes by *Neurospora crassa*. Arch. Biochem. Biophys 88: 287 - 293.

Mepham R. H., Lane G. R., 1969. Role of the tapetum in the development of Tradescantia pollen. Nature, 221: 282 - 284.

Risueño M. C., Giménez-Martin G., Lopéz-Sáez J. F., Garcia M. I. R. 1969. Origin and development of sporopollenin bodies. Protoplasma, 67: 361 - 374.

Rowley J. R., Southworth D., 1967. Deposition of sporopollenin on lamellae of unit membrane dimensions. Nature, 213: 703 - 704.

Shaw G., 1971. The chemistry of sporopollenin. In: Sporopollenin ed. J. Brooks et all., London.

Southworth D., 1973 a. Cytochemical reactivity of pollen walls J. Histochem. Cytochem. 21: 73 - 80.

Southworth D., 1973 b. Incorporation of radioactive precursors into developing pollen walls. In: Pollen, development and physiology, ed. J. Heslop-Harrison. London, Butterworths.

Treharne K. J., Mercer E. J., Goodwin T. W., 1966. Incorporation of (^{14}C) carbon dioxide and (2-^{14}C) mevalonic acid into terpenoids of higher plants during chloroplast development. Biochem. J., 99: 239 - 245.

Author's address:
Laboratory of Plant Cytology
and Cytochemistry, Institute
of Physiology and Cytology,
University of Łódź
Banacha Str. 12/16; 90-227 Łódź; Poland

Autoradiograficzny obraz włączania prekursorów lipidów do pylników Muscari comosum (L.) *Mill.*

Streszczenie

Badano włączanie następujących prekursorów lipidów do komórek pylników *Muscari comosum* (L.) Mill: DL-kwasu mewalonowego — 2 ^3H, kwasu palmitynowego ^3H oraz octanu sodu ^3H. Wykazano włączanie tych prekursorów lipidów do komórek macierzystych pyłku, ziaren pyłku oraz do komórek tapetum w kolejnych stadiach ich rozwoju. Wszystkie zastosowane izotopy wbudowują się do cytoplazmy badanych komórek, a w przypadku ziaren pyłku widoczne jest również wyznakowanie ściany komórkowej.

Najintensywniejsze włączanie do ściany komórkowej ziaren pyłku obserwuje się w przypadku octanu sodu ^3H, słabsze — kwasu palmitynowego ^3H, a najsłabsze kwasu mewalonowego — 2 ^3H. Znaczny wzrost radioaktywności cytoplazmy komórek tapetum, któremu towarzszy znakowanie ścian ziaren pyłku (głównie sporopolleninowego charakteru) w porównaniu do stadium tetrad zdaje się wskazywać na udział tapetum w tworzeniu egzyny. Dyskutowana jest ewentualna rola ciał Ubischa w tworzeniu egzyny.