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Intracellular RNA during microsporogenesis in plants: *Taxus baccata* as a model system

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Abstract

The fluorescent proble Acridine Orange has provided valuable information about changes in levels of insoluble RNA during meiosis in pollen mother cells of *Taxus baccata*, in which prophase is unusually prolonged. By combining the data offered by fluorescence microscopy with microdensitometry it has been possible to measure changes in the amounts of RNA within cytoplasm, nucleus and nucleolus individually. The data are generally in line with those arising from other techniques, but lend themselves to a wholly original interpretation of the controlling events in meiosis.

Key words: RNA, meiotic prophase, Acridine Orange, synaptonemal complexes

INTRODUCTION

Meiosis within anthers of flowering plants has now been studied in detail for almost twenty years and many features appear to be common to all genera. These include during prophase the transient dedifferentiation of the mitochondria and plastids (Dickinson and Heslop Harrison 1977) and the decline in countable ribosomes and levels of cytoplasmic RNA (Mackenzie et al. 1967), including mRNA (Porter et al. 1983). In *Lilium* the restoration of RNA to the cytoplasm appears to be via nucleoloids which are synthesized during prophase at the nucleolar organizing region of the chromosomes and excluded from the nucleus during the reformation of the envelope at telophases I and II. Dickinson (1981) has been associated with much of this work.

In conifers, however, the matter is believed to be somewhat different. In the pollen mother cells of *Pinus sylvestris*, for example, there is no

obvious depletion of ribosome numbers during prophase as there is in *Lilium* (Willemse 1972), and in *Pinus banksiana* only slight changes of this kind can be detected with microdensitometry (Dickinson 1981). Cytoplasmic changes during sporogenesis in the fern *Pteridium* (Sheffield and Bell 1979) and in the club moss *Lycopodium* (Pettitt 1978) appear to be of the *Lilium* kind, however.

Although the major groups of plants differ between themselves in respect of what has become known as the 'ribosome cycle', it has been held (Dickinson and Heslop Harrison 1977) that the decline in insoluble RNA in the cytoplasm of spore mother cells of plants during prophase is causally related to the elimination of sporophytic information-carrying molecules (essentially species of RNA) from the cell, preparatory to their replacement by specifically 'gametophytic' messages.

Acid phosphatases have been localized in prophase meiocytes of *Lilium* (Porter et al. 1983). These are thought to be associated with the destruction of ribosomes and soluble RNA's. Some translation however may occur during prophase (Porter et al. 1983), so it appears that certain regions of the cytoplasm are protected from attack by hydrolase enzymes. There is as yet no wholly satisfactory explanation of how this is accomplished.

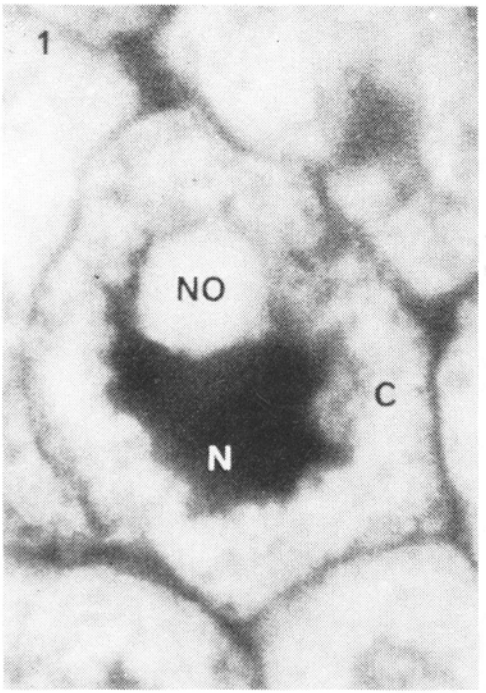
MATERIAL AND METHODS

We have used the dichroic fluorochrome Acridine Orange to investigate intracellular changes in RNA during male meiosis in the English Yew, *Taxus baccata* L. The binding of this probe is believed to be stoichiometric (Bucknall and Sutcliffe 1965). By removing the DNA from the cells with deoxyribonuclease we were able to produce photographs on black and white film of fluoresence attributable solely to RNA (Figs. 1-3). These images were then subjected to microdensitometry, the cytoplasm, nucleus and nucleolus being taken separately in each instance. These data were brought to a unit area basis, and subsequently total amounts of RNA were estimated (Fig. 5). We were then able to trace trends during prophase within each compartment of the pollen mother cells.

Figs. 1-4. Pollen mother cells of *Taxus baccata*

Figs. 1, 2, 3. Fluorescence micrographs of meiocytes fixed at preleptotene (1), zygotene (2) and telophase I (3), digested subsequently with DNAse and stained with Acridine Orange. Partitioning walls are conspicuous in the diads (3, arrow) and these may be used to stage the developmental sequence. C — cytoplasm, N — nucleus, NO — nucleolus. X 11 025

Fig. 4. Transmission electron micrograph of the meiotic cytoplasm fixed in zygotene. Ribosomes are abundant in the cytoplasm. M — mitochondrion, NE — nuclear envelope, NV — nuclear vacuole. X 26 000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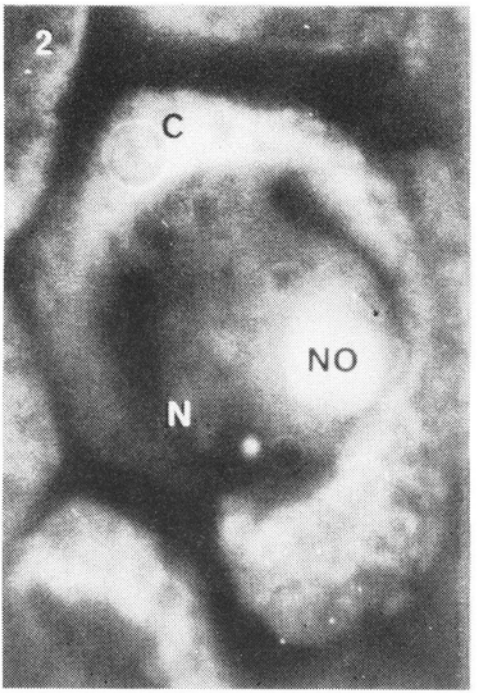
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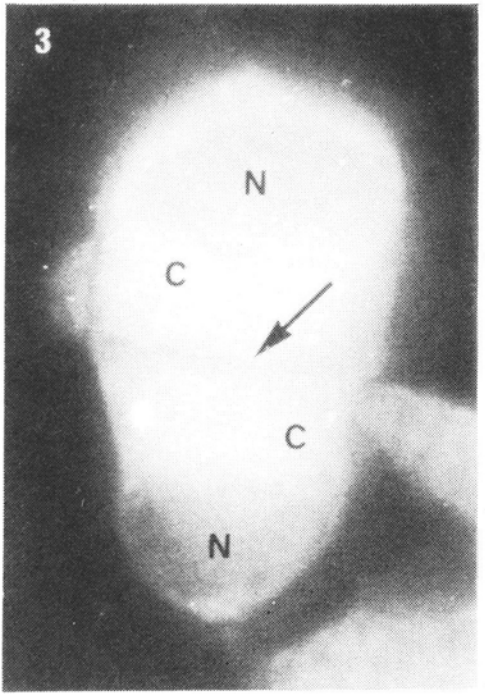
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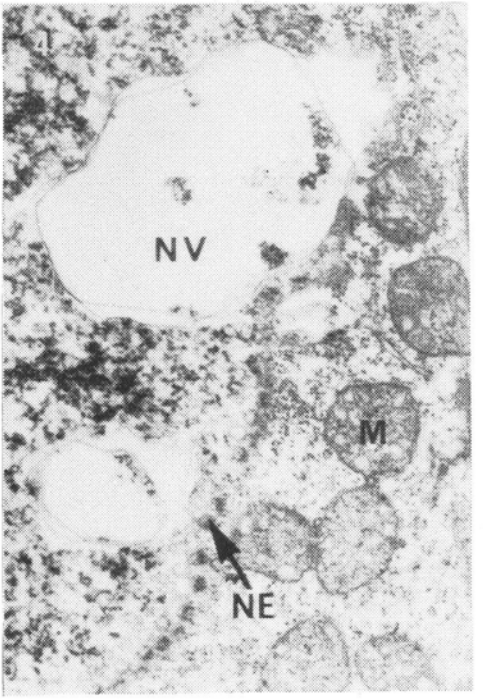
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M
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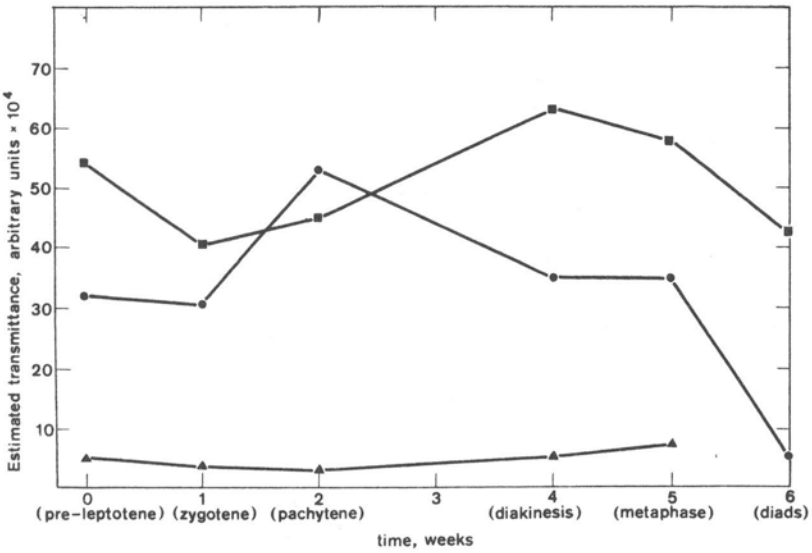

Fig. 5. Estimated fluorescence (based upon microdensitometry and expressed as transmittance) arising from microspore mother cells fixed at intervals during meiosis I. ■ – cytoplasm. ● – nucleus excluding nucleolus. ▲ – nucleolus alone

RESULTS AND DISCUSSION

THE DURATION OF MEIOSIS IN *TAXUS*

Meiosis in the microspore mother cells of *Taxus baccata* lasts for approximately six weeks, of which prophase of meiosis I occupies four weeks (Pennell and Bell 1985). During prophase leptotene, zygotene, and pachytene each persist for about one week.

In trees growing in Southern England prophase begins at the end of October and diads are formed by the end of November. Whilst it is impossible to stage the prophase meiocytes from their fluorescent images, diads are conspicuous (Fig. 3) and provide the means to identify accurately pre-leptotene (present six weeks before telophase I (Fig. 1)), and more approximately the various stages of prophase (Fig. 2).

OBSERVATIONS UPON *TAXUS* MICROSPORE MOTHER CELLS

Critical examination of the pollen mother cells of *Taxus* fails to reveal any substantial loss of ribosomes at any stage of prophase, and even at zygotene the cytoplasm remains osmiophilic (Fig. 4). This situation contrasts markedly with that in *Lilium*, for example, where the osmiophilia of the

cytoplasm during early prophase are strikingly low (Dickinson and Heslop Harrison 1977). Similarly, the Acridine Orange-induced fluorescence of the cytoplasm does not change visibly during meiosis I, even though that from the nuclei does alter (Figs. 1, 2).

When total amounts of RNA are estimated from microdensitometer readings it is evident that the amount within the cytoplasm falls only slightly at the beginning of prophase and this is accompanied by a reciprocal increase of similar magnitude in the fluorescence coming from the nuclei (Fig. 5). Subsequently both compartments of the meiocytes behave similarly, the total cellular fluorescence remaining constant until telophase I when it declines (Fig. 5). The values of nucleolar fluorescence vary little during prophase and, since there is no evidence of nucleolar budding or replication during prophase, this provides an internal control of our staining procedure.

THE SIGNIFICANCE OF THE EVENTS

The data obtained from these studies is difficult to reconcile with the view that large-scale removal of cytoplasmic RNA is essential for the change of phase in sporogenesis. There is no ambiguity about the change of phase during microsporogenesis in *Taxus*, even though there is no marked purging of RNA from the cytoplasm. It is also notable that in the aposporous production of gametophytes directly from sporophytes in controlled conditions, there is no evidence of any elimination of ribosomes (Sheffield 1985). Clearly, diminution of ribosome frequency is not an essential for the phase change. Further, recent studies of *Tradescentia* (Willing and Mascarenhas 1984) suggest that more than half the genes are active in both sporophyte and gametophyte. Consequently large scale cytoplasmic purging would seem to be unnecessary to remove the specifically 'sporophytic' moiety.

An alternative hypothesis may be proposed when the durations of prophase of *Taxus* and *Lilium* are compared. Whilst in *Lilium* (in which drastic changes do affect the cytoplasm) meiosis is completed in two or three days (Dickinson, personal communication), in *Taxus* (where such changes are not marked), prophase lasts for four weeks, the majority of this time being occupied by leptotene, zygotene and pachytene. It is therefore conceivable that cytoplasmic RNA mobilized early in meiosis enters the nucleus and accelerates the rate of which the meiocyte passes through prophase. It is significant that the increase in nuclear RNA occurs early in prophase (Fig. 5). Indeed synaptonemal complexes are known to contain RNA (Esponda and Stockert 1971, Westergaard and von Wettstein 1972) and it is possible that at least a part of the flux into the nucleus is involved in the construction of synaptonemal complexes. Further evidence

that ribonucleotides are causally involved in meiosis comes from observations on *Pterotheca falconeri* (*Compositae*). A range of nucleotides administered to growing root tips bring about reduction divisions, resulting in tetrads of nuclei, each containing the haploid set of chromosomes (Mehra 1981).

Taxus therefore repesents an organism in which it is possible to study intracellular levels of RNA over a prolonged meiotic prophase. Since meiosis in *Taxus* is completed before the onest of winter cold, the events in *Taxus* are comparable with those in any other seed plant at normal temperatures, and are not a consequence of chilling, known to have effects on meiosis (Andersson et al. 1969). The use of a fluorescent probe which is known to bind to RNA in conjunction with microdensitometry, has allowed us to examine RNA concentrations on an intracellular basis, and provided the data for a novel interpretation of many familiar features of meiosis in microsporangia.

REFERENCES

Adersson E., Ekberg I., Eriksson G., 1969. A summary of meiotic investigations in conifers. Stud. Forest. Suecica Nr. 70: 1-19.

Bucknall R. A., Sutcliffe J. F., 1965. The nucleic acids and cellular differentiation in the root apex of *Pisum sativum*. I. A fluorometric method for the estimation of nucleic acids in root cells. J. Exp. Bot. 16: 423-432.

Dickinson H. G., 1981. Cytoplasmic dedifferentiation during microsporogenesis in higher plants. Acta Soc. Bot. Pol. 50: 3-12.

Dickinson H. G., Heslop Harrison J., 1977. Ribosomes, membranes and organelles during meiosis in angiosperms. Phil. Trans. R. Soc. Lond. B 277: 327-342.

Esponda P., Stockert J. C., 1971. Localization of RNA in the synaptonemal complex. J. Ultrastruct. Res. 35: 411-417.

Mackenzie A., Heslop Harrison J., Dickinson H. G., 1967. Elimination of ribosomes during meiotic prophase. Nature (London) 215: 997-999.

Mehra P. N., 1981. Can meiosis be induced in somatic cells? In: Contemporary trends in plant sciences. S. C. Verma (ed.). Kalyani New Delhi. pp. 3-4.

Pennell R. I., Bell P. R., 1985. Microsporogenesis in *Taxus baccata* L.: The development of the archaesporium. Ann. Bot. 56 (in press).

Pettitt J. M., 1978. Regression and elimination of cytoplasmic organelles during meiosis in *Lycopodium*. Grana 17: 99-105.

Porter E. K., Parry D., Dickinson H. G., 1983. Changes in poly(A)$^+$ RNA during male meiosis in *Lilium*. J. Cell Sci. 62: 177-186.

Sheffield E., 1985. Cellular aspects of the initiation of aposporous outgrowths in ferns. Proc. Roy. Soc. Edin. 86B: 45-50.

Sheffield E., Bell P. R. 1979. Ultrastructural aspects of sporogenesis in a fern. *Pteridium aquilinum* (L.). Ann. Bot. 44: 395-405.

Westergaard M., von Wettstein D., 1972. The synaptonemal complex. Ann. Rev. Genet. 6: 71-110.

Willemse M. Th. M., 1972. Morphological and quantitative changes in the population of

cell organelles during microsporogenesis of *Pinus sylvestris* L. I. Morphological changes from zygotene until prometaphase I. Acta Bot. Neerl. 20: 261-274.

Willing P. P., Mascarenhas J. P., 1984. Analysis of the complexity and diversity of mRNAs from pollen and shoots of *Tradescantia*. Plant Physiol. 75: 865-868.

RNA wewnątrzkomórkowe podczas mikrosporogenezy u roślin: Taxus baccata jako system modelowy

Streszczenie

Badanie fluorescencji akrydyny orange dostarczyło cennych informacji o zmianach w ilości nierozpuszczalnego RNA, zachodzących podczas meiozy w komórkach macierzystych pyłku u *Taxus baccata*, u którego profaza trwa bardzo długo. Dzięki połączeniu wyników otrzymanych metodą mikroskopii fluorescencyjnej i mikrodensytometrii było możliwe zmierzenie zmian w ilościach RNA oddzielnie w cytoplazmie, jądrze i jąderku. Ogólnie, wyniki tej pracy są zgodne z wynikami otrzymanymi innymi metodami, ale nadają się one do całkowicie oryginalnej interpretacji zdarzeń zachodzących w meiozie.