CYTOCHEMICAL OBSERVATIONS ON CHANGES IN RNA CONTENT AND ACID PHOSPHATASE ACTIVITY DURING THE MEIOTIC PROPHASE IN THE ANTHER OF COSMOS BIPINNATUS CAV.

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SUMMARY

Cytophotometric methods have been used to demonstrate a fall in the acetic-alcohol fixable RNA of the cytoplasm of the meiocytes of *Cosmos bipinnatus* during the meiotic prophase. The decline occurs between zygotene and pachytene, and is probably attributable to a reduction in the ribosome population comparable with that already known to occur in *Lilium* and *Trillium*. Cytoplasmic acid phosphatase activity, estimated by cytochemical methods using naphthyl phosphates as substrates, rises from a low value in the preleptotene period to a maximum in zygotene and then subsequently declines. The possibility is suggested that the change in naphthyl cytochemically detectable acid phosphatase may be connected with the "autodigestive" processes concerned in ribosome elimination.

1. INTRODUCTION

It has been shown that the RNAase-extractable RNA in acetic-alcohol fixed meiocytes of *Lilium* and *Trillium* species declines sharply during the meiotic prophase to a minimum reached during diplotene-diakinesis, and that this fall is related to a reduction in the ribosome population of the cytoplasm (MACKENZIE, HESLOP-HARRISON & DICKINSON 1967; DICKINSON & HESLOP-HARRISON 1969). A reduction in cytoplasmic basiphilia during the meiotic prophase was first noted by PAINTER (1943) in *Rhoeo discolor*, and what is evidently a corresponding phenomenon has been recorded by SAUTER & MARQUARDT (1967) in the dicotyledon, *Paeonia tenuifolia*. It is to be expected that a degradative process in the cytoplasm involving a component so important as the ribosome population will be preceded, or accompanied, by conspicuous changes in enzyme activity, particularly of those enzymes likely to be concerned in "autodigestive" processes. However, the literature concerning enzyme activity in the anther during meiotic prophase does not provide any special evidence of this. LINSKENS (1966), for example, did not detect "acid phosphatase" activity in the midmeiotic prophase period in mechanically separated *Lilium* meiocytes, although he did observe a rise in thiamine pyro-phosphatase.

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We report here cytophotometric observations on changes in cytoplasmic RNA during prophase in the meiocytes of *Cosmos bipinnatus* Cav., and show that there are also marked variations in cytochemically detectable acid phosphatase over the same period. The special advantage of the use of a plant of the *Compositae* in cytochemical work on the anther arises from the fact that the radius of a single sectioned capitulum provides a developmental time axis. This facilitates the identification of meiotic stages, particulary when tapetal features and the state of the meiocyte cytoplasm and walls are available as criteria as well as chromosome behaviour. Furthermore, the presence of a developmental range in one section profoundly reduces errors due to variation in section thickness and processing procedures, inevitably high when a sequence has to be built up from several separate bud samples.

2. MATERIALS AND METHODS

Plants of *C. bipinnatus* were grown from seed in controlled environment cabinets under continuous illumination, and induced to flower as required by exposure to 8 h days. For RNA estimations, capitula of suitable ages (in the size range 3.5–4.0 mm in diameter) were fixed in acetic-alcohol (1:3, v/v) after removal of the involucral bracts. They were then dehydrated, wax embedded and sectioned at 6 μ. RNA was stained with azure B (Allied Chemical) or pyronin Y (E. GURR). The two procedures revealed essentially identical trends, and the quantitative data given here relate to measurements on pyronin Y stained material. The preparation procedure followed that adopted in a previous photometric study of the anther of *Zea mays* (MOSS 1967; MOSS & HESLOP-HARRISON 1967). The most reproducible staining was obtained using 0.25% pyronin in 0.2 M acetate buffer at pH 6.5.

Cytophotometry was carried out using a Canalco ultramicrospectrophotometer equipped with Olympus optics. For pyronin measurement, a Wratten 58 filter (peak transmission 530 nm) was used. Twenty readings were made for each developmental stage, and a mean absorption calculated. This value refers to a volume of cytoplasm determined by section thickness and aperture diameter, and since growth occurs during the prophase it does not give a direct estimate of amount of chromophore per cell. Mean meiocyte volumes were accordingly obtained for each developmental stage from *camera lucida* drawings of transverse and longitudinal sections of anthers, treating the meiocytes as regular cylinders to simplify calculation. The mean absorption figure for each stage was then multiplied by cell volume to give a value for relative total chromophore per cell.

For the enzyme study, capitula were segmented, and fixed in 2.8% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.4 for 6–8 h at 2 °C. The segments were then washed in several changes of buffer for 15–24 h at 2 °C, and encased in a medium containing 15% gelatine (w/w) and 1% dimethyl sulphoxide (v/v), and cooled rapidly to -15 °C (KNOX & HESLOP-HARRISON 1969). Sections were then cut in a cryostat at 6 μ. Enzyme tests were also carried out on capitula

freeze-sectioned fresh without prior fixation. Acid phosphatase activity was detected by the method of HANKER *c.s.* (1964) using 2-naphthyl-thiol phosphate salt in 0.2 M acetate buffer at pH 5.0 as substrate, or by that of BARKA & ANDERSON (1962) using α-naphthyl acid phosphate as substrate in 0.1 M veronal acetate buffer at pH 6.0. It proved difficult to measure the black osmiophilic reaction product densitometrically because of differences in the degree of dispersal, so activity was scored visually on a five-point scale ranging from no reaction to the heaviest observed throughout the sequence of stages examined. The numbers of cells scored for each stage were: preleptotene, 714; leptotene, 1096; zygotene 1056; pachytene, 1385, and diplotene, 813.

Large errors are undoubtedly involved in both the cytophotometric and enzyme activity determinations. However, the methods have the overwhelming advantage of permitting estimations to be made in individual meiocytes precisely identified for developmental stage, and they also eliminate any possibility of contamination from neighbouring tapetal tissue, itself in a highly active metabolic state during the meiotic prophase. Moreover, the differences observed are of a magnitude great enough to place their significance beyond doubt even given the high level of error.

3. RESULTS

The changes in total relative amount of cytoplasmic acetic-alcohol fixed RNA per cell are given in *fig. 1*. The trend is quite similar to that seen in the liliaceous meiocyte, with a sharp fall following zygotene to a low value in pachytene. On the basis of the pyronin absorption figures, the loss in fixable cytoplasmic RNA approaches 60%, somewhat greater proportionately than the loss in total fixable cell RNA measured by extraction methods in *Lilium* and *Trillium*.

The changes in RNA concentration in freeze-sectioned, glutaraldehyde-fixed and pyronin-stained meiocytes are apparent visually in the micrograph of *fig. 2*. The acid phosphatase reaction in this population of anthers is illustrated in *fig. 3*, of a neighbouring section from the same capitulum. The mean score for phosphatase activity in the cytoplasm during prophase up to diplotene is shown in *fig. 1*. From a low level in the preleptotene period, activity rises to a maximum in zygotene, then declines through pachytene into diplotene. Estimated on this basis, then, cytochemically detectable acid phosphatase activity in the cytoplasm of glutaraldehyde-fixed meiocytes reaches its peak just at the beginning of the period of decline of cytoplasmic RNA in the zygotene to pachytene interval. The enzyme activity in the chemically fixed and exhaustively buffer-washed cells must be held rather tenaciously, and presumably it is membrane bound, or bounded. However, in the unfixed, freeze-sectioned meiocytes, although detectable activity was higher and resolution within the cell poorer, a similar trend of increase from early to mid-prophase was observed.

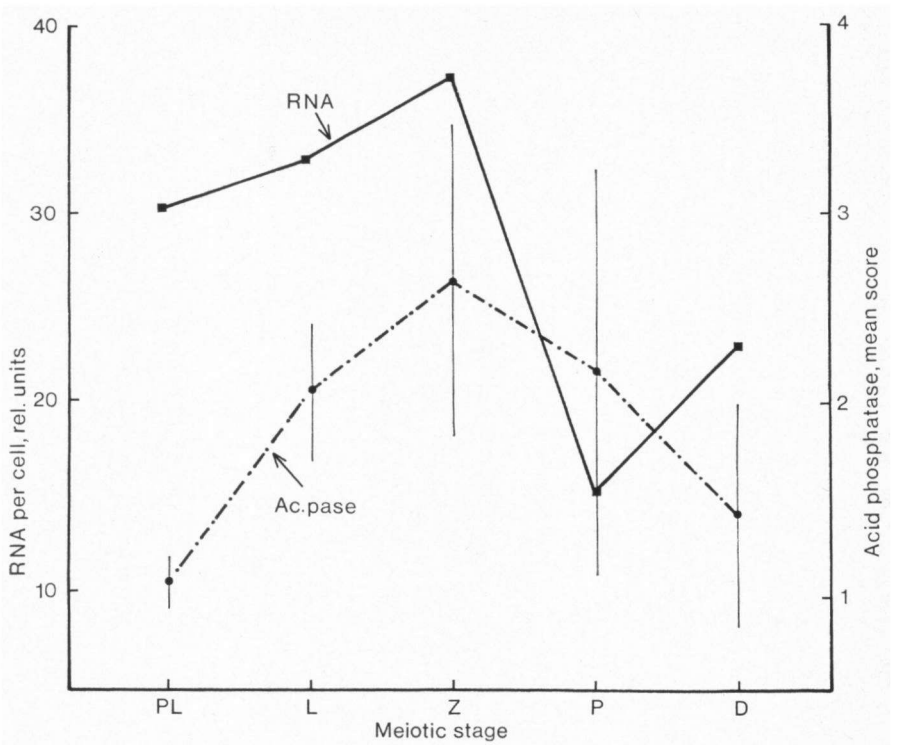
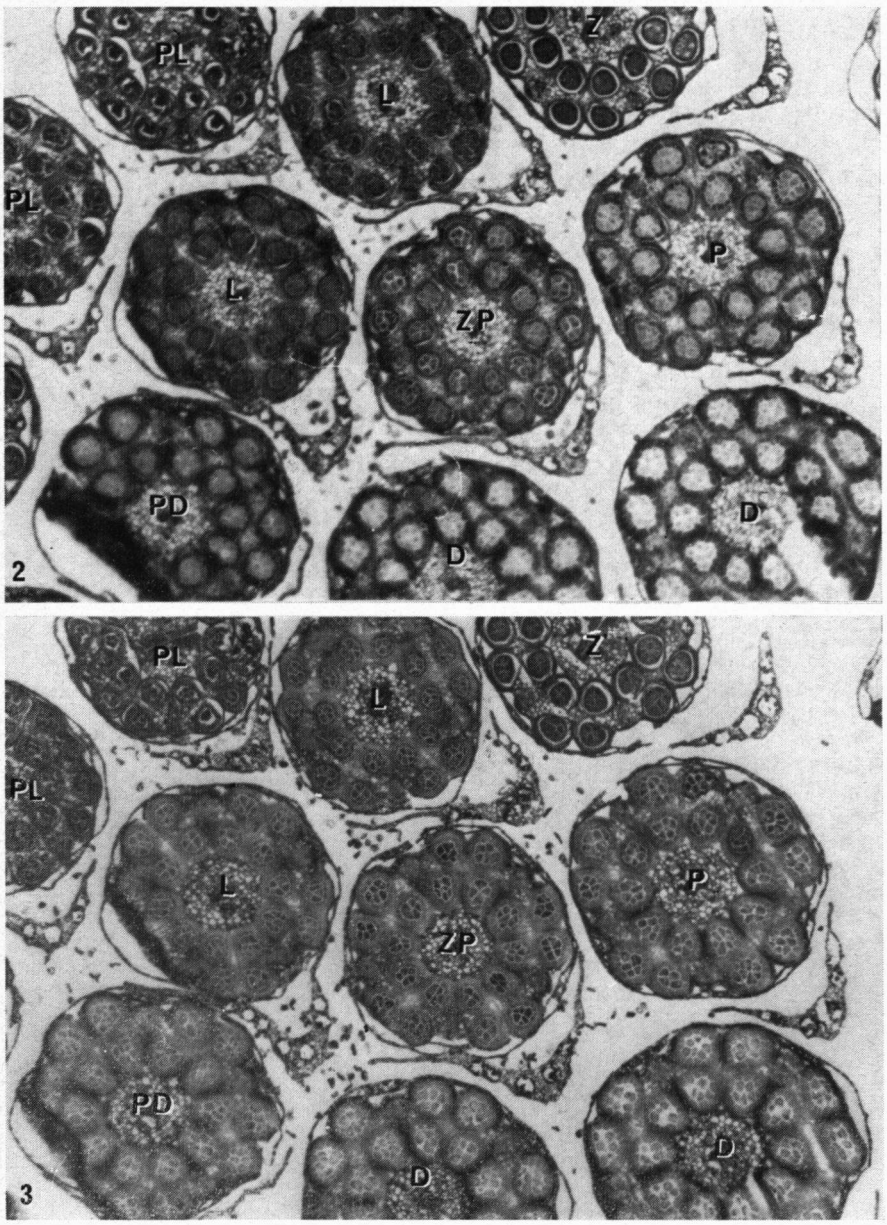


Fig. 1. Changes in RNA content and acid phosphatase activity in glutaraldehyde-fixed meiocytes of *Cosmos bipinnatus*. The RNA curve is derived from pyronin microdensitometry in the manner described in the text, and the acid phosphatase curve is based upon visual scoring of preparations in which enzyme activity was revealed using 2-naphthyl thiol phosphate as substrate. For the phosphatase curve, the vertical bars indicate ± standard error. Meiotic stages as in *figs. 2 & 3*.

4. DISCUSSION

These observations provide quantitative confirmation from a dicotyledonous species of the decline in meiocyte RNA during prophase previously observed in *Lilium* and *Trillium* (MACKENZIE *c.s.* 1967), and they further support the belief that the phenomenon is likely to be a general one among flowering plants. In *C. bipinnatus* also the observed decline will undoubtedly prove to be due to ribosome elimination, although fine-structural evidence has not yet been sought.

The data also appear to give a clear indication of phase specificity in the activity of the enzyme or enzymes cleaving naphthyl phosphates under the conditions of the cytochemical test. Although there is no secure indication of the functional significance of acid phosphatase in this sense in plant cells, differences in activity of the magnitude seen between preleptotene and zygotene must signify some rather substantial alteration in the status of the enzyme in the



Figs. 2 and 3. Sections of a sector of a capitulum of *Cosmos bipinnatus*, showing the same anther population, *c.* 75 ×. Meiotic stages as follows: PL, preleptotene; L, leptotene; Z, zygotene; ZP, transition from zygotene to pachytene; P, pachytene; PD, transition from pachytene to diplotene; D, diplotene. Fig. 2, pyronin stained, showing RNA distribution. The fall in concentration in the meiocyte cells between zygotene and pachytene may be seen. Fig. 3. acid phosphatase activity revealed by the methods of HANKER *c.s.* (1964) using 2-naphthyl thiol phosphate as substrate. The peak of activity in zygotene is followed by a decline through pachytene to diplotene.

meiocyte, presumably resulting either from accelerated synthesis, or activation, or both. Since the acid phosphatases are commonly accepted as being lysosomal enzymes, it may be that the peak in zygotene marks the activation of a group of hydrolases concerned with the "autodigestive" processes mentioned above which have as one consequence the reduction of the ribosome population of the prophase meiocyte.

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