LOCALIZATION OF ACID PHOSPHATASE ACTIVITY, PHOSPHATE IONS
AND INORGANIC CATIONS IN PLANT NUCLEAR COILED BODIES

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SUMMARY

In an attempt to clarify the physiology of plant nuclear coiled bodies, three different cytochemical tests related to nuclear RNA metabolism were made: 1) localization of an acid phosphatase activity; 2) presence of orthophosphate ions; and 3) presence of divalent cations.

The three cytochemical tests gave positive results, which are discussed in view of the present knowledge on nuclear RNA synthesis and metabolism.

INTRODUCTION

Many ultrastructural studies during the last years have revealed the existence of several nuclear components different from the chromatin and nucleolus. Some of these nuclear structures are common components of both animal and plant nuclei, as is the case with perichromatin fibrils and granules, and interchromatin granules (Bernhard, 1971, Jordan et al, 1980, Risueño and Moreno Díaz de la Espina, 1979). In previous papers (Moreno Díaz de la Espina et al, 1980, and in press) we reported the existence of coiled bodies, ultrastructurally homologous to those described in animal cell nuclei, in several plant tissues. They were characterized as being composed of ribonucleoprotein fibrils, having no detectable amounts of DNA. The autoradiographic experiments demonstrated that they do not incorporate tritiated uridine after pulses of 30 minutes. To attempt to clarify the role of coiled bodies in nuclear RNA metabolism, we tested, at the EM level, the localization of an acid phosphatase activity, as well as for the accumulation of inorganic phosphate ions and inorganic cations (Na^+ , Ca^{++} , Mg^{++}) in the coiled bodies. Nuclear acid phosphatase activity has been detected in plant nuclei by Sánchez-Pina et al (1980) in *Allium cepa* anthers and by Deltour et al. (1981) in *Zea mays* roots. In both cases a correlation was established between the localization of the acid phosphatase activity and the accumulation of orthophosphate ions, and nuclear RNA metabolism.

The aim of this work was to test, not only for the presence of acid phosphatase activity, but also for divalent cations which are

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engaged in the regulation of the activity of both RNA polymerases (Tres, et al, 1972, Grossmann and Seitz, 1979) and enzymes engaged in RNA processing. The presence of orthophosphate ions, which correlates to a high nuclear metabolic activity (Tandler, et al, 1973, Deltour, et al, 1981) was also tested using the method of Tandler and Solari (1969).

MATERIALS AND METHODS

Microspores, binucleate pollen and tapetal cells, corresponding to anthers of 1-3 mm in length, as well as meristematic root cells, from Allium cepa L. were used.

The detection of acid phosphatase activity was performed as previously described (Sánchez-Pina et al. 1980). The visualization of insoluble phosphates was done according to Tandler and Solari (1969). For detection of inorganic cations, anthers were fixed for 1h, in the pyroantimonate-osmium mixture according to Hardin and Spicer, (1970). Each cytochemical test was performed in an independent sample.

All samples were dehydrated in an ethanol series and embedded in Epon 812. Ultrathin sections were obtained in a LKB III ultratome and observed under a Phillips 300 electron microscope without additional staining.

RESULTS

As previously reported (Moreno Díaz de la Espina et al, in press) coiled bodies (cb's) appear in the interchromatinic nuclear regions, either free or showing connections with interchromatin granules and perichromatin fibrils. They have a spherical or irregular shape with a mean diameter of $0.4 \pm 0.15 \mu\text{m}$ and consist of densely packed ribonucleoprotein threads of 20-25 nm whose degree of packing varies from one body to another (fig. 1).

Cytochemical detection of acid phosphatase activity. The product of the phosphatase reaction is preferentially localized as a fine precipitate in the nucleolus and coiled bodies (fig.2). Within the nucleolus, the reaction is mainly located in the fibrillar component. The c.b.'s have a density of precipitate similar to that in the nucleolar granular component. We observed no significant precipitate on the condensed chromatin masses, in the interchromatinic regions of the nucleus, nor on any of the cytoplasmic structures.

We tested the specificity of the reaction in two series of controls: a) Using the incubation medium without substrate (fig.3); and b) by adding 0.02 M NaF to Gomori's medium (Gomori, 1952) as an inhibitor of the enzyme (fig.4). In both cases there is no precipitate, either on the nucleolus or in coiled bodies.

Localization of the pyroantimonate-osmium reaction products. After pyroantimonate-osmium fixation, the pattern of accumulation of antimonate precipitate is homologous to that shown in the case of the nuclear acid phosphatase activity (fig.5). The main locations for deposits of precipitate are the nucleolus and coiled bodies. In the

case of the nucleolus, the precipitate accumulates preferentially in the fibrillar component surrounding the fibrillar centres. In the coiled bodies the precipitate is homogeneous, the fine deposits forming cords in some cases.

Visualization of orthophosphate groups. Using the electron microscope for visualization of orthophosphate groups, we employed a method which involved their conversion into electron-opaque lead phosphates after fixation (Tandler and Solari, 1969). Within the nucleolus, the lead phosphate precipitate appears associated with the fibrillar component of the nucleolus, the coiled bodies and the interchromatinic nuclear region, while the condensed chromatin masses are practically free of them (fig.6). Within the coiled bodies, the precipitate occurs as fine granules similar to those found in the nucleolar granular component.

DISCUSSION

The first point to be discussed is the specificity of the cytochemical tests we have used. In the case of the nuclear acid phosphatase, the specificity of the reaction at pH 5.0 was demonstrated by us in different anther tissues in a previous work (Sánchez-Pina et al., 1980). The constant and specific localization of the reaction product, independent of the tissue we used, does not support the hypothesis of a cytoplasmic diffusion. In agreement with this location, Deltour et al (1981) have demonstrated an acid phosphatasic activity in both, whole cells and isolated nuclei of *Zea mays* with a similar pattern to our results. On the other hand, the results obtained in both series of controls (using either NaF as an inhibitor of the acid phosphatasic activity, or an incubation medium without substrate) provide evidence of the specificity of the reaction.

Electron microprobe analysis has demonstrated that the electron opaque antimonate deposits, formed after potassium pyroantimonate fixation, contain inorganic cations such as Na^+ , Mg^{++} and Ca^{++} (Tandler, et al, 1970; Kierszembau, et al, 1971). Previous studies by some of us, using a saturated solution of potassium pyroantimonate as a fixative, revealed the nucleolus and some loci in the nucleoplasm as the main sites of accumulation of antimonate deposits (Tandler, et al, 1973) which, according to several authors, correspond to the precipitate of the anion with Na^+ , Mg^{++} and Ca^{++} . Later experiments using the pyroantimonate-osmium mixture according to Hardin and Spicer (1970) which gives better preservation of the structures, confirm the previous pattern of antimonate deposits and allow us to identify the small deposits in the nucleoplasm with the coiled bodies. A similar pattern of distribution of precipitates was described by Rodríguez-García and Stockert (1979) in root tips and anthers of *Allium cepa*.

Some of us had previously studied the pool of inorganic orthophosphate ions in *Allium cepa* meristematic root cells (Fernández-Gómez et al, 1973) by using the method of Tandler and Solari (1969) which involved their conversion into lead-phosphate precipitate after fixation in lead acetate. The precise chemical composition of the pre-

precipitate as well as the specificity of the reaction was demonstrated by Tandler and coworkers in previous works (Tandler and Solari, 1969). In the present work, the soluble phosphate pool was partially eliminated by immersing the roots in glutaraldehyde and subsequently washing them with cold distilled water (Fernández-Gómez, et al, 1973). Nevertheless, the pattern of precipitation of lead orthophosphate in the nucleolus is similar to that obtained by other authors (Tandler and Solari, 1969; Deltour et al. 1981) using lead acetate mixed with different fixatives which avoid the elimination of water-soluble phosphates, localizing both, the soluble and insoluble pools. This fact could be due to two different reasons: either the elimination of soluble phosphates by the fixatives is not as considerable as thought, or the phosphates present in the nucleolus and coiled bodies correspond mostly to the insoluble pool, being associated to binder macromolecules as pointed out by other authors (Tandler and Solari, 1969).

From all the above, we can conclude the presence in coiled bodies of an acid phosphatasic activity, inorganic cations (mainly Na^+ , Ca^{++} and Mg^{++}), and a pool of phosphate ions probably loosely bound to binder macromolecules. The role that the detected ions, as well as the acid phosphatasic activity, could play in the functioning of coiled bodies is difficult to ascertain. In fact, most authors have correlated the accumulation of both the phosphate ions (Tandler and Solari, 1969, Deltour et al, 1981, Tandler et al, 1973) and the inorganic cations (Tres et al, 1977, Tandler et al, 1973) to the transcriptional activity of RNA polymerases. A correlation has been established, too, between the acid phosphatase activity and the accumulation of inorganic phosphate ions (Deltour et al. 1981) produced by its activity. Grossman and Seitz (1979) have demonstrated biochemically that RNA polymerase I activity in higher plants is regulated by both divalent cations and the nucleoside triphosphate pool (NTP) which, in turn, is controlled by a nuclear phosphatase.

According to all the above, coiled bodies would be engaged in nuclear transcription; but our previous autoradiographic data have demonstrated that coiled bodies do not incorporate tritiated uridine after pulses of 30 minutes (Moreno Díaz de la Espina et al. 1980 and in press) and would not be involved in transcription. The presence of the acid phosphatasic activity and the accompanying accumulation of orthophosphate ions could be related, in coiled bodies, to a process of trimming or degradation of the RNA that they contain, as was suggested by other authors (Dalgarno and Shine, 1977; Georgiev, 1967). The presence of inorganic cations in coiled bodies could be explained as cofactors of other enzymes engaged in RNA processing.

According to this interpretation, coiled bodies would be involved in RNA processing within the nucleus. This hypothesis fits completely with the results of the preliminary autoradiographic experiments, using chases of different duration after pulses of 30 minutes of tritiated uridine incorporation, which demonstrated that coiled bodies have a stable type of RNA (Moreno Díaz de la Espina, et al. 1980).

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Legends of figures

Fig.1.- Glutaraldehyde-Osmium fixation. Meristematic root cell. Coiled body (cb) in the interchromatinic nuclear region. The coiled structure of their threads is very conspicuous (arrow heads). A clump of interchromatin granules (ig) appear near the condensed chromatin masses (chr).

Fig.2.- Nuclear acid phosphatase localization. Tapetal cell. The precipitate due to the phosphatase reaction appears preferentially located in the nucleolus (nu) and coiled bodies (cb) which show a density of precipitate similar to that in the nucleolar granular component (g). The chromatin masses (chr) appear free of precipitate, but some scarce precipitation occurs in the inter- and perichromatin regions of the nucleus.

Fig.3 and 4.- Controls of the phosphatase reaction. (3) Cell incubated in gomori's medium without substrate and (4) using 0.025 M NaF as inhibitor of the enzyme. Microspore and vegetative cell of bicellular pollen grain respectively. In both cases there is no precipitate either in the nucleolus (nu in fig 4) or in coiled bodies (cb). Condensed chromatin masses (chr). Nuclear envelope (ne).

Fig.5.- Pyroantimonate-osmium-fixation. Tapetal cell. The fine deposits of antimonate precipitate in coiled bodies (cb) form cords in some zones of the sections (arrows). The chromatin show no significant precipitate.

Fig.6.- Visualization of orthophosphate groups. Meristematic root cell. After fixation in lead acetate a fine lead phosphate precipitate appears in coiled bodies (cb), similar to that in the nucleolar granular component (g). The condensed chromatin masses (chr) appear free of precipitate, but some precipitation occurs in the interchromatin spaces.

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