NUCLEAR COMPARTMENTALIZATION IN POLLEN MOTHER CELLS DURING MEIOTIC PROPHASE

M. I. Rodríguez-García¹, J. D. Alché¹, A. Majewska-Sawka², M. C. Fernández¹ and E. Jassèm²

¹ Estación Experimental del Zaidín (C.S.I.C.)
Profesor Albareda 1
18008 Granada
Spain

² Institute for Plant Breeding and Acclimatization
Weyssenhoffa 11
85-950 Bydgoszcz
Poland

INTRODUCTION

Nuclear vacuoles, which are expansions of the perinuclear space toward the karyoplasm, were first reported in animal cell by Rasmussen (1976) during meiotic prophase in Bombyx mori. Some years later, membrane limited spaces in nucleus formed by the invagination of the inner membrane of the nuclear envelope during meiotic prophase in different plant species, were also described by several authors (Sheffield et al., 1979; Karasawa and Ueda, 1983a; 1983b; Sangwan, 1986; Rowley and Walles, 1985; Rodríguez-Garcia et al., 1988; Majewska-Sawka et al., in press). Although these structures have been reported in many different species, the lack of information about the nature and function of the unusual compartments in the nucleus has made many scientists consider them with reservation, or identify them as artifacts, which is a ready explanation for anything new and unknown within a cell.

In this study we will present evidence that supports the existence of nuclear vacuoles as real, dynamic structures which originate in the cell at specific times and under specific circumstances, related to processes of nuclear reorganization during meiotic prophase.

MATERIALS AND METHODS

We based our work on different techniques including standard glutaraldehyde-osmium fixation, cryofixation and cryosubstitution of Olea europaea L. and Beta vulgaris L. anthers.

Cryofixation. The material was pretreated with the chemical fixation (3% glutaraldehyde at 4°C for 2h), followed by cryoprotection in 2.3M sucrose at 4°C overnight. Specimens were frozen by immersion in liquefied propane at -190°C in the Reichert Jung universal system. Cryosections were
obtained using the Reichert Jung FC4, stained by toluidine blue and observed by light microscopy.

Cryosubstitution. Following fixation in 3% glutaraldehyde for 3h, the material was cryosubstituted in 50% at -20°C, and then at -30°C. The anthers were embedded by low temperature medium Lowicryl K4M.

DEVELOPMENT OF NUCLEAR VACUOLES

Our present studies in Olea europaea show the pattern of formation of
nuclear vacuoles, which agrees with the results obtained earlier, by electron microscopy (Rodriguez-Garcia et al., 1988; Majewska-Sawka et al., in press). We will refer to the scheme in Figure 1a to 1d, which shows the formation, development, and disappearance of nuclear vacuoles during meiotic prophase in pollen mother cells (PMCs).

In early meiotic prophase (Figure 1a), the nucleus of the PMC shows a typical round contour, with no particular features of the nuclear envelope.

In leptotene/zygotene (Figure 1b) local dilations of the perinuclear space appear to be randomly distributed around the nuclear envelope. As zygote proceeds, the perinuclear dilations increase rapidly, growing toward the karyoplasm, while at the same time the inner nuclear membrane enlarges. In electron micrographs the nuclear envelope dilations show membrane limited spaces at the nuclear periphery (Figures 2a, 2b).

In pachytene (Figure 1c), the vacuolar expansions in the perinuclear space ramify progressively, penetrating deeper into the karyoplasm and becoming intercalated with the synaptonemal complexes of the paired chromosomes (Figure 2c). The increase in size of the nuclear vacuoles is accompanied by an increase in nuclear volume. Serial sections would be necessary to determine whether the vacuolar compartments formed in the nucleus maintain contact with the perinuclear space, or if on the other hand they are independent units, as the bidimensional images seem to suggest.
Figure 3. Pollen mother cells during meiotic prophase. Toluidine blue staining. (a-d): conventional glutaraldehyde-osmium fixation, (e): cryosubstitution, (f): cryofixation.

Toward the end of meiotic prophase, diplotene-diakinesis (Figure 1d), nuclear vacuoles are seen less frequently. They become steadily less numerous, and are always seen at the nuclear periphery, while the nucleus becomes noticeably smaller. By contrast, the number of cytoplasmic vacuoles rises. These observations may suggest that nuclear vacuoles are being transferred into the cytoplasm.

CONCLUSIONS

Because of the constant presence of nuclear vacuoles, observed with both conventional electron microscope techniques and with cryotechniques, we can conclude that these structures are real and not artifacts. The nuclear envelope clearly seems to be involved in their formation, together with enlargement of the inner nuclear membrane and the budding out of the perinuclear space into the karyoplasm.

REFERENCES


