Prenucleolar Bodies in the Cytoplasm of Meristematic Cells After Thermal Shock

Several authors have studied the effect of thermal shock on the structure and function of the nucleolus in the course of the last few years[1–4]. In plant cells, a thermal shock prevents nucleolar fusion during the prophase of meiosis in microsporocytes[5], as well as inducing nucleolar extrusion and additional nucleoli in suspensor cells[6].

We have not, however, found any references to the effects of thermal shock on the level of nucleolar reorganization in the telophase of mitosis. In the course of experiments on the thermosensitivity of the cell division cycle, we had occasion to observe a morphological characteristic of the cells in telophase which suggested the subject of this present study.

The material consisted of root meristems from *Allium cepa* bulbs, growing in tap-water at 15 °C with constant air-bubbling at the rate of 10–20 cm²/min. The thermal shocks, of 1, 2 and 3 h duration, were produced by applying temperatures of 30, 35 and 37.5 °C ± 0.5 °C with recovery of the roots after the shocks.

For observation under the light microscope a method of silver impregnation for nucleolar material was used[7]. For observation under the electron microscope, the roots were fixed in glutaraldehyde alone or in glutaraldehyde

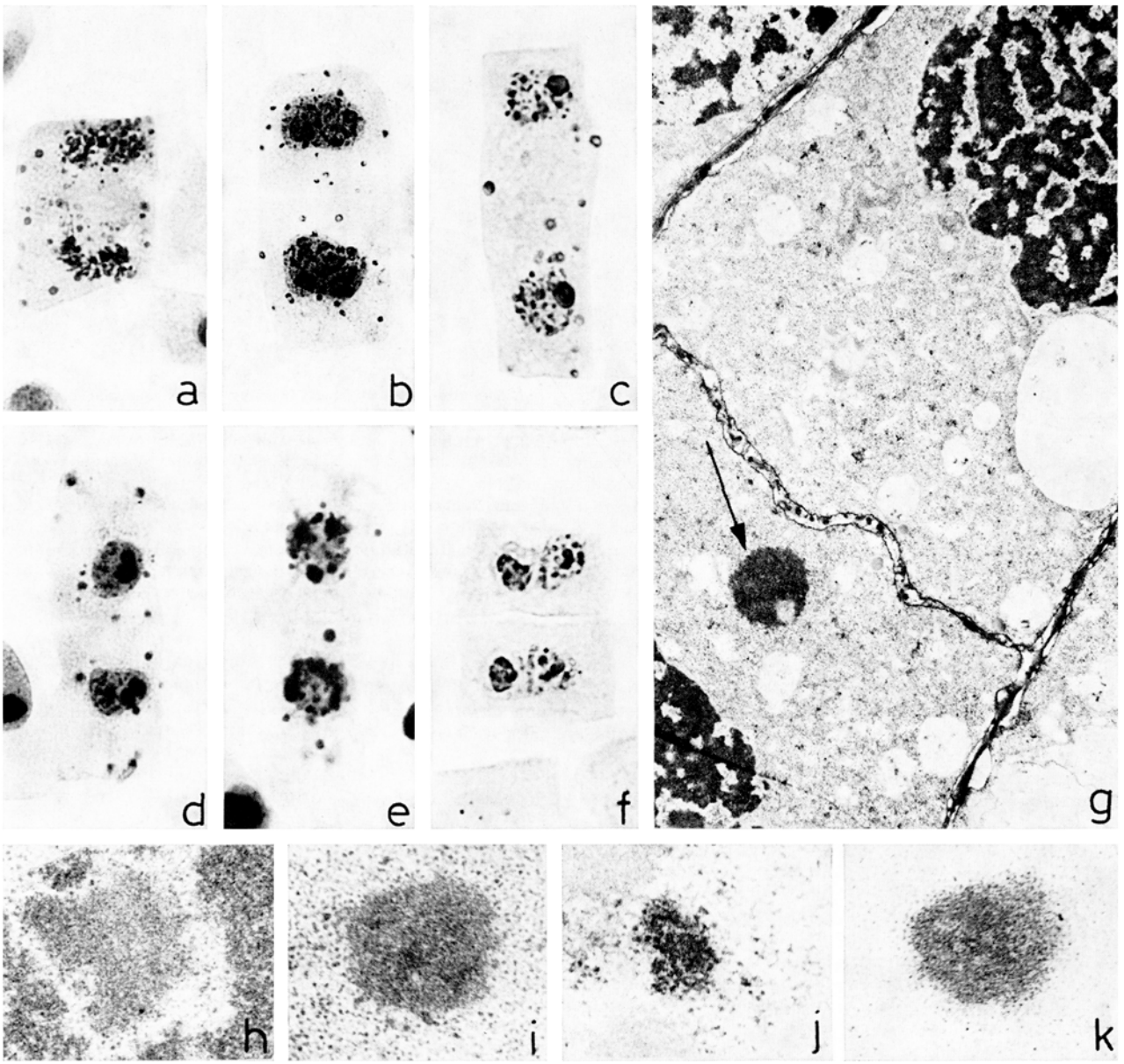

Fig. 1. a–e) Root-tip cells after thermal shock at 37.5 °C, with cytoplasmic prenucleolar bodies. f) Normal telophase with nucleoli and prenucleolar bodies within the nuclei. a–f) Silver impregnation. Bright-field lighting. ×710. g) Electron micrograph of a cell in which, near the new-formed cell wall, a cytoplasmic prenucleolar body can be observed (arrow). ×10,500. h) Telophase prenucleolar body in a normal cell, surrounded by chromosomal masses. ×63,000. i) High magnification of a cytoplasmic prenucleolar body. ×62,000. j) Telophase prenucleolar body, stained with the uranyl-EDTA-lead technique. ×78,000. k) Cytoplasmic prenucleolar body, stained as j). ×62,000.

followed by osmium tetroxide and then included in Epon 812. Ultrathin sections were stained with the uranyl-EDTA-lead technique[8], or with uranyl acetate and lead citrate respectively, and afterwards observed under a Philips 300 E.M.

Among the cytological effects produced by the various thermal shocks used, we wish to refer particularly to a phenomenon observed in the cells in telophase subjected to a shock of 15–37.5°C characterized by the appearance of spheroidal argyrophilic bodies in the cytoplasm, numbering 20–30, with staining peculiarities very similar to those of argyrophilic nucleolar and prenucleolar materials (Figure 1, a–e). The presence of argyrophilic bodies in the cytoplasm we also observed in a few anaphases and very early interphases. Normal telophases in control roots do not show any argyrophilic bodies in the cytoplasm (Figure 1, f).

Under the electron microscope the cells in telophase subjected to thermal shock showed roughly spherical bodies scattered about the cytoplasm, with a diameter of 0.3 to 0.6 μm (Figure 1, g). The predominantly fibrillary texture, the electron-density and the size of these bodies show very close correspondence with the characteristics of the prenucleolar bodies to be found normally within the nucleus in telophase (Figure 1, h and i). The cytoplasmic bodies have no limiting membranes, their periphery seems to be in close contact with the ribosomes, and with the uranyl-EDTA-lead staining technique[8], they show the same affinity as the normal prenucleolar bodies (Figure 1, j and k)[9].

The resemblance between these bodies observed in the cytoplasm of telophase cells and the normal prenucleolar bodies found in the nucleus at telophase in point of both size and argyrophilia, as well as ultrastructural appearance and staining properties suggests that they are identical. Thus the presence of prenucleolar bodies in the cytoplasm appears to represent a change in the reorganization of the nucleolar material in the telophase brought about by the thermal shock.

To provoke this phenomenon the roots were subjected to a temperature of 37.5°C for 2, 3 and 24 h, and in all cases it was observed that the appearance of prenucleolar bodies in the cytoplasm was reversible, very few cells showing such prenucleolar bodies 2 h after the initiation of the treatment. Figure 2 shows the percentage of these cells throughout a 2-hour period of shock. It may be observed that they reach a maximum of approximately 3% after one hour of treatment, and return to zero during recovery. The effect of the shock proved nonlethal in the meristematic population, since a normal rate of growth was observed in the roots after 24 h of recovery.

The reconstitution of the nucleolus from the telophase prenucleolar bodies is prevented by inhibitors of RNA synthesis[10]. On the other hand, it is known that nucleolar RNA synthesis is inhibited by temperatures above the optimum[1,3,4]. To explain the appearance of prenucleolar material in the cytoplasm, we suggested an interaction between 2 processes taking place in the telophase, possibly changed by the thermal shock: the reconstitution of the nucleolus and the re-formation of the nuclear membrane, of which the latter could be considered to be the factor which normally confines the prenucleolar bodies within the nucleus when the reconstitution of the nucleolus is prevented experimentally, as in the case of aneuploid nuclei induced by colchicine[10].

A process apparently resembling that which we have described occurs naturally in the course of meiotic divisions in the microsporocytes of Lilium[11,12].

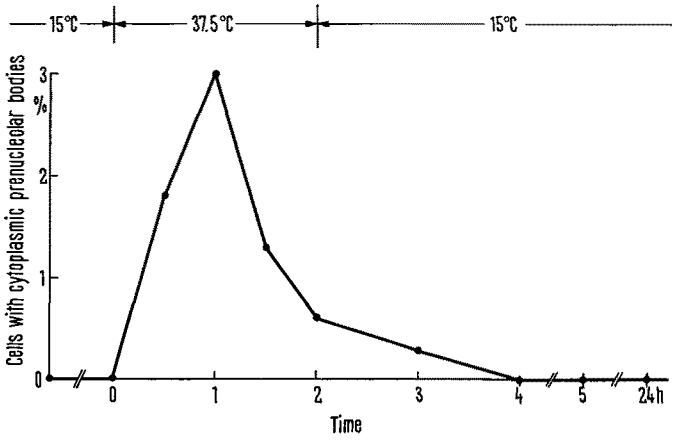

Fig. 2. Percentage of cells with cytoplasmic prenucleolar bodies (CPB) plotted against time, during the 2-h thermal shock and the recovery.

Resumen. Uno de los efectos producidos por el choque térmico, a temperaturas supraóptimas y subletales, sobre células meristemáticas de *A. cepa* es la aparición de cuerpos argentófilos en el citoplasma de las células telofásicas. Las características citoquímicas y ultraestructurales de estos cuerpos permiten identificarlos con los cuerpos prenucleolares.

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