Electron Microscopy of Synaptonemal Complexes in Semithin Sections

Ondina D. Colman and Juan C. Stockert

Departamento de Citología e Histología, Facultad de Ciencias, Universidad Autónoma de Madrid, and Instituto de Biología Celular, C. S. I. C., Madrid-34, Spain.

Z. Naturforsch. **34 c**, 299–300 (1979);
received December 11, 1978

Synaptonemal Complex, Osmium/*p*-Phenylenediamine, Mouse Spermatocytes, Semithin Sections, Electron Microscopy

The technique of osmium tetroxide fixation followed by a treatment with *p*-phenylenediamine was applied to mouse testes in order to analyze pachytene nuclei. Observation of Epon semithin sections (0.25–0.5 μm) in conventional electron microscopy revealed the presence of positively stained synaptonemal complexes surrounded by unstained chromatin. The present results show that by using this method a suitable contrast and resolution can be achieved for semithin sections, which facilitates the topographical study of synaptonemal complexes.

The ultrastructure of meiotic chromosomes and particularly of synaptonemal complexes (SC) in a variety of animal and plant species has been undertaken by means of different methods: a) conventional electron microscopy using ultrathin sections; b) serial sectioning for topographical reconstructions; c) techniques for ultrastructural cytochemistry; d) spreading and whole mounting methods, and e) high voltage electron microscopy for thick sections.

Application of some cytochemical staining techniques (*e. g.* ammoniacal silver [1], ethanolic phosphotungstic acid [2], uranyl-EDTA-lead [3, 5], and osmium tetroxide/*p*-phenylenediamine [6]), have revealed a characteristic composition for the lateral elements of SC. Osmium fixation followed by a treatment with *p*-phenylenediamine has allowed us to distinguish selectively ribonucleoprotein components within nuclei [7, 8]. In addition, the use of this staining technique has given us the opportunity to analyze SC in semithin sections under the electron microscope.

Mouse testes were fixed with 2% osmium tetroxide in distilled water for 30 minutes at room temperature. Samples were briefly washed in 6.5% sucrose solution and dehydrated in ethanol series. When dehydration reached the 70% ethanol step, the material was treated with 1% *p*-phenylenediamine (Merck) in 70% ethanol for 30 minutes, after which the dehydration was completed. Samples were embedded in Epon and sectioned using an LKB Ultrotome. Semithin sections (0.25 and 0.5 μm) were collected on copper grids and observed without additional staining under a Philips 300 electron microscope operated at 80–100 KV. For comparative purposes, other semithin sections (1 μm), were mounted on glass slides and examined in light microscopy.

As observed under phase contrast optics, the nuclei of pachytene spermatocytes show fine threads (Fig. 1, insert), which correspond to the light microscopical expression of the SC's [6]. The cores of the XY pair and the relationship of SC's with the nuclear envelope can easily be recognized. The analysis of semithin sections in electron microscopy reveals the occurrence of typical SC's in spermatocyte nuclei (Fig. 1, a and b). According to the orientation of cutting, structural features such as the coiling of lateral elements and their attachment to the nuclear membrane are also clearly observed. The central and lateral elements, as well as the XY cores appear specifically contrasted while the surrounding chromatin show scarce or no contrast. The nucleolar material, either located at the centromeric (heterochromatic) end of some bivalents or around the XY pair, and the cell membranes are also highly stained after this technique. Superimposed cytoplasmic organelles (endoplasmic reticulum, mitochondria) can be recognized in semithin sections. Although in 0.5 μm thick sections the superimposition of cytoplasmic structures impedes severely its detailed study, a suitable identification and topographical analysis of SC's can easily be made.

Application of semithin sections from osmium/*p*-phenylenediamine treated tissues for electron microscopy was introduced in an attempt to analyze SC's along greater distances, thus improving the cytological study of these structures. It was shown [9] that semithin sections from conventionally fixed and stained spermatocytes can be examined in a 80–100 KV electron microscope. Thicker sections, however, require employ of the high voltage electron microscope [10–12], which was also used to observe the osmium impregnated Golgi apparatus in thick sections [13, 14]. The present results show

Reprint requests to Dr. Ondina D. Colman.

0341-0382 / 79 / 0300-0299 $ 01.00/0

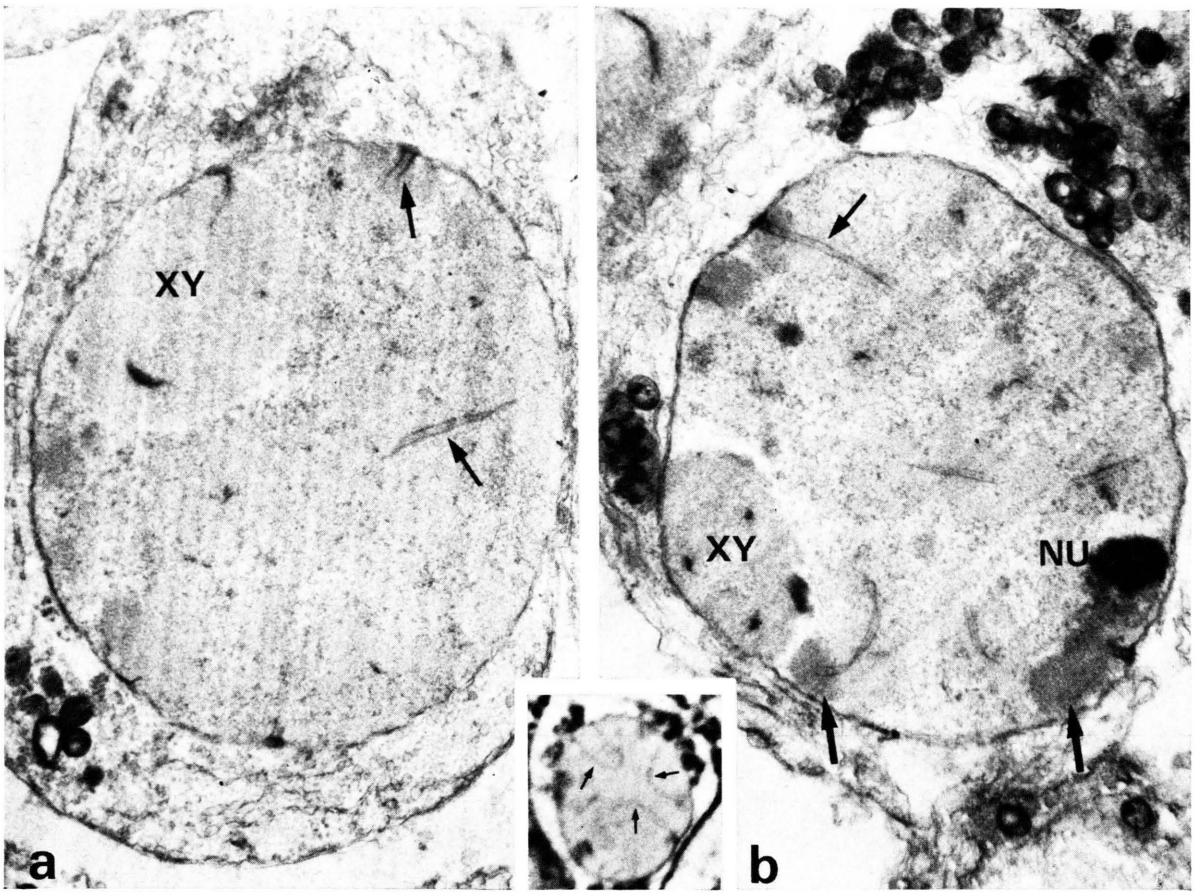

Fig. 1 a and b. Electron micrographs of pachytene spermatocytes from 0.25 (a) and 0.5 μm (b) thick sections of a mouse seminiferous tubule, after the osmium tetroxide/*p*-phenylenediamine technique. The nucleus and part of the cytoplasm are shown in both figures. Synaptonemal complexes (thin arrows) and heterochromatin masses in the form of basal knobs (thick arrows) are clearly visible. Nucleolar material (NU) and cores of the XY pair (XY) show high electron opacity, as well as membranous components. Some interchromatin materials are also recognizable. (a) and (b) : $\times$7.500.
Insert: pachytene spermatocyte from a 1 μm thick section as seen in the light microscope. Osmium/*p*-phenylenediamine, phase contrast optics. Dark granules around the nucleus are mitochondria. Arrows point out the synaptonemal complexes.

that suitable contrast and resolution can be obtained with relatively thick specimens by using the osmium/*p*-phenylenediamine technique and conventional electron microscopy. Since entire SC's may be included in a semithin section, this methodology seems to be especially promising for the study of pachytene nuclei.

This work was partially supported by a grant from the Comisión Asesora para la Investigación Científica y Técnica, Spain.

[1] M. Westergaard and D. von Wettstein, Comt. Rend. Trav. Lab. Carlsberg **37,** 239 (1970).
[2] W. F. Sheridan and R. J. Barrnett, J. Ultrastruct. Res. **27,** 216 (1969).
[3] P. Esponda and J. C. Stockert, J. Ultrastruct. Res. **35,** 411 (1971).
[4] P. Chevalier, J. Microscopie **19,** 147 (1974).
[5] J. C. Stockert, J. Histochem. Cytochem. **24,** 1207 (1976).
[6] P. Esponda and J. C. Stockert, Chromosoma **68,** 83 (1978).
[7] J. C. Stockert and O. D. Colman, Experientia **30,** 751 (1974).
[8] J. C. Stockert, Histochemistry **53,** 43 (1977).
[9] A. J. Solari, Chromosoma **29,** 217 (1969).
[10] H. Ris, J. Microscopie **8,** 761 (1969).
[11] R. C. King and H. Akai, J. Morphol. **134,** 181 (1971).
[12] R. C. King and H. Akai, Jeol News **9 e,** 2 (1971).
[13] A. Rambourg, Y. Clermont, and A. Marraud, Amer. J. Anat. **140,** 27 (1974).
[14] G. Thiery and A. Rambourg, J. Microsc. Biol. Cell. **26,** 103 (1976).