Attachment of Human Chromatin Fibers to the Nuclear Membrane, as Seen by Electron Microscopy

Fritz Lampert*
Kinderklinik der Universität München
und Institut für Hämatologie der GSF, Ass. Euratom, München

Received May 17, 1971 / July 29, 1971

Summary. Whole-mount preparations and thin sections of human interphase cells and metaphase chromosomes were examined by electron microscopy. Irregularly folded, 250 Å thick fibers, which is the basic substructure of inactive chromatin and mitotic chromosomes, were found to be firmly attached to the annuli of the inner nuclear membrane. At metaphase, fragments of the nuclear membrane were seen to adhere to the chromatids. Single fibers stretching out from the telomeres were observed connecting chromatids of nonhomologous chromosomes. A possible model of DNA replication at the nuclear pore complex is presented.


Introduction

The attachment of chromatin fibers to the annuli of the nuclear membrane has first been observed with the electron microscope in whole-mount preparations of honey-bee embryonic cells by Dupraw (1965), and later also in human cells by Comings and Okada (1970a). Studies with the ultracentrifuge also suggested a strong attachment of interphase chromatin to the nuclear envelope (Beams and Mueller, 1970). DNA, too, was found to be associated with the nuclear membrane, as investigated by its sedimentation behavior (Ormerod and Lehmann, 1971).

In this report electron micrographs are demonstrated — mainly of whole-mount preparations — confirming the attachment of human chromatin fibers to the nuclear membrane. Based on these morphological conclusions, a possible model of DNA replication at the nuclear pore complex is discussed.

Material and Methods

Human interphase cells or metaphase chromosomes were obtained from bone marrow or a 72-hr culture of blood lymphocytes. Cells used for thin sections were taken from a lymphoma tissue culture line (AL-1). Mitosis was blocked by a final incubation with Colcemid 0.1 µg/ml

* Supported by a grant (La 185/3) of the Deutsche Forschungsgemeinschaft.
for 2 to 6 hrs. After centrifugation at 600 × g for 5 min the cells were treated in a hypotonic medium (2.8 g KCl + 1.6 ml Heparin ad 500 ml distilled water) for 10 min, then washed once in distilled water and pelleted.

For spreading, one drop of the cell sediment was carefully applied on the clean surface of a modified Langmuir trough containing distilled water at pH 6.4. The surface forces on the trough spread and ruptured the cells releasing isolated nuclei and chromosome groups, which were picked up by touching the surface with a formvar-carbon-coated electron microscope grid. The grids were mounted directly in a plastic grid-holder under 30% ethanol, dehydrated through ascending concentrations to absolute ethanol, transferred to amyl acetate, and dried by the critical point method of Anderson (1951). No other fixatives or stains were used.

Thin sections were prepared by fixing the cell pellet in 5% phosphate-buffered glutaraldehyde for 12 hrs followed by postfixation in 1% buffered osmium tetroxide for 20 min, dehydration and embedding in Epon 812. Blocks were cut with a diamond knife in the LKB ultrotome III. Sections with gray interference colors were mounted on bare grids and stained with uranyl acetate.

Electron micrographs were made with a Siemens Elmiskop IA or 101 operating at 80 kV.

Fig. 1. Unstained, unsectioned human lymphocyte prepared for electron microscopy by surface spreading and critical point drying. The nucleus is composed of densely packed thin fibers which have loosened up at the periphery. A spiral arrangement of the chromatin mass can be imagined. × 6000. For comparison the inset at the upper left shows a Feulgen-stained lymphocyte with typical nuclear indentation as seen in the light microscope. × 1500