Protein-depleted Chromosomes

I. Structure of Isolated Protein-depleted Chromosomes

G. Hadlaczky, A.T. Sumner, and A. Ross
MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh, EH4 2XU, Scotland, U.K.; permanent address: Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, P.O.B. 521, Hungary; address for reprint requests

Abstract. Protein-depleted isolated Chinese hamster chromosomes have been obtained by different protein extraction procedures and examined by electron microscopy and SDS-polyacrylamide gel electrophoresis. Salt-resistant centromeric and telomeric structures are visible in protein-depleted chromosomes and the protein-depleted chromosomes appear to have a regular, longitudinal pattern in critical point dried preparations. The scaffold-like structure of protein-depleted chromosomes is highly affected by the ionic strength and composition of the extraction medium and by the spreading conditions. Nucleosomal histones of isolated chromosomes proved to be more sensitive to the sodium chloride treatment than histones of isolated chromatin. A small, but constant quantity of core histones was detected in 2 M salt extracted chromosomes and H3 and H4 histones of isolated chromosomes appeared to be resistant to the sodium deoxycholate treatment.

Introduction

Electron micrographs of chromosomes, prepared by conventional techniques, appear to consist only of nucleoprotein fibres (see Bostock and Sumner, 1978, pp. 271–274, for a discussion), and show no features that might maintain a predetermined linear arrangement of material on the chromosome. On the other hand, a fixed linear order of genes on the chromosome is well established, as is a characteristic pattern of stained bands. Thus the idea of some sort of chromosomal core which could hold the nucleoprotein fibres in a fixed pattern has attracted theoretical support (Sobell, 1973a, b; Dounce et al., 1973), although there is little actual evidence for such a structure (Stubbsfield and Wray, 1971). The technique of protein extracting isolated metaphase chromosomes to reveal a chromosome “scaffold” after surface spreading (Adolph et al., 1977a, b; Paulson and Laemmli, 1977) appears, therefore, to represent a notable advance in our understanding of chromosome structure. Essentially, when the histones and many of the non-histone proteins are extracted from an isolated chromosome, which is then spread on an aqueous surface, the
chromosome appears as numerous and extensive loops of DNA radiating from a coarsely fibrous structure having a shape resembling that of a typical chromosome and containing a characteristic set of proteins. Independent experiments have indicated that a proteinaceous "scaffold" may remain when chromosomal DNA is digested away (Jeppesen et al., 1978), while Mace et al. (1977) have shown by scanning electron microscopy that a chromosome-shaped body remains after extraction of the majority of chromosomal DNA and proteins.

In the experiments described here, we have confirmed and extended the observations made by Paulson and Laemmli (1977). Using a variety of solutions which supposedly extract different classes of histones selectively, we have attempted to gain some insight into the types of protein responsible for various aspects of the structure of protein-depleted chromosomes. It appears that chromosome scaffolds with a halo of DNA loops can be produced by a variety of procedures and that the spreading techniques are an important variable in determining the appearance seen under the electron microscope. In addition, we report certain aspects of the structure of chromosome scaffolds which have not previously been described.

Materials and Methods

Cell Culture and Chromosome Isolation

Chinese hamster (CHO) cells were grown in Ham's F-10 medium supplemented by 10 per cent Newborn Calf Serum (Sera-Lab) in a Nunc multitray. Mitotic cells were collected by shaking to dislodge the cells in mitosis, after 16 h incubation in 0.16 μg/ml colchicine. Chromosome isolation was performed by a modified Wray-Stubblefield procedure (Jeppesen et al., 1978). Mitotic cells were washed twice in cold Chromosome Isolation Medium [CIM: 1 mM CAPS, (3-cyclohexylamino-propane-sulphonic acid), 2 mM CaCl₂, 1% hexylene glycol adjusted to pH 9.6–10.0 with saturated Ca(OH)₂] and finally resuspended in warm CIM and incubated for 10–15 min at 37°C. Triton X-100 was added to the cell suspension to 0.1% final concentration and after 5 min incubation on ice the cells were disrupted by syringing through a 23 gauge needle. The released chromosomes were separated from the nuclei and cellular debris by a 10 min centrifugation at 180–200 × g. The supernatant containing the chromosomes was centrifuged at 1,000 × g and the chromosome pellet was washed once with cold CIM containing 0.1% Triton X-100 and subsequently several changes of cold Tris Chromosome Medium (TCM:10 mM Tris, 2 mM CaCl₂, 1% hexylene glycol and 0.1% Triton X-100 at pH 8.0).

To detect the proteolytic degradation of nucleoprotein "PMSF chromosome isolation" was also performed. After the disruption of cells and purification of chromosomes by differential centrifugation, the subsequent washes of chromosomes were made in TCM containing 1 mM PMSF (phenylmethylsulphonyl fluoride) in the presence of 1% isopropyl alcohol (Carter and Chae, 1976).

In both methods all procedures were performed on ice except if especially noted, and centrifugation was carried out using an MSE Chilspin centrifuge at 0–4°C.

The isolated chromosomes were stored in TCM at a DNA concentration of 1 mg/ml. The DNA concentration of the chromosome suspension was estimated at 260 nm, using a Pye Unicam SP 1750 ultraviolet spectrophotometer.

Although we failed to detect any quantitative or qualitative difference in protein composition of fresh PMSF isolated chromosomes or those stored for 3 weeks at 4°C, in the experiments only freshly isolated or 1–3 days stored chromosomes were used.

Protein Extraction of Isolated Chromosomes

Dextran sulphate extraction of chromosomes and purification of protein depleted chromosomes using step gradients were performed according to Paulson and Laemmli (1977).