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Metaphase chromosome and nucleoid differences between CHO-K1 and its radiosensitive derivative xrs-5

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Abstract

The Chinese hamster ovary (CHO) cell line xrs-5 is a radiation-sensitive mutant isolated from CHO-K1 cells. The radiation sensitivity is associated with a defect in DNA double-strand break rejoining. Chromatin structure also appears altered in xrs-5 cells compared to the parental CHO-K1 cells. Metaphase chromosomes from xrs-5 are more condensed in appearance than CHO-K1 chromosomes. The overcondensed look is not the result of colcemid sensitivity. Electron microscopy studies suggest that xrs-5 metaphase chromosomes have larger loops of chromatin extending out from the chromosome core. There are also differences between CHO-K1 and xrs-5 cells in the size and fluorescence pattern of ethidium bromide-stained nucleoid preparations. These results suggest that there is a fundamental difference between CHO-K1 and xrs-5 in either the organization of the supercoiled loops of DNA attached to the nuclear matrix or in the nature of the proteins that attach the DNA to the matrix. These alterations in chromosome structure may underlie, in part, the radiation sensitivity of xrs-5 cells.

Introduction

The multiple levels of organization of DNA in chromosomes are not only important for efficient packing of the genetic material but also are important for DNA function (van Holde, 1990). Recently we reported alterations in chromosome structure in a radiosensitive Chinese hamster ovary (CHO) cell line, xrs-5 (Schwartz *et al.*, 1990). The xrs-5 cell line is one of a group of six CHO cell lines isolated by P. Jeggo a number of years ago from an ethyl methanesulfonate-treated culture of CHO-K1 cells (Jeggo and Kemp, 1983). The xrs cell lines are all very sensitive to x-ray killing and chromosome aberration induction (Jeggo and Kemp, 1983; Kemp *et al.*, 1984; Shadley *et al.*, 1991; Jaffe *et al.*, 1990). The radiation sensitivity is associated with a reduced capacity and slower rate of DNA double-strand-break rejoining (Jeggo and Kemp, 1983; Kemp *et al.*, 1984; Jaffe *et al.*, 1990).

The xrs-5 cell line differs from CHO-K1 in that the metaphase chromosomes from xrs-5 are more condensed in appearance (Schwartz *et al.*, 1990). This overcondensed look could be a reflection of an unusual sensitivity to colcemid (the mitotic spindle poison used to collect cells in metaphase), or it might reflect some fundamental alteration in chromosome structure in xrs-5 cells. In the present study, we have expanded on our initial observations to further investigate the nature of this phenomenon.

Materials and methods

Cell lines and culture conditions

The CHO-K1 and xrs-5 cell lines used in this study were kindly provided by Dr. P.A. Jeggo. Both cell lines were cultured as monolayers in plastic T-75 tissue culture flasks at 37 °C. Cells were maintained in McCoy's 5A medium supplemented with 10% fetal calf serum, 25 mM HEPES buffer, 2 mM L-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin.

Metaphase chromosome preparation

The chromosome morphology of the cell lines was characterized by culturing exponentially growing cells for 2 h in 2×10^{-7} M colcemid. Mitotic cells were shaken off and treated with 0.075 M KCl for 15 min before being fixed in 3:1 methanol:acetic acid. Chromosomes were stained with 5% Giemsa in Gurr buffer. In some experiments, multiple sequential harvests (30-min intervals) were made with and without prior colcemid treatment. Fifty well-spread complete (21 ± 2 chromosomes) were analyzed for each cell line. As a measure of relative chromosome condensation, the ratio of relative length of the large acrocentric chromosome to its relative width was used. These measurements were made by eye micrometer.

Electron microscopy

Asynchronous or mitotic cells (selected by mitotic shake-off) were fixed in 4% glutaraldehyde, pelleted, post fixed in 1% OsO_4, and embedded in Epon-araldite for staining using conventional techniques (uranyl acetate and lead citrate).

Ultrathin sections were examined using a Phillips 100 Transmission Electron Microscope. To quantify differences in chromosome morphology, the relative widths (in arbitrary units) of chromosomes were measured in 10 sections from each cell line.

Nucleoid preparation and analysis

Nucleoids were prepared by suspending 5×10^5 cells in 0.5 ml of lysis buffer that contained 2 M NaCl, 10 mM Tris buffer, pH 8.0, 10 mM EDTA, and 0.5% Triton X-100. Nucleoids were stained with up to 50 μg/ml of ethidium bromide and analyzed either immediately or after a 10 min incubation at room temperature. Delaying analysis had no effect on results. Analysis was by flow cytometry using a Becton Dickinson FACS 440. The flow cytometric analysis allowed for measurement of nucleoid size and the degree of ethidium bromide intercalation. Both low-angle forward scatter (a measure of nucleoid size) and red (DNA) fluorescence intensity (a measure of ethidium bromide intercalation) were measured. Forward scatter and red fluorescence histograms were recorded for all samples. To analyze the light scatter response, the mean of the forward scatter distributions was used. Some cell samples were suspended in a solution of 0.6% Nonidet NP-40 and 0.2% bovine serum albumin in phosphate-buffered saline and stained with 20 μg/ml ethidium bromide, and then a cell cycle distribution was recorded. Data from 10,000 nucleoids were accumulated for each sample; triplicate samples were run. In some experiments, cells were irradiated with 12 Gy ^{60}Co gamma rays while on ice, prior to processing.

Results

Metaphase chromosome structure

As was previously reported (Schwartz *et al.*, 1990), metaphase preparations from CHO-K1 and xrs-5 cells showed distinct differences in chromosome morphology (Figure 1). The xrs-5 chromosomes are more condensed than CHO-K1 chromosomes. The overcondensed look was seen in almost all the cells examined. The distributions of length:width ratios for CHO-K1 and xrs-5 cells were distinctly different (Figure 2). The mean ± s.e.m. ratio of length:width for xrs-5 was 2.9 ± 0.2, while that for CHO-K1 was 6.2 ± 0.2.

◁ 1
◁ 2

Eliminating colcemid pretreatment yielded metaphases in which chromosomes were less condensed in both CHO-K1 and xrs-5; but even in this case, the chromosomes from metaphases of xrs-5 cells still had a more condensed appearance (Figure 2). The mean ± s.e.m. of chromosome length:width ratios for xrs-5 was 3.5 ± 0.3, while that for CHO-K1 was 9.6 ± 0.5.

Electron microscopy

To further investigate metaphase chromosome morphology, electron micrographs were prepared from mitotic cells. Once again, there were distinct differences between CHO-K1 and xrs-5 cells in metaphase chromosome appearance (Figures 3A and 3B). All the chromosome material was contained within sharply defined boundaries in CHO-K1 cells. In the xrs-5 cells, there were no sharp boundaries delineating individual chromosomes. Instead, there was a lot of chromosome material extending in what appeared to be loose arrays from the chromosome core. The relative width of the xrs-5 chromosomes, excluding the

extended chromosomal material, was actually slightly smaller than that seen in CHO-K1 cells (2.8 ± 0.1 units for CHO-K1 chromosomes and 2.4 ± 0.1 for xrs-5 chromosomes). When the extended chromosome material was considered, the width of xrs-5 chromosome nearly doubled to 4.5 ± 0.2 units. ◁ 3a ◁ 3b

Nucleoids and Nuclear Matrix Structure

Nucleoids are high-salt-extracted nuclei in which most of the proteins have dissociated from the cell, leaving supercoiled loops of DNA attached to the nuclear matrix (Cook and Brazell, 1975; Cook *et al.*, 1976). No differences in nucleoid size were found when unstained CHO-K1 and xrs-5 cells were analyzed (Figure 4). As nucleoids were stained with increasing concentrations of ethidium bromide, their size first increased as the supercoiled loops relaxed and unwound. The ethidium bromide concentration at which the maximum size is seen reflects the relative supercoiling level. There did not seem to be any difference between CHO-K1 and xrs-5 in the degree of DNA supercoiling, as the maximum relaxation was seen at about the same ethidium bromide concentration for both cell lines and the size of the CHO-K1 and xrs-5 nucleoids was similar at this ethidium bromide concentration. ◁ 4

As the concentration of ethidium bromide increased beyond the maximum relaxation concentration, the size of the nucleoids decreased as the ethidium bromide caused a positive winding of the DNA supercoiled loops. At high concentrations of ethidium bromide, CHO-K1 sizes were significantly smaller than similarly treated xrs-5 nucleoids. At these high concentrations, there appeared to be some constraint to the positive supercoiling in xrs-5 cells.

Ionizing radiation exposure will increase nucleoid size (Cook and Brazell, 1976; Kapiszewski *et al.*, 1989; Milner *et al.*, 1987). The increase (%) in nucleoid size following a 12 Gy gamma ray exposure was 33.3 ± 6.8 for CHO-K1 cells and 25.2 ± 5.9 for xrs-5. The difference was not significant.

Discussion

These studies, along with those previously reported (Schwartz *et al.*, 1990) suggest that there is a fundamental difference between CHO-K1 and xrs-5 cells in some aspect of chromosome structure or organization. Metaphase chromosomes from xrs-5 are overcondensed, being shorter and thicker than metaphase chromosomes from CHO-K1 cells. These differences are not due to any unusual colcemid sensitivity of xrs-5 cells. They also appear to be a characteristic of the entire xrs-5 population, not just an unusual subpopulation. The electron microscopy studies suggest that xrs-5 metaphase chromosomes have large loops of chromatin extending out from the chromosome core.

In addition to these differences in metaphase chromosome appearance, there are also differences between CHO-K1 and xrs-5 in their response as nucleoids to ethidium bromide treatment, which further supports the notion that these cells differ in some aspect of chromosome organization. The fluorescence profile seen in xrs-5 nucleoids stained with 50 μg/ml ethidium bromide is much broader and less resolved as compared to CHO-K1 nucleoids similarly stained (Schwartz *et al.*, 1990). No comparable differences are seen between CHO-K1 and xrs-5 cells in the fluorescence pattern of ethidium bromide-stained whole cells

(data not shown). The size of xrs-5 nucleoids stained with between 10 and 50 μg/ml ethidium bromide is also larger than that seen with CHO-K1 nucleoids (Figure 4), suggesting some constraint exists in xrs-5 nucleoids which inhibits the positive supercoiling measured in this assay.

There are other studies that suggest that xrs-5 differs from CHO-K1 in certain aspects of chromosome structure and nuclear organization. Iliakis and co-workers (Iliakis *et al.*, 1988; Iliakis and Pantelias, 1990) have reported that while they detect no difference between CHO-K1 and xrs-5 cells in DNA strand-break induction, as measured by DNA filter elution, the production of chromosome break damage per unit dose of radiation, measured immediately after irradiation by premature chromosome condensation (PCC) assay, is greater in xrs-5 cells than in CHO-K1 cells. The authors suggest that the discrepancy between DNA elution and PCC results might reflect an alteration in chromatin structure where more of the double-strand breaks are converted into chromosome breaks in xrs-5 cells than in CHO-K1 cells. Yasui *et al.* (1991) reported pronounced differences in nuclear structure in xrs-5 cells, which suggested that there was an alteration at the level of the nuclear matrix in these cells. We have recently reported faster rates of DNA unwinding under alkaline conditions in xrs-5 cells. As the rate of induction of DNA strand breaks is similar in CHO-K1 and xrs-5 cells, we suggested that the faster rate of unwinding in xrs-5 cells reflected an alteration in chromatin structure (Schwartz *et al.*, 1992).

Whether the alterations in chromosome structure are responsible for the radiation sensitivity and repair defect in xrs-5 cells remains an open question.

The xrs-5 cells were isolated from heavily mutagenized cultures of CHO-K1 cells. and Jeggo has suggested that they might contain additional mutations separate from the mutation affecting DNA repair (Jeggo, 1990). Similar differences in nucleoid patterns have been seen in other radiation-sensitive cell lines such as in V79 cells (Olive *et al.*, 1986; Milner *et al.*, 1987; Gordon *et al.*, 1990), the mouse cell line L5178Y (Kapiszewski *et al.*, 1989), cell lines from patients with ataxia telangiectasia (Taylor *et al.*, 1991), and human tumor cell lines (Schwartz and Vaughan, 1989; Vaughan *et al.*, 1992). Further study is required before conclusions concerning the role that chromosome structure differences play in xrs-5 radiation sensitivity can be made.

Acknowledgments

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Figure Legends

Fig. 1. Metaphase chromosome preparations from colcemid-treated cultures of CHO-K1 (top) and xrs-5 (bottom) cells.

Fig. 2. Distribution of metaphase chromosome length:width sizes for colcemid-treated (top) and non-colcemid-treated cell. Open symbols, CHO-K1. Filled symbols, xrs-5. Fifty metaphases analyzed for each distribution.

Fig. 3. A: Transmission electron micrographs (20,000 ×) of CHO-K1 (top) and xrs-5 (bottom) mitotic cells. Stained with uranyl acetate and lead citrate. B: Transmission electron micrographs (3,000 ×) of CHO-K1 (top) and xrs-5 (bottom) mitotic cells. Stained with uranyl acetate and lead citrate.

Fig. 4. Percent change in forward scatter in CHO-K1 (open circles) and xrs-5 (filled circles) nucleoids stained with increasing concentrations of ethidium bromide.

Figure 1 top

Metaphase chromosome and nucleoid differences.....

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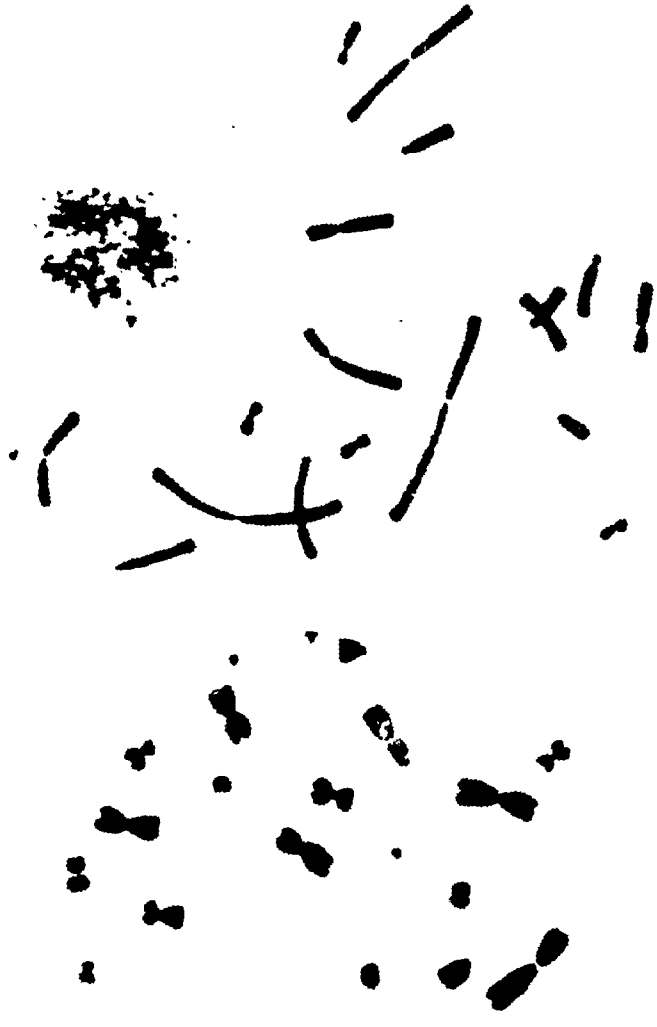

Figure 2 top

Metaphase chromosome and nucleoid differences.....

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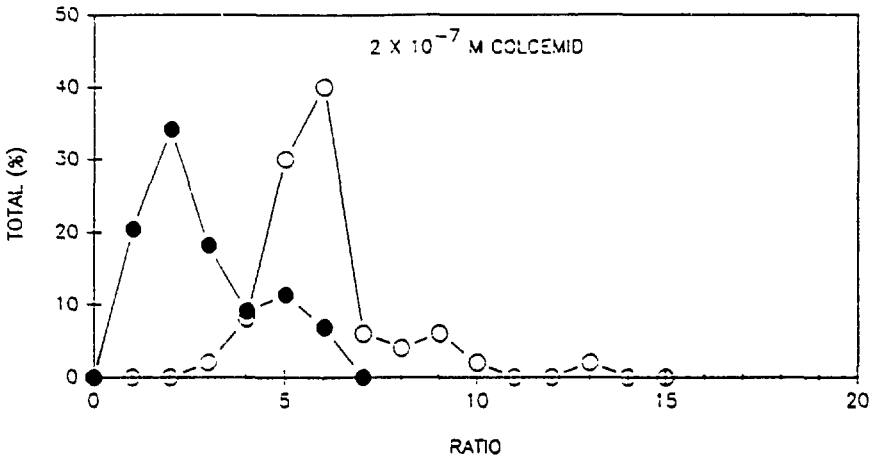

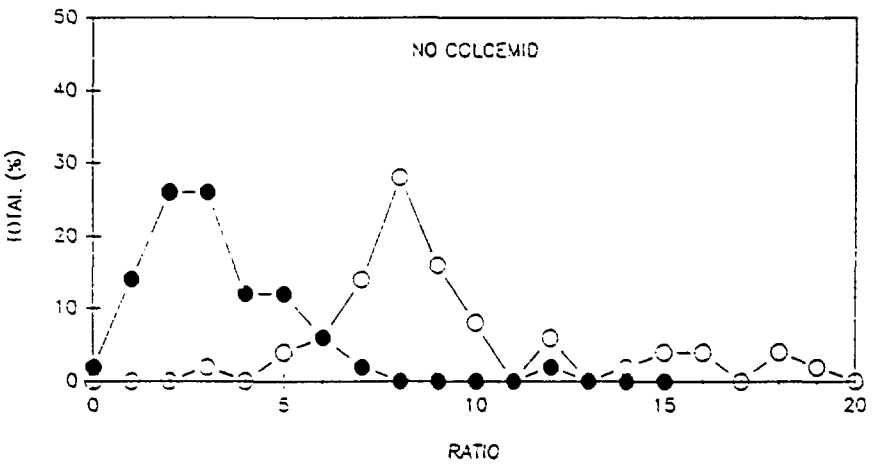

Figure 3a top

Metaphase chromosome and nucleoid differences...

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Figure 3b top

Metaphase chromosome and nucleoid differences.....

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Figure 4 top

Metaphase chromosome and nucleoid differences...

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