was also effective in enhancing SCE levels; however, the highest concentration of this compound was very toxic to the cells and hence no recognizable SCE was noticed. As seen in the table all compounds, overall, produced a dose response influence on the rates of SCE. Attempts were made to determine the effect of higher levels (than employed here) of these compounds on same parameter, but the concentrations proved to be too toxic to reveal clear cut SCE.

Discussion. For a long period of time the scientific community has been unsure of the possible role of steroid hormones or hormonal contraceptives in causing chromosome changes in mammalian cells. However, since the discovery of new, high resolution techniques for detecting subtle changes in chromosomes, there have been a few studies to resolve this controversy. Murthy and Prema [7, 8] showed that women using estrogen-progestogen combination contraceptive exhibited an increased frequency of SCE in cultured peripheral bloody lymphocytes compared to subjects consuming no oral contraceptives and pregnant women. These investigators suggested that alteration in SCE in the contraceptive-using women may be due to mutagenic activity of the estrogens or their metabolites. However, another group of workers [11] found no evidence at all of the elevated SCE in women using a similar combination of contraceptives.

The current study, utilizing the SCE technique, does indicate that the steroid hormones are capable of producing elevated SCE in cultured CHO cells. This is in complete agreement with our earlier study [6] which showed that estrogens, namely estradiol, estrone, estriol and ethynyl estradiol, were very effective in producing various types of chromosomal alterations in CHO cells. Also, an earlier study conducted on cultured HeLa cells by Rao and Engleberg [12] showed that similar estrogens showed a high degree of structural specificity in the induction of mitotic chromatid non-disjunction that resulted in undivided chromosomes in two daughter cells.

A study performed by Littlefield and coworkers [13] on the lymphocyte cultures of normal women, pregnant women and women taking oral contraceptives showed profound variations in the frequency of chromosome breakage among the consecutive cultures from the same person and among cultures from different individuals. However, a similar study [14] failed to show any significant increase in numerical or structural chromosome aberrations in women who had used the pill.

Despite existing controversies found in the literature, our studies do indicate that steroid hormones or the pill can contribute to chromosome changes in mammalian cells. These changes suggest an increased mutagenic environment in these cells, which may be due to steroids themselves or their metabolites. The mechanism by which these compounds bring about these changes is not clear, nor is the biological significance. Therefore, further studies are definitely needed to clarify the situation, and until such time it will be wise to view the current information with caution.

1 This work was supported by NIH-Minority Biomedical Research Support Program (MBRS), Grant No. RR 08124-11. The author is thankful for the technical assistance provided by NIH-MBRS student, Hoang Duong.

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0014-4754/88/010062-02$1.50 + 0.20/0

Heterochromatin associated with active versus inactive centromeres of mouse replicates at different times

B. K. Vig

Department of Biology, University of Nevada at Reno, Reno (Nevada 89557-0015, USA), 30 July 1987

Summary. A subline of mouse L-cells carries a dicentric chromosome in which one centromere always separates prematurely. This centromere is not involved in the dynamics of chromosome migration and is considered inactive. By use of anti-BRdU antibody binding to BRdU-treated chromosomes it is shown that the pericentric constitutive heterochromatin associated with the prematurely separating centromere replicates earlier than its counterpart associated with the active centromere and even before several euchromatic regions in the genome. These results point to a possible mechanism by which dicentric chromosomes segregate equationally.

Key words. Heterochromatin; dicentrics; DNA replication; L-cells; mouse L-cells; anti-BRdU antibody.

Certain mouse, rat and human cell lines have several dicentric and multicentric chromosomes. These chromosomes undergo orderly segregation in spite of the existence of more than one centromere along their length. It has been demonstrated that all but one of the centromeres in the dicentrics or multicentrics separate prematurely during prophase/early metaphase. This premature separation is related to the inactivity of the 'accessory' centromere [1–3]. By use of antikinetochore antibody found in the serum of scleroderma patients we demonstrated that the permanent dicentric and multicentric chromosomes in mouse carry only one kinetochore located at the site of the centromere which separates during late metaphase [4, 5]. It was therefore postulated that premature separation of a centromere precludes its binding to microtubules. However, this does not explain why premature centromere separation occurs. This report shows that premature separation is related to early or premature replication of the pericentric heterochromatin.

All genomes so far analyzed show a definite sequence of centromere separation. This sequence is dependent upon the

repetitive DNA found in the pericentric constitutive heterochromatin [6-8]. We therefore decided to look into the timing of replication of pericentric heterochromatin associated with the prematurely separating centromere in comparison to the block found around the functional centromere. This might help in understanding why premature centromere separation occurs and how a dicentric chromosome can behave as a monocentric.

The pericentric heterochromatin in mouse is made up of one main A : T rich satellite and a minor component which has considerable homology with the former [9]. It is therefore largely uniform in its structure. Under normal circumstances all heterochromatin replicates in the last part of the S phase. Any differences in the timing of replication may reflect qualitative differences. In a situation such as pericentric heterochromatin of mouse, which is composed of mostly similar types of repetitive DNA, one expects uniformity in the timing of synthesis of this fraction of chromatin. This communication reports that the DNA associated with pericentromeric heterochromatin in the vicinity of the prematurely separating centromere in a dicentric replicates consistently earlier than that associated with the active centromere which separates at its destined position at metaphase.

Materials and methods. A subline of mouse L-cells (ATCC CCL 91, NTC Clone 1469) kept in our laboratory for several years carries between 13 and 16 dicentrics and one octacentric chromosome [1, 2]. All but one dicentric appear to have originated from Robertsonian translocations. This places the two centromeres in the vicinity of each other in the middle of the chromosome. The exceptional dicentric has two centromeres placed about one-third of the way from the two ends of the chromosome (fig. 1 a). Upon C-banding one of the centromeres is found associated with a larger block of pericentric heterochromatin than is the other (fig. 1 b). The former is the functional centromere whereas the latter shows premature separation [8] (fig. 1 c, d).

Exponentially growing cells were treated with 10^{-5} or 10^{-6} M BUdR during the final phase of the culture and with 0.01 µg/ml Colcemid for 1 h before harvest. Cells were treated with 0.075 M KCl for 10 min and fixed in methanol: acetic acid (3 : 1) mixture. The flame dried slides were treated with 0.1 ml of IU 4 anti-BRdU antibody diluted 1 : 100 in HBSS for 20 min at 37 °C followed by goat anti-mouse IgG (US Biochemicals, Cleveland, OH) in 1 : 20 dilution in water for 10–15 min. These were stained with 1 : 1000 ethidium bromide solution in HBSS, and mounted in 5% solution of *n*-propylgallate in glycerol. Following every step the slides were rinsed with Dulbecco's buffer. The cells were examined under epifluorescence using a Zeiss microscope with BP 485/20 excitation filter. Color photographs were made using Kodak Kodachrome film, ASA 400, or Technical Pan black and white film. The exposure time ranged from 25 to 45 s.

Results and discussion. When exponentially growing cells were treated with BRdU (see 'Methods') for a period of up to 3.5 h, fixed and treated with anti-BRdU antibody, none of the metaphases showed any incorporation of the thymidine analog. The interphase cells, however, showed incorporation to varying amounts. Treatment of cells with the analog for up to 4.5 h showed only a little incorporation in certain chromosomes, both in the heterochromatin and euchromatin. All heterochromatic regions were, however, not labeled indicating that some heterochromatic regions in these cells replicate prior to late S. Additionally the results illustrate that some euchromatic segments do replicate at the end of S phase. These observations also show that the duration of the G2 period in this cell line is approximately 3–4 h.

Most cells for this study were obtained from the populations treated for 5 h during the terminal phase of culturing. Colcemid-treated cells showed varying degrees of incorporation of BRdU into metaphase chromosomes, ranging from those in which all chromosomes showed extensive label to those in which only some segments were labeled. However, attention was focused only on the labeled dicentrics. Out of 234 dicentrics analyzed 176 showed label in the pericentric heterochromatic region of both centromeres; the remaining 58 showed label in only one pericentric region which was identified as heterochromatin around the active centromere. Of these 58 dicentrics, 22 had separated at the prematurely separating centromere (fig. 2 a) while the active centromere and other chromosomes with apparently equivalent size C-bands had not yet separated into two daughter units. The label was, therefore, confined to the pericentric heterochromatic region of the active centromere which separates along with the rest of the genome (see refs 1, 4, 6 for detailed discusssion of active vs. inactive centromeres). The remaining 36 dicentrics, with only one pericentric region showing the label, were unseparated at both centromeres (fig. 2 b) as were many other chromosomes showing late replication of the pericentric heterochromatin. The criteria used to identify active or inactive centromeres used in our laboratory convinced us that the pericentric heterochromatin which had completed its replication before addition of BRdU to the cultures was indeed the one associated with the inactive centromere. These criteria are: late replicating euchromatic segment distal to the prematurely separating centromere (fig. 2 b), presence of label on both sides of the active centromere corresponding with the quantity, and pattern of localization of the pericentric heterochromatin and the differences in the degree of

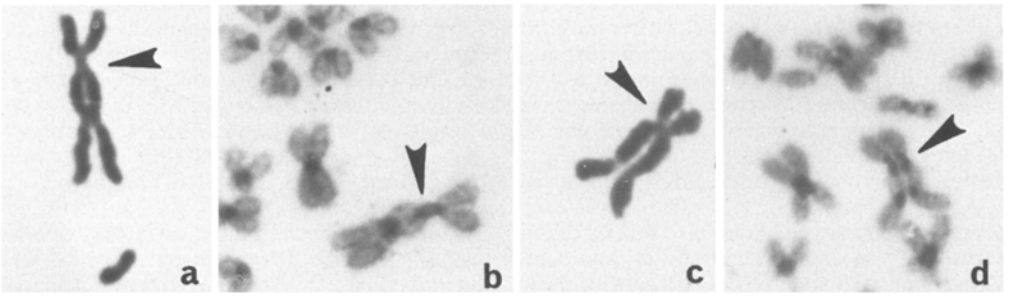

Figure 1. A set of Giemsa stained dicentrics showing the two primary constrictions (*a*) and corresponding blocks of pericentric heterochromatin (*b*); a dicentric showing premature separation of one centromere (*c*) and a C-banded dicentric in which both centromeres have separated (*d*). Configurations like the ones seen in (*a*) or (*c*) are found in cells in which most or all other chromosomes in the genome are still held at their respective centromeres testifying to the existence of premature centromere separation in the dicentrics. The prematurely separating centromere in this case is always the same and such separation is a regular feature of this and other stable dicentrics seen in all cell lines studied so far. The C-bands were produced by treating the cells with saturated solution of barium hydroxide. These show a larger block of heterochromatin associated with the active centromere. The arrow marks the active centromeres.

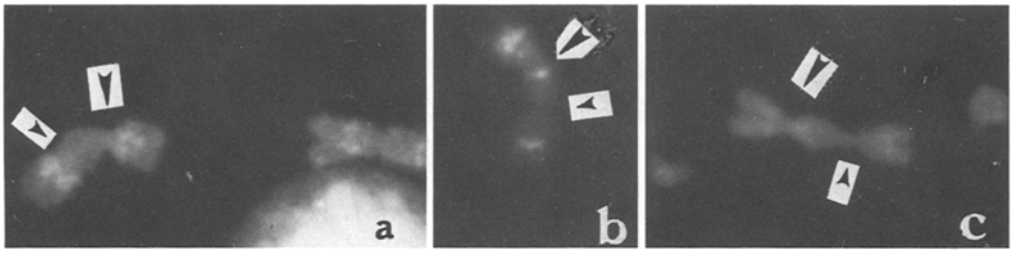

Figure 2. A panel of three dicentrics showing differential replication of pericentric heterochromatin associated with the two centromeres. The active centromeres are marked by arrows. (*a*) shows a dicentric in which one centromere is separated while the other is tightly holding the two sister chromatids. The prematurely separated centromere had completed replication of heterochromatin before BRdU was added to the culture. The segment showing BRdU incorporation below this centromere is late replicating euchromatin. The centromere that is not divided shows replication in the centromeric region as well as pericentric heterochromatin. (*b*) represents a dicentric showing replication of the region around the active centromere. The inactive centromere shows only a sign of slackening and there is no evidence of replication of either the centromeric region or the surrounding pericentric heterochromatin. Notice, however, a euchromatic block in the middle of the bottom third of this chromosome which replicates after the heterochromatin associated with the prematurely separating centromere has replicated. This pattern of replication was used to identify the position of the prematurely separating centromere in situations where neither of the two centromeres had separated (*c*).

tightness with which the two centromeres hold the chromatids together. Another 12 chromosomes showed localization of the label in only the central region of the late separating centromere. However, the mere dot-like location and weak fluorescence of the label did not appear on the photographs.

These results suggest that in mouse L-cells (i) all heterochromatin does not replicate late, (ii) there are regions of euchromatin which replicate later than certain regions of pericentric heterochromatin or regions of repetitive DNA (it is not known if the late replicating, apparently euchromatic, regions carry repetitive DNA in this cell line. It is unlikely though, that large blocks of euchromatin-like regions as seen in figure 2a would be entirely composed of repetitive DNA and yet show no C-bands), (iii) the timing of replication of heterochromatin blocks of more or less similar composition, as found in mouse, may possibly depend upon the location of the heterochromatin, and (iv) the prematurely separating centromere in the dicentric under discussion replicates its associated pericentric heterochromatin consistently earlier than that associated with the active centromere. For the present study it is immaterial whether or not the two blocks of heterochromatin have similar composition. It is entirely possible that the timing of centromere separation and the sequential separation observed in various genomes[6-8] is a reflection of the timing of replication of the pericentric heterochromatin, i.e., the sooner the pericentric heterochromatin completes its replication, the sooner a particular chromosome separates at its centromere. Thus, the timing of centromere separation may reflect the replication of this region and associated DNA sequences followed by its 'maturation' and resulting separation into daughter units. The heterochromatin of prematurely separating centromere apparently causes maturation of its centromere ahead of the maturation of equivalent region of the active centromere; hence, the former separates before the latter. A consistently sequential pattern of centromere separation in multicentric chromosomes may even suggest, a previously postulated but unpopular belief[10], that there exists some sort of interaction (or communication) between various centromeres in a given chromosome. This may result in the hierarchy leading to premature separation in dicentrics.

I am thankful to the Research Advisory Board of the University of Nevada at Reno and the Reno Cancer Center for supporting this study and to Dr L. Stanker, Lawrence Livermore Laboratory, for the generous gift of IU4.

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0014-4754/88/010063-03$1.50 + 0.20/0

Genotypic differences between the mouse adenovirus strains FL and K87[1]

C. Hamelin and G. Lussier

Centre de Recherche en Virologie, Institut Armand-Frappier, C.P. 100, Laval-des-Rapides (Québec, Canada H7N 4Z3), 18 May 1987

Summary. Restriction endonuclease analysis was used to compare the genome of mouse adenovirus (MAd) strains FL and K87. Large differences were found between the *Kpn* I, *Pae*R7, *Pvu* I, *Sal* I and *Sma* I restriction profiles of the prototype strains. MAd-FL and MAd-K87 thus represent two distinct species of mouse adenovirus.
Key words. Adenovirus; DNA; mouse; restriction endonuclease; virus disease.