The Relative Length and Arm Ratio of the Human Late-replicating X Chromosome

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The human chromosome complement at metaphase of mitosis may be divided into 10–12 sets. The chromosomes are conventionally assigned to the various groups according to the criteria of length and arm ratio. It has not yet proved possible, using these criteria, to separate the whole of the chromosome complement into 23 pairs of homologous chromosomes. The chromosomes of the X-6-12 group are particularly difficult to pair.

Recent developments in technique aid the identification of some of the chromosomes in the X-6-12 group. These techniques are the incorporation of ^3H thymidine and the enhancement of secondary constrictions. It has been shown, in the normal female, that one chromosome in the X-6-12 group incorporates ^3H thymidine at a later stage of DNA synthesis than the other members of this group (Morishima, Grumbach, and Taylor, 1962; Gilbert, Muldal, Lajtha, and Rowley, 1962; German, 1962). Evidence that this is an X chromosome is obtained also from studies on individuals with abnormal X chromosome complements (Gianelli, 1963; Rowley, Muldal, Gilbert, Lajtha, Lindsten, Fraccaro and Kaijser, 1963; Grumbach, Morishima, and Taylor, 1963; Atkins, Böök, Gustavson, Hansson, and Hjelm, 1963).

In this report we have studied the variation in length, arm ratio, and position within the X-6-12 group of the late replicating X chromosome (late X).

Material and Method

Investigations were carried out on blood culture preparations derived from 8 females, A–H, who were cytologically and clinically normal.

The late X was studied in three ways, as follows.

(1) As a heavily labelled chromosome in preparations obtained by the use of ^3H thymidine and Colcemid.

(2) As a heavily labelled chromosome in preparations obtained by the use of ^3H thymidine but without the use of Colcemid.

(3) As an unlabelled chromosome in preparations obtained by the use of ^3H thymidine and Colcemid.

Leucocytes from peripheral blood were cultured by a modification of the method of Moorhead, Nowell, Mellman, Battips, and Hungerford (1960). 'Long' labelling was performed on cultures from A–G by adding ^3H thymidine (SA.1.41 c/mM) to give a concentration of 0.2 $\mu\text{C}/\text{ml}$. of culture, four hours before termination. A repeat culture, but without the use of Colcemid, was performed on A.

'Flash-labelled' chromosome preparations were made from cultures derived from H by adding ^3H thymidine to give a concentration of 1 $\mu\text{C}/\text{ml}$. of medium. The cultures were incubated at 37°C. for 15 minutes. The cells were washed twice with medium containing excess thymidine ($100 \times$ the molar concentration of ^3H thymidine) and resuspended in this same medium. The cultures were terminated 6½ hours after reincubation. Autoradiographs were made (Bishop and Bishop, 1963) and the slides were scanned for metaphase plates in which the differentially labelled X chromosome was indisputably present. In the 'long labelled' preparations the late X was identified as the most heavily labelled chromosome in cells labelled at the end of DNA synthesis. In the 'flash-labelled' preparations the late X was identified as the only unlabelled chromosome in the X-6-12 group in cells labelled earlier in DNA synthesis. Between 10 and 20 cells which conformed to these criteria were selected from each of the 9 preparations. The cells were drawn with the X chromosome clearly marked and the location of the cells noted. The stripping film was then removed and the selected cells were re-examined. Only those cells in which the chromosomes were clear, reasonably free from distortion of shape and from overlaps were then chosen. There were 35 cells, consisting of 5 cells from each of 7 subjects, obtained from treatment (1), 15 from treatment (2), and 10 from treatment (3). The selected cells were photographed under uniform conditions and the chromosomes measured on enlarged prints.

The most satisfactory method of measurement was considered to be that made with an opisometer, and we used the type employed for map measuring. The wheel was run from the end of one chromatid to the end of the other chromatid, crossing at the centromere. This was repeated five times beginning on one chromatid and

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TABLE
POSITION OF LATE REPLICATING X CHROMOSOME IN X-6-12 GROUP

Position		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total Number of X Chromosomes Observed
Treatment (1)	(a)	0	1	3	0	4	1	5	0	1	0	1	0	0	0	0	0	16
	(b)	0	0	1	1	3	2	0	3	4	3	0	0	1	1	0	0	19
	(c)	0	1	4	1	7	3	5	3	5	3	1	0	1	1	0	0	35
Treatment (2)	(a)	2	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	6
	(b)	0	0	0	1	0	0	2	1	2	1	2	0	0	0	0	0	9
	(c)	2	0	1	2	0	1	3	1	2	1	2	0	0	0	0	0	15
Treatment (3)	(a)	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	3
	(b)	0	0	0	0	1	1	0	1	1	0	1	1	1	1	0	0	7
	(c)	1	0	1	0	1	2	0	1	1	0	1	1	1	1	0	0	10
(d)		3	1	6	3	8	6	8	5	8	4	4	1	2	1	0	0	60

- (a) The number of X chromosomes in each position, determined only by length.
 (b) The number of X chromosomes in each position, determined by length and arm ratio.
 (c) The total number of X chromosomes in each position.
 (d) The total for the three treatments.

five times beginning on the other chromatid, and the final cumulative reading was divided by 10 giving the length in cm. The 10 repeated measurements on the same chromosome varied from 1.85 cm. to 1.95 cm. The error on these measurements on the same chromosome was $\pm 3.5\%$. The length of each chromosome measured on the photograph was then divided by the magnification factor to give the actual length in microns.

Results

Chromosomes of X-6-12 group were arranged in descending order of length. They were not arranged in pairs, and for the purposes of this report the group was treated as a separate entity and the chromosomes were numbered 1 to 16 in descending order of length. When one or more chromosomes

had the same length as the labelled X they were arranged according to arm ratio, which was defined as the length of the long arm over the length of the short arm. The chromosome with the lowest arm ratio value was placed first. The Table shows the position of the labelled X in the 60 cells examined, arranged by treatments. The frequency distributions for the three treatments were tested and found not to differ significantly from each other ($p > 0.9$). These three distributions were then amalgamated (Fig. 1) to show a total distribution given in the bottom line of the Table. In this distribution the median position was 7 and the mean position 7 ± 6 (5% limits). For treatment (1) the correlation between the position and the length of the late X's

FIG. 1. The frequency distribution of the position of the late-replicating X chromosome in the X-6-12 group. Hatching indicates X chromosomes positioned by length only. Black indicates X chromosomes positioned by length and arm ratio.

gave a correlation coefficient, $r = -0.296$ and for treatment (1a), $r = -0.395$. Neither of the correlation coefficients is significant at the 5% level and there is no evidence for a correlation between the length of this chromosome and its position within the X-6-12 group.

The correlation between the length of each late X and the mean length of the other 15 chromosomes of the X-6-12 group gave a correlation coefficient, $r = 0.951$, which exceeds that required for 0.1% significance.

For the late X's the mean length was 4.71μ , and the estimate of the standard deviation of the population was 1.13. For the No. 3 chromosome, one of which was chosen at random from the same cells, the mean length was 6.29μ and the estimate of the standard deviation of the population was 1.60. Expressed as standard deviation for unit length of chromosome, the variability of the late X's is 0.24 compared with 0.25 for the No. 3 chromosomes. These ratios are not significantly different.

The mean arm ratio of the late X's for the three treatments was 1.56 (the lower 5% limit was 1.45 and the upper 5% limit was 1.70).

Discussion

German (1962) and Atkins *et al.* (1963) have commented on the relatively small size of the late labelling X chromosome in the preparations they studied. Extreme variability in the length of the late X was reported by Bader, Muller, and Mukherjee (1963) who observed it to be sometimes one of the largest chromosomes in the X-6-12 group and sometimes one of the smallest in the group. Saksela and Moorhead (1962), using a modified fixation technique for demonstrating negative heteropycnosis, placed the X 'between chromosome 7 and 8 in the X-6-12 group'. We have found (Table) that the late X in the X-6-12 group varies from position 1 to position 14 but has a mean position of 7. That is to say in terms of the Denver system, the mean late X is either indistinguishable from chromosome pair 8 if the other X is consistently larger than the late X, or from chromosome pair 9 if the other X is consistently shorter than the late X, or from pair 8 and 9 if the length of the two X chromosomes are similar. While it has been customary to place the X chromosome pair in the normal female karyotype as two of the longest four chromosomes in the X-6-12 group, there seems little justification for this in our data. Only in 14 out of 60 cells was the late X one of the 4 longest of the X-6-12 group.

Some of the observed variation in the position of the late X in the X-6-12 group may be due to differential contraction of this chromosome with

respect to other members of this group. Two factors that could cause a difference in degree of contraction of the X chromosome are treatment by Colcemid and treatment by ^3H thymidine. Though experiments were performed to investigate these possibilities, no evidence of heterogeneity (Table) was obtained. It was also considered that some of this variation in position might be due to the allocyclic behaviour of the late X which might be observed as a differential contraction during metaphase. No correlation between the position of the late X in the X-6-12 group and the absolute length of this chromosome was, however, obtained. The very strong positive correlation between the absolute length of the late X and the mean length of the remaining chromosomes in the X-6-12 group suggests that differential contraction of the late X was not observed in our data.

The mean length of the late X's was compared with that of one of the No. 3 chromosomes which were chosen as being morphologically recognizable. It was apparent that these two sets of chromosomes did not differ significantly in their variation in length. This indicates that the late X is not behaving differently from the other chromosomes in its total variation in length during that stage of mitosis which we have studied.

It is quite possible that chromosomes may show small differences in relative degrees of contraction depending on the stage of metaphase at which a cell is examined. A length difference within a homologous pair is often apparent in some cells of a preparation, particularly in the larger chromosomes. This probably represents a random difference in degree of spiralization. This variation in length of a chromosome would become more obvious when an identifiable chromosome is a member of a large group that contains others of similar length and could account for the observed randomness of the position of the late X within the group (Fig. 2).

Specific regions of late replication demonstrated by autoradiography have been identified with constrictions in certain chromosomes in the X-6-12 group (Saksela and Moorhead, 1962; Sasaki and Makino, 1963; Schmid, 1963). It may well be preferable to attempt the identification of individual members of the X-6-12 group by these newly recognizable features of labelling patterns and secondary constrictions.

Summary

The human late-replicating X chromosome, identified by autoradiographic methods, was studied in metaphases from leucocyte cultures from 8 normal females. The position of this X chromosome

FIG. 2. The X-6-12 groups from 10 cells reproduced at uniform magnification showing the variation in the position of the late-replicating X chromosomes (numbered). *Top row:* The pattern of tritium labelling of the chromosomes. *Second row:* The chromosomes from the same cell after removal of the autoradiographic film.

as defined by length and arm ratio was found to range over 90% of the X-6-12 group. The mean position, the median position, and the mean arm ratio were ascertained. There was no correlation between the position of the late-replicating chromosome in the X-6-12 group and its absolute length. This indicated that there was no demonstrable allocyclus at the stage of mitosis studied. A comparison of the late-replicating X chromosome and one of the No. 3 chromosomes showed that there was no significant difference in the variation in length of these two sets of chromosomes. It was emphasized that small differences in relative degrees of contraction of similar-sized chromosomes may cause large differences in their position within the group, as determined by length and/or arm ratio.

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