PERIPHERAL LOCATION OF THE HUMAN LATE X AND HOMOLOGOUS ASSOCIATION OF AUTOSOMES NUMBERS ONE, TWO AND THREE *

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Barton, David and Merrington (1964, 1965) have described a simple and elegant method for determining the "centroid" of a metaphase preparation. The centroid is a unique point which takes into account the distribution of the centromeres of all chromosomes. Using the centroid as the point of reference, they have, in these papers and in a subsequent report (Barton, David, Fix and Merrington, 1967) studied the distribution of human chromosomes in metaphase preparations. We have now performed this type of analysis on H^3-thymidine radioautographs, a procedure that permits unequivocal identification, by means of its characteristically late labelling, of the inactive X chromosome in the human female. The relative positions of chromosomes at metaphase is a topic of some interest (see Miller et al., 1963, p. 12 for a summary and references to earlier work), and an objective and quantitative study of the location of the inactive X has not to our knowledge yet been made.

Since the centromere of the late X gives a second unique point for each metaphase, we made use of the fact to verify the results of Schneiderman and Smith (1962), Barton and David (1962) and others, results which have indicated that homologous chromosomes tend to lie nearer to each other than would be expected on the basis of random distribution of chromosomes in the metaphase plate.

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This paper is dedicated to the late Jack Schultz, who to our knowledge was the first to have recognized the phenomenon of somatic association in human cells. Two of us (H. S. C. and D.A.H.) remain personally indebted to him for sound guidance, generously given, over an interval of many years.

Such studies, besides whatever intrinsic interest they might prove to have, could contribute further criteria useful in automated chromosome identification systems (see for example, Mendelsohn et al., 1966). Specifically, it might be possible to give some weight to the relative positions of chromosomes in analyses of data obtained with such systems.

METHODS

Radioautographs

H^3-thymidine radioautographs from three apparently normal females (accession numbers ICR 272H, 445H, and 447 H) were used. These had been prepared for an investigation of the morphology of the late-replicating X chromosome (Chandra and Hungerford, 1967). Preparative and other data are given in the earlier report. Photographic negatives of bleached radioautographs were examined and fifty were chosen (12 from 272 H, 20 from 445 H, and 18 from 447 H), the sole criteria being the presence of forty-six chromosomes and the quality of the metaphase. The choice of these metaphases was made without knowledge of the identity of the late X. Photographic enlargements ($8'' \times 10''$) of these negatives were made, and the late X was marked on each print after reference had been made to the radioautograph of the same metaphase. All measurements of distances referred to in this report were done on these enlargements.

Location of the late X

The centroid (symbol: G) of each metaphase was determined according to the method outlined by Barton, David and Merrington (1964). The x and y co-ordinates of the centromere of each chromosome were determined from arbitrary Cartesian co-ordinates. The means of the x's and y's were calculated to obtain the centroid $(\bar{x}, \bar{y})$, which was then used as the point of reference for the metaphase. The centroid method has the unusual advantage that distribution of chromosomes can be studied even in metaphases which are not circular. It thus appears to be superior to other methods which discard non-circular or irregularly-shaped metaphases (Miller et al., 1963; Ockey, 1969). On the other hand, it should be obvious that use of the centroid as centre introduces a small lack of independence between the centromere points, but, as Barton et al. (1964) have pointed out, this does not affect tests such as those to be described. Then, for each metaphase a circle was drawn with the centroid as centre and the distance between this point and the centromere of the late X as radius. The number of centromeres (not including that of the late X) lying outside this circle was counted. The late X was considered to be peripheral if the number of points outside the circle was 22 or fewer.

Proximity of Homologous Pairs 1, 2, *and* 3

With the use of simple random sampling methods a group of 25 metaphases was selected from the original population of 50 to be investigated regarding the

proximity of homologous chromosomes of pairs 1, 2, and 3. A number of correlation tests were done on this sample of 25 metaphases and these are described in the next section. Figure 1 gives details of the symbols used.

In the discussion which follows, whenever the distance between two chromosomes is mentioned, the distance referred to is that between the *centromeres* of those two chromosomes, standardised according to the procedure of Barton, David and Merrington (1964). Secondly, a chromosome whose centromere is farther away from the late X than is that of its homolog is identified by a prime (1′, 2′ or 3′) for the sake of convenience (Fig. 1). Also, the symbol X always refers to the late-replicating X chromosome and not to its unidentified homolog.

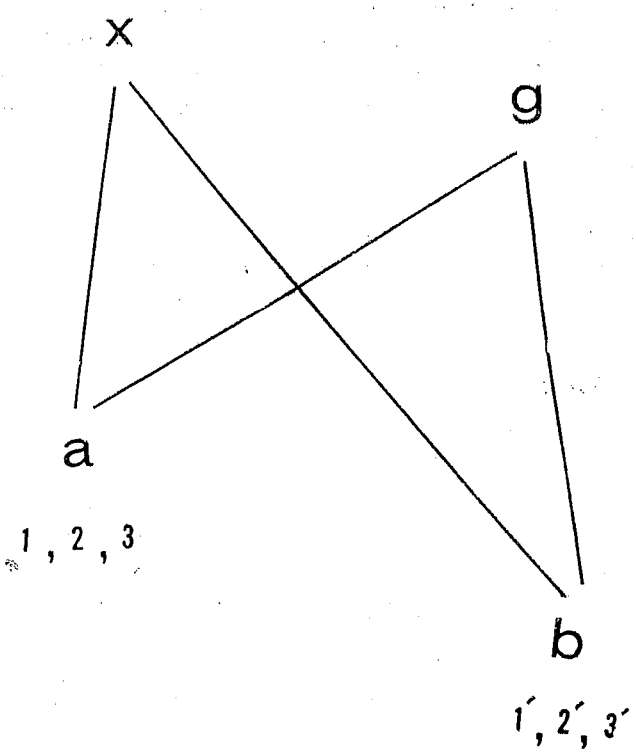

FIG. 1. Symbols used in the text and their relationships. x = centromere of late-replicating X chromosome; g = centroid; a = member of a homologous pair under discussion (1, 2 or 3) which is nearer to the centromere of the late X; b = o chromosome which is farther from the late X than its homolog (1′, 2′ or 3′).

RESULTS AND DISCUSSION

Location of the Late X

In 38 of the 50 metaphases the late X was considered to be peripheral according to the criteria outlined earlier (see Methods). Based on these data, confidence

intervals (95% level) for the proportion of metaphases in which the position of the late X could be considered peripheral were determined and found to lie between 64% and 88%, indicating that the late X is located peripherally in a significant number of cases. In terms of actual numbers, only 824 out of a total of 2,250 chromosomes were lying outside the circle defined above. Confidence intervals thus obtained were between 35%–38%, indicating that a minimum of 62% of the chromosomes lie within the circle, meaning again that the late X has a tendency to be situated away from the centre.

Proximity of Homologous Chromosomes of Pairs 1, 2, *and* 3

(*a*) *Distance from late X to homologous centromeres*: The null hypothesis that there is no correlation between the distances $(X - 1)$ and $(X - 1')$ was tested against its alternative. Similar tests were done for the remaining two pairs of chromosomes, and the results are given in Table 1. The correlation is highly significant for all three chromosome pairs, indicating that there is an association between the distances as measured from the late *X*.

Table 1. *Coefficients of correlation involving distances between the late* X *and homologous pairs* 1, 2, *and* 3

	Correlation for chromosome pairs		
	(1, 1′)	(2, 2′)	(3, 3′)
Distance from late *X*	0.613	0.580	0.555
$p =$	0.0016	0.0029	0.0063

(*b*) In order to assess the validity of the correlations observed in (*a*), above, we did the following further tests.

Correlation was determined between the distance from the late *X* to the centromere of the nearer member of a pair of homologous chromosomes (for example, distance $X - 1$) and the distance between the late *X* and a randomly chosen member of a non-homologous pair among the autosomes under consideration ($X - 2$ or $X - 2'$; $X - 3$ or $X - 3'$). Such correlation tests were done in all possible ways for the three pairs of chromosomes under consideration, and the results are summarized in Table 2 (a). These tests were repeated with use of the centroid rather than the late *X* as the point of reference (distance $G - 1$ compared with distance $G - 2'$ and so on) [Table 2 (b)].

In a second series of tests, the distance between the late *X* and a randomly chosen member of chromosome pair 1 (that is distance $X - 1$ or $X - 1'$) was compared

with the distance between the late X and a randomly chosen member of pair 2 or 3 ($X-2$ or $X-2'$; $X-3$ or $X-3'$). The one other possible combination, randomly chosen $X-2$ or $X-2'$ vs. randomly chosen $X-3$ or $X-3'$, was also tested and the results are given in Table 2 (c). As before, three further tests were done using the centroid rather than the late X as the point of reference (distance $G-1$ or $G-1'$ vs. distance $G-2$ or $G-2'$ and so on), and the correlation coefficients are given in Table 2 (d).

Table 2. *Correlation coefficients for all possible pairs of distances. In (a) and (b) one distance is kept constant, whereas in (c) and (d) neither distance is kept constant, i is chromosome 1 or 1', j is 2 or 2', k is 3 or 3'. See text for further details.*

	(*a*)				(*b*)		
	$X-i$	$X-j$	$X-k$		$G-i$	$G-j$	$G-k$
$X-1$	..	0.477*	0.392	$G-1$	..	0.045	0.148
$X-2$	0.086	..	0.247	$G-2$	−0.292	..	0.342
$X-3$	−0.120	0.464*	..	$G-3$	0.058	0.324	..
	(*c*)				(*d*)		
	$X-i$	$X-j$	$X-k$		$G-i$	$G-j$	$G-k$
$X-i$	..	0.097	0.231	$G-i$	..	0.266	0.475*
$X-j$	0.097	..	0.257	$G-j$	0.266	..	0.278
$X-k$	0.231	0.257	..	$G-k$	0.475*	0.278	..

* significant at the 5% level

It may be noted that the above tests, which may be looked upon as "controls" for the tests in (a), include a random choice of one or more chromosomes. In contrast to the highly significant correlations obtained for nearly all tests between homologous chromosomes [see (a), above] only three out of 18 tests involving non-homologous chromosomes are significant at the 5% level. The probability of this occurring under the null hypothesis that there is no correlation is 0·0583. In contrast, the probability of all three correlations in Table 1 being significant at the 0·63% level is $0{\cdot}25 \times 10^{-6}$. These results strengthen the validity of the results in (a) and thus seem overwhelmingly to confirm the conclusions of Schneiderman and Smith (1962), Barton and David (1962) and others that centromeres of homologous chromosomes tend to lie nearer to each other than expected. It is worth noting that only 2 out of the 18 correlations in Table 2 are negative

The explanation perhaps lies in the now well-known fact that the distribution of human chromosomes in metaphase is non-random.

Finally, it is worth recalling that the degree of homologous association seen in contemporary preparations is to some extent a function of hypotonic pretreatment. Therefore, association on the undisrupted spindle would be expected to be much more pronounced.

SUMMARY

By application of the "centroid" method of Barton, David and Merrington to a number of H^3-thymidine radioautographs, it was determined that the human late-replicating *X* chromosome lies towards the periphery of the metaphase in a significant number of cases. Further, in agreement with earlier findings by other authors, the centromeres of chromosomes 1, 2 and 3 were found to lie closer together than is to be expected on a random hypothesis.

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