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TRITIATED THYMIDINE INCORPORATION IN AN ISOCHROMOSOME FOR THE LONG ARM OF THE X CHROMOSOME IN MAN

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TRITIATED THYMIDINE INCORPORATION IN AN ISOCHROMOSOME FOR THE LONG ARM OF THE X CHROMOSOME IN MAN

Fraccaro et al.[1] in 1960 reported three sex-chromatin-positive females who exhibited primary amenorrhœa and other features of the Turner syndrome. Chromosome analysis revealed that these patients carried an abnormal chromosome which was interpreted as an isochromosome for the long arm of the X. Structural aberration of the X chromosome, which can be interpreted either as a deletion of the long arm or as an isochromosome for the short arm of the X, was first described by Jacobs et al.[2] The Barr bodies in this patient were reported to be unusually small. In 1961 Blank et al.[3] and Lindsten[4] described large iso-X,X/XO mosaicism in sex-chromatin-positive cases of Turner's syndrome.

Isochromosomes are generally believed to be formed as a result of misdivision of the centromere.[5][6] Strictly telocentric chromosomes arising from transverse fracturing of the centromere, are thought to be unstable owing to the centromere's irregular manner of division. The centric region heals by the rotation of one chromatid into the line of extension of the other, thus forming a metacentric chromosome with restituted centromere and with two identical arms. There are two possible isochromosomes from each normal chromosome, but it is doubtful whether both isochromosomes can be recovered from the same misdivision.

Isochromosomes may originate in somatic cells or develop from univalents in meiosis.[7] Formation of isochromosomes may also be a postmeiotic phenomenon—i.e., pollen mitosis.[8][9] With non-association and accom-

1. Fraccaro, M., Ikkos, D., Lindsten, J., Luft, R., Kaijser, K. *Lancet*, 1960, ii, 1144.
2. Jacobs, P. A., Harnden, D. G., Court Brown, W. M., Goldstein, J., Close, H. G., MacGregor, T. N., Maclean, N., Strong, J. A. *ibid.* 1960, i, 1213.
3. Blank, C. E., Gordon, R. R., Bishop, A. *ibid.* 1961, ii, 1059.
4. Lindsten, J. *ibid.* 1961, i, 1228.
5. Darlington, C. D. *J. Genet.* 1939, **37**, 341.
6. Darlington, C. D. *ibid.* 1940, **39**, 351.
7. McClintock, B. *Cold Spr. Harb. Symp. quant. Biol.* 1941, **9**, 72.
8. Rhoades, M. M. *Genetics*, 1936, **21**, 491.
9. Rhoades, M. M. *ibid.* 1940, **25**, 483.

panied failure of pulling out the centric region in the first division of meiosis, second anaphase misdivision may result. The fractured chromosome may then be carried by the fertilising spermatozoon, so that the isochromosome makes its appearance at the first cleavage division (fig. 1A).

Misdivision can also separate one of the chromatids of a chromosome arm from the remainder (fig. 1B; see also Upcott [10]) which should delay the appearance of the isochromosome until the second ensuing division and lead
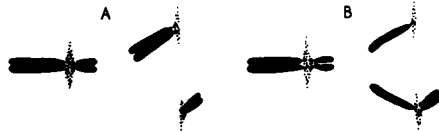

Fig. 1—(A) Misdivision with non-disjunction of chromatids, and (B) misdivision with partial disjunction of chromatids.

to the mosaic iso-X,X/XO. This mosaic is indistinguishable from misdivision at first cleavage with non-disjunction of sister chromatids (fig. 1A). If the misdivision takes place at any later cleavage division three stem-lines must be produced, one of them being the normal XX cell. The fact that both isochromosomes are not recovered suggests that the point of breakage is not exactly through the middle of the centromere.

Since metacentric chromosomes may be produced in several ways, it is difficult without exceptional genetic data, to prove that a particular metacentric chromosome is an isochromosome. The reason why the above-mentioned workers chose the isochromosome interpretation was, firstly, the consistency of the phenotypical and karyotypical appearance of this group of patients; secondly, that measurements of the isochromosomes were compatible with this hypothesis; and thirdly, the size of the Barr body. The Barr bodies have recently been investigated, with measurements of size and deoxyribonucleic acid (D.N.A.) content. [11]

By the method of D.N.A.-labelling of peripheral leucocytes with tritiated thymidine near the end of the S-period, statistical evidence may be produced for a delayed synthesis of the Barr-body-producing X chromosome in

10. Upcott, M. *Proc. roy..Soc. B*, 1937, **124**, 336.
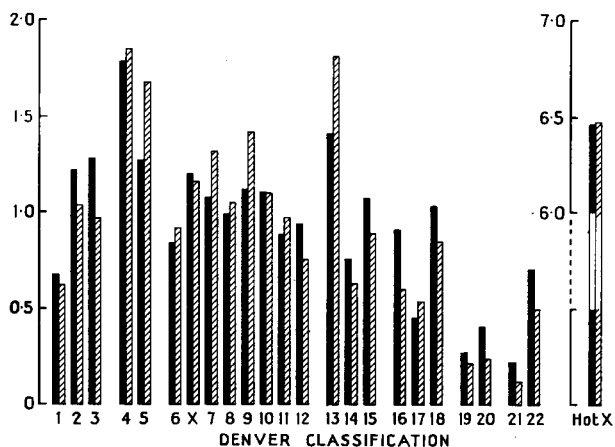11. Klinger, H. P., Lindsten, J., Fraccaro, M. (in preparation).

Fig. 2—Comparison of labelling of human chromosomes at the end of the S-period.

The ordinate shows the linear grain density of each autosome and X chromosome. The black bars are the mean figures from 33 cells in a normal female labelled at minus 4 hours, and the striped bars are from 25 cells in an iso-X, X female labelled at minus 3 hours.

man.[12] [13] Differential counts of grains over chromosome segments also showed that homologous chromosome parts did in fact synthesise their D.N.A. at the same time. By late S-labelling of leucocytes carrying the iso-X chromosome it should be possible to present evidence that (1) if the iso-X is responsible for the Barr body, then it should synthesise late in the S-period, and (2) if the chromosome is an isochromosome there should be evidence of symmetry of labelling in the two arms.

METHODS AND RESULTS

Peripheral blood-leucocyte cultures from a patient with one apparently normal X and one presumptive isochromosome for the long arm of X [1] [14] were labelled with tritiated thymidine at minus 3 hours, and autoradiographs were prepared as previously described.[12] A selection of 25 cells was chosen for complete grain analysis, the choice dependent on the morphological quality being sufficient for the positive identification of all the chromosomes. The grains over each chromosome were counted and the mean linear grain density for each chromosome was computed from the relation:

$$\text{Linear grain density} = \frac{s/l}{N/L}$$

12. Gilbert, C. W., Muldal, S., Lajtha, L. G., Rowley, J. *Nature, Lond.* 1962, **195,** 869.
13. Rowley, J., Muldal, S., Gilbert, C. W., Lajtha, L. G., Lindsten, J., Fraccaro, M., Kaijser, K. *ibid.* 1963, **197,** 251.
14. Lindsten, J., Fraccaro, M., Ikkos, D., Kaijser, K., Luft, R. *Ann. hum. genet.* (in the press).
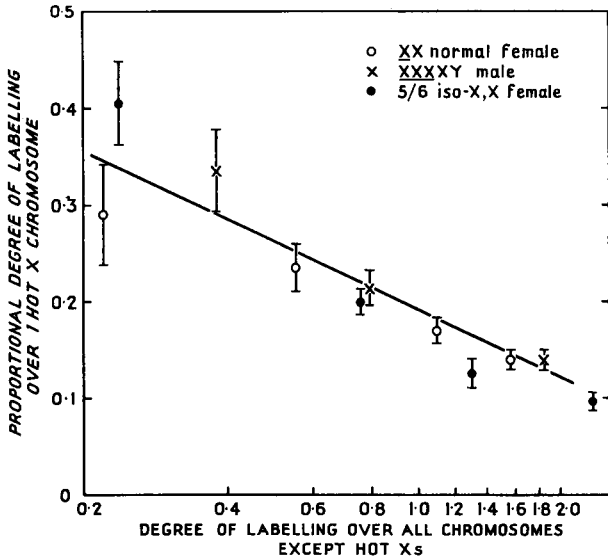
Fig. 3—Regression line relating the proportion of grains over the "hot" X chromosome to the grains over the other chromosomes in cells with different total label.

where s and l are the total grain-counts and the relative lengths of a chromosome, and N and L are the total grain-counts and the total lengths of all the chromosomes except the " hot " X. This is a slightly different definition of grain density from the one used in an earlier paper,[12] but by excluding the hot X from the totals a direct comparison can be made of materials with a hot X of different size or number. The distribution of grain density is shown in fig. 2 in comparison with the previously published results from a normal female. It is seen that the two materials show a completely similar pattern of labelling. The method of calculating the grain density makes allowance for the larger hot iso-X chromosome.

In a further 25 cells the iso-X chromosome was identified and the grains were counted. The total grains were also counted over all the other chromosomes without complete identification, thus 50 cells were available for detailed analysis of the iso-X chromosome. The proportions of grains over the iso-X to the grains over the other chromosomes were computed for cells with different total grain-counts. After allowance for the extra length of the iso-X, these ratios fitted the same regression line as for a normal female and an XXXXY male (fig. 3).[13]

Visual examination of the grain distribution over the iso-X chromosome shows a remarkable degree of symmetry over the two arms (fig. 4). A detailed distributional grain-count was made over forty-four isochromosomes (six were discarded

Fig. 4—Typical metaphase plate of a labelled iso-X,X cell. Below: average examples of less heavily labelled iso-X chromosomes.

owing to " shift " in the stripping film); each arm was divided into three equal lengths, and the grains were counted over each portion. The variance of the differences of the grain-counts over corresponding segments of the two arms of each iso-X were computed and compared with the variance of the sums of these counts. The ratio of these variances was compatible with the concept of the two arms being identical and symmetrical around the centromere.

DISCUSSION

Since no chromosome other than the large iso-X showed intense labelling at the end of the S-period, it is clearly this chromosome *only* which has a delayed synthesis. Although we always kept in mind the possibility that a normal X chromosome might sometimes be hidden under particularly heavy grain groups (when morphology is

obscured), the iso-X could not be found among the other chromosomes, so that the conclusion was unavoidable that the hot X chromosome always was the iso-X. The theory proposed by Lyon [15] to explain the variation in expression of genes on the X chromosome by regional inactivation of sometimes one, sometimes the other, of the two X chromosomes (in the form of Barr bodies) is not supported by our findings. On the other hand, the isochromosome is not a complete X chromosome.

Our results confirm the increased size of the Barr body in these cells. Since the arm-ratio of the X chromosome closely approximates to 2/3, the length of the large iso-X should be 6/5 of a normal X—an estimate which fits well with actual measurements from photographs. An increase of 20% may be visible as an increased size of the Barr body or, in the case of a small iso-X, as a reduction in size by the same amount.

It has been suggested [16–18] that the Barr body is formed by only a part of the X chromosome (" inactive "). This region is supposed to be adjacent to the centromere. If this is correct, there should be a certain relationship between the size of the Barr body and the region con-tributing to its structure. If both arms contribute equally, there should be no difference in size of the Barr body whether it be produced by a normal X or by either of the two isochromosomes. This proposition clearly does not fit the facts. The two arms evidently contribute dis-proportionately, and their contribution seems to be pro-portional to the length of the two arms. Since deletion in the long and the short arm of the X affects the size of the Barr body,[2 11] however, there seems to be a case for the view that the entire X chromosome is involved in the Barr body.

If we consider the size of the Barr body in relation to the interphase nucleus, this suggestion seems at first sight untenable. But if we consider the size of the chromosomes in anaphase/early telophase, the Barr body, developing later, could well contain the whole of the X chromosome provided that this chromosome remained in the anaphase state. Since the nuclear membrane is formed de novo in close contact with the chromosomes at the end of this stage, we have only to assume that the X and the adjacent

15. Lyon, M. *Lancet*, 1961, ii. 434.
16. Reitalu, J. *Acta Genet. med.* (Roma), 1957, **6**, 393.
17. Serr, D. M., Ferguson-Smith, M. A., Lennox, B., Paul, J. *Nature, Lond.* 1958, **182**, 124.
18. Sanderson, A. R., Stewart, J. S. S. 1960, (unpublished data).

membrane stay in this condition while the other chromosomes and the membrane surface expand. This clearly could account for the actual position of the Barr body as well as for its smallness and presumed inactive state. Surface contact with the membrane and the X chromosome might not always be achieved, and as a consequence an atypical chromocentre would be formed.

Different evidence is found in the fact that *all* of the X chromosome is heavily labelled and no significant zonal pattern can be distinguished along such chromosomes.[12] If part of the X chromosome only were involved in the Barr body, there is no reason why the remaining part should be delayed in D.N.A.-synthesis (this is evident from labelling of sex chromosomes in the Chinese hamster[19]). Furthermore, the iso-X shows a label which *closely* approximates to 6/5 of the label found over the normal hot X chromosome (fig. 3)—a fact which can be explained only by assuming a simultaneous synthesis along the whole length of the X. This shows also that the short arm must synthesise at the same time as the long arm, as was already clear from the normal female.[12] The simultaneous synthesis along the hot chromosome has created some difficulties in demonstrating the symmetry of labelling in the two arms of the iso-X. While lightly labelled iso-X chromosomes may show this symmetry in the clearest way possible (fig. 4), regional patterns become blurred when the data are pooled.

The distribution of grain density over the whole complement (fig. 2) shows a similar pattern of labelling as is found in the normal female, though there is a timing difference of an hour. The high activity over certain chromosomes—e.g., chromosome 13, is largely owing to high activity in one particular region of the chromosome. These regions are probably heterochromatic. If such regions were to behave like hot sex chromatin in relation to the total label of the nucleus a relative increase in the grain-counts over such regions would be expected when they were labelled nearer to the end of the S-period.

SUMMARY

Labelling of leucocytes from a large iso-X,X subject has lead to the following findings:

1. The iso-X is the *only* " hot " and Barr-body-producing X chromosome in this subject.

2. The symmetrical labelling pattern of the two arms of the

19. Taylor, J. H. *J. biophys. biochem. Cytol.* 1960, 7, 455.

hot X chromosome confirms the diagnosis that the metacentric chromosome is an iso-X chromosome.

3. A proportionality exists between the size of the Barr body, the size of the aberrant X chromosome, and the grain-count over this X, suggesting that the rate of synthesis is similar along the whole length of the hot chromosome. This implies that the entire sex chromosome makes up the Barr body.

4. The similarity in labelling of the iso-X and the normal X chromosome can be well demonstrated by the regression of the proportion of grains over the hot chromosome compared with the total label over the rest of the nucleus. If an allowance is made for the larger size of the iso-X (6/5) it becomes clear that the two regression lines overlap.

5. A very significant agreement is found between the relative labelling of this material and that of a normal female.

6. It is possible that heterochromatic regions (e.g., chromosome 13) may behave as the hot X with respect to the total label of the nuclei—i.e., that a similar regression line exists for such regions.

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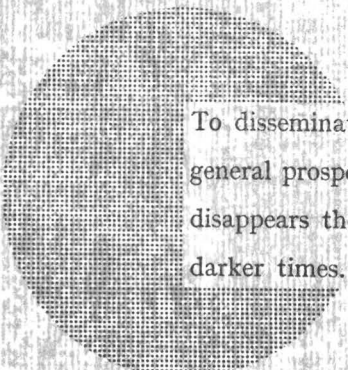
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To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel