Genet. Res., Camb. (1967), **9**, *pp.* 17–22
With 1 *text-figure and* 1 *plate*
Printed in Great Britain

Oocyte pachytene analysis of Cattanach's *fd* translocation

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(*Received* 13 *April* 1966)

1. INTRODUCTION

Cattanach (1961) has presented evidence to show that a chemically induced variegation of coat colour in the mouse is caused by a translocation between an autosome of linkage group I and the X-chromosome. This variegation—flecked (*fd*)—is normally limited to females and two types of such females occur. Type I has the complete balanced translocation, Type II an unbalanced chromosome complement possessing the translocated piece of the autosome as a duplication.

The preliminary cytological examination of the *fd* translocation made by the author in 1960 (Cattanach, 1961) on male meiotic metaphase chromosomes provided cytological evidence that it is a translocation between the X-chromosome and an autosome and suggested an attachment of a piece of autosome to the end of the X-chromosome. This was later changed by exact cytological analysis carried out by Ohno & Cattanach (1962) who reported that the translocation is insertional and that little or nothing of the X-chromosome was exchanged for a piece of autosome transposed into the X-chromosome. The present paper gives the results of an analysis of oocyte pachytene chromosomes of this translocation. Results from spermatocyte pachytene chromosomes will be published shortly.

2. MATERIAL AND METHODS

The material was obtained from Dr B. M. Cattanach in the form of Type I females 18–19 days pregnant. Shortly before the expected time of birth, the females were killed, the embryos dissected and ovaries from them were taken out. There were three types of embryo: chromosomally normal ones distinguishable by eye colour, and two translocation types—balanced and unbalanced—which could be distinguished only cytologically. Altogether there were twelve embryos of which four were normal, three were Type I, four of Type II and one was lost before the identification was possible. Besides that, casual observations were collected from nineteen different embryos of Type II.

On the microscopical slide some nuclei are unanalysable due either to too little

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squashing (there is much overlapping) or too much squashing (complicated figures are disrupted). One ovary was used to make one microscopic slide, which usually yielded twenty to thirty analysable figures.

Ordinary acetocarmine fixation followed by basic Fuchsin staining was generally employed. Microphotographs using a Baker phase-contrast microscope with Kodak Wratten filters Nos. 54 and 34 gave good resolution at about 1000× magnification.

3. RESULTS

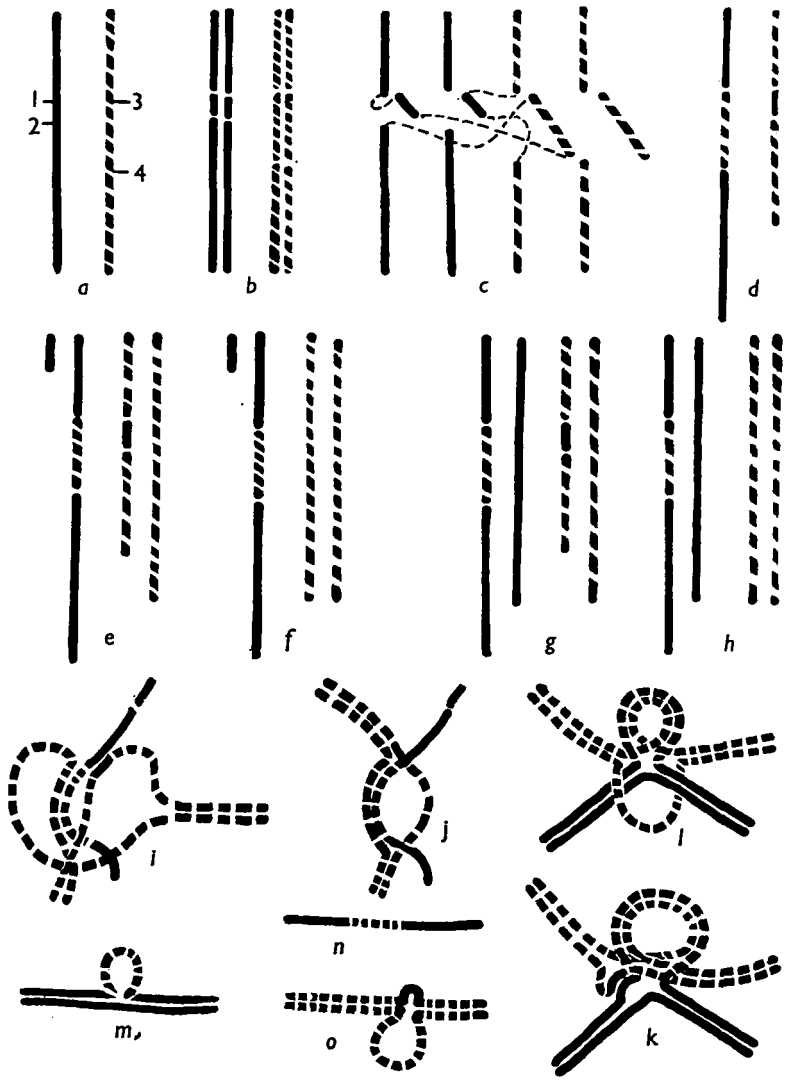
The results are illustrated by microphotographs (Plate I) and diagrams (Text-fig. 1). Oocyte pachytene configurations agree and confirm the conclusions of Ohno and Cattanach in principle. There is a piece of an autosome inserted in the mid-region of a chromosome (X–x on the photographs), which must be the sex chromosome. This chromosome is not present on the map of male pachytene chromosomes (Slizynski, 1949; Griffen, 1955). The donor autosome corresponding to linkage group I (Cattanach, 1961) is probably the chromosome mapped erroneously as the X-chromosome on both pachytene maps. On the photographs it is marked A–a. There is an urgent need for a revision of the pachytene chromosome map of the mouse.

Oocyte pachytene analysis revealed also that the translocation autosome of Type I animals contains an insertion of chromosomal material. The photographs show: (1) the identity of elements involved in the translocation configuration, namely a ring-like structure and four chromosome arms (X–x and A–a), which can be recognized in each of the five photographs (Plate I, Figs. 1–5); (2) all these photographs show at the base of the chromosome arms a heavy unresolved knob; (3) the translocation autosome separated by non-pairing from the rest of the translocation configuration is seen in Plate I, Fig. 8. It contains two loops—one in each of the paired homologues. The large loop is formed by normal autosome over the deficiency in its partner produced by translocation. The small loop is additional material (probably coming from the sex chromosome) inserted into the autosome with the deficiency; (4) the X-chromosome (separated by non-pairing) containing the insertion from the autosome is seen in Plate I, Fig. 9. The X-chromosome did not suffer any loss of material; (5) the autosome (broken off mechanically from the configuration) without the large loop shows the inserted material in non-expanded condition in Plate I, Fig. 10, and in an extremely expanded and understained condition in Plate I, Fig. 11. The expansion and loss of stainability occur in about 25% of cases.

(6) In another type of configuration found in animals, defined as Type II (Plate I, Figs. 6 and 7), the same four arms are connected to two ring-like structures, one of which is thinner; on the basis of the structural pattern it is probable that the thin ring-forming part is a homologue of the thick, paired ring-forming parts.

Normal chromosomes from a litter mate are shown in Plate I, Fig. 12.

Complete translocation quadrivalents appeared in about 20% of the 200 analysable oocytes.



Text-fig. 1. The origin of the *fd* translocation and some critical configurations. Sex chromosome, solid black; autosome, broken line. (*a*) Potential breaks induced by the treatment are marked on the chromosomes. (*b*) The chromosomes divided into chromatids and the breaks opened. (*c*) The broken ends joined in the new way. (*d*) New chromosomes: entire X-chromosome with an autosomal insertion (in inverted order) and the autosome with a deficiency of autosomal material and with an insertion of material from the sex chromosome. (*e*), (*f*), (*g*), (*h*) Constitution of Type I male, Type II male, Type I female and Type II female. (*i*) X/Y autosome quadrivalent of Type I male with all homologous regions paired. (*j*) X/Y autosome quadrivalent of Type II male. (*k*) X autosome quadrivalent of Type I female. (*l*) X autosome quadrivalent of Type II female. (*m*) Two X-chromosomes paired, one of them containing an autosomal insertion which is not paired with its homologue on the autosome. (*n*) Single X chromosome with an autosomal insertion. (*o*) Two autosomes paired. One has a deficiency and an insertion from the X-chromosome. The other, wild-type chromosome, forms a loop.

4. DISCUSSION

The finding in Type I animals of additional chromosomal material inserted into the autosome is interpreted as evidence for the reciprocality of the translocation. This point, partly suspected by Ohno & Cattanach (1962), is supported by the analysis of several favourable configurations in which the structure of the heavy knob could be partly resolved. This heavy knob cannot be due to the inversion of the inserted autosomal segment, because the whole of the autosomal segment is seen as a ring in all translocation configurations and particularly well in Plate I, Fig. 3, where it is separated from the knob. There can be no question of the inversion involving part of the ring and thus producing a knob. It must be concluded that the knob is produced by material other than the autosomal insertion. Good evidence for the reciprocality of the *fd* translocation has been found in twenty-two cases of incomplete pairing, such as shown in Plate I, Figs. 8, 9 and 10. In Plate I, Fig. 8, there are in the autosome two independent and overlapping loops described above. The material for the small loop has not come from any of the autosomes (see Plate I, Fig. 12), and it is assumed that it represents a piece of the X-chromosome reciprocally translocated and inserted into the autosome near the point of its translocation break.

In completely paired conditions the translocation configuration of Type I animals is resolved into its two components—the autosomal, seen separately in Plate I, Fig. 8, and the sex chromosomal, seen separately in Plate I, Fig. 9. When the large loop of the autosomal insertion inside the X-chromosome (seen in Plate I, Fig. 9) pairs with the large loop of the normal autosome (seen in Plate I, Fig. 8) the large ring with four chromosome arms of Plate I, Figs. 1–5, is produced. In addition, the small loop, that is the piece of X-chromosome inserted into the autosome (seen in Plate I, Figs. 8 and 10) pairs with its homologue in one or the other X-chromosome of the female, producing the heavy knob of the complete Type I configuration.

Figs. 6*a*, 7*a* and 6*b*, 7*b*, represent configurations in the oocytes of Type II females. There is no extra piece of the X-chromosome transferred into the autosome and there is no deficiency in the autosome; but a segment of the autosome is duplicated and, by not pairing with already paired homologous regions of the autosomes, it remains 'haploid'.

Figs. 8*a* and 8*b* represent the autosome bivalent (Type I) which in one chromosome contains an insertion from the X-chromosome and a deficiency; the other chromosome, which is a wild-type chromosome, forms a loop over the deficiency and over the insertion. This configuration is formed when there is no pairing of the autosomal segment inserted in the X-chromosome with the autosome.

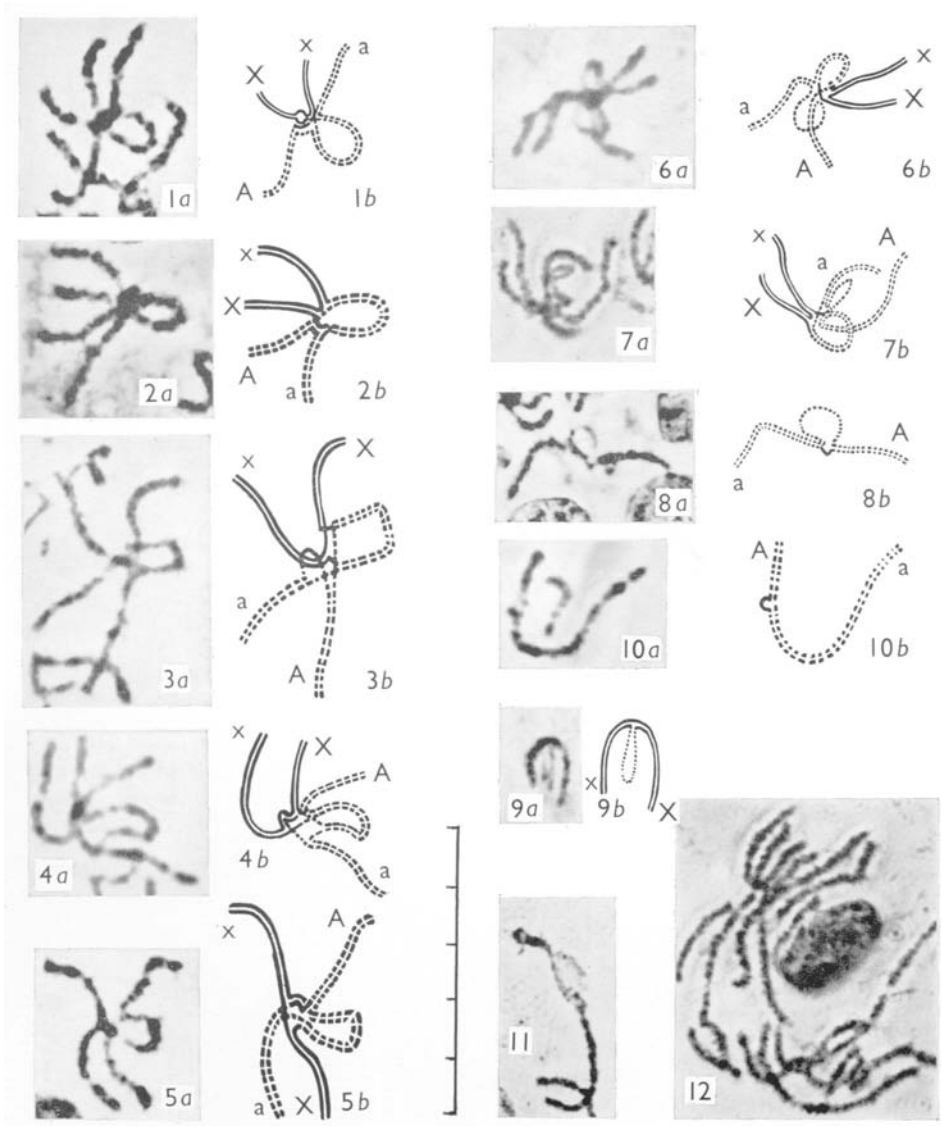
Fig. 9*a*, 9*b* of Type I shows the reverse condition. The intact X-chromosome contains the insertion which does not pair with its homologue.

Fig. 10*a* and 10*b* of Type I shows a piece of X-chromosome inserted into the autosome. The segment covering the deficiency is mechanically broken away.

Fig. 11 shows a configuration similar to that of Fig. 10, but the piece of X-chromosome inserted into the autosome is puffed and the puffing has spread over the neighbouring regions of the autosome.

Fig. 12 is a photograph of a wild-type litter mate from a mating in which Type I, Type II and wild-type females are produced.

In the diagrams, X-chromosome is represented by solid line, autosome by broken line, chromosomal elements not belonging to the translocation are omitted.



EXPLANATION OF PLATE

Figs. 1*a*, 2*a*, 3*a*, 4*a*, 5*a*, and 1*b*, 2*b*, 3*b*, 4*b*, 5*b*, are microphotographs and corresponding diagrams of the *fd* translocation in oocytes of Type I females. Note the presence of the unresolved knob at the base of the chromosome arms. The pachytene chromosomes show variation in length and thickness, but fundamental identification 'landmarks' remain unchanged. The drawings, which are simplified for clarity, present the most likely solutions of the configurations on the photographs. Figures 3 and 4 supply the evidence for the inverted nature of the autosomal insertion. (*Explanations continued on page* 20.)

https://doi.org/10.1017/S0016672300010272 Published online by Cambridge University Press

In the *fd* translocation the sex and the autosome bivalents have thus two points of association to form a quadrivalent in Type I animals. Complete association occurs in about one-fifth of analysable pachytene configurations in oocytes, being probably more frequent in females than in males, where Ohno & Cattanach (1962) observed the formation of a quadrivalent in less than 10% of meiotic metaphases.

One point of association is the small piece of the X-chromosome placed in the autosome; it pairs with its homologue in female pachytene in the oocytes. This inserted piece binds the autosome bivalent to the sex bivalent. Secondly there is the rather large piece of the autosome located within the intact sex chromosome (probably in an inverted order) by which when paired with the normal homologue received from the other parent the two bivalents are in association for the second time. The inverted order of the piece of the autosome transposed into the X-chromosome is indicated by configurations like that shown in Plate I, Figs. 3 and 4.

In Type II animals the translocation bivalents have one point of association in the form of autosomal material (Text-fig. 1, *j* and *l*, and Plate I, Figs. 6 and 7). In this case the heavy knob typical for the Type I animals is absent as in Plate I, Fig. 7. (Plate I, Fig. 6, does not show this point because of the overlapping autosome —this photograph is included in the plate solely to show two rings—one thin one thick—which are here only slightly out of focus.)

Corroboratory evidence for the reciprocality of the *fd* translocation may be found in Ohno & Cattanach's (1962) photograph (Plate I, No. 1, on page 133) of the Y/X/A/A quadrivalent in the male of Type I. This photograph was probably the reason why they partly suspected the reciprocality in this case. The configuration of this quadrivalent corresponds to Text-fig. 1*i* of the present paper. In this figure two points of association are clearly a necessity. In the absence of reciprocality any configuration involving Y/X/A/A would have only one point of association (as in Test-fig. 1*j*).

Since the translocation removed a piece of the X-chromosome but the X-chromosome does not show any resultant loss, it must be concluded that the translocation occurred at the transition from chromosome to chromatid. That is, the structural change is of the duplication/deficiency type (Slizynska, 1963).

Cattanach obtained the *fd* translocation in an experiment in which CBA males were given 0·2 mg. triethylenemelamine (TEM) per 1 kg. body weight. The first animal to show variegation was conceived 8 days after treatment of the father and thus the structural change was produced in the spermatozoon. The chemical mutagen used by Cattanach is known to produce in *Drosophila melanogaster* (Slizynska, unpublished) structural changes of the chromatid-break type with a high frequency comparable to that of formaldehyde which is a typical chromatid breaking mutagen. In the F_1 analysis Slizynska (1963) obtained about 71% of chromatid-type structural changes induced by TEM, which compares with about 8% in the X-ray experiments. The origin of the *fd* translocation, together with the resulting configurations, is suggested in Text-fig. 1.

Finally a point of interest which can be recorded here is the behaviour of the small piece of the X-chromosome inside the autosome. In about 25% of cases it becomes

expanded with a loss of stainability; there is a great deal of variation in the expression of this expansion. Two extreme cases are shown in the photographs. Superficially at least the expansion is very similar to the puff of the dipteran polytene chromosomes; however, its structure and behaviour is beyond the scope of the present paper.

The problem of *fd* is not yet solved. There are still difficulties in understanding exactly the nature of its structural changes.

SUMMARY

Oocyte pachytene analysis of embryos heterozygous for the *fd* translocation confirmed the facts of this translocation as described by Ohno and Cattanach. Besides that it revealed that a small piece of the X-chromosome has been transferred to the autosome where in about 25% of cases it shows less stainable expansion.

I wish to thank Dr B. M. Cattanach for the material and for discussion at the manuscript stage of this work.

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