Genet. Res., Camb. (1985), **47**, *pp.* 13–18 *With 2 text-figures Printed in Great Britain*

Assaying chromosome arrangement in embryonic interphase nuclei of *Drosophila melanogaster* by radiation induced interchanges

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(*Received 17 April 1985 and in revised form 25 June 1985*)

Summary

Despite recent advances in our understanding of chromatin ultrastructure, little is known of the arrangement of chromosomes during interphase, the portion of the cell cycle associated with somatic gene transcription. An experimental procedure is described which has allowed the determination of the nature of the relative arrangement during interphase of chromosomes in a specific diploid cell type of Drosophila, the salivary gland anlage of the 10–14-h-old embryo. At this stage of development the salivary gland cells have ceased mitotic divisions. Embryos of 10–14 h in age were irradiated with 12000 rads of gamma radiation and then allowed to develop into third instar larvae. The polytene chromosomes of these larvae were examined for radiation-induced interchanges. From the distribution of observed interchanges, three major features of interphase chromosome arrangement were inferred. (1) Each euchromatic chromosomal arm occupies a specific domain within the interphase nucleus which does not appreciably overlap with those of other arms. (2) Within these chromosomal domains DNA folding is very extensive. (3) The heterochromatic regions of each chromosomal arm are sequestered from the euchromatic regions. An additional point of interest concerns the nature of the interchanges observed. No reciprocal interchanges were observed – all appeared to be partial exchanges, possibly subchromatid interchanges involving only one DNA strand from each of the two exchange sites.

1. Introduction

Despite the significant recent advances in our understanding of chromatin ultrastructure (see Igo-Kemenes, Horz & Zachau, 1982 for a detailed review), we know little of the arrangement of chromosomes during interphase, the portion of the cell cycle to which most somatic gene transcription is confined. A recent review (Comings, 1980) argues that, on the basis of cytological evidence from a number of organisms, a case may be made for a non-random organization of the decondensed chromosomes of the interphase nucleus.

In 1885, C. Rabl suggested that chromosomes maintain their telophase orientation throughout interphase, occupying distinct domains within the interphase nucleus. Later T. Boveri, in his elegant studies of the nuclear cycle of *Ascaris megalocephala*, obtained evidence consistent with the Rabl hypothesis of chromosome territoriality (see Wilson, 1925 for discussion and citation of the work of Rabl and Boveri as well as relevant studies of other early workers, see especially pp. 890–895). However, this point could not be directly examined since individual chromosomes are not discernible within the interphase nuclei of most diploid cell types. It was possible that only certain chromosome regions (e.g. those associated with the centromeres and telomeres) maintained their telophase arrangement and that the intervening chromatin might intermingle with that of other chromosomes.

More recently Zorn *et al.* (1979) obtained evidence that in Chinese hamster cells synchronized in G1, the chromosomes occupy relatively compact territories within the interphase nucleus. A small segment of the nucleus was irradiated with laser – UV and then assayed for unscheduled DNA synthesis (UDS) utilizing radioactively labelled thymidine either before S phase or at metaphase (after a chase period with 'cold' thymidine followed by the induction of S phase and mitosis). The UDS was found restricted to the interphase region irradiated if cells were assayed still in G1 or in only a few chromosomes adjacent to each other on the metaphase plate if assayed at this later stage. It has since been documented that the same results are obtained if one irradiates this cell type in S phase or anaphase (Hens *et al.* 1983). Thus individual chromosomes would appear to occupy rather compact regions of the nucleus although perhaps overlapping appreciably with those of other chromosomes immediately adjacent. Attempts have been made in several cell types to determine whether

specific chromosomes associate with one another during interphase (see for example, Hager, Shroeder-Kurth & Vogel, 1982; Cremer *et al.* 1982). Indeed non-random association of specific chromosomal *regions*; namely, specific telomeres and centromeres, has been shown in some cell types (reviewed by Comings, 1980 and Avivi & Feldman, 1980).

I have developed an experimental procedure which permits determination of the relative arrangement of chromosomes in the interphase nucleus of a specific cell type of *Drosophila melanogaster*. These experiments involved the irradiation of 10–14-h-old *Drosophila melanogaster* embryos with 12000 rads of gamma radiation followed by the later examination of their third instar salivary gland polytene chromosomes for radiation induced abberations. At 8 h of embryonic development the larval salivary gland cells have been determined and undergo no further cell divisions (the first detectable salivary gland specific protein is synthesized prior to 12 h development) (see Berendes & Ashburner (1978) and Fullilove & Jacobson (1978) reviews of larval salivary gland development). These nuclei later undergo extensive endoreduplication of euchromatic DNA (Rudkin, 1969) resulting in the formation of giant polytene chromosomes by late third larval instar. We infer that any chromosomal interchange observed in the salivary gland polytene chromosomes (of third instar larvae which as embryos were irradiated at 10–14 h of development) indicates a physical association between the two chromosomal regions at the time of irradiation and/or repair. The breakpoints associated with the observed chromosomal interchanges are determined by the organization of the chromosomes of the interphase nucleus of this cell type. Regions close together at the time of irradiation can interchange and this can be visualized later in the salivary gland chromosomes of the third instar larvae following the transformation of the irradiated diploid cell into a cell containing polytene chromosomes. This chromosomal amplification allows one to observe readily the interchanges induced in the embryo. Even interchanges involving closely linked regions of DNA can be readily visualized in this system.

Two important features of polytene chromosome structure must be kept in mind. The first is that the intimate somatic pairing of the homologous chromosomes of the diploid interphase nucleus is maintained during polytenization. Indeed, each pair of homologous chromosome arms appears as a single chromosomal element in polytene nuclei. The second important feature is that the heterochromatic regions fuse to form a composite chromocentre and are exceedingly under-replicated during polytenization. Hence, heterochromatic–heterochromatic interchanges are not cytologically visible in polytene chromosome preparations.

2. Materials and Methods

(i) *Collection and irradiation of embryos*

Embryos were collected from population cages (Plexiglass) of Or-R maintained at 25 °C. The population cages were set up by adding approximately 10000 young adult Oregon-R flies. New adults were added weekly (approximately 5000–10000) and new cages were started on the third or fourth week. Prior to the egg-laying period, flies were 'prefed' fresh yeast slurry on grape juice-agar plates to avoid egg retention so that older embryos would not be collected. Drosophila were allowed to lay eggs on laying plates for 4 h. These plates were 8 in. plastic petri dishes filled to $\frac{1}{2}$ in. with grape juice-agar medium (40 g agar, 6 ml propionic acid, 6 ml 95% ethanol and 3 fl. oz. Welch's frozen grape juice concentrate plus water to a volume of 1 l). On the surface was placed a mound of, approximately, 2 g of live Brewer's yeast which was first wetted with 95% ethanol and then with water to form a yeast paste. (These plates were also used to maintain the cage populations, several fresh plates being supplied daily.)

After the egg-laying period, the embryos were maintained at 25 °C for 10 h. Ten hours after removal from the cage, the embryos were washed off the plates using lukewarm water with the aid of a paintbrush and laboratory test sieves. The sieves employed were brass framed with plated bronze mesh. Two sieves were placed together, a mesh no. 60 on a mesh no. 100 and lukewarm water used to wash the yeast off the embryos. Embryos were placed in small plastic petri dishes and irradiated in a Gamma cell with ^{60}Co gamma rays. (The embryos were placed near the centre of the irradiation chamber by placing them on top of an inverted 250 ml beaker.) Many more eggs were irradiated than were transferred to bottles of medium. This was done to ensure that egg samples did not dessicate. (It is our experience that small amounts tended to dry.) The irradiated embryos were then transferred to fresh bottles of Drosophila medium and incubated at 24–25 °C.

Samples of 10–14-h-old embryos were dechorinated and examined microscopically to determine the developmental stage. Embryos were found to fall within the developmental stages described by Bownes (1975) for 10–14-h-old embryos raised at 25 °C. This indicates that our collection conditions are appropriate.

By varying collection and post incubation times, 3–6-h-old embryos were irradiated as well.

(ii) *Cytological analysis*

Salivary glands were dissected from 3rd instar larvae in 45% acetic acid and transferred to a drop of stain (2% aceto-lacto-orcein) and immediately squashed. These temporary preparations were examined with

phase contrast optics employing a Zeiss photomicroscope.

Breakpoints of chromosomal aberrations were determined using the polytene chromosome maps of C. B. and P. N. Bridges (C. B. Bridges, 1935, 1938; Bridges & Bridges, 1939; P. N. Bridges, 1941*a*, *b*, 1942). In these maps each chromosome arm is subdivided into 20 numbered sections which, in turn, are subdivided into a variable number of subsections. All of the chromosomal arms are fused in the centromeric heterochromatin to form a common chromocentre. The heterochromatin is exceedingly underreplicated (Rudkin, 1969) and does not form polytene chromomeres. Sections 1 (telomeric) through 20 (centromeric) subdivide the *X* chromosome; sections 20 (telomeric) through 40 (centromeric), 2L; sections 41 (centromeric) through 60 (telomeric), 2R; sections 61 (telomeric) through 80 (centromeric) 3L; sections 81 (centromeric) through 100 (telomeric), 3R and the fourth chromosome consists of sections 101 (centromeric) and 102 (telomeric).

(iii) *Determination of radiation dosage*

Collections of 10–14-h-old embryos were subjected to a series of different radiation doses. At doses up to 4000 rad of gamma radiation there were decreasing proportions of adult survivors. At 8000 rad many embryos lived until the pupal stage. Similarly, at 12000 rad many larvae survived to early pupation. At a dosage of 16000 rad a decline in the proportion of embryos surviving to third instar was observed. At 60000 rad most embryos failed to hatch although in some collections a few very sluggish larvae were observed. The radiation dose chosen was 12000 rad. Although at this dosage many embryos survived to form healthy looking third instar larvae, upon dissection these had no visible imaginal discs (see Results).

At 4000 rad of gamma, few 10–14-h-old embryos survived to adults but many were morphologically abnormal and the sex ratio was distorted. In one collection of irradiated embryos distributed among 13 bottles, 4055 survived to pupation of which 143 died eclosing (or shortly thereafter) and 25 survived as viable adults. Among the 25 living adults there were 3 males, 3 intersexes and 19 females. Of the 3 males only one was morphologically normal. Of the 19 females, 10 were morphologically normal. Two of the three intersexes had additional morphological abnormalities. The most common abnormalities were misformed wings, eyes and abdomens and patches of short, thin bristles.

3. Results

With the radiation treatment utilized, 12000 rad at 10–14 h development (25 °C), third instar larvae appear, upon dissection, to have no imaginal discs although the salivary glands and most other larval tissues and organs appear relatively normal. (The brain, however, is markedly smaller and when examined in aceto-lacto-orcein squash preparations exhibits no mitotic figures and some cells and nuclei appear abnormal.)

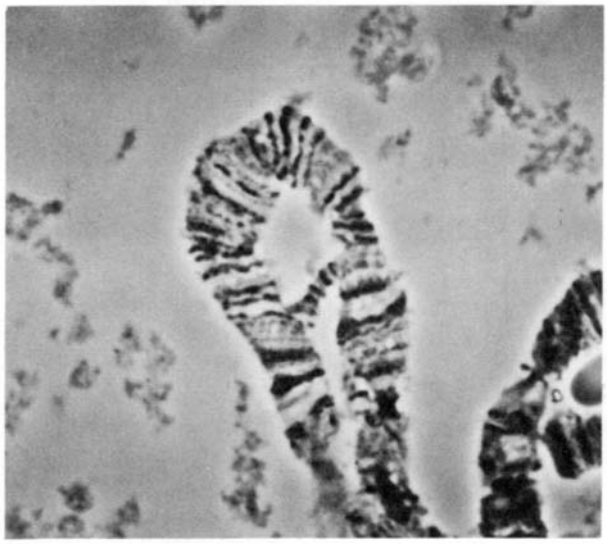

Fig. 1. Chromosomal interchange induced by irradiation of a 10–14-h-old embryo with 12000 rad of gamma radiation.

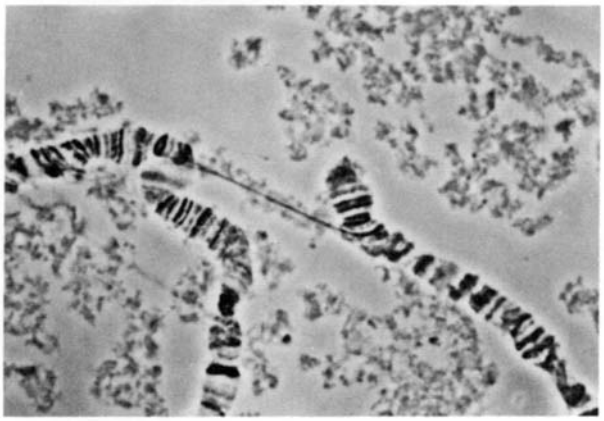

Fig. 2. An example of an ectopic strand. Unlike the interchanges, ectopic strands occur in the unirradiated controls. An additional difference exists. Crosslinks tend to occur exclusively within chromosome arms whereas ectopic strands often extend between arms.

Salivary gland squash preparations yielded essentially normal looking polytene chromosomes save for the presence of specific chromosomal interchanges in individual nuclei. The chromosomal interchange polytenized segments connecting different chromosomal regions (Plate 1, Fig. 1) are similar to those seen associated with polytene chromosomes from larvae with genotypes associated with heterozygosity for chromosomal abberations such as inversions, transpositions, translocation, etc. These crosslinks are distinct from ectopic strands which are thin, unbanded, thread-like connections between different chromosomal regions (Plate 1, Fig. 2). The polytenized

crosslinks were not seen in the polytene chromosomes of unirradiated controls. The unusual feature of these interchanges is that they do not appear to be reciprocal interchanges but rather resemble half-chromatid exchanges (i.e. a nonreciprocal interchange between two single strands of DNA) (see Fig. 1). No reciprocal interchanges were observed from the embryos irradiated at 10–14 h of development. (Recall that these nuclei undergo no mitotic divisions from the time of irradiation.)

These unusual interchanges were not observed in the salivary gland nuclei of third instar larvae irradiated at the earlier, 3–6 h stage of embryonic development, when the cells of the salivary gland anlage had further mitotic divisions to undergo. Very few chromosomal interchanges were observed in examining the nuclei of salivary glands of larvae irradiated at 3–6 h embryonic development with 1000 rad of gamma radiation. (This earlier stage exhibits much greater embryonic mortality than, following γ-irradiation, the 10–14-h-old embryos.) In over 1000 nuclei examined, only two interchanges were observed and both were reciprocal. One was a translocation between X and 3R (breakpoints: 2C–3A; 96B) and the other was a pericentric inversion of chromosome 2 (breakpoints: 39B; 51CD).

The major focus of this study was the analysis of interchanges induced in 10–14-h-old embryos. Table 1 summarizes the relative frequencies of intra- and inter-arm interchanges recovered from the irradiation of this stage. Of 74 interchanges, only 4 were between different chromosomal elements (arms). Although accurate breakpoints of the interchange cannot always be determined, approximate (or regional) breakpoints are often clear. Since each interchange was represented only by a *single* nucleus many observed interchanges could not be clearly mapped and included among the data set. Only those interchanges in which the breakpoints could be determined unambiguously to at least the polytene chromosome map subsection level were recorded. However, among those crosslinks not accurately mappable, the overwhelming majority were intra-arm interchanges. Thus, the paucity of interarm crosslinks observed represents a real phenomenon.

Table 1. *Distribution of chromosomal interchanges observed*

Chromosomal element(s)	Number
X	11*
2L	12
2R	22
3L	11
3R	14
X-2L	2
2L-3R	1
2R-3R	1
Total	74

* One of these crosslinks had a chromocentral proximal breakpoint and could not with certainty be termed an intra-arm crosslink.

Table 2 lists the cytological breakpoints of the interchanges. As noted above, the distribution of breakpoints is decidedly nonrandom with 70 of 74 interchanges (95%) being within chromosomal arms. Further, these interchanges often occur between distant sites within a chromosomal arm (Table 2). Thus, as each chromosomal interchange represents regions of close association, two major features of chromosomal arrangement during interphase can be inferred. First, each of the five chromosomal arms occupies a relatively exclusive domain within the interphase nucleus. Second, within these domains, regions that are separated by a large distance linearly may be associated, indicating that folding is very extensive; for example, DNA in the vicinity of the telomere may be in proximity to DNA associated with the proximal region of the same chromosomal arm (e.g. in 2L-23D and 40A, Table 2). Indeed, as inspection of Table 2 reveals, interchange breakpoints are generally spanned by at least several polytene sections, with very few interchanges occurring within the same section or between adjacent sections.

A third major feature of interphase chromosomal arrangement may be inferred from the paucity of heterochromatic-euchromatic interchanges. Heterochromatin constitutes approximately 25% of the major autosomal arms and 30–50% of the *X* chromosome. Hence, we might expect approximately 25% of interchanges with at least one euchromatic breakpoint to have a second breakpoint in heterochromatin. Thus among the 74 interchanges observed we would expect approximately 18 heterochromatic–euchromatic interchanges; whereas, we observe only one. Thus the euchromatic portions of each chromosomal arm appear to be relatively sequestered from the heterochromatic portions during diploid interphase. (We can make no inferences about the spatial relationships between heterochromatic regions of different chromosomal arms as heterochromatic–heterochromatic interchanges are not cytologically discernible in polytene nuclei.) It is possible that heterochromatic–euchromatic interchanges may be liable to rupture by the squashing procedure, given the underreplication of the heterochromatic DNA during polytenization. This would result in an underestimate of the proportion of euchromatic–heterochromatic interchanges. This consideration, to the extent that it is a valid one, qualifies the inference that the heterochromatin is sequestered from the euchromation.

4. Discussion

These results document that at least in one cell type of *Drosophila* (the salivary gland anlage of the 10–14-h-old embryo) the interphase chromosomes are

Table 2. *Breakpoints of chromosomal interchanges*

Element(s)	Proximal Breakpoint	Distal Breakpoint
X	Chromocentre*	2A
	19E	4C
	18BC	9BC
	15AB	7C
	12A	1A
	10D3-8	2B1-8
	8EF	6BC
	8B	1A
	5D	1A5-6
	3F	1F
	2F	2B7-8
2L	40A	23D
	35E	32BC
	34A	30AB
	34A	30C
	32AB	21B
	30A	21AB
	29D	21C
	28F	22A
	28A	25A
	25B5-C1	22B-4
	24EF	22B
	22DE	21C
2R	42A	42D
	42B1-2	46C
	42E1-2	47B
	42F	45B
	43B	57A
	43C	48D
	44C	53A
	44D	48C
	44F	47EF
	48E	53D
	49B	56B
	49F	54A
	52A	54B
	52F	54CD
	55A	58AB
	55C	60E
	55E	57E
	56D	57B
	56F	58E
	57A	59C
	58CD	60F
	60C7-D2	60E3-4
3L	76C	62B
	74A	71C1-2
	71B	69B
	70C	67DE
	68A1-2	63E
	67C1-4	66B
	64C6-8	63B
	64AB	61C
	64B	62CD
	63E5-9	62A1-5
	62B5-8	61C3-5
3R	82A11-14	85D17-21
	85A	86A
	85E	87A
	86C1-5	87F
	87F	89C
	88A	89A
	89F;90A	94D
	90A1-2	100F1-2
	92D	93D
	92F	94C
	93E	95C
	95E	98F1-2
	98B1-2	100B5-9
	99D1-2	100F1-3
X-2L	3A1-5	21E
	2A1-4	24D
2L-3R	30BC	90D
2R-3R	43CD	82E

* As the proximal breakpoint was chromocentral, it could not, with certainty, be assigned to the X chromosome heterochromatin, hence it may have been an inter-arm crosslink.

nonrandomly arranged. Each of the five major euchromatic chromosomal arms occupies a relatively exclusive domain within the interphase nucleus. Within each domain, regions quite distant in DNA linear dimensions are associated; however, we do not know if specific associations are regular within domains. Much more data must be accumulated to answer this question. Finally, the heterochromatic regions appear to be relatively sequestered from euchromatic chromosomal segments in the diploid interphase nucleus.

Similar results have been obtained from the analysis of Mathog *et al.* (1984) of the topological arrangement of chromosomes of fully polytenized salivary gland nuclei. Intact nuclei were stained with DNA-specific fluorescent dyes and examined in twenty-four optical planes. An image-processing computer program integrated the images to produce a three dimensional map of chromosomal arrangement. Fourteen nuclei were so analysed. Individual euchromatic chromosome arms exhibited extensive intra-arm folding but did not intertwine with one other. Further, intra-arm folding patterns did not appear to be limited to a specific configuration.

Thus in both diploid (embryonic) and fully polytenized salivary gland nuclei euchromatic chromosome arms are sequestered to individual domains of somatically paired elements exhibiting extensive intra-arm folding. This may be a feature of all interphase nuclei in *Drosophila*, diploid and polytene.

The organization of chromosomal arms into exclusive interphase domains may be of profound functional significance. Consistent with this notion are the earlier findings of Sturtevant & Novitski (1941) who noted that linkage groups, as defined by chromosome arms, are conserved within the genus *Drosophila*. Indeed, recent work (Foster *et al.* 1981) suggests that these major linkage groups have

remained intact during the evolution of the higher Diptera. Linkage group conservation may be related to the functional arrangement of chromosomes in the interphase nucleus. Each linkage group occupies an exclusive domain within the interphase nucleus and the arrangement of the chromosome arm within the domain may be crucial for the regulation of gene expression. Paracentric inversions, which are common within and between *Drosophila* species, would not upset the integrity of the chromosomal domain and compensatory intra-arm folding might allow important regional associations within the domain to be maintained.

Alternatively, the sequestering of somatically paired euchromatic chromosomal arms into individual domains may be of little or no functional significance. It may simply reflect the behaviour of the euchromatic DNA regions in solution, *i.e.* their physical behaviour upon decondensing at telophase. Perhaps, the uncoiling euchromatic DNA may tend to localize around the region defined by its position following its migration to the centriole.

I would like to thank Tannis Armstrong Neheli for her excellent technical assistance, Dr Rudi Appels for earlier discussions, and Dr Arthur Chovnick for his comments on the manuscript. This research has been funded from grants from the Research Advisory Board of the University of Guelph and the National Science and Engineering Research Council of Canada.

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