The *Drosophila melanogaster dodecasatellite* sequence is closely linked to the centromere and can form connections between sister chromatids during mitosis

Mar Carmena¹, Jose P. Abad², Alfredo Villasante² and Cayetano Gonzalez¹,*

¹Department of Physiology and Anatomy, Medical Sciences Institute, University of Dundee, Dundee DD1 4HN, Scotland
²Centro Nacional de Biotecnologia and Centro de Biologia Molecular, CSIC, facultad de Ciencias, Universidad Autonoma, 24049 Madrid, Spain
*Author for correspondence

SUMMARY

We have used fluorescence in situ hybridisation to wild-type and rearranged mitotic chromosomes to map the *Drosophila melanogaster dodecasatellite* sequence. It is located at a unique site, within the pericentric heterochromatin of the right arm of the third chromosome, closely linked to the primary constriction. In polytene chromosomes, *dodecasatellite* is found as one or a few dots in the central region of the chromocentre. In untreated diploid cells, *dodecasatellite* sequences are found as one or two dots throughout the cell cycle. This distribution can be altered in a cell cycle-dependent manner in two ways. Firstly, in interphase cells, hypotonic shock promotes the decondensation of the genomic region containing this satellite, resulting in a string-like structure. Secondly, some of the precociously separated sister chromatids produced by colchicine treatment show *dodecasatellite* within the intervening space connecting the main *dodecasatellite* signals of each chromatid. The distribution of *dodecasatellite* seems to be rather constant between individuals of the same species, as indicated by the lack of any detectable variations in its pattern amongst individuals from six geographically distant strains of *D. melanogaster*. On the other hand, the distribution of *dodecasatellite* shows a remarkable degree of variation amongst closely related species of the *melanogaster* subgroup ranging from a non-detectable signal in *Drosophila yakuba* and *Drosophila teissieri*, to staining in the X, second and third chromosomes of *Drosophila mauritiana*.

Key words: *Drosophila*, satellite, centromere, in situ

INTRODUCTION

In *Drosophila melanogaster*, heterochromatin accounts for about 29% and 35% of the haploid genome in females and males, respectively (Gatti et al., 1976). Apart from the Y chromosome, which is entirely heterochromatic, most of the heterochromatin is located at the pericentromeric regions of the chromosomes. Genetic analysis in this organism has revealed the presence of numerous functional sequences within the heterochromatin, such as: rDNA (Glover, 1981; Hawley and Marcus, 1989), a region that promotes pairing between the Y and the X chromosomes during meiosis I (Cooper, 1964; McKee and Karpen, 1990); fertility factors on the Y chromosome (Brosseau et al., 1961; Kennison, 1981; Gatti and Pimpinelli, 1983); the ABO regions (Pimpinelli et al., 1985; Tomkiel et al., 1991); *Rsp* (Wu et al., 1988); *E(SD)*, and a series of essential loci in the second and third chromosomes (Marchant and Holm, 1988; Hiliker et al., 1980). Furthermore, in *Drosophila*, as in many other organisms, heterochromatic regions are known to contain the centromeres, the sequences involved in kinetochore formation and pairing between sister chromatids.

Heterochromatin is largely made up of satellite DNA, which consists of a series of tandem repeats of a simple sequence (see Singer, 1982, for a review). About 21% of the haploid genome of *D. melanogaster* is satellite DNA (Lohe and Brutlag, 1986); until very recently, all satellite sequences identified in this organism had been isolated on the basis of their buoyant densities. As technologies, such as Yeast Artificial Chromosomes (Burke et al., 1987) and Pulsed Field Gel Electrophoresis (Schwartz and Cantor, 1984) have enabled the cloning and molecular characterisation of large genomic fragments, new sequences have been identified within the pericentric region. One such sequence is *dodecasatellite*, identified by Abad et al. (1992), who also reported that it maps to one of the major autosomes in *D. melanogaster*.

Here we present a cytological analysis of this satellite. The aims of this work are the following: (1) to identify the chromosome to which it maps and to determine whether...
dodecasatellite sequences are also found in euchromatic regions; (2) to define the region within the chromosome where this satellite is located, in particular its proximity to the primary constriction, the spindle fibre attachment region; (3) to analyse the extent of the conservation of the distribution of these sequences among individuals from different strains of *D. melanogaster*, and from different species of the *melanogaster* subgroup, and (4) to study the behaviour of dodecasatellite during different phases of the cell cycle.

**MATERIALS AND METHODS**

**Drosophila strains**

Six different wild-type strains of *D. melanogaster* were analysed in this work. These included two standard laboratory stocks (Oregon-R and Canton-S), and four strains chosen on the basis of geographical distance (Vallecas from Spain, Saltillo from Mexico, Hiroshima from Japan, and Crimea from The Ukraine). Four species of the *melanogaster* subgroup (*D. simulans*, *D. mauritiana*, *D. teissieri* and *D. yakuba*) were also studied.

The following chromosomal rearrangements, a detailed description of which is given by Ashburner (1989) and Lindsey and Zimm (1992), were used: C(2)EN, cn bw, which consists of two second chromosomes joined together to a single centromere; C(3L)P1; C(3L)P3, ri; C(3L)P6: C(3L), h; C(3L), st; C(3R)P2; C(3R)P3, sr; C(3R)P6, and C(3R), r, which are compound chromosome arms, each of which contains two copies of either the left, C(3L), or the right, C(3R), arm of the third chromosome, joined together. F(3L); F(3L), h; F(3R), c; F(3R)1, e<sup>+</sup> ro ca and F(3R)2, e<sup>+</sup> ro ca are free arms that contain only the euchromatin from either the left, F(3L), or the right, F(3R), arm of the third chromosome, plus an uncharacterised amount of heterochromatin. They are thereby acrocentric or telocentric derivatives of the metacentric third chromosome. T(Y;3)B197 is the result of a translocation between the Y and the third chromosome; the autosome breakpoint is on the right arm, in bands 96B1-10 of the polytene chromosomes. *TM6B*, *Hu e Tb ca* is a submetacentric derivative of a third chromosome that carries multiple overlapping inversions.

**DNA probes**

Probes were made using random oligonucleotide primers as described by Sambrook et al. (1989). Around 100 ng of an EcoRI/NorI fragment from plasmid pBK6E218 (Abad et al., 1992), purified from a low melting point agarose gel, were heat denatured, chilled on ice, and added to a solution containing 5 μl oligolabelling buffer, 1 μl biotin-16-UTP, 1 i.u. Klenow fragment, and water up to a final volume of 25 μl. After incubating overnight at room temperature, unincorporated nucleotides were removed in a Spin column (Pharmacia) and DNA was precipitated with ethanol. The precipitate was pelleted by centrifugation in a microfuge, the supernatant discarded, and the pellet air dried and resuspended in 50 ml water.

**In situ hybridisation**

**Mitotic chromosomes**

Brains from third instar larvae were dissected in saline (0.7% NaCl). Colchicine treatment was performed by incubating the brains in 0.5 mg/ml colchicine in saline for 1 to 2 hours. This treatment was carried out in a humid chamber, in darkness. Following colchicine treatment, the brains were given a hypotonic shock by incubation in 0.5% trisodium citrate. They were then placed on a microscope slide, fixed for 30 s in 45% acetic acid and 3 min in 60% acetic acid, covered with a siliconised cover-slip, and squashed between two sheets of blotting paper. Immediately after squashing, the preparation was frozen by immersion in liquid N<sub>2</sub>; the coverslip removed with a scalpel and the tissue dehydrated in 70% and 100% ethanol, for 5 min each, and air dried. Prior to denaturation, the slides were baked at 58°C for one hour in a dry oven. Denaturation was carried out by treatment with 70% formamide in 2× SSC at 70°C, for 2 min. After denaturation the slides were dehydrated as before. Hybridisation was carried out overnight at 58°C after applying 10 μl of a 1:1 solution of heat-denatured probe (prepared as described above) and 2× in situ hybridisation solution (2× Denhardt’s, 20% dextran sulphate, 0.4% salmon sperm DNA). Excess probe was washed off by treatment in 2× SSC at 53°C for 2 min, followed by 4× SSC for 5 min, 0.1% Triton X 100 in 4× SSC for 5 min, and 4× SSC for 5 min. The slides were then dipped in a solution containing 2% (v/v) FITC-avidin (Vector, cat. no. A2001) for 30 min and washed in 4× SSC for 5 min, 0.1% Triton X 100 in 4× SSC for 5 min, and 4× SSC for 5 min. All these treatments were carried out at room temperature. Slides were then mounted in a solution containing 1 mg/ml propidium iodide, 2.5% propylgallate, 85% glycerol, and sealed with nail varnish.

**Polytene chromosomes**

In situ hybridisation to polytene chromosomes was performed as described by Ashburner (1989) with the following modifications: probes were labelled with biotin-16-UTP and, after hybridisation, slides were processed exactly as described above for in situ hybridization to mitotic chromosomes. As an internal control, and to gain information on the degree of repetition of dodecasatellite in polytene chromosomes, in some instances around 100 ng of a 0.9 kb SalI fragment from plasmid pNB402a2, corresponding to the cDNA of the *polo* gene (Llamazares et al., 1991), was added to the oligolabelling reaction. This sequence is unique, and maps to the euchromatic region of the left arm of the third chromosome in band 77A3 (Llamazares et al., 1991).

Observations were made with a confocal MRC-600 microscope (BioRad). Image processing was performed following the instructions given by the manufacturer.

**RESULTS**

*dodecasatellite* sequences have previously been reported to map to the pericentric region of one of the major autosomes of *D. melanogaster* (Abad et al., 1992). To define further the localisation of this satellite we sought to determine whether it is restricted to the pericentric heterochromatin or can also be found interdispersed along euchromatic regions, and to identify the chromosome and the chromosomal region to which this satellite maps. To this end we performed in situ hybridisations to wild-type polytene chromosomes and a series of wild-type and rearranged mitotic chromosomes.

*dodecasatellite* is restricted to the middle part of the chromocentre of polytene chromosomes and maps to the right arm of the third chromosome

We were unable to detect the presence of *dodecasatellite* sequences within the euchromatic regions of any of the polytene chromosome arms (Fig. 1A). Instead, the satellite was only found in the middle of the chromocentre, either as a unique dot or as a group of up to four dots. In all cases, regardless of the number of dots, the hybridisation signal was compacted, its boundaries well defined, and always at
a distinct site, far away from the most proximal euchromatic regions of each chromosome. In those instances (as the one shown in Fig. 1A) in which a 0.9 kb fragment from a known euchromatic sequence (Llamazares et al., 1991) was included in the labelling reaction to provide an internal control, it was possible to appreciate that the intensity of the signal produced by the satellite is of about the same order of magnitude of that produced by the euchromatic probe.

To better identify the region of the heterochromatin to which dodecasatellite maps, we performed in situ hybridisation to mitotic chromosomes. The analysis of wild-type cells during mitosis, before chromosome condensation is fully achieved, made it possible to locate the dodecasatellite to the most proximal region of one of the arms of a pair of autosomes, very near to the primary constriction, but slightly shifted towards one arm (Fig. 1B). The signal produced by dodecasatellite occupies a region that accounts for a significant part of the whole heterochromatin content of that autosomal arm.

Although the presence of a secondary constriction in the second chromosome can be used to discriminate between the two major autosomes in D. melanogaster (Hinton, 1942), we positively identified the chromosome containing dodecasatellite by performing in situ hybridisation to a stock carrying a C(2)EN chromosome. This rearrangement contains two second chromosomes attached to the same centromere. C(2)EN chromosomes are thereby about twice as large as a wild-type second chromosome and lack a homologue, thus providing an easy cytological marker. In situ hybridisation to mitotic cells carrying such a rearrangement (Fig. 1C) showed that dodecasatellite maps to the third chromosome. Again, the hybridisation signal can be seen very near to the primary constriction, but slightly shifted towards one arm. To determine to which arm of the metacentric third chromosome these sequences map, we performed in situ hybridisation to mitotic chromosomes of Drosophila dodecasatellite

Fig. 1. Mapping the dodecasatellite to polytene (A) and mitotic (B to F) chromosomes. (A) In situ hybridisation to polytene chromosomes with probes against dodecasatellite (arrow), and the euchromatic gene polo (arrowhead). (B) Wild-type prometaphase, showing dodecasatellite staining in the pericentromeric region of one pair of major autosomes. Only one of the fourth chromosomes can be seen in this micrograph (IV). The arrows point to the primary constriction of the chromosomes to which dodecasatellite maps. (C) c-metaphase in a cell from an individual carrying a C(2)EN chromosome, a large derivative of two wild-type second chromosomes that can readily be identified. dodecasatellite sequences are restricted to the third chromosome (III). (D) Prometaphase from an individual carrying T(Y;3)B197/TM6B. The proximal element of the third chromosome in T(Y;3)B197 is labelled IIIp, and the TM6B chromosome is labelled TM6. Insets (E) and (F) show the hybridisation of the satellite to the short arm of the TM6B chromosome and to the arm that contains the euchromatic breakpoint of T(Y;3)B197, respectively. The images shown in (E) and (F) have been electronically processed to facilitate the identification of the arm to which dodecasatellite maps. X, Y; the X and Y chromosomes. Bar, 5 µm.
sation to cells carrying a translocation between the third and the Y chromosome. The Y chromosome is entirely made up of heterochromatin. Heterochromatin can be readily identified in squashed preparations stained with propidium iodide because of its high fluorescence signal, and because the corresponding heterochromatic segments within sister chromatids frequently remain paired following hypotonic shock, which separates the euchromatic regions. In situ hybridisation to cells carrying T(Y;3)B197 (Fig. 1D and inset 1F), showed that dodecasatellite sequences are located in the same arm that carries the autosomal translocation breakpoint (i.e. the right arm; see enlargement in Fig. 1F). This result is substantiated by the location of these sequences within the short arm of the submetacentric TM6B chromosome (Fig. 1D and inset 1E). This highly rearranged third chromosome carries a series of overlapping inversions that have transformed the original metacentric third chromosome into a submetacentric one, thus providing an additional cytological marker for defining the arm at which a heterochromatic sequence maps.

**dodecasatellite sequences are closely linked to the primary constriction and can be broken without compromising chromosome stability**

These results locate dodecasatellite close to the primary constriction in the right arm of the third chromosome. Unfortunately, heterochromatic sequences do not undergo polytenisation and so the extraordinary degree of resolution provided by in situ hybridisation to polytene chromosomes cannot be achieved. Mitotic chromosomes offer the only way to visualise and map heterochromatic sequences so that their distance to cytological landmarks, such as the primary constriction, can be estimated. This poses the problem that, as mitotic chromosomes are highly condensed and hence very small, the resolution that can be achieved is very low indeed. To get around this problem, we mapped dodecasatellite in a series of strains that carry different X-ray-induced chromosomal rearrangements with breakpoints within the heterochromatic region. Although some heterochromatic regions have been reported to be less sensitive to X-ray damage than others (Gatti and Pimpinelli, 1983), it is expected that the closer a sequence is to the centromeric region the fewer the breakpoints that map between that sequence and the centromere. In other words, by ascertaining whether any of these breakpoints would actually separate dodecasatellite from the primary constriction we could get an indication of the relative proximity between the centromere and the bulk of dodecasatellite sequences. In situ hybridisation to a series of nine compound arms, including five compounds of the left arm of the third chromosome, C(3L), and four compounds of the right arm, C(3R), revealed in all of them the presence of a single hybridisation signal per chromatid of about the same intensity as that found in the wild type (Fig. 2A), indicating that all the breakpoints in the right arm are distal to dodecasatellite. This result suggests that there is a very close proximity of dodecasatellite to the centromere.

The absence, amongst the collection of compounds described above, of any breakpoint within the bulk of dodecasatellite sequences was unexpected, as the size of the region occupied by this satellite is relatively large. This

![Fig. 2. Distribution of dodecasatellite in different chromosomal rearrangements. (A) C(3L)P3, ri; C(3R)P3, sr. dodecasatellite is found in both compound chromosomes (C) as a single dot per chromatid, indicating that their breakpoints are distal to the region that contains this satellite. (B) F(3L), h, F(3L); C(3R), sr, C(3R)). (C) C(3L), h2, C(3L)); F(3R)1, e ro ca; F(3R)2, e' ro ca, (both F(3R)). The arrow in (C) points to F(3R)1e ro ca in which the amount of dodecasatellite is greatly reduced. X, Y, II; X, Y and II chromosomes, respectively. Bar, 5 µm.]
could be interpreted as an indication of the instability of those chromosomes in which this region is disrupted. To examine this possibility further, we extended the analysis to a series of five free chromosome arms. These chromosomes carry at least two breakpoints, one within the heterochromatic region of the corresponding chromosome, and a second one somewhere else in the genome. Although the five free chromosome arms analysed showed positive *dodecasatellite* hybridisation (Fig. 2B), in one of them, *F(3R)1*, *e*^t ro ca*, the signal produced was much weaker than that shown by wild-type chromosomes (Fig. 2C). This result indicates that the amount of *dodecasatellite* is not critical, as it can be reduced without compromising chromosome stability.

**dodecasatellite is highly conserved amongst individuals of D. melanogaster, but is variable amongst species of the melanogaster subgroup**

To characterise *dodecasatellite* further we sought to analyse the distribution of this satellite in different species to determine the extent of its evolutionary conservation. Apart from *D. melanogaster* itself, this study included the following four species of the *melanogaster* subgroup, the closest relatives of *D. melanogaster*: *D. simulans, D. mauritiana D. yakuba* and *D. teissieri*. Comparative analyses of the organisation of the euchromatic and heterochromatic regions of the chromosomes of these species have been published by Lemeunier and Ashburner (1976) and Lemeunier et al. (1978), respectively. Before carrying out such a study, and concerned about a possible variability of satellite DNA sequences in both the second and the third chromosomes as it can be reduced without compromising chromosome stability.

Although the condensation state of the *dodecasatellite* region does not vary to any significant extent from prophase to telophase. After mitosis, and throughout interphase, *dodecasatellite* sequences are found as one condensed dot (Fig. 4B), whose size and shape are indistinguishable from those found in mitotic chromosomes. This result indicates that the chromosome region containing *dodecasatellite* does not decondense after mitosis, in agreement with the classical definition of heterochromatin.

Although the condensation state of the *dodecasatellite* region does not vary to any noticeable extent during the cell cycle, its sensitivity to hypotonic shock is intimately dependent upon the cell cycle stage. When interphase nuclei are treated with hypotonic shock, *dodecasatellite* is found as a thread-like structure with varying degrees of condensation, but invariably more spread than in untreated cells (compare Fig. 3A with B). Since no such effect has been observed in mitotic chromosomes, it seems reasonable to conclude that the nature of the condensation shown by the region that contains this satellite varies throughout the cell cycle. This observation might have considerable relevance to the mapping of heterochromatin as it provides a straightforward way to improve resolution.

**dodecasatellite sequences are often found connecting sister chromatids following colchicine treatment**

There is a second cell cycle-dependent characteristic of
sequences that we have observed by in situ hybridisation to colchicine-treated cells. Colchicine treatment depolymerizes microtubules and leads to cell cycle arrest with highly condensed chromosomes at a stage named c-metaphase. While in Drosophila most c-metaphase chromosomes show the two sister chromatids attached to one another by their centromeric region, in some instances the sister chromatids pop apart and remain very close to one another, aligned side by side, defining what Levan named ‘ski anaphases’ (Levan, 1938). None of the dyes used so far to visualise DNA has been reported to reveal any physical link between sister chromatids in ski anaphases. Nevertheless, in situ hybridisation with a dodecasatellite probe showed that in a fraction of these cells the hybridisation signal could be seen not only at its usual location within each chromatid, but also at the intervening space between them. The intervening signal was found as either a flat plaque (Fig. 4C) or as a string-like structure (Fig. 4D), spanning the distance between the two sister chromatids. As plaques and string-like signals were found between close and more distant chromatids, respectively, it is tantalising to suggest that plaque and string represent early and late stages of the same process.

**DISCUSSION**

Our knowledge about heterochromatin is scanty, despite the fact that it accounts for a large fraction of the whole genome and that it is known to contain vital functions. There are two major reasons that account for this lack of information. Firstly, its repetitive nature, which makes the molecular characterisation of these sequences extremely difficult. Secondly, as heterochromatin undergoes neither meiotic recombination nor polytenisation, these sequences are not amenable to conventional mapping techniques.

The development of techniques that allow for the manipulation of high molecular weight DNA has made it possible to identify hitherto unknown sequences within the pericentric region. We have applied high resolution in situ hybridisation to polytene and diploid cells to characterise further one of these sequences, known as dodecasatellite. Taking advantage of the availability of chromosomal rearrangements, with which it is possible to identify unequivocally the different chromosomes and their arms, we have mapped dodecasatellite to the right arm of the third chromosome near to the primary constriction. As judged by in situ hybridisation, this satellite accounts for a large frac-
tion of the heterochromatic content of the third chromosome. As with many other satellites in Drosophila, dodecasatellite is restricted to the heterochromatin and cannot be found interdispersed along the euchromatin. Nevertheless, unlike most Drosophila satellites, which are found on several chromosomes, no dodecasatellite sequences have been found in other heterochromatic regions, at the level of resolution provided by this technique. The dodecasatellite can therefore be considered a major third chromosome satellite in D. melanogaster. Other satellite sequences that show a unique chromosomal location are the X specific satellite (Hilliker and Appels, 1982), the Rsp satellite located in region h39 (Wu et al., 1988; Pimpinelli and Dimitri, 1989), and the Y specific satellite sequences (Bonaccorsi and Lohe, 1991).

The localisation of dodecasatellite in polytene chromosomes is quite striking. First of all, it always produces a well-defined signal, instead of the more dispersed one shown by other satellites (Peacock et al., 1973). Whether this is just the result of the different techniques used in each case (i.e. tritium versus fluorescence) or it actually reflects a particular feature of dodecasatellite we do not know. Perhaps it is revealing the location of the alpha-heterochromatin, which is not seen in D. melanogaster by conventional staining methods, but which can be seen in other species of Drosophila as a dark, centrally located region of the chromocentre (Gall et al., 1971), reminiscent of the kind of signal produced by dodecasatellite probes. The second feature that renders the staining pattern of dodecasatellite in polytene chromosomes striking is the variation in the number of dots that are produced. We do not understand the reasons for this. It could be due to the distortion imposed upon the polytene chromosomes by the squashing procedure, or it may reflect actual variations in the distribution of this satellite amongst each polytene nucleus, or both. The facts that no such variations are observed in diploid cells, that the number of dots on polytene chromosomes increases with the distortion of the chromocentre, and that the more dots that can be seen, the weaker they are, suggest that multiple dots are produced as a result of overstretching produced by the squashing procedure. Nevertheless, we cannot explain why overstretched should result in discrete dots, instead of a continuous signal. Whatever the structure of the dodecasatellite region within the chromocentre might be, it seems clear that its degree of polytenisation is very low indeed, in agreement with the published data regarding other heterochromatic sequences (Gall, 1973). This conclusion is drawn from the observation that in polytene chromosomes, dodecasatellite, whose size in the genome has been estimated to be around 1 Mb, appears as a dot whose intensity is of the same order of magnitude as that shown by a unique euchromatic 0.9 kb sequence, whose estimated number of copies is around 1000 times.

Their close proximity to the primary constriction is a
most important feature of dodecasatellite sequences. The primary constriction contains the spindle fibre attachment region, a key component of the mechanisms for chromosome segregation whose structure and molecular nature remain largely unknown. dodecasatellite sequences could therefore offer a valuable tool towards the characterisation of this region. Unfortunately, the degree of resolution that can be achieved by in situ hybridisation to mitotic chromosomes is rather low in terms of distances. Close proximity, therefore, means that the distance between dodecasatellite and the primary constriction can range from zero to a few megabases. It is formally possible to get a more precise estimate of this distance by applying in situ hybridisation to genetically induced breakpoints, although two limitations have to be considered when using this approach. The first one concerns those instances in which the sequences under study play an essential role for cell viability. In such cases, chromosomes that lack these sequences, or in which these sequences have somehow been disrupted, will not be recovered, thus biasing the result of such screening. Secondly, although there is no doubt that the distance between two markers and the probability of X ray induced chromosome breakages between them are inter-related, it is important to point out that these two parameter are not expected to correlate in a simple linear fashion, as examples of regions that show a lower than average sensitivity to X rays have been reported in the Y chromosome (Gatti and Pimpinelli, 1983) and similar regions may be present in other chromosomes. This approach is particularly amenable in Drosophila, where such chromosome rearrangements are often available or can be produced with relative ease, and has been exploited in the past by other authors (Bonaccorsi and Lohe, 1991).

We have applied this kind of analysis to nine compound arms and found that all of them carry breakpoints that are distal to dodecasatellite. This result indicates that, either there is a very close linkage between dodecasatellite and the primary constriction, or dodecasatellite sequences are essential for chromosome stability, or both. Indeed, the level of resolution attained by this approach can easily be improved by increasing the number of chromosomal rearrangements analysed. We are in the process of producing a large collection of such rearrangements with which to map this, and other, heterochromatic sequences in Drosophila.

One of the rearrangements studied in this work, F(3R)1, e^r ro ca, shows a very weak dodecasatellite signal. There are, essentially, two possible interpretations of this result. The first one would be instability of dodecasatellite sequences, leading to variations in the level of repetition of dodecasatellite between different individuals. The second explanation would be the generation of a partial deletion of dodecasatellite sequences in the F(3R)1, e^r ro ca chromosome, probably as a consequence of the X ray treatment by which this chromosome was derived. As far as the first possibility is concerned, although some satellite sequences have been reported to behave in this way (Verma and Dosik, 1980), the high conservation of the dodecasatellite sequences among geographically distant individuals reported in this work clearly weakens this interpretation. We therefore favour the second interpretation, i.e. that the free arm in question carries a partial deletion of dodecasatellite. If that is the case, the most likely possibility, although there are others, is that one of the breakpoints involved in the generation of this rearrangement is located within dodecasatellite itself in which case, as the dodecasatellite signal is located in the long arm of this chromosome, the small arm and the centromere of this subtelocentric chromosome could correspond either to the right arm of the X or to the left arm of the fourth chromosome. Experiments are in progress to test this hypothesis, which, if confirmed, would mean that the rearrangement that gave rise to F(3R)1, e^r ro ca has re-located dodecasatellite sequences near the proximal region of another chromosome. This should facilitate the cloning of the sequences of the other chromosome involved in the breakpoint, which is a task of major interest, as our data indicate that they are very near to its centromeric region. In addition, as dodecasatellite accounts for a noticeable amount of the heterochromatin present in the right arm of the third chromosome, it is expected that other rearrangements of this kind, with breakpoints within dodecasatellite, could be produced with relative ease.

The distribution of dodecasatellite seems to have undergone dramatic changes during the course of evolution, as judged by the results of in situ hybridisation analysis of five closely related species of the melanogaster subgroup. Several conclusions can be drawn from these observations. Firstly, that the chromosomal distribution of dodecasatellite is in agreement with the phylogenetic relationships that have been proposed between these species (Cariou, 1987), thus adding further support to them. Secondly, it can be concluded that, whatever the function of dodecasatellite might be, during the somatic cell cycle, the presence of different amounts of these sequences in the second and third chromosomes of D. simulans, as well as the lower amount of dodecasatellite in F(3R)1, e^r ro ca, indicate that the amount of this satellite is not critical. Finally, the presence of dodecasatellite in both the second and the third chromosomes of D. simulans, indicates that in this species, a multichromosomal location of this satellite is acceptable and does not disrupt chromosome behaviour. It seems clear, for instance, that dodecasatellite sequences do not lead to pairing during meiosis, which is in agreement with previous reports indicating that heterochromatin, as a whole, is not involved in meiotic pairing in D. melanogaster (Yamamoto, 1979; Hilliker et al., 1980; Hilliker et al., 1982). It is worth recalling that although D. yakuba and D. teissieri appear to have no detectable dodecasatellite, the absence of a signal in these in situ hybridisation experiments does not mean a complete absence of the sequences under study. The sequences could be present at a low level of repetition. Indeed, Salser et al. (1976) proposed that related species share a library of conserved satellite sequences, any of which could be amplified into a major satellite during the course of evolution.

As far as a possible functional role of dodecasatellite is concerned, and besides its close linkage to the primary constriction, the most compelling feature of the behaviour of these sequences is their presence in the intervening space between those sister chromatids that have popped apart, following colchicine treatment. This is the first case in which
a well-defined DNA sequence has been observed to behave in this way, although the presence of chromatid fibres between segregating chromatids has previously been reported. For instance, Suja et al. (1992) have reported that, during the second meiotic division in some species of grasshopper, silver staining reveals a strand that joins sister kinetochores, from metaphase to early anaphase. It would be interesting to see whether other Drosophila satellites show the same behaviour. A tantalising interpretation of the presence of dodecasatellite between sister chromatids is that this satellite plays some role in sister chromatid pairing. Nevertheless, the biological meaning of this observation is difficult to interpret, as the process of colchicine-induced chromatid separation is itself poorly understood. Some authors have proposed that the separation of sister chromatids in the presence of microtubule-depolymerising drugs reflects one of the actual stages of mitosis: the spindle-independent, complete individualisation of the chromatids prior to their segregation during anaphase (Levan, 1938), but other reports suggest otherwise (Gonzalez et al., 1991). There is not enough evidence to discard the possibility that sister chromatid separation following colchicine treatment is but an artefactual effect of the drug treatment, with no parallel in normal cell division. The situation is further complicated by the fact that, if this process did correspond to the actual process of chromatid separation at the onset of anaphase, one can expect it to be very fast, so that the fraction of cells that could be observed at this stage, at any given moment in untreated tissue, would be extraordinarily small, thus seriously compounding the difficulty of observing its occurrence.

We hope to be able to address the hypothesis of a functional role for dodecasatellite by a deletion and duplication analysis of these sequences. We are confident that the application of new molecular and cytological techniques to a genetically amenable higher eukaryote organism such as Drosophila will greatly facilitate the study of this, and other aspects, of the biological functions of heterochromatin.

The authors are indebted to D. M. Glover, in whose laboratory this work was carried out, for his sound criticism and advise, and constant support. The valuable comments from S. Llamazares, G. Maldonado-Codina, P. Ripoll, and an anonymous referee are much appreciated. We also acknowledge the contribution of the Drosophila Stocks Centres at Bloomington, Bowling Green, and Umea, which supplied most of the strains used in this work. M. C. and C. G. are supported by the Cancer Research Campaign (UK). J. P. A. and A. V. are supported by grants from the Direccian General de Investigacion Cientifica y Tecnica, the EC Science programme, and Fundacion Ramon Areces.

REFERENCES


(Received 16 November 1992 - Accepted 1 February 1993)