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Localisation of Reiterated Nucleotide Sequences in *Drosophila* and Mouse by *in situ* Hybridisation of Complementary RNA

KENNETH W. JONES and FORBES W. ROBERTSON
Department of Genetics, University of Edinburgh

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Abstract. The location of highly reiterated nucleotide sequences on the chromosomes has been studied by the technique of *in situ* hybridisation between the DNA of either *Drosophila melanogaster* salivary gland chromosomes or mouse chromosomes and tritium labelled complementary RNA (c-RNA) transcribed *in vitro* from appropriate templates with the aid of DNA dependent RNA polymerase extracted from *Micrococcus lysodeikticus*. The location of the hybrid material was identified by autoradiography after RNase treatment. — When *Drosophila* c-RNA, transcribed from whole DNA, was annealed with homologous salivary chromosomes in the presence of formamide the well defined labelling was confined to the chromocentre. With heat instead of formamide denaturation there was evidence of discontinuous labelling in various chromosome regions as well, apparently associated with banding. *Xenopus* ribosomal RNA showed no evidence of annealing to *Drosophila* chromosomes with the comparatively short exposure times used here. — When mouse satellite DNA was used as template the resulting c-RNA showed no hybridisation to *Drosophila* chromosomes but, when annealed with mouse chromosomes, the centromeric regions were intensely labelled. The interphase nuclei showed several distinct regions of high activity which suggested aggregation of centromeric regions of both homologous and non-homologous chromosomes. The results of annealing either c-RNA or labelled satellite DNA to homologous chromosomes were virtually indistinguishable. Incubation of *Drosophila* c-RNA with mouse chromosomes provided no evidence of localisation of grains. — It is inferred that both in mouse and *Drosophila* the centromeric regions of all chromosomes are enriched in highly reiterated sequences. This may be a general phenomenon and it might be tentatively suggested that the highly reiterated sequences play some role in promoting the close physical approximation of homologous and non-homologous chromosomes or chromosome regions to facilitate regulation of function.

Introduction

The technique of DNA-DNA and DNA-RNA hybridisation is well established as a method of measuring differences in nucleotide sequences. Recently the procedure has been adapted to anneal radioactively labelled nucleic acids to complementary sequences in the chromosomes directly (John, Birnstiel and Jones, 1969; Gall and Pardue, 1969) or to anneal and thereby localise mouse satellite DNA to homologous chromosomes

(Jones, 1970); Pardue and Gall (1969) reported unpublished evidence of a similar nature. The present paper deals with the *in situ* hybridisation of complementary RNA (c-RNA), transcribed with the aid of DNA dependent RNA polymerase from either whole DNA extracted from *Drosophila melanogaster* or from mouse satellite DNA, which is known to comprise the most highly reiterated fraction of the genome. The *Drosophila* chromosomes were derived from the salivary glands while the mouse chromosomes were prepared from primary embryonic cell cultures derived from an inbred line. Both direct and circumstantial evidence suggests that the highly reiterated sequences are responsible for the observed hybridisation in these experiments and also that they are concentrated in the centromeric regions of the chromosomes, according to the autoradiographic record of where the RNA-DNA hybridisation takes place.

Material and Methods

1. Preparation of c-RNA[1]

RNA polymerase was prepared from *Micrococcus lysodeikticus* by the method of Nakamoto, Fox and Weiss (1964); fraction V was used. RNA synthesis was carried out for 30—40 minutes at 30° C in 0.01 M tris-HCl, pH 7.5 at 30° C, 2.5 mM $MnCl_2$, 1.6 M spermidine, 0.8 mM each of GTP, UTP and CTP and 0.4 mM tritiated ATP with a specific activity of 18.0 ci mM (Schwarz). Approximately 1.2 times as much RNA as primer was obtained. After incubation the RNA was purified and recovered by the procedure described by Bishop and Robertson (1969), which involves DNase treatment, followed by phenol, ethanol precipitation, passage through SE Sephadex, to separate the RNA from nucleotides and oligonucleotides, followed by ethanol precipitation and centrifugation at 130,000 g for one hour. The pelleted RNA was taken up in a small volume of $2 \times$ SSC and stored at —20° C. The specific activity worked out at approximately 3×10^6 dpm per microgram RNA.

2. Preparation of DNA

The extraction of *Drosophila* DNA was the same as described by Robertson, Chipchase and Nguyen (1969) for the preparation of their labelled DNA.

Mouse DNA was prepared by the method described by Jones (1970). The satellite fraction was separated by centrifugation in cesium chloride (Flamm, Bond and Burr, 1966) and accumulated from a number of such fractionations. The pooled samples were refractionated, taking care to minimise sampling from the bulk of the DNA. The final preparation was then dialysed in a suitable buffer, 0.01 M tris-HCl, pH 7. 0.01 M NaCl, before use as primer.

3. Hybridisation Procedure

a) Preparation of Chromosomes. Drosophila chromosomes were prepared by dissecting the salivary glands of well fed, mature female larvae of a wild stock (Pacific) in 45% acetic acid, storing the glands in this fixative for 10 minutes and

1 *Abbreviations.* SSC. — 0.15 M sodium chloride, 0.015 M tri-sodium citrate, pH 7.0. ATP, UTP, GTP and CTP refer respectively to the 5′-triphosphates of adenosine, uridine, guanosine and cytidine.

then transferring them to siliconised slides on which the chromosomes were spread beneath a small non-siliconised cover slip in the usual way. The slides were then inverted in 70% ethanol, until the cover slips detached and the latter were then taken up increasing concentrations of ethanol to absolute, before drying in vacuo at room temperature.

The mouse chromosomes were prepared from cell cultures after treatment with colcemid (CIBA) at 0.4 μg/ml for 6 hours. The cells were removed from the culture flasks with trypsin, pelleted gently in hypotonic saline, repelleted and fixed in ice cold ethanol acetic acid (3:1). The fixed cells were resuspended in 45% acetic acid and drops of the cell suspension were air dried on cover glasses which were stored in vacuo at 4° C until use.

b) Denaturation of DNA. Alternative methods of denaturation have been used namely heat, pH and treatment with formamide. With heat denaturation, the cover slip carrying the chromosomes was held in boiling 0.1 SSC for 30 seconds, plunged quickly into ice cold 0.1 SSC and then transferred to 70% ethanol and taken up increasing concentrations to absolute before drying in vacuo. Alternatively, the chromosomes were treated with 0.07 M NaOH for 5 minutes at room temperature (Gall and Pardue, 1969), dehydrated with increasing concentrations of ethanol and then dried in vacuo. When formamide was used, the denaturation took place in the presence of 60% formamide and 3×SSC at 65° C (McConaughy, Laird and McCarthy (1969).

c) Annealing Procedure. The hybridisation of *Drosophila* c-RNA to homologous chromosomes was carried out in the presence of cold RNA, extracted from mouse liver, since it was found that this procedure reduced the background without interfering with the RNase resistant labelling. The dry denatured chromosome preparations were inverted over 1 μl of RNA solution containing approximately 0.1 μg c-RNA and 8 μg cold mouse RNA in 2×SSC and sealed to the slide with rubber solution. They were then incubated 20 minutes at 65° C, after which the cover slips were detached, washed in 2×SSC, incubated for 20 minutes at room temperature in a solution of a-amylase at a concentration of 6 μg/ml in 2×SSC and then incubated in a solution of p-RNase at 20 μg/ml in 2×SSC at 37° C for 20 minutes. After RNase treatment the cover slips were washed with gentle agitation for about 2—4 hours in a large volume (1—2 l), of 2×SSC at 4° C by inserting them upright into slits in silicone rubber discs which were transferred to the cold solution.

When formamide was used, the preparation including the RNA was held at 65° C for 30 minutes in 3×SSC for denaturation and then lowered 5° C per hour to 37° C at which temperature annealing was allowed to take place over a period of 18 hours before the cover slips were removed and treated as described above. Where *Xenopus* ribosomal RNA was used, this was prepared as described elsewhere (John, Birnstiel and Jones, 1969) although annealing was carried out in the way described in the present paper; cold RNA was not included. After annealing, RNase treatment, washing and drying, the preparations were mounted on slides and dipped into Ilford 14 emulsion diluted 1:1 with water and stored in the dark at 4° C. After an exposure time which varied between 10 and 20 days, the autoradiographs were developed in Kodak D19 developer, stained in 0.02% toluidine blue at pH 6.0 and photographed on Ilford R40 plates with a Zeiss Ultraphot II microscope, equipped with an interference filter. Naturally the appearance of the chromosomes is not as satisfactory as under the best cytological conditions but the structure is well enough preserved to leave no doubt about identification of regions.

Results

To examine the specificity of the *in situ* hybridisation and test alternative hypotheses several different types of annealing experiments have been carried out. They are as follows:

i) *Drosophila* c-RNA annealed with *Drosophila* salivary chromosomes after either heat or formamide denaturation of the DNA;

ii) *Xenopus* r-RNA annealed with *Drosophila* salivary chromosomes;

iii) Mouse satellite c-RNA annealed with either mouse or *Drosophila* chromosomes.

These experiments will be considered in turn.

I. The Annealing of Drosophila c-RNA

The most striking and highly reproducible result of annealing *Drosophila* c-RNA with heat denatured homologous salivary chromosomes is the focal labelling of the chromocentral region (Fig. 1). The label in autoradiographs shows no evidence of pattern in the grain distribution and this feature may be compared with the cytological evidence of apparent homogeneity of the heterochromatic chromocentral region. In some preparations a small, separate, highly labelled fragment may be seen lying near the chromocentral mass and this probably represents the short chromosome IV (Fig. 2). There is often evidence of sharp discontinuity between the labelled chromocentral mass and the distribution of label in the banded chromosome arms. The grains are apparently restricted in their distribution to the banded regions of the chromosomes, although the concentration of grains is not obviously correlated with the staining intensity, since some narrow bands may be more intensely labelled than other apparently larger bands (Fig. 2). So far we have found no evidence of intense labelling in puffed regions. Occasionally bands have been seen in which the labelling pattern extends over only part of the width of the band, while the rest is unlabelled.

Grains have been noted over the nucleolus region, when this can be detected, but the labelling is not particularly intense. This may be contrasted with the label pattern reported by Pardue, Gerbi, Eckhardt and Gall (1970), after labelling *Rhynchosciara* with r-RNA from *Xenopus*, where the label was concentrated over the nucleolus but not associated with the chromosome bands. Assuming that the structural organisation of the two species is similar we might infer that our c-RNA does not contain a disproportionate concentration of RNA complementary to the ribosomal cistrons and also that the chromocentral and band associated label is not due to r-RNA, a conclusion we shall presently reinforce by independent evidence. It should also be noted, in this context, that hybridisation between *Drosophila* c-RNA and homologous extracted

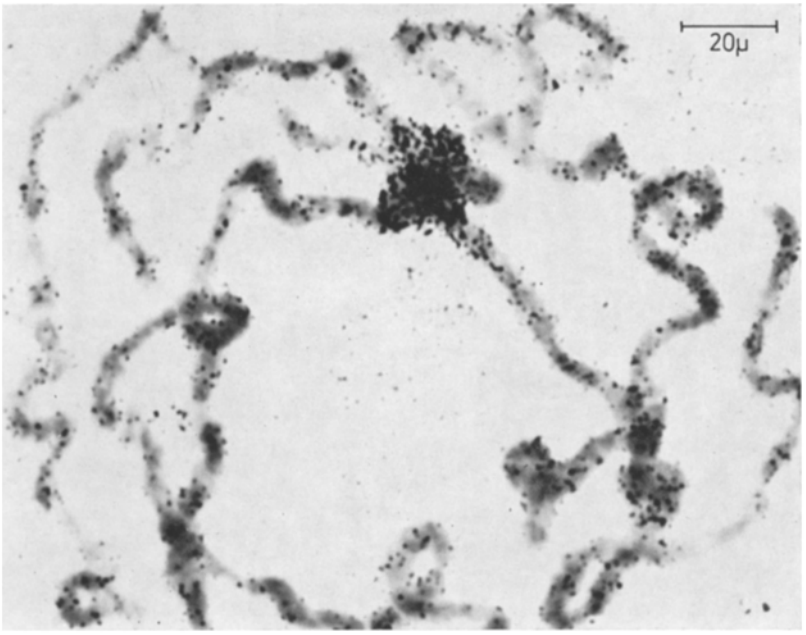

Fig. 1. The effects of incubating tritium labelled *Drosophila* c-RNA with heat treated salivary gland chromosomes. Note the densely labelled chromocentre. After fixing the chromosomes for 10 minutes in 45% acetic acid, alcohol dehydration and drying in vacuo, the cover slip carrying the chromosomes was immersed for 30 seconds in boiling 0.1 SSC and then quenched in ice cold 0.1 SSC. After alcohol dehydration and drying again in vacuo, the dry denatured preparation was inverted over 1 μl of RNA solution containing approximately 0,1 μg c-RNA and 8 μg cold mouse whole RNA in 2 × SSC and sealed with rubber solution to the supporting slide. The preparation was incubated for 20 minutes at 65° C after which the cover slip was removed, incubated for 20 minutes at room temperature in a solution of a-amylase (6 μg per ml) in 2 × SSC, followed by incubation for 20 minutes at 37° C in a solution of p-RNase (20 μg per ml) after which the preparation was washed for 2—4 hours with gentle agitation in 1—2 litres 2 × SSC at 4° C. After drying the preparations were mounted on slides and dipped into Ilford 14 emulsion diluted 1:1 with water and stored in the dark at 4° C. After an exposure time of 12 days autoradiographs were developed in Kodak D 19 developer, stained in 0.02% Toluidine blue at pH 6 and photographed on Ilford R 40 plates with a Zeiss ultraphot II photomicroscope equipped with an interference filter

DNA, showed that reiterated sequences in the *Drosophila* genome were responsible for the hybridisation (Robertson *et al.*, 1969).

We may next consider hybridisation in the presence of formamide, the use of which in annealing experiments has been recently considered

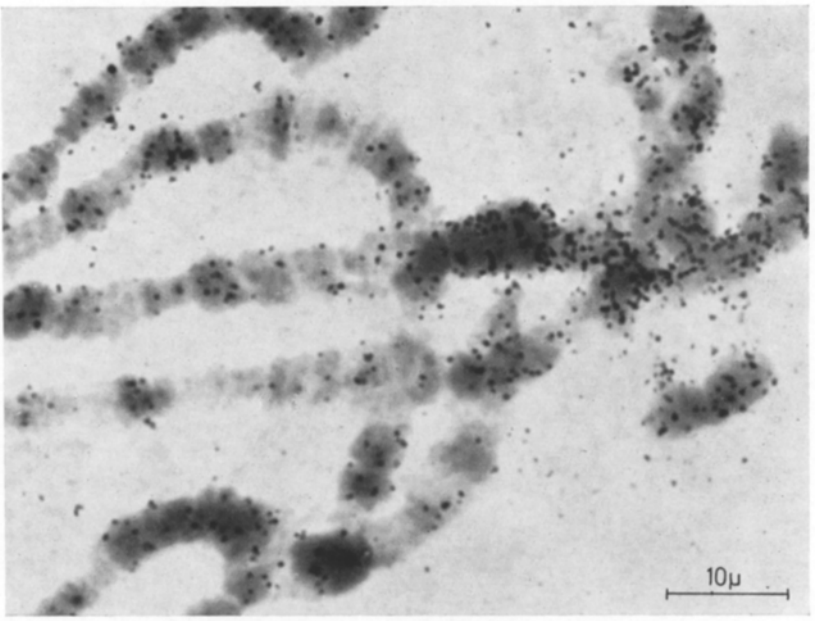

Fig. 2. The effects of incubating tritium labelled *Drosophila* c-RNA with heat treated salivary chromosomes. The experimental procedure was exactly as described in Fig. 1

by McConaughy, Laird and McCarthy (1969). This agent, which effectively denatures DNA, also allows renaturation of DNA and hybridisation between DNA and RNA to proceed at lower temperatures in its presence, and we thought that this might help to preserve the original structure of the chromosome. In any case, at this stage of development of the *in situ* hybridisation technique we have to compare the effects of alternative methods of both denaturation and renaturation.

When hybridisation was carried out in the presence of formamide (Fig. 3) we found hardly any evidence of label outside the chromocentral mass while the grain count was consistently lower than in the parallel experiments with heat denaturation. In addition there was virtual absence of background radioactivity associated with cellular non-chromosomal material. The absence of label on chromosome bands, when hybridisation is carried out in the presence of formamide, does not necessarily indicate the absence of corresponding complementary sequences in the c-RNA. Possibly hybridisation conditions in the presence of formamide provide a more stringent test of sequence complementarity. Or, on the other hand, less repetitive sequences may not hybridise in

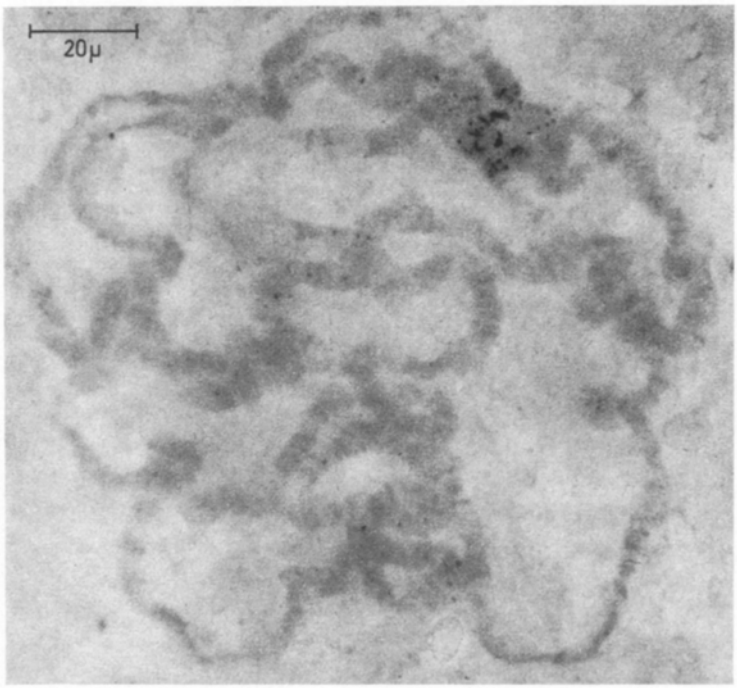

Fig. 3. The effects of incubating tritium labelled *Drosophila* c-RNA with formamide treated chromosomes. The chromosomes were fixed and dried as described in Fig. 1. The cover slip carrying the chromosomes was inverted over 1 μl of RNA solution containing approximately 0.1 μg c-RNA and 8 μg of mouse whole RNA in 60% formamide in 3 × SSC and sealed to the supporting slide with rubber solution. The preparation was held for 30 minutes at 65° C to effect denaturation and then the temperature was lowered at the rate of 5° C per hour to 37° C at which temperature annealing was allowed to take place for a period of 18 hours before the cover slip was removed for treatment with a-amylase, p-RNase, etc. From that point on the procedure was exactly as described under Fig. 1

the presence of formamide. The absence of background, together with the lower level of chromocentral label, compared with heat denaturation, are not inconsistent with the former suggestion. Clearly the origin of the differences is open to further study and interpretation but the main conclusion remains, namely that the chromocentral region is especially prone to hybridisation with c-RNA.

When slides were left for different periods before development of the autoradiographic film it was noted that the chromocentral label appeared in some regions of the chromocentre before it appeared in others, suggesting either that the concentration of DNA in the chromocentre is not

uniform or that the availability for hybridisation of sequences in the chromocentral region varies between regions.

Some preparations in this series, as well as in the earlier tests with heat denaturation, showed a splitting of the labelled chromocentral mass into two or more parts associated with distinct chromsomes, presumably due to excessive pressure during squashing of the chromosomes which led to the fragmentation of the normally fused mass into constituent blocks corresponding to separate chromsomes. We might infer that the DNA sequences which hybridise with c-RNA under these conditions, are present on most of the chromosomes at least.

Both in the formamide as well as the heat denaturation experiments it was noted that some of the radioactivity, not associated with the polytene chromosomes, nervertheless occurred as discrete foci, which turned out to be located over small nuclei, probably derived from fat body material, which had not been completely trimmed away during the preparation of the glands before squashing. These foci did not occupy the entire area of such nuclei but were restricted to one side. It may be suggested that the label is associated with the chromocentral mass in these nuclei. On the reasonable assumption that the same sequences are hybridised in these small nuclei as in the larger polytene nuclei, it appears that the intensity of the labelling is excessively high in the latter relative to their size.

II. The Annealing of Xenopus r-RNA

Xenopus r-RNA was hybridised with heat treated *Drosophila* salivary chromosomes for two reasons; firstly, to check on the general possibility of non-specific trapping of RNA and secondly, to test whether our c-RNA might have a disproportionate concentration of sequences complementary to ribosomal sequences, due to non-random transcription. A high level of cross hybridisation between DNA and r-RNA from different species has been reported (Brown, Weber and Sinclair, 1967) and this evidence provided the justification for using *Xenopus* r-RNA, which is more convenient to prepare than *Drosophila* r-RNA.

When we annealed such r-RNA of the same specific activity as the c-RNA used in the earlier tests, there was an entirely different distribution of grains over the salivary chromosomes. There was no suggestion of a focal labelling, the level of RNase resistant RNA retained on the preparations was low while there was no sign of any consistent association of grains with any particular chromosome region. This evidence reinforces the case for specificity in the c-RNA experiments and also makes it most unlikely that the chromocentral labelling represents binding to ribosomal sites.

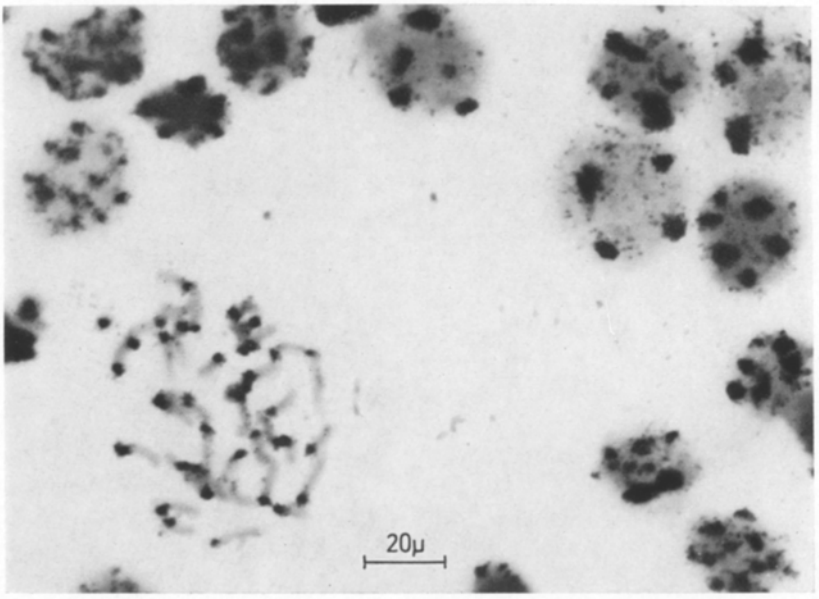

Fig. 4. The annealing of tritium labelled c-RNA, transcribed from mouse satellite DNA, with mouse chromosomes after pH denaturation. Note the centromeric localisation of the label and also the evidence for close physical approximation of centromeric regions in groups of chromosomes in interphase nuclei. The mouse chromosomes were prepared from cell cultures. The cells were removed from the culture flask with trypsin, pelleted gently in hypotonic saline, repelleted and fixed in ice cold ethanol acetic acid 3:1. The fixed cells were resuspended in 45% acetic acid and drops of the suspension were air dried on cover glasses and stored at 4° C. Denaturation was carried out by treatment with 0.07 M NaOH for 5 minutes at room temperature (Gall and Pardue, 1969), dehydrated with increasing concentrations of ethanol and dried *in situ*. The cover slip was inverted over 1 μl RNA solution containing approximately 0.1 μg c-RNA in 2 × SSC. Thereafter the procedure was exactly as described in Fig. 1

III. The Annealing of Mouse Satellite c-RNA

The possibility remains that the pattern of labelling in the hybridisation of *Drosophila* c-RNA to homologous chromosomes may reflect some relatively trivial property of the c-RNA compared with *in vivo* RNA. For example, c-RNA transcribed with *Micrococcus* polymerase may form RNase resistant duplexes due to symmetrical transcription (Bishop and Robertson, 1969) and so RNase resistance, coupled with non-specific sticking of the RNA to the chromosome could masquerade as hybridisation. To test this possibility we need to hybridise *Drosophila* chromosomes with some other c-RNA, prepared the same way, using

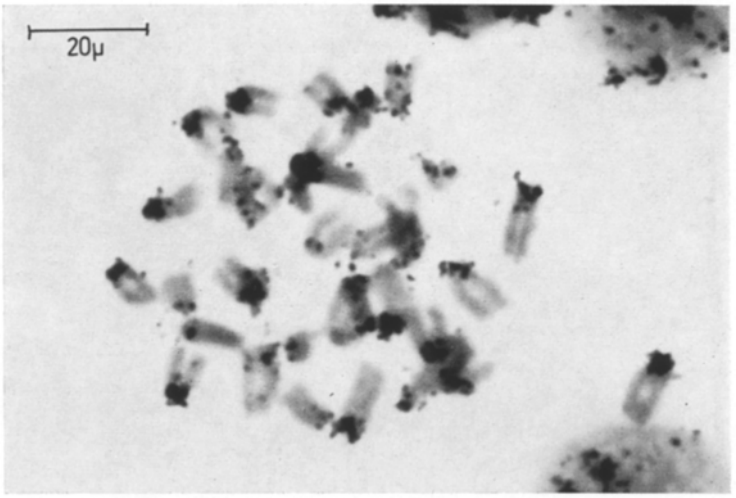

Fig. 5. The effects of annealing tritium labelled c-RNA transcribed from mouse satellite DNA, after pH denaturation. Note the localisation of label in the centromeric region and also the evidence for aggregation of centromeres in interphase nuclei. Experimental procedure exactly as described under Fig. 4

a DNA primer from a species sufficiently unrelated to *Drosophila* to make it unlikely that the c-RNA carries complementary sequences. Ideal material exists in the mouse satellite DNA which is known to show a high level of species specificity (Walker, Flamm and McLaren, 1969) and which is also known to be spatially located in the centromeric regions of the chromosomes (Fig. 6), from *in situ* DNA-DNA hybridisation experiments (Jones, 1970). Accordingly, c-RNA was synthesised on a template which consisted of purified, native mouse satellite DNA and this was annealed with both mouse and *Drosophila* chromosomes.

Mouse c-RNA annealed to mouse pH denatured metaphase chromosomes showed a strong localisation in the centromeric regions of all the chromosomes and confirms (Figs. 4 and 5) the finding from the DNA-DNA studies that the centromeric regions of the mouse chromosomes are enriched in the highly reiterated, satellite sequences. In interphase nuclei, after the same procedure, the pattern of grains showed a marked localisation over the nucleoli and the dense chromatin, in agreement with the earlier evidence from the DNA-DNA hybridisation experiments, that these regions are enriched in satellite sequences. The present experiment provides the first demonstration of the localisation of any satellite DNA by means of hybridisation to RNA transcribed *in vitro*. The annealing pattern of the c-RNA mirrors that encountered with the single satellite

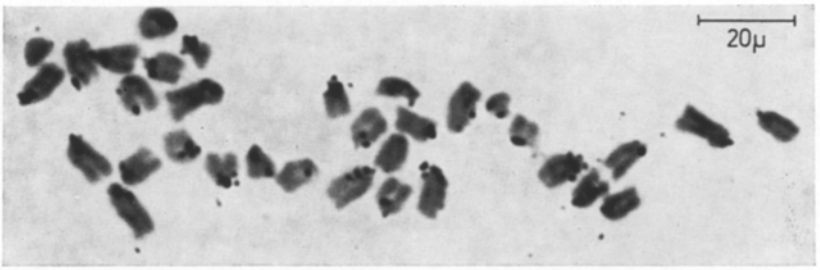

Fig. 6. The effects of annealing tritium labelled mouse satellite DNA to mouse chromosomes after pH denaturation. Experimental procedure the same as described under Fig. 4, except for the use of labelled DNA in place of c-RNA

DNA strands (Fig. 6) and this confirms, in a unique way, that the *Micrococcus* polymerase transcription product is a faithful copy of the mouse template DNA.

The annealing of mouse c-RNA to heat treated *Drosophila* chromosomes showed no evidence of focal labelling while the retained radioactivity was randomly distributed and not above background values. Thus there is no doubt that the distribution of grains found after annealing *Drosophila* c-RNA with homologous salivary chromosomes represents a specific reaction which indicates the distribution of those complementary sequences which are relatively enriched in the interacting polynucleotide strands. We conclude from this that the chromocentral, heterochromatic region of *Drosophila melanogaster* is enriched in reiterated sequences. In this respect it resembles the centromeric region of mouse chromosomes. Since two such widely separated species show the same features it may be a general rule that the centromeric region of chromosomes are rich in reiterated sequences. Both in mouse and *Drosophila* we have found evidence for these regions to be physically associated in interphase nuclei. This particular association may have important physiological significance.

The annealing of *Drosophila* c-RNA to mouse chromosomes led to the retention of some radioactivity which, however, was randomly distributed and showed no tendency to be localised at the centromeric region of the chromosomes or in the chromocentral regions of interphase nuclei (Fig. 7), from which we may infer that the retention is non-specific. It is not obvious why the retention between mouse satellite c-RNA and salivary chromosomes should result in little or no retention of radioactivity while the reciprocal combination shows a higher, although apparently random retention. One possibility is that the sequences present in the RNA complementary to the mouse satellite are greatly

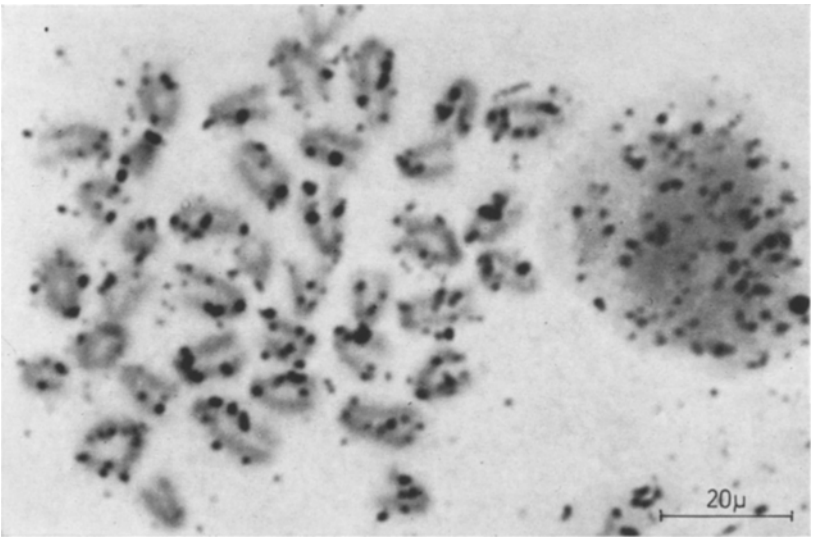

Fig. 7. The effects of incubating tritium labelled *Drosophila* c-RNA with mouse chromosomes after pH denaturation. This Figure should be compared with Figs. 4 and 5. Note the apparently random distribution of grains. Experimental procedure exactly as described under Fig. 4 except for the use of *Drosophila* c-RNA in place of mouse satellite c-RNA

restricted in variety, compared with the *Drosophila* c-RNA in which perhaps all or most of the genome is represented, with the consequent higher likelihood of encounter between partly complementary sequences distributed over the mouse chromosomes.

The mouse satellite annealing experiments provide an indication of the sensitivity of the technique. On the assumption that the mouse nucleus contains about 6.5 μμg DNA (Macgregor, 1968) and that the satellite represents about 10% of this, then each chromosome has, on average $\frac{6.5}{10\times 40}$ or 0.01625 μμg of such DNA. Assuming that the total satellite material is made up of some 10^6 sequences of 400 nucleotide pairs, we estimate each chromosome to carry about 25,000 of such sequences. Since, in the annealing experiments, a very strong signal was obtained after only 12 days exposure to film it is obvious that we are not working anywhere near the limits of sensitivity and so may confidently expect to detect between five and ten times less DNA than in these tests. These inferences are based on the use of c-RNA which can be synthesised with much higher specific activity than DNA labelled *in vivo* while they also assume DNA sequences with C_0t characteristics similar to mouse DNA.

IV. The Biological Role of Reiterated Sequences

Possible functions for the reiterated sequences localised in the centromeric region remain to be determined, although from what is already known certain possibilities may be considered. It is at present uncertain whether RNA complementary to mouse satellite DNA occurs *in vivo*. Harel, Hanania, Tapiero and Harel (1968) have claimed that this is so, although their evidence falls short of unambiguous proof. On the other hand, Walker, Flamm and McLaren (1969) failed to find evidence that the satellite DNA is transcribed *in vivo*. From what is known of the base sequence and estimated sequence length of satellite DNA (Southern, in preparation), it is rather unlikely that such DNA codes for the proteins with which we are familiar, and in this sense, such sequences may appear as genetically inert.

On the other hand they may play an important role in relation to the particular activity of the centromeres. Since the centromeric regions, with their reiterated sequences, are closely approximated at interphase, this could ensure a necessary correlation between the functions of different chromosomes. The reiterated sequences are intimately associated with the nucleolus, while the nucleolar fraction has been shown to be four times enriched for satellite sequences (Schildkraut and Maio, 1968).

Viewed in this light, highly reiterated sequences may serve to bring regions of non-homologous chromosomes together for the purpose of regulating their interaction in metabolism.

We cannot infer from these *in situ* hybridisation experiments whether or not other reiterated regions are scattered through the genome. Hybridisation of *Drosophila* c-RNA, after heat denaturation of the salivary chromosomes, provided evidence of label over a number of bands on different chromosomes. If renaturation in the presence of formamide, involves more stringent conditions, the absence of label in the presence of formamide could imply imperfect complementarity in the heat denatured chromosomes. Such possibilities are being examined in further experiments.

If identity or, at least, substantial similarity of the reiterated sequences of the centromeric region is an essential condition for their particular function, whatever that might be, this raises some interesting considerations. If there is no recombination between non-homologous chromosomes, apart from the rare translocation, it might be expected that mutation would lead to progressive divergence between the sequences on different chromosomes and this would tend to lower the average level of reiteration inferred from the measurement of rates of renaturation. In species like the mouse in which the highly reiterated fraction of the genome can be separated by centrifugation, this tends to encourage

the notion that we are dealing with a homogeneous distribution of reiterated sequences. Perhaps, the renaturation rates of such satellite DNA should be re-examined more closely, assuming it has been sufficiently purified and freed from contamination with non-reiterated material, to look for evidence of heterogeneity — admittedly a difficult property to describe quantitatively.

It is difficult to see how mutational drift between non-homologous chromosomes could be avoided, unless there is some mechanism to oppose it or minimise its effect. For example, if the particular function of such sequences could only be carried out by particular sequences of bases, for some special physical reason, then selection might discriminate against base changes. But if this were so we meet the problem that highly reiterated sequences appear to be particularly prone to divergence between closely related species (Walker *et al.*, 1969; Robertson *et al.*, 1969) which would imply that widely different sequences may perform the same role. But, on the other hand, the essential function may depend merely on a sufficient level of reiteration, irrespective of the base sequence which is repeated. If there were some mechanism for exchange of highly reiterated sequences between non-homologous chromosomes, without the occurrence of translocation, this could confer a statistical homogeneity on these regions generally. However, there is no evidence of this and so we have to be content for the time being with recognition of a rather intriguing problem.

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Dr. K. W. Jones
Department of Genetics
University of Edinburgh
Edinburgh EH9 3SN
Scotland

Professor F. W. Robertson
Department of Genetics
University of Aberdeen
Aberdeen AB9 1AS
Scotland