Disassembly of the mammalian metaphase chromosome into its subunits: studies with ultraviolet light and repair synthesis inhibitors

ANN M. MULLINGER and ROBERT T. JOHNSON

Cancer Research Campaign Mammalian Cell DNA Repair Group, Department of Zoology, University of Cambridge, Cambridge CB2 3EF, UK

Summary

Metaphase chromosomes of a simian virus-transformed Indian muntjac cell line have been examined by scanning electron microscopy of material in which the fully packed metaphase structure is progressively relaxed. Such chromosomes are seen in standard, spread preparations of ultraviolet light-irradiated, metaphase-arrested cells, which have been incubated in the presence of inhibitors of DNA synthesis; they are processed for electron microscopy by trypsinization, further fixation and osmium impregnation.

Decondensation is initially associated with a gradual elongation and loosening of the chromosome axis and, as loosening proceeds, the appearance of unexpected higher order structures – clusters of 20–40 nm diameter fibres. The arrangement of the clusters shows much variation between spreads. In the most fully extended chromosomes clusters are arranged in two longitudinal series with pairing between sister chromatids; the diameter of the majority of clusters in such chromosomes is in the range 0.4–0.6 μm. In the final stages of decondensation, clusters separate and individual chromosomes are no longer recognizable. Similar fibre clusters are found in interphase nuclei prepared by the same method.

We suggest that the clusters of chromatin fibres may assemble as intermediates in the construction of an axial structure, which is further compacted in the fully condensed metaphase chromosome.

Key words: chromosome structure, chromosome coiling, chromosome decondensation, DNA repair inhibition, chromatin higher order structure.

Introduction

The highly compact nature of metaphase chromosomes imposes severe limitations on the amount of information that can be obtained about their structure from electron micrographs of intact preparations. Studies of more-dispersed material, produced by spreading chromosomes into two dimensions on an air/water interface after removal of key chromosomal components, has been a useful approach (Paulson & Laemmli, 1977; Mullinger & Johnson, 1979, 1980; Earnshaw & Laemmli, 1983; Earnshaw & Heck, 1985) but is again limited since these procedures tend to destroy or obscure the higher levels of chromosome organization.

In this paper we establish a new approach to the understanding of metaphase chromosome organization, namely the analysis of material in which the fully packed metaphase structure is partly relaxed, but not reduced to two dimensions. Such chromosomes are produced from metaphase cells which are ultraviolet light (u.v.)-irradiated and treated with inhibitors of DNA synthesis. Under these conditions the process of excision repair of u.v.-induced damage is incomplete; DNA single-strand breaks accumulate at repair sites and chromosomes appear progressively decondensed, as viewed in standard hypotonically swollen, acid-fixed preparations in the light microscope (Schoreini et al. 1975; Collins et al. 1977; Johnson & Collins, 1978; Hittleman & Pollard, 1984). Restoration of compact chromosomal appearance is achieved rapidly by removal of the inhibitors and is accompanied by loss of single-strand breaks in the DNA (Johnson & Collins, 1978; Mullinger & Johnson, 1985). Examination of these attenuated chromosomes by scanning electron microscopy, as described in this paper, reveals a distinct family of higher order chromatin structures in the form of fibre
clusters. We believe that these represent an intermediate level of packing of chromatin fibres necessary for the construction of metaphase chromosomes.

**Materials and methods**

**Cell culture**

Cells were grown in monolayer in Eagle’s Minimal Essential Medium supplemented with non-essential amino acids and with 10% serum (Gibco, Europe; mixture of foetal and new-born calf serum). Simian virus 40 (SV40)-transformed cells of the Indian muntjac *Muntiacus muntjak* were kindly supplied by Dr K. Sperling, Free University of Berlin. Metaphase-arrested cells were obtained by a thymidine/nitrous oxide block regime, as described previously (Johnson et al. 1978). Such synchronized populations included occasional interphase cells.

**Experimental protocol**

The experimental protocol for the production of repair-inhibited cells has been fully described previously (Mullinger & Johnson, 1985). Metaphase arrested cells were pre-incubated in the presence of the DNA synthesis inhibitors hydroxyurea (HU, 10^{-3} M) and 1-β-D-arabinofuranosylcytosine (araC, 10^{-4} M) for 30 min, irradiated with 5–20 J m^{-2} u.v. in phosphate-buffered saline (PBS) containing inhibitors and then post-incubated for periods of 30–90 min in medium with inhibitors, followed in some cases by a further period of 15–60 min without inhibitors but in the presence of 10^{-3} M-deoxyribonucleosides (dXs). Colcemid (0.05 μg ml^{-1}) was present throughout except at the irradiation step. In control samples u.v. irradiation was omitted.

**Preparation of material for electron microscopy**

Chromosomes were treated with 0.56% KCl at 37°C for 20–60 min and fixed in three changes of Carnoy’s fixative (3:1 (v/v), methanol-acetic acid). After storage at 4°C overnight, samples were dropped onto glass coverslips and air dried. (For light microscopy some of these preparations were stained with Giemsa.) The following day material on coverslips was processed for scanning electron microscopy (SEM). Chromosomes were lightly trypsinized, fixed in phosphate-buffered glutaraldehyde, followed by osmium tetroxide, impregnated with alternate treatments of sodium thiocarbohydrazide and osmium tetroxide, dehydrated in acetone and critical point dried (Harrison 1983; Mullinger & Johnson, 1983). Chromosomes on fragments of coverslips were processed for scanning electron microscopy (SEM). Chromosomes were lightly trypsinized, fixed in phosphate-buffered glutaraldehyde, followed by osmium tetroxide, impregnated with alternate treatments of sodium thiocarbohydrazide and osmium tetroxide, dehydrated in acetone and critical point dried (Harrison et al. 1982; Mullinger & Johnson, 1983). Chromosomes on fragments of coverslips were examined without sputter coating in a Jeol 200 CX electron microscope operated at 80 kV in the secondary emission mode. Specimens were viewed at angles of tilt varying from +60° to −60°; stereo pairs were taken before and after tilting the specimen by 7°.

**Results**

The appearance of repair-inhibited metaphase chromosomes in the light microscope

Viewed in the light microscope (LM) in standard spread preparations, chromosomes from u.v.-irradiated metaphase cells incubated with HU and araC appear decondensed. The extent of decondensation increases with increase in the amount of u.v. and the length of the period of incubation with inhibitors (Mullinger & Johnson, 1985).

Various stages in the process of decondensation are illustrated for muntjac in Fig. 1. In control un-irradiated chromosomes a darkly stained, continuous axial structure or backbone is present in each chromatid (Fig. 1A). This axis appears to be in the form of a coil, with precise dimensions varying according to the state of contraction of the chromosome. Decondensation in the irradiated preparations (Figs 1B–F) is associated with a lengthening of the chromosome and a reduction in staining intensity. An axial structure is often still present, though it is reduced in thickness compared with control material. In the early stages of elongation the axis still appears coiled, though rather more loosely (Fig. 1B). As extension continues the coils are no longer apparent but the axis adopts a convoluted path (Fig. 1C); by this stage sister chromatids no longer lie parallel to each other. At an even more advanced stage, continuity of the axial structure cannot be traced and chromosomes appear as a tangle of fine threads (Fig. 1D); the width of such chromosomes is increased compared with control material. Finally, individual chromosomes are no longer recognizable but rather merge into a pale-staining, apparently featureless mass (Fig. 1E). Decondensation of some chromosomes involves relatively little change in length but an overall increase in width (Fig. 1F); axial continuity is hard to trace in such preparations.

Scanning electron microscopy of unirradiated metaphase chromosomes

The appearance of control chromosomes is shown in Fig. 2. Each chromatid is divided into blocks by a series of more or less pronounced transverse grooves, most of which run across the full width, usually at right angles to the main chromosome axis but sometimes diagonally. They also continue down the sides of the chromatid, as can be seen in micrographs of tilted specimens (Fig. 2C). A few shorter grooves are also found and these terminate partway across the chromatid (Fig. 2B,C). In some regions there is good matching between the grooves in sister chromatids. In others the matching is less than perfect; for example, a pronounced groove in one chromatid may correspond to a faint groove in the sister (Fig. 2A). Such features suggest that the precise appearance of grooves is sensitive to one or more steps in the preparative procedure. For example, differences in the degree of compaction may arise during chromosome spreading, and optimal trypsinization, necessary to reveal grooves in these preparations (cf. Harrison et al. 1983;
Mullinger & Johnson, 1983), may not always obtain throughout each chromosome.

The positioning of grooves along the chromosome is not absolutely regular and blocks vary in size (from about 0.4 to 1.4 μm along the chromatid length and from about 1.1 to 1.6 μm across its width). In some chromosomes there are short regions with an alternating pattern of grooves extending partway across the chromatid from opposite sides (arrows, Fig. 2C). Such features hint at a regular underlying folded axial structure that is obscured by close packing in most regions. Long centromeric regions characteristic of some chromosomes of this species also appear as a linear series of blocks, though these are smaller than elsewhere in the chromosome (Fig. 2B); short centromere regions of chromosomes appear as grooves between blocks of normal size.

At higher magnifications, blocks of all sizes are resolved into arrays of fibres (20–40 nm diameter) exposed at the surface of the chromatid and projecting radially outwards. In over-trypsinized preparations the fibres are matted so that transverse grooves are obscured.

**Scanning electron microscopy of repair-inhibited metaphase chromosomes**

The various stages of chromosome decondensation are apparent in scanning electron micrographs of u.v.-irradiated material incubated with inhibitors of repair synthesis. As chromosomes decondense the metaphase arrangement of chromatid blocks is progressively lost and the bulk of the chromatin is dispersed into smaller clusters of fibres. The diameter of fibres in these clusters is similar to that in controls.

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**Figs 2-11.** Scanning electron micrographs of muntjac chromosome or nuclear preparations. Figs 1A, 2, 11: unirradiated cells incubated with inhibitors for 60 min. Figs 1B–F and 3–10: irradiated cells exposed to 5 J m⁻² u.v. and post-incubated with inhibitors for either 30 min (Figs 1B–F, 3, 8, 9) or 90 min (Fig. 10). All SEMs taken at specimen tilts of 2° or 9° except Fig. 2C at 53° and 60°.

**Fig. 1.** LMs of control muntjac metaphase chromosomes (A) and decondensed, repair-inhibited chromosomes (B–F). Stained with Giemsa. X1200. Bar, 10 μm.
At the earlier stages of decondensation, when individual chromosomes are still recognizable, there is much variation in size and disposition of the fibre clusters. There are, however, two common patterns. In the elongated type (compare LM, Fig. 1B,C), the chromatid length increases as the width decreases, so

Fig. 2. Control metaphase chromosomes. A. Longitudinal series of fibre blocks separated by grooves, most of which run at right angles to the chromatid axis. Arrowed block in one chromatid corresponds to two in the sister chromatid. ×9000. B. Stereo pair of chromosome exhibiting hints of an underlying gyred structure: some grooves between blocks run at an angle to the chromatid axis and/or do not extend across the full chromatid width. Note smaller blocks in the long centromeric region. ×4900. C. An alternating pattern of grooves extending partway across the chromatid from opposite sides is particularly clear in one region (between arrows). ×8300. Bars, 1 μm.
that eventually a single cluster spans the width; in the
expanded type (compare LM, Fig. 1F), the major
change in chromatid appearance is an increase in
width and several clusters now span the chromatin.
Generally the same decondensation pattern is found
throughout a chromosome and among the chromo-
somes of one spread.

The first sign of the elongated pattern of deconden-
sation is an exaggeration of the grooves between
chromatin blocks. Diagonal grooves are more com-
monly seen and in addition there may be connecting
folds between adjacent chromatin blocks (Fig. 3). As
decondensation continues, each chromatid becomes
longer and thinner and its outline much more irregu-
lar, as shown in Fig. 4A, B. Closely packed metaphase
sized blocks are found only exceptionally. Elsewhere
the chromatids consist of smaller groupings of fibres,
which we call clusters, most of these being in the range
0.7-1.1 μm in diameter though some are as small as
0.5 μm. The arrangement of the clusters is irregular
and shows great variation, even in different parts of
the same chromosome; we interpret this as reflecting
various degrees of decondensation from the fully
packed metaphase organization. In a few regions
clusters form a more or less linear series. In most
parts, however, the arrangement is staggered, so that
the path joining clusters is convoluted or even a zig-zag
(e.g. see Fig. 5A, B). In such regions the junctions
between clusters are of two classes: one set (large
arrows, Fig. 5A) runs diagonally to the long chromo-
some axis, across the full chromatid width, whereas
the others (small arrows, Fig. 5B) are shorter and run
at right angles to the first set. On the basis of their size
and disposition we suggest that the former represent
vestiges of the original metaphase grooves, now separ-
ating blocks that are subdivided into smaller clusters of
fibres. A linear array of these smaller clusters in each
chromatid forms an axial structure, which is itself
partly unpacked from a folded metaphase arrange-
ment.

In the most grossly extended chromosomes
(Fig. 6A, B) sister chromatids are convoluted and
often separate, though they are similar in length and
overall appearance. Compared with control material,
each chromatid is smaller in diameter; it is also beaded
for most of its length, consisting of a chain of fibre
clusters (Figs 6B, 7). These clusters vary in size (from
about 0.3 to 1.1 μm average diameter), but in opti-
manally spread sister chromatids the patterns match well
(Fig. 7). The clusters forming one chromatid are
usually in contact with each other, but some are
isolated, and there may be breaks in the chromatids at
intervals, perhaps indicating differential stretching
along the chromosome; in still other cases clusters are
more closely packed so that the chromatid outline is
smoother. At this stage of extreme elongation chromo-
somes are some three to five times the metaphase
length, or longer, the most extended chromosome
observed having a length of about 130 μm. Estimates
of the numbers of clusters per chromatid for two
chromosomes, 130 and 70 μm long, are 170 and 130,
respectively. In these two highly extended chromo-
somes, clusters are reasonably similar in size, with
about 70 % having a diameter in the range 0.4-0.6 μm.

In the expanded chromosomal pattern, the first
signs of decondensation involve one or more of the
large metaphase blocks, either confined to the telo-
meres or occurring in several regions of the chromo-
some. Blocks appear to have split laterally across
the width into two smaller groupings; these may be
aligned or slightly staggered with respect to the
chromatid axis. As decondensation continues the
chromatids increase in width and an increasing pro-
portion of the blocks appear subdivided into smaller
clusters of fibres. Usually sister chromatids are affec-
ted similarly, but sometimes a group of small clusters

Fig. 3. Stereo pair of part of a slightly decondensed, repair-inhibited chromosome showing loosely packed blocks and
revealing hints of underlying gyres: many grooves run diagonally to the long chromosome axis, and connecting folds
(arrow) are seen between adjacent chromatin blocks. ×13000. Bar, 1 μm.
in one chromatid corresponds to a single, larger cluster in its sister (Fig. 8). In the most extreme situation, whole chromosomes consist of a mass of clusters (Fig. 9), all smaller than the blocks of control chromosomes. The width of these chromatids is now considerably increased (up to 7 µm), though their length is only 1.5–3 times that of the control and not more than 60 µm. In one example, there are about 100 clusters per chromatid, two-thirds of which have an average diameter from 0.4–0.6 µm, though the chromosome length is only 32 µm. At this stage the relation between the pattern of clusters and the original metaphase blocks is not apparent, although sometimes clusters lie in groups.

At the stage of decondensation corresponding to an amorphous mass in the light microscope (e.g. see Fig. 1E), preparations are not structureless in the SEM, but consist of an assembly of clusters with an average diameter in the range 0.2–0.9 µm (Fig. 10). By this stage clusters cannot be attributed to particular chromosomes, although often there are regions where clusters lie in chains reminiscent of less completely decondensed chromosomes. There are about 2000 clusters per spread originating from a single cell. In over-trypsinized preparations the organization into clusters is completely lost and all that remains is a tangled mass of fibres.

**Scanning electron microscopy of interphase nuclei**

In unirradiated populations of mitotic cells there are a few interphase cells and these give rise to nuclear spreads. Each nuclear area is clearly delimited and relatively smooth in outline (Fig. 11A) but the nuclear envelope is not recognizable, perhaps because it has been digested away during trypsin treatment. Nuclei appear as a mass of fibre clusters ranging in diameter between about 0.3 and 1 µm (Fig. 11B). While these are similar in size and appearance to clusters in the highly decondensed, repair-inhibited metaphase chromosomes, they are more closely packed and not obviously arranged in chains or other groupings.

**Discussion**

By means of scanning electron microscopy we have examined the disassembly of the metaphase chromosome making use of the progressive decondensation induced by u.v. irradiation and subsequent incubation with inhibitors of repair synthesis (Mullinger & Johnson, 1985). In this way in spread preparations

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Fig. 4. A. Repair-inhibited chromosomes showing partial decondensation of the elongated pattern. ×1400. Bar, 10 µm.
B. Enlargement of part of A showing heterogeneous organization of chromatids. ×4200. Bar, 1 µm.
Fig. 5. Enlargements of part of 4B. A shows large diagonal grooves (large arrows) and, running normal to these, smaller subdivisions (small arrows) between fibre clusters of the loosely packed axial structure in each chromatid. ×13000. B. Stereo pair of part of A. ×13000. Bars, 1 μm.
Fig. 6. A. Highly elongated, repair-inhibited chromosomes. ×1500. Bar, 10 μm. B. Enlargement of part of A. Chromatids are composed of chains of clusters that generally lie in close juxtaposition within the chromatid, though are sometimes detached. ×4600. Bar, 1 μm.

Fig. 7. Stereo pair of part of another highly elongated, repair-inhibited chromosome in which matching of clusters between sister chromatids is particularly clear. ×12000. Bar, 1 μm.
of Carnoy-fixed, hypotonically swollen cells we can detect a progressive loosening of the tightly packed chromosome. As the chromatids extend the axis is revealed in the form of a series of discrete chromatin fibre clusters. Further attenuation is associated with the separation of clusters and loss of chromosome identity. We suggest that these features show aspects

![Image](image.jpg)

**Fig. 8.** Repair-inhibited chromosome exhibiting expanded pattern of decondensation. The extent of decondensation varies within the chromosome, being greatest at the telomeres. In one region large fibre blocks, separated by narrow links (arrow), correspond in the sister chromatid to several smaller clusters. ×9000. Bar, 1 µm.

![Image](image.jpg)

**Fig. 9.** A. Highly decondensed chromosomes of the expanded type. Almost the entire chromosome is dispersed into clusters and the distinction between separate chromatids is lost. ×1200. Bar, 10 µm. B. Part of one expanded chromosome showing fibre clusters. Arrow marks a telomere. ×10000. Bar, 1 µm.

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of the higher order packing of the metaphase chromosome.

Packing of the chromatid axis: evidence for gyres?
A spiral or, more accurately, a gyred axial structure has been inferred by a number of authors on the basis of light microscope (LM) observations of mitotic and meiotic chromosomes, viewed after treatment with various hypotonic salt solutions, and sometimes in untreated material (e.g. see Manton, 1950; Bajer, 1965; Ohnuki, 1968). The putative gyres appear fairly uniform in amplitude and frequency along the chromosome axis; but they also vary in size and number according to the state of contraction of the chromosomes (Manton, 1941; Ohnuki, 1968; Ruzicka & Schwarzacher, 1974), and are no longer visible in highly extended material (Ohnuki, 1968). The appearance of a gyred axial structure is particularly clear

Fig. 10. A. Extreme stage of decondensation of repair-inhibited metaphase cell in which individual chromosomes are no longer recognizable. ×1600. Bar, 10 μm. B. Stereo pair of part of A, showing arrays of fibre clusters. ×9100. Bar, 1 μm.
in standard, hypotonically treated, fixed LM preparations of control chromosomes stained with Giemsa (Mullinger & Johnson, 1985). Can scanning electron microscopy (SEM) help to resolve the structural basis or reality of the light microscope images of axial gyration?

Fig. 11. Nuclear spreads from unirradiated interphase cell, showing fibre clusters. A. One nucleus. ×7400. Bar, 1 μm. B. Stereo pair of part of one nucleus. ×11,000. Bar, 1 μm.
The transverse grooves of control SEM material may possibly reflect an underlying gyre. However, while the images are persuasive the evidence is not yet compelling, and there are various reasons why grooves may not all correspond in a simple manner to the spaces between adjacent gyres. For example, geometrical considerations dictate that the turns of a helix would run diagonally in a direction determined by the dimensions of the axial structure and the coils. But there is a tendency for many of the SEM grooves in control chromosomes to run at, or close to, a right angle with respect to the long axis of the chromatin in both top and side views. Furthermore, we find the average number of LM axial turns is rather less than the number of SEM grooves (Mullinger & Johnson, unpublished data). This is also true for human chromosomes; for example, at metaphase the number of grooves along chromosome 5 is similar to the number of LM gyres; at prometaphase, however, the increase in the number of grooves is much greater than the increase in LM gyres (data compared are from Harrison et al. 1985; Ohnuki, 1968).

In control material, one of the problems of analysis is the high degree of chromatin compaction and extreme sensitivity of grooves to optimal chromosome preparation and trypsinization. Clearly the final metaphase compaction largely subsumes evidence of linear organization along the axis, gyred or otherwise. An additional problem is that the SEM provides only a surface view of any object whereas the contorted LM axial structure appears to form a core (Howell & Hsu, 1979). In the more relaxed, repair-inhibited chromosome, there is clearer evidence of diagonal grooves and there are also regions where the chromatid diameter is reduced, providing reasonable evidence of a continuous folded axis (Figs 2, 3, 8). In even more decondensed material, the convoluted path of the chromatid axis can be interpreted as evidence of a partially unwound helical gyre (Figs 4, 5). It seems to us that while the SEM helps to identify the pattern of axial convolution, particularly in relaxed chromosomes, it does not provide definitive proof for the existence of the LM helical structure suggested by many authors. A detailed comparison between LM and SEM images of a specific chromosome examined in different stages of unpacking may help to resolve the problem. However, what the SEM images confirm is that in relaxed chromosomes there is indeed an axial structure of relatively uniform diameter from which the convolutions have been removed.

Both LM helices and SEM grooves have been invoked as a basis for G-banding (Kato & Yosida, 1972; Harrison et al. 1981). The frequency of G-bands varies with the stage of division (Yunis et al. 1978), and in Chinese hamster chromosomes there is imperfect mapping between turns of the LM gyres and G-bands (Kato & Yosida, 1972). The matching of gyres and G-bands holds good for human chromosomes only when G-band resolution is poor, and the well-resolved G-bands of prometaphase are far more numerous than the helical turns (e.g. see Haapala & Nokkala, 1982). It seems likely that conditions used to reveal both gyres and bands amplify, perhaps in a slightly different manner, the same underlying structure. The SEM grooves of human chromosomes clearly correspond with the G-band pattern (Harrison et al. 1981, 1985), and both features are revealed by trypsin treatment; grooves correspond to darkly staining bands. However, the match though good is not exact; a single G-band may include several neighbouring grooves, or a groove may correspond to an inter-G-band region. Also, the increase in number of grooves between metaphase and prometaphase is again much greater than the increase in number of G-bands (data from Harrison et al. 1985). Nevertheless, we assume that differential adherence of fibre blocks along the highly contracted metaphase chromosome is at least partly responsible for the irregular G-banding pattern and that latent grooves become visible in more extended chromosomes where G-bands are more numerous. The reason for heterogeneous adhesion of fibre blocks is not clear, though the species specificity of G-banding patterns points to an underlying sequence dependence.

**Fibre clusters**

Regardless of the details of the final stages of chromosome packing, an important new characteristic of the chromatid axis is revealed in the present paper, namely the presence of regular subdivisions in the form of clusters of chromatid fibres. Unlike the apparent discontinuities associated with the metaphase grooves, the junctions between the clusters of the extended axis are likely to separate distinct units of DNA packaging. In the partially unwound chromatid (Fig. 5) these cluster-junctions are distinguishable from the grooves and run at a different angle to the axis. In the fully extended chromatid the junctions between clusters appear as periodic constrictions of the axis.

The main issue is whether the chromatin clusters reflect the true underlying chromosomal substructure. Since chromosomes of repair-inhibited cells are not decondensed in the untreated intact cell seen in the light microscope (Mullinger & Johnson, 1985), there is a possibility that clusters are promoted by one or more stages in the preparation. There is evidence that trypsinization, and/or possibly Carnoy fixation, remove certain proteins from metaphase chromosomes (Sumner et al. 1973; Burkholder & Duzech, 1982), and it is possible that chromosomes containing incomplete repair sites are more extensively affected by these treatments or by exposure to hypotonic solutions.
Although some artifacts are likely to be generated by stages of disassembly of the final metaphase structure; boundaries or planes of weakness in chromatin are these means, it is equally possible that pre-existing structure are: (1) there is a progressive series of the preparations revealing units of chromosome sub-data). (Brinkley et al. 1980), spreading or air drying. Variations, and is relatively greater in muntjac than in chromatids, though the extent of length increase with clusters of interphase chromatin seen in nuclei and also in prematurely condensed chromosomes (PCC) (Mullinger & Johnson, 1983, and unpublished data).

Decondensation involves an increase in length of chromatids, though the extent of length increase varies, and is relatively greater in muntjac than in HeLa cells (Mullinger & Johnson, unpublished). In the most extreme case (muntjac elongated pattern) an increase to five times or more the metaphase length is achieved. This is far greater than the normal twofold increase from prometaphase to metaphase in this cell (Sen & Sharma, 1985). It is, however, very similar to the maximum average difference in length of interphase compared with metaphase muntjac chromosomes. Interphase chromatin can be induced to condense into recognizable chromosomes prematurely by fusion of interphase and mitotic cells; the length of such chromosomes (PCC) depends on the phase of the cell cycle but, except for the very earliest G1 chromosomes, is always greater than in the mitotic partner. The longest G1 chromosomes are on average five times the metaphase length (Sperling, 1982); the longest muntjac chromosome can extend by late G1 to 126 μm, a figure close to the maximum decondensed length recorded in repair-inhibited material. This suggests that there may be some critical stage of disassembly corresponding to the maximally unravelled, but still intact, metaphase chromosome axis. Beyond this stage clusters detach from each other, as in the most highly decondensed material seen in this study. The total loss of cluster organization associated with excess trypsin treatment suggests that the grouping of fibres is dependent on one or more protein components.

The most extended muntjac chromosomes are made up of a chain of small, contiguous clusters, some 70% of which have an average diameter of 0.4–0.6 μm. There are some 150 clusters per chromatid and, assuming this is an estimate for muntjac chromosome 1, the total number per haploid set would correspond to some 450 clusters. This figure as expected exceeds the number of G-bands at metaphase (75–89) and prometaphase (220–283), but it does not reach the G1 PCC number (935) (Rohme & Heneen, 1982; Ved Brat et al. 1979; Sen & Sharma, 1985). The features underlying G-banding in G1 PCC are even less well established than in metaphase chromosomes, but it is possible that in these decondensed chromosomes there is a different basis, namely the accumulation of stain at the boundaries between loosened adjacent fibre clusters, instead of at grooves. If so, the shortfall in numbers of clusters in repair-inhibited, extended metaphase chromosomes would be explained if perhaps they do not unpack as completely into clusters.

Clusters are not only found in the elongated chromosomes. First, they occur in the less-extended, though laterally expanded and decondensed, chromosomes described in this paper; here clusters are similar in size and abundance in sister chromatids, even if they do not match exactly, presumably because of spatial constraints in spreading. Similar fibre clusters are also found in a more-dispersed, less-orderly array in the final stage of decondensation of repair-inhibited metaphase chromosomes, as well as in interphase nuclei and in S-phase PCC (Mullinger & Johnson, 1983). The possibility that clusters are in some way a special feature of SV40-transformed muntjac cells, which have a post-replication repair-defective phenotype and are, therefore, hypersensitive to u.v. (Pillidge et al. 1986), is excluded by the observation that essentially similar structures are observed in repair-inhibited, decondensed metaphase chromosomes of HeLa cells (Mullinger & Johnson, unpublished data). All this suggests that clusters represent a basic higher order assembly of chromatin fibres. The identity of this assembly is uncertain but it may correspond to a replication structure (Lau & Arrighi, 1980a,b; Mullinger & Johnson, 1983; Hameister & Sperling, 1984), to a chromomere, or to clusters of loops such as those seen in largely deproteinized DNA of metaphase HeLa chromosomes, spread by the Kleinschmidt technique on an air/water surface (Mullinger & Johnson, 1980).

If fibre clusters do represent a higher order unit of chromatin packing, how are these arranged in the fully contracted metaphase chromosome? Our previous work with S-phase PCC (Mullinger & Johnson, 1983) suggested that as replication proceeds paired fibre aggregates appear in chains to produce the G2 chromatids, and that during this process smaller aggregates are assembled into larger groupings in an unknown manner. On the basis of the likely number of replicon clusters per genome we suggested that the smaller fibre aggregates possibly represent replicon clusters. Much the same conclusions had been drawn by Lau & Arrighi (1980a,b) from an analysis of differentially stained sister chromatids in PCC preparations. Our observations on repair-inhibited chromosomes lend support to this view. For instance, as chromosomes become more decondensed the size of the fibre assemblies decreases and their number increases. This applies irrespective of the overall change in length.
of the chromosome. The observation that in some expanded chromosomes a large block in one chromatid seems to correspond to a group of several smaller clusters in the sister chromatid, together with the apparent grouping of clusters in other chromosomes, also adds weight to the argument.

Molecular events underlying decondensation

Turning to the molecular changes that underlie decondensation, we have little information about the events that predispose such massive chromosome-wide behaviour. We have shown previously that the speed and degree of decondensation are closely correlated with the number of DNA breaks accumulated during incubation with repair synthesis inhibitors. The majority of these breaks are likely to be single-strand, though with increasing time a proportion are converted to the double-strand form (Mullinger & Johnson, 1985). We have also calculated that, for extensive chromosome-wide decondensation of human chromosomes to occur, around 15 or more breaks per 10⁶ daltons of DNA (up to 2000 for the largest human chromosome) are necessary (Mullinger & Johnson, 1985). It is worth pointing out that this speed corresponds to about one break per 10⁹ base pairs, or one per 500 nucleosomes, so the chromosomal changes reflect an enormous amplification of relatively rare inhibited repair sites in chromatin. It is possible that in the repair-inhibited state chromosome structural proteins are much more readily removed or displaced by one or more steps in the preparative methods. Whatever the nature of the changes they are on the whole readily reversible on removal of inhibitors (Mullinger & Johnson, 1985). The extent to which they mimic molecular events associated with the normal condensation/decondensation chromosomal cell cycle remains to be established. Further dissection of subchromosomal higher order structures awaits the development of suitable molecular probes.

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