INTRANUCLEAR ELECTROPHORESIS OF THE
CHROMATIN OF LIVING CELLS

R. J. SKAER, SUSAN WHYTOCK AND J. P. EMMINES
Department of Haematological Medicine, Hills Road,
Cambridge CB2 2QL, England

SUMMARY

Intranuclear electrophoresis of living cells under appropriate conditions causes the chromatin and nucleoli to move rapidly into the anodal side of the nucleus. In pig kidney cells, chromatin lengths attached to the nuclear envelope are oriented by the current and freed from surrounding non-oriented chromatin. Individual chromatin strands isolated in this way are often long and have not been subjected to the trauma of isolation from the nucleus. This has allowed us to demonstrate oriented lines of up to 8 chromomeres in a strand, linked by fine single fibres. These chromomeres of chromatin have the same linear dimensions as the bands and interbands of polytene chromosomes. A very wide range of morphology of chromatin is revealed— from lines of nucleosomes in open array, to strands uniformly 25 nm thick. Doublet strands and multiple strands— often embedded in darkly staining material— are also seen. All morphological types may be seen in the same nucleus. Many of the oriented threads appear to be transcriptionally active. The variable morphology of these sites and their relation to peripheral heterochromatin is discussed. Histone nucleosomes are present in these apparently transcriptionally active regions. The method is useful for investigating the relationships between chromatin and the nuclear envelope. Approximately 1500 attachment sites per nucleus are found in these cells. Some nucleoli are attached to the nuclear envelope.

INTRODUCTION

Intranuclear electrophoresis is a powerful tool for orienting lengths of chromatin in nuclei and revealing these oriented lengths free from non-oriented chromatin (Skaer & Whytock, 1976a).

This paper describes 4 features of nuclear organization that have become clearer as a result of the technique: (1) the thickness and fine structure of chromatin threads; (2) the morphology of what are apparently sites of RNA transcription and their location in relation to the nuclear envelope; (3) the linear organization of the chromatin thread with particular reference to any organization into a linear pattern that could correspond to the bands and interbands of polytene chromosomes; and (4) physical links between nearby chromatin threads, between the oriented chromatin and the nuclear envelope, and between the nucleolus and the nuclear envelope.

These aspects of the organization of chromatin are discussed in relation to the structure of chromosomes from other tissues—with particular reference to lampbrush chromosomes and polytene chromosomes. Possible artifacts of the technique are also discussed. However since intranuclear electrophoresis can be applied directly to living cells rather than to isolated nuclei or isolated chromatin—techniques normally necessary for the examination of long lengths of chromatin—artifacts resulting from isolation techniques are avoided.
MATERIAL AND METHODS

A primary culture of pig kidney epithelial cells (a gift from Dr M. Daniel) was grown on elongate pieces of Falcon plastic Petri dish in Dulbecco's medium supplemented with glutamine and 5% calf serum. The cells were electrophoresed in situ using 2 parallel platinum wire electrodes 1 cm long separated by 1-1.5 cm with a P.D. of 140 V/cm. Current was passed for 2-3 s only. Longer electrophoresis caused acid and alkaline products of electrophoresis to accumulate and damage the cells. Three per cent glutaraldehyde in 0.1 M cacodylate buffer pH 7.4 was dropped on to the cells before the current was switched off. The direction of the current was along the length of the strip of plastic dish; the end nearer the cathode was marked. Cells were fixed for 1 h at room temperature and were then treated with 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h. They were dehydrated in a graded series of ethanol and embedded in Spurr resin directly from absolute ethanol. The plastic from the Falcon dish, though rendered opaque by this treatment could be sectioned with the cells parallel to the direction of the current. Thin sections doubly stained with uranyl acetate and lead citrate were examined at 60 kV in an AEI EM6B electron microscope.

Conditions for electrophoresis. Although this paper deals only with pig kidney cells, we have applied the method successfully so far to HeLa cells and the salivary glands of Drosophila larvae. The response varies with the medium surrounding the cells and also with the arrangement of the cells in relation to the electric field. Thus the giant chromosomes of Drosophila move to the anodal side of the nucleus if the salivary glands are electrophoresed at 10-30 V/cm in Hanks' solution, pH 7.5 or Hanks' solution diluted 1:4 with distilled water. On switching off the current they move back nearly to their original positions. On the other hand they do not move significantly if the glands are electrophoresed in the same way in Lezzi's solution (90 mM KCl, 60 mM NaCl and 1 mM CaCl₂, pH 6.3). With a culture of pig kidney cells grown as small clumps of cells attached to a coverslip, extensive movement occurs in all cells electrophoresed at 140 V/cm. If the culture becomes confluent and is electrophoresed under the same conditions only occasional cells throughout the culture are affected - most commonly those at the periphery. Lower voltages produce less movement.

Isolated nuclei. The method also works on isolated nuclei provided the nuclear envelope is not destroyed. Philpot & Stanier (1954) observed the movement of chromatin towards the anode resulting in the production of eclipse forms of nucleus by 10-50 V/cm electrophoresis of isolated rat liver nuclei in solutions buffered to pH 6.9 or 8.0. We find that the contents of isolated nuclei of HeLa cells move to the anode if electrophoresed in Hanks solution even in the presence of 5% Nonidet P40. No movement occurs, however, if the isolated nuclei are first treated with phospholipase C for a few minutes until the nuclear margin becomes slightly indistinct.

RESULTS

Electrophoresis results in the great majority of the chromatin, nucleoli and components of the nuclear sap becoming arranged in the half of the nucleus nearest the anode (Fig. 1). Remaining in the half of the nucleus nearest the cathode are the oriented chromatin lengths, nucleoli that are attached to the nuclear envelope.
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and some components of the nuclear sap. A potential difference strong enough to orient the chromatin (Fig. 2) does not remove ribosomes from their attachment to the endoplasmic reticulum (Fig. 2 and its enlargement Fig. 3). Unattached ribosomes are moved to the cathodal end of the cell.

Nuclear sap

Most of those components of the nuclear sap that become fibrous on fixation move into the anodal side of the nucleus. This can be shown by comparing pig kidney cells that have been electrophoresed with cells of the same line that have been centrifuged at 140,000 g for 2 h at 4 °C—a process that packs chromatin into the centrifugal side of the nucleus. Fixation in cacodylate-buffered glutaraldehyde in the usual way after these treatments shows that the artifactual network produced in the nuclear sap by glutaraldehyde fixation (Skaer & Whytock, 1976b, c) is much denser and more prominent in the centripetal half of the nuclei of centrifuged specimens than in the cathodal side of the nuclei in the electrophoresed specimens.

Thickness and fine structure of chromatin threads

Most of the chromatin threads oriented by this technique are extremely thin—less than 5 nm thick and of very variable morphology. They are often coated with beads and granules of varying sizes. The smallest beads seen in oriented chromatin are approximately 10-12 nm in diameter—the size of nucleosomes (Oudet, Gross-Bellard & Chambon, 1975). In some regions (Figs. 5, 9, 10, small arrows) they are separated along the thread by slightly more than their own diameter. In other regions of the same thread the beads are packed so that they touch each other (Fig. 5). In these regions the thread is 20-25 nm thick (Fig. 5). It is not clear to what extent the more open arrangement might be a result of slight stretching of the thread during orientation.

In some places the thread itself is coated with beads larger than nucleosomes but

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Fig. 2. Nucleus of a similar cell showing a nucleolus attached to, and distorting, the profile of the nuclear envelope. At the top of the nucleus a cisterna of the endoplasmic reticulum arises from the nuclear envelope. × 12,500.

Fig. 3. Enlargement of the endoplasmic reticulum shown in Fig. 2. Despite electrophoresis ribosomes are attached to both the anodal and cathodal faces of the cisterna. × 50,000.

Fig. 4. Clump of tangled threads of what may be hnRNA (R) containing a granule the size of a perichromatin granule (large arrow). The chromatin thread to which the clump is attached is arranged in an open V shape and at each end shows a site of ectopic pairing (small arrows) with long chromatin threads oriented by electrophoresis. Enlargement from Fig. 1. × 40,000.

Fig. 5. Enlargement of part of the V-shaped chromatin thread shown in Fig. 4. The thread ends in a cluster of granules the size of interchromatin granules (large arrow). Nucleosomes (small arrows) are linked by fine threads. At the double-headed arrow the thread is 25 nm thick and is apparently made of packed nucleosomes. × 100,000.
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in a fairly open packing; some are approx. 16 nm in diameter (large arrowheads in Fig. 10, small arrows in Fig. 6) but most are 20–25 nm (Fig. 10, large doubleheaded arrow) (fig. 5 in Skaer & Whytock, 1976a).

Some parts of the thread are free from granules – at z in Fig. 10 some 360 nm of thread is arranged in what may be a helix or a zigzag with a pitch of 18–20 nm. What may be a similar structure is present in Fig. 6(z). In some places the threads appear smooth and approximately 10 nm thick (fig. 5 in Skaer & Whytock, 1976a) or 22–25 nm thick (doubleheaded arrow in Fig. 5) but most commonly a darkly staining nebulous material coats the threads and obscures their structure (Figs. 6, 8, 9). A similar nebulous material encloses clusters of 20–24-nm diameter interchromatin granules (large crossed arrow in Fig. 6).

RNA material

Clusters of interchromatin granules – the latter identified on the basis of their 20–25-nm size and strong staining – occur elongated in a direction approximately normal to the long axis of the thread (Fig. 6, large arrows). Others are mushroom-shaped with the stalk normal to the thread (Fig. 6, large crossed arrow and fig. 2 in Skaer & Whytock, 1976a). Granules of approximately 35 nm – the size of perichromatin granules but without the pale halo typical of these granules (Vazquez-Nin & Bernhard, 1971), are occasionally seen associated with the thread (arrows in Fig. 1). Many typical perichromatin granules are found among the unoriented chromatin packed into the anodal side of the nucleus. Sometimes, complexly tangled threads of darkly staining material are seen as approximately cone-shaped clouds (R on Fig. 4) with the point of the cone often apparently associated on the thread with a cluster of granules the size of interchromatin granules (Fig. 4). There is a striking resemblance between these tangled threads and those identified as hnRNA revealed by the centrifugation technique of Miller & Bakken (1972), Kierszenbaum & Tres (1975), Angelier & Lacroix (1975). The resemblance is strengthened by the presence of large (approx. 40-nm) and small (approx. 20-nm) granules on the tangled threads in both electrophoresed and centrifuged preparations. The larger granules have a densely stippled substructure resembling that of perichromatin granules (large arrow in Fig. 4) (Puvion & Bernhard, 1975). The tangled threads may also correspond to

Fig. 6. Two oriented long lengths of chromatin; that on the left is double (doubleheaded large arrow), the one in the centre is single but has chromomeres (crossed small arrows) joined by a fine thread (z). Further down this thread are elongate clusters of interchromatin granules (large arrows) that occur as lateral extensions, approximately normal to the long axis of the thread. Similar (mushroom-shaped) clusters (large crossed arrow) occur near the point of maximal separation of the doublet thread. Granules 16 nm in diameter lie in approximate register on the 2 threads (small arrows). Fibrous components produced by the effect of the glutaraldehyde fixative on the nuclear sap form a background to the threads. x 52,000.

Fig. 7. Long single length of oriented chromatin with a region of 3 parallel fibres (arrow). x 50,000.
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the perichromatin fibrils found by Nash, Puvion & Bernhard (1975) to be the site of rapid incorporation of tritiated uridine when starved, isolated hepatic cells are subjected to step-up conditions.

Length of transcribing chromatin

The clump of tangled fibrils shown in Fig. 4 and regarded on morphological grounds as hnRNA is associated with a length of chromatin drawn by electrophoresis into the shape of an open V, with the transcribed material lying at the point of the V nearest the anode. The V is suspended between 2 regions (arrows in Fig. 4). In serial sections these points of attachment can be seen to be threads that lie more or less along the direction of the current (Fig. 1). The points of attachment themselves are very granular and coated with densely staining nebulous material. The length of chromatin in the V is presumably the length available for transcription. On one side of the V it is 0.8 μm and on the other approximately 0.6 μm. The length of hnRNA in the clump appears to be much longer than this.

Double threads

Oriented lengths of chromatin are quite commonly double over at least part of their length (Fig. 6, large doubleheaded arrow) (fig. 5 in Skaer & Whytock, 1976a) and the double regions have a surprisingly similar morphology wherever they are found. Two regions of maximal separation of the threads are joined by a short region where the 2 threads cross over each other. The maximum distance between the 2 threads is rarely more than 60 nm. Clusters of interchromatin granules embedded in darkly staining material commonly occur near the regions of maximal separation (crossed large arrow in Fig. 6). Distal to the region of maximum separation the 2 threads approach each other gradually over a distance of up to 0.4 μm, and can often be seen to intertwine over a distance of at least 0.5 μm. Granules approximately 16 nm in diameter on each thread sometimes appear to be in register across the double threads (small arrows in Fig. 6).

Multiple threads

Threads with 3 parallel dark lines for part of their length (large arrow in Fig. 7) and multiple threads with 4 or more parallel strands embedded in darkly staining material (arrow in Fig. 8) occur occasionally.

Fig. 8. Several long lengths of chromatin, each consisting of several parallel fibres (arrow) embedded in a darkly staining nebulous material. x 30000.
Fig. 9. Enlargement of Fig. 8 showing lines of nucleosomes in open array (arrows) embedded in darkly staining material. x 126000.
Fig. 10. Enlargement of the ends of 3 long oriented chromatin threads shown in Fig. 1. Nucleosomes (small arrows), 16-nm granules (arrow heads) and 20–25-nm granules (double-headed arrow) all occur on oriented threads. At 2 is a long length of thread free from granules or beads but showing a zigzag or helix with a wavelength of approximately 17 nm. x 70000.
Regions of heterochromatin

Heterochromatin, fixed in glutaraldehyde, is commonly coarsely granular, containing granules 20–25 nm in diameter (Fawcett, 1966). Peripheral heterochromatin in electrophoresed cells is typically rich in granules of this size, and is not reoriented by the current since it is firmly attached to the nuclear envelope. Very small regions of heterochromatin isolated from the main aggregates by intranuclear electrophoresis are therefore difficult to distinguish by conventional electron microscopy from clusters of interchromatin granules. As in most heterochromatin it is difficult to see the chromatin threads owing to the presence of the granules and darkly staining nebulous material. Linearly arranged local differentiations of the chromatin into irregular-shaped regions each 0.1–0.3 μm across are occasionally seen. These contain non-oriented threads and 20–25-nm diameter granules and would appear to be heterochromatin aggregates joined together by single oriented threads (small crossed arrows in Fig. 6). The linking threads, each 0.05–0.2 μm long, join the centres of each darkly staining, granular region together – so they look like clouds on a string. Up to 8 such clouds have been seen on a single string.

On the other hand in some regions of oriented threads, aggregates of what are apparently interchromatin granules are packed tightly together (large arrow in Fig. 6) as lateral extensions from the thread and might conceivably contain lateral loops of chromatin. These regions occur at apparently irregular intervals along the thread. Each occurs on one side of the thread only but since they are distributed in 3 dimensions they may be more numerous and closely spaced than they appear to be in a single section.

Associations between different chromatin threads

Figs. 1 and 3 show a length of chromatin thread that has not oriented to lie between anode and cathode; rather, because of its association with 2 chromatin threads that have oriented in the current, it has come to form the cross-piece of an M-shape. Other similar associations have been seen. These seem to occur naturally rather than as aggregation products of electrophoresis, for both oriented thread and cross-piece are bound together by darkly staining nebulous material.

Associations between chromatin threads and peripheral heterochromatin or the nuclear envelope

Almost all the oriented threads arise from patches of heterochromatin that are firmly attached to the peripheral lamina of the nuclear envelope. Oriented threads of chromatin are of 2 types: short (0.5–1 μm) and long (2–5 μm). The short lengths usually span between a clump of peripheral heterochromatin on the nuclear envelope and a clump of peripheral heterochromatin in the nuclear sap on the anodal side of the origin. These short lengths are more numerous than the long lengths. It seems unlikely that the short lengths are produced by breakage of the chromatin fibre during orientation. It seems more likely that they are short loops of chromatin extending to and from the nuclear envelope but that only one side of the loop is
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usually seen owing to the effects of sectioning. Whether or not some threads are broken by electrophoresis does not affect the calculation of number of attachments. On the average 1–2 long (2–4 μm) and 2–3 short (0.5–1 μm) oriented chromatin threads can be seen in each 50-nm-thick section. Attachments of such threads to peripheral heterochromatin can be seen in approximately one-third of each nuclear profile. Since each nucleus is approximately spherical and 9–10 μm in diameter the surface area of such a nucleus (4πr²) must be at least 250 μm², so there must be 600–1200 oriented long lengths and 1200–2000 short lengths present. From serial sections of one particular nucleus 9 μm in mean diameter we find a mean of 1.3 long lengths and 2.88 short lengths (or 26.6 and 57.6 long and short lengths per 9.5 μm nuclear perimeter per μm thickness of section). This gives 717 long lengths and 1552 short lengths per nucleus. From the sections it looks as if each of these arises from a single clump of heterochromatin on the nuclear envelope. A figure of 2269 attachment points to the nuclear envelope per nucleus, however, is likely to be an overestimate since many of the attachment points are likely to be intercalary in the chromatin rather than terminal. A figure of perhaps one-half to two-thirds of this estimate might be nearer the true number of attachment points per nucleus.

Quite commonly the clump of heterochromatin from which an oriented thread arises can be seen to be attached, by a single strand of chromatin, or sometimes by a bridge of heterochromatin, to a second clump of heterochromatin on the nuclear envelope. Occasionally a single chromatin strand appears to be attached directly to the peripheral lamina of the nuclear envelope. These always extend from the nuclear envelope to a patch of heterochromatin that is also attached to the nuclear envelope. Sometimes a single loop of 20-nm-thick chromatin spans between a clump of heterochromatin on the nuclear envelope and the peripheral lamina adjacent to a nuclear annulus. Chromatin fibres that end on the nuclear annuli (DuPraw, 1965) have not been seen. In the pore of each nuclear annulus on the nuclear side is a clump of at least 6 very fine fibrils 2–4 nm thick and 100 nm long that may arise from the inner annular granules as suggested by Franke & Scheer (1974). These fine fibrils are quite distinct from chromatin fibres. It is possible that there are associations between chromatin and the nuclear envelope that do not involve heterochromatin but that these are displaced by electrophoresis. This will be tested by using lower voltages.

Associations between the nucleolus and the nuclear envelope

Most nucleoli are found in the anodal half of the nucleus (Fig. 1). Some nucleoli, however, are firmly attached to the nuclear envelope (Fig. 2). In the living cell the nucleoli can be seen to move towards the anode during electrophoresis; in Fig. 2 the nucleolus attached to the nuclear envelope has distorted the nuclear profile, pulling the envelope itself slightly towards the anode. Some nucleoli (Fig. 2) are attached to the nuclear envelope directly by perinucleolar heterochromatin. After electrophoresis this appears frayed – unlike its compact appearance normally and in those nucleoli found in the anodal end of the nucleus. In each section of an attached nucleolus several short lengths (0.2–0.5 μm) of chromatin thread extend from the nucleolar surface and do not appear to be oriented by the current. Interspersed among these
short lengths are long lengths (2–4 μm) of chromatin that arise from the perinucleolar heterochromatin and are oriented by the current.

Some nucleoli are attached to heterochromatin on the nuclear envelope by several strands of chromatin 2–3 μm long. It is possible that some nucleoli in the anodal half of the nucleus may actually be attached on the cathodal side of the nucleus but by chromatin threads longer than half the nuclear diameter.

**DISCUSSION**

*Morphology of the chromatin thread*

Chromatin threads prepared by intranuclear electrophoresis are strikingly variable in structure. In their thinnest regions the threads can often be seen to be coated with nucleosomes, sometimes in regions that appear to be active transcriptionally. The assumption of transcriptional activity is based on the resemblance between complexly tangled fibres with granules (R in Fig. 4) and similar structures prepared by centrifugation techniques (Miller & Bakken, 1972; Angelier & Lacroix, 1975), spreading at an air/water interface (Kierszenbaum & Tres, 1974) or sectioning (Nash, Puvion & Bernhard, 1975). In the latter 2 studies the distribution of a pulse label of tritiated uridine coincides approximately with the regions of supposed hnRNA.

It is generally assumed that nucleosomes of histones are absent from regions that are active transcriptionally (News and Views (1975), *Nature, Lond.*, 258, 662) for they are not seen in active ribosomal RNA genes or on the loops of lampbrush chromosomes. Bonner's (1975) 'template-active fraction' of chromatin is impoverished in histones. Our results suggest that histone nucleosomes probably do occur on active genes. Kierszenbaum & Tres (1975) show beaded chromatin in transcriptionally active regions of mouse early spermatids, but in these cells protamines may, partially at least, replace histones.

The range of morphology of chromatin threads shown up by intranuclear electrophoresis is from what may be naked DNA (z in Fig. 10), through strands of approximately uniform thickness at 10 nm (Skaer & Whytock, 1976a) or 22–25 nm thickness (Fig. 5) to thick aggregates up to 0.15 μm across in which threads are embedded in darkly staining amorphous material of unknown composition (Figs. 8, 9). This range of structure exists even without the possible interference of artifactual fibres from the nuclear sap (Skaer & Whytock, 1976b) and after fixation with only one fixative method. It is possible that some of the variability in structure is artifactual, perhaps due in some cases to stretching by the electrophoretic orientation or the destruction of organization by electrophoresis or fixation. The open array of nucleosomes in Fig. 9, however, is possibly not due to stretching, as the whole fibre is coated with a thick layer of darkly staining, amorphous material. Finch, Noll & Kornberg (1975) suggest that extended arrays of nucleosomes may sometimes be a denaturation phenomenon and that the packing of nucleosomes may be dependent on the level of free ions in the nucleus—a situation that might be upset by electrophoresis. However, this is unlikely to be a cause of small, local variations in packing of the
Intranuclear electrophoresis of chromatin nucleosomes, and overall changes in packing of the nucleosomes in the nucleus depending on nearness to anode or cathode have not been observed. Solari (1974) has stressed the dependence of the size and shape of chromatin fibres on the specific physiochemical conditions of their surrounding medium and fixative up to and through the dehydration stage of preparative techniques for electron microscopy. Skaer & Whytock (1976c) have pointed out that all fixatives in present use for electron microscopy destroy a substantial amount of the organization of polytene chromosomes. Loss of organization on fixation presumably also happens in chromatin where the structure is not supported by a semi-crystalline array of chromatids as occurs in polytene chromosomes. To that extent chromatin would seem to be more vulnerable to fixation.

The significance particularly of the doublet fibres (Fig. 6, double arrow) cannot be discovered until intranuclear electrophoresis has been performed on synchronized populations of cells, and a range of fixatives in both chelating and non-chelating buffers has been used. Despite the relatively common occurrence of these doublet arrays and their relatively uniform morphology it is not clear if they are a naturally occurring feature of chromatin – perhaps associated with some stages of replication or transcription – or whether they represent an artificial unwinding of an originally thicker fibre owing to chelation of divalent cations by cacodylate buffer in the fixative. Ris (1957, 1975) has claimed for many years that divalent cations are important stabilizers of transcriptionally inactive chromatin fibres with a thickness of 25 nm, and that in the presence of chelating buffers the chromatin thread reduces to 10 nm thickness, perhaps by 2 halves of the thread separating. This separation has never been directly demonstrated. However, the doublet fibres we have seen make up only a small proportion of fibres in each nucleus, so may well not be due to a process such as fixation, applied to the whole nucleus.

Morphology of RNA material

A detailed description of this must be deferred until the results of combining intranuclear electrophoresis with autoradiography become available. However, what may be perichromatin fibrils that presumably contain hnRNA (R in Fig. 4), interchromatin granules (large arrows in Fig. 6) and perichromatin granules (arrow in Fig. 1) have been identified on morphological grounds. The significance of the relatively tight packing of what may be interchromatin granules into elongate arrays oriented normal to the chromatin thread (large arrows and crossed large arrow in Fig. 6) is not at present clear.

The linear organization of the chromatin thread

Crick (1971) has assumed that chromatin has an organization into bands and interbands like a single chromatid from a polytene chromosome. Until now such an organization has not been seen in chromatin. Neither the centrifugation technique (Miller & Bakken, 1972), nor the spreading technique at an air/water interface give a convincing indication of a division into bands and interbands that is such a strikingly clear-cut feature of polytene chromosomes. Collapsed loop structures seen as
occasional interruptions of the normally linear chromatin thread in spread preparations of nuclei from mouse liver could be artifacts of the spreading technique (Comings & Okada, 1974) as these authors suggest. Since, however, both the spreading technique and the centrifugation technique destroy a great deal of the higher orders of chromosome organization (Skaer & Whytock, 1976d) a linear subdivision into bands and interbands might be destroyed by these preparative techniques. Even with intranuclear electrophoresis it is not a striking feature of the organization of the chromatin thread—though this may be due to the effects of fixation as explained earlier. It may also be due to the fraying effect of electrophoresis as shown by the perinucleolar heterochromatin of nucleoli attached to the nuclear envelope. What is revealed by the technique at present are periodic, linearly oriented, ‘clouds on a string’ (small crossed arrow in Fig. 6) up to 8 per string, in which the clouds consist of darkly staining material containing non-oriented chromatin and some granules. The size of these ‘clouds’ and the length of their linking threads is directly comparable to the size of bands and interbands in polytene chromosomes. These ‘clouds’ can presumably be called ‘chromomeres’ since this is a wide, relatively non-specific term. On the one hand it is used for the condensed portions on the axis of a lampbrush chromosome, each of which gives rise to a pair or 2 pairs of loops (Mott & Callan, 1974); on the other hand it is applied to the whole cluster of loops and condensed material on the axis of other types of meiotic chromosomes that correspond numerically to the relatively few bands on mitotic chromosomes (Okada & Comings, 1974). These bands are generally regarded as heterochromatin. The term ‘chromomere’ is also applied to the bands of polytene chromosomes (Sorsa, 1974) though in these Beermann (1972) distinguishes between the majority of bands and those made of heterochromatin. The chromomeres we have seen in the chromatin of pig kidney cells do not differ markedly from clumps of peripheral heterochromatin attached to the nuclear envelope.

Although it is tempting to argue that this demonstration of chromomeres is evidence for the comparability of organization of the hereditary material in all its forms—chromatin, and polytene, lampbrush and mitotic chromosomes, we do not know enough about the fine-structural organization of unsquashed, well fixed polytene chromosomes to decide how similar in detail these are to chromatin. So far we have not demonstrated any lateral loops of chromatin that could be compared with those on lampbrush chromosomes.

Physical links between chromatin threads and the nuclear envelope

The significance of associations between chromatin and the nuclear envelope is not clear—whether it is a mechanism for holding chromatin into some complex arrangement within the nucleus or whether it is related to the activities of transcription or replication is not known. Skaer & Whytock (1975) have shown that the pattern into which polytene chromosomes are held by their associations with the nuclear envelope varies from cell to cell in the same tissue. The small number of attachments between polytene chromosomes and the nuclear envelope, even during replicative stages, contrasts with the large number of initiation sites for replication.
Intranuclear electrophoresis of chromatin on polytene chromosomes. It seems unlikely that an association with the nuclear envelope is necessary for replication (Franke & Scheer, 1974).

It is not known whether actively transcribing genes or puffs move nearer the nuclear envelope as they become active - associations of chromosomes with each other in apparently functionally logical ways have been postulated by Steffensen, Duffey & Prensky (1974). It is important to remember, however, that evidence for this in Drosophila melanogaster is based on occasional associations seen in squashed preparations of polytene chromosomes. Thus the band coding for 5S RNA (a component of ribosomes) though localized on chromosome 2 is occasionally near the nucleolus. The nucleolus organizer, however, is on the X chromosome (Steffensen & Wimber, 1972). Nevertheless, the regions of polytene chromosomes that tend to be permanently attached to the nuclear envelope - the chromocentre and the telomeres - are regions that are likely to be transcriptionally inactive and contain few puffs.

There is certainly a very wide variation from cell type to cell type in the numbers of associations between chromatin and the nuclear envelope - from 10—12 in the polytene chromosomes of Drosophila to the 1000—2000 in these pig kidney cells. It may be significant that many of the oriented chromatin threads - that must be relatively close to the nuclear envelope since they have become oriented - appear to be transcriptionally active. If one divides the several hundreds of thousands of microns of DNA present in each nucleus by the 1000-2000 connexions between chromatin and the nuclear envelope it is clear that even an oriented length that is transcriptionally active 4—5 μm from the nuclear envelope is still relatively close to the nuclear envelope, quite apart from the possibility that electrophoresis might have displaced the active region from a position close to the nuclear surface. However, since the nuclear envelope is lined with peripheral heterochromatin, and autoradiography suggests that transcription occurs mainly at the junction between heterochromatin and euchromatin (Bouteille, Laval & Dupuy-Coin, 1974) it may be that the apparent association between transcription and the nuclear envelope is fortuitous and reflects rather an association between heterochromatin and euchromatin. A transcriptionally active region of chromatin would thus consist of an extended region of chromatin together with a piece of heterochromatin. It is unfortunately not clear whether one can relate such a unit to a band plus its interband. One cannot easily use the lengths of euchromatin and heterochromatin as a guide to similarity, for in a puff the dimensions of bands and interbands are unknown and probably change as the puff enlarges. If one could relate such a unit of euchromatin and heterochromatin to bands and interbands, it would be the extended region or euchromatin that corresponded to the interband as Crick (1971) suggested, although both might at some stage be transcribed.

Associations between chromatin threads

A surprising feature revealed by intranuclear electrophoresis is the association between chromatin threads that presumably represents sites of ectopic pairing. This is particularly striking where, as in Fig. 4, so short a length of chromatin fibre spans
from one attachment point to another that the fibre cannot orient itself between the cathode and anode, but is strung across the direction of current flow.

Transcriptionally active lengths of chromatin

The length of such a thread of chromatin as that in Fig. 4 – strung between what would appear to be fixed points of attachment, may well be one way in which the maximum length of a transcribable unit can be defined. The length in Fig. 4 is clearly many times greater than would be needed merely to code for a single polypeptide chain, even one with a molecular weight as high as 32000. Such a polypeptide would be coded for by approx. 1000 base-pairs (1 kb), and with 200 base pairs per nucleosome (Finch et al. 1975) information specifying the full amino-acid composition could be contained within approximately 5 nucleosomes. However it is well known that the genetic units of chromatin are usually much larger than this – 30–40 kb (Cedar, 1975) or 60 kb (Meilhac & Chambon, 1973) in calf thymus DNA as measured by the number of initiation sites for RNA polymerase. The bands of Drosophila may contain 25–100 kb (Beermann, 1972; Wensink, Finnegian, Donelson & Hogness, 1974), and it seems likely that the transcription product of the Chironomus BR2 gene is very large – 758 and reaches the cytoplasm (Daneholt, 1975). On the other hand the interbands contain much less DNA – a mean of 5% of that in the bands (Beermann, 1972) but still apparently enough to specify the proteins produced – as Crick (1971) has pointed out.

It is not clear just how many nucleosomes are contained in the thread in Fig. 4 – especially where the thread becomes thicker (doubleheaded arrow in Fig. 5) but it is unlikely that as many as 125 nucleosomes could be packed into it, and that is the number that would be associated with a medium sized (25 kb) band of DNA. The DNA content of the V is thus rather less than that contained in a medium-sized band and interband, though the linear extent (1.4 μm) is much greater than the mean thickness of a combined band and interband (0.24 μm) (Beermann, 1972). It may be that electrophoresis has caused this V-shaped thread to become abnormally extended, but even the shortest distance between the 2 points of attachment is almost 1 μm so any artifactual extension must be less than 50%, unless, as seems unlikely, the separation between attachment points has altered significantly during electrophoresis. It seems not unreasonable that chromatin in a cell in tissue culture should be in a more extended form than in a polytene chromosome. In this case the linear extent may also be large because of transcriptional activity.

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