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ATTACHMENT OF INTACT SUPERHELICAL DNA TO THE NUCLEAR CAGE DURING REPLICATION AND TRANSCRIPTION

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Summary

Using conventional methods it is impossible to isolate nuclear DNA without breaking it. However, the DNA may be freed of most cellular material by lysing living cells in a non-ionic detergent and 2M salt. Then structures are released which resemble nuclei and which contain naked histone-free DNA. The DNA of such nucleoids is supercoiled and so intact: the linear DNA must be organised into loops by attaching it to a nuclear cage.

3 different kinds of attachment are described. One is structural, remaining throughout the cell cycle, and a method is described for mapping sequences relative to these points of attachment. The other 2 types are functional: pulse-labelling studies show that sequences which are being replicated or transcribed are closely associated with the cage. We argue that every gene is in a precisely defined place in the nucleus and attachment to the cage determines whether or not it is replicated or transcribed.

1. Isolating intact nuclear DNA

We know very little about the way DNA is folded within the nucleus. This is so, for two reasons. First, it is impossible to isolate nuclear DNA unbroken by conventional methods: DNA is very fragile, merely pipetting it shears it to fragments of about 10^7 dalton (Burgi and Hershey, 1961; Levinthal and Davison, 1961). Second, we have few methods of analysing structure at levels of organisation above that of the nucleosome. A few years ago we solved the problem of isolating nuclear DNA unbroken and we now describe our attempts at developing methods to analyse the way the DNA is folded. We argue that every gene is in a precisely defined place within the nucleus and that its place determines its function.

If one wants to isolate and manipulate intact DNA from eukaryotes, there are two things one must not do. One should not first make nuclei. Most methods involve swelling cells in hypotonic buffers — this activates nucleases

which degrade the DNA (Warren, 1977) – and ultimately this yields what most biochemists would call "high molecular weight DNA", but this is, by our criteria, extensively broken. The second thing one must not do is to use ionic detergents like sodium dodecyl sulphate: they strip too many protective proteins from the DNA, decondensing it to release long strands which inevitably get sheared. Despite these restrictions, it proves to be very easy to free nuclear DNA of most cellular material without breaking it. If living cells are lysed in a non-ionic detergent (e.g. Triton X-100), 2 M salt and a concentration of chelating agent sufficient to completely inhibit nucleases, structures are released which resemble nuclei (Cook and Brazell, 1975, 1976a, 1978; Cook et al., 1976; McCready et al., 1979). Such nucleoids contain naked histone-free DNA packaged within a flexible cage of RNA and protein (Fig. 1). The RNA is concentrated in the nucleolus and a perinuclear rim and some of the proteins resemble those of subnuclear structures isolated by others and called variously the nuclear "pore complex", "lamina", "envelope" or "matrix" (Berezney and Coffey, 1974; Riley et al., 1975; Aaronson and Blobell, 1975; Comings and Okada, 1976). In addition, nucleoids contain cytoskeletal elements (i.e. actin and intermediate filaments) which have condensed onto the basic subnuclear structure (Levin, 1978). We have made nucleoids from a wide variety of cells (e.g. fibroblasts, lymphocytes, erythroblasts, teratocarcinoma and epithelial cells of men, mice, birds, frogs and insects) and each cell type yields cages of characteristic morphology and strength. Some cages (e.g. those from HeLa cells) are so robust that the fragile DNA is almost completely protected from shearing forces so that they can be pipetted freely without breaking DNA.

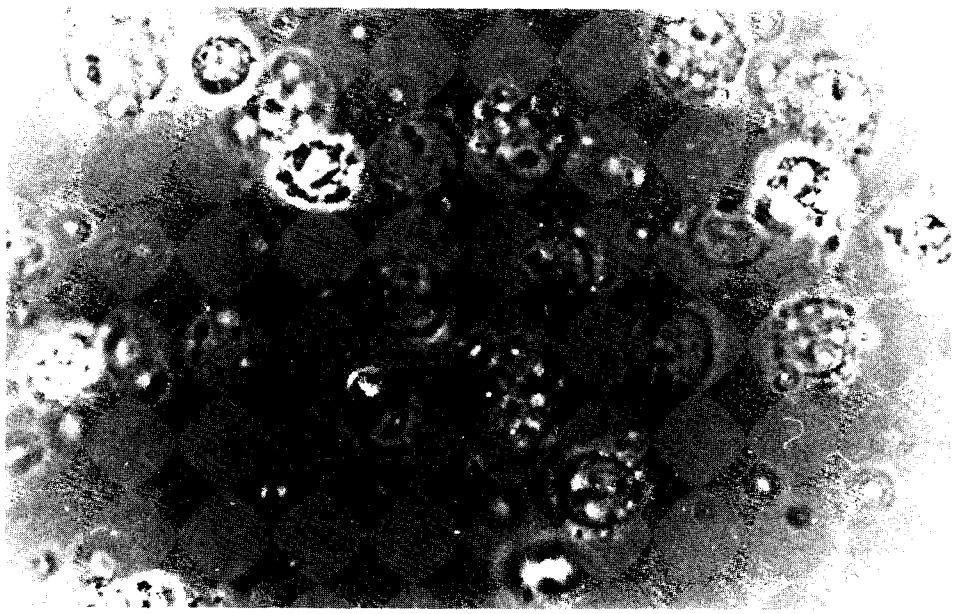

Fig. 1. HeLa nucleoids isolated in 1.95 M NaCl and photographed in the phase-contrast microscope. The diameter of the nuclear region is about 12 μm (see Cook et al., 1976).

2. Supercoils in nuclear DNA

We have stressed that nucleoid DNA is unbroken. What is the evidence for this? It comes from a variety of experiments that demonstrate supercoiling in nucleoid DNA. Supercoiling can only be maintained in small circles of pure DNA whilst they remain intact. Supercoiling is a high energy state and breaking (nicking) even one phosphodiester bond anywhere in the molecule releases the supercoils. The energy associated with supercoiling gives superhelical molecules quite distinctive properties (Bauer and Vinograd, 1974) and these are shared by nucleoid DNA. For example, they sediment in gradients containing ethidium in a biphasic manner (Cook and Brazell, 1975, 1976a). Others who are probably working with similar structures, have since confirmed this distinctive sedimentation pattern (e.g. see Benyajati and Worcel, 1976; Hartwig, 1978; Pinon and Salts, 1977). Nucleoids also exhibit the distinctive ethidium-binding capacity (Cook and Brazell, 1978) and light-scattering properties (P.R. Cook, unpublished observations) of circular DNA. Perhaps the most striking demonstration of supercoiling in nuclear DNA comes from electron microscopy of nucleoids prepared using Kleinschmidt's procedure (McCready et al., 1979). The DNA, initially confined within the cage, is spread to form a huge skirt which is attached to, and surrounds, the collapsed cage (Fig. 2). Individual DNA fibres can be resolved from the tangled network only at the very edge of the skirt and they appear highly twisted and coiled in the manner characteristic of superhelical DNA (Fig. 3).

All this suggests that nuclear DNA is circular. However, genetic maps are

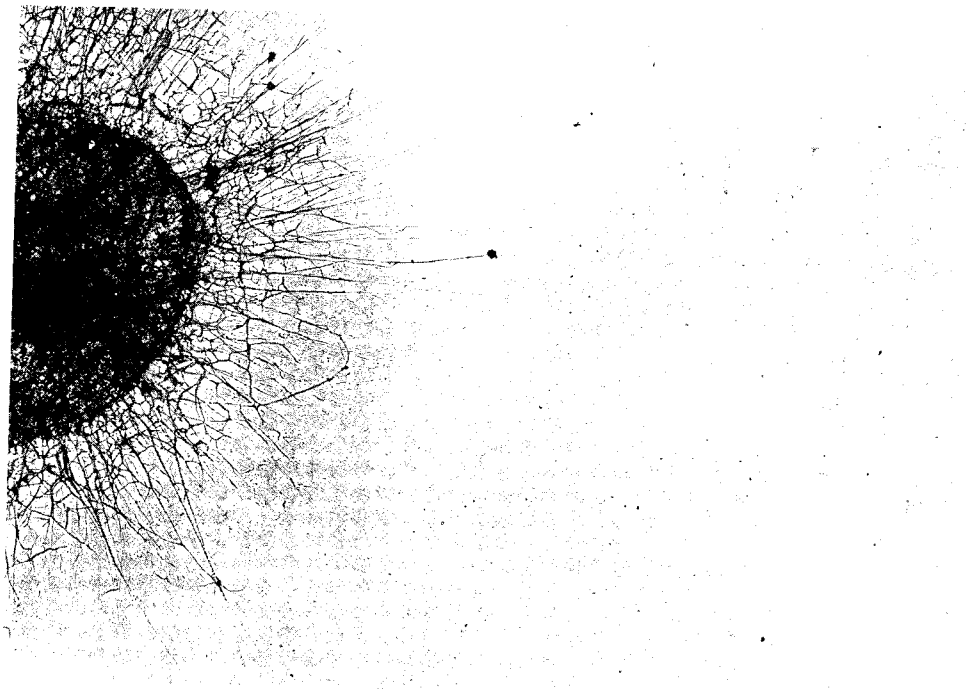

Fig. 2. Part of a spread of a HeLa nucleoid. The central region contains the collapsed cage and radiating from it are highly superhelical fibres. The diameter of the cage is about 15 μm (see McCready et al., 1979).

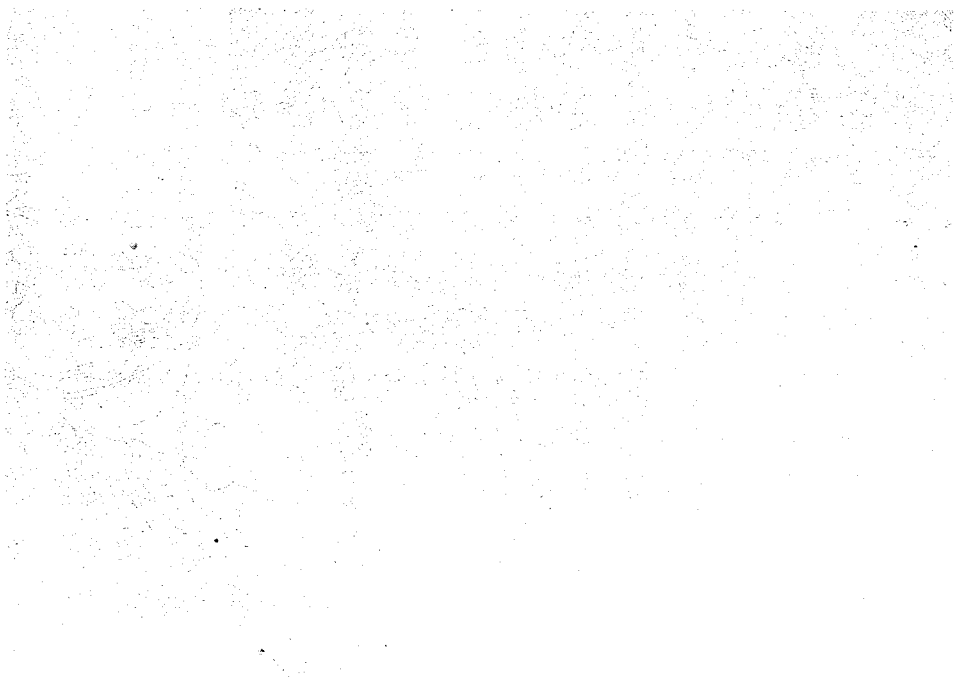

Fig. 3. Superhelical DNA at the edge of the skirt. Magnification X 20 000. (see McCready et al., 1979).

linear — rather than circular — and we reconcile this by assuming that the linear DNA is organised into a number of loops. Nicking in one loop relaxes supercoils in that loop, but not in adjacent loops (Cook and Brazell, 1975). We estimate there to be, on average, 1 supercoil every 90—180 base pairs (Cook and Brazell, 1977) in loops of 220 kb (Cook and Brazell, 1975, 1978) whose size remains unchanged throughout the cell cycle, including mitosis (Warren and Cook, 1978). [These loop sizes are very large. Since only 1 nick in a loop releases all supercoiling in that loop, this means that we can detect breaks in DNA and their repair with great sensitivity. Furthermore we can do this in non-dividing cells which cannot be radiolabelled with thymidine (Cook and Brazell, 1976b; Cook et al., 1978.]

3. Mapping sequences in loops of DNA

Over the years many microscopists have suggested such a loop-like organisation for chromosomal DNA but there was no evidence for attachment of specific sequences to specific sites within the nucleus. Now we have developed a method which enables us to map the position of genes within a loop relative to the point of attachment (Cook and Brazell, 1980). The fact that we can do this implies that sequences cannot be attached at random.

Our approach is to isolate nucleoids from HeLa cells and partially digest their DNA to various degrees with a restriction endonuclease and sediment the cages — and any associated DNA — free from unattached DNA. Next the DNA

associated with the cage is purified and completely fragmented by the same restriction endonuclease. Equal weights of fragmented DNA are separated by gel electrophoresis, transferred to a filter and the relative amounts of any sequence on the filter determined after hybridisation to the appropriate probe. If sequences are associated at random with cages, detachment of DNA will lead neither to an enrichment nor a depletion of a particular sequence. On the other hand, if the association is specific a sequence close to the point of attachment of the DNA will resist detachment: those remote from the attachment site will be detached at a rate which depends on the number of restriction sites between them and the attachment site. We established the feasibility of the method of mapping the relative positions of the α-, β- and γ-globin genes in the loops of the HeLa cell using the restriction endonuclease, Eco RI. (Of course, more detailed maps can be derived by using different restriction enzymes.) It turns out that the α-globin genes are closely associated with the cage (Fig. 4) whereas the β- and γ-genes are less so. Our approach permits us to map the position of any gene for which we have a probe, relative to its point of attachment to the nuclear cage. The precise nature of the attachment remains obscure but we have shown that it is resistant to extremes of temperature and alkali (Cook and Brazell, 1978). The success of such mapping experiments necessarily means that the attachment is specific. Are there any other kinds of attachment?

4. Attachment during replication

Whether or not DNA is replicated at a fixed site — for example, at the nuclear membrane or matrix — has been the subject of controversy for a number of years (Edenberg and Huberman, 1975; Pardoll et al., 1980). Two approaches — biochemical and using autoradiography — have both given variable results. Biochemists have demonstrated association of more or less pulse-labelled DNA either with the nuclear membrane or with the matrix, and we believe that this variation is partly caused by the use of broken templates. Generally nuclei

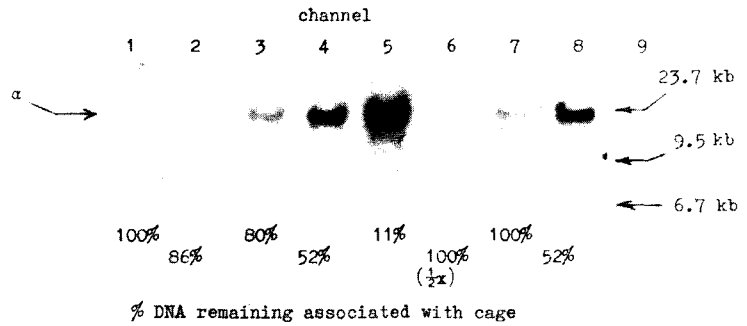

Fig. 4. α-Globin genes are enriched by detaching DNA with Eco RI. Nucleoids were digested with Eco RI and the percentage of DNA remaining associated with cages was determined. After purifying the DNA, redigestion, electrophoresis, blotting and hybridization with α-cDNA probe, an autoradiograph was prepared and photographed. Equal amounts of DNA were applied to all channels except 6 which contained half the amount. The position of flanking size markers is indicated on the right. The 23-kb α-globin band is arrowed: its intensity increases as more DNA is detached from the cage (channels 1—5). Channels 6—8 contain control samples which illustrate the uniformity of blotting and hybridisation (see Cook and Brazell, 1980).

are prepared as a first step, leading to nicking, so that some DNA inevitabl becomes detached and may artifactually associate with sticky membranes. Th results from autoradiography of cell sections are also conflicting. DNA synth sis is so extremely rapid (~ 1 μm/min) and the radius of the nucleus so sma (~ 3 μm) that pulses long enough to give significant labelling in the autoradic graphs used for electron microscopy also permit ample time for the movemer of the pulse-labelled DNA far from the site of synthesis (Edenberg an Huberman, 1975). We have applied both approaches to the problem (McCread et al., 1980). Nucleoids have obvious advantages for these experiments. W can isolate the DNA unbroken and free of sticky membrane and for autoradic graphy we can spread the DNA over an area many times greater than that c the nucleus.

4.1. Biochemical experiments

For the biochemical experiments, cells were grown for more than 1 gener tion (i.e. 24 h) in [^{14}C]thymidine to label their DNA uniformly and then fc a much shorter period in [^{3}H]thymidine. After nucleoids had been isolatec they were digested to different degrees with Eco RI, and the nucleoid cage with associated DNA filtered free of any unattached fragments. Finally th amounts of cage-associated radioactivity on the filters were determined an expressed as a percentage of radioactivity in undigested controls. If replicatio takes place at sites scattered at random throughout the DNA, then the ^{14} and ^{3}H labels will be removed equally from the cages as the DNA in the loop is cut with the endonuclease. For the cage-associated DNA, the normalize ratio of pulse label to bulk label [i.e. (% ^{3}H) ÷ (% ^{14}C)] will remain at unit independently both of pulse length and degree of digestion. On the othe hand, if DNA is replicated at sites close to the cage, then after digestion rela tively more pulse label will remain associated with the cage. The ratio will b greater than unity and will critically depend upon the length of the pulse an the degree of digestion. It will only approach unity when the length of th pulse enables all the DNA in the loops to become labelled with ^{3}H.

The ratio is sensitive to both pulse length and degree of digestion (Fig. 5 For example, after a very short ^{3}H pulse of 2.5 min all but 20% of the ^{14}C removed, whereas 50% of the ^{3}H remains: therefore, the ratio of 50% ÷ 20 or 2.5. As the pulse time increases the maximum ratio decreases and is reache when less ^{14}C has been removed. When pulsed for 24 h both labels becom equally digested and the ratio is unity at all degrees of digestion.

The preferential association of pulse label with cages is not due to a labellin artifact since newly synthesised DNA can be chased away from the cage to giv a ratio approaching unity at all degrees of digestion, quite unlike the patter obtained with a pulse alone (Fig. 5). Furthermore it cannot simply reflect th geometry of the cell and the effects of diffusion — the DNA at the outside o the nucleus being labelled first. Cytosine arabinoside — an inhibitor of DN synthesis — reduced the amount of DNA made in 20 min to the level foun after about 2 min in the absence of the drug. The label has plenty of time t diffuse to the centre of the nucleus, yet what little DNA is made resists diges tion — up to 70% of the ^{14}C can be removed without detachment of any ^{3}H.

We have compared our biochemical results with computer-generated predic

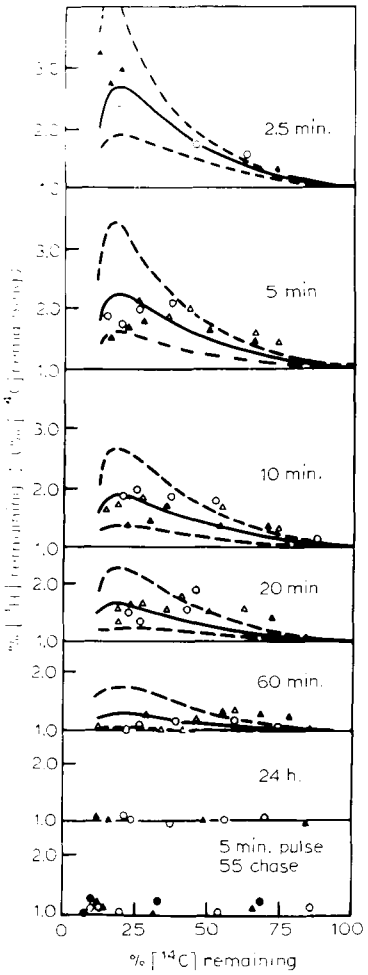

Fig. 5. Newly-synthesised DNA resists detachment by Eco RI from the cage. Cells were labelled with [^{14}C]thymidine for 24 h and then for different periods with [^{3}H]thymidine (top 6 panels) or a 5-min pulse followed by a 55-min chase (bottom panel). Nucleoids were isolated, digested with varying amounts of Eco RI and the DNA remaining associated with nucleoid cages filtered free of detached DNA. The radioactivity remaining associated with cages was measured and expressed as a percentage of that associated with undigested nucleoids. The lines were generated by computer (see McCready et al., 1980).

tions based on a number of models that involve a constant rate of replication within a loop, random initiation and termination amongst loops and a random cutting and consequent detachment of DNA. Only models involving replication at, or close to, the cage are consistent with our results. Curves of best fit were obtained using this model and we calculate the time taken to replicate the loop to be ~11 min.

4.2. Autoradiographic experiments

Autoradiography confirms the association of newly synthesised DNA with the nucleoid cage. Nucleoids were labelled for different periods, spread using Kleinschmidt's procedure and autoradiographs prepared. The radial distribution

of label was then found by counting silver grains in 2.5-μm sections of a 3(sector extending from the centre of the cage. After 24-h labelling, the radi distribution reflects the radial concentration of the uniformly labelled DN (Fig. 6). 45% of the grains in the sector lie over the cage; the remainder a almost entirely within 15 μm from the cage edge, with <2% between 15 μ and the edge of the skirt (~30 μm). After a 5-min pulse, 90% of the grai lie over the cage (Fig. 7). As the pulse time increases this proportion pr gressively falls and the grains spread out over the skirt (Fig. 8). Few grai are found beyond 5 μm after a 5-min pulse (Fig. 8) or beyond 10 μm af a 10-min pulse (Fig. 8), consistent with a rate of elongation of 1 μm/m Again controls involving pulse-chases and inhibitors rule out trivial explan tions of these results.

We can derive the time taken to replicate a loop from the pulse time need to approach the radial distribution of randomly-labelled DNA. This tur out to be about 10 min — in excellent agreement with the biochemical resul If we assume bidirectional replication, this gives an average replicon size ~20 μm — again this agrees with published results (Edenberg and Huberma 1975).

4.3. A model for replication

How is the unit of replication — the replicon — related to the unit of stru ture, the loop? We estimate the average loop to be ~75 μm or 2.2×10^5 ba

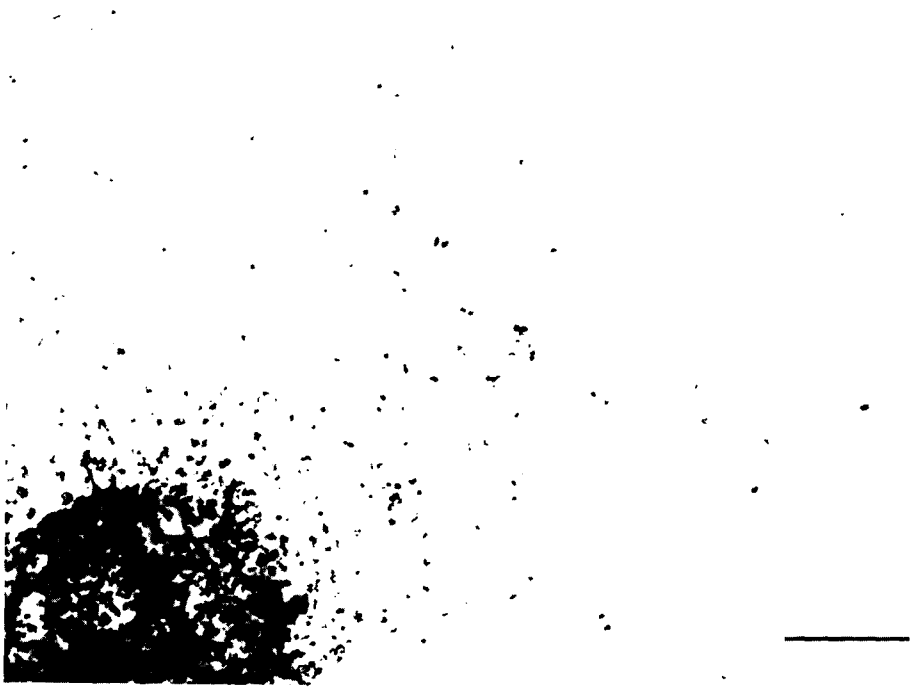

Fig. 6. Autoradiograph of a spread nucleoid isolated from cells labelled for 24 h with [^{3}H] thymidi Label lies over both cage and skirt. In order that silver grains can be easily seen and counted, DN shadowed but not stained and so can only be seen at higher magnification than that represented h DNA in the skirt fills the field. The bar represents 5 μm (see McCready et al., 1980).

Fig. 7. Autoradiograph of a spread nucleoid isolated from cells labelled for 5 min with [^{3}H]thymidine. Label is concentrated over the cage even though DNA in the skirt fills the field. The bar represents 5 μm (see McCready et al., 1980).

pairs (Cook and Brazell, 1975, 1978) and this basic structure remains throughout the cell cycle (Warren and Cook, 1978). When replication is initiated, sequences remote from the cage in one structural unit (~75 μm) must become attached to the cage to form about 4 smaller loops or replicons (~20 μm)

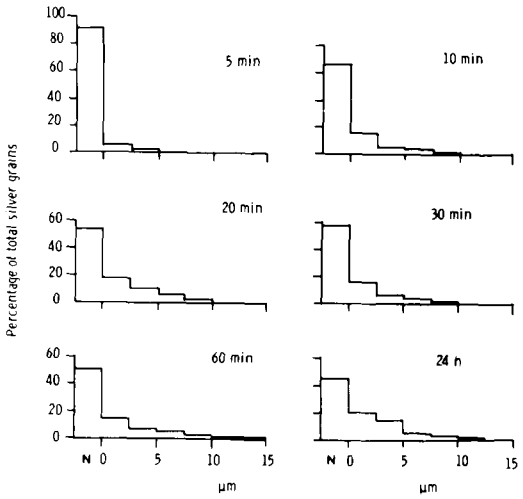

Fig. 8. The radial distribution of silver grains over spread nucleoids isolated from cells labelled with [^{3}H]-thymidine for the periods shown. The histograms show the distribution of grains over the nucleoid cage (N) and in 2.5-μm sections of a 30° sector extending from the centre of the cage. 0 marks the edge of the cage (see McCready et al., 1980).

(Fig. 9). Results from autoradiography of DNA fibres, differential staining c late-replicating regions and inhibition of synthesis by very low doses of radi tion, all point to synchronous initiation in adjacent replicons (Edenberg an Huberman, 1975; Jasny and Tamm, 1979; Willard and Latt, 1976; Painter an Young, 1975, 1976; Povirk, 1977), suggesting that different sites in one stru tural loop attach together.

4.4. Attachment during replication of herpesvirus and repair of UV-damaged DNA

We are currently using both approaches to see whether the DNA of herpe virus is replicated at the cage: preliminary results suggest that it is. In additic we are examining the site of repair of UV-damaged DNA. (This we can do present only by autoradiography, since so little label is incorporated.) Do repa enzymes operate like the fire brigade — rushing to the site of the fire (i.e. t damaged DNA, wherever it be on the loop) — or are they, too, stuck to t cage, requiring the attachment of the damaged sequences?

5. RNA is synthesised at the cage

It is now widely assumed that eukaryotic genes are transcribed by moveme of an RNA polymerase along the DNA. The beautiful photomicrographs "genes in action" taken by Miller and colleagues strikingly illustrate this vie (Miller and Beattie, 1969; McKnight and Miller, 1976). Although both electr microscopists (for a review see Franke, 1974) and biochemists (Miller et a 1976; Cook et al., 1976; Herman et al., 1978; Harlan et al., 1979) have not an association of newly synthesised RNA with various fixed sub-nuclear stru tures, the view that a mobile polymerase processes along the DNA remai

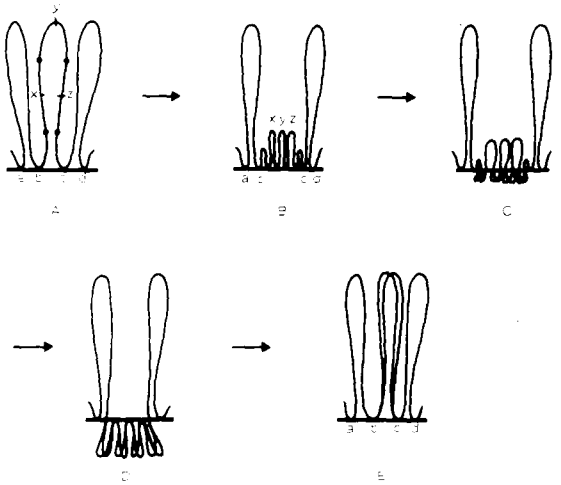

Fig. 9. A model of the replication of nuclear DNA. (A) 3 adjacent structural loops (~75 μm) are attacl at the nuclear cage by specific base sequences (a, b, c, d). (B) Prior to initiation, there is synchron attachment, perhaps provoked by a change in degree of supercoiling in the loop, of 4 sequences (⊣) form 4 replicons (bx, xy, yz and zc) each of about 20 μm. (C, D) Bidirectional replication from each the initiation points takes place as replicon loops move through the replication complex at the nucl cage. (E) When DNA synthesis is complete, the DNA detaches to form 2 newly replicated structural loo The rate of replication could be controlled by varying the number of initial attachments.

unchallenged. We now argue that transcription also occurs at the nuclear cage (Jackson, McCready and Cook, 1981).

5.1. Newly-synthesised RNA is closely associated with the cage

When HeLa cells are incubated with [^{3}H]uridine for 1 or 15 min to label only nuclear RNA, >95% of the radioactivity initially present in the cells and insoluble in trichloroacetic acid subsequently cosediments with nucleoids (Cook et al., 1976). It might do so, not because it is attached to the cage, but because it is entangled in DNA. We tested this possibility in two ways. In the first, we transferred DNA — by spreading — from the inside to the outside of the cage and asked whether or not RNA was also transferred. When cells are pulse labelled with [^{3}H]uridine for 2.5 min and nucleoids isolated and spread (Fig. 10c), >95% of the grains lie over the cage: the distribution of RNA does not reflect the distribution of spread DNA (cf. Figs. 10a and b).

A second experiment confirms that nascent RNA is not simply entangled in DNA. Cells were labelled with [*methyl*-^{14}C]thymidine for 24 h, followed by [^{3}H]uridine for 2.5 min. [Actinomycin D (0.08 μg/ml) was present during, and 30 min prior to, the ^{3}H pulse to suppress ribosomal RNA synthesis]. Nucleoids were isolated, incubated with the restriction endonuclease Eco RI and the amount of the 2 labels remaining associated with cages determined after filtration. In one typical experiment, when 90% of ^{14}C (i.e. DNA) was detached, <15% of the ^{3}H (i.e. RNA) was lost.

The following control experiments demonstrate that RNA which is entangled in DNA can escape with the DNA from the cage. Entangled RNA was synthesised in vitro in incubating nucleoids with *E. coli* RNA polymerase, [^{3}H]uridine and the appropriate precursors (Colman and Cook, 1977): presumably the transcripts are initiated at sites scattered around the loops of naked DNA. In this case, subjecting the nucleoids to Kleinschmidt's procedure spreads the labelled RNA: 34% of the autoradiographic grains are found over the skirt (Fig. 10d). Furthermore, digestion with Eco RI detached this RNA labelled in vitro since the labelled RNA can be filtered free of the cages. This RNA made in vitro is not so tightly associated with the cage as that synthesised in vivo. We next determined whether this tight association was specific.

5.2. Attachment of the 5′ end of nascent RNA

A "cap" containing methylated bases is attached at the 5′ end of nascent RNA very soon — if not immediately — after transcription begins (Furuichi, 1978; Salditt-Georgieff et al., 1980; Babich et al., 1980). Therefore the 5′ end of such transcripts can be labelled in vivo by incubating cells in [^{3}H]methionine. This label is also incorporated into proteins, DNA and other methylated bases within RNA chains, especially into those within ribosomal RNA molecules, but the latter labelling can be suppressed almost completely by low levels of actinomycin D (Salditt-Georgieff et al., 1980). We first established what proportions were incorporated into these different fractions by labelling cells for 15 min with [^{3}H]methionine in the presence of actinomycin D as before (Table 1). Of the label recoverable in nucleoids, 75% was solubilised by proteinase K and S.D.S. and so must be in protein and 23% was recovered in RNA. The distribution of label in caps and internal nucleotides was determined using an established

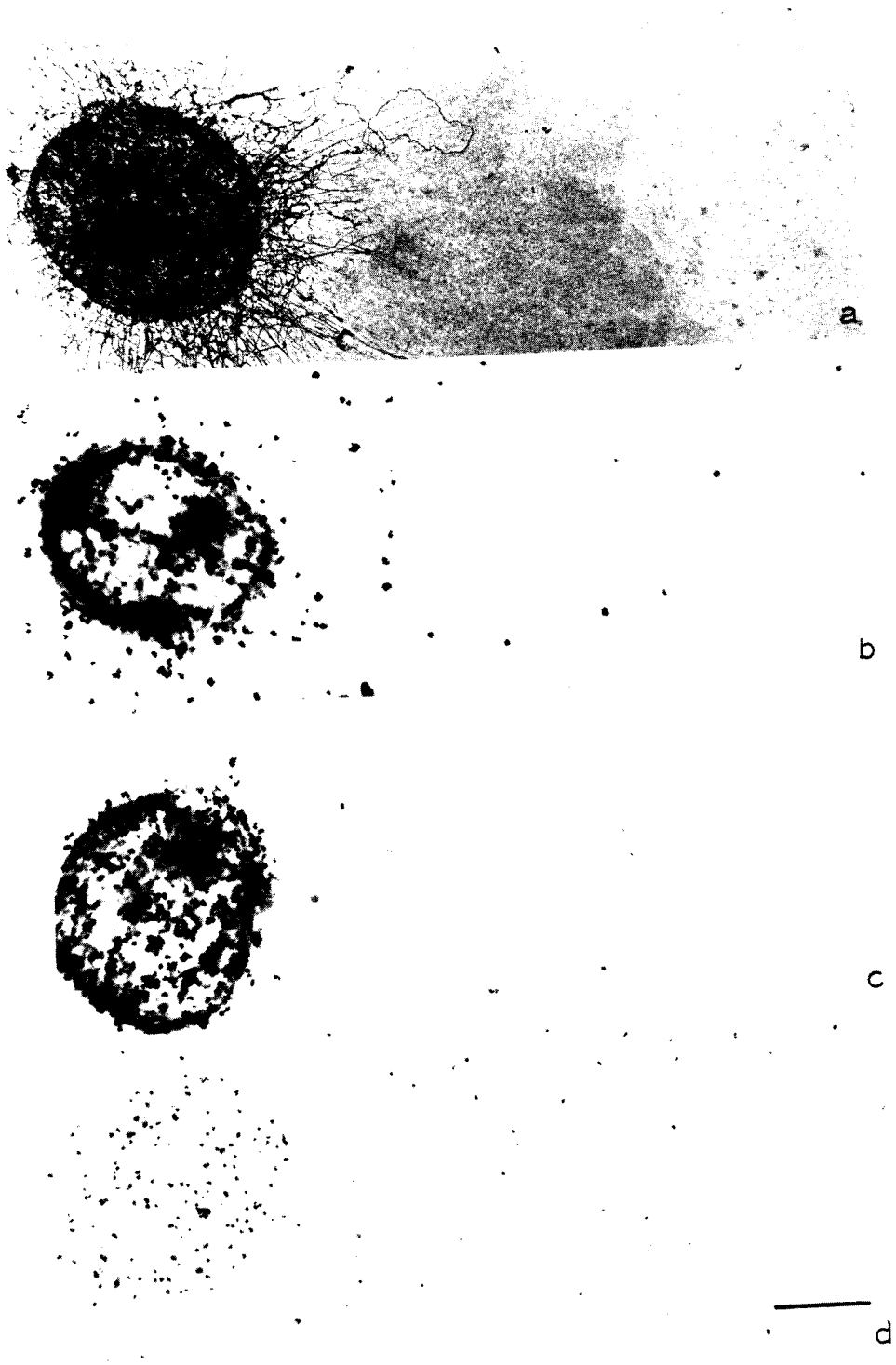
a
b
c
d

TABLE 1

	Amount of label remaining	
	Control (%)	RNAase treated (%)
Initially in nucleoids	100	100
In RNA	23	11
In internal residues in RNA	15	4.1
In caps in RNA	8.3	8.0

Caps resist detachment by ribonuclease.

procedure which involves digestion with RNase A and T2 followed by column chromatography (Salditt-Georgieff et al., 1980). As others have found, 36 and 64% of the label in RNA was recoverable in the cap and purine mononucleotide fractions respectively, the latter being derived from methylated adenines within the original RNA chains (Perry et al., 1975; Wei et al., 1975).

If nascent RNA is attached at its 5′ end, caps should resist detachment by pancreatic ribonuclease. Therefore we labelled cells with [^{3}H]methionine as above, isolated nucleoids and incubated them with ribonuclease. A parallel experiment showed that ribonuclease detached 75% of the RNA labelled with [^{3}H]uridine under similar conditions. Next cages and any associated RNA were filtered free of detached RNA and the amounts of label in the different fractions were determined (Table 1). The ribonuclease treatment did not affect the labelling of protein or DNA but it halved the amount of label recovered in RNA. This reduction was almost entirely due to the loss in internal residues with negligible loss of label in caps. Therefore removal of 75% of the body of the chain detaches few, if any, caps.

5.3. Attachment of the 3′ end of nascent RNA

Any attachment at the 3′ end of the chain is technically much more difficult to demonstrate. Nevertheless, we have attempted to do so using doubly-labelled nucleoids as follows. Cells were incubated — as before in the presence of actinomycin D — for 2 min with [^{14}C]uridine; then [^{3}H]uridine was added and the incubation continued for 1 min. The 3′ end will be richer in ^{3}H than ^{14}C and if attached, inspection of simple models indicates that the ^{3}H in nucleoids should be more resistant to detachment by ribonuclease than is the ^{14}C (Fig. 11a, b). Therefore cells were doubly labelled as described above, nucleoids prepared and incubated with ribonuclease, the detached RNA removed by filtration and the percentage of ^{14}C and ^{3}H remaining associated with the cages determined. If RNA is attached at random to the cage, then ^{14}C and ^{3}H will be detached in equal proportions from the cages i.e. the ratio of (% ^{3}H remaining) ÷ (% ^{14}C remaining) will remain at unity independently of the amount of ^{14}C remaining.

Fig. 10. Nascent RNA is closely associated with the cage. (a) An electron micrograph of a control nucleoid spread to illustrate the extent of the skirt surrounding the cage. (b—d) Autoradiographs of spreads after labelling (b) HeLa cells in vivo for 24 h with [^{3}H] thymidine, (c) HeLa cells in vivo for 2.5 min with [^{3}H]-uridine or (d) nucleoids in vitro by transcribing them for 15 min with *E. coli* RNA polymerase. So that silver grains can be easily seen, DNA is shadowed but not stained in b—d and can be seen only at higher magnifications. The bar represents 5 μm.

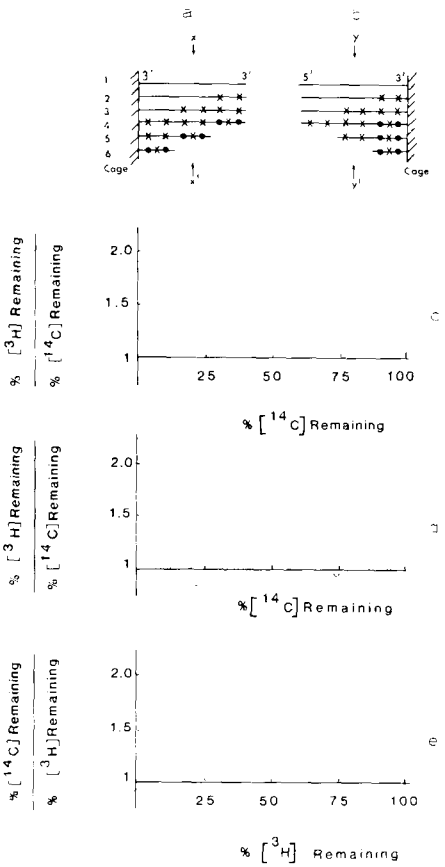

Fig. 11. Nascent RNA is attached at the 3′ end. (a) A simple model for labelling and cutting RNA a… at the 5′ end. (1) A completed strand of RNA (—) is attached at its 5′ end to the cage. Cells are … for 2 min with [^{14}C]uridine (X) followed by 1 min with [^{3}H]uridine (•). (2—3) Some nascent RN… cules complete synthesis after the addition of ^{14}C but before the addition of ^{3}H, so becoming o… labelled, whilst others (4—6), which initiate during the pulses, become labelled with both ^{14}C … Digestion with ribonuclease (i.e. cutting between x and x′ detaches ^{14}C and ^{3}H in roughly equal … tion. Therefore on digestion, the ratio (% ^{3}H remaining) ÷ (% ^{14}C remaining) remains at about un… An array of molecules labelled as in (a) are attached at the 3′ end. Cutting between y and y′ deta… but not ^{3}H: therefore the ratio is greater than unity. (c) Cells were labelled with [^{14}C]uridine f… followed by 1 min with [^{3}H]uridine, nucleoids isolated, incubated with ribonuclease and the per… of each label remaining associated with cages was determined. (d) As (c), except both labels were … together for 3 min. (e) As (c), except labelling was for 2 min with [^{3}H]uridine followed by 1 m… [^{14}C]uridine.

On the other hand, if the 3′ end is attached, then detachment of RNA wi… to a relative enrichment of ^{3}H i.e. the ratio will increase above unity a… amount of ^{14}C remaining decreases. [Attachment at both ends would also … this behaviour but we cannot distinguish such double attachment fr… attachment using this labelling regime.] The results are consistent with a… ment at the 3′ end since the ^{3}H resists detachment: for example removal … but 30% of the ^{14}C leaves 54% of the ^{3}H (i.e. the ratio is 54 ÷ 30 = 1.8… 11c). When both labels are present together for 3 min there is no such var… in the normalised ratio so that these results cannot be due to a labelling ar…

(Fig. 11d). This is confirmed by reversing the labels: in this case it is ^{14}C — the label added last — which is enriched (Fig. 11e).

5.4. Transcribed genes lie close to the cage

If nascent RNA is so closely associated with the cage, then so too must be the genes from which it is transcribed: DNA close to the cage should be richer than total DNA in transcribed sequences. We therefore prepared 4 types of DNA by incubating nucleoids with various amounts of Eco RI; then cages, and cage-associated DNA, were sedimented free of detached fragments to yield pellets which retained 100, 35, 14 and 5% of the initial amount of cage-associated DNA. This cage-associated DNA was purified, labelled by "nick-translation", denatured and the percentage of the DNA forming a hybrid with an excess of total nucleoid RNA determined (Fig. 12). 13% of nucleoid RNA hybridised with total DNA (i.e. sample 1). All the other samples of cage-associated DNA hybridised to greater extents, showing that they were richer in sequences complementary to

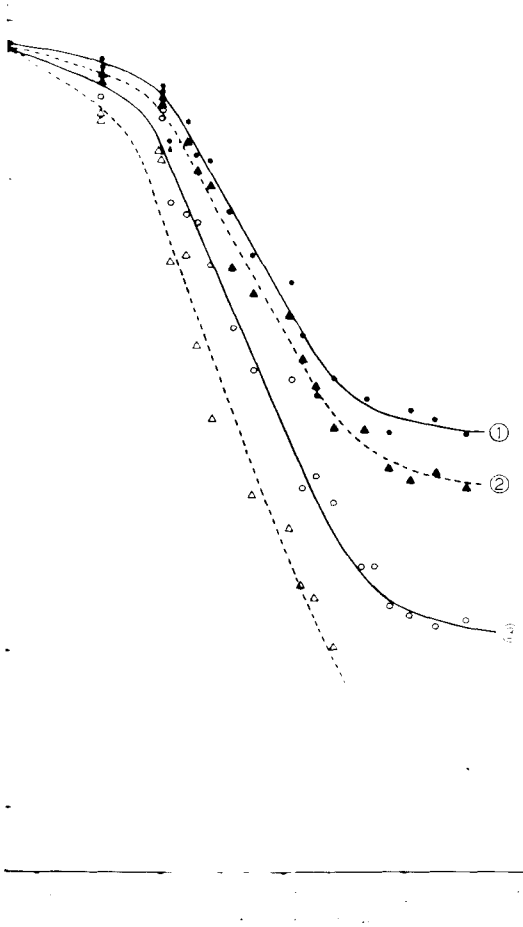

Fig. 12. Cage-associated DNA is enriched in sequences complementary to nucleoid RNA. 4 different samples of cage-associated DNA containing 100% (1), 35% (2), 14% (3) and 5% (4) of the DNA associated with undigested nucleoids were labelled by nick-translation, denatured and the percentage of the DNA forming a hybrid with an excess of nucleoid RNA was determined.

nucleoid RNA. The result with the cages which retained 5% of the total DNA (i.e. sample 4) is quite striking: 23% of this DNA is complementary to nucleoid RNA. If we assume that only 1 strand is transcribed, then about half this sample of cage-associated DNA contains transcribed sequences — a remarkable enrichment.

We conclude from these experiments that both ends of nascent transcripts are attached to the cage and that transcribed sequences lie close to the cage. If so, transcripts must be generated as DNA passes through a fixed transcription complex at the cage (Fig. 13).

6. Conclusions

We have discussed 3 kinds of specific association of DNA with the nuclear cage. One, which might be called structural, is an attachment that forms the linear DNA into loops. These loops are large (~220 kb) and present throughout the cell cycle, including mitosis. The 2 other kinds of attachment are functional — involved in replication and transcription. We suggest that the replication and transcription machinery are attached to the cage and that DNA can only be replicated or transcribed when it becomes attached to, and passes through this fixed machinery. It then becomes attractive to suppose that genes are activated by attaching them to the cage. For example, cellular differentiation might be established during development by the acquisition of higher-order structural polymorphism of the DNA (Cook, 1973, 1974). Earlier, we have demonstrated

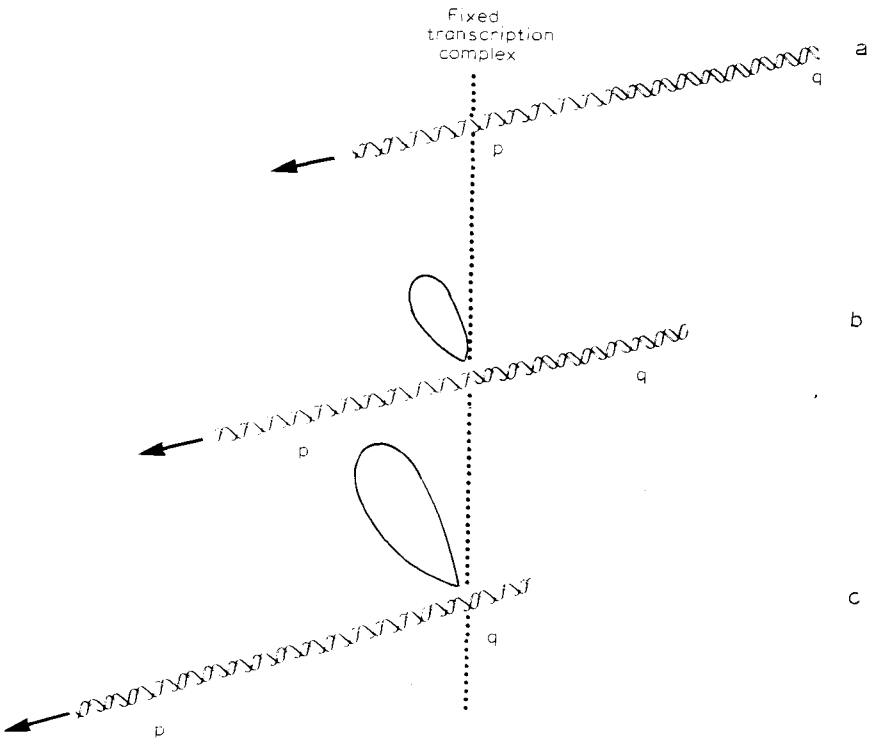

Fig. 13. A model for transcription. (a) A fixed complex transcribes DNA between p and q. (b, c) RNA is synthesised as DNA passes through the complex, the nascent RNA chain being attached at both ends forming a loop.

the inverse correlation — gene inactivation coupled with sequence detachment — during the differentiation of the avian erythrocyte (Cook and Brazell, 1976a, b). Now we have preliminary evidence that the human γ- and ϵ-globin genes are more closely associated with the cage in cells in which they are expressed than in HeLa cells (P.R. Cook, unpublished observations).

Perhaps the most surprising result of these experiments is not that replication or transcription takes place at the nuclear cage, but that the mechanism that holds the DNA is stable in the detergent and 2 M salt used during the isolation of nucleoids. Therefore nucleoids, with their intact RNA and superhelical DNA held in the appropriate manner, should prove useful in studies of both structure and function.

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