J. Cell Sci. Suppl. 1, 59–79 (1984)
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REPLICATION AND TRANSCRIPTION DEPEND ON ATTACHMENT OF DNA TO THE NUCLEAR CAGE

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SUMMARY

When living cells are lysed in a non-ionic detergent and 2 M-NaCl, structures are released that resemble nuclei. They contain naked nuclear DNA packaged within a flexible cage of RNA and protein. Since the DNA is supercoiled, it must be intact and looped by attachment to the cage. It is argued that this cage is the active site of the key nuclear functions, transcription and replication: outlying sequences are activated by attachment to polymerases at the cage. This thesis is supported by the close and specific association of nascent RNA with cages, the attachment of active viral sequences (in transformed and productively infected cells) and the attachment of nascent DNA during both normal and repair synthesis.

INTRODUCTION

It is an old idea that DNA and chromosomes are ordered within the eukaryotic nucleus (Comings, 1968; Dupraw, 1970) and that gene position affects function (Baker, 1968). However, it has only recently become possible to analyse the precise biochemical basis for this order, largely because methods have now been devised for isolating DNA in association with a sub-nuclear structure.

Since the first reports 20 years ago (Zbarsky, Dmitrieva & Yermolayeva, 1962; Smetana, Steele & Busch, 1963) many different sub-nuclear structures have been extracted from nuclei using high concentrations of salt. They share many basic constituents and include nuclear pore complexes, envelopes, ghosts, matrices, lamins, scaffolds and folded chromosomes (for reviews see Agutter & Richardson, 1980; Hancock, 1982; this volume). More or less degraded nucleic acid is attached to them. They can be isolated in association with *intact* DNA if cells – rather than nuclei – are lysed directly in a non-ionic detergent (e.g. Triton X-100), 2 M-NaCl and sufficient chelating agent to inhibit nucleases completely; then, structures are released that resemble nuclei (Fig. 1; Cook & Brazell, 1975, 1976; Cook, Brazell & Jost, 1976). These nucleoids can be made from a wide variety of cells (e.g. fibroblasts, lymphocytes, erythroblasts, teratocarcinoma and epithelial cells of men, rats, mice, birds, frogs and insects). They contain all the nuclear RNA and DNA but few proteins characteristic of chromatin (e.g. they contain no histones). They do contain elements of the matrix, the pore-complex/lamins and attached cytoskeletal actins and keratins. Four different approaches indicate that their DNA is intact; all involve the demonstration of supercoiling in nucleoid DNA.

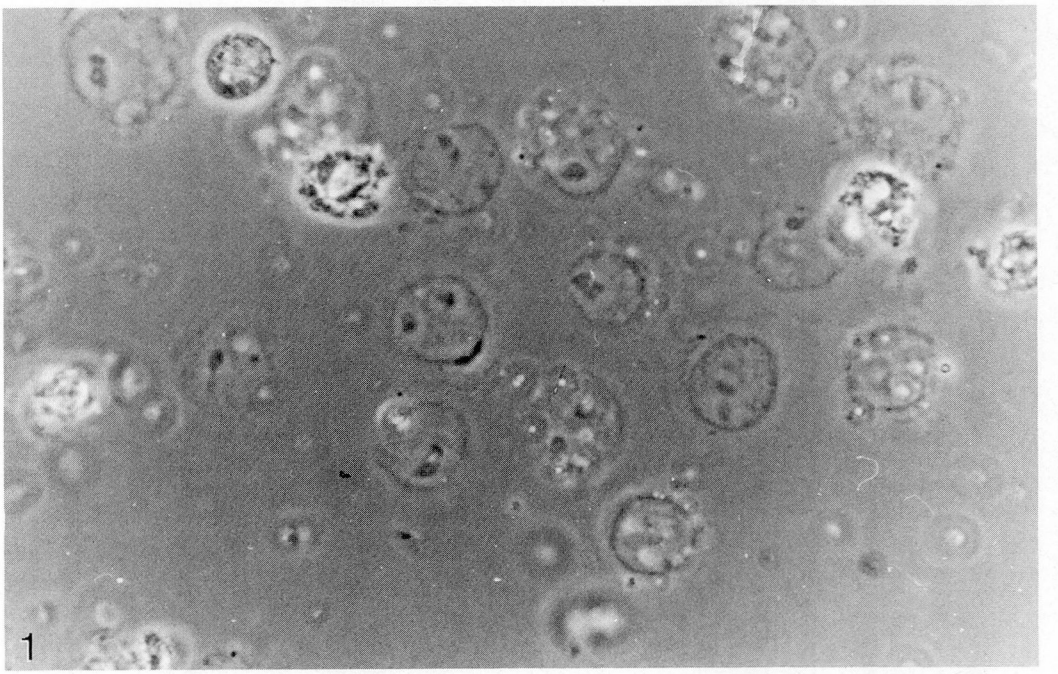
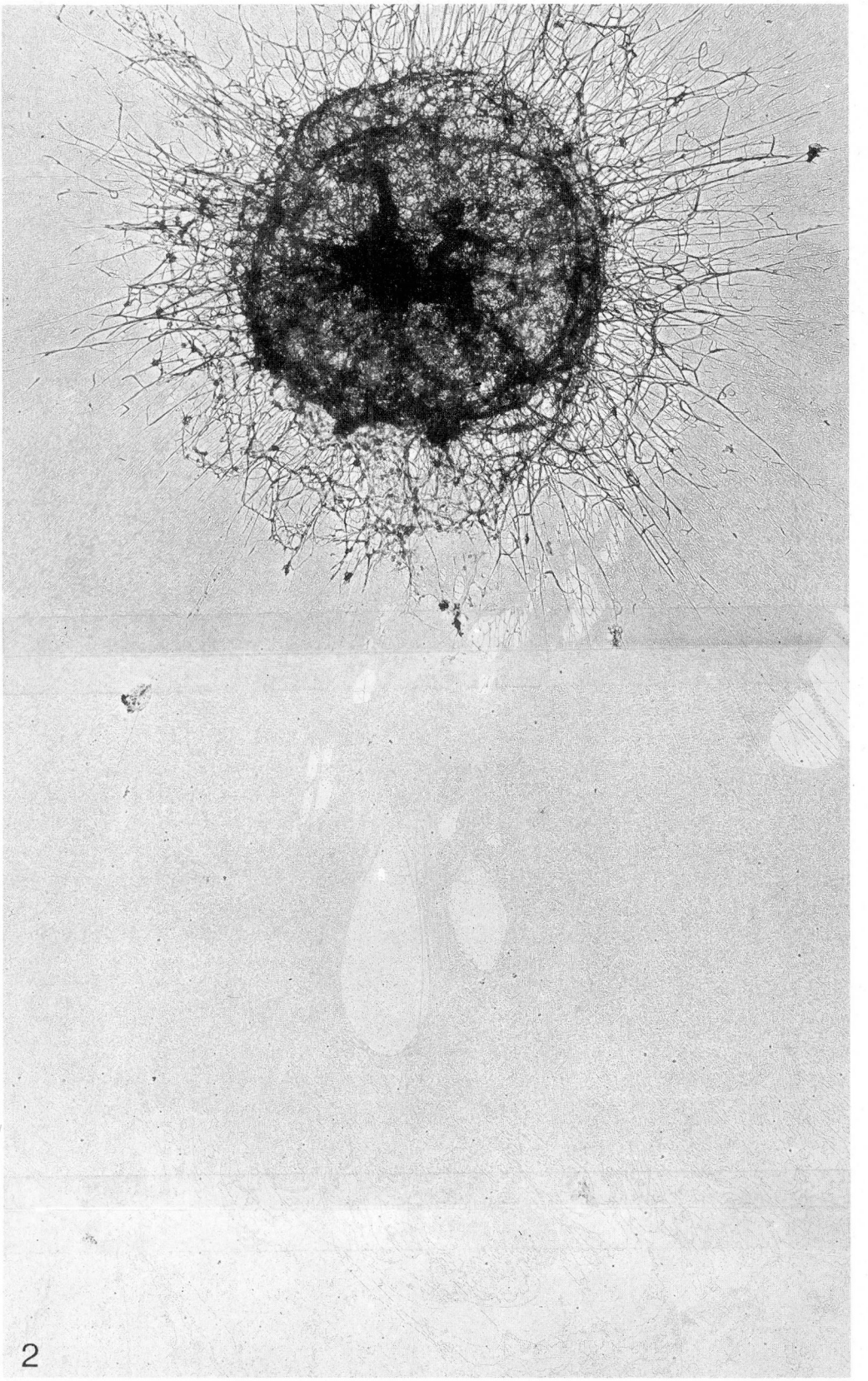


Fig. 1. HeLa nucleoids isolated in 1·95 M-NaCl and photographed in the phase-contrast microscope. The diameter of the nuclear region is about 12 μm (from Cook *et al.* 1976).

Supercoiling in nucleoid DNA

Supercoils can be maintained in circular, but not in free linear, DNA. Supercoiled molecules have distinctive properties (Bauer & Vinograd, 1974) and these are shared by nucleoids. For example, they sediment in gradients containing intercalating agents in a biphasic manner (Cook & Brazell, 1975, 1976) and they bind ethidium (Cook & Brazell, 1978) and scatter light (unpublished observation) like superhelical DNA. Perhaps the most striking demonstration comes from electron microscopy of nucleoids prepared using Kleinschmidt's procedure (Mullinger & Johnson, 1979; McCready, Akrigg & Cook, 1979). DNA, initially confined within a residual structure that we call the nuclear cage, is spread to form a huge skirt of supercoiled fibres attached to the collapsed cage (Fig. 2). All this evidence suggests that nuclear DNA is circular; however, as we believe that chromosomal DNA is linear, it must be looped, presumably by attachment at the base of the loops to the cage. Nicking one loop releases supercoils in that loop, but not in adjacent loops. We estimate there to be, on average, one supercoil every 90–180 base-pairs (Cook & Brazell, 1977) in loops of 220×10^3 base-pairs (Cook & Brazell, 1975, 1978). We have been unable to detect

Fig. 2. Part of a spread of a HeLa nucleoid. A tangled mass of superhelical fibres stretches from the collapsed cage to the edge of the field. The diameter of the cage is about 15 μm (see McCready *et al.* 1979).



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biophysically any changes in loop size as cells progress through mitosis (Warren & Cook, 1978). These loop sizes are very large – too large to represent one unit of transcription or replication.

The cage

The isolation of intact DNA packaged within the nucleoid cage has the important practical consequence that long and fragile DNA molecules can be pipetted without shearing them. Unfortunately, most primary diploid cells possess weak cages that break easily, releasing the DNA, which then shears (Cook & Brazell, 1976 and unpublished). Therefore, we have worked mainly with nucleoids that have robust cages and so contain DNA that remains superhelical on pipetting.

We will argue that the cage serves another – and most important – function: namely, that it is the *active site* of the key nuclear functions, replication and transcription. As a result, genes are positioned precisely within loops of nuclear DNA and their positions relative to the cage determine whether they are replicated or transcribed.

RNA IS SYNTHESIZED AT THE CAGE

It is now widely assumed that eukaryotic genes are transcribed by movement of an RNA polymerase along the DNA. The beautiful photomicrographs of 'genes in action' taken by Miller and colleagues strikingly illustrate this view (Miller & Beattie, 1969; Miller, 1975, and this volume), which is reinforced by the isolation of soluble polymerases. However, we hardly ever see transcription complexes in the skirts of nucleoid spreads; rather, all nascent RNA remains associated with the cage (Jackson, McCready & Cook, 1981). Furthermore, it is worth remembering that: (1) polymerases are only solubilized by sonication or incubation in the presence of Mg^{2+} (Beebee, 1979); (2) even then, the majority remain intractably associated with pelletable material (Beebee, 1979; Weil, Luse, Segall & Roeder, 1979; Kaplan, Kleinman & Horwitz, 1977; Klempnauer, Fanning, Otto & Knippers, 1980); (3) soluble RNA polymerases initiate inefficiently (e.g. in one relatively efficient system, the crude 'Manley' extract, RNA polymerase II polymerizes correctly initiated transcripts at <10 nucleotides/h (Manley *et al.* 1980) or 0·01 % of the rate *in vivo* (Cox, 1972). Our work with nucleoids led us to examine an alternative view: namely, that transcription occurs by movement of DNA past a fixed polymerase (Jackson *et al.* 1981).

Newly synthesized RNA is closely associated with the cage

When HeLa cells are incubated with [^{3}H]uridine for 1 min to label only nuclear RNA, >95 % of the radioactivity initially present in the cells and insoluble in trichloroacetic acid subsequently cosediments with the nucleoids. 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. First, when cells are pulse-labelled with [^{3}H]uridine for 2·5 min, nucleoids are isolated and spread, and autoradiographs are prepared, >95 %

of the grains lie over the cage; even though DNA is spread, nascent RNA is not. A second experiment confirms that nascent RNA is not simply entangled in DNA. Cells were labelled with [^{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 before, the ^{3}H pulse to suppress ribosomal RNA synthesis.) Nucleoids were isolated, incubated with the restriction endonuclease, *Eco*RI, and the amounts of the two labels remaining associated with cages were determined after filtration. In one typical experiment, when 90 % of ^{14}C (i.e. DNA) was detached, <15 % of the ^{3}H radioactivity (i.e. RNA) was lost.

The following control experiments demonstrate that RNA that *is* entangled in DNA *can* escape with the DNA from the cage. Entangled RNA was synthesized *in vitro* by incubating nucleoids with *Escherichia coli* RNA polymerase, [^{3}H]uridine and the appropriate precursors; presumably, the transcripts are initiated at sites scattered around the loops of naked DNA. In this case, spreading nucleoid DNA spreads labelled RNA; 34 % of the autoradiographic grains are found over the skirt. Furthermore, digestion with *Eco*RI detached this RNA. The RNA made *in vitro* is not so tightly associated with the cage as that synthesized *in vivo*. We next determined whether this tight association was specific.

Attachment of the 5′ end of nascent RNA

A 'cap' containing methylated bases is attached at the 5′ end of nascent RNA immediately transcription begins (Furuichi, 1978; Salditt-Georgieff, Harpold, Chen-Kiang & Darnell, 1980; Babich, Nevins & Darnell, 1980) so the 5′ end of such transcripts can be labelled with [^{3}H]methionine. This label is also incorporated into proteins, DNA and other methylated bases within RNA chains; only 8·3 % being incorporated into caps. If nascent RNA is attached at its 5′ end, caps should resist detachment by pancreatic ribonuclease. Therefore, we labelled cells with [^{3}H]-methionine for 15 min, isolated nucleoids and incubated them with sufficient ribonuclease to detach 75 % of nascent RNA. Now 8 % of the label was recovered with cages in caps. Therefore, removal of 75 % of the body of the chain detaches few, if any, caps.

Attachment of the 3′ end of nascent RNA

Any attachment at the 3′ end of the growing 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. 3A,B). Therefore, cells were doubly labelled, nucleoids were prepared and incubated with ribonuclease, the detached RNA was removed by filtration and the percentages of ^{14}C and ^{3}H remining associated with cages were 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. On the other hand, if the 3′ end is attached, then detachment of RNA will lead to a relative enrichment of ^{3}H, i.e. the ratio will increase above unity as the amount of ^{14}C remaining decreases. (Attachment at both ends would also cause

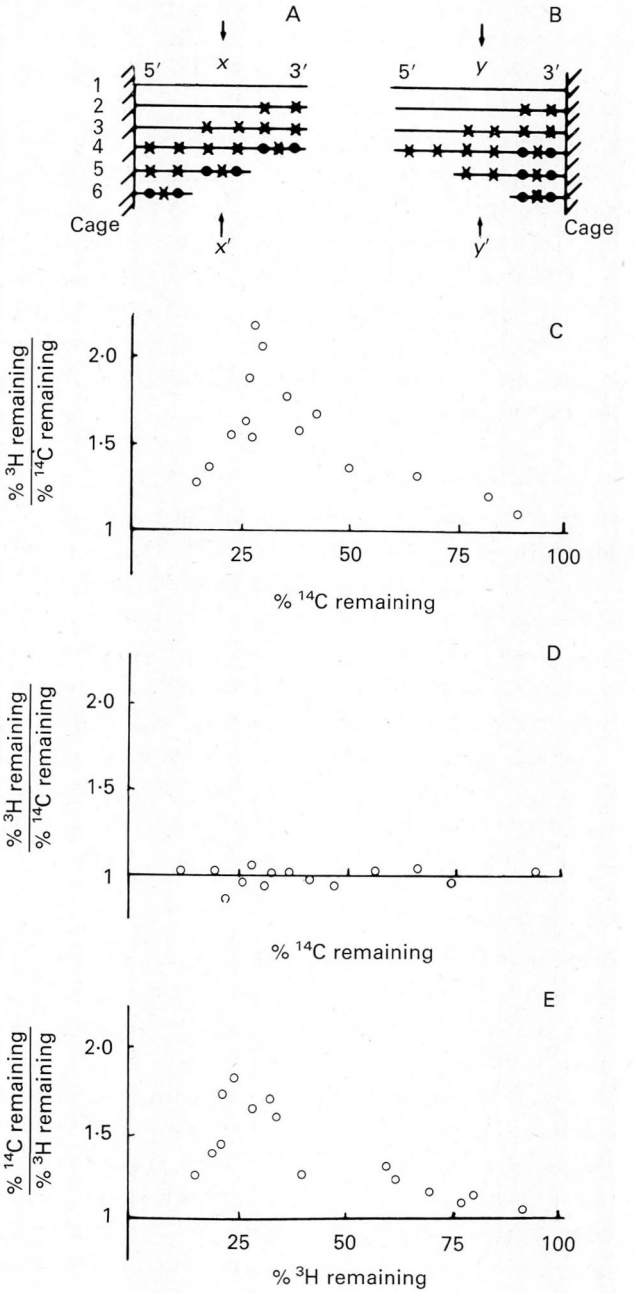


Fig. 3

this behaviour but we cannot distinguish such double attachment from 3′ attachment using this labelling regime.) The results are consistent with attachment at the 3′ end since the ^{3}H resists detachment; for example, removal of all but 30 % of the ^{14}C leaves leaves 54 % of the ^{3}H (i.e. the ratio is $54 \div 30 = 1{\cdot}8$) (Fig. 3c). When both labels are present together for 3 min there is no such variation in the normalized ratio so that these results cannot be due to a labelling artefact (Fig. 3D). This is confirmed by reversing the labels: in this case it is ^{14}C (the label added last) that is enriched (Fig. 3E).

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 in transcribed sequences than total DNA. Therefore, we prepared four types of DNA by incubating nucleoids with various amounts of *Eco*RI; then cages, and any associated DNA, were sedimented free of detached fragments to yield pellets that 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 was determined (Fig. 4); 13 % of nucleoid RNA hybridized with total DNA (i.e. sample 1). All the other samples of cage-associated DNA hybridized to greater extents, showing that they were richer in sequences complementary to nucleoid RNA. The result with the cages that 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 one strand is transcribed, then about half this sample of cage-associated DNA contains transcribed sequences – a remarkable enrichment.

These experiments suggest 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. 5).

ATTACHMENT OF TRANSFORMING GENES

This model for transcription suggests that only attached genes can be transcribed.

Fig. 3. Nascent RNA is attached at the 3′ end. A. A simple model for labelling and cutting RNA attached at the 5′ end. 1. A completed strand of RNA (——) is attached at its 5′ end to the cage. Cells are labelled for 2 min with [^{14}C]uridine (×) followed by 1 min with [^{3}H]uridine (●). 2–3. Some nascent RNA molecules complete synthesis after the addition of ^{14}C but before the addition of ^{3}H, so becoming only ^{14}C-labelled, whilst others (4–6), which initiate during the pulses, become labelled with both ^{14}C and ^{3}H. Digestion with ribonuclease (i.e. cutting between x and x') detaches ^{14}C and ^{3}H in roughly equal proportions. Therefore, on digestion, the ratio (% ^{3}H remaining)÷(% ^{14}C remaining) remains at about unity. B. An array of molecules labelled as in A are attached at the 3′ end. Cutting between y and y' detaches ^{14}C but not ^{3}H; therefore, the ratio is greater than unity. C. Cells were labelled with [^{14}C]uridine for 2 min followed by 1 min with [^{3}H]uridine, nucleoids were isolated, incubated with ribonuclease and the percentage of each label remaining associated with cages was determined. D. As C, except both labels were present together for 3 min. E. As C except labelling was for 2 min with [^{3}H]uridine followed by 1 min with [^{14}C]uridine (from Jackson *et al.* 1981).

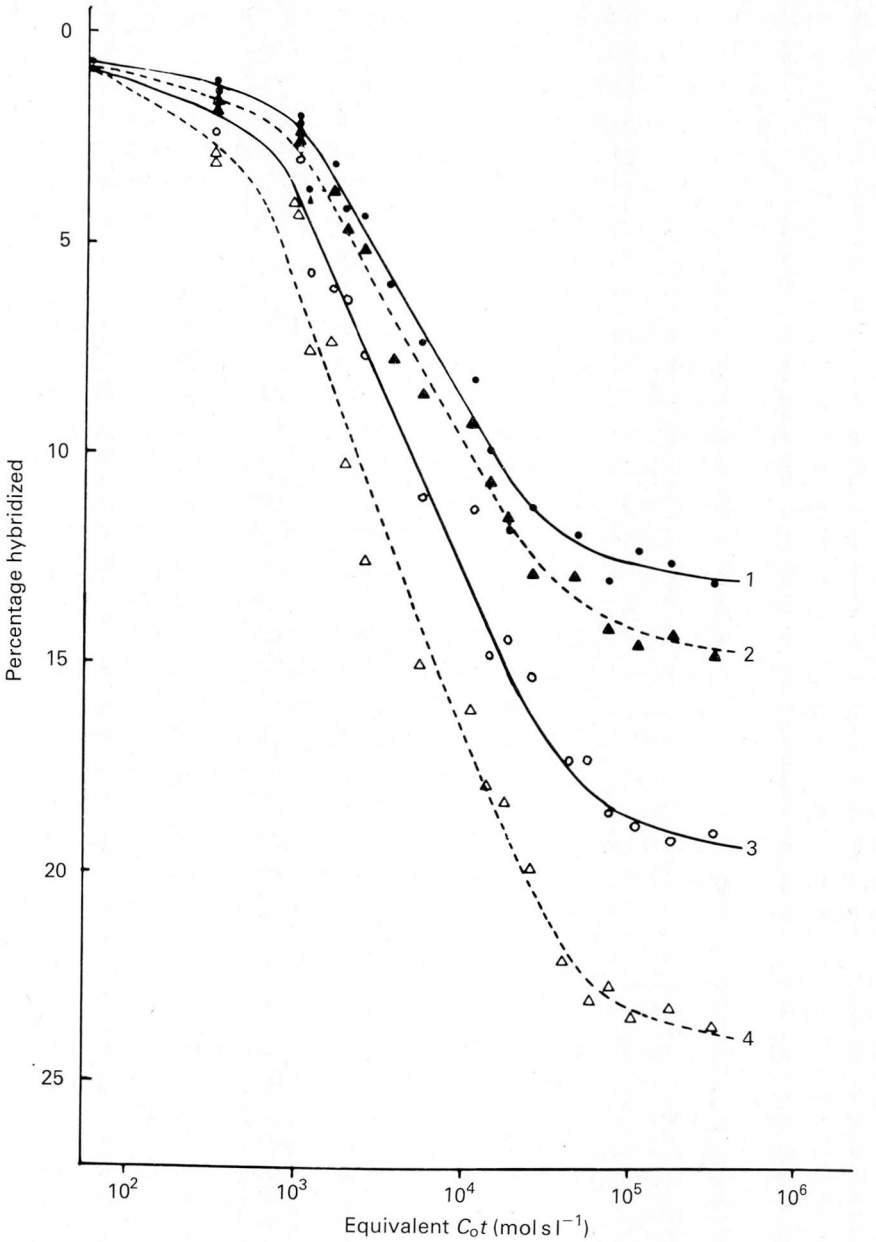


Fig. 4. Cage-associated DNA is enriched in sequences complementary to nucleoid RNA. Four different samples of cage-associated DNA containing 100% (1), 35% (2), 14% (3) and 5% (4) of the DNA associated with undigested nucleoids were labelled by nick-translation, denatured and the percentage of the DNA forming a hybrid with an excess of nucleoid RNA was determined (from Jackson *et al.* 1981).

We tested this using a series of rat cells transformed by polyoma and avian sarcoma virus (ASV) (Cook *et al.* 1982). On transformation these viruses integrate randomly within the genome so that we might expect viral sequences to integrate initially at

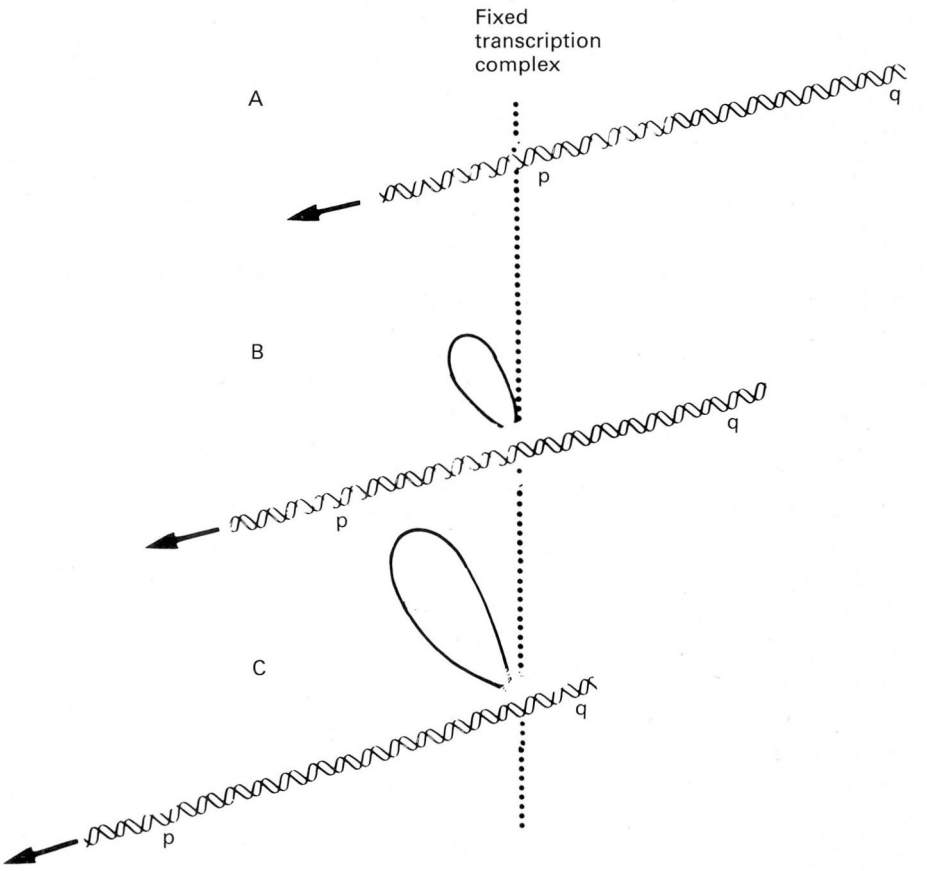


Fig. 5. A model for transcription. A. A fixed complex transcribes DNA between p and q. B,C. RNA is synthesized as DNA passes through the complex, the nascent RNA chain being attached at both ends, forming a loop (from Jackson *et al.* 1981).

random in the loops. However, if the cells express the transformed phenotype, we would predict that the integrated viral sequences, being transcribed, would lie close to the polymerase and so to the cage.

We map sequences relative to their point of attachment using our established procedure (Cook & Brazell, 1980). Nucleoids are partially digested with a restriction endonuclease; then cages, and any associated DNA, are sedimented free of detached DNA. The cage-associated DNA is purified and completely redigested using the same restriction endonuclease. Equal weights of this DNA are resolved into discrete fragments by gel electrophoresis and these are transferred to a filter and the relative amounts of any sequence on the filter are determined by autoradiography after

hybridization with the appropriate probes. Following the first partial digestion, sequences lying close to the point of attachment will tend to co-sediment with the cages and so will be present in relatively greater abundance on the filter; therefore, they yield bands of greater intensity on autoradiography. The degree of enrichment is determined by reference to known amounts of total DNA run in adjacent channels in the gel.

Fig. 6 illustrates the mapping of polyoma sequences integrated into the cellular DNA in one transformed line, 82. Control DNA, undigested during the first partial digestion, yields three bands when subsequently digested completely with *Eco*RI and hybridized with a polyoma probe (Fig. 6A, channel 2; i.e. 100% remaining). These correspond to the left and right-hand arms of the integrated virus – which also contain cellular sequences – and to an internal, and purely viral, sequence. If these nucleoids are partially digested with *Eco*RI to leave only 6% of their DNA remaining attached to the cage and then their DNA is purified and completely fragmented with *Eco*RI, the bands obtained subsequently are three times more intense than those obtained with an equal weight of control DNA (compare channel 2 with 3, and 4 with 5). In contrast, hybridization of an albumin probe to the DNA of nucleoids that retain 6% of the total DNA yields four bands, none of which are more intense than those obtained with the control DNA (Fig. 6B: compare channel 2 with 3, and 4 with 5; one band is probably a doublet). The enrichment of the viral, but depletion of the albumin, sequences can be highlighted by hybridizing a mixture of the two probes to the same filter (Fig. 6C). We conclude that there are fewer restriction sites between the integrated viral sequences and the point of attachment to the cage than there are between the albumin gene and its adjacent attachment sequence; i.e. the expressed viral sequences lie 'closer' to the cage than albumin sequences, which are – as far as we can judge – unexpressed in these fibroblasts.

The results obtaind with the other transformed lines are summarized in Table 1. (Of course, comparisons between different cells should be made at the same levels of detachment.) In no case was the concentration of albumin sequences in the DNA that is closely associated with cages richer than that in the control. In every case the integrated viral sequences were enriched in the fraction of DNA that pellets with the cages.

Line 82 was analysed more extensively. In general, detaching more DNA from the nucleoids, whether with *Eco*RI or *Bam*HI, enriches the viral sequences to a greater extent (Table 1). We can assign the point of attachment to the left or right side of the viral sequence in line 82 by reference to the relative enrichments of each of the three viral bands. The left-hand junction sequence, which contains both cellular flanking sequences and viral sequences, is enriched more than the internal, and purely viral, sequence; both are enriched more than the right-hand junction sequence (Table 2 and fig. 7; compare channel 5 with the others). These differences increase as more DNA is detached. We interpret this as indicating that the left-hand fragment is closest to the attachment site or is attached more strongly. We note that the left-hand, internal and right-hand fragments contain 2, 1 and 0 enhancers, respectively.

Does the virus integrate selectively in sequences lying close to the cage or does it

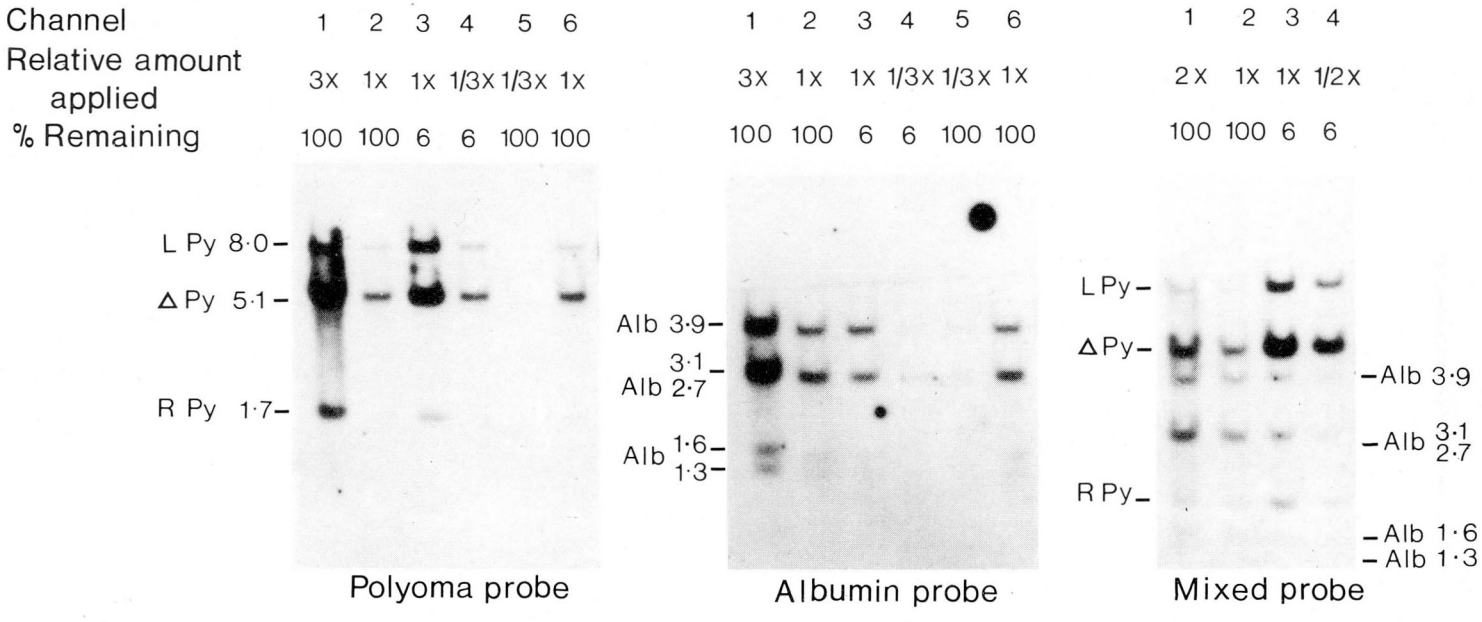


Fig. 6. Detachment mapping of albumin and polyoma viral sequences in the polyoma transformant, 82. Samples of total DNA (100 % remaining) and DNA that resists detachment by *Eco*RI (6 % remaining) were completely digested with *Eco*RI and various amounts were applied to three gels. After electrophoresis, blotting and hybridization with polyoma or albumin probes, autoradiographs were prepared and photographed. The sizes of the three polyoma bands (LPy, ΔPy, and RPy corresponding to the left-hand, internal and right-hand fragments, respectively) and the five albumin bands (two are not clearly resolved under these conditions) are given in base-pairs $\times 10^{-3}$. The polyoma, but not the albumin, sequences are enriched in the nucleoid samples that retained 6 % of the total DNA (from Cook *et al.* 1982).

Table 1. *Detachment mapping of albumin, viral and junction sequences in various cell lines*

Cell	Percentage DNA remaining (relative enrichment)			
	Albumin	Polyoma	ASV	Polyoma junctions
Parent				
Rat–1	4 (1×)	100 (no bands)	100 (no bands)	9 (0·8×)[82J1] 4 (1·6×)[53C1] 9 (1·0×)[7TL] 4 (1·2×)[7TR]
Polyoma-transformed				
82	6 (0·9×)	14 (1·7×) 6 (3·5×) 5 (6·9×)* 4 (4·6×)† 1 (6·7×) 0·8 (18·0×)		
53		6 (2·3×)		
7axT	4 (0·9×)	4 (4·0×)		
Tsa 3T3	5 (0·7×)	5 (3·0×)		
ASV-transformed				
A+11	13 (0·6×)		13 (2·1×)	
A+22	3 (0·8×)		3 (3·9×)	
A23	6 (0·5×)		6 (>3·0×)	6 (0·6×)[7TR]
B31	5 (0·6×)		5 (3·9×)	5 (0·5×)[7TL]
A11 V1T	14 (0·8×) 2 (1·0×)		17 (2·1×)‡ 14 (2·0×) 9 (3·0×)§ 7 (3·0×) 5 (3·1×)‡ 5 (>9·0×)† 4 (7×) 2 (>9·0×)	
Flat revertants of A11 VIT				
13N			3 (1·4×)	
21N	10 (1×)		17 (1·0×)† 10 (0·9×) 10 (1·5×) 4 (0·8×) 4 (2×)	
Aza-cytidine selected retransformants				
21 aza-C *trans* 1			8 (5·6×) 7 (5·1×)	
21 aza-C *trans* 3			8 (7·7×) 7 (6·0×) 6 (4·5×)	

Autoradiographs like those illustrated in Fig. 6 were prepared for each cell-line using polyoma, ASV, albumin or polyoma junction probes, scanned using a microdensitometer and peak heights were measured. The relative intensities of one of the strongest bands (and hence the relative enrichments) were determined by reference to similar bands obtained with varying weights of total DNA.

* Nucleoids were obtained from a population containing 75% mitotic cells obtained by successive thymidine and colcemid blocks.

† *Bam*H was used instead of *Eco*RI in both digestions.

‡ Nucleoids were incubated with ribonuclease to remove all but 4% or less of the RNA labelled in 15 min with [^{3}H]uridine (10 μCi/ml) before the first *Eco*RI digestion.

§ Nucleoids were isolated from cells that had been subjected to 45 °C for 10 min, a procedure that reduced incorporation of pulse-labelled [^{3}H]uridine into RNA by >95% (largely from Cook *et al.* 1982).

Table 2. *The left-hand* Eco *RI fragments of the integrated virus in cell line 82 lie closest to the cage*

Percentage DNA remaining	Relative enrichment of various fragments		
	Left	Internal	Right
14	1·7×	1·7×	1·6×
14	2·0×	1·4×	1·0×
6	3·5×	3·0×	2·2×
1	6·7×	5·5×	
0·8×	18·0×	13·7×	10·0×

Band intensities in autoradiographs prepared like those in Figs 6 and 7 were measured and the relative enrichments were determined (from Cook *et al.* 1982).

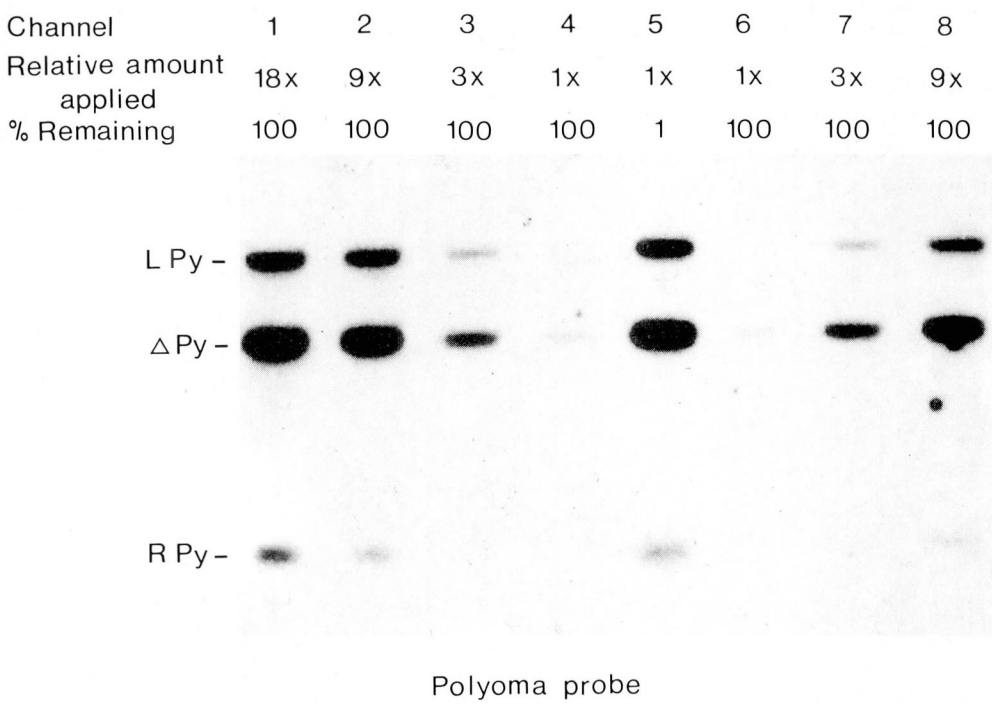


Fig. 7. Mapping the polyoma proviral sequence in cell line 82 with high levels of detachment. Various amounts (from 1 to 18x) of total DNA (100% remaining) or DNA that resisted detachment by *Eco*RI (1% remaining) were applied to the gel as indicated and an autoradiograph was prepared using the polyoma probe. The left-hand polyoma band (LPy) is enriched more than the right-hand band (RPy) (from Cook *et al.* 1982).

integrate randomly, inducing new attachments? Various viral sequences and contiguous cellular sequences have been cloned; therefore, we can test these possibilities by seeing whether cellular sequences that flank the inserted virus lie close to the cage in the parental Rat-1 cells (Table 1). Cellular sequences homologous to all four such polyoma junction probes tested (i.e. 82J1, 53C1, 7TL and 7TR) are readily detached from untransformed Rat-1 cages and cages prepared from ASV-transformants (i.e. the relative enrichments are $<1{\cdot}6$; Table 1). By contrast, in the polyoma transformants these cellular sequences are attached to the integrated viral DNA and so are clearly associated with the cage. The attachment of outlying cellular sequences induced by viral integration can be highlighted as follows. The junction probe from the right side of the virus in 7axT (7TR) hybridizes with one major *Eco*RI fragment of $5{\cdot}0 \times 10^3$ bases from parental Rat-1 cells. When the virus integrates, it does so into only one of the two homologous chromosomes, so that the junction probe now hybridizes to two fragments from the transformant 7axT: one of $5{\cdot}0 \times 10^3$ base-pairs from the unaffected chromosome and another of $5{\cdot}1 \times 10^3$ base-pairs, which contains viral sequences. With total DNA, the $5{\cdot}0 \times 10^3$ base-pair band is the more intense (Fig. 8, channels 1, 2 and 5); however, when all but 4 % of the DNA is detached from

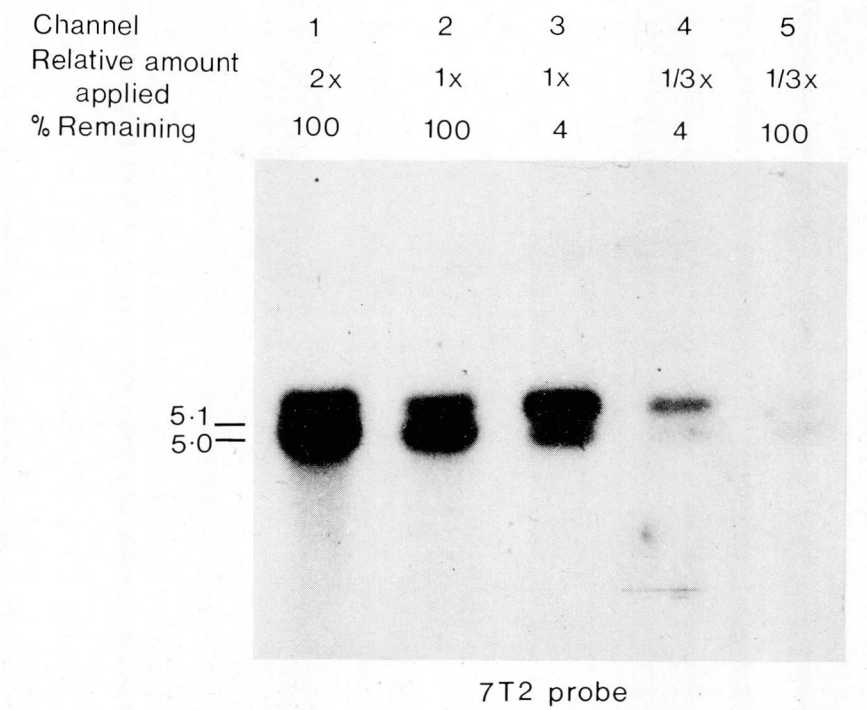


Fig. 8. Detachment mapping a junction sequence (7TR) in 7axT. Various amounts of total DNA or DNA that resisted detachment by *Eco*RI (4 % remaining associated with cages) were applied to the gel. Autoradiographs were prepared using the junction probe 7TR. The 5·1 and $5{\cdot}0 \times 10^3$ base-pair bands are enriched 2·4 and 0·6 ×, respectively (from Cook *et al.* 1982).

7axT nucleoids, the band intensities are reversed (channels 3 and 4). The purely cellular $5{\cdot}0 \times 10^3$ base-pair band is depleted whilst the viral $5{\cdot}1 \times 10^3$ base-pair band is enriched. A similar enrichment of the viral bands but depletion of the purely cellular band is obtained when the junction probe 82J1 is used with 82 nucleoids.

Sub-clones of one of the ASV transformants (i.e. A11 VIT) present us with an opportunity to test the strength of this correlation between gene activity and proximity to the cage. Two sub-clones (i.e. 13N and 21N) have lost the transformed phenotype and contain no detectable viral transcripts. When these 'flat revertants' are treated with the antimetabolite, aza-cytidine (aza-C), and recloned, transformed colonies containing viral transcripts emerge at a high frequency. Two such clones derived from 21N (i.e. 21 aza-C *trans* 1 and 3) were analysed. As far as can be judged by restriction enzyme mapping, all cells in this series contain unchanged proviral sequences inserted in the same cellular sequence. However, they differ in whether or not the proviral sequence is expressed. Detachment mapping indicates that the ASV sequences, which are closely associated with the cage in the transformed VIT, are much less so in the untransformed 'flat revertants' (13N and 21N) but have regained their close association with the cage in the aza-C treated derivatives (Table 1). Again, gene activity correlates with proximity to the cage (see also Robinson, Nelkin & Vogelstein, 1982).

One trivial explanation of all these results is that nascent transcripts, which are presumably closely associated with their templates, prevent access of *Eco*RI to potential cutting sites in transcribing DNA. This possibility is unlikely since similar enrichments are seen when: (1) *Bam*HI replaced *Eco*RI in both digestions; (2) nascent RNA was detached before *Eco*RI digestion; (3) transcription is suppressed by heat-shock or during mitosis.

INFLUENZA VIRAL TRANSCRIPTS ARE ATTACHED

Our model also suggests that when a nuclear virus *productively* infects a cell it must first plug into the cage. Therefore, we studied the association of nascent transcripts of influenza, a virus known to require host nuclear activity (Jackson *et al.* 1982). Influenza virus is an RNA virus: infecting negative strands are first transcribed and the resulting positive strands then replicated into new virion RNA. After labelling infected chicken fibroblasts for 2·5 min with [^{3}H]uridine, we showed by hybridization that both nascent positive and negative strands were closely associated with the cage. In contrast, nascent RNA of a control rhabdovirus, whose reproduction is cytoplasmic, was not so associated.

ATTACHMENT DURING REPLICATION

It is now believed that DNA is replicated at the matrix or cage in the nucleus (Dijkwel, Mullenders & Wanka, 1979; Pardoll, Vogelstein & Coffey, 1980; McCready *et al.* 1980). Are sequences that initiate replication (i.e. origins) usually out in the loop, attaching only during *S*-phase (Fig. 9), or are they attached but

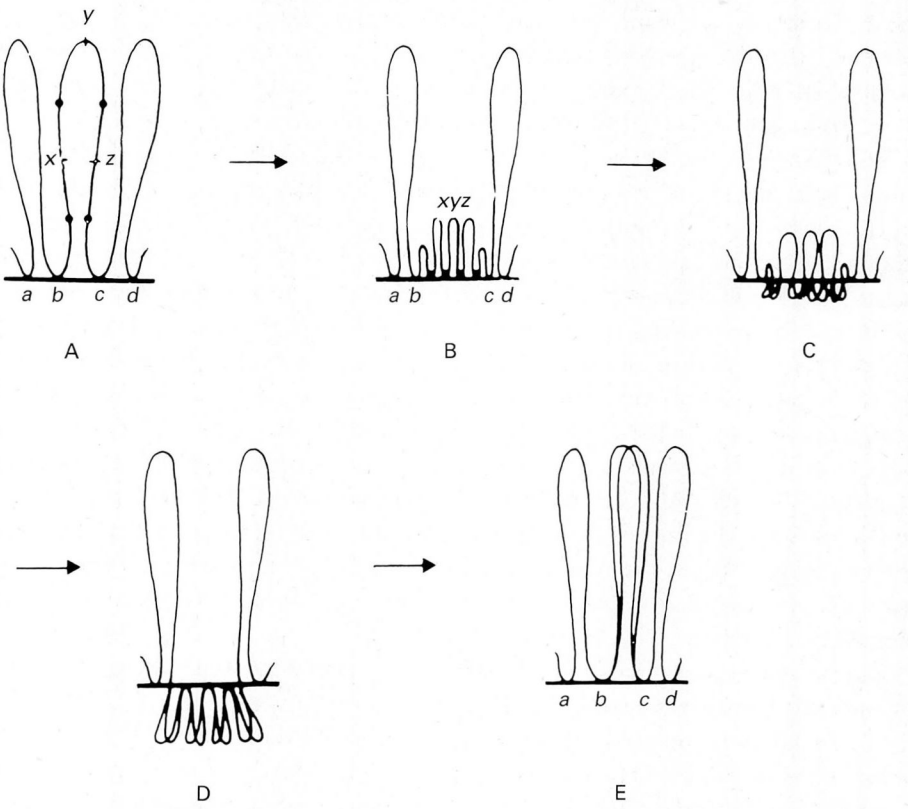
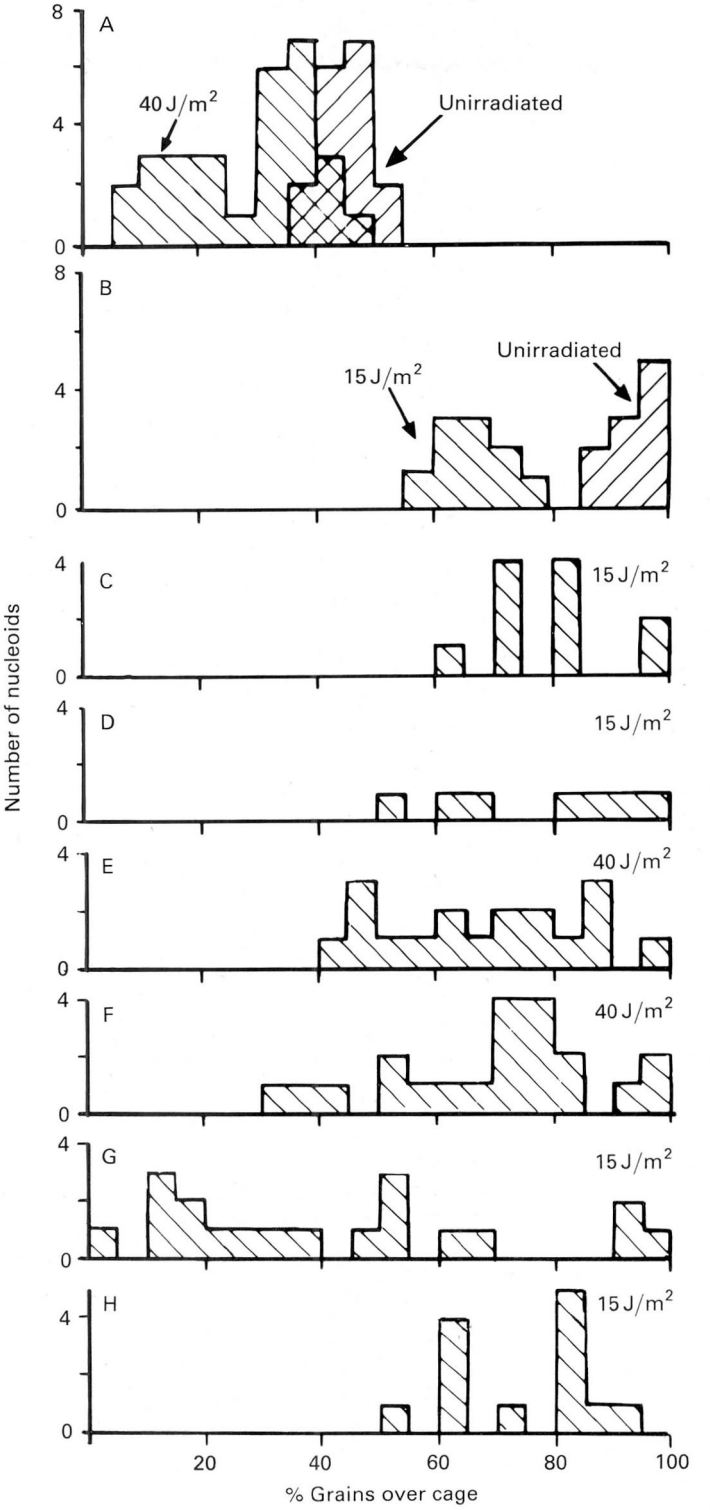


Fig. 9. A model for replication of nuclear DNA. A. Three adjacent structural loops (75 μm) are attached at the nuclear cage by specific base sequences (*a*,*b*,*c*,*d*). B. Before initiation, there is synchronous attachment, perhaps provoked by a change in degree of supercoiling in the loop, of four sequences (•) to form four replicons (*bx*, *xy*, *yz* and *zc*) each of about 20 μm. C,D. Bidirectional replication from each of the initiation points takes place as replicon loops move through the replication complex at the nuclear cage. E. When DNA synthesis is complete, the DNA detaches to form two newly replicated structural loops. It follows naturally from this model that the rate of replication could be controlled by varying the number of initial attachments and that adjacent replicons within a loop might attach and initiate replication synchronously (from McCready *et al.* 1980).

Fig. 10. Histograms of grain distributions over nucleoid spreads. A. 24 h label with [^{3}H]thymidine, then ± irradiation; the unirradiated distribution reflects the total DNA distribution that is redistributed by irradiation. B. ± Irradiation (15 J/m^2), then 5 min pulse, *S*-phase spreads; *S*-phase incorporation is cage-associated and somewhat redistributed by irradiation. C–F. Irradiation (15 (C,D) or 40 J/m^2 (E,F)), then 2·5 (C,E) or 5 min (D,F) pulse, non-*S* spreads; label is associated with cages. G. 15 J/m^2 irradiation, then 2·5 min pulse followed by 57·5 min chase, non-*S* spreads: some label remains associated with cages. H. *S*-phase spreads from G; label remains associated with cages. For C–H, medium was removed from HeLa cells growing logarithmically in Petri dishes, the cells were u.v.-irradiated and warm medium was added. After 30 min, [^{3}H]thymidine was added for 2·5 or 5 min, the cells detached and lysed, nucleoids isolated and spread, autoradiographs prepared and photographed, and grains over each cage expressed as a percentage of the total over skirt and cage. Spreads were easily categorized as *S* or non-*S* according to grain density (from McCready & Cook, 1984).


A
40 J/m²
Unirradiated
B
15 J/m²
Unirradiated
C
15 J/m²
D
15 J/m²
E
40 J/m²
F
40 J/m²
G
15 J/m²
H
15 J/m²
Number of nucleoids
% Grains over cage
0
4
8
20
40
60
80
100

quiescent during most of the cell-cycle, awaiting activation during *S*-phase?

If origins are generally attached, even in the absence of DNA synthesis, they should be enriched in the fraction of total DNA that resists detachment by a nuclease from the sub-structure. The sites involved in initiating replication in nuclear DNA are poorly characterized but sequences that permit plasmids to replicate autonomously in yeast (i.e. ARSs) are likely candidates (see Monteil *et al.* 1984, for a review). Therefore, we compared the relative concentration of ARSs in total DNA and in DNA that resisted detachment and found that they were roughly similar. We also mapped the positions of four human ARSs relative to attachment points and found that all were located out in the loops (Cook & Lang, 1984) consistent with the model described in Fig. 9.

Repair of ultraviolet light-induced lesions

In principle, we can introduce damage randomly into DNA loops by ultraviolet (u.v.) irradiation of cells. Do repair enzymes operate like the fire brigade – rushing to the site of the fire (i.e. the damaged DNA) wherever it be – or are they, too, stuck to the cage requiring attachment of the damaged sequences?

We examined which is so by u.v.-irradiating HeLa cells (15 or 40 J/m^2), then growing them for 30 min before pulse-labelling (2·5 or 5 min) with [^{3}H]thymidine. Next, nucleoids were isolated and spread, autoradiographs were prepared, silver grains were counted over each spread and the proportion over the cage was calculated. The histograms of label distributions given in Fig. 10C–F for cells involved in unscheduled DNA synthesis (i.e. repair synthesis) clearly show that pulse-label is associated with the cage. Its distribution is quite unlike that of total DNA shown in Fig. 10A. Once again, enzymes work on DNA only when it is attached (McCready & Cook, 1984).

ACTIVE SITES OR ARTEFACTS?

Most of the experiments we have described involve associations of nucleic acids – usually nascent molecules – with cages. Might not these associations simply be artefacts induced by exposure to extreme conditions (i.e. detergents and 2 M-NaCl)? Simple calculations show that RNA and DNA are present at extraordinarily high concentrations in the nucleus (~100 mg/ml). Furthermore, single-stranded nucleic acids aggregate in high salt concentrations (Asano, 1975), so one might expect RNA and nascent DNA to precipitate onto any sub-nuclear structure. As only a fraction of the genome is being replicated or transcribed at any time only this fraction will precipitate, apparently specifically. If so, it would not be surprising to find that nascent DNA and RNA are associated, or that sequences can be mapped relative to these association points. However, nascent DNA cannot be solely responsible for the attachments since both loop size and the relative positions of genes within a loop remain constant even when no DNA is replicating during mitosis or G_1 (Warren & Cook, 1978; Cook & Brazell, 1980). The criticism that transcribing complexes are the mediators of artefactual attachments is more difficult to eliminate, but some telling

observations on the *specificity* of the attachments of nascent RNA make this possibility unlikely. First, *all* the nascent RNA is attached specifically at its 5′ end. Second, only RNA made *in vivo* is attached; pulse-labelled RNA does not associate with cages if first 'chased' into the cytoplasm, nor does nascent RNA if synthesized *in vitro* within the isolated nucleoid. Third, nascent transcripts of a nuclear virus, influenza virus, are all associated with cages, whereas those of a cytoplasmic rhabdovirus, are not. Fourth, the proximity of active viral genes in transformed cells to the cage cannot be an immediate consequence of transcription since they remain close to the cage when transcription stops during mitosis or heat-shock.

CONCLUSION

We have argued that a nuclear sub-structure that we call the cage not only organizes the DNA within the nucleus but is also the active site of transcription and replication. Attached polymerases generate nascent molecules, which are themselves attached. Replication might be initiated by attachment of origins or thymine dimers, transcription by attachment of 'enhancers' and viral infection by attachment of the incoming genome. It is easy to imagine how selective attachment might underlie selective gene activity during development or oncogenesis. Indeed, gross detachment and loss of supercoiling is correlated with total inactivation of the avian erythrocyte nucleus (Cook & Brazell, 1976) and oncogene attachments with transformation.

As anyone who has ever sailed on a boat knows, ropes of any length must be tied down at all times – otherwise they get tangled, preventing orderly operation; the same must be true of the long nucleic acid polymers in the nucleus. Then what is really remarkable – and fortunate – is that these attachments can remain stable during isolation in detergents and 2 M salt.

We thank the Science Research Council and the Cancer Research Campaign for their support. Figures and tables are reproduced with permission from *J. Cell Sci.*, the *E.M.B.O. J.* and *Nature*.

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