

## Transcription occurs at a nucleoskeleton

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**Native chromatin aggregates under isotonic conditions so it is generally studied using higher or lower salt concentrations. This has led to different interpretations of how transcription might occur. Studies using hypertonically-isolated preparations suggest that DNA functions in close association with a skeletal nuclear substructure, the matrix or cage, but such a structure is not usually seen under hypotonic conditions (e.g., in 'Miller-spreads'). Using a novel method for preparing chromatin under isotonic conditions we have investigated the site of transcription. We find that all three constituents of the transcription complex, nascent transcripts, active RNA polymerase and genes being transcribed are all closely associated with some structure too large to be electroeluted from the nucleus. Hypotonic treatment partly disrupts this association. We suggest a model for transcription that involves the participation of a nucleoskeleton at the active site and reconcile the contradictory results obtained using different salt concentrations.**

*Key words:* chromatin/mechanism/microbeads/nucleoskeleton/transcription

### Introduction

The question of whether the nucleus of the living cell possesses the same skeletal substructure as that isolated after treatment with hypertonic salt concentrations remains controversial (for a review, see Hancock, 1982). Such structures – called, amongst others – the nuclear matrix, scaffold or cage are often associated with DNA being replicated (Dijkwel *et al.*, 1979; Pardoll *et al.*, 1980; McCready *et al.*, 1980) and transcribed (Jackson *et al.*, 1981, 1982; Cook *et al.*, 1982; Robinson *et al.*, 1982) as well as nascent RNA as it is synthesized (Jackson *et al.*, 1981, 1982) and during its subsequent processing (Miller *et al.*, 1976; Van Eekelen and Van Venrooij, 1981; Ciejek *et al.*, 1982; Mariman *et al.*, 1982). In sharp contrast, no skeletal structures are ever seen in the photomicrographs of 'genes in action' obtained by Miller and colleagues using hypotonic conditions (Miller and Beattie, 1969). These powerful images resembling Christmas trees are generally – but not always (McKnight and Miller, 1979) – obtained with complexes containing polymerase I and are readily interpreted in terms of a mobile polymerase which processes along the DNA and is unattached to any larger structure. This model is extended to include complexes containing polymerase II and is now included in most standard textbooks (see, for example, Alberts *et al.*, 1983). Such a view is reinforced by the isolation of soluble polymerases that work in the absence of any skeletal elements. As a result, we have two paradoxical views of DNA function: in the one, the skeletal substructure is the essential active site; in the other, it is not required – at least for transcrip-

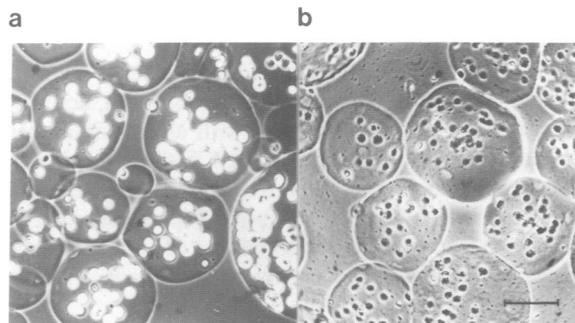
tion – and may not even exist.

In the accompanying paper we describe a method for isolating chromatin using a physiological salt concentration (Jackson and Cook). The resulting preparation can be manipulated freely without aggregation and it contains intact DNA since removal of the histones releases it in superhelical form. Living cells are encapsulated in 0.5% agarose microbeads by homogenizing an aqueous phase containing cells in molten agarose with an immiscible phase of liquid paraffin: on cooling, suspended agarose droplets gel into microbeads (Figure 1a, Nilsson *et al.*, 1983). The pores in the beads are large enough to allow free exchange of protein  $< 1.5 \times 10^8$  daltons but not chromosomal DNA (Cook, 1984). Therefore, when the encapsulated cells are immersed in Triton X-100 at a physiological salt concentration, most cytoplasmic proteins and RNA diffuse out through the pores in the beads to leave encapsulated nuclei (Figure 1b). If cells are lysed in EDTA, the resulting nuclei contain unbroken DNA and can be manipulated freely in a variety of different salt or detergent concentrations. The chromatin within the bead is well-protected from aggregation and shearing but is nevertheless completely accessible to enzymes and other probes. This chromatin has been characterized in the accompanying paper (Jackson and Cook). Using this preparation, we have investigated whether nascent RNA, RNA polymerase II and active genes are associated with a skeletal substructure. We use the term nucleoskeleton to describe the structure found under isotonic conditions to distinguish it from others isolated in 2 M NaCl.

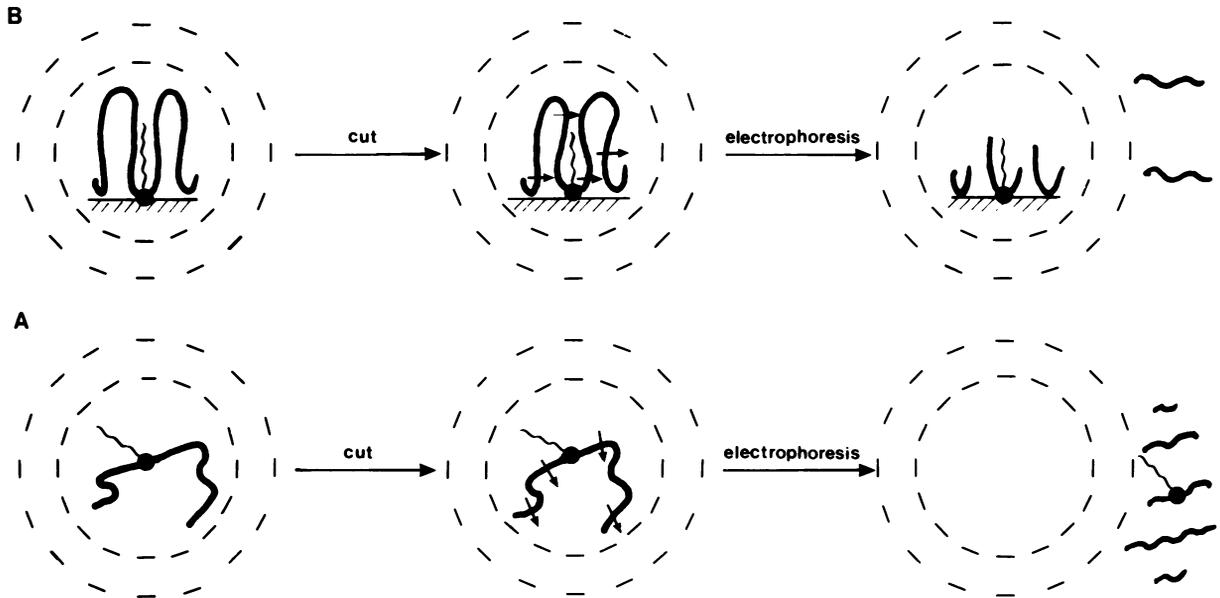
### Results

#### *Two models for transcription*

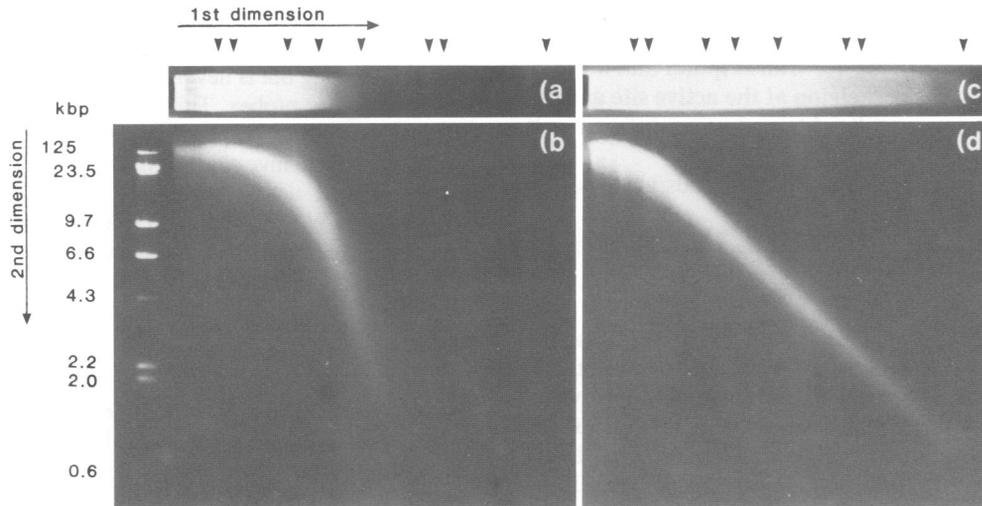
Two extreme views of how transcription might occur are presented in Figure 2: A is based largely on images seen after chromatin is spread under hypotonic conditions (i.e., in 'Miller spreads'), and B on work on nuclear cages isolated in 2 M NaCl. The essential difference is the participation of a larger nuclear substructure in the active site of the transcription complex. They can be distinguished using encapsulated chromatin prepared under isotonic conditions, then fragmenting the chromatin with an endonuclease and finally removing any unattached chromatin by



**Fig. 1.** Phase contrast micrographs of 0.5% agarose beads containing HeLa cells before (a) and after (b) lysis. Bar, 100  $\mu$ m. About 75% of the beads have diameters between 25 and 75  $\mu$ m.



**Fig. 2.** Two models for transcription. **A.** RNA polymerase (●) processes along the DNA (—) synthesizing a nascent transcript (---). **B.** Transcripts are generated as DNA moves past a polymerase associated with the nuclear skeleton (hatched area). A variant of **B** is that the 5' end of the nascent transcript is also attached to the nuclear skeleton (Jackson *et al.*, 1981). After cutting DNA with an endonuclease (arrow) and electrophoresis, the transcribed sequence, nascent RNA and polymerase should be retained within the bead (broken circles) in **B** but not **A**.



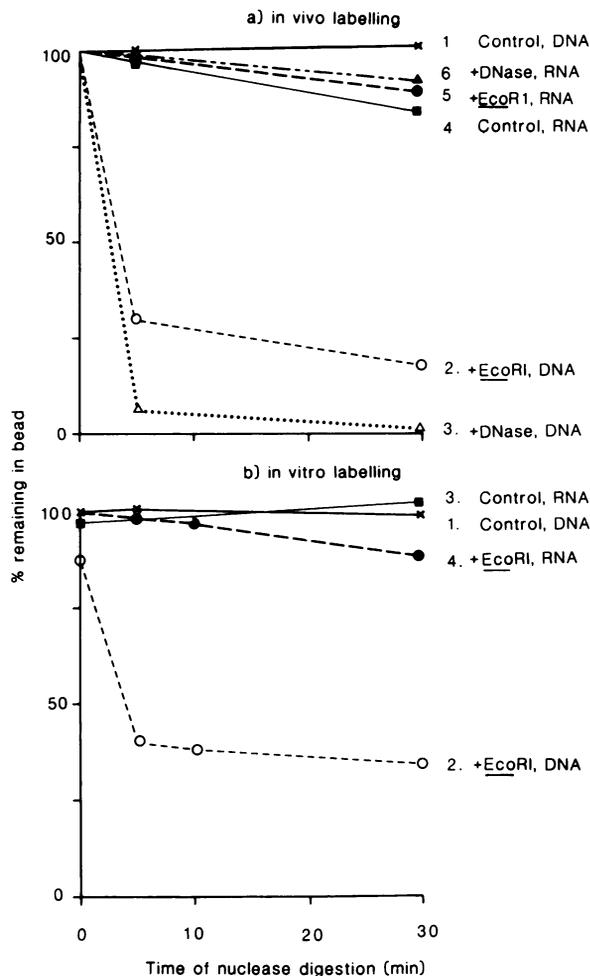
**Fig. 3.** The relative mobilities of DNA fragments, free and in chromatin. Encapsulated nuclei were incubated with *EcoRI* and subjected to electrophoresis. After staining with ethidium and photography (a), the channel was removed from the gel, soaked in proteinase K and SDS, laid on a second slab gel, and this gel electrophoresed, stained and photographed (b). Photographs of *EcoRI* fragments of pure DNA run in the first (c) and second dimensions (d) are included for comparison. The positions of marker DNA fragments (kbp) from uncut T5 and  $\lambda$  cut with *HindIII* are shown. In both dimensions markers were run in a different channel in the same gel as shown on the left in b. The short arrows along the top show the positions of the same markers in the first dimension. The long arrows show the direction of migration.

electrophoresis. If view B is correct then the three constituents of the transcription complex, the nascent transcript, active RNA polymerase and the transcribed gene will all remain associated with the larger structure and so trapped in the bead: if view A is correct, they should all escape from the bead on electrophoresis. Indeed, if A is correct, we might expect even low levels of digestion to lead to the preferential loss of all three, since active genes are preferentially cut by endonucleases (Weisbrod, 1982).

*Electrophoretic migration of chromatin in agarose gels*

The experiments outlined above require the electrophoretic

removal of large chromatin fragments from the beads. Perhaps surprisingly, chromatin fragments migrate electrophoretically in agarose gels about as rapidly as the free DNA that they contain (Weintraub, 1984). We have determined the minimum size of the largest chromatin particle that can migrate out of the beads under our conditions as follows. Encapsulated nuclei were incubated with a little *EcoRI* and subjected to electrophoresis. The range of resulting chromatin particles can then be visualized by staining the gel with ethidium (for DNA; Figure 3a) or Coomassie blue (for proteins). Next the size of DNA fragments within the particles was determined by cutting the track out of the gel, soaking it in proteinase K and SDS, and laying it sideways on a second



**Fig. 4.** Detaching chromatin does not detach nascent RNA labelled *in vivo* (a) or *in vitro* (b). (a) Cells labelled overnight with [ $^{14}\text{C}$ ]thymidine were encapsulated, regrown and pulse-labelled for 2.5 min with [ $^3\text{H}$ ]uridine and lysed. Beads were incubated with *EcoRI* or DNase and at various times, samples removed and after electrophoresis the radioactivity remaining in beads counted and expressed as a percentage of the label found in undigested controls. Curves 1–3 show the % DNA remaining in the undigested control (1), *EcoRI*-treated (2) and DNase-treated samples (3) and curves 4–6 the % nascent RNA remaining in the undigested control (4), *EcoRI*-treated (5) and DNase-treated samples (6). (b) Cells were labelled overnight with [ $^3\text{H}$ ]thymidine (0.01  $\mu\text{Ci/ml}$ ), encapsulated, lysed and washed. The encapsulated nuclei were incubated with [ $^{32}\text{P}$ ]UTP (5  $\mu\text{Ci/ml}$ ) for 2.5 min to label nascent RNA as described in Materials and methods. The doubly-labelled beads were then treated as in (a) and the  $^3\text{H}$  (i.e., DNA) and  $^{32}\text{P}$  (i.e., RNA) determined and expressed as a percentage of the label found in undigested controls. The curves show the % DNA (1–2) and nascent RNA (3,4) remaining in undigested (1,3) and *EcoRI*-digested (2,4) samples.

slab gel. After electrophoresis and staining with ethidium, some pure DNA fragments with lengths greater than that of the T5 DNA marker (i.e., > 125 kb) are found, indicating that they had migrated – as chromatin – out of the 0.5% agarose bead and about 1 cm into the first 0.66% agarose gel (Figure 3b). [Such a chromatin particle, if half DNA and half protein, would have a weight of  $150 \times 10^6$  daltons.] Since such particles have a weight at least ten times greater than the weight of the mammalian multienzyme complex involved in replication (Noguchi *et al.*, 1983) they are much larger than other known ‘soluble’ nuclear complexes. Furthermore, as they migrated a substantial distance into the first gel, the pores in the beads must permit egress of even larger particles.

The mobilities of free DNA fragments were compared with

**Table I.** Attachment of nascent RNA after various treatments

Pulse time (s)	% pulse-label remaining attached			
	Isotonic		Hypotonic	
	–DNase	+DNase	–DNase	+DNase
10	92	88	90	76
30	80	82	64	49
60	79	80	46	32
180	82	88	44	33
300	86	93	47	33

Cells were labelled with [ $^3\text{H}$ ]thymidine overnight (0.01  $\mu\text{Ci/ml}$ ), encapsulated ( $50 \times 10^6/\text{ml}$ ), and pulse-labelled for various times with [ $^3\text{H}$ ]uridine (50  $\mu\text{Ci/ml}$ ). Labelling was stopped by the addition of excess ice-cold PBS supplemented with 1 mM uridine, the encapsulated cells pelleted and lysed in the isotonic lysis mixture. Some beads were hypotonically treated. Each sample was incubated  $\pm$  DNase (100  $\mu\text{g/ml}$ ) for 10 min at 37°C, and subjected to electrophoresis. >94% of the  $^{14}\text{C}$  was lost from beads on DNase treatment and electrophoresis. The  $^3\text{H}$  remaining in beads is expressed as a percentage of the label in isotonic controls that had not been DNased or electrophoresed.

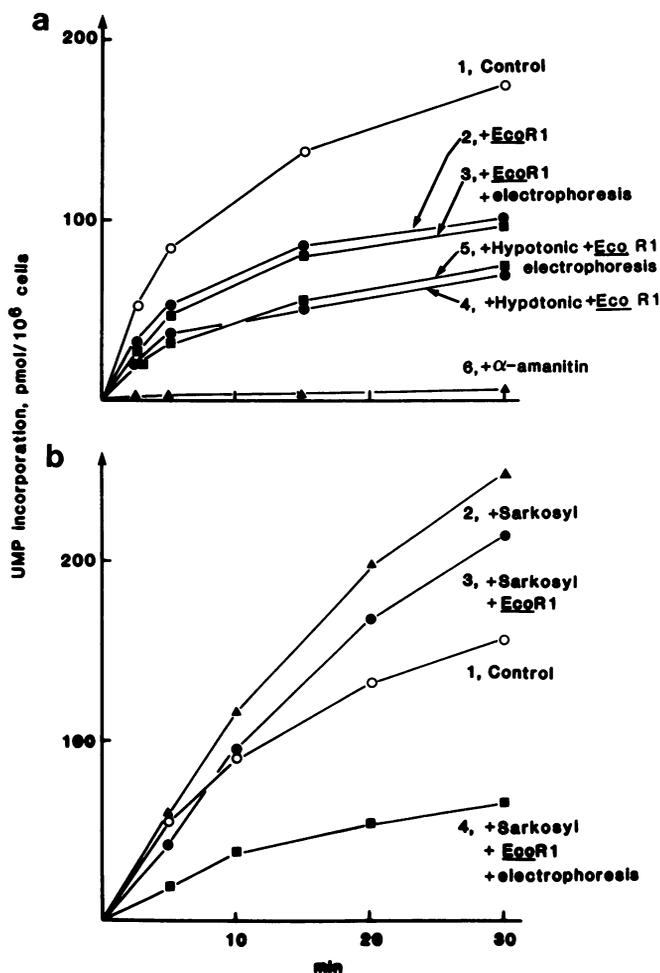
those of similarly-sized fragments in chromatin by running pure DNA on an adjacent track in the first gel (Figure 3c): after electrophoresis in the second dimension, pure DNA fragments of < 20 kb are found along the diagonal (Figure 3d) whereas those from chromatin are along a line with half the slope. Therefore such fragments in chromatin run at half the rate as free DNA. Above 20 kb the relationship ceases to be linear.

RNA readily passes through the agarose pores. For example, cytoplasmic RNA is immediately lost by diffusion on lysis and nearly all nuclear RNA can escape after treatment with sarkosyl (see later) or lithium dodecyl sulphate (Cook, 1984; Jackson and Cook, accompanying paper).

#### Detaching chromatin does not detach nascent RNA

We first determined whether nascent RNA was retained within beads after removing most of the chromatin (Figure 4a). Cellular DNA was uniformly labelled with  $^{14}\text{C}$ , the cells encapsulated and then incubated with [ $^3\text{H}$ ]uridine for 2.5 min to label nascent RNA. After lysis, the encapsulated nuclei were treated with *EcoRI* or deoxyribonuclease for different times and the bulk of the chromatin removed by electrophoresis. In untreated controls, all the DNA (Figure 4a; curve 1) and 84% of the nascent RNA (curve 4) remain associated with the beads throughout this extensive series of manipulations, confirming the remarkable stability of the encapsulated chromatin. Nearly all the DNA – and so chromatin – is lost on digestion with *EcoRI* or deoxyribonuclease (curves 2,3) without *any* further loss of nascent RNA (curves 5,6). Thus 92% of the nascent RNA remains when all but 2% of the chromatin has been removed (curves 3 and 6). This retention of pulse-labelled RNA despite loss of nearly all chromatin is true for pulse-labels of between 10 and 300 s (Table I).

Since transcription complexes are generally visualized after hypotonically swelling nuclei, we investigated the effect of such a treatment on the stability of the nascent RNA and polymerase in the complex (Table I). As shown above, nascent RNA cannot be removed by electrophoresis from isotonic preparations of chromatin. In contrast, after hypotonic treatment electrophoresis removes about half the transcripts labelled in pulses of 60–300 s from intact chromatin and combined DNase treatment and electrophoresis removes two-thirds. Intriguingly the remaining one-third remains stably associated even after longer hypotonic treatments (results not shown) and shorter pulses (i.e., 10–30 s) increase



**Fig. 5.** *EcoRI* treatment and electrophoresis do not remove active RNA polymerase (a) unless sarkosyl is present (b). (a) Cells were labelled overnight with [<sup>3</sup>H]thymidine (0.01  $\mu$ Ci/ml; 50 Ci/mmol), encapsulated, lysed and washed. Sample 1, beads were kept on ice; sample 2, incubated with *EcoRI* (4000 units/ml; 10 min) and then kept on ice. Sample 3, incubated with *EcoRI*, subjected to electrophoresis. Sample 4, as 2 with hypotonic treatment preceding *EcoRI* digestion. Sample 5, as 3, with hypotonic treatment preceding *EcoRI* digestion. The samples were then incubated with [<sup>32</sup>P]UTP (10  $\mu$ Ci/ml) and appropriate cofactors for various times and the amount of label incorporated into RNA determined. 100% of the <sup>3</sup>H initially present was recovered in samples 1, 2 and 4, 25% in sample 3 and 20% in sample 5. In a parallel experiment, beads were also incubated with 10  $\mu$ g/ml  $\alpha$ -amanitin (sample 6). (b) An experiment like that in (a), except that beads were incubated  $\pm$  0.5% sarkosyl after *EcoRI* digestion and during polymerase assay. (1) control, as sample 1 above. (2) as 1, sarkosyl treated. (3) *EcoRI* cut and sarkosyl treated. (4) *EcoRI* cut, sarkosyl treated and subjected to electrophoresis. 100% of the <sup>3</sup>H initially present was recovered in samples 1–3, but only 60% in sample 4.

the proportion of this resistant fraction (Table I). Whatever the basis for this differential stability of the two fractions, it is clear that hypotonic treatment does destabilize some of the nascent RNA.

Loss of chromatin without loss of any nascent RNA can also be demonstrated by pulse-labelling the RNA *in vitro* rather than *in vivo* (Figure 4b). Cellular DNA was labelled with [<sup>3</sup>H]thymidine and then cells encapsulated and lysed. The resulting encapsulated nuclei contain a highly active RNA polymerase which uses the endogenous template and is sensitive to  $\alpha$ -amanitin (Figure 5a, sample 6). On incubation with [<sup>32</sup>P]UTP for 2.5 min, nascent RNA was synthesized *in vitro*: after incubation with *EcoRI* most of this RNA resisted electro-

**Table II.** Active transcription complexes cannot be removed electrophoretically from beads following treatment with *EcoRI* and RNase

Treatment	% Remaining		
	DNA	RNA <sup>a</sup>	Polymerase
Control	100	100	100
+ <i>EcoRI</i>	30	100	85
+ RNase <sup>b</sup>	100	<5	86
+ <i>EcoRI</i> and RNase <sup>b</sup>	27	<5	70

After various treatments, the incorporation of [<sup>32</sup>P]UTP into RNA in 30 min was expressed as a percentage of the control. Conditions were as described in Figure 5a.

<sup>a</sup>% RNA remaining after pulse-labelling cells for 2.5 min with [<sup>3</sup>H]uridine.

<sup>b</sup>50  $\mu$ g/ml RNase A for 10 min.

phoretic removal, unlike the chromatin. [In a variety of different experiments (unpublished data) the nascent RNA synthesized *in vitro* is not as resistant to nucleolytic degradation as that made *in vivo*, presumably because it is not properly packaged into ribonucleoprotein.]

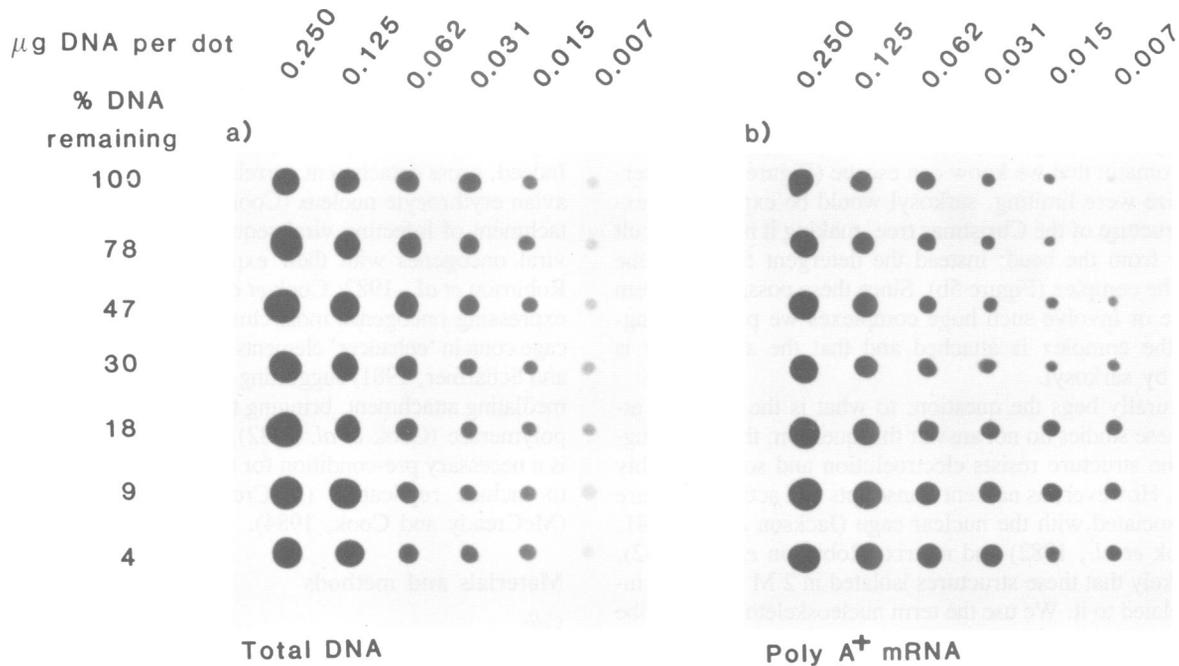
#### Detaching chromatin does not detach RNA polymerase

We next determined whether RNA polymerase II activity was retained within beads after removing most of the chromatin (Figure 5a). Again, the activity within the beads is stable. Cellular DNA was uniformly labelled with <sup>3</sup>H, the cells encapsulated, lysed and RNA polymerase activity assayed by incorporation of [<sup>32</sup>P]UTP into acid-insoluble material. [Since there is no initiation under these conditions (unpublished data), note that we deal only with endogenous polymerase halted by lysis during elongation on the endogenous template.] *EcoRI* digestion of the encapsulated nuclei reduces both the initial rate of RNA synthesis and the total amount of RNA made to ~60% of that of the control (Figure 5a, curves 1 and 2), presumably because the template is truncated or its conformation is otherwise changed. Removing 75% of the chromatin by electrophoresis reduces the activity no further (curve 3).

Although the chromatin fibre has been cut into pieces small enough to escape, perhaps the nascent RNA and associated protein makes the transcription complex too bulky. Therefore beads were incubated with sufficient RNase A to detach >95% nascent RNA and hence RNP. With or without electrophoresis this reduces the polymerase activity by ~15% (Table II and unpublished data). Treatment with RNase and *EcoRI*, followed by electrophoresis, removes >95% nascent RNA and 73% of the DNA. In contrast, 70% of the polymerase activity remained (Table II), and this reduction to 70% can be explained by the additive effects of *EcoRI* and RNase treatments. Clearly little – if any – active polymerase escapes with the chromatin, degraded RNA and associated ribonucleoprotein. This suggests that the transcribing complex must remain very large and cannot be tethered to the substructure solely through RNA.

Just as hypotonic treatment disrupts nascent RNA in the transcription complex (Table I), it also disrupts polymerase. *EcoRI* digestion, with or without electrophoresis, reduced the amount of RNA synthesized to 60% of the control (Figure 5a, samples 2,3): an additional hypotonic treatment reduces it further to 35% of the control (Figure 5a, samples 4,5). These results are again consistent with about half the transcription complexes being sensitive to disruption and detachment by hypotonic treatment.

Rather surprisingly, the ionic detergent, sarkosyl, stimulates RNA polymerase II (Gariglio *et al.*, 1974). This allows us to



**Fig. 6.** Active genes resist detachment. Encapsulated chromatin was incubated with 0–2000 units/ml *Eco*RI for 10–45 min and subjected to electrophoresis. The beads were recovered and DNA remaining expressed as a percentage of that initially present. After purification, different dilutions were dot-blotted on to filters and duplicate filters hybridized with <sup>32</sup>P-labelled total cell DNA (a) or poly(A)<sup>+</sup> cytoplasmic RNA (b).

detach the active transcription complex from the nuclear substructure so that now it can be removed electrophoretically (Figure 5b). Addition of sarkosyl to encapsulated chromatin removes all but ~3% of protein (Jackson and Cook, accompanying paper) and increases the amount of RNA made (Figure 5b, curves 1 and 2), presumably because histones have been stripped from the template. As before, *Eco*RI digestion reduces the rate of synthesis (curve 3). On pelleting the beads, some polymerase activity is found in the supernatant (unpublished data) and electrophoresis removes 40% of the chromatin but 70% of the activity (curve 4). This shows that an active transcription complex can escape from the beads. Note that in this experiment, less *Eco*RI was used than in the others, enabling us to detect the preferential sensitivity of active chromatin to digestion.

#### Active genes resist detachment

The transcribed template constitutes the third element of the transcription complex and so following *Eco*RI digestion and electrophoresis it, too, should behave like the other two elements. Cells were labelled with [<sup>3</sup>H]thymidine to allow quantitation of the DNA, encapsulated, lysed and incubated with different amounts of *Eco*RI. After electrophoresis, DNA retained within the bead was purified and a series of dilutions dotted on to filters and their concentrations of active genes determined by hybridization with <sup>32</sup>P-labelled poly(A)<sup>+</sup> cytoplasmic RNA. The resulting autoradiographs show that the concentration of hybridizing sequences (i.e., active genes) in the sample that retained only 9% of the total DNA is about four times that in total DNA (Figure 6b). Intermediate enrichments are found in the samples that retained more DNA. Essentially similar results are obtained when nuclear RNA is used as a probe (unpublished data). Duplicate filters hybridized with <sup>32</sup>P-labelled total DNA demonstrate that these enrichments are not due to faulty DNA loadings (Figure 6a). Clearly, active genes are less likely than others to be removed by this procedure from the beads.

## Discussion

### The utility of encapsulated nuclei

When cells are encapsulated in agarose and then lysed in Triton X-100 and an isotonic solution, most soluble cytoplasmic proteins and RNA immediately diffuse out through the pores in the gel to leave a nucleus and residual cytoskeleton. The nuclei contain intact DNA associated with nuclear protein and this chromatin is accessible to a variety of probes (e.g., proteins as large as 150 x 10<sup>6</sup> daltons). The microbeads can be manipulated freely without aggregation and are readily pelleted using a bench centrifuge from one solution for transfer to another. Some of the experiments described here involve several enzyme digestions or assays, treatment with hypotonic solutions or detergents and electrophoresis overnight – manipulations that would be impossible using free nuclei or chromatin which aggregate and jellify so readily. It seems likely that this chromatin, packaged in an accessible yet manipulable form, will prove useful for studies on both structure and function.

### Association of transcription complexes with a larger nuclear structure

When encapsulated chromatin is incubated with endonucleases and subjected to electrophoresis, the bulk of the chromatin escapes from beads; in striking contrast, nascent RNA, active RNA polymerase II and active genes cannot. We believe this is most simply interpreted by association of transcription complexes with the nucleoskeleton. Before discussing this possibility we discuss others involving an unattached complex. First, the complex might have no net charge and so be unable to migrate electrophoretically. However, it seems highly improbable that the complex can exactly neutralize the charge on each of the wide range of randomly-sized chromatin fragments that are generated. Second, an unattached complex might be too large to escape through the densely packed material in the nucleus or through the pores in the bead. If so, it must remain too large even when 73% of the

chromatin and >95% of the nascent RNA is removed (Table II). Even if the transcription complex has an extended 'Christmas-tree' structure, such an RNase treatment effectively removes all the 'branches' (i.e., the RNP). Then the residual complex must still be very much larger than  $150 \times 10^6$  daltons, which is the size of chromatin that we know can escape (Figure 3). Furthermore, if size were limiting, sarkosyl would be expected to extend the structure of the Christmas tree, making it more difficult to remove from the bead: instead the detergent facilitates the escape of the complex (Figure 5b). Since these possibilities seem improbable or involve such huge complexes we prefer to suggest that the complex is attached and that the attachment is destroyed by sarkosyl.

This naturally begs the question: to what is the complex attached? These studies do not answer this question; they only suggest that the structure resists electroelution and so is probably very large. However, as nascent transcripts and active genes are closely associated with the nuclear cage (Jackson *et al.*, 1981, 1982; Cook *et al.*, 1982) and matrix (Robinson *et al.*, 1982), it seems likely that these structures isolated in 2 M NaCl are intimately related to it. We use the term nucleoskeleton to describe the structure found under isotonic conditions to distinguish it from the others isolated in 2 M NaCl, and suggest that it is the structure with which the transcription complex is associated. Obviously, further work is required to define the nature of the nucleoskeleton and its interactions with the transcription complex.

#### *Different artefacts in hypertonic and hypotonic solutions*

Transcription complexes seen in spreads after exposure to hypotonic media are unattached to a larger structure whereas their counterparts isolated in 2 M NaCl are attached. These apparently paradoxical results can be reconciled if it is accepted that both are artefacts. In 2 M NaCl, nucleosome structure and polymerase activity are destroyed, whilst attachments of active nascent transcripts to the nucleoskeleton are retained. Indeed, new attachments may be generated (Mirkovitch *et al.*, 1984; Jackson and Cook, accompanying paper). On the other hand, in hypotonic media, nucleosome structure and polymerase activity are retained but the transcription complex is disrupted and some transcripts are detached from the nucleoskeleton. Since hypotonic treatment removes one quarter of the protein of our preparations (accompanying paper) and since no skeletal structure can be seen in 'Miller' spreads, it may even be destroyed. Of course, it may turn out that polymerase II transcription units are attached whilst the polymerase I transcription units usually seen in spreads are not.

#### *An attachment hypothesis for transcription*

We envisage the nucleoskeleton as one part of the active site of the transcription complex, organizing the template – the DNA – in three-dimensional space into close proximity to the polymerization site. Transcription proceeds by passage of the DNA through the complex to yield attached transcripts.

This suggestion seems to conflict with many observations that soluble polymerases work. However, >95% of polymerase II pellets with broken nuclear fragments (Weil *et al.*, 1979) and can be released only by prolonged incubation in  $Mg^{2+}$  ions or by sonication (Beebe, 1979). Furthermore, it is not widely appreciated that 'semi-soluble' polymerases initiate extraordinarily inefficiently: one of the most efficient systems, a crude 'Manley' extract, polymerizes correctly-initiated transcripts at average rates of <10 nucleotides/h (Manley *et al.*, 1980) or 0.01% of the rate *in vivo* (Cox, 1976).

If the polymerase is tethered to the nucleoskeleton, then only

genes closely associated with this skeleton will be transcribed: those that are remote from it will not. Then it becomes easy to imagine how selective attachment of genes to the nucleoskeleton might underlie selective gene activity during development or oncogenesis (McCready *et al.*, 1982; Jackson *et al.*, 1981, 1984). Indeed, gross detachment correlated with total inactivation of the avian erythrocyte nucleus (Cook and Brazell, 1976) and the attachment of infecting viral sequences, the ovalbumin gene and viral oncogenes with their expression (Jackson *et al.*, 1982; Robinson *et al.*, 1982; Cook *et al.*, 1982). The sequences within expressing oncogenes most closely associated with the nuclear cage contain 'enhancer' elements (Moreau *et al.*, 1981; de Villiers and Schaffner, 1981) suggesting that they might be the sequences mediating attachment, bringing the transcription unit close to the polymerase (Cook *et al.*, 1982). A model in which attachment is a necessary pre-condition for transcription is readily extended to include replication (McCready *et al.*, 1980) and repair (McCready and Cook, 1984).

## Materials and methods

### *Cells*

HeLa cells were grown, labelled and encapsulated in 0.5% agarose (Sigma type VII) as described (Cook, 1984).

### *Radiochemicals*

Labels included [methyl- $^{14}C$ ]thymidine (~60 mCi/mmol), [methyl- $^3H$ ]thymidine (~50 Ci/mmol), [5,6- $^3H$ ]uridine (~50 Ci/mmol), uridine 5'-[ $\alpha$ - $^{32}P$ ]triphosphate (~400 Ci/mmol), adenosine 5'-[ $\gamma$ - $^{32}P$ ]triphosphate (~3000 Ci/mmol), deoxyadenosine 5'-[ $\alpha$ - $^{32}P$ ]triphosphate (~3000 Ci/mmol).

### *Cell lysis*

Encapsulated cells were lysed by mixing 1 vol beads in phosphate-buffered saline or growth medium with 3 vol ice-cold 0.5% Triton X-100, 100 mM KCl, 10 mM Tris (pH 8.0), 25 mM  $Na_2EDTA$ , 1 mM dithiothreitol. After 20 min on ice, beads were washed in 'isotonic buffer' [100 mM KCl, 25 mM  $(NH_4)_2SO_4$ , 10 mM Tris (pH 8.0), 1 mM EDTA, 1 mM dithiothreitol, 5% glycerol] at least three times.

### *Hypotonic treatment*

The treatment is based on that used in the preparation of 'Miller' spreads (McKnight and Miller, 1979). Encapsulated nuclei in isotonic buffer were diluted to 1.5 mM salt by two washes in 10 volumes 100  $\mu M$  borate buffer (pH 8.5), and incubated on ice for 15 min prior to rewashing in isotonic buffer.

### *Electrophoresis*

DNA was subjected to electrophoresis (2 V/cm; 6 h) through 0.8% agarose gels in 40 mM Tris (pH 8.3), 2 mM EDTA and 20 mM sodium acetate (TEA). Encapsulated chromatin was electrophoresed similarly except that the sample and electrophoresis buffers were equal mixtures of isotonic buffer and TEA. After electrophoresis beads were recovered and washed in the appropriate buffer.

The 2-D gel in Figure 3b was run as follows. Encapsulated chromatin in isotonic buffer was incubated with *EcoRI* (2000 units/ml; 15 min), an equal volume of TEA added and applied to a 0.66% agarose gel. After electrophoresis (0.7 V/cm; 16 h) the gel was stained with ethidium, photographed, washed (2 h; 20°C) in TEA supplemented with 1% SDS and 12.5  $\mu g/ml$  proteinase K and rewashed in TEA (1 h; 20°C). A 2 mm strip was cut from the centre of the channel, placed along the top of another 10 cm wide 0.66% agarose gel containing 0.1% SDS and subjected to electrophoresis as above in TEA.  $\lambda$ DNA cut with *HindIII* and T5 DNA served as markers. Encapsulated nuclei, washed with 2 M NaCl and treated with *EcoRI* were applied to the control gel (Figure 3c,d).

### *Digestion and electrophoresis of encapsulated nuclei*

A typical procedure (i.e., the one used for Figure 4a) is described. Cells were labelled overnight with [ $^{14}C$ ]thymidine (0.01  $\mu Ci/ml$ ), encapsulated (40  $\times 10^6/ml$  agarose), regrown briefly and pulse-labelled for 2.5 min with [ $^3H$ ]uridine (50  $\mu Ci/ml$ ). Labelling was stopped by adding 10 vols ice-cold phosphate-buffered saline, the encapsulated cells were pelleted, lysed, washed in isotonic buffer and then suspended in isotonic buffer supplemented with 2 mM  $MgCl_2$ . Some samples (500  $\mu l$ ) were incubated at 37°C with *EcoRI* (2000 units) or DNase (20  $\mu g$ ) and at various times, 150  $\mu l$  samples removed and subjected to electrophoresis. (With high levels of *EcoRI*, addition of glycerol with the enzyme activates the *EcoRI*\* activity.) After electrophoresis, beads were recovered, pelleted and SDS added to a final concentration of 2%. Finally the radioactivity in 100  $\mu l$  samples containing 5  $\times 10^5$  cells was measured.

*Polymerase assay*

Beads were washed in isotonic buffer, an equal volume of a twice concentrated transcription mixture added, pre-incubated (37°C; 10 min) and synthesis initiated by addition of triphosphates, S-adenosyl methionine and MgCl<sub>2</sub>. At various times samples were removed, SDS added to 2%, diluted 5-fold and spotted onto glass fibre discs (Whatman GF/C). After washing in 5% trichloroacetic acid the discs were counted. During transcription the final concentrations were 50 mM Tris (pH 8.0), 25 mM KCl, 100 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgCl<sub>2</sub>, 0.5 mM dithiothreitol, 1 mM EDTA, 5 mM spermidine, 5% glycerol, 50 μM S-adenosyl methionine, 500 μM ATP, 500 μM CTP, 500 μM GTP, 50 μM UTP (supplemented with 10 μCi/ml [<sup>32</sup>P]UTP at ~400 Ci/mmol).

*Hybridization*

DNA dissolved in SSC, purified from chromatin that remained within beads following electrophoresis, was 'dot-blotted' onto a Biotodyne filter (Pall) and the filter baked (75°C; 16 h), pre-hybridized (41°C; 4 h) and hybridized (41°C; 24 h) with <sup>32</sup>P-labelled probes prepared either by 'nick-translating' EcoRI cut total HeLa DNA (10<sup>8</sup> c.p.m./μg) or by polynucleotide kinase treatment of HeLa poly(A)<sup>+</sup> cytoplasmic RNA (10<sup>7</sup> c.p.m./μg). After hybridization, filters were washed in 2 x SSC, 0.1% SDS (three times; 20 min; 20°C) and then 0.1 x SSC, 0.1% SDS (twice; 15 min; 55°C) and the autoradiograph prepared. All procedures are given in Maniatis *et al.* (1982).

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