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The Nucleoskeleton: Active Site of Transcription and Replication

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A. Introduction

Nuclei and chromatin are rarely studied at a physiological salt concentration since they aggregate so readily [16]. As a result, they are generally studied in the presence of "stabilizing" divalent cations under hyper- or hypotonic conditions. Such conditions are unsatisfactory for several reasons. The "stabilizing" cations activate nucleases, destroying template integrity and supercoiling, and unphysiological salt concentrations may introduce artefacts. It has been suggested that structures called variously the nuclear matrix, cage or scaffold, are the site of replication and transcription [8], but they are not seen in the micrographs of "genes in action" obtained by Miller and colleagues using hypotonic conditions [15, 14]. These powerful images resembling Christmas trees are interpreted in terms of a mobile polymerase which processes along the DNA and is unattached to any larger structure. Such models are now included in most standard textbooks [1]. 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 and may not even exist.

We have described a method for isolating chromatin using a physiological salt concentration. Living cells are encapsulated in agarose microbeads. The bead pores are large enough to allow free exchange of protein as large as 1.5×10^8 daltons but not of chromosomal DNA [3, 9]. Therefore, when encapsulated cells are immersed in Triton X-100 at a physiological salt concentration, most cytoplasmic proteins and RNA diffuse out through the pores to leave encapsulated chromatin. If cells are lysed in the presence of EDTA, the resulting DNA remains intact. The procedure yields essentially a preparation of encapsulated nuclei (Fig. 1). However, these nuclei differ from their unencapsulated counterparts in that they contain unbroken DNA and can be manipulated freely. The chromatin within the bead is well pro-

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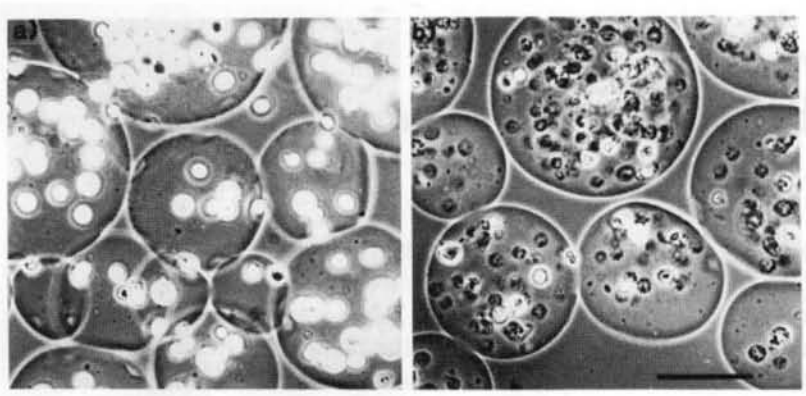

Fig. 1. Phase contrast micrographs of 0.5% agarose beads containing HeLa cells before (**a**) and after (**b**) lysis. *Bar* = 100 μm. (From Jackson and Cook [10])

tected from aggregation and shearing but is nevertheless completely accessible to enzymes and other probes used in modern molecular biology.

I. Two Models for Transcription

Two extremely different views of how transcription might occur are presented in Fig. 2. The essential difference is the participation of a larger nuclear substructure in the active site of the transcription complex. They can be distinguished by fragmenting the chromatin with an endonuclease and removing any unattached chromatin by electrophoresis. If view B is correct, then the transcription complex will remain associated with the larger structure and so trapped in the bead; if view A is correct, it should escape from the bead on electrophoresis [10].

The encapsulated nuclei contain a very active RNA polymerase which is sensitive to α-amanatin, a specific inhibitor of RNA polymerase II, and which synthesizes RNA at a rate roughly equivalent to that found in vivo. *Eco*RI digestion reduces both the initial rate of RNA synthesis and the total amount of RNA made to ~60% of the control (Fig. 3, curves 1 and 2), presumably because the template is truncated. Removing 75% of the chromatin by electrophoresis reduces the activity no further (Fig. 3,

Table 1. Active transcription complexes cannot be removed electrophoretically from beads following treatment with *Eco*RI and RNase (from Jackson and Cook [10])

Treatment	% Remaining		
	DNA	RNA[a]	Polymerase
Control	100	100	100
*Eco*RI	30	100	85
RNase	100	< 5	86
*Eco*RI and RNase	27	< 5	70

After various treatments, the incorporation of [^{32}P] UTP into RNA in 30 min was expressed as a percentage of the control.
[a] RNA remaining after pulse-labelling cells for 2.5 min with [^{3}H] uridine.

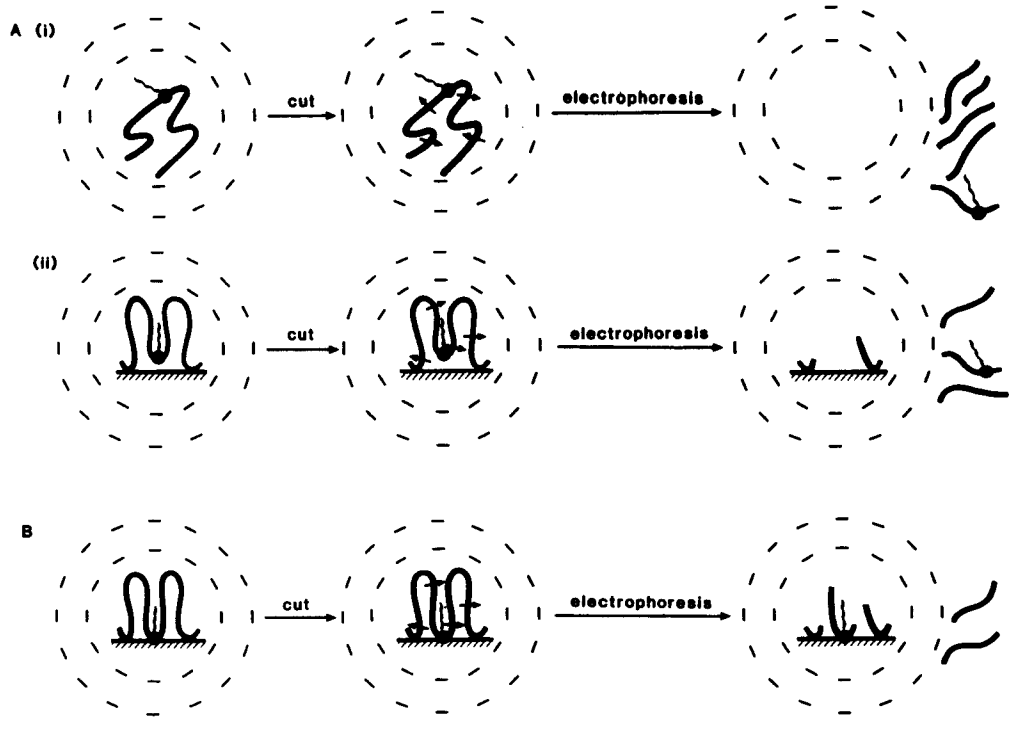

Fig. 2. Two models for transcription. **A**, RNA polymerase (●) processes along the DNA (—) synthesizing a nascent transcript (~). **B**, Transcripts are generated as DNA moves past a polymerase associated with the nuclear skeleton (*hatched area*). After cutting DNA with an endonuclease (*arrow*) and electrophoresis, the transcribed sequence, nascent RNA and polymerase should be retained within the bead (*broken circles*) in B but not A. (From Jackson and Cook) [10])

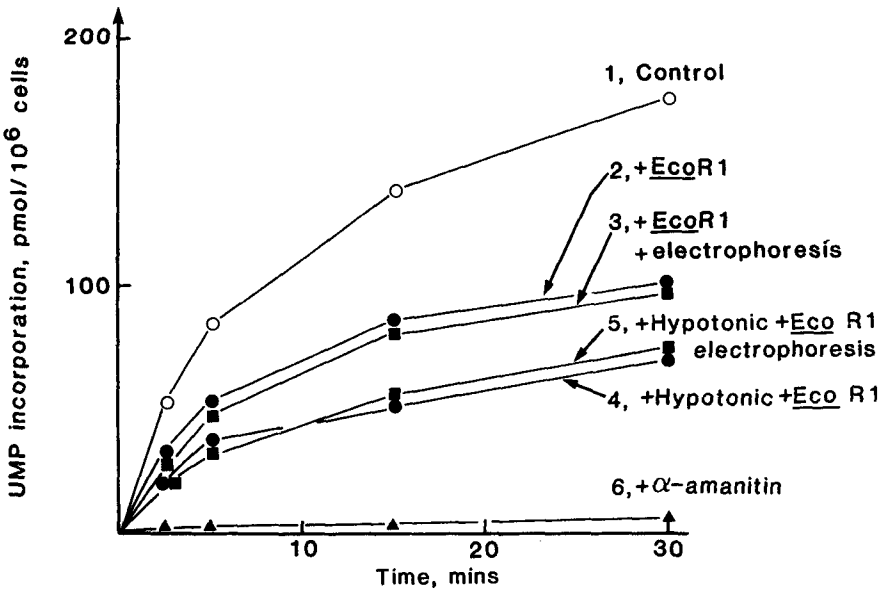

Fig. 3. *Eco*RI treatment and electrophoresis do not remove active RNA polymerase. Cells were labelled overnight with [^{3}H]thymidine, encapsulated, lysed and washed. Sample *1*, beads were kept on ice; sample *2*, incubated with *Eco*RI and then kept on ice; sample *3*, incubated with *Eco*RI, subjected to electrophoresis; sample *4*, as 2, with hypotonic treatment preceding *Eco*RI digestion; sample *5*, as 3, with hypotonic treatment preceding *Eco*RI digestion. The samples were then incubated with [^{32}P]UTP and appropriate cofactors for various lengths of time and the amount of label incorporated into RNA was determined; 100% of the ^{3}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 μg/ml α-amanitin (sample *6*). (From Jackson and Cook [10])

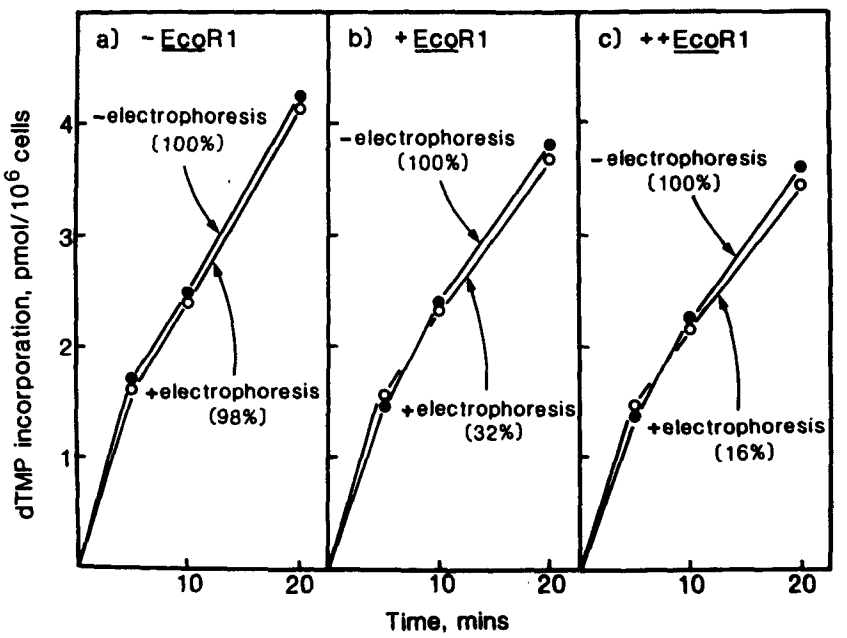

Fig. 4. The replication complex is closely associated with the nucleoskeleton. Cells labelled for 24 h with [^{3}H] thymidine were encapsulated and lysed, and beads were washed. $MgCl_2$ was added and samples incubated with (*a*) 0, (*b*) 1000 and (*c*) 5000 units/ml *Eco*RI. Half of each set of beads was subjected to electrophoresis in isotonic buffer. After recovering beads, the rate of incorporation of [^{32}P] dTTP into DNA was determined. The amount of [^{3}H] in equal volumes of each sample was determined and expressed as a percentage (*brackets*) of the sample in (a) that had not been treated with *Eco*RI or subjected to electrophoresis. (From Jackson and Cook [11])

curve 3). A combined treatment with RNase and *Eco*RI, followed by electrophoresis, removes >95% nascent RNA (and so RNP) and 73% of the DNA (and so chromatin) but only 30% of the polymerase (Table 1). Clearly, little – if any – active polymerase escapes with the chromatin, degraded RNA and associated ribonucleoprotein.

Nascent RNA and the transcribed template constitute two other elements of the transcription complex and we have shown that following *Eco*RI digestion they, too, resist electroelution [10].

II. Two Models for Replication

Replication might also involve attached or unattached polymerases [11]. Encapsulated nuclei contain a DNA polymerase α which is found only in S-phase cells and which is not stimulated by added "activated" templates, preferring the endogenous chromatin; most importantly, it is extremely efficient. For example, under the suboptimal concentration of dTTP that we use here, the initial rate of incorporation is 9% of that in vivo; under more optimal concentrations it exceeds 75%. It is relatively stable at 4 °C and resists electroelution, with about 90% of the activity being recovered in beads after electrophoresis for 5 h in isotonic buffer. However, this activity is relatively unstable at 37 °C, becoming soluble, able to escape from beads and more like the activities studied by others (e.g. it is now stimulated by added activated templates or by nicking or cutting the endogenous template). These aberrant activities easily obscure the authentic activity if broken templates are available. *Eco*RI treatment of encapsulated nuclei followed by electroelution removed up to 84% of the chromatin but no activity (Fig. 4); the active polymerizing complex also resists electroelution.

B. Discussion

Some of the experiments described here involve several enzyme digestions or assays in physiological salt concentrations, treatment with 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.

When encapsulated chromatin is incubated with endonucleases and subjected to electrophoresis, the bulk of the chromatin escapes from beads; in striking contrast, the three elements of the transcription complex (i.e. nascent RNA, active RNA polymerase II and active genes) and the two elements of the replication complex (i.e. polymerase α and nascent DNA) cannot. We believe this is most simply interpreted by association of transcription and replication complexes with the nucleoskeleton. This naturally begs the question: To what is the complex attached? As nascent transcripts, DNA, and active genes are closely associated with the nuclear cage [5–7] and matrix [17], it seems likely that these structures isolated in 2*M* NaCl are intimately related to it. We use the term "nucleoskeleton" to describe the analogous structure found under isotonic conditions and envisage it as one part of the active site of the transcription and replication complex, organizing the template in three-dimensional space into close proximity to the polymerization site. Passage of the DNA through the complex would then yield attached transcripts or nascent DNA.

This suggestion seems to conflict with many observations that soluble polymerases work. However, they do so very inefficiently. For example, crude "Manley" extracts polymerize correctly initiated transcripts at less than 0.01% of the rate in vivo [13], and DNA polymerases also initiate very inefficiently [2, 12].

If the polymerase is tethered to the nucleoskeleton, then only genes closely associated with this skeleton will be transcribed or replicated: 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. Indeed, gross detachment correlated with total inactivation of the avian erythrocyte nucleus [4] and the attachment of infecting viral sequences, the ovalbumin gene and viral oncogenes with their expression [5, 7, 17].

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