Attachment of transcriptionally active DNA sequences to the nucleoskeleton under isotonic conditions

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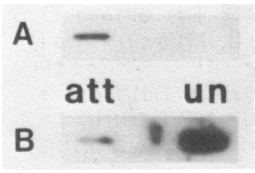
We have modified the method described by Jackson and Cook (1) for studying interactions between the nucleoskeleton and DNA sequences in relation to transcription. Jackson and Cook shake a suspension of cells in molten agarose with liquid paraffin, forming droplets of agarose-encapsulated cells suspended in the paraffin, chill to gel the agarose, wash away the paraffin, and treat the beads with Triton, which lyses the cells and allows restriction enzymes to digest their chromatin. We first treat cells with lysolecithin which, unlike Triton, makes them permeable without adversely affecting transcription (2). We then suspend them in isotonic buffer (15 mM Hepes pH 7.4, 140 mM KCl, 5 mM $MgCl_2$, 1.3 mM DTT, 0.2 mM EDTA, 0.5 mM spermidine tetrahydrochloride, 0.2 mM spermine trihydrochloride), add an equal volume of molten agarose (SeaPlaque, low melting point, 1% w/v in isotonic buffer at 37°) and pipette the suspension into the wells of a microtitre dish. On treating the agarose plugs with restriction enzymes, the chromatin yields 2 fractions. One can be immediately eluted from the agarose by electrophoresis (we use an LKB Extraphor, which facilitates subsequent purification of the DNA), and contains fragments of DNA which are not attached to the nucleoskeleton. The other can be eluted only after treatment with 0.2% SDS (15 minutes suffices), and contains DNA fragments which are attached to the nucleoskeleton.



The figure shows the results of such an analysis. Human cells (A431) were processed as described, and the attached and unattached DNA was slot-blotted and probed with end-labelled polyadenylated RNA from A431 cells. Panel A shows that active genes were associated with the attached DNA. The same filter was then re-probed with a probe made from A431 DNA (panel B), which shows that very little of the total DNA is attached to the nucleoskeleton.

Using a different, and somewhat simpler, technique from Jackson and Cook, we reach the same conclusion, namely that DNA which is attached to the nucleoskeleton is greatly enriched in sequences which are being transcribed.

REFERENCES

1. Jackson, D.A. and Cook, P.R. (1985) EMBO J. 4, 919-925.
2. Gurdon, J.B. (1976) J. Embryol. Exp. Morph. 36, 523-540.