COMMENTARY

The nucleoskeleton: artefact, passive framework or active site?

P. R. COOK

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK

"As seen in the living cell the nucleus most commonly appears as a clear, rounded, sac-like body bounded by a delicate membrane and often showing no visible structure save for the presence within it of one or more smaller rounded bodies, the nucleoli. After coagulation by fixing agents, the nucleus offers a much more complicated appearance containing ... a net-like framework...."

"Very often no trace of the framework is seen before coagulation sets in; and this has led to a skeptical attitude concerning it on the part of some observers."

The problem of artefacts

The quotations above are taken from E. B. Wilson's classic review of cell biology written in 1892–1893 and revised during the first quarter of this century (Wilson, 1928). Then, as now, many observers felt that a nucleoskeleton must organize the complicated contortions of the chromatin in the nucleus. What Wilson pointed out, however, still remains the central problem in the field – are the different preparations of the nucleoskeleton that have been isolated, or seen microscopically, any more than artefactual coagula created during fixation or deconstruction of the cell? Nuclear RNA, DNA and protein are all so highly concentrated (each at about $0{\cdot}1\,g\,ml^{-1}$) that one might expect them to coagulate as soon as water or ion concentrations are altered. Criteria by which artefacts might be recognized are crucial in helping us evaluate the relevance of these preparations. Unfortunately, rather loose criteria have been applied to many of the isolates, but more stringent criteria based on biological function have recently become available and this should allow us to assess which of the various isolates are more than artefacts. Here I will outline some difficulties in identifying isolated structures with a nucleoskeleton that exists *in vivo* and I will summarize recent evidence suggesting that a nucleoskeleton is the active site of the major nuclear functions, replication and transcription.

Isolation procedures

Given the problem of artefacts, one must try to deconstruct the cell gently using conditions as close as possible to the physiological (e.g. pass along the thick arrows in Fig. 1). (Note the terms 'mild' and 'gentle' are invariably used in this field in a loaded sense.) However, it comes as a great surprise to most people outside the field to discover that conditions even remotely approaching the physiological are rarely used. This is largely because chromatin aggregates in isotonic salt concentrations into a gelatinous and unworkable mess (Fredericq, 1971). Nuclei are usually isolated by homogenizing cells in buffers containing about one tenth of the physiological salt concentration (MacGillivray & Birnie, 1986), but this destroys the 30 nm chromatin fibre, decondenses heterochromatin, extracts a quarter of the nuclear protein, including polymerases (Jackson & Cook, 1985*a*, 1986*a*) and converts ribonucleoprotein particles into fibres that cannot be redissolved in 2 M-NaCl (Lothstein *et al.* 1985). 'Stabilizing' cations are also added, but these activate degradative nucleases. As these preparations are soluble and because intuition suggests that low salt concentrations are 'mild', such isolation procedures are not carefully standardized, with every laboratory using slightly different protocols. Another problem is associated with heat-shock. When cells are incubated 3–5 deg. C above the normal, a characteristic set of proteins associates with karyoskeletal elements. This also happens to nuclei isolated by conventional procedures, but is triggered by physiological temperatures; isolation sensitizes nuclei (Evan & Hancock, 1985; Littlewood *et al.* 1987; McConnell *et al.* 1987). Therefore our skeptic is unimpressed with structures like scaffolds seen only after pretreating isolated nuclei at 37 °C (Mirkovitch *et al.* 1984).

Sub-nuclear structures are also extracted using high concentrations of salt (e.g. 2 M-NaCl), dextran sulphate or the detergent, lithium diiodosalicylate (LIS). They range from essentially pure preparations of a few polypeptides like lamins, through nuclear pore complexes and envelopes to complex structures like ghosts,

Journal of Cell Science 90, 1–6 (1988)

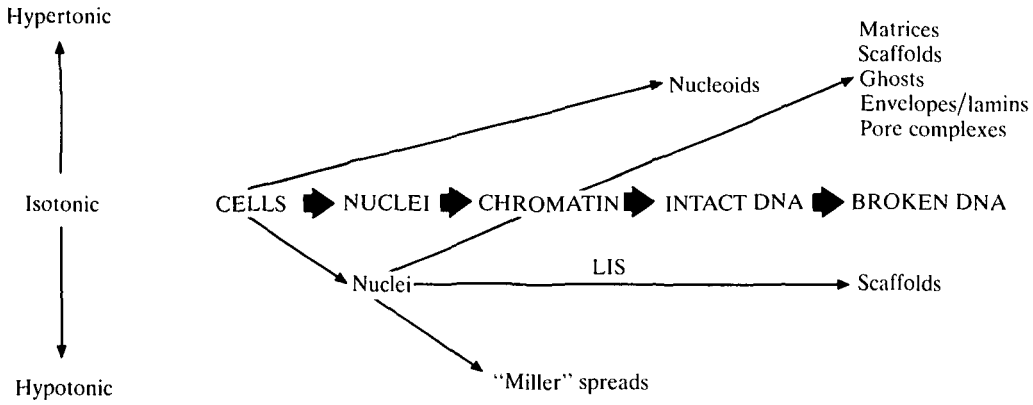

Fig. 1. Pathways for the deconstruction of living cells. The thick arrows show an idealized path, the thin arrows the path *via* hypo- and hypertonic conditions to the various nuclear substructures discussed. They are derived by extracting living cells in hyper- or hypotonic salt concentrations. Simpler structures tend to be on the right. LIS, lithium diiodosalicylate.

matrices, scaffolds, folded chromosomes and nucleoid cages (Fig. 1; for reviews, see Agutter & Richardson, 1980; MacGillivray & Birnie, 1986). How far any of these structures (isolated using such non-physiological conditions) are related to natural ones is open to argument. Recently it has become possible to use isotonic salt concentrations, avoiding aggregation by first encapsulating cells in agarose microbeads (Jackson & Cook, 1985*a*). (Of course, we know only very roughly the precise ionic constitution of the cell.) As agarose is freely permeable to small molecules, the cells can be regrown or extracted using almost any conditions.

Evaluation of isolated sub-nuclear structures

Once structures are isolated by any of the methods in general use, by what criteria can we judge the extent to which they are authentic? One approach has been to see if structures isolated in different ways are roughly similar. However, skeptics point to the differences. Thus, Berezney & Coffey (1974) and Aaronson & Blobell (1975) extracted rat liver cells in roughly similar ways but obtained very different structures: an internal matrix or an external envelope. Some differences have been traced to the precise sequence of operations (Kaufmann *et al.* 1981), oxidative cross-linking of protein (Berezney & Coffey, 1977) and perhaps to metal binding by mercaptoethanol (Lebkowski & Laemmli, 1982). Striking differences are also found when nuclei are treated with very high or very low salt concentrations (as in 'Miller' spreads). In the former case we obtain matrices or scaffolds, in the latter no skeleton at all (Miller, 1984). Such differences undermine any confidence in the search for similarities.

Preservation of microscopic appearance is an attractive criterion for natural structure, but is by no means free of problems of interpretation. The following observation highlights one problem. When mitotic cells are encapsulated in agarose microbeads, strong ionic detergents (e.g. sodium dodecyl sulphate) extract nearly all protein and RNA, leaving DNA, which retains its chromosomal morphology (Cook, 1984). Perhaps chromosome structure is not maintained by a protein skeleton at all, but by forces resistant to strong detergents; for example, by hydrogen bonds between duplexes, stabilized by supercoiling (Cook & Brazell, 1978). Then pure chromosomal DNA might share with so many other biological polymers the ability to fold itself into its three-dimensional shape! Alternatively, the structure might be maintained by tightly bound molecules, but these cannot be scaffold/matrix/lamin/cage proteins, which are all soluble in sodium dodecyl sulphate. A third possibility is that on deproteinization long chromosomal DNA remains so entangled with itself that it retains its original shape. According to this jaundiced view, the tangle *in vitro* would naturally reflect the structure *in vivo*, long chromosomes giving elongated tangles and short chromosomes shorter ones. Tangles from less well-extracted cells are even more likely to nucleate the precipitates that we call matrices, scaffolds and nucleoids. Nucleation by single-stranded (and therefore sticky) nucleic acids seems even likelier, so we might expect nascent DNA and RNA to be at the centre of the precipitate and so resist nucleolytic detachment. The precipitate, inevitably containing some proteins like topoisomerase II, would bind *in vitro* some sequences but not others (Cockerill & Garrard, 1986). Above all, different precipitates prepared by different methods would be different.

The use of morphological criteria has another drawback: any nucleoskeleton has dimensions between those best analysed by light and electron microscopy. Electron microscopy of thin sections supported in resins is not particularly suited to analysing filamentous structures in three dimensions. More depth can be provided using whole mounts (Capco *et al.* 1982),

deeply etched (Heuser & Kirschner, 1980) or thicker, but resinless, sections (Fey *et al.* 1986) of freeze-dried or gently extracted samples. Then cytoskeletons are seen intimately associated with nucleoskeletons after extractions in 500 mM and 50 mM monovalent cation (Capco *et al.* 1984; Fey *et al.* 1986), but only after drying specimens using conditions known to be prone to form artefactual networks (Ris, 1985). However, this association may well be real, as cytoplasmic intermediate filaments are homologous to nuclear lamins (Aebi *et al.* 1986; McKeon *et al.* 1986).

Given these difficulties, how can those of us who believe in a nucleoskeleton convince Wilson's skeptics that these structures reflect more than a coagulum?

Specific interactions

One essential requirement is to show that any interactions found *in vitro* reflect those *in vivo*, both in quantity and in specificity. At least this means that recoveries of any hypothetical 'scaffolding' molecules must be carefully monitored during fractionation. It is insufficient to show that a protein is associated with a structure. Even cytoplasmic enzymes copurify to some extent with nuclei, especially if basic; we must know what percentage copurifies. This is rarely done and can be a difficult task. For example, proteins are often identified immunologically, but during fractionation cryptic antigens may be unmasked, others destroyed, still others (being only identifiable by immunofluorescence) are difficult to quantify. Where proteins are identified by enzymic activity, this may be very different *in vivo* and *in vitro*; thus polymerases are assayed *in vitro* using broken and naked templates and not native chromatin. Despite these difficulties, some proteins (notably the lamins and scaffold protein I, which turns out to be topoisomerase II) have been shown to copurify quantitatively with certain substructures (Gerace *et al.* 1984; Berrios *et al.* 1985; Earnshaw *et al.* 1985; Earnshaw & Heck, 1985; Gasser *et al.* 1986).

Currently, interest is rightly focused on whether specific sequences attach DNA in loops to a skeleton. I will exemplify some problems in demonstrating this by discussing work on nucleoid cages, chosen because they were the preparation in which supercoiling (and so looping) was first demonstrated and because they alone of the preparations illustrated in Fig. 1 contain unbroken DNA, so ends and nicks cannot attach artefactually during isolation (Cook & Brazell, 1975). If attachments are specific, attached sequences should resist nucleolytic detachment and so should pellet with the skeleton; if not, they will vary from one structure to another in the population so that any given sequence will be neither enriched nor depleted in the pellet (Cook & Brazell, 1980). Integrated viral sequences (if transcribed) are enriched 18 times in pellets from transformed rat cells, implying that they attach on activation (Cook *et al.* 1982). However, high enrichments correlate with high levels of protein in the pellet/coagulum and we might expect sticky transcription complexes to form its core.

This mapping technique has been extended to 'matrix' DNA, but the enrichments are less convincing (i.e. 2–5 times) and sometimes cannot be demonstrated (Robinson *et al.* 1982; Kuo, 1982). This might reflect uncontrolled detachment of DNA during isolation or, equally, no specific attachment. Scaffolds prepared by extraction with lithium diiodosalicylate provide striking (but unquantified) enrichments, the most tightly attached regions containing topoisomerase II binding sequences. In contrast to results with nucleoid cages, transcribed regions are never attached (Mirkovitch *et al.* 1984; Gasser & Laemmli, 1986). Partisans suggest that this reflects different subsets of the many attachments found *in vivo*; skeptics note that these scaffolds are only seen after isolated nuclei are 'stabilized' by heat under conditions that might allow topoisomerase II to trap hitherto unbound DNA. Even more suggestive of an artefact is the observation that glutaraldehyde, far from fixing the structure, prevents its formation (Mirkovitch *et al.* 1984)!

Specific association has also been demonstrated by incubating matrices with pure DNA fragments: the sequence next to the immunoglobulin kappa enhancer binds preferentially (Cockerill & Garrard, 1986; Cockerill *et al.* 1987). However, as this sequence contains topoisomerase II sites, and matrices contain this enzyme, our skeptic will find the binding *in vitro* of an enzyme to its substrate unsurprising. Moreover, the region does not bind to nuclei, implying that sites are only created during isolation. Nevertheless, this powerful approach must be prosecuted because the existence of a nucleoskeleton will probably only be accepted when pure skeletal components are shown to bind specific DNA sequences *in vitro*.

Function

Many feel that a skeleton is required to organize nuclear structure, but skeptics see little need for any additional *functional* role now that cell-free replication and transcription systems have been developed. Nevertheless, it is as well to remember that if these systems involve pure proteins and template they are inefficient compared with the living cell and, conversely, those systems that are efficient generally involve long preincubations in crude extracts, when skeletal elements may well reassemble. (For example, see Ariga & Sugano, 1983; Li & Kelly, 1984; Stillman, 1986; Wobbe *et al.* 1985.) Thus, one of the most efficient transcription systems, crude 'Manley' extracts, polymerize correctly initiated transcripts at average rates of

<10 nucleotides h^{-1} or 0·01% of the rate *in vivo* (Manley *et al.* 1980). Chromatin templates are transcribed even less efficiently (Knezetic & Luse, 1986; Lorch *et al.* 1987) but appropriate preincubations improve rates slightly (Workman & Roeder, 1987). The most efficient replication system similarly involves crude extracts (from frogs' eggs) but again synthesis only begins after a nuclear membrane (and perhaps a nucleoskeleton) has re-formed around the template (Blow & Laskey, 1986; Blow & Watson, 1987; Newport, 1987).

Evidence for a role for a nucleoskeleton in replication (Pardoll *et al.* 1980; McCready *et al.* 1980), transcription (Jackson *et al.* 1981) and the repair of damage in DNA (McCready & Cook, 1984) stems from observations that nascent DNA and RNA and topoisomerase II (Berrios *et al.* 1985; Earnshaw *et al.* 1985; Earnshaw & Heck, 1985; Gasser *et al.* 1986) are associated with substructures isolated using hypertonic or hypotonic conditions (Fig. 1). Recently, however, it has become possible to isolate and manipulate chromatin containing intact DNA (encapsulated in agarose) in salt concentrations that are the closest to the physiological that have been used to date. This material, which has never been exposed to abnormal salt concentrations, is not sensitized to heat (Jackson & Cook, 1985*a*; D. A. Jackson & P. R. Cook, unpublished data). After nuclease treatment, most chromatin can be removed by electrophoresis in the physiological buffer to leave a skeleton and nearly all the replicative and transcriptional activities of the living cell (Jackson & Cook, 1985*b*, 1986*a*,*b*). Thus, polymerase activities, which are the best markers for nuclear function (at least when they are the major, authentic activities rather than minor, aberrant ones), are associated with a skeleton seen *in vitro*. If artefactual, then these attachments must be generated without affecting central functions!

It is easy to imagine how complicated nuclear functions might be controlled by siting them at a nucleoskeleton *in vivo* (Jackson *et al.* 1984). Such an attachment hypothesis views the skeleton as much more than a passive framework holding DNA in loops; rather, it is the active site to which polymerases are attached and at which DNA functions. Gene activation and inactivation are then seen as attachment and detachment to a skeleton, which (like its counterpart in the cytoplasm) is dynamic, turning over during differentiation and division. Topoisomerases stand at the strategic interface between skeleton and loop, transducing structural information (Cook, 1973; Cockerill & Garrard, 1986). If this view is correct, it is the loop, rather than the gene and adjacent controlling sequences, that is the unit of function and it becomes prudent to preserve its integrity when studying function. Grosveld and colleagues (1987) may well have done this when they created a 'mini' locus of 38 kb from the 90 kb β-globin locus; it is expressed tissue-specifically in transgenic mice, independent of its site of integration. It is functional studies like these, rather than merely structural ones, that are leading to an increasing acceptance of a nucleoskeleton. But probably Wilson's artefact will only be laid to rest when a nucleoskeleton is seen functioning in the *living* cell.

I thank the Cancer Research Campaign for their continued support and R. D. Cole for help in drafting the manuscript.

References

Aaronson, R. P. & Blobell, G. (1975). Isolation of nuclear pore complexes in association with a lamina. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1007–1011.

Aebi, U., Cohn, J., Buhle, L. & Gerace, L. (1986). The nuclear lamina is a meshwork of intermediate filaments. *Nature, Lond.* **323**, 560–564.

Agutter, P. S. & Richardson, J. C. W. (1980). Nuclear non-chromatin proteinaceous structures: their role in the organization and function of the interphase nucleus. *J. Cell Sci.* **44**, 395–435.

Ariga, H. & Sugano, S. (1983). Initiation of simian virus 40 replication *in vitro*. *J. Virol.* **48**, 481–491.

Berezney, R. & Coffey, D. S. (1974). Identification of a nuclear protein matrix. *Biochem. biophys. Res. Commun.* **60**, 1410–1417.

Berezney, R. & Coffey, D. S. (1977). Nuclear matrix: isolation and characterization of a framework structure from rat liver nuclei. *J. Cell Biol.* **73**, 616–637.

Berrios, M., Osheroff, N. & Fisher, P. (1985). *In situ* localization of DNA topoisomerase II, a major polypeptide of the *Drosophila* nuclear matrix. *Proc. natn. Acad. Sci. U.S.A.* **82**, 4142–4146.

Blow, J. J. & Laskey, R. A. (1986). Initiation of DNA replication in nuclei and purified DNA by a cell-free extract. *Cell* **47**, 577–587.

Blow, J. J. & Watson, J. V. (1987). Nuclei act as independent and integrated units of replication in a *Xenopus* cell-free DNA replication system. *EMBO J.* **6**, 1197–2002.

Capco, D. G., Krochmalnik, G. & Penman, S. (1984). A new method of preparing embedment-free sections for transmission electron microscopy: applications to the cytoskeleton framework and other three-dimensional networks. *J. Cell Biol.* **98**, 1878–1885.

Capco, D. G., Wan, K. M. & Penman, S. (1982). The nuclear matrix: three dimensional architecture and protein composition. *Cell* **29**, 847–858.

Cockerill, P. N. & Garrard, W. T. (1986). Chromosomal loop anchorage of the kappa immunoglobulin gene occurs next to the enhancer in a region containing topoisomerase II sites. *Cell* **44**, 273–282.

Cockerill, P. N., Yuen, M.-H. & Garrard, W. T. (1987). The enhancer of the immunoglobulin heavy chain locus is flanked by presumptive chromosomal loop anchorage elements. *J. biol. Chem.* **262**, 5394–5397.

COOK, P. R. (1973). Hypothesis on differentiation and the inheritance of gene superstructure. *Nature, Lond.* **245**, 23–25.

COOK, P. R. (1984). A general method for preparing intact DNA. *EMBO J.* **3**, 1837–1842.

COOK, P. R. & BRAZELL, I. A. (1975). Supercoils in human DNA. *J. Cell Sci.* **19**, 261–279.

COOK, P. R. & BRAZELL, I. A. (1978). Spectrofluorometric measurement of the binding of ethidium to superhelical DNA from cell nuclei. *Eur. J. Biochem.* **84**, 465–477.

COOK, P. R. & BRAZELL, I. A. (1980). Mapping sequences in loops of nuclear DNA by their progressive detachment from the nuclear cage. *Nucl. Acids Res.* **8**, 2895–2905.

COOK, P. R., LANG, J., HAYDAY, A., LANIA, L., FRIED, M., CHISWELL, D. J. & WYKE, J. A. (1982). Active viral genes in transformed cells lie close to the nuclear cage. *EMBO J.* **1**, 447–452.

EARNSHAW, W. C., HALLIGAN, B., COOKE, C. A., HECK, M. M. S. & LIU, L. F. (1985). Topoisomerase II is a structural component of mitotic chromosome scaffolds. *J. Cell Biol.* **100**, 1706–1715.

EARNSHAW, W. C. & HECK, M. M. S. (1985). Localization of topoisomerase II in mitotic chromosomes. *J. Cell Biol.* **100**, 1716–1725.

EVAN, G. I. & HANCOCK, D. C. (1985). Studies on the interaction of the human c-*myc* protein with cell nuclei: p62c-*myc* as a member of a discrete subset of nuclear proteins. *Cell* **43**, 253–261.

FEY, E. G., KROCHMALNIK, G. & PENMAN, S. (1986). The non-chromatin-sub-structures of the nucleus: the RNP-containing and RNP-depleted matrices analyzed by sequential fractionation and resinless section electron microscopy. *J. Cell Biol.* **102**, 1645–1665.

FREDERICQ, E. (1971). The chemical and physical properties of nucleohistones. In *Histones and Nucleohistones* (ed. D. M. P. Phillips), pp. 135–186. London: Plenum Press.

GASSER, S. M. & LAEMMLI, U. K. (1986). Cohabitation of scaffold binding regions with upstream/enhancer elements of 3 developmentally regulated genes of *D. melanogaster*. *Cell* **46**, 521–530.

GASSER, S. M., LAROCHE, T., FALQUET, J., BOY DE LA TOUR, E. & LAEMMLI, U. K. (1986). Metaphase chromosome structure: involvement of topoisomerase II. *J. molec. Biol.* **188**, 613–629.

GERACE, L., COMEAU, C. & BENSON, M. (1984). Organization and modulation of nuclear lamina structure. *J. Cell Sci. Suppl. 1*, 137–160.

GROSVELD, F., ASSENDELFT, G. B., VAN GREAVES, D. R. & KALLIOS, G. (1987). Position independent, high-level expression of the β-globin gene in transgenic mice. *Cell* **51**, 975–985.

HEUSER, J. E. & KIRSCHNER, M. W. (1980). Filament organization revealed in platinum replicas of freeze-dried cytoskeletons. *J. Cell Biol.* **86**, 212–234.

JACKSON, D. A. & COOK, P. R. (1985*a*). A general method for preparing chromatin containing intact DNA. *EMBO J.* **4**, 913–918.

JACKSON, D. A. & COOK, P. R. (1985*b*). Transcription occurs at a nucleoskeleton. *EMBO J.* **4**, 919–925.

JACKSON, D. A. & COOK, P. R. (1986*a*). Replication occurs at a nucleoskeleton. *EMBO J.* **5**, 1403–1410.

JACKSON, D. A. & COOK, P. R. (1986*b*). A cell-cycle-dependent DNA polymerase activity that replicates intact DNA in chromatin. *J. molec. Biol.* **192**, 65–76.

JACKSON, D. A., MCCREADY, S. J. & COOK, P. R. (1981). RNA is synthesised at the nuclear cage. *Nature, Lond.* **292**, 552–555.

JACKSON, D. A., MCCREADY, S. J. & COOK, P. R. (1984). Replication and transcription depend on attachment of DNA to the nuclear cage. *J. Cell Sci. Suppl. 1*, 59–79.

KAUFMANN, S. H., COFFEY, D. S. & SHAPER, J. H. (1981). Considerations in the isolation of rat liver nuclear matrix, nuclear envelope and pore complex lamina. *Expl Cell Res.* **132**, 105–123.

KNEZETIC, J. A. & LUSE, D. S. (1986). The presence of nucleosomes on a DNA template prevents initiation by RNA polymerase II *in vitro*. *Cell* **45**, 95–104.

KUO, M. T. (1982). Distribution of tightly bound proteins in the chicken ovalbumin gene region. *Biochemistry* **21**, 321–326.

LEBKOWSKI, J. S. & LAEMMLI, U. K. (1982). Non-histone proteins and long-range organization of HeLa interphase DNA. *J. molec. Biol.* **156**, 325–344.

LI, J. J. & KELLY, T. J. (1984). Simian virus 40 replication *in vitro*. *Proc. natn. Acad. Sci. U.S.A.* **81**, 6973–6977.

LITTLEWOOD, T. D., HANCOCK, D. C. & EVAN, G. I. (1987). Characterization of a heat-shock induced insoluble complex in the nuclei of cells. *J. Cell Sci.* **88**, 65–72.

LORCH, Y., LAPOINTE, J. W. & KORNBERG, R. D. (1987). Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. *Cell* **49**, 203–210.

LOTHSTEIN, L., ARENSTORF, H. P., CHUNG, S.-Y., WALKER, B. W., WOOLEY, J. C. & LESTOURGEON, W. M. (1985). General organization of protein in HeLa 40S nuclear ribonucleoprotein particles. *J. Cell Biol.* **100**, 1570–1581.

MANLEY, J. L., FIRE, A., CANO, A., SHARP, P. A. & GEFTER, M. L. (1980). DNA-dependent transcription of adenovirus genes in soluble whole-cell extract. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3855–3859.

MACGILLIVRAY, A. J. & BIRNIE, G. D. (1986). *Nuclear Structures: Isolation and Characterization*. London: Butterworths.

MCCONNELL, M., WHALEN, A. M., SMITH, D. E. & FISHER, P. A. (1987). Heat shock-induced changes in the structural stability of proteinaceous karyoskeletal elements *in vitro* and morphological effects *in situ*. *J. Cell Biol.* **105**, 1087–1098.

MCCREADY, S. J. & COOK, P. R. (1984). Lesions induced in DNA by ultraviolet light are repaired at the nuclear cage. *J. Cell Sci.* **70**, 189–196.

MCCREADY, S. J., GODWIN, J., MASON, D. W., BRAZELL, I. A. & COOK, P. R. (1980). DNA is replicated at the nuclear cage. *J. Cell Sci.* **46**, 365–386.

MCKEON, F. D., KIRSCHNER, M. W. & CAPUT, D. (1986). Homologies in both primary and secondary structure

between nuclear envelope and intermediate filament proteins. *Nature, Lond.* **319**, 463–468.

MILLER, O. L. (1984). Some ultrastructural aspects of genetic activity in eukaryotes. *J. Cell Sci. Suppl. 1*, 81–93.

MIRKOVITCH, J., MIRAULT, M.-E. & LAEMMLI, U. K. (1984). Organization of the higher-order chromatin loop specific DNA attachment sites on nuclear scaffold. *Cell* **39**, 223–232.

NEWPORT, J. (1987). Nuclear reconstitution *in vitro*: stages of assembly around protein-free DNA. *Cell* **48**, 205–217.

PARDOLL, D. M., VOGELSTEIN, B. & COFFEY, D. S. (1980). A fixed site of DNA replication in eukaryotic cells. *Cell* **19**, 527–536.

RIS, H. (1985). The cytoplasmic filament system in critical point-dried whole mounts and plastic embedded sections. *J. Cell Biol.* **100**, 1474–1487.

ROBINSON, S. I., NELKIN, B. D. & VOGELSTEIN, B. (1982). The ovalbumin gene is associated with the nuclear matrix of chicken oviduct cells. *Cell* **28**, 99–106.

STILLMAN, B. W. (1986). Chromatin assembly during simian virus 40 DNA replication *in vitro*. *Cell* **45**, 555–565.

WILSON, E. B. (1928). *The Cell in Development and Heredity*, 3rd edn, with corrections pp. 78, 80. New York: Macmillan, Inc.

WOBBE, C. R., DEAN, F., WEISSBACH, L. & HURWITZ, J. (1985). *In vitro* replication of duplex circular DNA containing the simian virus 40 DNA origin site. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5710–5714.

WORKMAN, J. L. & ROEDER, R. G. (1987). Binding of transcription factor TFIID to the major late promoter during *in vitro* nucleosome assembly potentiates subsequent initiation by RNA polymerase II. *Cell* **51**, 613–622.