Cell, Vol. 66, 627–635, August 23, 1991, Copyright © 1991 by Cell Press

The Nucleoskeleton and the Topology of Replication

Review

Peter R. Cook
Sir William Dunn School of Pathology
University of Oxford
South Parks Road
Oxford OX1 3RE
England

The primosome . . . moves like a locomotive down the template track.
—A. Kornberg (1987)

This statement summarizes the view held by most molecular biologists of how replication occurs: soluble polymerases bind to the "origin" and then track along the template as they synthesize DNA (reviewed by Alberts, 1990; Hurwitz et al., 1990). However, convincing evidence now suggests that active polymerases are bound to a skeleton in the eukaryotic nucleus, with replication occurring as DNA is reeled through a fixed complex (e.g., Jackson, 1990). Therefore, it seems an appropriate time to discuss the topological problems posed by fixed polymerases; these problems are compounded if the nucleoskeleton to which they are attached must also be duplicated. I concentrate on eukaryotic polymerases, but much of the discussion also applies to prokaryotic enzymes. Finally, I update old models for chromosome duplication and present a model for replication in which key activities are all attached.

Active Polymerases Are Attached

If polymerases run "down the template track," we might expect sites of replication to be scattered diffusely throughout nuclei, reflecting the template concentration. But we now know that synthetic sites are not diffusely scattered; they are focally concentrated, with the many polymerases within one focus firing coordinately. This provides powerful, albeit circumstantial, evidence for attachment of active polymerases to some integrating structure.

When rat fibroblasts are briefly exposed to bromodeoxyuridine during S phase and sites of incorporation are then visualized using fluorescently labeled antibodies directed against the analog, a number of discrete foci—each of which must contain ~20 replication units—can be seen scattered throughout nuclei. Cells at different stages during S phase give characteristic patterns (Nakamura et al., 1986; Nakayasu and Berezney, 1989). Similar beautiful foci are also seen using biotin–dUTP and FITC–streptavidin after incubating demembranated sperm in extracts of frogs' eggs (Blow and Laskey, 1986; Hutchison et al., 1987, 1988; Mills et al., 1989). A membrane re-forms, and DNA is replicated efficiently in 100–300 foci distributed throughout the nuclei. Since replication occurs so quickly in eggs, each of these foci must contain 300–1000 replication forks, and it is difficult to see how so many unattached polymerases could act so coordinately and so focally without being integrated in space by some structure. Moreover, particles containing the requisite number of polymerases can be extracted from somatic nuclei (Tubo and Berezney, 1987); even if unattached, such "megacomplexes" are large enough to be immobile.

A second series of observations point more directly to attachment of polymerases. Berezney and Coffey (1975) first showed that nascent DNA was tightly associated with the nuclear "matrix," an observation that subsequently led to a vast literature on the subject (e.g., Pardoll et al., 1980; McCready et al., 1980; Nelson et al., 1986; Verheijen et al., 1988; Jackson, 1991). But all results on the matrix (and other structures like it) have been dogged by the problem of artifacts: nascent DNA and polymerases are, of course, very sticky and do aggregate artifactually during extraction in the high salt concentrations used for isolation (Martelli et al., 1990). As a result, the role of the nuclear matrix remains controversial (Cook, 1988). Now, however, conditions very close to the physiological have been used to demonstrate that essentially all active polymerases are attached to some very large structure in the nucleus (Jackson and Cook, 1986a, 1986b; Jackson et al., 1988).

Unphysiologically high salt concentrations have been used traditionally to isolate the matrix because chromatin tends to aggregate under isotonic conditions. However, problems of aggregation can be sidestepped by encapsulating HeLa cells in agarose microbeads ($r \approx 25$ μm) before lysing membranes in a "physiological" buffer (Jackson and Cook, 1985; Jackson et al., 1988). The resulting encapsulated nuclei are protected by the agarose yet accessible to molecular probes. Most chromatin can be removed by treatment with an endonuclease followed by electrophoresis to leave residual clumps of chromatin attached to an intermediate filament–like skeleton (Jackson and Cook, 1988; see also He et al., 1990; Wang and Traub, 1991). Despite removal of most chromatin, no polymerizing activity is lost. Nascent DNA, whether synthesized in vitro or in vivo, also resists electroelution, presumably because the replication complex is attached to some skeleton. The use of physiological conditions throughout the experiment and recovery of essentially all activity (the best marker for replication) and not a minor fraction make explanations based on an artifactual aggregation of the polymerase difficult to sustain. (A soluble polymerase—this is the polymerase purified by most biochemists—is also found. Its activity does not change during the cell cycle, so it may represent an inactive pool awaiting activation by attachment [Jackson and Cook, 1986c].)

Soluble Polymerases

These results pointing to an attachment of polymerases seem to be incompatible with a wealth of evidence that shows that pure DNA can be replicated in vitro without any added skeleton. However, when systems are efficient (e.g., Xenopus egg extracts), they are crude and may contain skeletal elements, and when they are highly purified, they are very inefficient, with the pure enzyme preferring

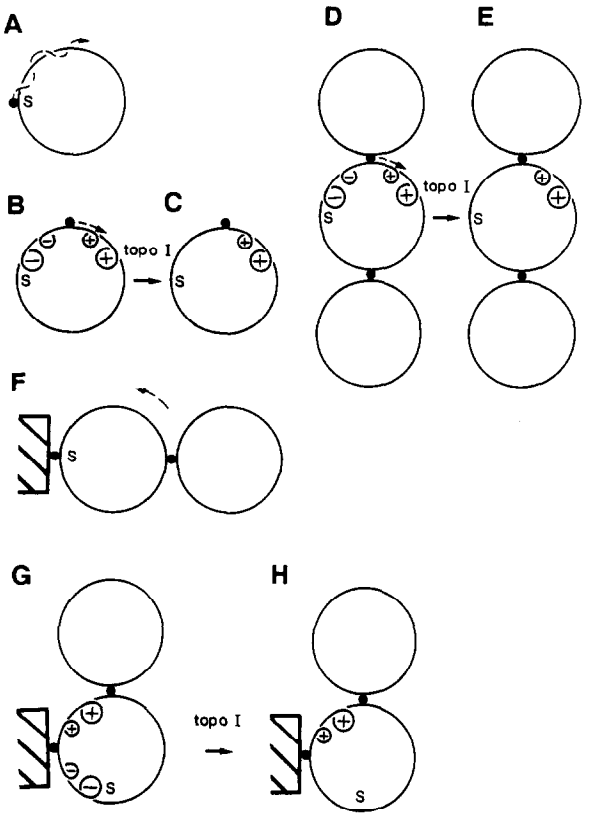

Figure 1. How T Antigen Supercoils

Circles represent relaxed circular DNA, filled dots multimeric T antigen, and hatched areas an immobilizing complex. Arrows indicate movements.

(A) T antigen binds at S and moves laterally, rotating about the helix axis as it does so; no changes in supercoiling result.

(B, C) The twin-domain model, in which T antigen moves along while DNA rotates. T antigen bound at S and then moved a quarter of the way along a relaxed template without rotating around the template axis; instead, the template rotates. This leads to a compensatory accumulation of right-handed (+) supercoils ahead of the protein and left-handed (−) supercoils behind it. Addition of topoisomerase I then removes negative supercoils, leaving positive supercoils.

(D, E) A variant of the twin-domain model in which the moving T antigen is complexed to another circle (top) to prevent it from rotating. As in (B), T antigen bound at S and tracked a quarter of the way along the middle circle, but as it is also bound to another circle (top), it cannot rotate around the template axis; DNA also rotates, so generating twin domains of supercoiling that are prevented from diffusing around the middle circle and annihilating each other by the stalled T antigen–template complex at the bottom. Topoisomerase treatment then leaves positive supercoils.

(F–H) Supercoiling by an immobilized T antigen. (F) S, part of a complex consisting of an inactive T antigen on the right and two circles, binds to an active T antigen (left) immobilized in an aggregate. The complex of two circles plus inactive T antigen then begins to move past the immobile T antigen. (G) The template has moved past the immobile T antigen, rotating as it did so, leading to compensatory supercoiling. The twin domains are stably segregated by the inactive complex, which is now at the top. (H) Topoisomerase treatment then leaves positive supercoils.

to initiate incorrectly at nicks (Kornberg, 1974). Nevertheless, pure soluble activities supplemented with T antigen can replicate pure SV40 DNA in vitro in the absence of any skeletal elements (Wobbe et al., 1987; Ishimi et al., 1988; Tsurimoto et al., 1990). If the DNA of SV40—which codes for only one protein involved in replication (i.e., T antigen) and so must be replicated by the cellular machinery—can be replicated in vitro without a skeleton, then why invoke a role for one in vivo?

The result of an elegant experiment on the "tracking" of T antigen (used at very high concentrations in these replication systems) suggests how this contradictory evidence might be reconciled. A relaxed plasmid circle was incubated with pure T antigen and ATP. Subsequent treatment with topoisomerase I, which removes negative supercoils but not positive supercoils, gave a surprising result: a positively supercoiled template (Yang et al., 1989).

T antigen and helix must move relative to each other, both along and around the helix axis. With two players and two movements, there are four possibilities. The smaller T antigen might move around the static circle, rotating about the helical axis as it does so; however, this cannot give the supercoiling that was found (Figure 1A). It might also rotate while DNA moves laterally; but, again, no supercoiling results. Supercoiling arises only if DNA—counterintuitively—rotates. So the "twin-domain" model has T antigen moving along the template axis as DNA rotates. This inevitably generates positive and negative domains of supercoiling (Figure 1B); then topoisomerase removes negative supercoils, leaving the positive coils (Figure 1C).

But what stops T antigen from rotating, and why do the positive and negative supercoils not diffuse around the circle and annihilate each other before the topoisomerase acts? The authors plausibly suggest that T antigen aggregates; then it cannot thread through the circle—and so rotate—and other aggregates might segregate twin domains, preventing their annihilation (Figures 1D and 1E). A variant of this model involves two (or more) aggregated T antigen complexes bound to opposite sides of one circle, with the complex moving laterally. The last possibility has DNA moving both laterally and rotationally past T antigen immobilized by aggregation (Figures 1F–1H). I suspect this most closely mimics what happens in vivo. Again, a likely variant involves two aggregated and immobile T antigen complexes attached to only one moving circle. Whatever the details, positive supercoils result only if T antigen is partially or completely immobilized. Since replication systems use similar conditions, perhaps the polymerase coaggregates with T antigen, immobilizing both on an artificial "skeleton." In any event, evidence from viruses is unlikely to be decisive, when their role is to subvert normal processes.

Duplicating Helices

The topological problems of ensuring that chromosomal DNA is correctly duplicated and segregated to daughter cells are mind-boggling enough without the extra problems posed if an associated skeleton must also be duplicated and segregated. But despite the complexities, we can establish some limits within which any mechanism must operate.

The general problem of duplicating a helix and then separating the intertwined daughters was discussed long be-

fore the correct structure for DNA was proposed (Manton, 1945, 1950). Although the precise description of helices is complicated (White et al., 1988), the problem can be demonstrated simply by winding a ribbon on a rod so the ribbon lies flat against the rod throughout its length (as in a barber's pole). If the ribbon is now split down its length and the rod removed, the two helical half-ribbons are interlocked, once per gyre of the original spiral. There is an added problem if the rod is itself helical; the half-ribbons describe a superspiral, giving additional interlocks spread more globally.

Of the two formal solutions, one involves breaking the backbone of one (or both) helical strand(s), movement of one (or both) strand(s) through the break to separate the strands, followed by a mending of the break(s); this occurs during replication of DNA and is catalyzed by topoisomerases. If neither backbone is broken, the end of one helix—whether it be DNA or a chromatid—must rotate about the other, once for every gyre. Where rotation about ends is impossible (e.g., in circles or linear duplexes looped by attachment), untwining must involve breakage. Therefore it becomes important to know whether DNA is attached to, and entwined about, a skeleton and whether the ends of DNA and skeleton are free to rotate. Presumably even one persistent interlock, in DNA or skeleton, is lethal.

Note that double-helical strands are not inevitably interlocked. This can be demonstrated by winding without rotating the edges of the ribbon about each other; this means that the ribbon is twisted about itself and cannot lie flat against the rod. After cutting the ribbon and removing the rod, the two half-ribbons are not interlocked. However, twists in the ribbon must exactly equal gyres in the spiral, and I argue below that nature cannot arrange things so exactly.

Resolving Helical DNA: Topoisomerases

Genetic studies point to an essential role for topoisomerases during both replication and chromosome segregation (Brill et al., 1987). There are at least three enzymes in yeast; one of them, topoisomerase II, is also a major structural component of mitotic chromosomes, matrices, and scaffolds (Berrios et al., 1985; Earnshaw et al., 1985; Gasser et al., 1986). Inactivation of topoisomerase I in temperature-sensitive *top1* mutants shows it to be unessential (Uemura and Yanagida, 1984), even though DNA elongation is temporarily delayed (Kim and Wang, 1989). Chain synthesis is not significantly affected in *top2* mutants (Kim and Wang, 1989), but nuclear division is arrested because daughter duplexes are intertwined (DiNardo et al., 1984; Uemura et al., 1987). *top1 top2* double mutants immediately stop growing (Uemura and Yanagida, 1984, 1986; Goto and Wang, 1985; Kim and Wang, 1989). Studies with inhibitors (e.g., camptothecin and VM26) confirm that both topoisomerases I and II assist elongation of infecting SV40 or adenoviral DNA, but only topoisomerase II is required later during infection (Richter et al., 1987; Snapka et al., 1988; Schaak et al., 1990). Antibodies against the two topoisomerases also inhibit DNA synthesis by cell-free systems, and addition of either topoisomerase restores synthesis; however, only topoisomerase II gives decatenated daughters (Richter et al., 1987; Wobbe et al., 1987; Snapka et al., 1988).

These results imply that either enzyme can act during elongation, but topoisomerase II is uniquely required later. The double-helical interlocks ahead of the polymerase must be undone during elongation. Either enzyme can act as such a swivel by cutting strands. Topoisomerase I transiently breaks one DNA strand and rotates a cut end about the uncut strand; topoisomerase II transiently cuts both DNA strands and passes another duplex through the cut (Liu, 1989). Topoisomerase II is also required after S phase, both to resolve more global interlocks (Ishimi et al., 1988) and to drive chromosome condensation (Newport and Spann, 1987; Wood and Earnshaw, 1990; Adachi et al., 1991).

Resolving Helical Nucleoskeletons

Unfortunately, there is little agreement as to the nature of any nucleoskeleton. Therefore, it is as well to begin any discussion with some disclaimers. First, the various synonyms of nucleoskeleton (e.g., matrix, scaffold) imply stability, but the true skeleton is probably disassembled and then reassembled. Second, we talk of one skeleton: there may be many, some related (e.g., mitotic and interphase skeletons) and others unrelated, both structurally and functionally, like the different cytoskeletal elements. Third, there are methodological problems in visualizing a nucleoskeleton. For example, immunofluorescence pictures of antibodies against structural nuclear components almost always show speckles, and not a filamentous network analogous to the cytoskeleton. But the skeleton might be too diffuse to be detected in this way, with epitopes too weakly immunogenic or inaccessibly buried in chromatin. Sections generally used for electron microscopy are also too thin to image clearly a diffuse skeleton, but the preparation of thicker (resinless) sections is technically more demanding (He et al., 1990). However, the main reason why the nucleoskeleton remains so elusive and controversial is because candidate structures (e.g., matrices, scaffolds, cages) are isolated using such markedly unphysiological conditions that they may simply be isolation artifacts, with no counterparts in vivo (Cook, 1988).

Ultimately, the controversy can be resolved only by studying structure in vivo or using physiological conditions. Fortunately, meiotic lampbrush chromosomes of living newt cells do provide undisputable proof of chromatin loops attached to a skeletal core (Callan, 1977). The intermediate filament–like skeleton described above was seen using a "physiological" buffer and was associated with loops of ~86 kb—roughly the size of replicons—that are maintained throughout the cell cycle. Structures like matrices, scaffolds, and cages all have very different loop sizes, highlighting how real the problem of artifacts is in this field (Jackson et al., 1990).

Because there is no agreement as to the molecular constitution of the skeleton, it is never included in models of replication drawn by molecular biologists. But the existence of a cytoskeleton was once controversial, and that controversy evaporated when antibodies were obtained that decorated the various cytoskeletal systems. We can

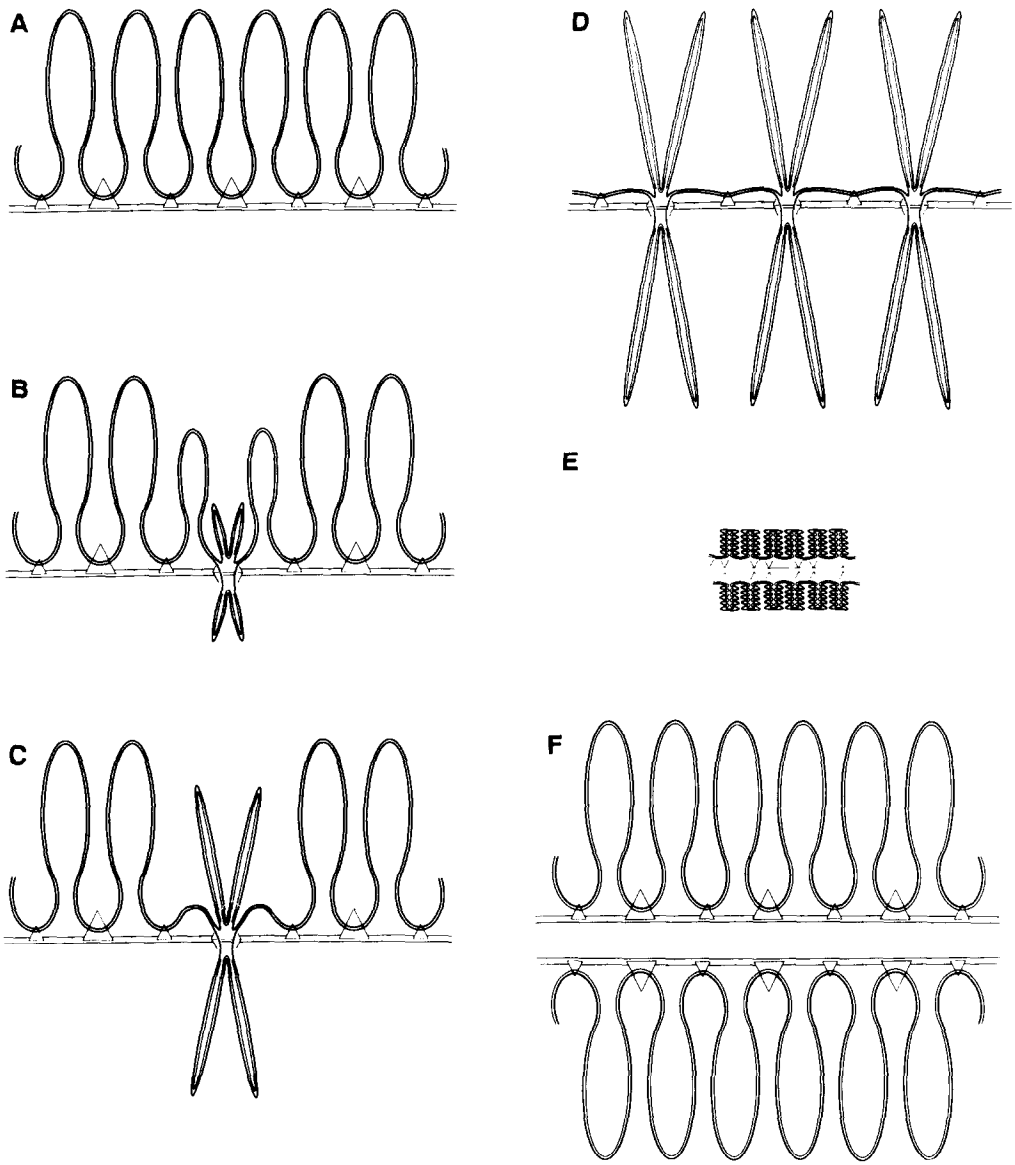

Figure 2. A Macroscopic Model

(A) Three replicons are shown attached at origins (in large triangles) to a helical interphase skeleton (rod). Replication termini also permanently attach DNA at other sites (small triangles).

(B) On entry into S phase, a polymerizing site assembles around the central origin from a pool of soluble, and hitherto inactive, polymerase. Replication then occurs as DNA is reeled through the fixed complex. The origin remains attached. Nascent DNA is shown as a thinner line.

(C) As parental loops diminish in size, replicated DNA is extruded in four new loops.

(D) Firing of other replicons results in duplicated loops connected by unreplicated DNA, which will be duplicated later.

(E) At mitosis, the interphase skeleton dissolves to allow complete resolution of parental DNA strands, leaving duplicated loops still associated with attachment points; the two chromatids also condense, with a few attachment points of the old interphase skeleton now forming helical mitotic skeletons (shown as linear structures). Condensation ensures that all interlocks between parental strands are resolved and that none of the rod-like elements of the interphase skeleton remain undissolved.

(F) After segregation to daughter cells, chromatids decondense as new interphase skeletons are assembled on each daughter during G1.

Origins and termini may not be attached throughout the cell cycle as shown here (Marilley and Gassend-Bonnet, 1989; Dijkwel and Hamlin, 1989); perhaps origin attachment triggers initiation (McCready et al., 1980). Here, attachment sites are the only part of the skeleton to be duplicated (concurrently with DNA) and then conserved through mitosis. They might be duplicated earlier (the complete remodeling seen in early S phase PCCs hints at this), with each skeleton binding one daughter duplex to ensure neither can be reduplicated. Alternatively, little interphase skeleton might be conserved, with assembly of a completely new mitotic skeleton; then skeletons seen in PCCs would be derived from the mitotic cell.

only hope that a nucleoskeleton—or, more probably, several of them—will soon be defined in similar molecular detail. But even in ignorance of the nucleoskeleton's molecular constitution, we can deduce some general principles governing how it might be duplicated.

It is a truism that extended biological complexes are helical (e.g., actin, tubulin, DNA); assembly into nonhelices requires that subunits be positioned next to neighbors too perfectly for nature to achieve, i.e., without any axial rotation. Therefore, we might expect the nucleoskeleton

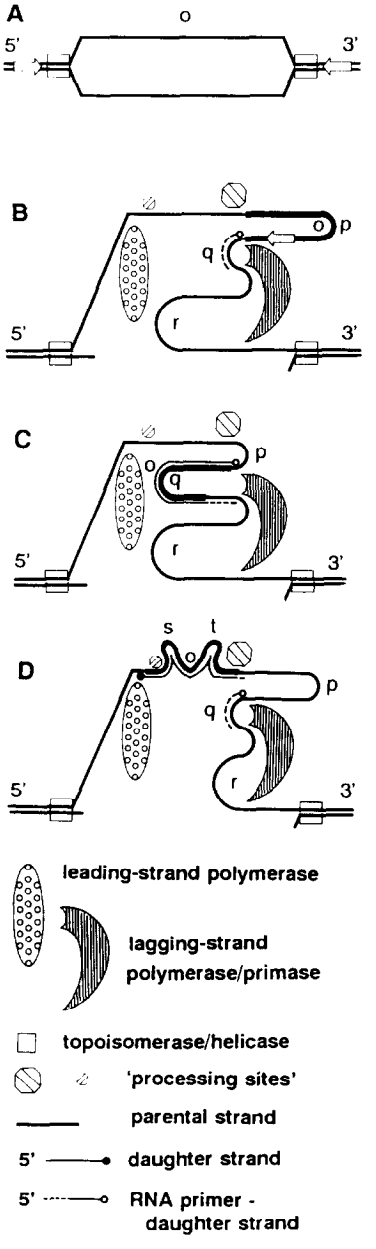

Figure 3. Initiation

All activities are attached to the skeleton (the page surface) and probably to each other, but are shown spread out for convenience. Only the upper half of the symmetrical complex is illustrated in (B)–(D); one segment of DNA is shown as a thickened line to emphasize its passage through the complex.

(A) DNA around the origin (o) melts as it slides (arrows) through two topoisomerases/helicases.

(B) The resulting single strands assemble around the polymerases into loops p and r. Then o slid toward the upper tip of the lagging-strand polymerase as an RNA primer was synthesized and extruded into loop q; its ends remain attached. DNA synthesis now begins at its 3′ end (open circle) as the thickened segment (p) slides to the left (arrow).

(C) The newly synthesized DNA has been extruded into q; its ends remain attached.

(D) Loop q detaches and reattaches to two "processing sites" (which remove primers and seal gaps), o, and the leading-strand polymerase. This creates loops s and t (the "rabbit ears" in Figure 2B) and allows the nascent 3′ end (now shown as a filled circle) to prime leading-strand synthesis. Loop r also rearranges, becoming loop p. Synthesis of the second RNA primer (in loop q) has just been completed. Leading- and lagging-strand synthesis now begin (at the closed and open circles, respectively).

at the local level to be helical, with its sense and pitch precisely defined by the relationship between monomers. However, at the macromolecular level this helicity will be distorted by the action of growth forces on the flexible skeleton (see Galloway, 1990, for a discussion). So when skeletons are duplicated, daughters will be locally and globally entwined, unless the total number of right-handed twists exactly—and fortuitously (because it depends on the growth conditions)—equals the number of left-handed twists.

Intuition suggests that a new skeleton might be assembled next to an old one without interlocking, if only the two could be kept apart. In practice, however, separation would be achieved through some molecular spacer; as its binding sites on the old skeleton must be arranged helically, inevitably the spacer will direct the synthesis of a new skeleton that winds around the old one. Even if not duplicated until after segregation of daughter DNA molecules, the parental skeleton would still remain interlocked with one or other (or both) daughter DNA, as DNA-binding sites on the old skeleton must be arranged helically. Therefore, interphase skeletons cannot simply contract like a spring at mitosis, because daughters would remain interlocked. The interlocks can only be resolved in the ways described above.

Resolution by rotation of chromatid ends seems unlikely in view of the stable inheritance of ring chromosomes, which lack ends (Strathern et al., 1979; Wong et al., 1989). The appearance of prematurely condensed chromosomes (PCCs) also makes it unlikely. When fused with a mitotic cell, the interphase chromatin prematurely condenses into extended chromosome-like structures. G1 nuclei yield single chromatids and G2 nuclei double ones; those from mid-S are a mixture, often separated by diffuse replicating regions (Rao and Johnson, 1974; Gollin et al., 1984). If duplicated skeletons were usually resolved by rotation (presumably driven by the condensation that occurs early in prophase), we should see intermediates in the unwinding of sister chromatids among PCCs. We do not; they always seem perfectly resolved.

Therefore, resolution must be achieved by breaking backbones. As no enzyme like a topoisomerase that decatenates interlocked protein filaments (or protein filaments and DNA) is known, the filaments must depolymerize. Then nature adopts the same strategy to resolve all catenanes—backbones are broken—but the tactics differ: topoisomerases cut and rejoin, whereas skeletons disassemble and reassemble.

Chromosome Condensation Drives Resolution

Special topological problems are encountered at termination. Topoisomerases acting ahead of the polymerase can remove the torsional barrier to elongation, but they cannot remove the last few double-helical interlocks at the termi-

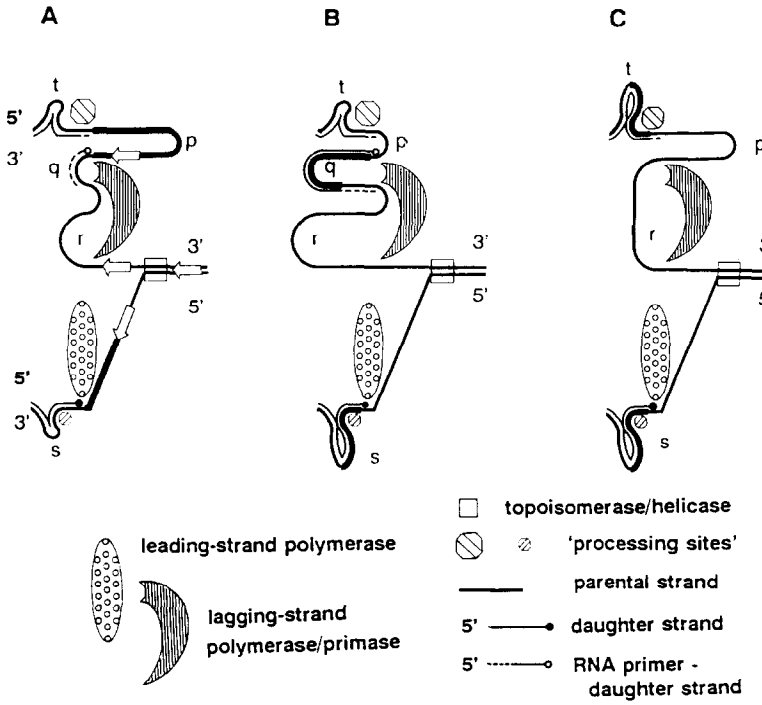

Figure 4. Elongation

Only the replication fork on the right-hand side of a symmetrical complex is shown, so Figure 3D and (A) here represent different views of the same structure. Two DNA segments are shown as thickened lines to emphasize their passage through the complex. Leading-strand synthesis is straightforward: in (A), DNA slides through the topoisomerase/helicase and (thickened segment) past the polymerizing site (closed circle) on the leading-strand polymerase (B), enlarging loop s. Even if synthesis is discontinuous, the same path can be followed. Lagging-strand (discontinuous) synthesis requires DNA flow forward through loops r and p, backward into q, and forward again into t. Some of these flows are shown together.

(A) DNA enters the complex, enlarging loop r (arrows). The thickened segment in loop p also moves (arrow) past the polymerase/primase as DNA is made at the 3′ end of the RNA primer (open circle).

(B) This twin flow enlarges r and extrudes nascent DNA into q (its ends remain attached).

(C) Loop q then detaches and flows into t past the large processing site (where gaps are sealed and primers removed). Loop r also rearranges, becoming p. A structure like that in (A) is reformed from the one shown here as p moves leftward, extruding a primer. Synthetic cycles continue until all DNA (except at the termini) is replicated.

nus because the duplex to which they are bound will inevitably contain some interlocks. Therefore, Sundin and Varshavsky (1980, 1981) suggested that the unreplicated terminus is denatured to transform its interlocks into the topologically equivalent interlocks of a catenane; these are resolved by a topoisomerase acting as a decatenase and not as a swivel. As catenanes with 10–30 interlocks are found late during replication, perhaps 100–300 bp are denatured like this (Sundin and Varshavsky, 1981; DiNardo et al., 1984). (Such interlocks are removed well before anaphase, so they cannot alone hold sister chromatids together [Koshland and Hartwell, 1987].)

But this does not ensure that all of the interlocks are resolved; decatenases act reversibly to yield a Boltzmann distribution of products centered around zero interlocking, but very few are completely unlinked. Moreover, they act locally and cannot immediately sense—and so eliminate—an interlock spread over thousands of base pairs. Chromosome condensation is the obvious way to ensure complete resolution; it both creates the substrate for the topoisomerase and removes its product. Even extended interlocks would soon condense to give the crossed duplexes that topoisomerases recognize (Zechiedrich and Osheroff, 1990); after resolution, further condensation would remove the unlinked product by packing it into a dense chromosome, so driving the various products in the equilibrium mixture toward zero interlocking. Some topoisomerases might be diffusible, so they could seek out crossed duplexes; others might play a structural role by organizing and condensing loops.

Condensation also ensures that the skeleton is completely depolymerized. "Depolymerases" must also be reversible, yielding mixtures of products. Condensation, again by removing products, will shift the equilibrium toward complete depolymerization—and zero interlocking. Condensation, then, is the mechanism that enables even one interlock spread over a whole chromatid to be detected and removed, ensuring that resolution of both DNA and skeleton is complete; there is no room for any catenanes or still-polymerized interphase skeleton in the tightly packed mitotic chromosome.

Models Involving Replication by Attached Polymerases

Our intuition that polymerases track along DNA stems from a perception of relative size: the smallest object moves. But if polymerases are attached and the template is subdivided into independent loops, the template becomes relatively small and so could move instead (Dingman, 1974). The topological considerations discussed above impose considerable constraints on any models for replication involving attached polymerases. For example, they must incorporate a mandatory depolymerization of the interphase skeleton to allow resolution, coupled with condensation to ensure it is complete. Moreover, because synthesis occurs 5′→3′ on strands of opposite polarity, the two strands must move locally past the relevant synthetic sites in opposite directions.

Specific models incorporating these features are illus-

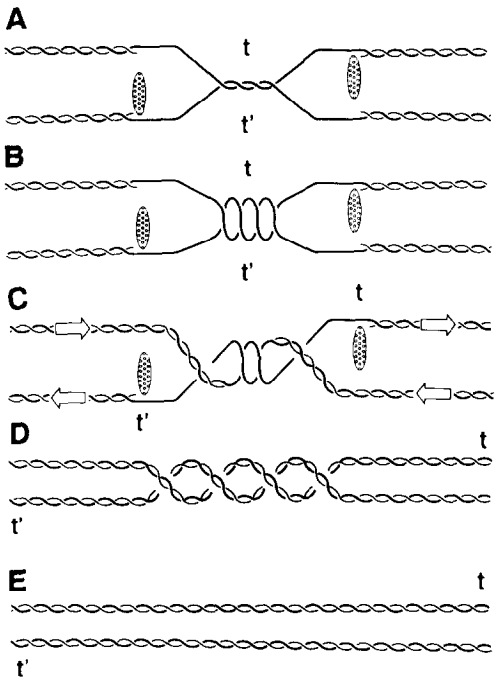

Figure 5. Termination

(A) Two replication forks flank the parental helix at the terminus (t, t'), which lies between two (simplified) complexes like those in Figure 4; only the leading-strand polymerases (ellipses) are shown. An analogous model involving lagging-strand polymerases is also possible.
(B) Remaining parental duplex is denatured.
(C) Daughter strands (thin lines) are extended as t slides rightward and t' leftward (arrows) past the polymerases.
(D) Complete replication and ligation of ends (not shown) yield interlocked daughters. Attachments to the skeleton (not shown) restrict diffusion of interlocks along the chromosome.
(E) Catenanes are resolved either by attached topoisomerases (not shown) or soluble activities.

trated in Figures 2–5. All the main activities (i.e., ligases, topoisomerases, helicases, polymerases, etc.) are attached; others (e.g., single-strand-binding proteins, polymerases, and ligases awaiting activation) might be soluble. It is difficult to imagine how the lagging strand can reverse its movement relative to the leading strand unless it performs a lagging-strand "shuffle" like that described in Figure 4. DNA first flows into loop r (Figures 4A and 4B), which rearranges to enlarge loop p (Figure 4C), then reverses past the polymerizing site into loop q (Figures 4A and 4B) before finally moving forward again into loop t (Figure 4C). The models for initiation and termination (Figures 3 and 5) are obviously speculative, but they are included to show that they can be drawn. In Figure 3, the first Okazaki fragment synthesized 3' to the origin acts as a primer for continuous synthesis 5' to the origin, as in current models for initiation of SV40 DNA synthesis (Tsurimoto et al., 1990; Hurwitz et al., 1990). At first sight these models appear topologically complicated, but they are not markedly more so than the traditional ones with which we have all grown familiar. In Figures 3 and 4 it is the lagging strand, and not its polymerase, that does the pirouetting, and inevitably it takes a little time to learn the new steps, especially when we know the old ones so well.

The models have several general advantages. Each complex is dedicated to replicating one (or at most a few) replicon(s); then it is easy to imagine how a complex could be prevented from acting more than once per cell cycle and how groups of adjacent replicons might initiate synchronously and so give focal synthesis. Obviously, the number of complexes must change as replicon size changes during development. Moreover, position in a loop could be the "chromosomal context" that determines which of several potential origins of replication are in fact used (Umek et al., 1989; Linskens and Huberman, 1990). Second, a symmetrical complex coordinates initiation on each side of the origin as well as leading- and lagging-strand synthesis. Third, inclusion of topoisomerases in the complex automatically couples local resolution of parental strands with synthesis during initiation, elongation, and termination. Interlocks persisting at termination cannot diffuse down the chromosome; even if the terminus is not permanently attached, as shown here, interlocks are restricted to a region between two attached complexes (i.e., between two topoisomerases) where they can be resolved easily. Any interlocks remaining after S phase might be removed by different topoisomerases that diffuse to their targets as chromosomes condense. Still other topoisomerases might be involved directly in chromosome condensation. Fourth, ends of nascent DNA are generally tied down and so cannot become entangled or substrates for recombination. Fifth, the models allow inheritance of specific attachments (with conservative or semiconservative inheritance of skeletal elements) and hence specific patterns of gene expression (Cook, 1989).

Conclusions

The quotation that heads this piece summarizes one view of replication: the polymerizing locomotive moves down the template track unattached to any skeleton. Like many received ideas, this has little supporting evidence. Accurate and efficient replication by a soluble system in vitro would provide strong evidence, and the SV40 system seems to provide it. However, recent results using this system are only explicable if one of its essential ingredients, T antigen, aggregates and becomes—at the very least—partially immobile. This means that this apparently decisive evidence is compromised.

Original evidence for immobile polymerases all depended on the use of unphysiological conditions, but now similar evidence has been obtained using more physiological conditions. Attachment of active polymerases to a skeleton is also consistent with the focal clustering of polymerizing complexes. Then nuclear architecture becomes a key determinant of function and the nucleoskeleton much more than a structural framework: it becomes the active site at which replication occurs. But all the evidence for this view is circumstantial. We must now define the skeleton in molecular detail, map attachments of the polymerase to it, and determine when it is duplicated. This poses a difficult challenge for biochemists, who generally discard insoluble material and study the supernatant. Perhaps it is time to shift interest from partial activities in the supernatant to

the authentic activities in the pellet. Fortunately, several cell-free systems that allow dissection of the chromosome cycle in vitro are now available (see Lohka and Masui, 1983; Burke and Gerace, 1986; Hutchison et al., 1987; Newport, 1987).

Acknowledgments

I thank the Cancer Research Campaign for support, Dean Jackson for helpful discussions, and Shirley McCready and Bob Johnson for comments.

References

Adachi, Y., Luke, M., and Laemmli, U. K. (1991). Chromosome assembly in vitro: topoisomerase II is required for chromosome condensation. Cell *64*, 137–148.

Alberts, B. M. (1990). Recipes for replication. Nature *346*, 514–515.

Berezney, R., and Coffey, D. S. (1975). Nuclear protein matrix: association with newly synthesized DNA. Science *189*, 291–293.

Berrios, M., Osheroff, N., and Fisher, P. (1985). In situ localization of DNA topoisomerase II, a major polypeptide component of the Drosophila nuclear matrix fraction. Proc. Natl. Acad. Sci. USA *82*, 4142–4146.

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

Brill, S. J., DiNardo, S., Voelkel-Meiman, K., and Sternglanz, R. (1987). Need for topoisomerase activity as a swivel for DNA replication and for transcription of ribosomal RNA. Nature *326*, 414–416.

Burke, B., and Gerace, L. (1986). A cell free system to study reassembly of the nuclear envelope at the end of mitosis. Cell *44*, 639–652.

Callan, H. G. (1977). Lampbrush chromosomes. Proc. R. Soc. London (B) *214*, 417–448.

Cook, P. R. (1988). The nucleoskeleton: artefact, passive framework or active site? J. Cell Sci. *90*, 1–6.

Cook, P. R. (1989). The nucleoskeleton and the topology of transcription. Eur. J. Biochem. *185*, 487–501.

Dijkwel, P. A., and Hamlin, J. (1989). Matrix attachment regions are positioned near replication initiation sites, genes, and interamplicon junctions in the amplified dihydrofolate reductase domain of Chinese hamster ovary cells. Mol. Cell. Biol. *8*, 5398–5409.

DiNardo, S., Voelkel, K. A., and Sternglanz, R. (1984). DNA topoisomerase II mutant of Saccharomyces cerevisiae: topoisomerase II is required for segregation of daughter molecules at the termination of DNA replication. Proc. Natl. Acad. Sci. USA *81*, 2616–2620.

Dingman, C. W. (1974). Bidirectional chromosome replication: some biological considerations. J. Theor. Biol. *43*, 187–195.

Earnshaw, W. C., Halligan, B., Cooke, C. A., Heck, M. M. S., and Liu, L. F. (1985). Topoisomerase II is a structural component of mitotic chromosome scaffolds. J. Cell Biol. *100*, 1706–1715.

Galloway, J. (1990). Putting a twist in the tail. Nature *343*, 513–514.

Gasser, S. M., Laroche, T., Falquet, J., Boy de la Tour, E., and Laemmli, U. K. (1986). Metaphase chromosome structure—involvement of topoisomerase II. J. Mol. Biol. *188*, 613–629.

Gollin, S. M., Wray, W., Hanks, S. K., Hittelman, W. N., and Rao, P. N. (1984). The ultrastructural organization of prematurely condensed chromosomes. J. Cell Sci. (Suppl.) *1*, 203–221.

Goto, T., and Wang, J. C. (1985). Cloning of yeast TOP1, the gene encoding DNA topoisomerase I, and construction of mutants defective in both DNA topoisomerase I and DNA topoisomerase II. Proc. Natl. Acad. Sci. USA *82*, 7178–7182.

He, D., Nickerson, J. A., and Penman, S. (1990). Core filaments of the nuclear matrix. J. Cell Biol. *110*, 569–580.

Hurwitz, J., Dean, F. B., Kwong, A. D., and Lee, S.-H. (1990). The in vitro replication of DNA containing the SV40 origin. J. Biol. Chem. *265*, 18043–18046.

Hutchison, C. J., Cox, R., Drepaul, R. S., Gomperts, M., and Ford, C. C. (1987). Periodic DNA synthesis in cell-free extracts of Xenopus eggs. EMBO J. *6*, 2003–2010.

Hutchison, C. J., Cox, R., and Ford, C. C. (1988). The control of DNA replication in a cell-free extract that recapitulates a basic cell cycle in vitro. Development *103*, 553–566.

Ishimi, Y., Claude, A., Bullock, P., and Hurwitz, J. (1988). Complete enzymatic synthesis of DNA containing the SV40 origin of replication. J. Biol. Chem. *263*, 19723–19733.

Jackson, D. A. (1990). The organization of replication centres in higher eukaryotes. BioEssays *12*, 87–89.

Jackson, D. A. (1991). Structure–function relationships in eukaryotic nuclei. BioEssays *13*, 1–10.

Jackson, D. A., and Cook, P. R. (1985). A general method for preparing chromatin containing intact DNA. EMBO J. *4*, 913–918.

Jackson, D. A., and Cook, P. R. (1986a). Replication occurs at a nucleoskeleton. EMBO J. *5*, 1403–1410.

Jackson, D. A., and Cook, P. R. (1986b). A cell-cycle-dependent DNA polymerase activity that replicates intact DNA in chromatin. J. Mol. Biol. *192*, 65–76.

Jackson, D. A., and Cook, P. R. (1986c). Different populations of DNA polymerase α in Hela cells. J. Mol. Biol. *192*, 77–86.

Jackson, D. A., and Cook, P. R. (1988). Visualization of a filamentous nucleoskeleton with a 23nm axial repeat. EMBO J. *7*, 3667–3677.

Jackson, D. A., Yuan, J., and Cook, P. R. (1988). A gentle method for preparing cyto- and nucleo-skeletons and associated chromatin. J. Cell Sci. *90*, 365–378.

Jackson, D. A., Dickinson, P., and Cook, P. R. (1990). The size of chromatin loops in HeLa cells. EMBO J. *9*, 567–571.

Kim, R. A., and Wang, J. C. (1989). Function of DNA topoisomerases as replication swivels in Saccharomyces cerevisiae. J. Mol. Biol. *208*, 257–267.

Kornberg, A. (1974). DNA Synthesis (San Francisco: W. H. Freeman).

Kornberg, A. (1987). Enzyme systems initiating replication at the origin of the Escherichia coli chromosome. J. Cell Sci. (Suppl.) *7*, 1–13.

Koshland, D., and Hartwell, L. H. (1987). The structure of sister minichromosome DNA before anaphase in Saccharomyces cerevisiae. Science *238*, 1713–1716.

Linskens, M. H. K., and Huberman, J. A. (1990). The two faces of higher eukaryotic DNA replication origins. Cell *62*, 845–847.

Liu, L. F. (1989). DNA topoisomerase poisons as antitumor drugs. Annu. Rev. Biochem. *58*, 351–375.

Lohka, M. J., and Masui, Y. (1983). Formation in vitro of sperm pronuclei and mitotic chromosomes induced by amphibian ooplasmic components. Science *220*, 719–721.

Manton, I. (1945). Comments on chromosome structure. Nature *155*, 471.

Manton, I. (1950). The spiral structure of chromosomes. Biol. Rev. *25*, 486–508.

Marilley, M., and Gassend-Bonnet, G. (1989). Supercoiled loop organization of genomic DNA: a close relationship between loop domains, expression units and replicon organization in rDNA from Xenopus laevis. Exp. Cell Res. *180*, 475–489.

Martelli, A. M., Gilmour, R. R., Falcieri, E., Manzoli, F. A., and Cocco, L. (1990). Temperature-dependent association of DNA polymerase α activity with the nuclear matrix. Exp. Cell Res. *190*, 227–232.

McCready, S. J., Godwin, J., Mason, D. W., Brazell, I. A., and Cook, P. R. (1980). DNA is replicated at the nuclear cage. J. Cell Sci. *46*, 365–386.

Mills, A. D., Blow, J. J., White, J. G., Amos, W. B., Wilcock, D., and Laskey, R. A. (1989). Replication occurs at discrete foci spaced throughout nuclei replicating in vitro. J. Cell Sci. *94*, 471–477.

Nakamura, H., Morita, T., and Sato, C. (1986). Structural organisation of replicon domains during DNA synthetic phase in the mammalian nucleus. Exp. Cell Res. *165*, 291–297.

Nakayasu, H., and Berezney, R. (1989). Mapping replication sites in the eukaryotic cell nucleus. J. Cell Biol. *108*, 1–11.

Nelson, W. G., Pienta, K. J., Barrack, E. R., and Coffey, D.S. (1986). The role of the nuclear matrix in the organization and function of DNA. Annu. Rev. Biophys. Chem. *15*, 457–475.

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

Newport, J., and Spann, T. (1987). Disassembly of the nucleus in mitotic extracts: membrane vesicularization, lamin disassembly, and chromosome condensation are independent processes. Cell *48*, 219–230.

Pardoll, D. M., Vogelstein, B., and Coffey, D. S. (1980). A fixed site of DNA replication in eukaryotic cells. Cell *19*, 527–536.

Rao, P. N., and Johnson, R. T. (1974). Regulation of cell cycle in hybrid cells. In Control of Proliferation in Animal Cells, B. Clarkson and R. Baserga, eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 785–800.

Richter, A., Strausfeld, U., and Knippers, R. (1987). Effects of VM26 (teniposide), a specific inhibitor of type II DNA topoisomerase, on SV40 chromatin replication in vivo. Nucl. Acids Res. *15*, 3455–3468.

Schaak, J., Schedl, P., and Shenk, T. (1990). Topoisomerase I and II cleavage of adenovirus DNA in vivo: both topoisomerase activities appear to be required for adenovirus DNA replication. J. Virol. *64*, 78–85.

Snapka, R. M., Powelson, M. A., and Strayer, J. M. (1988). Swivelling and decatenation of replicating simian virus 40 genomes in vivo. Mol. Cell. Biol. *8*, 515–521.

Strathern, J. N., Newlon, C. S., Heskowitz, I., and Hicks, J. B. (1979). Isolation of a circular derivative of yeast chromosome III: implications for the mechanism of mating type interconversion. Cell *18*, 309–319.

Sundin, O., and Varshavsky, A. (1980). Terminal stages of SV40 DNA replication proceed via multiply intertwined catenated dimers. Cell *21*, 103–114.

Sundin, O., and Varshavsky, A. (1981). Arrest of segregation leads to accumulation of highly intertwined catenated dimers: dissection of the final stages of SV40 DNA replication. Cell *25*, 659–669.

Tsurimoto, T., Melendy, T., and Stillman, B. (1990). Sequential initiation of lagging and leading strand synthesis by two different polymerase complexes at the SV40 DNA replication origin. Nature *346*, 534–539.

Tubo, R. A., and Berezney, R. (1987). Identification of 100 and 150S DNA polymerase alpha-primase megacomplexes solubilized from the nuclear matrix of regenerating rat liver. J. Biol. Chem. *262*, 5857–5865.

Uemura, T., and Yanagida, M. (1984). Isolation of type I and II DNA topoisomerase mutants from fission yeast: single and double mutants show different phenotypes in cell growth and chromatin organisation. EMBO J. *3*, 1737–1744.

Uemura, T., and Yanagida, M. (1986). Mitotic spindle pulls but fails to separate chromosomes in type II DNA topoisomerase mutants: uncoordinated mitosis. EMBO J. *5*, 1003–1010.

Uemura, T., Ohkura, H., Adachi, Y., Morino, K. Shiozaki, K., and Yanagida, M. (1987). DNA topoisomerase II is required for condensation and separation of mitotic chromosomes in S. pombe. Cell *50*, 917–925.

Umek, R. M., Linskens, M. H. K., Kowalski, D., and Huberman, J. A. (1989). New beginnings in studies of eukaryotic DNA replication origins. Biochim. Biophys. Acta *1007*, 1–14.

Verheijen, R., Van Venrooij, W., and Ramaekers, F. (1988). The nuclear matrix: structure and composition. J. Cell Sci. *90*, 11–36.

Wang, X., and Traub, P. (1991). Resinless section immunogold electron microscopy of karyo-cytoskeletal frameworks of eukaryotic cells cultured in vitro: absence of a salt-stable nuclear matrix from mouse plasmacytoma MPC-11 cells. J. Cell Sci. *98*, 107–122.

White, J. H., Cozzarelli, N. R., and Bauer, W. R. (1988). Helical repeat and linking number of surface-wrapped DNA. Science *241*, 323–327.

Wobbe, C. R., Weissbach, L., Borowiec, J. A., Dean, Y., Murakami, Y., Bullock., P., and Hurwitz, J. (1987). Replication of simian virus 40 origin-containing DNA in vitro with purified proteins. Proc. Natl. Acad. Sci. USA *84*, 1834–1838.

Wong, C., Kazazian, H. H., Stetten, G., Earnshaw, W. C., van Keuren, M. L., and Antonarakis, S. E. (1989). Molecular mechanism in the formation of a human ring chromosome 21. Proc. Natl. Acad. Sci. USA *86*, 1914–1918.

Wood, E. R., and Earnshaw, W. C. (1990). Mitotic chromatin condensation in vitro using somatic cell extracts and nuclei with variable levels of endogenous topoisomerase II. J. Cell Biol. *111*, 2839–2850.

Yang, L. Jessee, C. B., Lau, K., Zhang, H., and Liu, L. F. (1989). Template supercoiling during ATP-dependent DNA helix tracking: studies with simian virus 40 large tumor antigen. Proc. Natl. Acad. Sci. USA *86*, 6121–6125.

Zechiedrich, E. L., and Osheroff, N. (1990). Eukaryotic topoisomerases recognise nucleic acid topology by preferentially interacting with DNA crossovers. EMBO J. *9*, 4555–4562.