

COMMENTARY

Does transcription by RNA polymerase play a direct role in the initiation of replication?

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SUMMARY

RNA polymerases have been implicated in the initiation of replication in bacteria. The conflicting evidence for a role in initiation in eukaryotes is reviewed.

Key words: cell cycle, initiation, origin, replication, transcription

PRIMERS AND PRIMASES

DNA polymerases cannot initiate the synthesis of new DNA chains, they can only elongate pre-existing primers. The opposite polarities of the two strands of the double helix coupled with the 5'→3' polarity of the polymerase means that replication occurs relatively continuously on one (leading) strand and discontinuously on the other (lagging) strand. The continuous strand probably needs to be primed once, usually at an origin. Nature has found many different ways of doing this, including the use of RNA primers made by an RNA polymerase (e.g. during replication of the plasmid ColE1 and mitochondrial DNA), preformed tRNA (by retroviral reverse transcriptases), DNA primers generated by endonucleolytic cleavage (by gpA of ϕ X174) and even a serine OH group (adenoviral 55 kDa terminal protein). The discontinuous strand must be primed repeatedly to generate short nascent chains (Okazaki fragments) and the multiple RNA primers needed are invariably synthesized by a special primase, which is an integral part of the replication machinery (see Kornberg and Baker, 1992, for a comprehensive review). This means that RNA synthesis, by RNA polymerases or primases, is inevitably involved in primer synthesis. This review will concentrate on the additional roles played by RNA polymerases in initiating DNA synthesis, especially in eukaryotes. Unfortunately no clear view as to their role emerges.

The current model for the initiation of replication at origins unifies the findings from many different organisms (Bramhill and Kornberg, 1988). A *trans*-acting initiator protein first binds to, and transiently unwinds, a specific site in the origin, before a helicase extends the unwound region. After recruitment of a primase-polymerase complex, RNA-primed DNA synthesis initiates on the leading strand. Next a processive polymerase captures the leading-strand primer and the primase-polymerase

complex becomes exclusively involved in the synthesis of Okazaki fragments on the lagging strand. A critical step in this process is the unwinding of the duplex.

RNA POLYMERASES AND THE INITIATION OF REPLICATION IN BACTERIA

The first evidence of a role for RNA polymerase came from the demonstration that the initiation of replication in *Escherichia coli* was sensitive to rifampicin, an inhibitor of RNA polymerase, independently of any requirement for protein synthesis (Lark, 1972). Genetic analysis also pointed to a direct role (reviewed by Kornberg and Baker, 1992). Replication begins at *oriC*, a ~245 bp region that is highly conserved among enteric bacteria; it contains four binding sites for *dnaA*, the initiator protein, and back-to-back promoters. Temperature-sensitive mutations in *dnaA* are generally lethal at the non-permissive temperature but can be suppressed by additional mutations elsewhere in the genome. Such suppressors map to *rpoB*, the gene encoding the β subunit of RNA polymerase, indicating that altered RNA polymerases can assist defective *dnaA* proteins. Some mutations in both the β and β' subunits of the polymerase also elevate the copy number of *oriC*-bearing chromosomes, again implicating this polymerase in the regulation of initiation.

Fortunately, templates containing *oriC* can be replicated *in vitro* and direct experiments show that RNA polymerase is required, but only under conditions that make unwinding difficult (e.g. a reduced negative superhelicity of the naked template or a reduced temperature). The RNA polymerase does not make primers as full activation still occurs when they are terminated with 3'-dATP and so lack 3'-OH groups. Transcription from one of the back-to-back promoters probably

generates a RNA-DNA hybrid that stimulates origin unwinding (Baker and Kornberg, 1988).

Another example of helix-opening mediated by transcription is provided by phage λ (reviewed by Keppel et al., 1988). Again, a promoter, P_R , lies close to the origin and mutants with no transcription from P_R generally activate new promoters that restore synthesis close to, but not necessarily across, the origin. And again replication in vitro under conditions that make unwinding difficult depends upon transcription. As the cI protein represses both P_R and lytic growth, the logic of this circuitry ensures that replication quickly stops during lysogenization.

These examples show that the act of transcription can regulate initiation of replication in prokaryotes. In eukaryotes, the situation is less clear, largely because their origins are so ill-defined (reviewed by Fangman and Brewer, 1992; DePamphlis, 1993) and no in vitro system is available that can initiate efficiently, other than a crude *Xenopus* extract (reviewed by Laskey et al., 1989). Fortunately, the origins of several viruses that grow in eukaryotic cells have been defined and systems that replicate them in vitro established. Nevertheless, it is clear that, like their bacterial counterparts, eukaryotic origins are always closely associated with transcription units. They are also rich in binding sites for transcription factors and those factors directly influence initiation (reviewed by Heintz, 1992; DePamphlis, 1993). What is unclear is whether binding alone or binding plus transcription is necessary. The evidence will now be reviewed and its limitations highlighted.

TRANSCRIPTION FACTORS AND ORIGINS

We begin with the incontrovertible evidence: origins bind transcription factors. For example, initiation at the adenoviral origin in vitro requires three viral proteins, including the DNA polymerase, plus three host proteins; two of the latter turned out to be transcription factors (CTF and OTF-1) and one binds directly to the DNA polymerase (Jones et al., 1987; O'Neill et al., 1988; Chen et al., 1990). Similarly, transcriptional transactivator proteins VP16 and GAL4 stimulate replication of bovine papilloma virus in vitro, again binding directly to a replication factor (i.e. RPA; Li and Botchan, 1993; He et al., 1993). The one chromosomal origin that has been analyzed in molecular detail, yeast *ARS1*, also binds the transcription factor ABF1 and binding sites for other transcription factors (i.e. RAP1 and GAL4) can substitute for the ABF1 site (Marahrens and Stillman, 1992).

We might imagine that these transcription factors all 'open' or 'mark' chromatin, so that adjacent origins become accessible to replication factors (Heintz, 1992). Then, transcription factors contribute to a chromosomal 'context' that can determine when an origin fires. Indeed, the timing of firing depends on chromosomal position; *ARS1* usually replicates early during the cell cycle, but when translocated to a late-replicating region it replicates late and, conversely, *ARS501*, which usually replicates late, replicates early when transferred to a circular plasmid (Ferguson and Fangman, 1992).

Initiation at the origin of SV40 virus has been analyzed in perhaps the greatest detail and provides strong evidence against a more direct role for transcription (Challberg and Kelly, 1989). This virus uses only host factors for replication, except

for the viral T antigen (a *dnaA* analogue). Like the viruses discussed above, an RNA polymerase II promoter with six Sp1-binding sites abuts and partly overlaps the origin core. This overlap unfortunately compromises attempts to assess the effects of transcription on replication by deleting the promoter. However, soluble extracts from monkey cells still replicate exogenously-added origins even when transcription is eliminated by 250 μ g/ml α -amanitin, an RNA polymerase inhibitor (Li and Kelly, 1984). Moreover, replacing some or all of the Sp1 sites with binding sites for different transcription factors varies the transcription rate with relatively little effect on the replication rate, unless multiple binding sites are present (Guo and DePamphlis, 1992; Hoang et al., 1992; Cheng et al., 1992). A worry here is that the in vitro system that replicates *oriC* is also sensitive to an RNA polymerase inhibitor only under certain conditions (see above), and the same may be true of the SV40 system.

EMBRYOGENESIS IN *XENOPUS* AND *DROSOPHILA*

Embryogenesis in frogs and insects initially provided apparently decisive evidence that initiation occurred in the absence of transcription. After fertilization, the *Xenopus* egg divides rapidly 12 times to give ~4000 cells; then, at the midblastula transition, the rate of division slows (Newport and Kirschner, 1982a,b). No RNA synthesis, measured by incorporation of [³H]uridine (by whole cells) or [³²P]UTP (after microinjection), was initially detected prior to the midblastula transition. Furthermore, injecting α -amanitin to give an internal concentration of ~50 μ g/ml had no effect on the first 12 divisions but then prevented the appearance of transcripts generated by polymerase II and III. Similar results were also obtained with *Drosophila* eggs, which divide 10 times without detectable RNA synthesis or inhibition by α -amanitin (Edgar et al., 1986; Edgar and Schubiger, 1986).

These results imply that DNA can be duplicated without transcription but recent results provide some important caveats. First, endogenous UTP pools, ~4 mM in *Drosophila* embryos (Edgar and Schubiger, 1986), inevitably dilute added radiolabel and make it difficult to detect transcription in the few nuclei present earlier during embryogenesis. However, nascent frog transcripts have now been detected at the 7th cleavage division using higher concentrations of radiolabel (Kimelman et al., 1987). Moreover, some paternal *Drosophila* genes are expressed at the third cleavage division (i.e. at the 8 cell stage) and so must be transcribed even earlier (Brown et al., 1991). Second, 50 μ g/ml α -amanitin does not completely inhibit frog RNA polymerase III and the *Drosophila* enzyme is even more resistant (Kornberg and Baker, 1992); therefore, it is perhaps not surprising that residual transcription from injected plasmid templates can be detected in the presence of these concentrations of α -amanitin (Newport and Kirschner, 1982b). But even despite this caveat, we might expect to see at least some effects of α -amanitin on development if transcription is required for initiation, but we do not.

CELL CYCLE MUTANTS

Various cell cycle mutants arrest during G₁ and characteriza-

tion of their defects provides some surprises. For example, one temperature-sensitive mutant of BHK cells (i.e. tsAF8) arrests in G₁ at the non-permissive temperature, but microinjection of RNA polymerase II allows progression into S-phase (Waechter et al., 1984). A phenotypically similar mutant can be rescued by transfection of a cDNA encoding the cell cycle gene 1 (*CCG1*; Sekiguchi et al., 1991) and this gene turns out to encode a transcription factor associated with the TATA-binding protein (i.e. TAF_{II}250; Ruppert et al., 1993; Hisatake et al., 1993; Kokubo et al., 1993). Another mutant can be rescued from G₁ arrest by a human cell cycle gene, *BN51*; this is homologous with the yeast *RPC53* gene that encodes a subunit of RNA polymerase III. Moreover, yeast containing the corresponding temperature-sensitive allele also arrest in G₁ at the non-permissive temperature (Mann et al., 1992). It can hardly be fortuitous that so many cell cycle mutants are deficient in polymerases and transcription factors.

The use of α -amanitin also suggests transcription is involved (Adolph et al., 1993). Addition of the inhibitor (at 3–30 μ g/ml) to unsynchronized NIH3T3 cells specifically arrests those late in G₁. As cells already in S-phase continue to cycle normally, transcription by RNA polymerase II cannot play any role during replicational elongation. However, as about half the activity of RNA polymerase III is resistant to these concentrations of α -amanitin, residual activity of this polymerase could still play a role in initiation by late-replicating origins.

These surprising results with mutants and inhibitors again link RNA polymerases with the initiation of replication. However, both could act indirectly by reducing transcription of genes that encode proteins involved in progression into S-phase. (See Andrews (1992) for a review of genes containing the *MluI* box that are transcribed specifically at the G₁/S boundary.)

COINCIDENCE OF SITES OF TRANSCRIPTION AND REPLICATION

The colocalization of eukaryotic replication sites relative to transcription sites also suggests one process depends on the other. Immunolabelling shows that DNA precursors (e.g. biotin-dUTP) are incorporated into discrete foci (Fig. 1; Nakamura et al., 1986; Nakayasu and Berezney, 1989; Mills et al., 1989; Kill et al., 1991), which cannot be fixation artifacts because they are seen in unfixed cells (Hassan and Cook, 1993). In the electron microscope, these foci appear as dense bodies strung along a skeleton. Simple calculations suggest each body contains ~40 forks. After a short incubation with biotin-dUTP, incorporated analogue is associated only with the bodies, but after longer incubations it spreads into adjacent chromatin (Hozák et al., 1993). This suggests that nascent DNA is extruded as templates slide past polymerases fixed in the bodies. Therefore textbooks that depict tracking polymerases may be misleading: rather than a polymerase that moves along a stationary template, the template slides past an enzyme fixed to a body.

Analogous transcription sites, which also contain ~40 active polymerases, are immunolabelled after incubation with Br-UTP (Fig. 1; Jackson et al., 1993; Wansink et al., 1993) or by hybridization with appropriate probes (Carter et al., 1993). Polymerizing activity and these foci remain when most

chromatin is removed, suggesting that transcription also occurs as templates slide past enzymes fixed to the skeleton.

As transcriptionally-active genes are generally replicated before inactive genes (e.g. see Hatton et al., 1988; O'Keefe et al., 1992), we might expect that some sites of transcription would overlap sites of replication at the beginning of S-phase. However, the overlap was better than expected; all replication sites were transcribing and all transcription sites were replicating (Hassan et al., 1994). This near-perfect overlap is illustrated in the confocal image of a section through a HeLa cell at the G₁/S border (Fig. 1); almost no pure red (i.e. transcription alone) or pure green (i.e. replication alone) is visible. Rather all sites appear white or blue, indicating that both transcription and replication occur together. Later during S-phase, we might also have expected that heterochromatin, which is widely assumed to be transcriptionally inert (e.g. see Hatton et al., 1988; O'Keefe et al., 1992), would be replicated in transcription-free sites (i.e. we should see green during mid-to-late S-phase). But contrary to expectation, replication sites were again transcriptionally active (i.e. in Fig. 1 there is little bright green and nearly all sites appear blue or white, indicating that transcription and replication are co-localized). This RNA synthesis that occurs at replication sites is unlikely to be primase-dependent because there is so much of it and it is sensitive to α -amanitin. This co-localization has now been confirmed by electron microscopy (P. Hozák and P. R. Cook, unpublished results).

The finding that replication always takes place at sites that are transcriptionally active again points to a functional relationship between the two processes. We might imagine that replication foci or 'factories' are assembled at the G₁/S border around pre-existing transcription factories simply because the latter are in open euchromatin. But this does not explain why transcription is so intimately associated with replication later during S-phase.

A RECONCILIATION

We therefore have a set of conflicting data. In bacteria, genetic evidence points to a role for transcription in the initiation of replication, but biochemical evidence shows that cell-free systems will initiate in the absence of active RNA polymerases under certain conditions. Things are no clearer in eukaryotes: genetic evidence points to a role for transcription, and transcription factors are implicated in initiation at both viral and cellular origins, but active RNA polymerases do not seem to be required by cell-free systems nor, perhaps, by early embryos. How can the conflicting data be reconciled?

One answer is that different organisms initiate replication in different ways, as exemplified by bacteriophages (Kornberg and Baker, 1992); then transcription might be involved in some cases and not in others. But it is also attractive to suppose that variety is superimposed upon a basic theme. The following speculations provide one way of reconciling the data; it involves active polymerases fixed in 'factories' of the type described above (Fig. 2; Cook, 1989, 1991).

We imagine that the G₁ chromatin fibre is looped by attachment to transcription factories, either through transcription factors at promoters/enhancers, or through active RNA polymerases (Jackson and Cook, 1993). Ties involving poly-

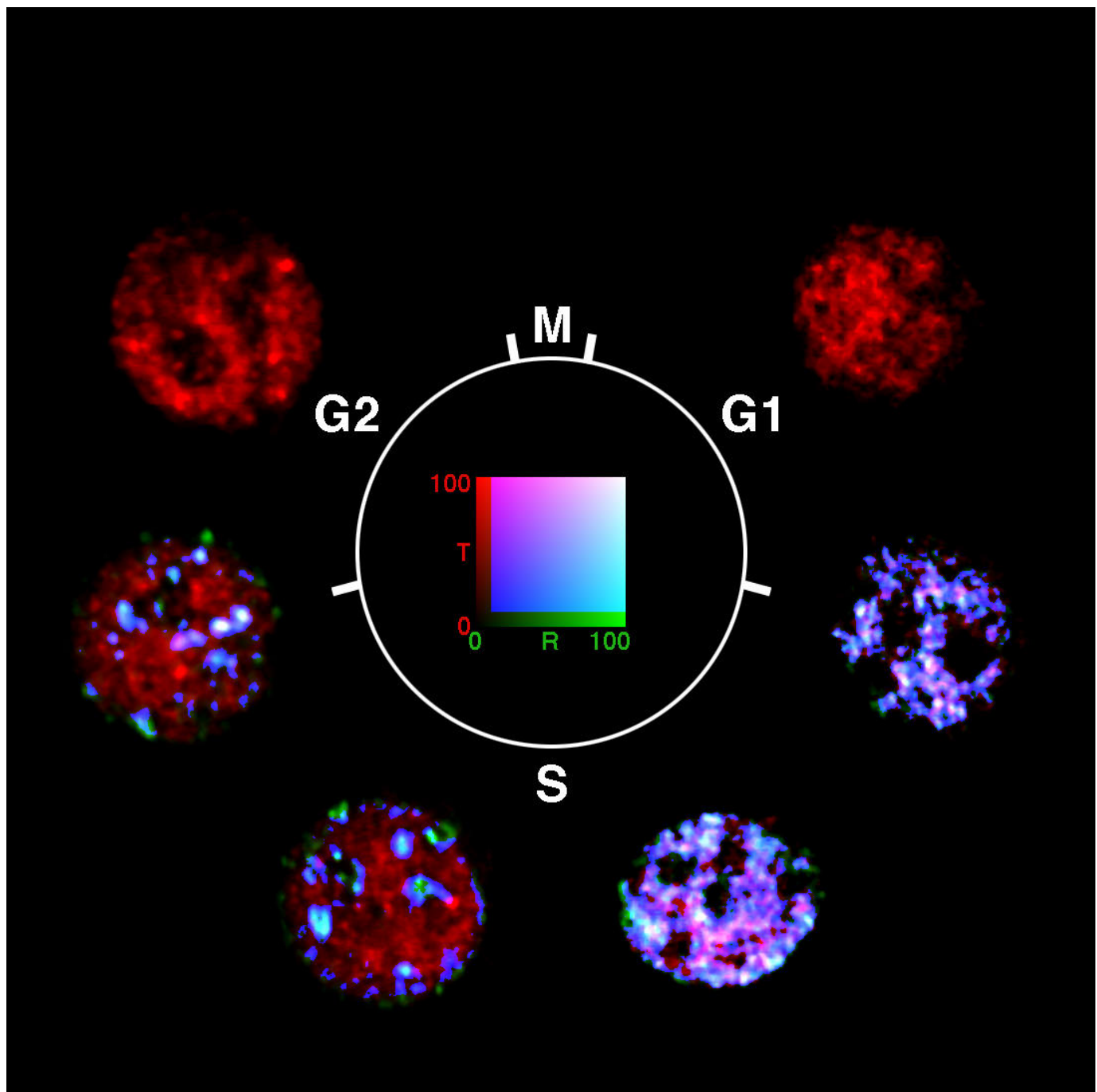


Fig. 1. Seven optical sections through HeLa cells synchronized at different stages of the cycle. Permeabilized cells were incubated with biotin-dUTP and Br-UTP, sites of DNA and RNA synthesis were indirectly immunolabelled with fluorescein and Texas Red before an optical section was taken through the centre of 7 different cells using a confocal microscope. The different colours reflect different amounts of replication and transcription. (See central graph. R, replication (fluorescein); T, transcription (Texas Red); axes, 0-100%. Sites where replication or transcription occur alone range from black (0%) to pure green or red (100%); sites where they occur together (above the threshold indicated) range from purple to white. During mitosis (M), the cell is black as no replication or transcription occurs. Nuclei then increase in size as a transcription and a replication cycle run in parallel. In G₁, transcription (red) begins at ~300 extra-nucleolar sites which, on entry into S-phase, aggregate into ~150 discrete sites that are also replicating (i.e. purple-white; large black holes are nucleoli). Then transcription sites quickly disperse to recreate the original (red) pattern that is maintained into G₂. At no stage does intense replication occur without transcription (which would appear bright green). At the G₁/S boundary, replication initiates in transcriptionally-active foci (purple-white). Early during S-phase, DNA synthesis occurs maximally at most transcription sites (blue-white). During mid S-phase, heterochromatin around nucleoli and the nuclear periphery is replicated in fewer, larger, sites that are nevertheless transcribing (purple/blue). At the end of S-phase, dense heterochromatin is replicated in large, but still transcriptionally-active, sites (blue-white) (see Hassan et al. (1994) for details).

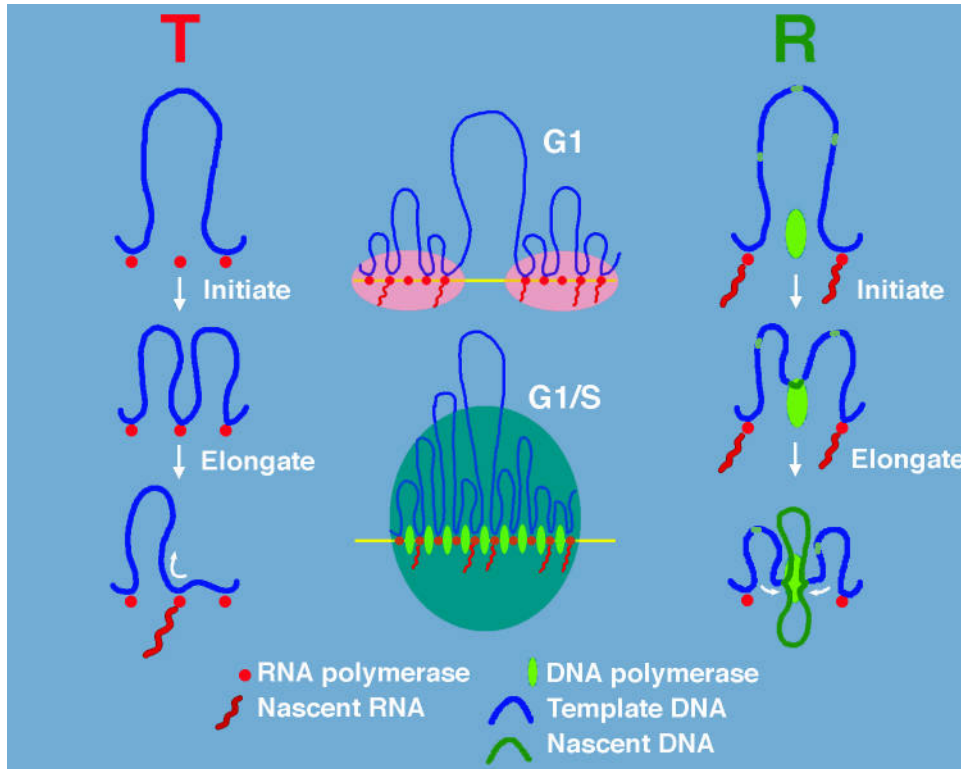


Fig. 2. Model for the initiation of replication. Left: high magnification view of transcription (T). A chromatin loop is attached through transcription factors or RNA polymerases. Transcription initiates as the promoter in the loop attaches to an unengaged polymerase; then the template slides (curved arrow) past the fixed enzyme as a transcript (shown attached only through its 3' end for clarity) is extruded. Centre: low magnification view illustrating assembly of a replication factory. Two G₁ transcription factories (ovals with attached loops in upper panel) on a skeleton (straight line) seed assembly at the G₁/S boundary of a replication factory (oval in lower panel) containing DNA polymerases. Right: high magnification view of replication (R). Three potential origins in a loop are now close to a DNA polymerase in the factory. Replication initiates as one origin attaches; then templates slide (curved arrows) past the fixed DNA polymerase as nascent DNA is extruded.

merases are dynamic in the sense that attached DNA sequences change as templates slide past. Late during G₁, some as yet undefined signal (mediated by a kinase?) triggers assembly of a replication factory around ~2 transcription factories (Hassan et al., 1994). Here the structures involved in transcription nucleate the assembly of replication factories.

Transcription may also bring an origin close to a factory so that it can attach. Consider a loop bearing three potential origins, O₁₋₃, buried in a cluster of heterochromatic loops. Origins in peripheral loops in the cluster are more likely than O₁₋₃ to attach to a factory and initiate. Replication of peripheral loops, which involves dragging them past fixed polymerases, will inevitably expose our inner loop, increasing the probability that a promoter within it can attach to RNA polymerases in the joint transcription and replication factory. When it does, our loop is subdivided into smaller loops and O₁₋₃ become more closely tethered to the DNA polymerases that are intermingled with the RNA polymerases on the surface of the factory. Whether O₂ now competes more effectively than O₁ or O₃ for bound initiating proteins will depend on their relative proximities. Therefore, different origins will be used, even in cells of one particular type. Here transcription from a promoter adjacent to an origin generates a mini-loop, shortening the length of an origin's tether and enhancing the chances that the origin can bind.

This probabilistic model has various consequences. (1) As the first replication factories are assembled around transcription factories, transcribed genes are likely to be replicated earlier than heterochromatin. (2) Genes that are adjacent on a chromosome will tend to be clustered into the same transcription factory and so replicated together. Then the bands seen in mitotic chromosomes (Craig and Bickmore, 1993) may be vestiges of transcription factories still associated with genes

that are replicated together. (3) Replication initiates close to transcription units, as in viruses (see above) and in the rDNA cluster (Little et al., 1993; Hyrien and Mechali, 1993). (4) Once transcription has stimulated origin binding, the chromatin fibre becomes locally attached through both an RNA polymerase and a DNA polymerase and it seems unlikely that such an RNA polymerase could then maintain its activity (Brewer, 1988). In yeast, binding of the origin recognition complex does indeed inhibit or silence transcription from the adjacent promoter (Bell et al., 1993; Foss et al., 1993). (5) Nature faces a dilemma when designing the tie for the base of a loop: it must be stable enough to maintain looping, but not so stable that it prevents replication. Sliding ties involving polymerases allow attachments (and so looping) to be maintained during replication. For example, replication of the 5' end of a transcription unit, held through an RNA polymerase at the 3' end, generates two promoters that are close to a transcription/replication factory and so likely to reattach.

According to this model, transcription promotes the initiation of replication in three ways: (1) transcription factories seed formation of replication factories; (2) transcriptional attachments create small loops that bring origins closer to binding sites in factories; and (3) the act of transcription itself unwinds adjacent origins. In somatic cells, where transcription and replication occur together, all three mechanisms operate. In frogs' eggs, replication has a much greater priority than transcription, and this is reflected by a concentration of replication factors that is so high that even added λ DNA can be organized into replication foci and forced to replicate (Cox and Laskey, 1991). The few transcription factors and RNA polymerases, perhaps already organized into factories, which are present may be insufficient to unwind every origin but sufficient to nucleate the formation of the necessary replication

factories and to bring origins close to binding sites on them. Viruses, with templates that are specially adapted to compete effectively with cellular promoters/origins for scarce binding-sites, may represent a different kind of special case. After attaching to a factory, early transcription of SV40 virus eventually generates sufficient T antigen to displace cellular initiator proteins from factories. Then, the proximity of the viral origin, plus its affinity for T antigen, ensure that the origin engages. But even in these special cases, at least one of the three mechanisms is involved in the initiation of replication. Only in vitro, where factories are disassembled and the system optimized for replication, can replication initiate in the complete absence of RNA polymerases and transcription factors.

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REFERENCES

- Adolph, S., Brusselbach, S. and Muller, R. (1993). Inhibition of transcription blocks cell cycle progression of NIH3T3 fibroblasts specifically in G₁. *J. Cell Sci.* **105**, 113-122.
- Andrews, B. J. (1992). Dialogue with the cell cycle. *Nature* **335**, 393-394.
- Baker, T. A. and Kornberg, A. (1988). Transcriptional activation of initiation of replication from the *E. coli* chromosomal origin: an RNA-DNA hybrid near *oriC*. *Cell* **55**, 113-123.
- Bell, S. P., Kobayashi, R. and Stillman, B. (1993). Yeast origin recognition complex functions in transcription silencing and DNA replication. *Science* **262**, 1844-1849.
- Bramhill, D. and Kornberg, A. (1988). A model for the initiation at origins of replication. *Cell* **54**, 915-918.
- Brewer, B. J. (1988). When polymerases collide: replication and transcriptional organization of the *E. coli* chromosome. *Cell* **53**, 679-686.
- Brown, J. L., Sonoda, S., Ueda, H., Scott, M. P. and Wu, C. (1991). Repression of the *Drosophila fushi tarazu* (*ftz*). *EMBO J.* **10**, 665-674.
- Carter, K. C., Bowman, D., Carrington, W., Fogarty, K., McNeil, J. A., Fay, F. S. and Lawrence, J. B. (1993). A three-dimensional view of precursor messenger RNA metabolism within the nucleus. *Science* **259**, 1330-1335.
- Challberg, M. D. and Kelly, T. J. (1989). Animal virus DNA replication. *Annu. Rev. Biochem.* **58**, 671-717.
- Chen, M., Mermoud, N. and Horwitz, M. S. (1990). Protein-protein interactions between adenovirus DNA polymerase and nuclear factor I mediate formation of the DNA replication preinitiation complex. *J. Biol. Chem.* **265**, 18634-18642.
- Cheng, L. Z., Workman, J. L., Kingston, R. E. and Kelly, T. J. (1992). Regulation of DNA replication *in vitro* by the transcriptional activation domain of GAL4-VP16. *Proc. Nat. Acad. Sci. USA* **89**, 589-593.
- Cook, P. R. (1989). The nucleoskeleton and the topology of transcription. *Eur. J. Biochem.* **185**, 487-501.
- Cook, P. R. (1991). The nucleoskeleton and the topology of replication. *Cell* **66**, 627-635.
- Cox, L. S. and Laskey, R. A. (1991). DNA replication occurs at discrete sites in pseudonuclei assembled from purified DNA *in vitro*. *Cell* **66**, 271-275.
- Craig, J. M. and Bickmore, W. A. (1993). Chromosome bands - flavours to savour. *BioEssays* **15**, 349-354.
- DePamphilis, M. L. (1993). Eukaryotic DNA replication: anatomy of an origin. *Annu. Rev. Biochem.* **62**, 29-63.
- Edgar, B. A., Kiehle, C. P. and Schubiger, G. (1986). Cell cycle control by the nucleocytoplasmic ratio in early *Drosophila* development. *Cell* **44**, 365-372.
- Edgar, B. A. and Schubiger, G. (1986). Parameters controlling transcriptional activation during early *Drosophila* development. *Cell* **44**, 871-877.
- Fangman, W. L. and Brewer, B. J. (1992). A question of time: replication origins of eukaryotic chromosomes. *Cell* **71**, 363-366.
- Ferguson, B. M. and Fangman, W. L. (1992). A position effect on the time of replication origin activation in yeast. *Cell* **68**, 333-339.
- Foss, M., McNally, F. J., Laurenson, P. and Rine, J. (1993). Origin recognition complex (ORC) in transcriptional silencing and DNA replication in *S. cerevisiae*. *Science* **262**, 1838-1844.
- Guo, Z.-S. and DePamphilis, M. L. (1992). Specific transcription factors stimulate simian virus 40 and polyomavirus origins of DNA replication. *Mol. Cell. Biol.* **12**, 2514-2524.
- Hassan, A. B. and Cook, P. R. (1993). Visualization of replication sites in unfixed human cells. *J. Cell Sci.* **105**, 541-550.
- Hassan, A. B., Errington, R. J., White, N. S., Jackson, D. A. and Cook, P. R. (1994). Replication and transcription sites are colocalized in human cells. *J. Cell Sci.* **107**, 425-434.
- Hatton, K. S., Dhar, V., Brown, E. H., Iqbal, M. A., Stuart, S., Didamo, V. T. and Schildkraut, C. L. (1988). Replication program of active and inactive multigene families in mammalian cells. *Mol. Cell. Biol.* **8**, 2149-2158.
- He, Z., Brinton, B. T., Greenblatt, J., Hassell, J. A. and Ingles, C. J. (1993). The transactivator proteins VP16 and GAL4 bind replication factor A. *Cell* **73**, 1223-1232.
- Heintz, H. H. (1992). Transcription factors and the control of DNA replication. *Curr. Opin. Cell Biol.* **4**, 459-467.
- Hisatake, K., Hasegawa, S., Takada, R., Nakatani, Y., Horikoshi, M. and Roeder, R. G. (1993). The p250 subunit of native TATA box-binding factor TFIID is the cell cycle regulatory protein CCG₁. *Nature* **362**, 179-181.
- Hoang, A. T., Wang, W. and Gralla, J. D. (1992). The replication activation potential of selected RNA polymerase II promoter elements at the simian virus 40 origin. *Mol. Cell. Biol.* **12**, 3087-3093.
- Hozák, P., Hassan, A. B., Jackson, D. A. and Cook, P. R. (1993). Visualization of replication factories attached to a nucleoskeleton. *Cell* **73**, 361-373.
- Hyrien, O. and Mechali, M. (1993). Chromosomal replication initiates and terminates at random sequences but at regular intervals in the ribosomal DNA of *Xenopus* early embryos. *EMBO J.* **12**, 4511-4520.
- Jackson, D. A. and Cook, P. R. (1993). Transcriptionally-active minichromosomes are attached transiently in nuclei through transcription units. *J. Cell Sci.* **105**, 1143-1150.
- Jackson, D. A., Hassan, A. B., Errington, R. J. and Cook, P. R. (1993). Visualization of focal sites of transcription within human nuclei. *EMBO J.* **12**, 1059-1065.
- Jones, K. A., Kadonaga, J. T., Rosenfeld, P. J., Kelly, T. J. and Tjian, R. (1987). A cellular DNA-binding protein that activates eukaryotic transcription and DNA replication. *Cell* **48**, 79-89.
- Keppel, F., Fayet, O. and Georgopoulos, C. (1988). Strategies of bacteriophage DNA replication. In *The Bacteriophages*, vol 2 (ed. R. Calendar), pp. 145-262. Plenum Press, New York.
- Kill, I. R., Bridger, J. M., Campbell, K. H. S., Maldonado-Codina, G. and Hutchison, C. J. (1991). The timing of the formation and usage of replicase clusters in S-phase nuclei of human diploid fibroblasts. *J. Cell Sci.* **100**, 869-876.
- Kimelman, D., Kirschner, M. and Scherson, T. (1987). The events of the midblastula transition in *Xenopus* are regulated by changes in the cell cycle. *Cell* **48**, 399-407.
- Kokubo, T., Gong, D.-W., Yamashita, S., Horikoshi, M., Roeder, R. G. and Nakatani, Y. (1993). *Drosophila* 23-kD TFIID subunit, a functional homologue of the human cell cycle gene product, negatively regulates DNA binding of the TATA box-binding subunit of TFIID. *Genes Dev.* **7**, 1033-1046.
- Kornberg, A. and Baker, T. (1992). *DNA replication*. 2nd edition. W. H. Freeman, New York.
- Lark, K. D. (1972). Evidence for the direct involvement of RNA in the initiation of DNA replication in *Escherichia coli* 15P-. *J. Mol. Biol.* **64**, 47-60.
- Laskey, R. A., Fairman, M. P. and Blow, J. J. (1989). S phase of the cell cycle. *Science* **246**, 609-614.
- Li, J. J. and Kelly, T. J. (1984). Simian virus 40 DNA replication *in vitro*. *Proc. Nat. Acad. Sci. USA* **81**, 6973-6977.
- Li, R. and Botchan, M. R. (1993). The acidic transcriptional activation domains of VP16 and p53 bind the cellular replication protein A and stimulate *in vitro* BPV-1 DNA replication. *Cell* **73**, 1207-1221.
- Little, R. D., Platt, T. H. K. and Schildkraut, C. L. (1993). Initiation and termination of DNA replication in human rRNA genes. *Mol. Cell. Biol.* **13**, 6600-6613.

- Mann, C., Micouin, J. Y., Chiannikulchai, N., Treich, I., Buhler, J. M. and Sentenac, A.** (1992). RPC53 encodes a subunit of *Saccharomyces cerevisiae* RNA polymerase C (III) whose inactivation leads to a predominantly G₁ arrest. *Mol. Cell. Biol.* **12**, 4314-4326.
- Marahrens, Y. and Stillman, B.** (1992). A yeast chromosomal origin of DNA replication defined by multiple functional elements. *Science* **255**, 817-823.
- 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.
- Newport, J. and Kirschner, M.** (1982a). A major developmental transition in early *Xenopus* embryos: I. Characterization and timing of cellular changes at the midblastula stage. *Cell* **30**, 675-686.
- Newport, J. and Kirschner, M.** (1982b). A major developmental transition in early *Xenopus* embryos: II. Control of the onset of transcription. *Cell* **30**, 687-696.
- O'Keefe, R. T., Henderson, S. C. and Spector, D. L.** (1992). Dynamic organization of DNA replication in mammalian cell nuclei: spatially and temporally defined replication of chromosome-specific α -satellite sequences. *J. Cell Biol.* **116**, 1095-1110.
- O'Neill, E. A., Fletcher, C., Burrow, C. R., Heintz, N., Roeder, R. G. and Kelly, R. G.** (1988). Transcription OTF-1 is functionally identical to the DNA replication factor NF-III. *Science* **241**, 1210-1213.
- Ruppert, S., Wang, E. H. and Tjian, R.** (1993). Cloning and expression of human TAF_{II}250: a TBP-associated factor implicated in cell cycle regulation. *Nature* **362**, 175-179.
- Sekiguchi, T., Nohiro, Y., Nakamura, Y., Hisamoto, N. and Nishimoto, T.** (1991). Human *CCG1* gene, essential for progression of the G₁ phase, encodes a 210-kilodalton nuclear DNA-binding protein. *Mol. Cell. Biol.* **11**, 3317-3325.
- Waechter, D. E., Avignolo, C., Freund, E., Riggenbach, C., Mercer, W., McGuire, P. M. and Baserga, R.** (1984). Microinjection of RNA polymerase II corrects the temperature-sensitive defect of tsAF8 cells. *Mol. Cell. Biochem.* **60**, 77-82.
- Wansink, D. G., Schul, W., van der Kraan, I., van Steensel, B., van Driel, R. and de Jong, L.** (1993). Fluorescent labelling of nascent RNA reveals transcription by RNA polymerase II in domains scattered throughout the nucleus. *J. Cell Biol.* **122**, 283-293.