DNA replication in eukaryotes: a model for the specific involvement of chromatin subunits.

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ABSTRACT

A model is proposed whereby eukaryotic DNA replication is specifically directed by the 200 base pair repeat structure of the DNA-histone complex. The model proposes a mechanism for the sequential, bidirectional replication of DNA from initial origin points on the chromatin fibre and is consistent with the known properties of eukaryotic DNA replication. Several predictions can be made from the model which are amenable to testing.

Recent studies of eukaryote chromatin¹⁻⁷ have resulted in the proposal of basic structure in which the DNA is coiled on the exterior of a protein backbone^{3-5,7,8}. The backbone appears to be composed of repetitive histone oligomeric subunits^{3,4}. Sites susceptible to hydrolysis by some DNases are situated at a repeat distance of approximately 200 base pairs on the DNA. This 200 base pair periodicity is presumed to represent the repeat distance between chromatin protein subunits^{1,2} and correlates with estimates of periodicity obtained from X-ray diffraction⁴, neutron scattering^{3,5} and electron microscopy⁶. The amount of DNA exposed between the subunits is not precisely known but must comprise less than 15% of the total nuclear DNA².

The extent of enzymatic digestion of chromatin DNA depends to a large extent on which DNase is used to probe the chromatin structure. The endogenous nuclear $\text{Ca}^{++}\text{-Mg}^{++}$ endonuclease digests chromatin DNA at the 200 base pair repeat only¹, whilst micrococcal nuclease attacks the same site but will digest also at one further site within the 200 base pair periodicity². DNase I is only partially specific and on extended exposure attacks virtually all of the DNA^{1,7}. Analyses of partial digests of chromatin by DNase I show that the enzyme

digests the chromatin DNA at a repeat distance of 10 base pairs⁷. It has been proposed that the 10 base pairs repeat represents successive turns of the DNA helix⁷ but it is possible that the repeat reflects some other aspects of the DNA-protein conformation within the protein subunits themselves⁷. In intact nuclei, the number of sites accessible to some endonucleases is reduced, compared with isolated chromatin, and a second order of complexity has been proposed whereby access to some of the available sites is blocked^{1,9}. It has been proposed that histone H1 is located between the oligomeric subunits^{3,8} and may be responsible for this reduced access. Nonhistone protein could also block the access of certain enzymes to these regions but the possible contribution of these proteins is uncertain. Thus, the extent of steric inhibition of various enzymes by the chromosomal proteins depends on the nature and properties of the particular enzyme studied. Some enzymes acting on DNA appear to be almost completely inhibited by the structure of the nuclear DNA-protein complex, whilst others exhibit only marginal inhibition, and act on virtually all of the nuclear DNA.

For the purposes of the model proposed below it is postulated that the enzymes acting on DNA within the eukaryotic cell nucleus should be inhibited to varying degrees by the structural proteins of the chromatin fibres. For convenience the enzymes have been divided into three general classes with intermediate sub-classes also possible.

The first class consists of those proteins and enzymes for which access to the nuclear DNA is not restricted by the chromatin proteins. The existence of this class is a function of the location of the DNA on the outside of the protein subunits^{3,7,8}, the DNA being available for association with those enzymes possessing the appropriate tertiary or quaternary structure, such as DNase I. Since the majority of eukaryotic nuclear DNA is organized in a repeating subunit structure²⁷, 'transcriptional' RNA polymerases and DNA binding proteins involved in the regulation of gene expression must belong to this

class of proteins. In the model below it is assumed that the replicative DNA polymerase is a member of this class also.

The second class comprises those enzymes which, because of their tertiary or quaternary structure, are restricted in action to the sites between the chromatin protein subunits. Several nucleases belong to this class, for example DNase II²⁴ and the endogenous nuclear $\text{Ca}^{++}\text{-Mg}^{++}$ DNase^{1,19}. Micrococcal nuclease is intermediate between this and the first class^{2,9}.

The third class is an extension of the second class and comprises the proteins and enzymes which have very restricted access to the DNA. These enzymes act on the DNA between the protein subunits at a very limited number of sites, where the DNA is exposed by the removal or modification of a secondary structural protein. A Mg^{++} dependent DNase has been observed in nuclear preparations which is possibly a member of this enzyme class²⁹. The nuclear $\text{Ca}^{++}\text{-Mg}^{++}$ DNase behaves in a manner suggesting this class of enzymes when acting on nuclear DNA in situ^{1,19}.

In the proposed model, the replication of nuclear DNA is regulated and directed by the properties of the enzymes involved at each stage, relative to these three classifications.

Mammalian DNA replication is bidirectional¹⁰ and proceeds by the synthesis of short fragments of DNA which are subsequently joined into high molecular weight DNA^{11,12}, in a manner similar to bacterial replication. The nascent DNA fragments are, however, an order of magnitude shorter in mammalian systems than bacterial systems^{13,14,15} and, in mammalian cells, are within the size of the 200 base repeat of nuclease susceptibility in the chromatin structure^{14,15}. Recently it has been demonstrated that the nascent DNA fragments of replicating eukaryote DNA possess a short segment of RNA at their 5' ends which presumably acts as a primer for DNA polymerase action^{16,17}.

A simple model for eukaryote DNA replication has been constructed from these observations. It is assumed that on initiation of DNA synthesis a

Figure I.

Diagrammatic representation of the initiation of DNA replication in a chromatin fiber.

A. RNA polymerase initiates priming at an accessible site (---->). B. DNA synthesis begins from the primer (--->). Rotation of the helix is transmitted to the flexible sites above and below the origin (↺) which causes these sites to be exposed (represented by displacement of the blocking protein). C. Further sites are exposed. The initial RNA primer is excised and RNA priming initiates on the displaced strand. D. The nascent DNA chains are ligated. DNA synthesis and further primers are initiated on the displaced strand. The original chromatin conformation is restored in the replicated region and the initial site is blocked to further replication (→■). For simplicity, the helical structure of the DNA, coiling of the DNA around the proteins and base pairing between strands of DNA have been omitted.

DNA == ; RNA ; Protein subunit ● ; Secondary blocking protein ■.

specific RNA polymerase is induced or activated which is sterically inhibited to a very large degree by the various chromatin proteins. The RNA polymerase would be a member of the third class of enzyme proteins defined above. The majority of the DNA would be unavailable as a template for this RNA polymerase because of the primary protein backbone and the secondary blocking of sites between the histone oligomers. Thus, initiation of RNA synthesis would occur on the DNA at the relatively few available sites between the protein oligomers. The spacing of these sites would be determined by the binding of particular

proteins to specific positions on the chromatin or the modification of proteins bound to these inter-subunit sites, as proposed elsewhere^{3,20}. The RNA primer synthesized would be of a short length and would not necessarily possess a unique base composition (Figure 1A).

DNA synthesis would be initiated on the RNA primers by a replicative DNA polymerase. It is assumed that this DNA polymerase would not be inhibited by the chromatin proteins but possess the ability to synthesize DNA on the surface of the histone protein oligomer. A transient release of the DNA from the histones of the subunit may occur as replication proceeds but extensive dissociation would not be required. Secondary sites for RNA primer synthesis would then become available on either side of the first initiation site.

A simple mechanism by which this could occur is proposed. It is assumed that the DNA is strongly bound to a specific site or groove in the histone oligomer. In this case, the DNA bound to the protein subunits would be held in a relatively rigid conformation and torsion or flexion of the chromatin fibre would occur at the more flexible sites between the protein subunits, where the DNA is less strongly bound to the protein.

The initiation of RNA priming and DNA synthesis in the inter-subunit sites would tend to generate supercoiling in the parental DNA helix as the two daughter strands are separated. Because the DNA-subunit complexes would tend to behave as single units, this supercoiling could not be expressed in DNA bound to the protein oligomers but would be transmitted to the more flexible regions between the protein oligomers immediately on either side of the replication initiation site. This transmission of supercoiling along the chromatin fibre would cause the protein subunits on either side of the initiation site to alter their orientation with respect to the next pair of subunits. The change in orientation would result in the DNA between the pairs of subunits on either side of the initiation site becoming more accessible to RNA polymerase (Fig. 1B). The extent of relative displacement of the protein subunits could

be either an overall rotation of the subunits relative to their nearest neighbours, creating open sites for enzyme action, or a minor shift which would cause secondary blocking proteins to displace and expose the DNA which they normally protect (Fig. 1B). This mechanism would ensure that replication proceeded sequentially in either direction from the primary initiation point. The replication forks would move along the chromatin fibre, preceded by a series of minor conformational changes in the chromatin fibre substructure.

The sites for synthesis of RNA primers on the antiparallel complementary DNA strand may not be located as specifically as those on the strand remaining associated with the parental chromatin fibre, but would probably tend to occur on the displaced strand at or near the initial sites of RNA polymerase action (Fig. 1C). This region of DNA would be the first to be exposed to RNA polymerase when it is displaced from the parental chromatin fibre (Fig. 1C). The displaced strand could possibly associate with a low affinity site on the histone oligomer until sufficient replication has occurred, whereupon it would associate with new histone oligomers, and be effectively removed and isolated from the parent chromatin fibre²³. When a region of the DNA has been replicated, restoration of the original chromatin structure and blocking of the initial priming site would prevent the initiation of further replication cycles (Fig. 1D).

One feature of this model is that one can predict that the basic chromatin structure would not be extensively disrupted during replication. Studies of chromatin structure during DNA replication in normal and protein depleted nuclei have not demonstrated any alteration in the basic chromatin repeat structure during replication^{21,22} and it has been proposed that DNA is not extensively dissociated from chromatin proteins during replication²². Secondly, it is not assumed or required that there is any DNA base specificity for either the primary initiation of RNA primer synthesis or for any subsequent primer initiation. The initiation of DNA polymerisation can also be independent

of base sequence. All template selectivity is conferred by proteins bound to the DNA between the histone oligomer subunits and by the properties of the subunits themselves. Thus the base composition of the RNA primers would reflect only the average base composition of the total DNA. Although the primer RNA has not been extensively studied in eukaryotic systems, preliminary observations suggest that the base composition of RNA primers may be random¹⁷. This lack of base specificity has been observed during polyoma virus replication¹⁸. Polyoma virus replication takes place in the nucleus of mammalian cells and it has been proposed that the viral DNA replicates via a mechanism similar to that of eukaryote chromosomal DNA¹⁸. Replicating DNA of the closely related virus, simian virus 40, exists as a chromatin-like complex with mammalian histones²⁶.

The inevitable consequence of the model is that the replication proceeds sequentially and in both directions from the initial origin of replication. The replicon size observed by Huberman and Riggs¹⁰, is represented in this model by the average distance between the primary initiation events.

It can be predicted from the model that the DNA strand displaced during DNA synthesis would be less protected from DNase action than the DNA strand which remains associated with the chromatin subunit. Newly replicated DNA has been observed to possess enhanced nuclease susceptibility^{21,23} which decreases to resting levels of susceptibility within a short time after replication. In the model proposed, one strand of the DNA helix would always remain associated with pre-existing chromosomal proteins during and after replication (assuming minimal exchange of subunit components) while its complementary strand would associate with new proteins, either from a pre-existing pool or from proteins synthesized during replication. This has been observed in mammalian cells in tissue culture²³. It is predicted that DNA synthesized on this complementary strand would be the DNA which exhibits the greatest nuclease susceptibility.

In this model, as with other models of DNA synthesis, the size of the

nascent DNA fragments would be determined by the relative rates of initiation, polymerization, primer removal and ligation.

In systems where polymerization is rate limiting, all monomeric fragments should be less than 200 bases long, as is the apparent situation in vivo^{14,15}. If RNA primer removal or joining of the nascent fragments becomes rate limiting, accumulation of fragments of 200 base length should be observed, which would indicate the existence of a periodicity of primer initiation at the 200 base repeat site. In systems deficient in initiation, fragments greater than the unit length would predominate as the DNA polymerase would continue synthesis past the inter-subunit regions.

As presented, the model is highly simplified and omits many of the processes which must act on the replicating chromosomal DNA. For example, although limited supercoiling of the DNA during replication is a necessary feature of the model, some mechanism must be provided for the eventual relief of the DNA supercoils generated, in order that the daughter DNA strands could separate and the initial chromatin conformation be restored. An enzyme similar to the " ω " protein of E. coli²⁵ could accomplish this unwinding and can be very easily accommodated by the model. Enzymes equivalent to the " ω " protein have been isolated from mammalian cells²⁸. The model explains several features of mammalian DNA replication and makes several predictions which can be tested. These predictions are summarized:

1. The nascent DNA fragments produced during DNA replication should be associated with chromatin subunits and possess lengths which are less than, or equal to the 200 base subunit repeat.
2. The DNA polymerase responsible for mammalian DNA replication should not be extensively inhibited by chromatin proteins.
3. A specific RNA polymerase should be present during replication which would not use native chromatin readily as a template in vitro.
4. The RNA primers attached to nascent DNA fragments should possess a

random base composition and have lengths less than that of the DNA between the chromatin subunits.

5. The replicating DNA strands should exhibit an asymmetry of nuclease susceptibility.

6. One strand of the replicating DNA helix should remain associated with pre-existing chromatin subunits during and after replication. This phenomenon has been observed to occur during replication in the presence of cycloheximide²².

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REFERENCES

1. Hewish, D.R. and Burgoyne, L.A. (1973) *Biochem. Biophys. Res. Comm.* 52, 504-510.
2. Noll, M. (1974) *Nature* 251, 249-251.
3. Baldwin, J.P., Bosely, P.G. and Bradbury, E.M. (1975) *Nature* 253, 245-249.
4. Kornberg, R.D. (1974) *Nucleic Acids Res.* 1, 1573-1578.
5. Bram, S., Butler-Browne, G., Baudy, P. and Ibel, L. (1975) *Proc. Natl. Acad. Sci.* 72, 1043-1045.
6. Olins, A.L. and Olins, D.E. (1974) *Science* 183, 330-332.
7. Noll, M., (1974) *Nucleic Acids Res.* 1, 1573-1578.
8. Van Holde, K.E., Sahasrabudhe, C.G. and Shaw, B.R. (1974) *Nucleic Acids Research* 1, 1579-1586.
9. Sollner-Webb, B. and Felsenfeld, G. (1975) *Biochemistry* 14, 2915-2920.
10. Huberman, J.A. and Riggs, A.D. (1968) *J. Mol. Biol.* 32, 327-341.
11. Schandl, E.K. and Taylor, J.H. (1969) *Biochem. Biophys. Res. Commun.* 34, 291-300.
12. Painter, R.B., Shaeffer, A. (1969) *Nature* 221, 1215-1217.
13. Okazaki, R., Okazaki, T., Sakabe, K., Sugimoto, K. and Sugino, A. (1968) *Proc. Natl. Acad. Sci. U.S.A.* 69, 598-605.
14. Nuzzo, F., Brega, A. and Falaschi, A. (1970) *Proc. Natl. Acad. Sci. U.S.A.* 65, 1017-1024.
15. Gautschi, J.R. and Clarkson, J.J. (1975) *Europ. J. Biochem.* 50, 403-412.
16. Waqar, M.A. and Huberman, J.A. (1973) *Biochem. Biophys. Res. Commun.* 51, 174-179.
17. Waqar, M.A. and Huberman, J.A. (1975) *Biochim. Biophys. Acta* 383, 410-420.
18. Reichard, P., Eliasson, R. and Soderman, G. (1974) *Proc. Natl. Acad. Sci. U.S.A.* 71, 4901-4905.
19. Burgoyne, L.A. and Mobbs, J. (1975) *Nucleic Acids Res.* 2, 1551-1558.
20. Stein, G.S., Spelsberg, T.C., and Kleinsmith, L.J. (1974) *Science* 183, 819-823.

21. Searle, R.L. (1975) *Nature* 255, 247-249.
22. Searle, R.L. and Simpson, R.T. (1975) *J. Mol. Biol.* 94, 479-501.
23. Weintraub, H. (1973) *Cold Spring Harbor Symp. Quant. Biol.* XXXVIII, 247-256.
24. Spadafora, C. and Geraci, G. (1975) *FEBS Letters* 57, 79-82.
25. Wang, J.C. (1971) *J. Mol. Biol.* 55, 523-533.
26. Griffith, J.D. (1975) *Science* 187, 1202-1203.
27. Burgoyne, L.A., Hewish, D., and Mobbs, J. (1974) *Biochem. J.* 143, 67-72.
28. Keller, W. (1975) *Proc. Natl. Acad. Sci. U.S.A.* 72, 2550-2554.
29. Hewish, D.R. and Burgoyne, L.A. (1973) *Biochem. Biophys. Res. Comm.* 52, 475-481.