Acta Biochimica Polonica

Vol. 42 No. 2/1995

127–132

QUARTERLY

Minireview

The paper was presented at the "First International Seminar on Nuclear Matrix"

Functional aspects of the nuclear matrix

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Key words: nuclear matrix, DNA replication, DNA unwinding, MAR, SAR

A model is proposed of the way in which the unwinding of the chromosomal DNA loops is controlled during DNA replication. It is based on the observation of a permanent binding of replication origins to the nuclear matrix and of a transient attachment of replicating DNA regions to sites in the immediate neighbourhood. DNA unwinding is controlled while the replicating loops are reeled through the replication binding sites.

Also a mechanism is proposed to explain how the once-per-cycle replication of individual replicons can be controlled. DNA synthesis is initiated at single-stranded loops exposed by tandemly repeated DNA sequences at the replication origins. The single-stranded loops turn into fully double-stranded DNA during replication, becoming inaccessible for a second initiation during the same cell cycle. The configuration competent for initiation is restored by specific protein-DNA rearrangements coupled to mitotic condensation of the matrix into chromosomal scaffolds and its reversal.

According to the original definition proposed by Berezney & Coffey in 1974 [1] the nuclear matrix consists of a residual nuclear envelope and an internal fibro-granular structure primarily composed of proteins. If we deal with the nuclear matrix we always have to be aware that, depending on the specific nuclear process which is studied, different preparation methods have to be used. This unavoidably leads to more or less different results with regard to the ultrastructure, and even more, to the protein composition. Such differences have been the cause of some disagreement in early matrix research. At the present stage I would say the nuclear matrix consists of a basic protein skeleton to which a number of structural components are bound in a more or less ordered fashion. The latter may be responsible for nuclear activities like DNA transcription, its regulation, RNA processing and others. These structures become removed by more rigorous preparation procedures.

ORDERING OF DNA REPLICATION

We have been interested in eukaryotic DNA replication for some time. There are two specific features by which eukaryotic genomes differ from prokaryotic ones. One is that the large mass of DNA is subdivided into many thousands of replicons that replicate more or less independently from each other. To warrant genetic continuity it is strictly necessary that each replicon is duplicated just a single time per cell cycle. The other difference results from the fact that the genome is subdivided into a species-specific number of chromosomes, some of which contain a DNA molecule of up to several centimeters in length equivalent to several millions of helical turns. These molecules

have to become completely unwound during replication and the two daughter molecules must be positioned side by side for proper separation in mitosis.

The diagram in Fig. 1 shows how the unwinding can be conceived. Part 1a shows a domain of the genome with consecutive origins of replication bound in close succession to the protein backbone. Part 1b shows the same domain after its replication in a position ready for mitotic separation. Details of the unwinding of the double helix and proper positioning of the daughter molecules are shown in parts 1 c–g. Soon after replication is initiated the fork regions become bound to adjacent sites called replication binding sites. During DNA synthesis the DNA strands are reeled through the binding sites and unwound as if separated by a wedge. Of course, the unwinding must be made possible by transient single-strand nicks ahead of the replication fork. Finally, when the replication of the loop is completed its terminal region is released from the backbone which sooner or later must also be duplicated by assembly of a new set of proteins.

Evidence for the existence of such a structure has been obtained by isolating mammalian cell nuclei in the presence of nonionic detergents and extracting them with 2 M NaCl in the absence of divalent cations. If the residual material is digested exhaustively with DNase and spread on a water surface for electron microscopy, the internal matrix appears as a filamentous network [2]. When microsections are prepared from matrix material that has been digested only briefly with DNase one finds short tracks of filaments from which fragments of DNA threads emerge, showing that the filaments serve as protein backbones for the DNA attachment. Permanent attachment of replication origins to the matrix filaments was documented by pulse-labelling synchronous cells with [^{3}H]dThd at the beginning of the S phase followed by a chase into G_2 or the next G_1 phase. The label was recovered in the matrix-associated DNA regions in *Physarum* and mammalian and sea urchin cells cultured *in vitro* [3–5]. Similar results were obtained by other techniques [6].

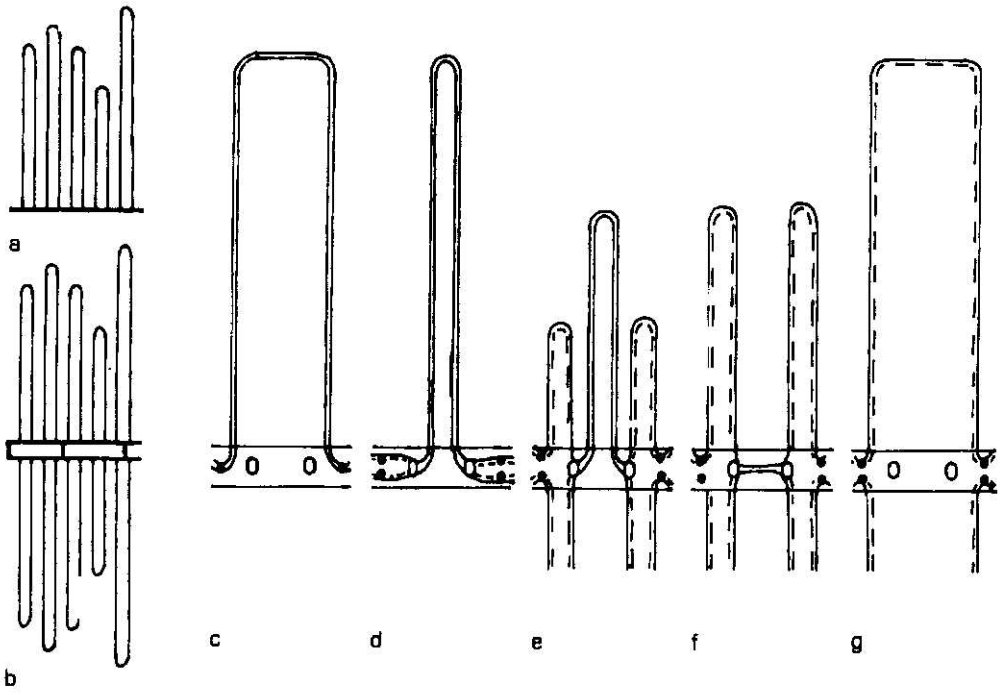

Fig. 1. Diagram of a model of the untwining of replicating DNA.
a, Part of a chromosome with DNA bound to a protein backbone by consecutive origins of replication; b, the same part after its duplication and ready for mitotic separation; c, magnified segment of (a) with replication origins bound to origin attachment sites (filled circles); d, after the onset of replication the duplicated origins separate from each other and DNA regions close to the replication forks become attached to replication attachment sites (empty ovals); e, the replicating DNA loop is untwined by beeing reeled through the replication attachment sites; f, and g, fully duplicated and untwined DNA loop before and after release from the replication binding site.

Table 1

Repetetive sequence in the cloned matrix associated regions (MAR) of Physarum DNA.

MARs, of *Physarum polycephalum* $M_{3c}IV$ were cloned into M13 mp10 [8]. Tandem repeats of 12 to 16 bp length are underlined. The lines above the sequences indicate the longest repeats. Numbers preceding and following the sequences stand for random base pairs.

Clone number

PhyMAR 1 0-CATTGTTGTTGTTGTTGTTGTTGTTGTTGTTG-0

PhyMAR 2 0-CATGCTCATGCTCATGCTCATGCTCATGCTCATGCTCATGCTCATGCTCATGCTCATGCTCATGCTCATGCTCATGCTCATGTCAAT-0

PhyMAR 3 0-AGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGAAGAGA-0

PhyMAR 4 0-GGTCATTTGGTCATTTGGTCATTTGGTCATTTGGTCATTTGGTCATTTGGTCATTTGGTCATTTGGTCATTTGGTCATTTGGTCATTT

GGTCATTTGGTCATTTGGTCATTTGGTCATTTGGTCATTT-35

PhyMAR 5 0-TTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTATTA

TTATTATTATTATTATTATTATTATTATTATTATTATTA-0

PhyMAR 6 0-GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG-0

PhyMAR 7 0-CTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAAC-0

PhyMAR 8 116-CACACACACACACACACACACACACACACACACACACACACA-0

PhyMAR 9 77- ACCACACCACACCACACCACACCACACCACACCACACCACACCACACCACACCACACCACACCACACC-43

PhyMAR 10 0-TGTTTTGTTTTGTTTTGTTTTGTTTGTTTTGTTTTGTTTTGTTTTGTTTTGTTTTGTTTTGTTTTGTTTTGGTGGG-0

PhyMAR 11 0-TTATTTATTTATTTATTTATTTATTTATTTATTTATTTATTTAGATATTTATTTAGATATTTATTTATTTATTTA-13

PhyMAR 12 0-TCCTTTCCTTTCCTTTCCTTTCCTTTCCTTTCCTTTCGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAA

GGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGGAAAGG

AAAGGAAAGGAAA-0

PhyMAR 13 0-GTTTTTGTTTTTGTTTTTGTTTGTTTTTGTTTTTGTTTTGTTTTTGTTTTCTTTTTCTTTTTGTTTTTGTTTT-19

PhyMAR 14 16-TGAGAAGAGAGAGAAGTGAGAAGAGAGAGAAGTGAAAGTGAGAAGAGAGAGAAGTGAAGAGAGAGAA->100

PhyMAR 15 17-AAATAAAATAAAACAAAATAAAATAAA-67

PhyMAR 16 15-GTTTTTGTCTTTGTTTTTGTCTTT-110

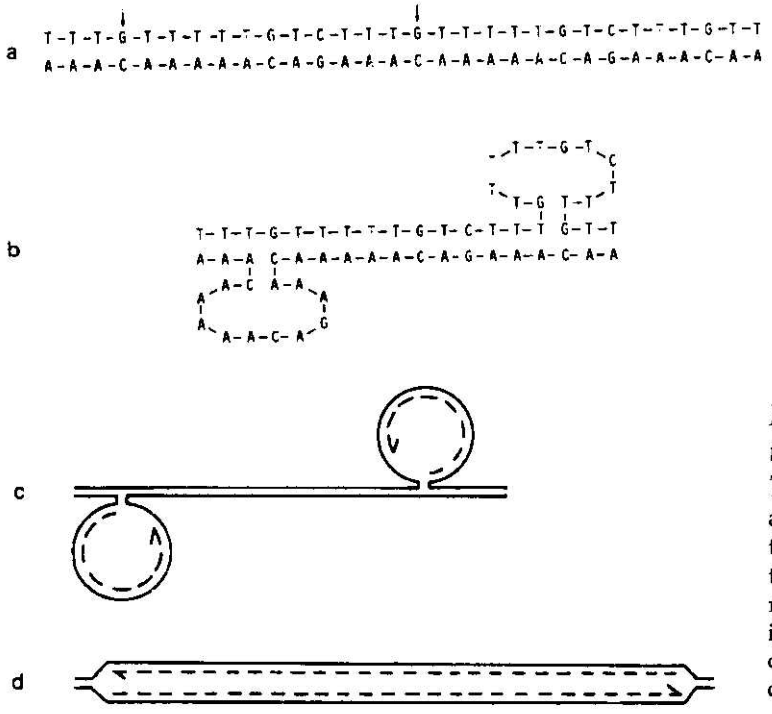

Fig. 2. Possible conformations of a tandem repeat (PhyMAR 16 of Table 1).

a, Fully double-stranded conformation; b, partly single-stranded alternative resulting from a base-pair shift: c, replication of b automatically results in two fully double-stranded repeats; d, the new chain is indicated by the dashed line.

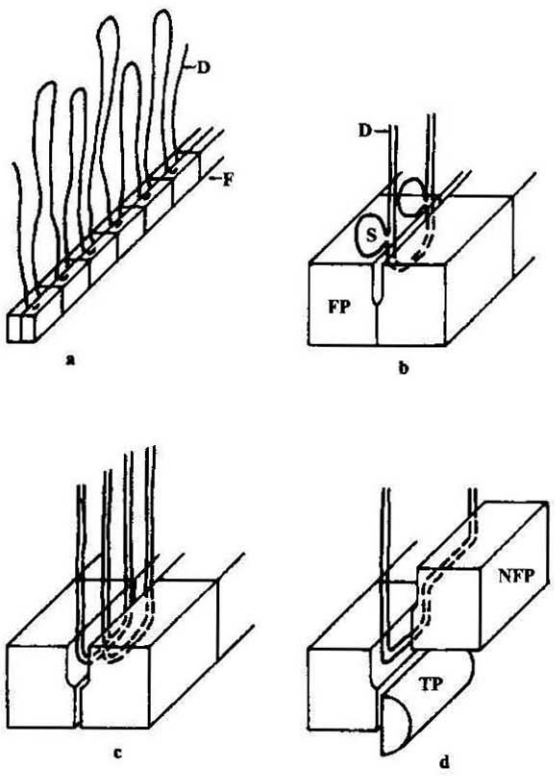

Fig. 3. Diagram of the putative origin reorganization during the cell cycle.
a, G_1-phase situation. DNA (D) is attached to matrix filaments (F) by replication origins; b, magnified segment of (a) showing the single-stranded loops; c, the same segment after DNA replication; d, one half of the symmetrical segment with transiently associated proteins in mitosis. The second half of the segment is being assembled from new proteins (NFP). The original situation (b) is recovered by dissociation of the transiently associated proteins (TP) and a final structural alteration.

Evidence for the attachment of replication forks has been obtained by labelling both synchronous and asynchronous cultures with [^{3}H]dThd and analysing the label distribution immediately after a brief pulse [3, 7]. Positive results have been reported by several investigators. This indicates that replication origins are permanently fixed to the nuclear matrix while replicating DNA loops are reeled through a second binding site during DNA synthesis as required by the hypothesis proposed.

REGULATION OF DNA REPLICATION

The second problem that awaits an explanation is how does the cell manage that each replicon is duplicated just a single time during the cell cycle. Some authors propose the involvement of a so-called licencing factor, but what this means in molecular terms remains enigmatic. So far it is known that replication can be initiated at any time during G_1 phase but never in G_2. We reasoned that the competence to become initiated may be lost as a consequence of the replication itself and that it may be regained by some structural alteration that takes place at the replication origins during mitosis. Therefore, we cloned short fragments of matrix-associated DNA into M13 and analysed the nucleotide sequences. DNA of *Physarum* plasmodia in G_2 was chosen in order to exclude contamination by aspecific DNA domains derived from matrix-associated replicating regions. Sequence analysis showed that more than 25% of the clones contained direct repeats of variable length (Table 1). The sequences of all repeats differed from each other but all contained at least one tandem repeat of between 12 and 16 base pair length [8]. Such repeats can occur either as fully double-stranded helices or, by a shift of base pairing, with single stranded loops, as shown in Fig. 2.

According to the generally accepted view, priming of a new DNA chain requires a piece of unpaired parental strand. One might therefore envisage the possibility that the partly single-stranded configuration of the repeats exists in the G_1 phase. It is important that the origin region is turned into a full-length double helix by its replication (Fig. 2 c and d) and thus becomes inaccessible for a new round of replication. This provides an explanation for the fact that each replicon duplicates only once per cell cycle. The more difficult question is how the initiation-competent configuration is restored in mitosis. For thermodynamical reasons the rearrangement cannot occur spontaneously. One could imagine, however, that it is coupled to a reorganization of the filamentous protein matrix. In our view such a reorganization is inherent to the conversion of the internal nuclear matrix into the chromosomal scaffold. The reasons are as follows: (1) Replication origins remain attached to the chromosomal scaffolds as well; (2) in *Physarum* which undergoes a closed mitosis the condensing matrix filaments follow the movement pattern of the chromosomes; (3) in mammalian cells at least two proteins of 47 kDa and 53 kDa, but very likely a couple more, are components of the nuclear

matrix as well as of the chromosomal scaffold [9]. Two other proteins of 37 kDa and 83 kDa are only found in chromosomal scaffolds. We suppose that their interaction with the matrix filaments leads to a condensation into the chromosomal scaffold.

Theoretically, the recovery of the property to become initiated should coincide with the coiling and uncoiling of the chromosomal scaffold. How this may be achieved is shown diagrammatically in Fig. 3. The protein filament (F) shown in parts 3a and 3b may be split into two symmetrical halves sooner or later after the DNA has been replicated (Fig. 3c). Both halves become associated with transiently bound proteins (TP) and are coiled up into solenoids typical of chromosomal scaffolds. New filament proteins are then assembled (Fig. 3d) and a final protein rearrangement leads to the dissociation of the transiently associated proteins, uncoiling of the solenoid and reappearance of the original structure. The shift in base pairing involved in the restoration of the single-stranded DNA loops is supposed to be coupled to this process. In *Physarum* plasmodia DNA replication is initiated at the late telophase, i.e. at the time when the uncoiling of the chromosomal scaffold becomes completed.

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