Association of newly replicated DNA with the nuclear matrix of *Physarum polycephalum*

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ABSTRACT

We have studied the role of the nuclear matrix in DNA replication in a naturally synchronized eucaryote, *Physarum polycephalum*. When *P. polycephalum* macroplasmodia were pulse labeled with ^3H -thymidine, the DNA remaining tightly associated with the matrix was highly enriched in newly synthesized DNA. This enrichment was found both in nuclei that had just initiated DNA replication as well as in nuclei isolated later during S phase. Pulse chase experiments showed that the association of newly replicated DNA with the matrix is transient, since most of the newly replicated DNA could be chased from the matrix by incubating pulse labeled macroplasmodia in media containing unlabeled thymidine. Studies measuring the size distribution of the matrix DNA supported the hypothesis that replication forks are attached to the nuclear matrix. Reconstitution controls indicated that these results were unlikely to be due to preferential, nonspecific binding of nascent DNA to the matrix during the extraction procedures. These results with *P. polycephalum*, in combination with previous studies in non-synchronized rodent cells, suggest that the association of newly replicated DNA with the nuclear matrix may be a general feature of eucaryotic DNA replication.

INTRODUCTION

Based on suggestions made by Jacob, Brenner and Cuzin¹, several investigators have attempted to show that DNA replication occurs in association with a structural support. In procaryotes, it has been shown by both genetic and biochemical techniques that the cell wall provides such a support²⁻⁵. In eucaryotes, the nuclear membrane was at first envisioned to be the structural support analogous to the procaryotic cell wall. However, the evidence for this has been controversial; ultrastructural studies have demonstrated that DNA replication does not preferentially occur on the nuclear membrane during most of S phase⁶⁻¹¹, while many biochemical studies have indicated that the nuclear membrane (or at least a large hydrophobic structure) is associated with newly replicated DNA¹²⁻¹⁷.

Recently, the presence of another structural entity has been discovered

within the nucleus, the nuclear matrix^{18, 19}. The nuclear matrix is a skeletal structure remaining after the nucleus has been extracted with detergents and high salt solutions. It is composed of at least 3 structural components: a residual nucleolus (nucleolar matrix), a residual peripheral lamina²⁰, and an interconnecting fibrillar component reminiscent of the ribonucleoprotein network previously described by Zbarsky, Georgiev and Busch²¹⁻²⁵. The nuclear matrix reflects the shape and basic structural organization of the nucleus¹⁹ and contains a distinct set of nuclear proteins²⁶. It has been described in diverse types of eucaryotic cells including rat liver¹⁹, human HeLa cells²⁷, mouse Friend cells²⁸, Tetrahymena²⁹ and Physarum³⁰.

Several investigators have shown that newly replicated DNA is intimately associated with the nuclear matrix³¹⁻³⁵. These studies have suggested that the nuclear matrix provides a structural support for DNA synthesis in eucaryotes analogous to the cell wall of procaryotes. However, all studies to date have dealt exclusively with rodent cells. We show here that DNA synthesis in Physarum polycephalum also occurs in association with the nuclear matrix. We chose to examine P. polycephalum because its cell cycle is naturally synchronized³⁶, allowing us to examine DNA replication at several points during S phase, and also because there is a great evolutionary distance between Physarum and rodents. Our results suggest that the involvement of the nuclear matrix with DNA replication in eucaryotes is a general one.

MATERIALS AND METHODS

P. polycephalum (Strain M₃C VIII) was generously provided by M. J. Maher, Univ. of Texas, Richardson, Texas. Macroparasitoidia were grown in semi-defined media (SDM) at 26°C on filter paper as described³⁷. The DNA of the plasmodia was uniformly labeled with ¹⁴C-thymidine (40 mCi/mmol, Amersham, Arlington Heights, Ill.) by its inclusion (0.2 µCi/ml) in SDM. Progression through the cell cycle was determined by phase contrast microscopy of ethanol fixed plasmodia³⁷.

During the second synchronous S phase, cultures were pulse labeled by changing the medium to one containing ³H-thymidine (Amersham, 1.0 mCi/ml, 50 Ci/mmol). For pulse chase experiments, filters containing pulse labeled plasmodia were immediately washed thrice (5 seconds each) in chase SDM (SDM containing unlabeled thymidine (3.0 mM) and deoxycytidine (0.01 mM)), and then placed in chase SDM at 26°C for 2 hours.

Nuclei were prepared by homogenization in a Waring blender for 30 seconds in SMN buffer (250 mM sucrose, 10 mM tris pH 7.0, 4 mM MgCl₂)

containing 0.5% diethylpyrocarbonate, 0.5 mM phenylmethanesulfonyl fluoride and 1% NP-40. After low speed centrifugation to remove debris, nuclei were purified by centrifugation through a cushion containing 1.0 M sucrose in SMN buffer. This procedure represents a slight modification of that described by Mohberg and Rusch³⁸.

Nuclear matrices were then prepared from these nuclei by suspension in SMN at 10^7 nuclei/ml followed by slow addition of an equal volume of high salt buffer (HSB), composed of 4 M NaCl, 4 mM $MgCl_2$, 10 mM Tris-HCl, pH 7.0. These nuclear matrices contained 100% of the nuclear DNA (see Results). To cleave DNA from these nuclear matrices, DNase I (DN-CL, Sigma, St. Louis, Mo. or DPFF, Worthington, Freehold, N. J., were used with equivalent results) was added at 20 U/ml and the matrices incubated at 37°C for various time periods. Nuclear matrices were recovered by centrifugation through a 5 cm cushion of a solution containing 42.5% SMN, 42.5% HSB and 15% glycerol at 10,000 g for 15 minutes at 4°C.

To determine the amounts of ^{14}C or 3H labeled DNA associated with the matrix, one of two procedures was used. In the first, nuclear matrices pelleted through glycerol as described above were suspended in a solution of 1% SDS and 10 mM EDTA, pH 7.0 and split into 3 parts. One part was boiled for 15 min in 12% trichloroacetic acid (TCA) and one part was boiled for 15 min in 0.4 M NaOH. Then all three parts were treated with 12% TCA at 4°C and collected on glass fiber filters (GF/C, Whatman, Clifton, N. J.). After drying, the filters were counted in Liquifluor (New England Nuclear, Boston, Mass.). The amount of labeled DNA was calculated after correcting for labeled protein (i.e., hot acid resistant) and labeled RNA (i.e., hot alkali sensitive). Alternatively, nuclear matrices were suspended in TE (10 mM NaCl, 20 mM EDTA, 50 mM Tris, pH 9.0) containing boiled RNase A (100 µg/ml) (Sigma, St. Louis, Mo.) at 37°C for 5 hours, followed by addition of 1% SDS and 500 µg/ml pronase (Boehringer-Mannheim, Indianapolis, Ind.) and further incubation at 37°C for 16 hours. The amount of labeled DNA was then determined by acid precipitation of these samples at 4°C and collection on glass fiber filters as described above. The chemical and enzymatic techniques for determining labeled DNA gave equivalent results.

DNA was purified from the nuclear matrices by SDS-proteinase K digestion followed by phenol-chloroform extraction³⁹. After ethanol precipitation and subsequent RNase treatment, another cycle of SDS-proteinase K digestion and organic extraction was performed. Purified DNA was electrophoresed through a 1.5% agarose gel; the gel was stained and each slice boiled in

1 M HCl before scintillation counting in Aquasol II (New England Nuclear, Boston, Mass.).

For fluorescence microscopy, nuclei (in SMN) or nuclear matrices (in 50% SMN, 50% HSB) were stained with acridine orange (100 µg/ml, BioRad, Richmond, Cal.). They were viewed with a Zeiss Photo III microscope and photographed with Kodak Ektochrome 400 film at 945 X magnification.

RESULTS

The biochemical and ultrastructural features of P. polycephalum nuclei treated with 2 M NaCl and nucleases have been previously described³⁰. When Physarum nuclei are incubated in high salt solutions containing an intercalating agent, much of the DNA can be seen to protrude in the form of a halo surrounding the nuclear matrix (Fig. 1b). In mouse nuclei, a similar halo has been shown to represent unwound supercoiled loops of DNA^{35, 40-44}; we assume that the halo seen around Physarum matrices also represents unwound loops of DNA anchored at their base to the nuclear matrix (see Discussion). This halo is green when viewed under the fluorescence microscope after staining with acridine orange, while the residual nucleolus is red, reflecting the high concentration of RNA in Physarum nucleoli³⁸. If DNase I is added to these structures, the halo radius can be observed to gradually decrease over time. In this way, the amount of DNA left associated with the nuclear matrices (recovered by pelleting through a glycerol cushion) can be controlled (Fig. 2). When 85-90% of the DNA has been removed, no visible DNA halo remains (Fig. 1c).

We next investigated whether the distribution of newly replicated DNA differed from that of the total DNA with respect to the nuclear matrix-halo organization. To this end, we cleaved various amounts of DNA from nuclear matrices of P. polycephalum. The DNA in these plasmodia had been uniformly labeled with ¹⁴C by growth in media containing ¹⁴C-thymidine for one full generation. The plasmodium was then pulse labeled with ³H-thymidine for 30 seconds during the peak of S phase. The data in Figure 3 shows that even when most (95%) of the DNA was removed from the matrix (represented by ¹⁴C counts), about 40% of the newly replicated DNA (³H counts) remained associated with the matrix. As more DNA was cleaved from the matrix, the enrichment for newly replicated DNA increased (Fig. 3). This suggests that the replication forks are very close to the nuclear matrix-DNA attachment points.

It should be noted that in P. polycephalum, nucleotide precursors are extensively degraded^{45, 46}. Hence, plasmodia labeled with ¹⁴C- or ³H-

Figure 1. Fluorescence Micrographs of Acridine Orange Stained Nuclei and Nuclear Matrices of *P. polycephalum*

(a) Purified nuclei, in SMN at 21°C. The diameter of the nucleus shown is 4.5 microns. (b) *Physarum* nuclear matrix, in 50% SMN, 50% HSB at 21°C, not DNAase treated. Note the (green) halo surrounding the residual nucleus. (c) *Physarum* nuclear matrix, DNAase I treated (20 U/ml, 37°C for 15 minutes) in 50% SMN, 50% HSB, so that approximately 95% of the nuclear DNA was removed. Note the absence of the halo. The exposure time for the photograph was 12 fold longer than for those of (a) and (b).

thymidine contain significant acid precipitable radioactivity in RNA and protein (up to 30% of the total) in addition to that in DNA. For these reasons, it is necessary to account for labeled RNA and protein in determinations such as those in Figure 3 (see Materials and Methods). Failure to do so results in significantly different results.

One great advantage of *P. polycephalum* as an experimental tool is its natural synchrony³⁶. We have exploited this synchrony to analyze whether DNA synthesis is associated with the nuclear matrix at various times during S phase. S. phase begins in *P. polycephalum* during the unwinding of telophase chromosomes, within 5 minutes after metaphase⁴⁷⁻⁴⁹; we have found that nuclear matrices can be prepared from nuclei at this stage of the cell cycle.

Figure 2. Preparation of Nuclear Matrices with Varying Amounts of DNA Remaining

Nuclear matrices were prepared from ^{14}C -labeled macroplasmodia 30 minutes past M2, as described in Materials and Methods, and treated with DNAase I for the indicated time periods. The matrices were recovered by centrifugation through a glycerol cushion. ^{14}C -labeled DNA remaining with the nuclear matrices was determined as described in Materials and Methods. The mean and standard deviation from 5 separate experiments is shown. The ordinate represents the percentage of total nuclear ^{14}C -labeled DNA recovered with the matrix.

These matrices exhibit a halo of DNA similar to that shown in Figure 1b, but the nucleolus is not yet formed. Within 10-20 min past metaphase, the rate of DNA synthesis reaches a maximum level which it sustains for approximately 2 hours before tapering off⁴⁷⁻⁴⁹. We performed pulse labeling experiments with *P. polycephalum* at various times during the second synchronous S phase. Table 1 shows that at 5 min past the second synchronous metaphase (M2), as well as 30 and 90 min past M2, newly synthesized DNA is preferentially associated with the nuclear matrix. The data in Figure 3 and Table 1 also shows that the newly synthesized DNA that is matrix associated does not represent a small subfraction of the newly synthesized DNA; on the contrary, a large fraction (40-65%) of the newly synthesized DNA is associated with the nuclear matrix at points when only 5-20% of the total DNA remains with the matrix.

Figure 3. Extent of Association of Newly Synthesized DNA with the Nuclear Matrix as a Function of Its Total DNA Content.

Nuclear Matrices were prepared from plasmodia which had been uniformly labeled with ^{14}C -thymidine and pulse labeled with ^3H -thymidine for 30 seconds at 30 minutes past M2. The extent of DNA remaining with the matrices was controlled by DNAase I digestion (Fig. 2). The percentage of ^{14}C - and ^3H -labeled nuclear DNA remaining with the matrix preparations was determined as described in Materials and Methods.

Pulse chase experiments were performed to examine the time course of association of the newly replicated DNA with the nuclear matrix. In these experiments, macroplasmodia were pulse labeled with ^3H -thymidine for 30 seconds, and then chased with an excess of unlabeled thymidine for 2 hours. During the first several minutes of the chase period, ^3H -thymidine which had been integrated into the pools during the pulse period is incorporated into DNA, so the chase is not a perfect one. Nevertheless, the data in Table 2 clearly indicate that the newly replicated DNA (which was originally associated with the nuclear matrix immediately after the pulse) travels from the nuclear matrix during the chase period. The enrichment of newly replicated DNA in the matrix decreased from 6-8 fold immediately after the pulse to 0.7 fold after the chase. Furthermore, 72-85% of the ^3H -thymidine counts originally associated with the nuclear matrix immediately following the pulse became dissociated from the matrix during the chase period. These results were obtained both during the beginning and peak of DNA synthesis (Table 2).

Table 1

Enrichment of Newly Replicated DNA on the Nuclear Matrix During S Phase

Time of Pulse ^a	DNase Treatment Time (mins) ^b	% Newly Replicated DNA ^c	%Total DNA ^d	Enrichment ^e
5 min past M2	10	37.1	4.7	7.9
	30	13.4	1.4	9.6
30 min past M2	10	41	5.5	7.4
	30	24.5	2.1	11.7
90 min past M2	10	18.9	3.1	6.1
	30	12.4	0.9	13.8

^aMacroplasmidia, previously labeled with ^{14}C -thymidine, were pulse labeled for 30 seconds with ^3H -thymidine at the indicated times, and nuclei were immediately prepared.

^bNuclear matrices were prepared from purified nuclei using DNase I digestion for the indicated time periods.

^cpercent of the total nuclear ^3H -labeled DNA which remained with the matrix.

^dpercent of the total nuclear ^{14}C -labeled DNA which remained with the matrix.

^eComputed as the ratio of ^3H -labeled DNA to ^{14}C -labeled DNA in the nuclear matrices relative to the ratio in the nuclei from which the matrices were made.

To characterize further the nascent DNA bound to the matrix, we utilized gel electrophoresis to determine its size distribution. Nuclear matrices were prepared from ^{14}C -thymidine labeled macroplasmidia which were pulse labeled with ^3H -thymidine for 30 to 60 seconds at 30 minutes past M2. The average length of the DNA fragments associated with the matrix (number average) was 2740 bp (^{14}C labeled DNA) and 1540 bp (^3H labeled DNA) in the 30 second pulse and 3129 bp (^{14}C labeled DNA) and 1890 bp (^3H labeled DNA) in the 60 second pulse. In Figure 4, it can be seen that the specific activity ($^3\text{H}/^{14}\text{C}$ ratio) increased with decreasing DNA size until it reached a plateau at approximately 500-550 base pairs (30 second pulse) or 950-1100 base pairs (60 second pulse).

If DNA replication were occurring on the matrix, one would predict that all matrix bound DNA fragments shorter than the number of bases replicating during the pulse labeling period should be uniformly labeled and thus have a constant $^3\text{H}/^{14}\text{C}$ ratio. It has been shown by biochemical and electron microscopic techniques that the average rate of DNA chain elongation in *P. polycephalum* is 600-1500 base pairs per replicon per minute⁵⁰⁻⁵². Hence

TABLE 2

Pulse Chase Experiments

<u>Time of Pulse</u> ^a	<u>Newly Replicated DNA Remaining with the Matrix (³H CPM)</u>	<u>% Newly Replicated DNA on the Matrix^d</u>	<u>Total DNA Remaining with the Matrix (¹⁴C CPM)</u>	<u>% of Total DNA Remaining with the Matrix^e</u>	<u>Enrichment^f</u>
5 min past M2	Pulse only ^b	34.1	284	3.9	8.7
	Pulse chase ^b	2.5	274	3.8	0.66
30 min past M2	Pulse only ^c	22.1	792	3.6	6.1
	Pulse chase ^c	1.8	625	2.8	0.65

^aMacroplasmidia, previously labeled with ¹⁴C-thymidine, were pulse labeled with ³H-thymidine at the indicated time. The plasmidia was then divided in half; one half was homogenized immediately and the other half was incubated in chase media containing excess unlabeled thymidine for 2 hours before homogenization. After homogenization, nuclei were purified as described in Materials and Methods, and nuclear matrices were prepared from these nuclei using DNase I digestion for 2 minutes.

^bDuring the pulse, approximately 1480 ³H-cpm were incorporated into the DNA of each half of the plasmidia. During the chase period, another 4080 cpm were incorporated into the half that was "chased".

^cDuring the pulse, approximately 12,100 ³H-cpm were incorporated into the DNA of each half of the plasmidia. During the chase period, another 9750 cpm were incorporated into the half that was "chased".

^dBased on the number of ³H-cpm in DNA of the nuclear matrices compared to the ³H-cpm in the nuclei from which the matrices were made.

^eBased on the number of ¹⁴C-cpm in DNA of the nuclear matrices compared to the ³H-cpm in the nuclei from which the matrices were made.

^fComputed as the ratio of ³H-labeled DNA to ¹⁴C-labeled DNA in the nuclear matrices relative to the ratio in the nuclei from which the matrices were made.

Figure 4. Relative Enrichment for Newly Replicated DNA on Matrix DNA Fragments of Various Size.

^{14}C -thymidine labeled macroplasmodia were pulse labeled with ^3H -thymidine for 30 or 60 seconds at 30 minutes following M2. Nuclear matrices were then prepared from the purified nuclei of these macroplasmodia using a 10 minute DNAase I digestion. Purified DNA from these nuclear matrices was then electrophoresed on a 1.5% agarose gel, and gel slices were counted as described in Materials and Methods. DNA markers were bacteriophage lambda cleaved with Hind III and ϕX174 RF cleaved with Hae III. ● = 60 second pulse label; ○ = 30 second pulse label.

the results in Figure 4 are in concordance with a matrix bound DNA replication site.

It was possible that the results recorded above were due to the artifactual binding of newly replicated DNA to the nuclear matrices during their preparation. To examine this possibility, we performed reconstitution experiments by incubating nuclear matrices with exogenous DNA (either purified or together with nucleoproteins) and determining the extent of association of the exogenous DNA with the matrices. The source of the exogenous DNA was P. polycephalum nuclei uniformly labeled with ^{14}C -thymidine and pulse labeled with ^3H -thymidine for 30 seconds at 30 minutes past M2. DNA was prepared from these nuclei in 2 ways. In the first, DNA

was purified by proteinase K treatment followed by phenol-chloroform extraction (see Materials and Methods). In the second, the nuclei were incubated with 2 M NaCl to form matrices; these matrices were then briefly sonicated (Heat Systems sonicator, Plainview, N. Y.), and large particles were removed by centrifugation at 10,000 g for 20 minutes. Most of the DNA (72% of the ^3H label, 74% of the ^{14}C label) was recovered in the supernatant. This DNA (HS DNA) was not further purified, but was more likely to mimic the state of DNA during the preparation of nuclear matrices. Either purified DNA or HS DNA was mixed with nuclear matrices prepared from unlabeled Physarum cultures 30 minutes after the second synchronous mitosis. Nuclear matrices that had not been treated with DNase, and also matrices that had been treated with DNase to remove approximately 95% of the total DNA, were used. Purified DNA or HS DNA were added to these nuclear matrices in SMN buffer containing 2 M NaCl at either 37°C or 4°C, and then the matrices were pelleted through 15% glycerol as described previously. In all these reconstitution experiments, insignificant quantities of exogenous DNA (less than 2% of the ^3H label or ^{14}C label) became associated with the matrices.

DISCUSSION

The results described above show that newly synthesized DNA is associated throughout S phase with the nuclear matrices of P. polycephalum. Mitchelson et al.³⁰ have previously shown that a nuclear matrix can be prepared from P. polycephalum, and have described its chemical and ultrastructural features. We have used a similar method to prepare nuclear matrices, and have used various amounts of DNase I to control the amount of DNA remaining associated with the matrices. When no DNase was used, a halo of DNA was found surrounding each individual matrix. This halo was probably DNA, since it was green upon staining with acridine orange and was sensitive to DNase I. Hence it was similar to the halo of DNA surrounding the nuclear matrix of mouse cells previously described³⁵. There was an important difference, however. The halo of DNA loops surrounding the mouse nuclear matrix was seen to reversibly change diameter upon changing the ethidium bromide concentration, indicating that the halo was comprised of supercoiled loops of DNA³⁵. Supercoiled loops had previously been detected in many organisms by biophysical techniques⁴⁰⁻⁴⁴. With high concentrations of ethidium bromide, the loops were positively supercoiled and there was no halo unless the supercoiled loops were nicked, allowing them to relax and extend³⁵. In Physarum,

however, we found that the halo was always present, regardless of the concentration of intercalating agents or whether the DNA had been purposefully nicked. Our interpretation of the results with *P. polycephalum* is that the halo represents loops of DNA that have been nicked by endogenous nucleases during the purification of the nuclei. Our attempts to limit endogenous nuclease activity with additional nuclease inhibitors (polyvalent anions) and chelators of divalent cations were not successful, however, since nuclear morphology grossly changed under these conditions. Hence the equivalence of the DNA halo and the supercoiled loops remains conjectural at this time.

The results in Figure 2 show that all of the nuclear DNA is associated with the nuclear matrix when no DNase is used in its preparation. In addition to some of the usual criteria for establishing true association of DNA with a subcellular structure (co-sedimentation and lack of association in reconstitution experiments), data such as that provided in Figure 1 show that the association of DNA with the nuclear matrix can be directly visualized using fluorescence microscopy. As the DNA is cleaved from its matrix attachment sites, the diameter of the halo gradually decreases. Ionic detergents or proteolytic enzymes destroy the structural integrity of the nuclear matrix; visually, no nuclear-matrix-halo structure can be observed, and the background fluorescence is increased; upon sedimentation, all DNA remains in the supernatant (BFH and BV, unpublished data).

The results in Figure 3 and Table I show that newly replicated DNA is less easily cleaved from the nuclear matrix than is total nuclear DNA. This matrix associated, newly replicated DNA does not represent a small subfraction of the newly synthesized DNA; on the contrary, the majority of newly synthesized DNA is associated with the nuclear matrix when only 20% of the total DNA remains matrix associated. These results are unlikely to be due to protection of newly replicated DNA from the action of DNase I since the sizing experiments reported show that the size of the newly replicated, ^3H labeled matrix associated DNA is smaller, rather than larger, than the size of the total ^{14}C labeled matrix associated DNA.

The simplest interpretation of these results is that the newly replicated DNA is preferentially located in the nuclear matrix at the base of the DNA halo. Since similar results were found for replicated DNA in newly initiated replicons (5 min past M2) and more mature replicons (30 min past M2), the data indicates that elongation, not simply initiation, occurs on the nuclear matrix. According to this interpretation, the DNA would travel

through the nuclear matrix as it is replicated; hence replication complexes, containing the many enzymes required for DNA synthesis, would be situated on the nuclear matrix with the DNA in motion, rather than the DNA fixed and the replication complex in motion. DNA replication complexes containing several enzymes have been demonstrated on eucaryotic cells⁵³, but as yet there is no direct evidence for their association with the nuclear matrix.

We have previously proposed a model wherein replication complexes are situated at the anchorage site for the DNA loops within the nuclear matrix, and the DNA loops are reeled through these replication complexes as they are replicated. This model would account for the bidirectional replication known to take place on Physarum⁵² and other eucaryotes⁵⁴, and would also allow for the preservation of the looped structure of chromatin throughout S phase. The data presented here for Physarum support this model. Other interpretations are possible, however. For example, the site for replication of DNA may be different from the anchorage sites for the DNA loops, although both may lie within the nuclear matrix. The reconstitution experiments show that our results are unlikely to be due to nonspecific binding of released newly replicated DNA to the nuclear matrices. However, we cannot rule out the possibility that the nascent DNA becomes trapped within or bound to the nuclear matrices during the extraction procedures and that this association did not really exist in situ.

The previous studies in rodent cells³¹⁻³⁵, together with the studies reported here in P. polycephalum, suggest that the association of newly replicated DNA with the nuclear matrix may be a general feature of eucaryotic DNA replication.

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