Attachment of origins of replication to the nuclear matrix and the chromosomal scaffold

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We have investigated the attachment of DNA to the nuclear matrix and chromosomal scaffold in synchronized bovine liver cells. Label incorporated at the onset of the S phase remained preferentially associated with the matrix during the subsequent G_1 phase and with a residual protein structure from dehistonized chromosomes during mitosis. On the other hand label incorporated during mid or late S phase was about equally distributed over the DNA molecule after a chase into the G_1 phase. These results suggest that DNA is attached to the nuclear matrix and chromosome scaffolds by the origins of replication.

Replication origin *Nuclear matrix* *Chromosomal scaffold* *Mammalian cell*

1. INTRODUCTION

Considerable progress has been made in the understanding of eukaryotic DNA replication since it has been shown that the nuclear matrix plays a significant role in the structural organization of DNA, replication forks being preferentially associated with it [1–4]. However, studies on the regulation of DNA synthesis are seriously hampered by our ignorance of the origins of replication. Recently evidence was obtained indicating that in nuclei of the slime mold *Physarum polycephalum* origins of replicons are preferentially attached to the nuclear matrix during the entire interphase [5]. This could explain why average lengths of DNA loops emanating from nuclear matrix preparations are close to replicon lengths [6,7]. As similar loop lengths have been found between attachment sites of DNA to chromosomal scaffolds it seems reasonable to suppose that the latter are formed by a partial rearrangement of the nuclear matrix [8,9].

In support of this we show here that in mammalian cells label incorporated in replication origins at the onset of the S phase is associated with DNA sites that are bound to the nuclear matrix during G_1 phase as well as to the chromosome scaffold during mitosis.

2. MATERIALS AND METHODS

2.1. *Cell synchronization and labelling*

Monolayers of bovine liver cells were grown as in [3]. Cells were synchronized by a double blockade with 2 mM thymidine [10] of 20 h each. DNA was randomly pre-labelled with 0.25 μCi/ml [2-^{14}C]dThd (52.8 mCi/mmol; NEN) during the 15 h interval between thymidine blocks. Cells were pulse labelled for 3 h with 20 μCi/ml [*Me*-^{3}H]-dThd (20 Ci/mmol; NEN) in the presence of 250 μg/ml FdUrd and 10 μg/ml Urd.

2.2. *Preparation of nuclear and chromosomal lysates*

Membrane-deprived nuclei were prepared in 50 mM Tris buffer (pH 8) containing 0.1% Triton X-100 as in [2]. After the addition of an equal volume of 4 M NaCl, nuclear lysis was allowed to proceed at room temperature for 15 min.

For chromosome preparations mitotic cells, accumulated by a 4 h block with 0.1 mg/l colcemid, were detached from the glass surface by gentle

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shaking. Chromosomes were released by hypotonic treatment at pH 8 and 0.5% Triton X-100 in the presence of spermine and spermidine [11]. To obtain high-salt-resistant scaffold–DNA complexes chromosomes were lysed in the same way as for nuclei.

2.3. *Enzymatic DNA digestion and sucrose gradient analysis*

Lysates of nuclei or chromosomes were incubated with various concentrations of DNase I (Sigma, electrophoretically pure) in the presence of 7.5 mM $MgCl_2$ for 30 min at 37 or 0°C, respectively. Enzyme treatments were terminated by adding EDTA at a final concentration of 10 mM. Subsequently rapidly sedimenting protein–DNA complexes were separated from the released DNA by centrifugation through sucrose gradients [5] in a Beckman 27-2 rotor for 1 h at 25000 rpm and 20°C. All gradients were fractionated starting from the bottom of the tubes. Fractions were processed and radioactivities determined as in [12].

3. RESULTS

We studied the position of replication origins with respect to the nuclear matrix by selectively labelling them at the onset of the S phase and analysing the label distribution in nuclear lysates by graded DNase digestion. For this purpose in vitro cultured bovine liver cells were synchronized by two successive thymidine blocks. Fig.1 shows that the first free-running S phase starts at about 19 h after release from the second block. At this point ^{3}H-labelled thymidine was added together with FdUrd to a final concentration of 250 μg/ml to reduce the rate of DNA synthesis and enhance label incorporation. At the end of a 3 h pulse the reduced chain growth was checked by preparing nuclear lysates and detaching the DNA from the matrix by incubation with graded concentrations of DNase I. Fig.2 shows that the $^3H/^{14}C$ ratio increases as less DNA remains attached to the rapidly sedimenting nuclear matrix. This means that the matrix-attached DNA regions are enriched in newly replicated DNA. The enrichment was about the same as found previously after a 20 min pulse, indicating a limited length of the ^{3}H-labelled DNA regions [3].

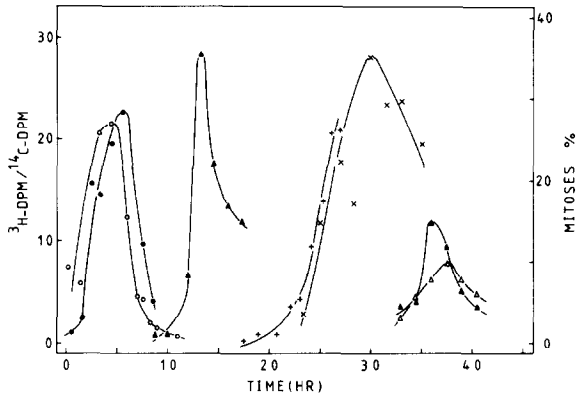

Fig.1. Synchronous growth after double thymidine block. Cells were grown and synchronized on coverslips in petri dishes. At the indicated times starting with the release from the second block (t = 0) samples of 2 coverslips each were labelled for 10 min in growth medium containing 5 μCi/ml [^{3}H]dThd. Label incorporation was determined as in [12] and average data are presented as ^{3}H incorporated per ^{14}C bulk DNA. First (○, ●) and second S phase (×, +) from 4 independent experiments; mitotic index (▲). In one experiment the mitotic index was determined after exposure to 250 μg/ml FdUrd and 10 μg/ml Urd from 19 to 22 h after release (△).

To determine whether the pulse-labelled DNA regions remained attached to the nuclear matrix in the G_1 phase, a simultaneously labelled culture was chased for 20 h and the DNA attachment was then analysed similarly. In fig.3A the relative $^3H/^{14}C$ ratios of the matrix-bound DNA corresponding to fractions 1–3 in fig.2 are plotted as a function of the residual amount of bulk DNA remaining at the matrix after DNase treatment. The inverse relationship between the $^3H/^{14}C$ ratio and the amount of attached DNA indicates that the short-pulse-labelled DNA regions that include those replication origins that are initiated at the beginning of the S phase remain associated with the nuclear matrix during the G_1 phase.

In a similar experiment pulse label was incorporated in the mid S phase from t = 26 to 29 h and subsequently chased until the G_1 phase. At this time label should be incorporated at random into replicon sites. Analysis showed that the pulse-labelled DNA regions were indeed randomly distributed over the DNA loops as can be inferred from the finding that the relative $^3H/^{14}C$ ratio remained close to 1 when the amount of residual

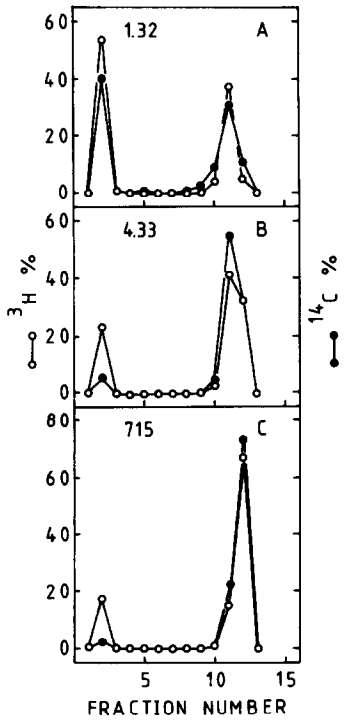

Fig.2. Matrix attachment of replicative DNA during early S phase. Pre-labelled cells, grown in a Carrel flask, were pulse labelled at the beginning of the S phase. A nuclear lysate was prepared and divided into 3 portions. DNase I was added to final concentrations of 0 (A), 4 (B) and 8 units/ml (C). After incubation the samples were analysed by sucrose gradient centrifugation. Direction of sedimentation is from right to left. The numbers in each panel represent the ratios of the percentages of ^{3}H and ^{14}C dpm in the matrix-associated DNA.

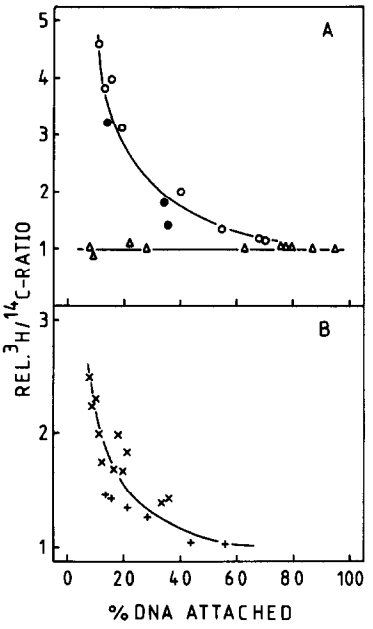

Fig.3. Association of replication origins with the nuclear matrix and chromosomal scaffolds. (A) Pre-labelled cells were pulse labelled at the beginning (○, ●) or middle of the S phase (△) and chased until 42 h after release from the block (see fig.1). Nuclear lysates were analysed by graded digestion with DNase I (0–100 units/ml) and sucrose gradient centrifugation. Relative ^{3}H/^{14}C ratios are plotted as a function of the residual amount of DNA attached to the matrix. (B) Pre-labelled cells were pulse labelled at the beginning of the S phase and chased until mitosis. Mitotic cells were collected and the label distribution was analysed as in A. × and + are from independent experiments.

DNA in the rapidly sedimenting matrix–DNA complex was reduced by DNase digestion (fig.3A). Essentially the same result was obtained when the pulse label was given late in the first S phase, i.e., 8 h after release from the thymidine block (see fig.1), and analysed after a chase until the subsequent G_1 phase (not shown).

To determine whether origins of replicons are also attached to the chromosomal scaffolds we isolated chromosomes from mitotic cells of a synchronized culture, pulse labelled at the onset of the first free-running S phase. Scaffold–DNA complexes have been shown to sediment rapidly through sucrose gradients [9]. Therefore scaffold-bound DNA and DNA released by graded DNase digestion were separated in the same way as shown in fig.1. The results of such analyses are summarized in fig.3B. The ^{3}H/^{14}C ratio was again found to increase very markedly in the residual DNA when its amount decreased. This indicates that origins of replicons are attached to the chromosome scaffold as well.

4. DISCUSSION

The results described above show that label incorporated during the initiation of DNA synthesis in mammalian cells is present in DNA sites that are bound to the chromosomal scaffolds during mitosis and to the nuclear matrix during the subsequent G_1 phase. Previously a similar situation was found in *Physarum*, where label incorporated at

the onset of one S phase remained associated with the matrix during the subsequent G_2 phase and the next S phase [5]. Our tentative conclusion from these data is that origins of replication are the sites of attachment of the DNA to the nuclear matrix and chromosomal scaffold, respectively. This concurs with the finding that DNA loops between successive attachment sites are of the order of replicon lengths [6–8] and also with the similarity of the type of DNA observed at the sites of attachment to both the nuclear matrix and chromosome scaffold [13].

The apparent conservation of the DNA-binding sites during interphase and mitosis supports the suggestion that chromosome scaffolds are formed by a reversible rearrangement of parts of the nuclear matrix [9] without interruption of the DNA attachment.

The described structure also lends support to previous attempts to explain the separation of newly replicated DNA molecules and their correct distribution to the daughter nuclei during mitosis [3,4,15]. At the same time it shows new possibilities to isolate and study the structure of origins [16] and of components associated with them.

REFERENCES

[1] Berezney, R. and Coffey, D.S. (1975) Science 189, 291–293.
[2] Wanka, F., Mullenders, L.H.F., Bekers, A.G.M., Pennings, L.J., Aelen, J.M.A. and Eygensteyn, J. (1977) Biochem. Biophys. Res. Commun. 74, 739–747.
[3] Dijkwel, P.A., Mullenders, L.H.F. and Wanka, F. (1979) Nucleic Acids Res. 6, 739–747.
[4] Pardoll, D.M., Vogelstein, B. and Coffey, D.S. (1980) Cell 19, 527–536.
[5] Aelen, J.M.A., Opstelten, R.J.G. and Wanka, F. (1983) Nucleic Acids Res. 11, 1181–1195.
[6] Vogelstein, B., Pardoll, D.M. and Coffey, D.S. (1980) Cell 22, 79–85.
[7] Buongiorno-Nardelli, M., Micheli, G., Carri, M.T. and Marilley, M. (1982) Nature 298, 100–102.
[8] Paulson, J.R. and Laemmli, U.K. (1977) Cell 12, 817–828.
[9] Lewis, C.D. and Laemmli, U.K. (1982) Cell 29, 171–181.
[10] Bootsma, D., Budke, L. and Vos, O. (1964) Exp. Cell Res. 33, 301–309.
[11] Pieck, A.C.M., Van der Velden, H.M.W., Rijken, A.A.M. and Wanka, F., in preparation.
[12] Wanka, F. (1974) Exp. Cell Res. 85, 409–414.
[13] Razin, S.V., Mantieva, V.L. and Georgiev, G.P. (1979) Nucleic Acids Res. 7, 1713–1735.
[14] Dingman, C.W. (1974) J. Theor. Biol. 43, 187–195.
[15] Wanka, F., Pieck, A.C.M., Bekers, A.G.M. and Mullenders, L.H.F. (1982) in: The Nuclear Envelope and the Nuclear Matrix (Maul, G. ed) pp.199–211, Alan R. Liss, New York.
[16] Goldberg, G.I., Collier, I. and Cassel, A. (1983) Proc. Natl. Acad. Sci. USA 80, 6887–6891.