Organization of DNA replication in *Physarum polycephalum*. Attachment of origins of replicons and replication forks to the nuclear matrix

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ABSTRACT

We have investigated the attachment of the DNA to the nuclear matrix during the division cycle of the plasmodial slime mold *Physarum polycephalum*. The DNA of plasmodia was pulse labelled at different times during the S phase and the label distribution was studied by graded DNase digestion of the matrix-DNA complexes prepared from nuclei isolated by extraction with 2 M NaCl. Pulse labelled DNA was preferentially recovered from the matrix bound residual DNA at any time of the S phase. Label incorporated at the onset of the S phase remained preferentially associated with the matrix during the G₂ phase and the subsequent S phase. The occurrence of the pulse label in the matrix associated DNA regions was transiently elevated at the onset of the subsequent S phase. Label incorporated at the end of the S phase was located at DNA regions which, in the G₂ phase, were preferentially released from the matrix by DNase treatment. From the results and previously reported data on the distribution of attachment sites it can be concluded that origins of replicons or DNA sites very close to them are attached to the matrix during the entire nuclear cycle. The data further indicate that initiations of DNA replication occur at the same origins in successive S phases. Replicating DNA is bound to the matrix, in addition, by the replication fork or a region close to it. This binding is loosened after completion of the replication.

INTRODUCTION

Considerable progress in the understanding of the DNA replication in procaryotes has been made in recent years. However, specific problems turn up at the organizational level of eucaryotic cells. For example, it is not known how the cell ensures that each chromosomal DNA molecule is duplicated a single time between 2 cell divisions. It is also but poorly understood how the daughter cells obtain one partner of each pair of sister chromatids that arises from the replication. Now, evidence is accumulating that a scaffold-like structure, the nuclear matrix, may play a significant role in these processes.

The nuclear matrix is a protein structure which can be obtained from mammalian cell nuclei by successive treatments with apolar detergents and 2 M NaCl (1, 2). It has also been obtained by similar procedures from other

organisms (3, 4). Ideas on the possible role of the matrix in the spatial organization of DNA replication have been corroborated by the observation that replication forks are preferentially associated with it (5 - 12). To explain the process of untwining and separation of DNA daughter molecules it has been suggested that, beside the temporary binding of the replication forks, replication origins might be attached to the scaffold as well (13, 14).

Convincing evidence of the binding of the origins is still lacking. It requires the specific labelling of at least part of the origin regions. This can be achieved only by adding label to highly synchronous cell systems at the very beginning of the S phase. Plasmodia of the slime mold Physarum polycephalum are particularly useful for such studies because of the extreme synchrony of mitoses and S phases in all nuclei of a plasmodium. We here report results which indicate that origins of replicons are bound to the nuclear matrix during the entire nuclear cycle and that replication points are bound in the S phase but are released from the binding sites after termination of the replication.

MATERIAL AND METHODS

Growth and labelling of Physarum plasmodia. Microplasmodia of Physarum polycephalum, strain M_{3C} IV, were grown as described by Daniel and Baldwin (15). Macroplasmodia were prepared according to Güttes and Güttes (16) and the stages of the nuclear cycle were determined as described by Schel and Wanka (17).

Plasmodia were pre-labelled by adding 0.2 $\mu\text{Ci/ml}$ [$2\text{-}^{14}\text{C}$] deoxythymidine (about 50 mCi/mmol) to the growth medium. To terminate the pre-labelling the plasmodia were transferred to label-free medium at least 1 h before the pulse labelling experiments were performed. For pulse experiments the plasmodia were placed on 0.5 ml medium containing 200 $\mu\text{Ci/ml}$ [$\text{Me-}^3\text{H}$] deoxythymidine (20 Ci/mmol) for the times indicated. For chase experiments the plasmodia with the supporting filter discs were briefly put on sterile filter paper to remove excess fluid, then briefly washed and placed for 3 min on unlabelled medium containing 20 $\mu\text{g/ml}$ thymidine and subsequently grown on medium without thymidine addition for the time required.

Preparation and analysis of the nuclear lysates. Nuclei were isolated essentially as described by Mohberg and Rusch (18) using an isolation medium containing 0.25 M sucrose, 5 mM MgCl_2 , 10 mM Tris-HCl pH 7.2 and 0.1% Triton X-100. Nuclear lysates were prepared by suspending the isolated nuclei in an appropriate volume of 50 mM Tris-HCl pH 7.2 and mixing the suspension with

an equal volume of 4 M NaCl in the same Tris buffer. Digestions with DNase I (Sigma Corp., electrophoretically pure) were carried out at 37°C in the presence of 7.5 mM MgCl₂. The digestions were terminated by adding EDTA at a final concentration of 10 mM.

15 to 40% sucrose gradients containing 2 M NaCl were prepared on 65% sucrose cushions containing 2 M NaCl and 0.4 g/ml CsCl. Samples of 4 ml were placed on top of the gradients and centrifuged for 2 h at 20 000 r.p.m. and 15°C in a Beckman 27-2 rotor, if not stated otherwise.

Determination of the radioactivities. To determine the label incorporation into plasmodia, samples were removed and washed in 70% ethanol. The material was then dissolved in 1% SDS (sodium dodecyl sulphate) solution. Salmon sperm DNA was added at a final concentration of 0.2 mg/ml. The DNA was precipitated by mixing with 0.5 vol of cold 20% trichloroacetic acid and the hot-acid soluble radioactivity of the sediment was determined as described (19). Radioactivities of sucrose gradient fractions were precipitated and determined in the same way.

When DNA and protein were labelled simultaneously, in addition to DNA, bovine serum albumin was added as carrier at a final concentration of 0.4 mg/ml and the acid insoluble precipitate was redissolved in 0.2 ml 1 N NaOH for the determination of the radioactivities.

RESULTS

Deoxythymidine incorporation into plasmodia in pulse and chase experiments. The particular advantage of *Physarum* plasmodia lies in the excellent synchrony of the nuclear events and the predictability of the onset of DNA replication (20). DNA synthesis starts between 5 and 13 min after metaphase (21). However, the duration of the S phase as reported by different authors varies considerably (22 - 25). This may be due to minor differences in culture conditions. We therefore determined the thymidine incorporation into the hot-acid soluble material during successive 10 min intervals following the 3rd mitosis after plasmodial fusion. As shown in fig. 1 DNA synthesis started between 5 and 15 min after metaphase and gradually ceased 2 hours later. Thymidine incorporation into plasmodia continues at a low rate during the G₂ phase, due to mitochondrial and nuclear DNA synthesis (26, 27). The duration of the total nuclear cycle from mitosis to mitosis varied between 7 and 8 hours.

The abrupt increase of the thymidine incorporation at the beginning of the S phase makes it possible to specifically label at least that population

Figure 1. Rate of thymidine incorporation during the S phase.

Parts were cut out of a pre-labelled plasmodium at successive times during the S phase and placed on medium containing 10 $\mu\text{Ci/ml}$ [^3H]dThd. Hot-acid soluble counts were determined in each sample after 10 min of incorporation. The points represent amounts of [^3H] label incorporated per unit of [^{14}C] labelled bulk DNA. Data are averaged from 3 experiments.

of replication origins which is initiated at the beginning of the S phase. To assess the specificity of the origin labelling in pulse-chase experiments we determined the incorporation of label during the chase after a pulse. This is of particular importance in view of the large pools of thymidine nucleotides (28, 29) and the long transportation distances in the voluminous plasmodia. Representative results of these experiments are summarised in fig. 2. They show that a rapid increase of acid insoluble radioactivity continues for 2 - 3 min after the termination of the pulses and is followed by a further incorporation at a negligible rate. The total radioactivity incorporated into the DNA during the chase was usually equal to that of a 6 to 7

Figure 2. Continuation of label incorporation in pulse-chase experiments. Pre-labelled plasmodia were pulse labelled either from 0 - 20 min (+), 25 - 30 min (x) or 35 - 40 min (o) after metaphase. The plasmodia were then chased in the presence of 25 $\mu\text{g/ml}$ unlabelled thymidine. Samples were removed for the determination of hot-acid soluble label at successive times. The increase of [^3H] label per unit [^{14}C] DNA is expressed in % of that found at the end of the pulse.

min pulse. This incorporation is due to the presence of labelled thymidine nucleotides accumulated in the plasmodium during the pulse period which is only gradually diluted by the addition of unlabelled thymidine at the beginning of the chase. The incorporation of label during the chase was about twice as high when the unlabelled thymidine was omitted from the chase medium (results not shown). For obvious reasons the relative increase of the acid insoluble radioactivity was higher after a short pulse than after a long one.

We would like to mention that the apparent label increase was irregular after short pulses given at the very beginning of the S phase (fig. 2). This irregularity is due to the fact that different areas of a plasmodium enter the S phase at slightly different times and therefore incorporate different amounts of label during the pulse period (21). These differences add up to the label incorporated during the subsequent chase and contribute to the final amount of label in the various parts of a plasmodium. It is therefore not possible to determine reliably the radioactivity incorporated during the chase which follows a pulse at the beginning of the S phase. It may be presumed, however, that it will not differ much from that incorporated after a pulse applied at a later time.

The DNA-matrix complex. Treatment of *Physarum* nuclei, isolated in the presence of 0.1% Triton X-100, with 2 M NaCl results in a residual protein structure. The high-salt insoluble structure is analogous to the nuclear matrix of higher eucaryotes (1, 2, 4). In electron micrographs thin DNase sensitive threads are found to be associated with the matrix (30). To estimate the proportions of total nuclear protein and DNA associated with the matrix-DNA complex we grew macroplasmodia of *Physarum* for 24 h on a medium containing [^3H]-leucine and [^{14}C]-thymidine. The nuclei were isolated 4 h after metaphase and the nuclear lysate prepared in 2 M NaCl was analysed by sedimentation through sucrose gradients. Fig. 3 shows that 36% of the protein label and almost 90% of the DNA label were associated with the rapidly sedimenting salt-insoluble structure. Such high proportions of matrix-associated DNA were only found in carefully prepared salt lysates. Detachment of up to 40% of the DNA, due to unavoidable shear of endogeneous nucleolytic digestion, were not uncommon. More than 99% of the [^{14}C] label were released from the matrix by a moderate DNase treatment (fig. 3B) indicating that virtually all [^{14}C] was present in the DNA. Obviously, the proportions of total plasmodial protein and RNA present in the matrix are so small that aspecific incorporation of radioactivity, derived from thymidine degradation, does not impair the interpretation of the results (31).

Figure 3. Association of DNA and protein with a rapidly sedimenting matrix structure. A plasmodium was labelled with 2 $\mu\text{Ci/ml}$ [^3H] leucine and 0.2 $\mu\text{Ci/ml}$ [^{14}C]dThd for 20 hours. A nuclear lysate was prepared 4 h after metaphase and centrifuged through a 2 M NaCl containing sucrose gradient for 16 h at 20,000 r.p.m. (A). Parts of the lysate were digested for 20 min with 10 $\mu\text{g/ml}$ DNase at 37°C (B) or 1 mg/ml proteinase K at 45°C (C) prior to centrifugation. Sedimentation direction is from right to left.

Fig. 3 also shows a decrease of matrix associated protein label from 36 to 27% upon DNase treatment. This could mean that a minor part of the labelled proteins is attached to the DNA loops which are removed from the complex by the DNase treatment. But less or no loss of protein label has been observed in other experiments. On the other hand, exhaustive proteolytic digestion of the matrix always released the DNA from the rapidly sedimenting structure (fig. 3C) indicating that the protein matrix functions as a scaffold to which the DNA is bound. Digestion of nuclear lysates with RNase had no effect of the sedimentation pattern.

It should be noticed that the nuclei used for this particular experiment have been isolated 4 h after the metaphase. This means that the attachment of the DNA is not limited to the S phase but continues into the G_2 . This question of a continuous binding of the DNA has not been considered in detail in previous studies (7, 12).

Binding of the DNA at the replication forks. To investigate the attachment of specific DNA regions to the matrix we first labelled these regions specifically and then analysed the label distribution by graded DNase digestion of the nuclear lysates. The released DNA fragments were subsequently separated from the matrix by centrifugation. A typical experiment for pulse labelled replicating DNA regions is shown in fig. 4. About 60% of the total randomly labelled DNA and 95% of the newly synthesized DNA were associated with the rapidly sedimenting matrix. After further release of DNA by diges-

Figure 4. Attachment of newly replicated DNA to the matrix. A plasmodium pre-labelled with [^{14}C] was pulse labelled with [^3H] from 15 to 25 min after metaphase. Nuclei were isolated from one half of the plasmodium immediately (A and B) and from the other half after a chase of 1 h (C and D). Nuclear lysates were prepared and half of each lysate was incubated with 2.5 $\mu\text{g/ml}$ DNase for 15 min (B and D). The samples were analysed by sucrose gradient centrifugation. Sedimentation is from right to left.

tion with DNase I 15% of the total and 70% of the newly synthesized DNA remained with the matrix (fig. 4B). The relative increase of pulse label in the matrix associated DNA residues indicates that the replicating DNA molecules are attached to the matrix by the fork region (8) and, consequently, pass through the binding sites along with the movement of the replication fork. On continuing replication, a once labelled DNA region should be displaced into a position more or less remote from the binding site. This is supported by the finding that the enrichment of pulse label in the matrix associated DNA residues becomes negligible after a 1 h chase (fig. 4C and D).

The preferential association of the newly replicated DNA regions with the matrix is characterized by a progressive increase of the [^3H]/[^{14}C] ratio in the residual material when the DNA is detached from the complex by graded DNase digestion (8, 10). Fig. 5 summarizes several representative experiments performed at different times during the S phase. Clearly, the most recently replicated DNA parts are associated with the nuclear matrix at any time of the S phase. However, pulse label incorporated at the very beginning of the S phase was always less accessible to DNase than pulse label incorporated at a later time (upper and lower curve in fig. 5 respectively).

Permanently bound DNA sites. As already mentioned the attachment of the

Figure 5. Matrix attachment of newly replicated DNA during the S phase. Plasmodia were pulse labelled at different times of the S phase. Lysates were prepared immediately after the pulse and digested with graded concentrations of DNase. The concentration range in this and the following experiments was between 0.3 and 10 $\mu\text{g/ml}$. The digested samples were analysed by sucrose gradient centrifugation as described in fig. 4. Relative ratios are % of [^3H] label per % of [^{14}C] label associated with the rapidly sedimenting matrix (corresponding to fractions 1 to 5 in fig. 4). They are plotted as a function of the % bulk DNA remaining at the matrix after the DNase treatment. The pulse times of the 5 experiments were as follows: x and + from 0 to 10 min after metaphase, o from 30 to 35 min after metaphase, • from 80 to 85 min after metaphase and Δ from 100 to 110 min after metaphase.

DNA to the matrix is not limited to the S phase but continues into the G_2 (see fig. 3). We next tried to find out whether the attachment in the G_2 phase is random or whether it is in some way related to the replication process. For example one might imagine that either origin or termination sites of the replication remain permanently bound to the matrix. We therefore pulse labelled plasmodia at various times of the S phase and then chased until the late G_2 phase. In such experiments label incorporated in the middle of the S phase did not appear at a distinct position with regard to the attachment site. The relative [^3H]/[^{14}C] ratios upon DNase digestion were close to 1 with only insignificant and irregular deviations (results not shown).

To label origins of replicons preferentially [^3H] thymidine was added at metaphase in order to ensure a high specific activity of the dTTP pool at the onset of DNA synthesis. The plasmodia were transferred to chase medium 10 min later. This results in a very efficient labelling of the newly initiated DNA which, by this time, varies in length from below 1 to about 4 μm (21, 32). Attempts to reduce the length of the labelled regions by a shorter pulse were not successful because the radioactivity incorporated was too low for a reliable analysis of the label distribution. Fig. 6 shows that the pulse label appeared preferentially in DNA residues not detached from the matrix by DNase digestion. The inverse relationship between [^3H]/[^{14}C] ratio and

Figure 6. Attachment of the origins of replicons to the matrix. Pre-labelled plasmodia were pulse labelled with [^3H]dThd from 0 to 10 min after metaphase. The plasmodia were then chased for various times and nuclear lysates were analysed as described in fig. 5. x and + chase until late G_2 (6 h after metaphase), o, ● and Δ chase until 15 min after the subsequent metaphase, Δ chase until 90 min after the subsequent metaphase. The upper curve approximates the data obtained with the chase until the onset of the next S phase, the lower curve those of the other chase times.

amount of attached DNA indicates that the binding is at the pulse labelled regions even when they are very short, including little more than the origins of replicons. This preferential association of the initially labelled DNA regions to the matrix was observed during the G_2 and the subsequent S phase and was slightly, but reproducibly elevated during the onset of the latter (fig. 6).

To label termination sites and/or adjacent regions we pulse labelled plasmodia at the end of the S phase and subsequently chased into the late G_2 phase. Label incorporated into this region was more rapidly released from the matrix than bulk DNA. This is shown by the progressive decrease of the [^3H]/[^{14}C] ratio in the matrix bound DNA residues remaining upon digestion

Figure 7. The detachment of the termination sites after the replication. Pre-labelled plasmodia were pulse labelled with [^3H]dThd from 90 to 100 (o) and 100 to 110 (●) min after metaphase and chased until 6 h after metaphase. Nuclear lysates were then analysed as described in fig. 5.

with increasing DNase concentrations (fig. 7). Apparently the labelled DNA regions are at a remote position from the G_2 attachment sites.

We conclude from these experiments that a DNA site at or close to the origins of replicons remains attached to the matrix in the G_2 phase, while the attachment of replication points is released after completion of the replication. This conclusion was supported by the result of a final experiment. An unlabelled plasmodium was pulse labelled with [^3H] thymidine at the beginning of the S phase and a second time with [^{14}C] thymidine 100 to 115 min after metaphase. The label was then chased in the usual way and the label distribution was analysed again in the late G_2 phase. As shown in fig. 8 the label incorporated in the late S phase was more rapidly removed by the DNase treatment than the label incorporated during the initiation of the DNA synthesis. This supports the conclusion that termination sites are remote from the attachment sites.

DISCUSSION

A residual protein structure, usually named nuclear matrix, can be obtained from isolated cell nuclei by extraction with 2 M NaCl (1 - 4). In careful preparations almost 100% of the nuclear DNA remain associated with the nuclear matrix (6 - 8). Graded digestion of the complex by DNase has

Figure 8. Different release of early and late pulse labelled DNA by digestion with DNase.

An unlabelled plasmodium was pulse labelled from 0 to 10 min after metaphase with 200 $\mu\text{Ci}/\text{ml}$ [^3H]/dThd and from 100 to 115 min after metaphase with 10 $\mu\text{Ci}/\text{ml}$ [^{14}C]/dThd. The plasmodium was then chased into the subsequent G_2 phase (6.5 h after metaphase) and the nuclear lysates were analysed by digestion with DNase. The percentages of [^3H] and [^{14}C] label remaining associated with the matrix were investigated as shown in fig. 4. The dashed line indicates the result that should be obtained with random attachment sites.

revealed that, after a short pulse label, the radioactivity is preferentially localized in the matrix associated DNA regions (6 - 12). This is indicated by the increase of the [^3H]/[^{14}C] ratio which is observed when the [^{14}C]dTh pre-labelled and [^3H]dThd pulse-labelled DNA-protein complex is subjected to a graded DNase digestion. A higher ratio increase, up to 12, was observed when the pulse duration was reduced to 2 min. This is in agreement with previous results (8, 12) and according to the theory (10). Actually, the higher ratios observed at the onset of the S phase (fig. 6) are also due at least in part, to shorter incorporation times. This is because the plasmodia, although exposed to the label for 10 min, start with the replication about half way this time or even later.

The possibility that the association results from artificial precipitation of nascent DNA to the matrix can be disregarded as shown previously. Purified native and denatured DNA, added to the nuclei 15 min prior to the 2M salt treatment does not become bound to the matrix (6, 7, 9). The non-specific attachment of some hypothetical replication complex is also unlikely because there is no evidence for the existence of such a salt resistant complex independent of the matrix. Also it has been shown that Okazaki fragments hydrogen bonded to parental DNA can be excised from the fork region by single strand specific nuclease. The same results are obtained with isolated nuclei and with the salt resistant DNA-matrix complex (8, 33). Although the possibility of an artifact cannot be excluded with absolute certainty, we do not consider it to be a realistic possibility.

The experiments described above show that the binding of replication forks exists during the entire S phase (12). This implies that the matrix contains specific binding sites through which the DNA molecules move during replication. Such a movement has been illustrated by autoradiography (10, 11). The data presented here also indicate that the DNA is released from the replication binding sites after completion of the DNA synthesis but, owing to additional binding, remains still associated with the matrix. This is concluded from the observation that label incorporated at the end of the S phase is initially enriched in the matrix attached DNA regions, but can be preferentially detached from the matrix-DNA complex by DNase digestion after a chase into the G_2 phase. Apparently the label is now at a position remote from the binding site, for example at the distal part of the DNA loop shown diagrammatically in fig. 9B. This means that the additional binding sites are located at a certain distance from the termination sites of consecutive replicons, possibly at their origins.

A major difference between the two types of binding is that binding of the fork region is transient and therefore random, while origin binding, by definition should be non-random and most probably permanent. Non-random binding of the DNA has also been inferred from the observation that α , β and γ globin gene loci are at distinct distances from the attachment sites (34). It is further supported by the predominance of middle repetitive nucleotide sequences in the matrix associated DNA regions (35). There is also circumstantial evidence for a relation between the distribution of the binding sites and the organization of the genome: 1) In 3T3 cells the DNA loops emerging from the residual nuclear structure are close to 90,000 base pairs (11), which is about the length of replicons (36). 2) In mammalian cell nuclei estimates of domains between chromatin attachment sites are in the range of replicon size (37). Interestingly, mammalian chromosomes partially deproteinized by 2 M NaCl treatment also reveal a protein scaffold to which the DNA is attached in such a way that the loops between attachment sites are of the order of replicon size (38). These findings suggest that nonreplicating DNA has one permanent binding site per replicon.

More direct proof of a permanent binding of replicon origins to the nuclear matrix is provided by the present results. The evidence is that label specifically incorporated into the origins at the onset of the S phase is found preferentially in the matrix bound DNA regions during the G_2 phase and the next S phase. The accessibility of the pulse label to the action of DNase is considerably lower at the end of the pulse than after the chase. The main reason for this is the binding of the replication forks present at both ends of the pulse labelled regions. As outlined in fig. 9 a single discontinuity in the newly synthesized part is not sufficient to release pulse labelled DNA. It will result, however, in a release of pulse label in the G_2 phase because of the absence of a second binding close to the origin. Another cause for the increased release of pulse label in the G_2 is the fact that additional radioactivity is incorporated into the DNA beyond the original pulse labelled region during the chase. This additional label is more exposed to the action of DNase (fig. 9B).

The neighbourhood of additional binding sites after the initiation of the replication leads to another interesting point. In procaryotes, origins of replication are specific nucleotide sequences at which DNA synthesis is initiated in successive replication cycles (39). It is generally assumed that this is also the case in eucaryotes although evidence on this point is scarce. It has been shown by fiber autoradiography that DNA synthesis is

Figure 9. Diagram showing how the DNA release from the matrix by DNase digestion depends on the binding conditions.

A DNA loop between 2 origin binding sites (o) is shown shortly after the initiation of replication (A and C) and in the G_2 phase (B). At the end of the pulse (A) the label, indicated by dots, is confined to the parts between the origin and replication binding sites (o), but owing to post-pulse incorporation it extends beyond this region in the G_2 and subsequent S phase (B and C). Random breaks by DNase are represented by discontinuities. A limited DNase digestion leads to a preferential release of poorly labelled, distal parts of the loop. The relative amount of pulse label in the attached residues is highest in case A because the release of pulse label requires at least 2 discontinuities between the attachment sites. Pulse label will not be released by a single strand discontinuity in the pulse labelled DNA region in cases A and C but it will be released in the G_2 phase (case B) or late S phase (not shown). In addition the apparent enrichment in cases B and C will be reduced owing to the release of post-pulse label.

initiated at the same site in successive replication cycles (49). If this is the case label incorporated at the very beginning of one S phase should be temporarily less accessible to the DNA action at the onset of the subsequent one, because the release by randomly distributed discontinuities will be less effective due to the presence of the additional binding sites (fig. 9C). A corresponding elevation of the $[^3\text{H}]/[^{14}\text{C}]$ ratio in the residual matrix bound DNA fragments has been observed repeatedly.

Origins of replicons which are active later in the S phase can not be labelled selectively. Their attachment to the matrix may be inferred, however, from the observation that termination sites of late replicons are remote from the site of attachment to the matrix. On the other hand there is some recent evidence that in *Physarum* all replication origins are initiated at the beginning of the S phase (41, 42). These findings, together with those on the

lengths of DNA loops between successive attachment sites (11, 37, 38, 43) suggest that each replicon is permanently attached to the nuclear matrix at its origin or a site very close to it. Significantly, viral DNA in polyoma infected 3T6 cells is also attached to the nuclear matrix by the origins of replication or adjacent regions (44).

We have isolated the residual DNA fragments remaining after extensive DNase digestion. Their average length was 200 to 300 base pairs. From our preliminary results it seems that the fragments are similar to bulk DNA in base composition and content of repetitive nucleotide sequences.

Our results give no evidence for a temporal or permanent binding of additional DNA sites, not related to the replicon organization. But the presence of a limited number of such binding sites cannot be excluded either.

REFERENCES

1. Berezney, R. and Coffey, D.S. (1974) *Biochem. Biophys. Res. Commun.* 60, 1410-1417.
2. Berezney, R. and Coffey, D.S. (1977) *J. Cell Biol.* 73, 616-637.
3. Herlan, G. and Wunderlich, F. (1976) *Cytobiologie* 13, 291-296.
4. Mitchelson, K.R., Bekers, A.G.M. and Wanka, F. (1979) *J. Cell Sci.* 39, 247-256.
5. Berezney, R. and Coffey, D.S. (1975) *Science* 189, 291-293.
6. Wanka, F., Mullenders, L.H.F., Bekers, A.G.M., Penning, L.J., Aelen, J.M.A. and Eygensteyn, J. (1977) *Biochem. Biophys. Res. Commun.* 74, 739-747.
7. Wanka, F. and Mitchelson, K.R. (1979) in: *Current Research in Physarum*, Vol. 120 (Sachsenmaier, W. ed.) pp. 59-63, Univ. of Innsbruck press.
8. Dijkwel, P.A., Mullenders, L.H.F. and Wanka, F. (1979) *Nucl. Acids Res.* 6, 219-230.
9. Pardoll, D.M., Vogelstein, B. and Coffey, D.S. (1980) *Cell* 19, 527-536.
10. McCready, S.M., Godwin, J., Mason, D.W., Brazell, J.A. and Cook, P.R. (1980) *J. Cell Sci.* 46, 365-386.
11. Vogelstein, B., Pardoll, D.M. and Coffey, D.S. (1980) *Cell* 22, 79-85.
12. Hunt, B.F. and Vogelstein, B. (1981) *Nucl. Acids Res.* 9, 349-363.
13. Dingman, C.W. (1974) *J. Theor. Biol.* 43, 187-195.
14. 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.
15. Daniel, C.W. and Baldwin, H.H. (1964) *Methods in Cell Physiology* 1, 9-41.
16. Gütttes, E. and Gütttes, S. (1964) *Methods in Cell Physiology* 1, 43-53.
17. Schel, J.H.N. and Wanka, F. (1973) *Exp. Cell Res.* 79, 287-294.
18. Mohrberg, J. and Rusch, H.P. (1971) *Exp. Cell Res.* 66, 305-316.
19. Wanka, F. (1974) *Exp. Cell Res.* 85, 409-414.
20. Nygaard, O.F., Gütttes, S. and Rusch, H.P. (1960) *Biochim. Biophys. Acta* 38, 298-306.
21. Beach, D., Piper, M. and Shall, S. (1980) *Exp. Cell Res.* 129, 211-221.
22. Braun, R., Mittermaier, C. and Rusch, H.P. (1966) *Biochim. Biophys. Acta* 114, 527-535.
23. Braun, R. and Willi, H. (1969) *Biochim. Biophys. Acta* 174, 246-252.
24. Sachsenmaier, W. (1964) *Biochem. Zeitschr.* 340, 541-547.
25. Solao, P.B. and Shall, S. (1971) *Exp. Cell Res.* 69, 295-300.

26. Braun, R. and Evans, T.E. (1969) *Biochim. Biophys. Acta* 182, 511-522.
27. Holt, C.G. and Gurney, E.G. (1969) *J. Cell Biol.* 40, 484-496.
28. Bersier, D. and Braun, R. (1974) *Biochim. Biophys. Acta* 340, 463-471.
29. Fink, K. (1975) *Biochim. Biophys. Acta* 414, 85-89.
30. Bekers, A.G.M., Gijzen, H.J., Taalman, R.D.F.M. and Wanka, F. (1981) *J. Ultrastruct. Res.* 75, 353-362.
31. Fink, K. and Nygaard, P. (1978) *Eur. J. Biochem.* 89, 417-424.
32. Funderud, S., Andreassen, R. and Haugli, F. (1978) *Cell* 15, 1519-1526.
33. Opstelten, R.J.G., Dijkwel, P.A. and Wanka, F. (1981) *Biochem. Biophys. Res. Commun.* 101, 807-813.
34. Cook, P.R. and Brazell, J.A. (1980) *Nucl. Acids Res.* 8, 2895-2906.
35. Razin, S.V., Mantieva, V.L. and Georgiev, G.P. (1979) *Nucl. Acids Res.* 7, 1713-1735.
36. Huberman, J.A. and Riggs, A.D. (1968) *J. Mol. Biol.* 32, 327-341.
37. Igo-Kemenes, W.T. and Zachau, H.G. (1977) *Cold Spring Harbor Symp. on Quant. Biol.* 42, 109-118.
38. Paulson, J.R. and Laemmli, U.K. (1977) *Cell* 12, 817-828.
39. Kornberg, A. (1980) *DNA Synthesis*, W.H. Freeman, San Francisco.
40. Amaldi, F., Buongiorno-Nardelli, M., Carnevali, F., Leoni, L., Mariotti, D. and Pomponi, M. (1973) *Exp. Cell Res.* 80, 79-87.
41. Funderud, S., Andreassen, R. and Haugli, F. (1978) *Nucl. Acids Res.* 5, 3303-3313.
42. Haugli, F., Andreassen, R. and Funderud, S. (1982) *J. Cell Biol.* 95, 323-331.
43. Buongiorno-Nardelli, M., Cari, M.T. and Marilley, M. (1982) *Nature* 298, 100-102.
44. Buckler-White, A.J., Humphrey, G.W. and Pigiet, V. (1980) *Cell* 22, 37-46.