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# Nuclear organization and dynamics of DNA replication in eukaryotes

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## 1. ABSTRACT

Replication foci are the units of DNA replication in the nucleus. They harbor the replication machinery and activereplicons. Spooling of individual replicons appears to occur at replication foci of budding yeast. The dynamics associated with fork progression in mammalian nuclei are largely unclear but large-scale spooling of DNA does not occur. Replication foci are arranged into spatio-temporal higher-order patterns. Different kinds of such patterns have been observed in different eukaryotic taxa but the characteristic patterns observed in mammals are highly conserved. The changes of higher-order patterns of replication foci during S phase progression are in mammals due to the sequential association of the replication machinery with neighboring stably positioned chromatin domains, which leads to the sequential formation of replication foci at neighboring nuclear sites. These findings suggest that the spatio-temporal patterns of replication foci are determined by the underlying genome architecture. Future studies will have to address the question in which way exactly genome architecture is involved in the spatial and temporal regulation of DNA replication.

## 2. INTRODUCTION

Genomes are multiplied by the process DNA replication. DNA replication initiates in viral, bacterial, and eukaryotic genomes at so-called origins of DNA replication (1-3). Here, unwinding of the two parental DNA strands is initiated, which serve as templates for the synthesis of the new daughter strands. Unwinding and synthesis of new DNA strands proceeds at the so-called replication fork, which is found at increasing distances from the origin of replication as DNA replication progresses (4, 5).

Viral and bacterial genomes are under the control of only one origin, from which the whole genome is replicated. In contrast, larger eukaryotic genomes possess multiple origins (1, 4, 5). The DNA unit that is under the control of one origin is called replicon and in most cases one replicon is replicated by two replication forks emanating bi-directionally from a central origin (2, 4, 5).

The process of DNA replication, which takes place during the S phase of the cell cycle, covers extended time periods in eukaryotes and is only finished after about 1

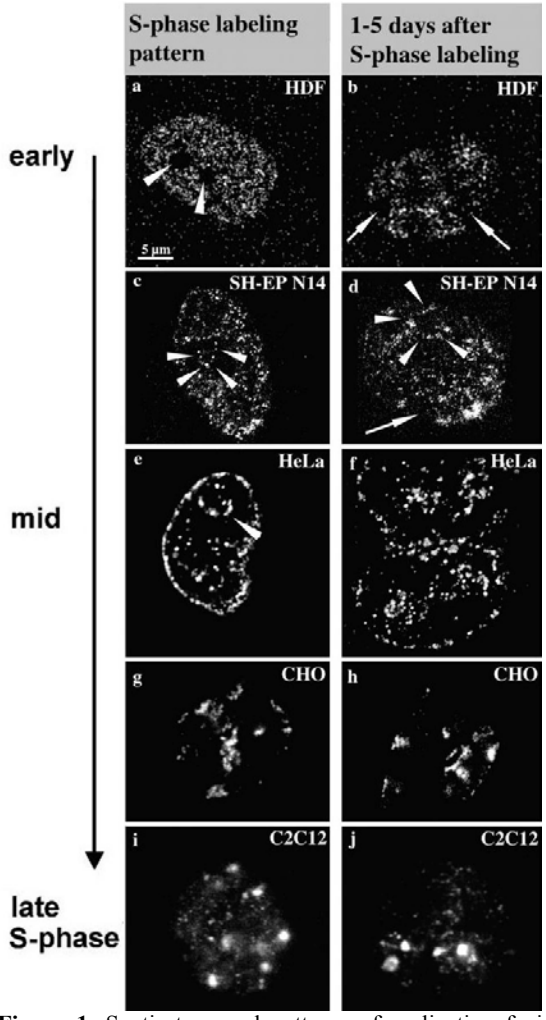


**Figure 1.** Spatio-temporal patterns of replication foci in mammalian cells and stable positioning of chromatin with a defined replication timing. The panels show nuclei of different mammalian cell types (HDF: human primary diploid fibroblasts, SH-EP N14: human neuroblastoma cell line, HeLa: human cervix carcinoma cell line, CHO: chinese hamster ovary cells, C2C12: murine myoblast cell line). Replicating DNA has been pulse-labeled during S phase with labeled nucleotides. Cells were fixed immediately (left-hand panels) or 1-5 days after labeling (right-hand panels). Nuclei display the characteristic labeled foci, which show the typical changes in numbers and sizes and of spatial higher-order patterns during S phase progression (left-hand panels, temporal progression of S phase as indicated on the left). Several days after labeling (right-hand panels) cells and their descendants (note daughter nuclei with similar labeling patterns in f) still show the same patterns, indicating the stable spatial positioning of chromatin domains with a defined replication timing during the interphases of subsequent cell cycles. Arrowheads point to nucleoli and arrows point to unlabeled chromosome territories, which appear after the second mitosis after labeling due to the segregation of labeled and unlabeled chromosomes. Scale bar: 5 micrometer. Reproduced with slight changes from (34) with permission ofThe Rockefeller University Press.

hour in budding and fission yeast and after about 8-10 hours in mammalian cells. During these extended time periods not all origins fire simultaneously but only certain origins fire at specific time points of S phase (6-9). The temporal control of origin firing is currently not very well understood but appears to be related in somatic cells of higher eukaryotes to transcriptional regulation as well as to DNA sequence composition, histone modifications, and the nuclear positioning of a given chromosomal domain (6, 7, 10). Despite of these difficulties in understanding the temporal control of DNA replication, it is obvious that partitioning of genomes into distinct regions that replicate sequentially at defined time points of S phase is a fundamental principle of eukaryotic genome organization, which applies to metazoans (6, 7, 10-12) as well as to budding and fission yeast (8, 9).

In mammalian cells groups of neighboring origins fire simultaneously together at defined time points of S phase, as already observed in early experiments performed in the 1960s using fiber autoradiography of DNA that was labeled with radioactive DNA precursors (4, 5, 13). Such groups of simultaneously replicating neighboring DNA units are called replicon clusters. Although not every replicon appears to be organized into a cluster (14, 15), recent analyses revealed that chromosomal regions that replicate at a given time point during S phase are relatively broad and cover about 0.1-2.0 mega basepairs on human chromosome 22 (16).

Together, these findings show that the process of DNA replication in eukaryotes is highly organized at the structural as well as at the temporal level. In addition, DNA replication is a dynamic process involving fork progression and sequential activation of distinct genomic regions. This raises the questions how this process is spatially and temporally organized in the nucleus of eukaryotic cells, where DNA replication takes place, and which kinds of nuclear dynamics are involved. These questions will be addressed in the following sections.

## 3. SPATIAL ARRANGEMENTS AND DYNAMICS OF REPLICATION FOCI

### 3.1. Replication foci and their nuclear higher-order patterns in metazoans

A fundamental observation with regard to mammalian S phase is that DNA replication does not take place uniformly throughout the nucleus but occurs at so-called replication foci (RF), which have been observed in fixed as well as in living cells (14, 17-20) (Figure 1). RF are also called replication sites, domains, or factories. One way to visualize RF is to perform pulse-labeling during S phase with labeled DNA precursors, which become incorporated into the newly synthesized DNA. For example, halogenated, biotinylated or fluorescently labeled nucleotides like bromodeoxyuridine or Cy3-dUTP have been used (19-21). As replicons and replicon clusters are the physical units of DNA replication at the level of the DNA fiber and as RF are the physical entities of DNA replication in the nucleus it is generally believed that most RFs represent three-dimensionally (3D) organized replicon

clusters (7, 14, 21, 22). This view is supported by the finding that the DNA content of RF is in the range of about 1 Mb (19), which corresponds to the average size of a replicon cluster. Some RF may also represent single large replicons, as not every replicon appears to be organized into a cluster (14, 15).

Furthermore, studies combining replicational pulse-labeling of DNA and detection of replication proteins revealed that all proteins involved in DNA replication, like the DNA polymerases, DNA ligase I, and the proliferating cell nuclear antigen (PCNA) are assembled at replication foci, where they form large complexes (17, 23-25). These complexes also harbor cell cycle proteins (26) as well as proteins important for the replication of epigenetic marks (27), such as DNA methyltransferase (28) and histone deacetylase (HDAC) 2 (29). HDAC2 is recruited to RF during late S phase and while the protein composition of RF might remain similar throughout S phase with regard to proteins involved in DNA replication, the association with proteins involved in the replication of epigenetic marks might differ at distinct temporal stages, at which chromatin with different epigenetic mark replicates.

DNA pulse-labeling studies revealed that the average lifetime of mammalian RF is in the range of about 45 minutes up to 1 hour (30). Live cell studies using GFP-tagged PCNA revealed a considerable variability in the lifetime of RF, ranging from about 30 minutes to 3 hours (18). Live cell studies of mammalian RF also revealed that DNA replication continuously ceases at earlier replicating RF during S phase progression, while it becomes initiated at later replicating RF (18, 31, 32).

In addition to constant changes in replicational activity, the numbers and sizes of RF change during S phase progression, as well as their nuclear higher-order patterns (Fig 1) (from the extensive literature on replication patterns see, for example (19, 20, 33, 34). Thus, during early S phase numerous small RF are distributed over large parts of the nucleus. Such early RF display diameters in the range of about 300 nm and are excluded from nucleoli and heterochromatic areas. As S phase proceeds RF progressively disappear from interior nucleoplasmic regions but appear instead at perinuclear and perinucleolar regions, where most RF are concentrated during mid S phase. During the second half of S phase RF progressively disappear from these regions and only relatively few but large RF are typically present during late S phase, which can be found at the periphery but also at other nuclear regions. Such late RF can have diameters of more than 1 micrometer.

Although it is obvious that the numbers of RF decrease during S phase progression while their sizes increase and many data concerning numbers and sizes of RF at different temporal stages of S phase can be found in the literature, it is actually very difficult to determine these parameters accurately, particularly the numbers of RF. The problems are mostly due to the fact that determining the overall nuclear numbers of RF requires the application of techniques compatible with the generation of 3D reconstructions of imaged nuclei and light microscopy including confocal microscopy has been extensively applied. However, especially during early S phase the small RF are so densely packed in the nuclear interior that also the application of high-resolution confocal microscopy does not appear to be sufficient to resolve all individual foci. Under such conditions also subsequent image analysis necessarily yields suboptimal results, also if sophisticated algorithms are applied. Furthermore, it is very difficult to perform appropriate controls for the results obtained by the image analysis. In the light of such problems it is not surprising that the numbers of RF counted during early S phase in mammalian cells increased from about 126 (19) via 250 (35), 600 (36), and 750 (22) to about 1100 (30), in parallel with the improvement of imaging and image analysis techniques. Whether the numbers of counted foci will further be adjusted in the future remains to be seen.

Apart from these problems with counting the numbers of RF during early S phase, also determining the exact numbers and sizes of RF during late S phase appears to be relatively problematic. This is suggested by a more recent live cell study, where replication foci have been visualized by GFP-tagged PCNA. The findings obtained by imaging live cells suggested that one large late replicating RF might consist of several smaller RF that are clustered (18) and such clusters appear be difficult to resolve in fixed cells, which have been mostly studied.

Given these uncertainties in determining numbers and sizes of RF at different S phase stages and given the variability in their lifetimes it is highly problematic to use such data for calculating the average numbers of replicons and replication forks per RF, as frequently performed. The question is anyway how useful it is to calculate the average numbers of replicons per focus, as the numbers of replicons per cluster and their sizes appear to be highly variable (14, 22). Genome-wide analyses of replication timing, as recently performed (16, 37, 38), might be more useful in order to learn more about numbers and sizes of mammalian genomic regions that replicate together at a particular time point, although also with such data the genome-wide numbers of origins and replication forks remain difficult to determine. Nervertheless, there appear to be recent advances in this field (39).

Despite of these uncertainties with regard to numbers and sizes of mammalian RF, it is obvious that they are arranged into defined spatial higher-order patterns, which are characteristic for a given temporal stage of S phase (Figure 1). These, characteristic spatio-temporal higher-order patterns of DNA replicating at defined time points during S phase are highly conserved in metazoans. Thus, similar patterns as observed in mammals can be found in fruit flies and in hydra (40, 41). Furthermore, corresponding spatio-temporal patterns are not only restricted to animals, but can be also found in plants (41).

It should be noted that the typical spatial arrangements of DNA domains replicating at a defined time point during S phase are not S phase-specific (Figure 1).

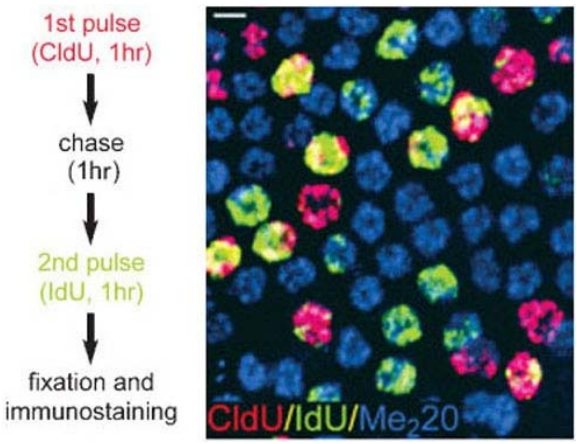


**Figure 2.** Spatio-temporal patterns of replication foci in the chordate *Oikopleura dioica.* Mitotically cycling diploid cells of the intestinal epithelium of *O. dioica* have been pulse- labeled according to the labeling scheme indicated on the left. The two different thymidine analogs chlorodeoxyuridine (CldU, red) and iododeoxyuridine (IdU, green) can be differentially detected with specific antibodies. Cells that had incorporated only IdU were in early or mid S phase, whereas cells that had incorporated both thymidine analogs were in late S phase at the time point of fixation. Accordingly labeled nuclei are shown on the right-hand panel. In addition, immunostaining of histone H4 di-methylated at lysine 20 ($Me_2$20) is displayed in blue, which is more broadly distributed in chromatin during S phase. The different patterns showing "patchy" as well as almost overall incorporation of thymidine analogs indicate spatio-temporal changes of replication patterns during S phase. This is also indicated by differential incorporation of IdU and CldU into partially non-overlapping chromatin zones (red and green "patches"). However, the patterns are clearly distinct from the spatio-temporal patterns observed in mammals. Reprinted with slight changes from (55) with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc...

This characteristic type of chromatin architecture, where chromatin domains with a defined replication timing occupy characteristic nuclear regions, becomes established rapidly after cell division and is from then on maintained for the rest of interphase (34). Thus, the typical and highly conserved spatio-temporal patterns of DNA replication observed during S phase reflect the underlying chromatin architecture (31, 34).

Although corresponding patterns of chromatin architecture and DNA replication appear to be highly conserved, garden peas (*Pisum sativum)* (41) and the chordate *Oikopleura dioica* (42, 43), which belongs to the sister group of vertebrates, display different spatio-temporal patterns of DNA replication and underlying genome organization (Figure 2). This suggests that some metazoan taxa have evolved independently different patterns of genome organization and DNA replication.

### 3.2. Replication foci in ciliates and yeast

How is DNA replication organized in the nuclei of unicellular eukaryotes and particularly in yeast, where many other features of DNA replication have been extensively studied? A recent study investigated the nuclear organization of DNA replication in the ciliate *Stylonychia lemnae,* which harbours a micro- and a macronucleus, like other ciliated protozoa. Micro- and macronuclei are functionally different and the germline micronucleus is transcriptionally inert. In both distinct nuclei DNA replication occurs at RF (44). In the micronucleus RF are arranged into spatio-temporal higher-order patterns displaying some similarity to those observed in mammals and other metazoans, suggesting evolutionary conservation (44). In contrast, RF are arranged into a replication band in the macronucleus, which progresses through the nucleus during DNA replication. Thus, RF are also present in this unicellular eukaryote, but they are arranged into different higher-order patterns in micro- and macronuclei.

Recent studies taking advantage of replication factors fused to fluorescent proteins confirmed the presence of RF in living cells of budding yeast (45) and fission yeast (46). In budding yeast it was observed that DNA replication occurs at RF harbouring DNA polymerase (45). Furthermore, live cell imaging of two fluorescently tagged genomic sites, which are located 60 kb apart on opposite sites of an origin on chromosome IV of budding yeast, revealed that these sites replicating at similar time points during S phase were closely spaced during their replication (45) (Figure 3). This suggests assembly of two forks belonging to one replicon at a common RF and is in accordance with the idea that RF comprise whole replicons at the DNA level.

Based on the numbers of replicons present in budding and fission yeast (8, 9) and the numbers of RF counted in living cells of these organisms (45, 46) it was suggested that each RF harbours about 10 replicons in budding yeast (45), and 14 replication forks originating from 7 bidirectional origins in fission yeast (46). However, counting of RF in the relatively small nuclei of these yeast species might not be a trivial task and, as with regard to mammals, such estimations should be interpreted carefully. Also, the highly variable fluorescence intensity of GFP-labeled RF in budding yeast suggests that the numbers of replicons at individual RF might be highly variable (45), which parallels the findings obtained with mammalian cells (14, 22).

Spatio-temporal patterns of RF have been further investigated in fission yeast (46). Also in this unicellular eukaryote RF formed different nuclear higher-order patterns at different temporal stages of S phase. The numbers of foci decreased progressively during S phase and typically two bright RF in or around the nucleolus were observed at the end of S phase (46). These changes of patterns are reminiscent to the spatio-temporal changes observed in metazoans.

In summary, RF appear to be conserved in eukaryotes and have been observed in all unicellular and

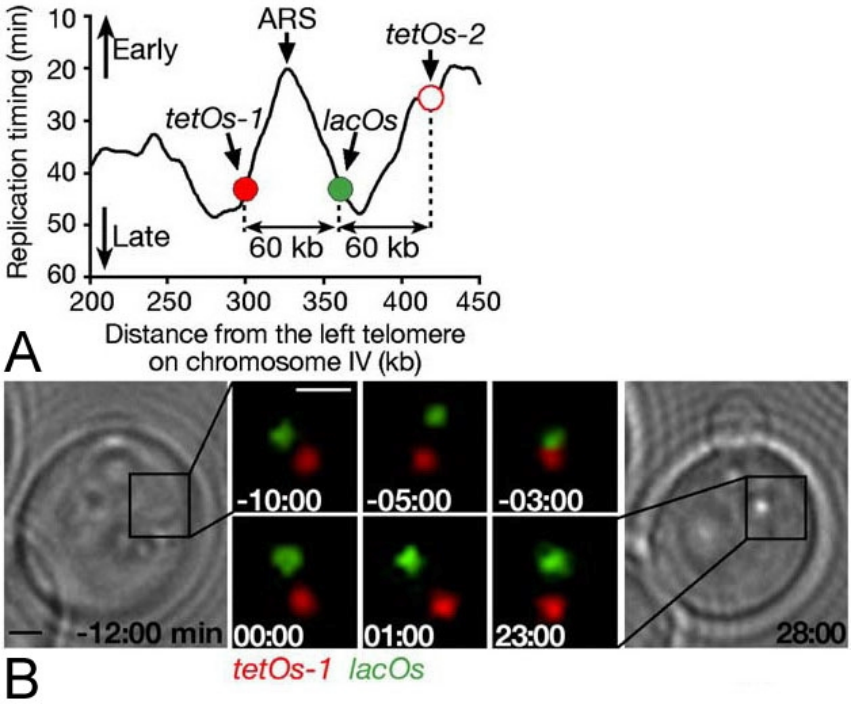


**Figure 3**. Replicon dynamics in budding yeast. A) Two loci 60 kb apart and on opposite sides of a replication origin (ARS) were marked with an array of 224 *tet* operators (*tet*OS-1) or 256 *lac* operators (*lac*OS), respectively. Alternatively, *tet* operators were integrated at the other site of *lac*OS (*tet*OS-2), also at a distance of 60 kb. The replication timing is indicated on the y-axis. *Tet*OS-1 and *lac*OS replicate at about similar time points during S phase (difference 2.0 minutes +/- 1.4 minutes; mean of the replication time of the two loci is defined as mid-replication time (45)), whereas *tet*OS-2 replicates earlier. Using strains expressing TetR-3CFP and GFP-lacI the different integrations could be visualized as CFP and GFP dots. B) A time-lapse series of CFP- and GFP-tagged *tet*OS-1 and *lac*OS loci in a haploid synchronized cell progressing through S phase is shown on the panels in the middle. The mid-replication time is indicated in minutes. Briefly before replication (-03:00, replication at time point 00:00) the two loci come closely together. Bright field images of the cell are shown by the left- and right-hand panels. *Tet*OS-2 dots did not show a frequent close localization with *lac*OS sides around the mid-replication time (45). Scale bar: 1 micrometer. Reproduced from (45) with permission of Elsevier Limited.

multicellular eukaryotes investigated so far. They represent in the nucleus the structural unit of DNA replication and harbour complexes of proteins required for the replication of the genetic and epigenetic information. Furthermore, they harbor the replicating DNA. The findings suggest that most RF harbor several replicons although the numbers of replicons at individual RF might be variable. RF are arranged into spatial higher-order patterns characteristic for defined temporal stages of S phase and the higher-order patterns found in mammals are highly conserved and represent the underlying chromatin organization.

### 3.3. Nuclear dynamics of DNA replication

As outlined in the beginning, DNA replication is not only a structurally highly organized process, but also a very dynamic process involving fork progression as well as the subsequent initiation at different genomic regions. So, what are the nuclear dynamics of DNA replication and how are they related to fork progression and the changing patterns of active replicons?

The dynamics of DNA replication have first been studied in fixed mammalian cells. Early studies suggested the extrusion of newly replicated DNA from fixed replication sites and for such sites, representing assemblies of replication proteins, the term “replication factory” has been coined, emphasizing their fixed and static nature (47-49). A corresponding model has been proposed suggesting the spooling of DNA through fixed replication factories (47-49). Thus, according to this model the DNA would move and would be spooled through the replication machinery.

Experimental evidence suggests that spooling of DNA through fixed factories occurs during the replication of bacterial genomes, which are replicated from a single origin (50-54). But what about eukaryotic genomes with their multiple origins? This question has been addressed by the recent elegant live cell study using budding yeast (45). As described above, in this study dynamic relocalizations of two forks belonging to one replicon and close proximity of both forks around the time point of replication have been demonstrated (Figure 3). These observations would be consistent with DNA spooling through the replication machinery. However, currently other types of dynamics leading to transient fork assembly cannot be excluded.

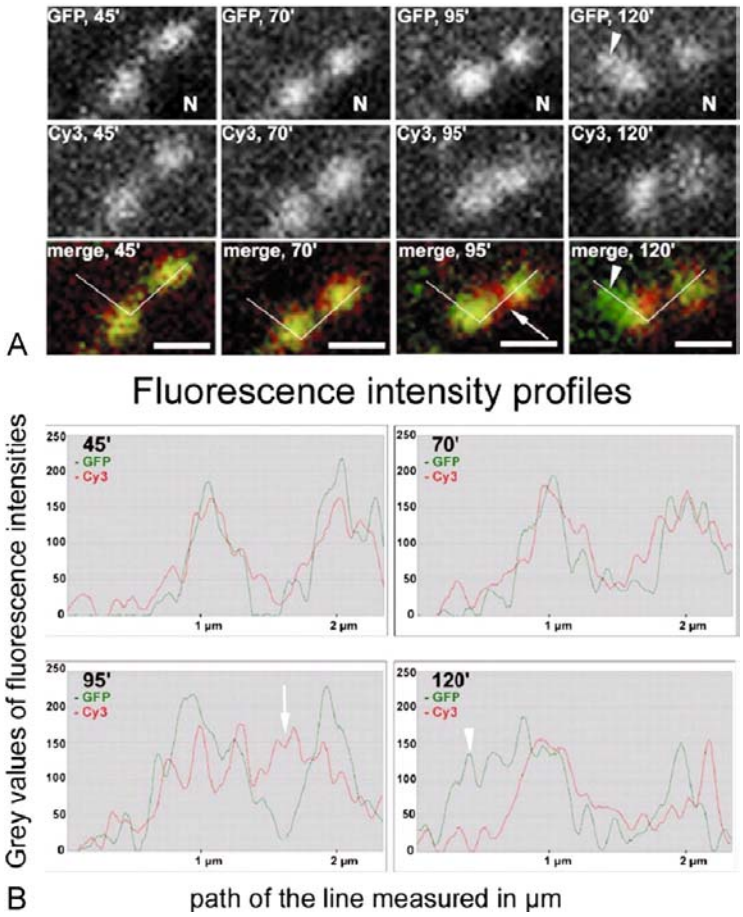


**Figure 4.** Local dynamics at replication foci in living HeLa cells. The replication machinery has been visualized by expression of a fusion between the central replication factor PCNA and GFP (green fluorescence). Nascent DNA has been labeled by microinjection of Cy3-dUTP (red fluorescence) and the time points (45´- 120´) indicate the minutes after microinjection. A) The panels show a time-lapse series of two replication foci associated with the periphery of the nucleolus (N). GFP- and Cy3-fluorescences are shown separately as well as merged. B) Fluorescence intensity profiles along the lines displayed on the merged panels in A. The x-axis indicates the path of the line in micrometers (0: left end of the line). Arrows and arrowheads point to the same regions as on the panels in A. At 45 and 70 minutes after microinjection two separate replication foci are present, at which nascent DNA (red) and GFP-PCNA (green) colocalize. Locally confined reorganizations of DNA led to a transient relocalization of parts of the nascent DNA between the two foci (arrows) at 95 minutes. At 120 minutes the nascent DNA occupies its previous positions and is represented by two well-separated DNA foci associated with the nucleolar periphery. GFP-fluorescence at the sites occupied by the labelled DNA ceases and at the lower replication site the bulk of GFP-fluorescence occupies now a neighbouring site (arrowheads) located farther away from the nucleolar periphery. Scale bars: 1 micrometer. Reproduced with permission from (31).

Also, so far, only very limited conclusions can be drawn concerning the behavior of other replicons and no direct evidence is available with regard to the questions whether and how multiple replicons might be processed at single RFs. Nevertheless, the methodology recently developed for studying DNA replication in living budding (45) and fission (46) yeast cells is very powerful and using this methodology it might be possible to further address the still open questions.

What has been observed in living mammalian cells? It is obvious that the model of DNA spooling through fixed sites of DNA replication is not compatible with the fact that the patterns of RF change during S phase progression. Thus, RF cannot be fixed, at least not for extended time periods. But how do the patterns of RF change during S phase progression and which dynamics of DNA and proteins are involved? These questions have been extensively addressed by using living mammalian cells (18, 31, 32). Simultaneous labeling of replicating DNA using Cy3-dUTP and of the replicating machinery by expressing GFP-tagged PCNA showed that DNA remains stably positioned during its replication in mammalian cells (31). DNA performed only minor local rearrangements in the range of about 1 micrometer and large-scale spooling of DNA in and out of RF as defined by GFP-PCNA was not observed (Figure 4). This study further revealed that DNA remains stably positioned during S phase progression,

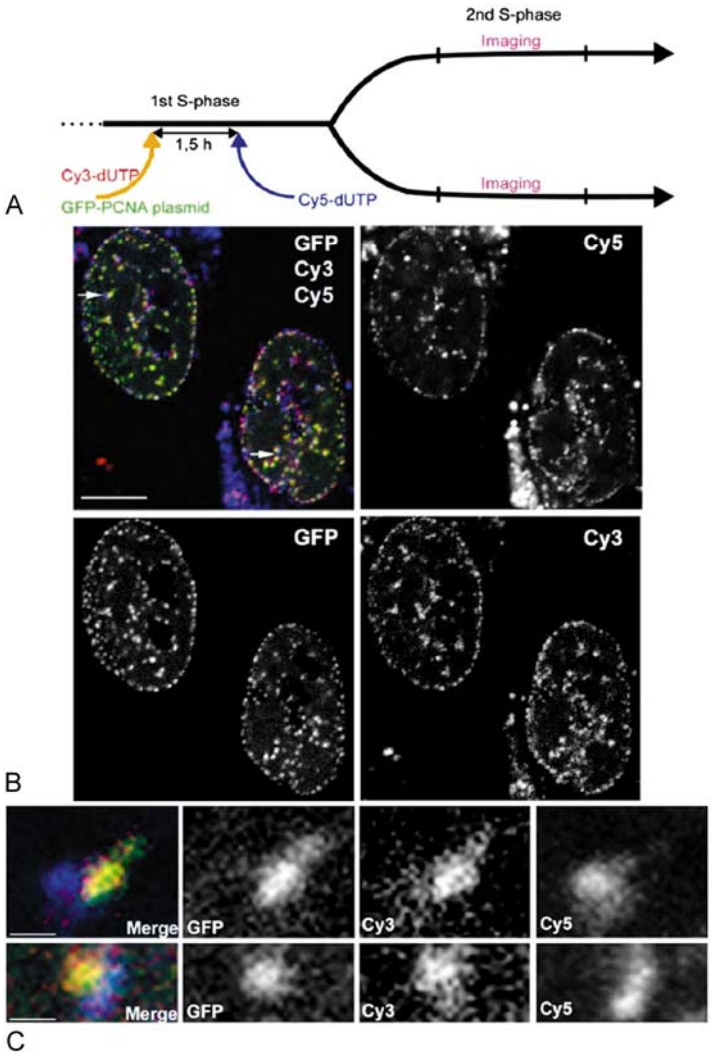


**Figure 5.** Replication foci and stably positioned DNA domains in living HeLa cells. A) Labeling scheme. Unsynchronized HeLa cells were microinjected during the first S phase with Cy3-dUTP and the GFP-PCNA expression plasmid. 1.5h later the same cells were microinjected with Cy5-dUTP, which (like Cy3-dUTP) becomes stably incorporated into nascent DNA. Imaging was performed during the next of S phase of labeled daughter cells. B) Live image of two triple-labeled sister cells. The mother cell has been labeled according to the scheme outlined in A. C) Enlargements of two individual triple-labeled sites marked with arrows in B. In B and C the fluorescences of the different fluorochromes are displayed separately as well as merged (GFP: green, Cy3: red, Cy5: blue). Both sister cells shown in B display similar patterns of Cy3- and Cy5-labeled DNA, which are typical for mid S phase (enrichment of foci at nuclear and nucleolar peripheries, see Figure 1) and reflect the time point of labeling of the mother cell. Cy3- and Cy5-labeled domains, which replicated at consecutive time points in the mother cell (average lifetime of a replication focus is in the range of 1 hour), occupy separate but closely adjacent sites in the daughter cells (see enlargements in C). At the time point where the sister cells have been imaged the replication machinery (GFP-labeled) occupied in almost all cases only the Cy3-labeled foci, which replicated earlier in the mother cell, but not the neighboring later replicating Cy5-labeled foci (see enlargements in C). The observations suggest on the one hand that the replication timing of chromatin domains and the nuclear positioning of chromatin domains with a defined replication timing is stably maintained during subsequent cell cycles. On the other hand, the findings suggest that the patterns of chromatin domains determine the spatial patterns of replication foci at a given temporal stage of S phase. In the example shown active replication foci are represented by the GFP-labeled replication machinery, which occupies at the given time point defined patterns of stably positioned Cy3-labeled chromatin domains. Scale bars: 10 micrometer (B) and 1 micrometer (C). Reproduced with permission with slight changes from (31).

while complexes of replication proteins appear at new sites. Photobleaching studies demonstrated that the positional changes of complexes of replication proteins are not due to movements of whole complexes, but are due to disassembly at previously replicating sites finishing replication and new assembly at newly initiated sites (32). Intriguingly, both live cell studies discussed here used a different methodology but showed consistently that newly

assembled complexes of replication proteins always appear at sites closely adjacent to previously replicating sites (Figure 4). Although it is currently difficult to explain how neighboring chromosomal domains become sequentially activated, it is obvious that DNA replication proceeds during S phase progression through such neighboring chromatin domains, which occupy defined and stable positions in the nucleus in agreement with their replication timing (Figs. 1 and 5).

From these findings the following conclusions can be drawn: extensive spooling of DNA in and out of fixed replication factories does not take place. Although spooling of DNA within RF and at the level of single replicons cannot be excluded and is suggested by the results obtained with budding yeast (45), the question which dynamic movements of protein complexes and DNA are associated with fork progression remains largely unclear.

Furthermore, the concept of fixed replication factories is not in accordance with the observations in mammalian cells and therefore the use of the term replication factory should be carefully considered. Rather than being fixed at given nuclear sites, complexes of replication proteins transiently interact with stably positioned chromatin domains and disassemble when finishing local DNA replication. Subsequently, new complexes of replication proteins assemble on a neighboring chromatin domain.

These kinds of dynamics and the corresponding changes in the higher-order patterns of replication foci are obviously related to the sequential activation of genomic regions replicating at different time points during S phase. The finding that the DNA remains stably positioned for the whole interphase while RF proceed sequentially through neighboring chromatin domains during S phase progression suggests that it is the spatial organization of distinct chromosomal domains in a mammalian nucleus that determines the characteristic spatio-temporal patterns of RF during S phase (31). Furthermore, the findings imply that defined spatial arrangements of neighboring chromatin domains "guide" the replication machinery through the nucleus during S phase.

Is this similar in fission yeast, where also changing spatio-temporal patterns of RF have been observed during S phase progression? Here, it has been found that single RF can traverse the nucleus in less than 1.5 min (46), suggesting that RF are highly mobile. However, fluorescently tagged DNA loci displayed similar dynamics as RF visualized by fluorescently labelled PCNA (46). Thus, RF mobility likely reflects underlying chromatin dynamics. Furthermore, movements were constraint and the corresponding radius of constraint was 0.65 micrometer for DNA loci as well as for RF (46). It is important to note that local DNA dynamics at RF were also observed in mammalian cells and these were within a range of about 1 micrometer (31) (Figure 4). Comparing these numbers it appears that the ranges of dynamic relocalizations are relatively similar in fission yeast and in mammalian nuclei and that the dynamics observed in fission yeast appear to be more dramatic due to the much smaller size of nuclei.

Furthermore, it has been pointed out that mobile RF in fission yeast have been observed to fuse and re-segregate (46). This has not been observed with regard to mammlian RF (18). However, in mammalian cells it has been observed that large late replicating RF are composed of clustered smaller RF (18), which can not always be resolved as individual foci by the imaging techniques applied. Thus, it remains to be shown whether "split" and "merge" events observed in the small nuclei of fission yeast might simply reflect relative movements of closely spaced independent foci, that could not be always resolved as independent RF during imaging period, or whether they indeed represent structural changes at RF that do not occur in mammalian cells.

## 4. SUMMARY AND PERSPECTIVE

In the nucleus DNA replication occurs at replication foci (RF), which are highly conserved in eukaryotes. Although RF are the basic cellular units of DNA replication, which have been investigated for many decades now, there is still only very little direct evidence with regard to the questions what the DNA content of individual RF is and how DNA and the replication machinery are structurally organized at single RF. It is also not well understood which kinds of dynamics occur at RF and to what extent DNA might be spooled at these structures in association with fork progression. The powerful yeast experimental systems developed recently (45, 46) and further advances in imaging methodologies might help to answer these questions in the future.

RF are arranged in the nucleus into higher-order patterns and the spatial patterns of RF change during the temporal progression of S phase, which has been observed in unicellular eukaryotes as well as in metazoans. The dynamic changes of higher-order patterns of RF reflect the sequential replication of different genomic regions during S phase progression. In mammals the patterns of RF change by disassembly of the replication machinery at earlier replicating sites and new assembly at later initiating sites. DNA remains stably positioned during this process and during S phase progression the replication machinery sequentially occupies neighboring stably positioned chromatin domains. This suggests that the underlying patterns of chromatin domains determine the spatio-temporal patterns of RF. Currently, it is not understood why newly initiated domains are always closely adjacent to previously replicating sites. It is also not known how the spatial organization of chromatin domains in the nucleus is regulated and how exactly it is related to the spatio-temporal organization of S phase. It is obvious that answering these questions requires further understanding of nuclear chromatin organization and genome architecture.

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## 6. REFERENCES

1. Gilbert, D. M.: In search of the holy replicator. *Nat. Rev. Mol. Cell Biol.*, 5, 848-855 (2004)

2. Jacob, F., S. Brenner & F. Cuzin: On the regulation of DNA replication in bacteria. *Cold Spring Harb Symp Quant Biol*, 28, 329-348 (1964)

3. Newlon, C. S. & J. F. Theis: The structure and function of yeast ARS elements. *Curr. Opin. Genet. Dev.*, 3, 752-758 (1993)

4. Edenberg, H. J. & J. A. Huberman: Eukaryotic chromosome replication. *Annu. Rev. Genet.*, 9, 245-284 (1975)

5. Hand, R.: Eucaryotic DNA: Organization of the genome for replication. *Cell*, 15, 317-325 (1978)

6. Goren, A. & H. Cedar. *Nat. Rev. Mol. Cell Biol.*, 4, 25-32 (2003)

7. Zink, D.: The temporal program of DNA replication: new insights into old questions. *Chromosoma*, 115, 273-87 (2006)

8. Kim, S. M. & J. A. Huberman: Regulation of replication timing in fission yeast. *Embo J*, 20, 6115-26 (2001)

9. Raghuraman, M. K., E. A. Winzeler, D. Collingwood, S. Hunt, L. Wodicka, A. Conway, D. J. Lockhart, R. W. Davis, B. J. Brewer & W. L. Fangman: Replication dynamics of the yeast genome. *Science*, 294, 115-21 (2001)

10. Schwaiger, M. & D. Schubeler: A question of timing: emerging links between transcription and replication. *Curr Opin Genet Dev*, 16, 177-83 (2006)

11. Gilbert, D. M.: Replication timing and metazoan evolution. *Nat Genet*, 32, 336-7 (2002)

12. Aladjem, M. I.: Replication in context: dynamic regulation of DNA replication patterns in metazoans. *Nat Rev Genet*, 8, 588-600 (2007)

13. Huberman, J. A. & A. D. Riggs: Autoradiography of chromosomal DNA fibers from Chinese hamster cells. *Proc. Natl. Acad. Sci. USA*, 55, 599-606 (1966)

14. Berezney, R., D. D. Dubey & J. A. Huberman: Heterogeneity of eukaryotic replicons, replicon clusters, and replication foci. *Chromosoma*, 108, 471-484 (2000)

15. Yurov, Y. B. & N. A. Liapunova: The units of DNA replication in the mammalian chromosomes: evidence for a large size of replication units. *Chromosoma*, 60, 253-267 (1977)

16. White, E. J., O. Emanuelsson, D. Scalzo, T. Royce, S. Kosak, E. J. Oakeley, S. Weissman, M. Gerstein, M. Groudine, M. Snyder & D. Schubeler: DNA replication-timing analysis of human chromosome 22 at high resolution and different developmental states. *Proc Natl Acad Sci U S A*, 101, 17771-6 (2004)

17. Cardoso, M. C., J. Cuthbert, H. P. Rahn, R. Reusch, B. Nadal-Ginard & H. Leonhardt: Mapping and use of a sequence that targets DNA Ligase I to sites of DNA replication *in vivo*. *J. Cell Biol.*, 139, 579-587 (1997)

18. Leonhardt, H., H.-P. Rahn, P. Weinzierl, A. Sporbert, T. Cremer, D. Zink & M. C. Cardoso: Dynamics of DNA replication factories in living cells. *J Cell Biol*, 149, 271-279 (2000)

19. Nakamura, H., T. Morita & C. Sato: Structural organizations of replicon domains during DNA synthetic phase in the mammalian nucleus. *Exp. Cell Res.*, 165, 291-297 (1986)

20. Nakayasu, H. & R. Berezney: Mapping Replicational Sites in the Eucaryotic Cell Nucleus. *J. Cell Biol.*, 108, 1-11 (1989)

21. Zink, D., T. Cremer, R. Saffrich, R. Fischer, M. F. Trendelenburg, W. Ansorge & E. H. K. Stelzer: Structure and dynamics of human interphase chromosome territories *in vivo*. *Hum. Genet.*, 102, 241-251 (1998)

22. Jackson, D. A. & A. Pombo: Replicon Clusters Are Stable Units of Chromosome Structure: Evidence That Nuclear Organization Contributes to the Efficient Activation and Propagation of S Phase in Human Cells. *J. Cell Biol.*, 140, 1285-1295 (1998)

23. Bravo, R. & H. Macdonald-Bravo: Existence of two populations of cyclin/proliferating cell nuclear antigen during the cell cycle: association with DNA replication sites. *J. Cell Biol.*, 105, 1549-1554 (1987)

24. Leonhardt, H., A. Sporbert & M. C. Cardoso: Targeting regulatory factors to intranuclear replication sites. *Crit Rev Eukaryot Gene Expr*, 10, 127-33 (2000)

25. Montecucco, A., E. Savini, F. Weighardt, R. Rossi, G. Ciarrocchi, A. Villa & G. Biamonti: The N-terminal domain of human DNA ligase I contains the nuclear localization signal and directs the enzyme to sites of DNA replication. *EMBO J*, 14, 5379-5386 (1995)

26. Cardoso, M. C., H. Leonhardt & B. Nadal-Ginard: Reversal of terminal differentiation and control of DNA replication: cyclin A and Cdk2 specifically localize at subnuclear sites of DNA replication. *Cell*, 74, 979-992 (1993)

27. McNairn, A. J. & D. M. Gilbert: Epigenomic replication: linking epigenetics to DNA replication. *Bioessays*, 25, 647-56 (2003)

28. Leonhardt, H., A. W. Page, H.-U. Weier & T. H. Bestor: A targeting sequence directs DNA methyltransferase to sites of DNA replication in mammalian nuclei. *Cell*, 71, 865-873 (1992)

29. Rountree, M. R., K. E. Bachman & S. B. Baylin: DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. *Nat Genet*, 25, 269-77 (2000)

30. Ma, H., J. Samarabandu, R. S. Devdhar, R. Acharya, P.-C. Cheng, C. Meng & R. Berezney: Spatial and temporal dynamics of DNA replication sites in mammalian cells. *J. Cell Biol.*, 143, 1415-1425 (1998)

31. Sadoni, N., M. C. Cardoso, E. H. Stelzer, H. Leonhardt & D. Zink: Stable chromosomal units determine the spatial and temporal organization of DNA replication. *J Cell Sci*, 117, 5353-65 (2004)

32. Sporbert, A., A. Gahl, R. Ankerhold, H. Leonhardt & M. C. Cardoso: DNA polymerase clamp shows little turnover at established replication sites but sequential de novo assembly at adjacent origin clusters. *Mol. Cell*, 10, 1355-1365 (2002)

33. O´Keefe, R. T., S. C. Henderson & D. L. Spector: Dynamic Organization of DNA Replication in Mammalian Cell Nuclei: Spatially and Temporally Defined Replication of Chromosome-specific a-Satellite DNA Sequences. *J. Cell Biol.*, 116, 1095-1110 (1992)

34. Sadoni, N., S. Langer, C. Fauth, G. Bernardi, T. Cremer, B. M. Turner & D. Zink: Nuclear organization of mammalian genomes: polar chromosome territories build up functionally distinct higher order compartments. *J. Cell Biol.*, 146, 1211-1226 (1999)

35. Fox, M. H., D. J. Arndt-Jovin, T. M. Jovin, P. H. Baumann & M. Robert-Nicoud: Spatial and temporal distribution of DNA replication sites localized by immunofluorescence and confocal microscopy in mouse fibroblasts. *J Cell Sci*, 99, 247-253 (1991)

36. Berezney, R., H. Ma, C. Meng, J. Samarabandu & P.-C. Cheng: Connecting Genomic Architecture and DNA Replication in Three Dimensions. *Zool. Stud.*, 34, 29-32 (1995)

37. Watanabe, Y., A. Fujiyama, Y. Ichiba, M. Hattori, T. Yada, Y. Sakaki & T. Ikemura: Chromosome-wide assessment of replication timing for human chromosomes 11q and 21q: disease-related genes in timing-switch regions. *Hum Mol Genet*, 11, 13-21 (2002)

38. Woodfine, K., H. Fiegler, D. M. Beare, J. E. Collins, O. T. McCann, B. D. Young, S. Debernardi, R. Mott, I. Dunham & N. P. Carter: Replication timing of the human genome. *Hum Mol Genet*, 13, 191-202 (2004)

39. Lucas, I., A. Palakodeti, Y. Jiang, D. J. Young, N. Jiang, A. A. Fernald & M. M. Le Beau: High-throughput mapping of origins of replication in human cells. *EMBO Rep*, 8, 770-7 (2007)

40. Alexandrova, O., I. Solovei, T. Cremer & C. N. David: Replication labeling patterns and chromosome territories typical of mammalian nuclei are conserved in the early metazoan Hydra. *Chromosoma*, 112, 190-200 (2003)

41. Mayr, C., Z. Jasencakova, A. Meister, I. Schubert & D. Zink: Comparative analysis of the functional genome architecture of animal and plant cell nuclei. *Chromosome Res.*, 11, 471-484 (2003)

42. Spada, F., J. Koch, N. Sadoni, N. Mitchell, P. Ganot, U. De Boni, D. Zink & E. M. Thompson: Conserved patterns of nuclear compartmentalization are not observed in the chordate Oikopleura. *Biol Cell*, 99, 273-87 (2007)

43. Spada, F., M. Vincent & E. M. Thompson: Plasticity of histone modifications across the invertebrate to vertebrate transition: histone H3 lysine 4 trimethylation in heterochromatin. *Chrom. Res.*, 13, 57-72 (2005)

44. Postberg, J., O. Alexandrova, T. Cremer & H. J. Lipps: Exploiting nuclear duality of ciliates to analyse topological requirements for DNA replication and transcription. *J Cell Sci.*, 118, 3973-3983 (2005)

45. Kitamura, E., J. J. Blow & T. U. Tanaka: Live-cell imaging reveals replication of individual replicons in eukaryotic replication factories. *Cell*, 125, 1297-308 (2006)

46. Meister, P., A. Taddei, A. Ponti, G. Baldacci & S. M. Gasser: Replication foci dynamics: replication patterns are modulated by S-phase checkpoint kinases in fission yeast. *Embo J*, 26, 1315-26 (2007)

47. Cook, P.: The organization of replication and transcription. *Science*, 284, 1790-1795 (1999)

48. Hozak, P., A. B. Hassan, D. A. Jackson & P. R. Cook: Visualization of Replication Factories Attached to a Nucleoskeleton. *Cell*, 73, 361-373 (1993)

49. Hozak, P., D. A. Jackson & P. Cook: Replication factories and nuclear bodies: the ultrastructural characterization of replication sites during the cell cycle. *J. Cell Sci.*, 107, 2191-2202 (1994)

50. Lemon, K. P. & A. D. Grossman: Localization of bacterial DNA polymerase: evidence for a factory model of replication. *Science*, 282, 1516-1519 (1998)

51. Lemon, K. P. & A. D. Grossman: Movement of replicating DNA through a stationary replisome. *Mol. Cell*, 6, 1321-1330 (2000)

52. Jensen, R. B., S. C. Wang & L. Shapiro: A moving DNA replication factory in Caulobacter crescentus. *Embo J*, 20, 4952-63 (2001)

53. Lau, I. F., S. R. Filipe, B. Soballe, O. A. Okstad, F. X. Barre & D. J. Sherratt: Spatial and temporal organization of replicating Escherichia coli chromosomes. *Mol Microbiol*, 49, 731-43 (2003)

54. Migocki, M. D., P. J. Lewis, R. G. Wake & E. J. Harry: The midcell replication factory in Bacillus subtilis is highly mobile: implications for coordinating chromosome replication with other cell cycle events. *Mol Microbiol*, 54, 452-63 (2004)

55. Spada, F., M. Chioda & E. M. Thompson: Histone H4 post-translational modifications in chordate mitotic and endoreduplicative cell cycles. *J Cell Biochem.*, 95, 885-901 (2005)

**Abbreviations:** RF: replication foci; Cy3-dUTP: deoxyuridine-triphosphate conjugated to the fluorochrome Cy3; GFP: green fluorescent protein; CFP: cyan fluorescent protein; PCNA: proliferating cell nuclear antigen; 3D: three-dimensional; kb: kilo basepairs; IdU: iododeoxyuridine; CldU: Chlorodeoxyuridine

**Key Words:** DNA replication, Replication Focus, Replication Factory, Replicon, Nucleus, Nuclear Architecture, Nuclear Dynamics, Chromatin Organization, Review

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