

Telomeric chromatin modulates replication timing near chromosome ends

Jeffery B. Stevenson^{1,3}
and Daniel E. Gottschling^{2,4}

¹Department of Molecular Genetics and Cell Biology,
The University of Chicago, Chicago, Illinois 60637 USA;

²Division of Basic Sciences, Fred Hutchinson Cancer Research
Center, Seattle, Washington 98109

***Saccharomyces cerevisiae* telomeric DNA replicates late in S phase, and telomeric genes are transcriptionally silent. Transcriptional repression of telomere-proximal genes results from silent chromatin initiating at the chromosome end, but the relationship between telomeric chromatin and DNA replication is unknown. Mutations in *SIR3*, a silent chromatin component, cause telomeric DNA on chromosome V to replicate much earlier because of earlier initiation of a nearby replication origin, the Y' ARS. A second telomere-proximal ARS, from an X element, does not act as an origin in a wild-type strain, whereas in a *sir3* cell it does. We conclude that telomeric chromatin has a Sir3-dependent inhibitory effect on DNA replication.**

Received October 6, 1998; revised version accepted December 1, 1998.

Different regions of the eukaryotic genome are known to replicate at distinct, reproducible times within the period of S phase. This is observed in plants, insects, and vertebrates, as well as in the yeast *Saccharomyces cerevisiae* (Hand 1978; Fangman and Brewer 1992). In species with large genomes, extensive regions of the chromosomes replicate early in S phase, whereas other domains do not initiate replication until after the early domains have completed synthesis. Frequently, late replication of DNA is associated with its assembly into heterochromatin, which is highly condensed chromatin that often contains repeated DNA sequences such as those found at centromeres and telomeres (John 1988). This condensed chromosome replicates much later in S phase than its transcriptionally active homolog.

Telomeric chromatin in *S. cerevisiae* has several traits typical of heterochromatin (Grunstein 1998). In particular, telomeres confer epigenetic silencing of nearby genes (position effect variegation), and they replicate late in S phase (McCarroll and Fangman 1988; Gottschling et al. 1990). The special chromatin found near telomeres is

composed of hypoacetylated core histones as well as the SIR proteins, which are required for silencing telomeric genes (Grunstein 1998). Of the SIR proteins, Sir3p is probably the key component that defines a telomeric domain of transcriptional repression. It interacts with the tails of histones H3 and H4, spreading from the telomere inward along the chromosome, and its abundance in the cell determines how far a silent domain extends from the telomere (Renauld et al. 1993; Hecht et al. 1996).

In yeast, chromosome replication initiates at ARS (autonomous replicating sequences) elements (Fangman and Brewer 1991). ARS elements were originally identified as sequences that permit high-efficiency transformation of plasmids in yeast by serving as origins of DNA replication. However, in their normal chromosomal context, only a subset of the ARS elements initiate DNA replication within the ~30 min duration of S phase (Fangman and Brewer 1992). Specific chromosomal origins of *S. cerevisiae*, like *ARS1* on chromosome IV, initiate replication relatively early in S phase. Other ARS elements initiate later in S phase, such as *ARS501* on chromosome V, which replicates ~10 min after *ARS1* (Ferguson et al. 1991).

It appears that telomeres can confer late replication on proximal origins. For instance, telomere-proximal middle repetitive sequences replicate relatively late in S phase (McCarroll and Fangman 1988; Louis 1995). In addition, whereas ARS elements on circular plasmids initiate replication early in S phase, they replicate late in S phase when the plasmid is linearized and telomeric sequences are added to its ends (Ferguson and Fangman 1992). In this study we investigated the role of silent chromatin in imposing late replication on origins near telomeres.

Results and Discussion

We asked whether silent telomeric chromatin was responsible for the relatively late replication time of telomere-proximal DNA by measuring the replication kinetics of sequences near the right telomere of chromosome V in a wild-type strain and in a strain in which the *SIR3* gene was deleted. This region of the genome is represented schematically at the top of Figure 1A, and its late replication has been documented previously (McCarroll and Fangman 1988; Ferguson et al. 1991). Located adjacent to the telomeric repeats at the extreme end of the chromosome are members of two families of subtelomeric, middle repetitive sequences, the X and Y' elements (Louis 1995). The X element is found at all native yeast telomeres, and the Y' at most but not all. However, neither is essential for linear chromosome stability (Wellinger and Zakian 1989). Although each of these elements contains an ARS, it is not known whether either has origin activity at the right telomere of chromosome V (Ferguson et al. 1991). The regions examined for their replication kinetics are the restriction fragment R6, which contains the late-replicating chromosomal origin

[Key Words: *Saccharomyces cerevisiae*; heterochromatin; DNA replication; telomeres; silencing]

³Present address: Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah 84112 USA.

⁴Corresponding author.

E-MAIL Dgottsch@fhcrc.org; FAX (206) 667-5894.

ARS501 and is ~25 kb from the telomere, and R2, the penultimate unique DNA sequence adjacent to the telomeric X element.

Replication times were measured by a density transfer protocol (McCarroll and Fangman 1988). The replication time of a chromosomal fragment (t_{rep}) is defined as the time when that fragment is replicated in 50% of the cells in a culture. Replication times are expressed as minutes after the replication time of the nontelomeric, early replicating fragment *ARS1* (Δt_{rep}).

SIR3 delays replication of a telomere-adjacent fragment

In a *SIR*⁺ strain (AR120), the R6 restriction fragment containing *ARS501* replicated at approximately +10 min after *ARS1* (Fig. 1A,B), consistent with earlier reports (Ferguson et al. 1991), and in the *sir3* strain (UCC4403), this same fragment replicates at +8 min (Fig. 1A, B). In contrast, R2 replicated at +13 min in the *SIR*⁺ strain but at +4 min in *sir3* (Fig. 1A,B). Because *ARS501* (R6) replicated at essentially the same time in *SIR*⁺ and *sir3* strains, the difference in the replication times at R2 was likely the result of changes in an origin firing distal to it. To verify whether this is true, the direction of replication forks passing through the R2 fragment in the two strains was determined by a two-dimensional gel method (Friedman and Brewer 1995). In both the *SIR*⁺ (AR120) and *sir3* (UCC4403) strains, a fork originating from its telomeric side replicates the R2 fragment in >95% cells (Fig. 1A,C). This suggests that in the majority of cells, R2 is replicated by origins that initiated at or near the telomere and that the *SIR3*-dependent delay in replication timing is the result of changes in a telomere-proximal origin.

Although the replication timing of *ARS501* was not affected by the absence of Sir3p, it was possible that deletion of the *SIR3* gene might have global effects on late replication. To test this idea, the replication time of a fragment within a nontelomeric, late-replicating region was measured in both *SIR*⁺ and *sir3* strains. This region on chromosome XIV near the *KEX2* gene contains *ARS1412*, which lies ~200 kb from the nearest telomere and is late replicating (Brewer et al. 1993; Friedman et al. 1996). The Δt_{rep} of this fragment in a *SIR*⁺ strain was +11 min (s.d. = 1.7 min; data not shown). In a *sir3* strain, the replication time of this fragment was +12 min (s.d. = 2.5 min; data not shown), essentially unchanged. These results, in combination with the fact that *ARS501* (R6) did not respond to the Sir3-dependent change in replication timing, suggested that Sir3 was not responsible for late replication throughout the genome. This also suggested that the late firing of *ARS501* (reported previously) is not under *SIR3* control (Ferguson and Fangman 1992).

Which telomere-proximal origin was responsible for R2 replication and responded to *SIR3* changes? It was likely to be the ARS in either the X or Y' element, although in principle it could have been the end of the chromosome itself. To distinguish between these possibilities, the X and Y' sequences at the right end of chromosome V were deleted, thus bringing the telomere ad-

acent to the R2 sequence (middle of Fig. 1A). This truncated chromosome was then analyzed by the same methods used to study the intact chromosome.

There were two notable differences in replication effects between this truncated chromosome and the normal chromosome. First, the R2 sequence in the truncated chromosome replicated even later in S phase (cf. +20 min of UCC4431 to +13 min of AR120; Fig. 1A,B) by a fork now originating primarily from the centromeric side of R2 (Fig. 1A,C). This reversal of fork directions suggested that either the X or the Y' element contained the origin of replication that was normally responsible for most of R2's replication.

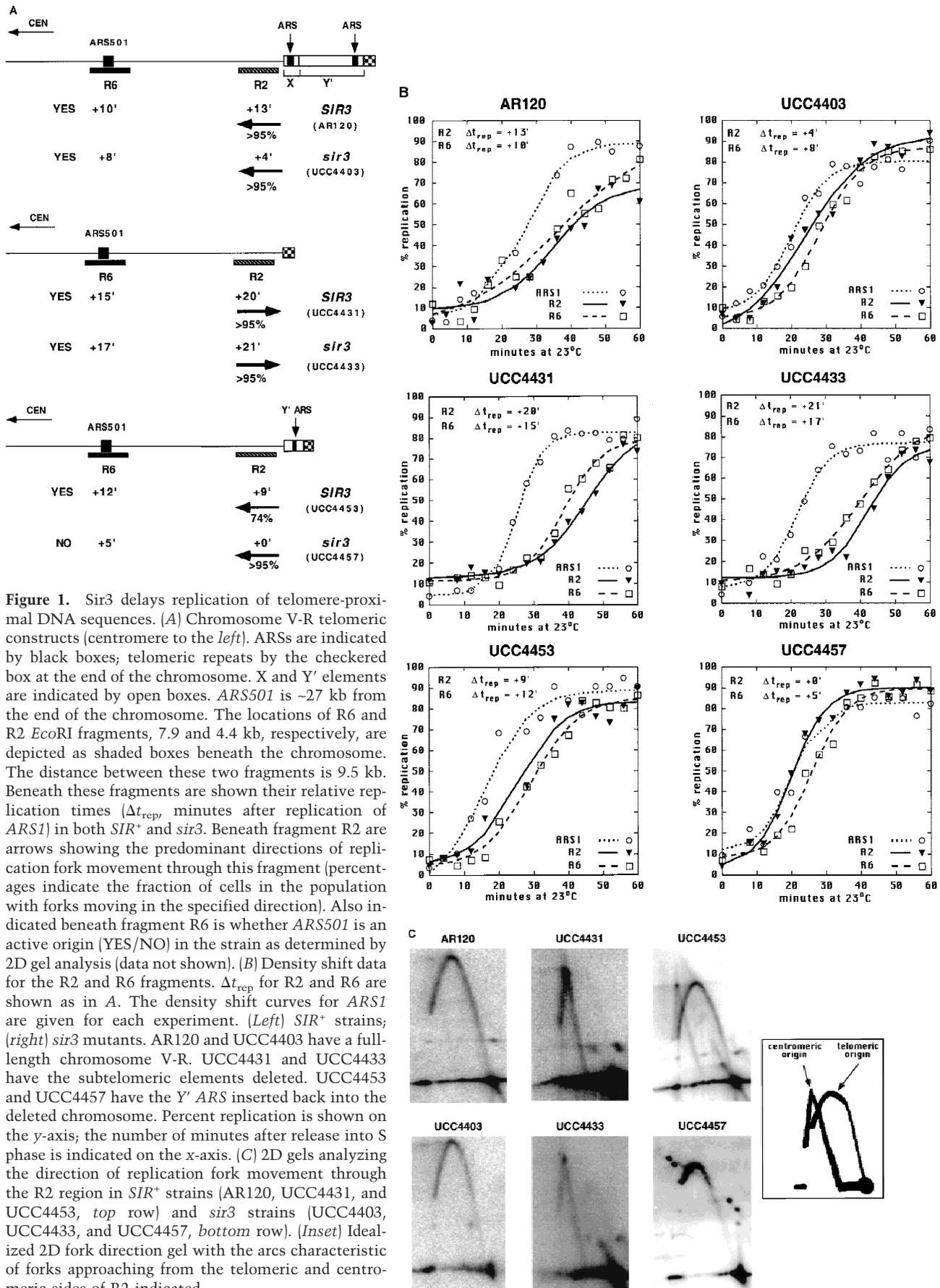
Second, there was no difference between the replication timing of R2 (or R6) when comparing *SIR*⁺ (UCC4431) and *sir3* (UCC4433) versions of the truncated chromosome (Fig. 1A,B). This suggested that the Sir3-dependent change in timing of R2 seen in the normal chromosome acted through origins in the X or Y' elements.

Telomeric Y' ARS initiation is delayed by Sir3

Using the truncated chromosome as a starting point, we examined whether the ARS within the subtelomeric Y' element was responsible for the Sir3-dependent delay in replication timing at R2. The Y' ARS was a good candidate for being affected because it is located <1 kb from the junction of the terminal TG₁₋₃ DNA tract, well within the normal domain of silent chromatin extending from the end of the chromosome known to be influenced by *SIR3* (Renauld et al. 1993). The telomeric fragment of Y' containing this ARS was reinserted into the truncated chromosome such that the sequences and spacing from the telomere to the Y' ARS were maintained as in the normal chromosomal configuration. With this modified chromosome, the Sir3-dependent late replication of the R2 fragment was once again observed; R2 replicated at +9 min in *SIR3* cells, whereas in *sir3* cells it replicated at 0 min (Fig. 1A,B). This strongly suggests that silent chromatin delayed replication of the Y' ARS.

The R2 fragment with the reinserted Y' ARS was replicated approximately 4 min earlier in this chromosome compared to the normal chromosome, for both the *SIR3* and *sir3* versions of the strains (+9 min vs. +13 minutes for *SIR*⁺ and 0 min vs. +4 min for *sir3*). This 4-min difference can be explained by considering that ~9 kb of DNA is missing between R2 and the Y' ARS in the altered chromosome and that the estimated rate of DNA replication fork movement in yeast is 1–3 kb/min (Rivin and Fangman 1980). In the final comparison of this altered construct, *ARS501* does not act as an origin of replication in the *sir3* version (Fig. 1A). Most likely, R6/*ARS501* is passively replicated by a much earlier replicating fork from the Y' ARS.

Next we asked whether the *SIR3*-dependent delay was restricted to the V–R telomere by measuring the replication kinetics of the total Y' family of subtelomeric elements. The time of replication for the Y' family shifted from +10 min in the *SIR*⁺ strain, to +2 min in the *sir3*



strain (data not shown). Although it was not possible to ascertain whether this shift was a result of all, or only a subset of the Y' elements replicating earlier, as a group they replicated significantly earlier in the *sir3* strain. Hence, the timing effect was not specific to the V-R telomere and suggested that replication timing of most telomeres was under *SIR3* control.

Sir3 delays initiation of ARS1 near a telomere

The above results led us to ask whether other *ARS* elements, when placed near a telomere within the normal silencing domain of Sir3p, responded to the Sir3-dependent delay in firing. To answer this question, a fragment containing the early replicating *ARS1* was inserted at the same location that the Y' *ARS* fragment had been integrated, in both *SIR*⁺ and *sir3* strains (Fig. 2). In this case, as with the integration of the Y' *ARS*, R2 undergoes a shift in replication timing from late, in the *SIR*⁺ strain (UCC4451, +21 min), to 8 min earlier in the *sir3* strain (UCC4455, +13 min). *ARS1* was acting as an origin in both the *SIR*⁺ and *sir3* strains as evidenced by forks coming from the telomeric side of R2 (Fig. 2C). Thus the Sir3-dependent delay in replication of R2 was not specific to the Y' *ARS*, but can be extended to at least one other origin, *ARS1* when it is located within 1 kb of the telomere.

There is also a Sir3-independent delay in the replication of the telomeric *ARS1*. In the *sir3* strain, the R2 fragment adjacent to the telomeric *ARS1* replicated significantly later (13 min) than a fragment next to *ARS1* at its normal location on chromosome IV. Because the telomeric *ARS1* does not act as an origin in all the *sir3* cells (only 58%), we cannot determine whether the delay is the result of a later firing of the telomeric *ARS1*, a lack of its use, or a combination of both effects.

Sir3 prevents initiation of a telomeric X ARS

Finally, the fate of the X *ARS* near the normal chromosome V-R telomere (*ARS5RX*) was examined. X elements are much more heterogeneous in sequence, and their ARSs are more variable in distance from the telomere than is the case for Y' elements (Louis 1995). Nonetheless we wished to determine whether *ARS5RX* was affected by *SIR3*. Specifically we examined whether *ARS5RX* acted as an origin of replication in a native chromosome in the *SIR*⁺ and *sir3* strains. In the *SIR*⁺ strain the *ARS5RX* does not appear to act as an origin, just as has been reported for *ARS3LX* (Fig. 3; data not shown; Newlon et al. 1993). Thus, *ARS5RX* is normally passively replicated by a fork originating at the Y' *ARS*. In contrast, *ARS5RX* was able to act as an origin when *SIR3* was deleted, as shown by the replication bubble arc in Figure 3. Thus, we conclude that Sir3p prevents X *ARS* from acting as an origin of replication.

How does SIR3 cause late replication of telomeric DNA?

In this study we have shown that Sir3p causes a significant delay (8–9 min in an S phase that lasts ~30 min) in

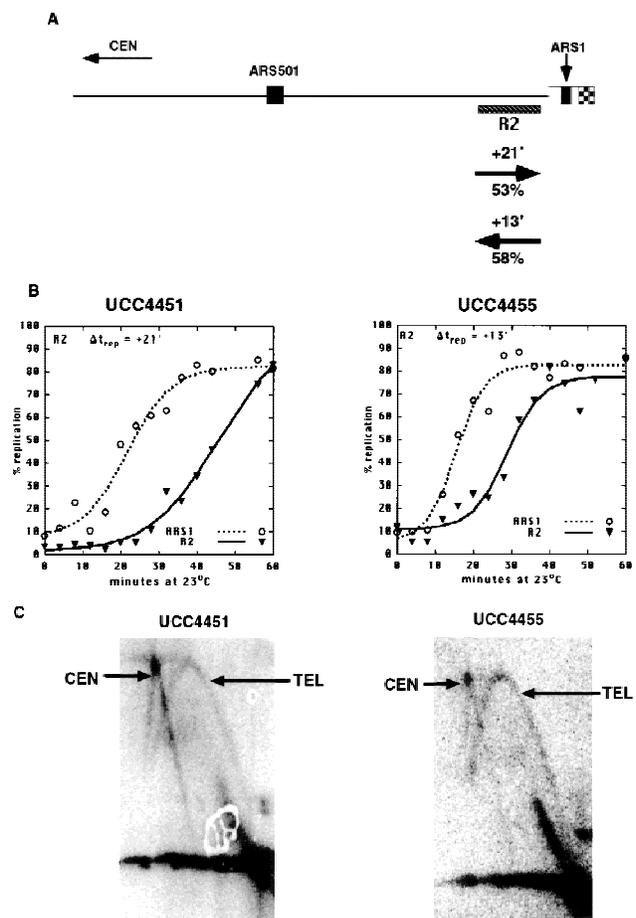


Figure 2. *ARS1* integrated at the V-R telomere is delayed in replication by Sir3. (A) Diagram of the chromosome V-R telomeric *ARS1* integration construct (centromere to the left). (Rightward arrow) *SIR3* (UCC4451); (leftward arrow) *sir3* (UCC4455). Details are as in Fig. 1A. (B) Density shift data for the R2 fragment. Details are as in Fig. 1B, except only replication times (Δt_{rep}) for R2 and *ARS1* (at its natural location on chromosome IV) are presented. (C) 2D gels analyzing the direction of replication fork movement through the R2 region in wild-type (UCC4451) and *sir3* (UCC4455) strain. Details are as in Fig. 1C. (Left) *SIR3*; (right) *sir3* (B,C).

the apparent initiation of replication at telomeric *ARS* elements, for both a Y' *ARS* and *ARS1*. This is an 'apparent' delay, because given the limitation of the assays, we cannot distinguish between a true delay in initiation of DNA synthesis at the *ARS* and a localized initiation event at the *ARS* in which subsequent fork elongation is severely inhibited or slowed.

Regardless of the precise mechanism, we propose that silent telomeric chromatin prevents key components of the replication machinery from readily gaining access to the underlying DNA. Most chromatin is comprised of nucleosomes, whereas silent chromatin has nucleosomes that are stabilized by molecules such as Sir3p. In essence, there is a reduced off-rate of chromatin components from the DNA for silent chromatin compared to 'normal' chromatin. A reduced off-rate means that the effective on-rate for limiting replication factors is re-

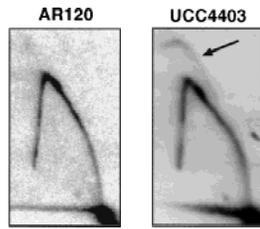


Figure 3. The *ARS5RX* becomes active in a *sir3* strain. 2D gels analyzing activity of *ARS5RX* in *SIR*⁺ strain (AR120) and *sir3* (UCC4403). The arrow points to the arc characteristic of an active replication origin in UCC4403. The shapes of the arcs are somewhat atypical because the fragment containing *ARS5RX* is relatively large (5.2 kb) and the origin is asymmetrically located on the fragment.

duced. Studies on the activation of a silenced telomeric gene support such an idea. Higher concentrations of a transcriptional activator are required to express a telomeric gene to a given level than when the same gene is located at an internal, nonsilenced locus (Aparicio and Gottschling 1994). The on-rate for any binding factor is a function of the factor's concentration and the amount of time it has to bind to its substrate. Thus, in an event like S phase that has a defined starting point, the delay in replication seen in silent chromatin may be the result of a need to synthesize sufficient levels of a limiting component during S phase to achieve the necessary concentration for binding. Alternatively, the concentration of the factor may not change significantly during S phase; however, more time is required for it to have a chance to bind.

It is thought that all active origins of replication have a prereplication complex assembled on them (Santocane and Diffley 1996). This consists of the Origin Recognition Complex (ORC), which binds the ARS DNA, Cdc6p, the Mcm proteins, Cdc45p, and possibly other proteins (Pasero and Gasser 1998). Assuming that active telomeric origins have fully assembled prereplication complexes, then components like the Mcm proteins and Cdc45p, which are known to be recruited to chromatin and act at or near the replication fork, are reasonable candidates for the limiting replication factor in the model we present (Aparicio et al. 1997). The competition may occur either in the factor's initial binding or in its ability to perturb nucleosomes for fork progression.

Why do telomeres replicate late?

It is tempting to speculate that late replication of telomeric DNA serves a special function in the cell; it may be required to re-establish silent chromatin, help coordinate replication of the chromosome end by telomerase, or facilitate sister chromatid cohesion. However, it is also likely that the Sir3-dependent late replication is a secondary consequence of telomeric chromatin. Telomeric chromatin may have evolved to serve in canonical roles associated with telomeres: distinguishing telomeres from broken chromosome ends, and protecting telomeres from degradation and end-to-end fusion.

In considering this latter idea, it is worth noting that the Y' element causes the telomeric region of chromosome V-R to replicate earlier than when the Y' element was not present (Fig. 1). Although Y' elements have been suggested to play a role in telomere maintenance in the absence of telomerase (Lundblad and Blackburn 1993), we propose that Y' elements may reside at telomeres because they provide a selective advantage in duplicating the genome more rapidly. X elements, which do not have the ability to initiate replication at V-R or III-L (Fig. 3; Newlon et al. 1993), are much more heterogeneous in sequence and size and therefore appear to be much older in evolutionary history than Y' elements. Thus, in laboratory strains of *S. cerevisiae*, we may be spectators to the evolutionary expansion of a recently acquired DNA element, under the selective pressure of completing S phase more quickly.

Materials and methods

DNA plasmids

pSC4120, pL110, and pR151 were gifts from W. Fangman (University of Washington, Seattle), and plasmid pYP1-L2 was a gift of E. Louis (Louis and Haber 1992). Plasmids pVRINTY'ARS, pY'H3/XbaI, pUCR2, pR2X-2, pUCR2X/P, pUCK/N, pUCR6, pVRAXY'TEL, and pVRINTARS1 were created in this study. Detailed maps of these plasmids are available upon request.

Yeast strains and methods

Media used for the growth of *S. cerevisiae* were described previously (Gottschling et al. 1990). All yeast strains in this study were grown at 23°C in YEPD unless noted otherwise. Standard methods were used for genetic manipulation of *S. cerevisiae* (Renauld et al. 1993). Methods for DNA preparation and analysis have been described previously (Gottschling et al. 1990; Aparicio et al. 1991).

The strain AR120 (*HMLa MATa HMRa cdc7-1 bar1 trp1-289 ura3-52 leu2-3,112 his6*) was a gift from W. Fangman. The strain UCC4403 was created by transforming AR120 with *Bam*HI-*Hind*III-digested pJH107.1 (Ivy et al. 1986). *Leu*⁺ transformants were selected and checked by Southern blot to confirm the loss of *SIR3*.

The strains UCC4431 and UCC4433 were created by transforming the strains AR120 (*SIR*⁺) and UCC4403 (*sir3*), respectively, with *Pvu*II-digested pVRAXY'TEL. *Ura*⁺ transformants were selected to give strains UCC4430 (*SIR*⁺) and UCC4432 (*sir3*). These strains were then patched to 5-FOA, and 5-FOA resistant colonies were selected to give the strains UCC4431 (*SIR*⁺) and UCC4433 (*sir3*). The resulting strains have all sequences distal to the R2 fragment replaced with telomeric TG₁₋₃ repeats.

The strains UCC4453 and UCC4457 were created by transforming the strains AR120 and UCC4403, respectively, with *Pvu*II-digested pVRINTY'ARS and processed as described above. These strains have all sequences distal to the R2 fragment replaced with the Y' ARS and telomeric TG₁₋₃ repeats.

The strains UCC4451 and UCC4455 were created by transforming the strains AR120 and UCC4403, respectively, with *Pvu*II-digested pVRINTARS1 and processed as described above. These strains have all sequences distal to the R2 fragment replaced with *ARS1* and telomeric TG₁₋₃ repeats.

Determination of replication times

Replication times were determined by a density transfer protocol as described previously (McCarroll and Fangman 1988). Isolated genomic DNA was digested with *Eco*RI for the replication timing protocols and probed with the following fragments to determine replication timing: a 2.8-kb *Eco*RI fragment from pSC4120, which hybridizes adjacent to *ARS1*; a 3.4-kb *Eco*RI fragment from pL110 was used for *ARS1412*; a 3.1-kb *Xba*I fragment from pR151 (Ferguson et al. 1991) was used for R6; a 3.9-kb *Eco*RI-*Nde*I fragment from pUCR2 for the R2 fragment and a 0.3-kb *Hind*III-*Xba*I fragment from pY'H3/*Xba*I was used as a probe for the Y' family.

Slot blots were exposed for 2–7 days on a PhosphorImager cassette, and the images were quantified using ImageQuant software (Molecular Dynamics). For each time point, the unreplicated peak and the replicated peak were identified, and the relative hybridization for each peak was determined using NIH Image 1.61 software. Percent replication for each time point was calculated with the following equation: % replicated = $(0.5 \times \% \text{ hybridization in the replicated peak}) / [(0.5 \times \% \text{ hybridization in the replicated peak}) + (\% \text{ hybridization in the unreplicated peak})]$.

The percent replication was plotted on the y-axis versus the number of minutes after release from *cdc7* arrest on the x-axis using Sigma Plot graphics software (Jandel Scientific). The equation for calculating a best fit curve for percent replication data was $f(x) = [a/1 + e^{b(x-c)}] + d$, where $a = 100$, $b = -0.1$, $c = 50$; and $d = 0$.

Representative data sets were used for Figures 1–3. Each Δt_{rep} value reported for *ARS1412* were the average of three independent experiments, for *SIR** and *sir3*, from strains AR120, UCC4431, UCC4403, and UCC4433. The values for the total Y' elements were an average of two independent experiments from strains UCC4403, UCC4431, and UCC4433.

2D gel methods

Cell collection and isolation of nuclei were performed as described (Marahrens and Stillman 1994). The collection of DNA by CsCl gradient and subsequent digestion, BND–cellulose chromatography, and DNA precipitation were done as described (Liang et al. 1993). 2D and fork direction gels were performed as described (Brewer and Fangman 1987; Friedman and Brewer 1995).

Firing of the *ARS501* origin was determined on 2D gels with genomic DNA that was cleaved with *XbaI* to generate a 3.1-kb fragment containing *ARS501*. Blots were probed with this same 3.1-kb *XbaI* fragment isolated from plasmid pUCR6. Firing of *ARS5RX* was determined similarly, except the DNA was cleaved with *HindIII* and *SaII* to yield a 5.2-kb fragment containing *ARS5RX*. Blots were then probed with a 500-bp fragment from pRX2-2.

For all fork direction gels, except UCC4457, DNA was digested with *EcoRV* and *XbaI* before being run in the first dimension. In-gel digests were done with *SpeI*. Southern blots were probed with a 1.5-kb *BamHI*–*HindIII* fragment from pUCR2X/P and a 1.0-kb *KpnI*–*NdeI* fragment from pUCR/N. For UCC4457, DNA was digested with *EcoRI* and *XhoI* before being run in the first dimension. The in-gel digest was done with *PstI*. The blot was probed with a 1.5-kb *BamHI*–*HindIII* fragment from pUCR2X/P.

For quantification of fork direction gels, the intensity of hybridization was measured for each arc from approximately the point where the two curves, intersect to the apex of each curve, using ImageQuant software. The background signal was subtracted and the percent hybridization for each arc was calculated.

Acknowledgments

We are indebted to B. Brewer, W. Fangman, and M.K. Raghuraman for gifts of plasmids, strains, and their invaluable advice and patience during this work. E. Louis for plasmids, B. Stillman for technical advice, and members of the Gottschling laboratory and J. Roberts for comments on the manuscript. This work was supported by National Institutes of Health grant GM43893.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked 'advertisement' in accordance with 18 USC section 1734 solely to indicate this fact.

References

Aparicio, O.M. and D.E. Gottschling. 1994. Overcoming telomeric silencing: A *trans*-activator competes to establish gene expression in a cell cycle-dependent way. *Genes & Dev.* **8**: 1133–1146.
 Aparicio, O.M., B.L. Billington, and D.E. Gottschling. 1991. Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. *Cell* **66**: 1279–1287.
 Aparicio, O.M., D.M. Weinstein, and S.P. Bell. 1997. Components and dynamics of DNA replication complexes in *S. cerevisiae*: Redistribu-

tion of MCM proteins and Cdc45p during S phase. *Cell* **91**: 59–69.
 Brewer, B.J. and W.L. Fangman. 1987. The localization of replication origins on ARS plasmids in *S. cerevisiae*. *Cell* **51**: 463–471.
 Brewer, B.J., J.D. Diller, K.L. Friedman, K.M. Kolor, M.K. Raghuraman, and W.L. Fangman. 1993. The topography of chromosome replication in yeast. *Cold Spring Harbor Symp. Quant. Biol.* **58**: 425–434.
 Fangman, W.L. and B.J. Brewer. 1991. Activation of replication origins within yeast chromosomes. *Annu. Rev. Cell Biol.* **7**: 375–402.
 ———. 1992. A question of time—Replication origins of eukaryotic chromosomes. *Cell* **71**: 363–366.
 Ferguson, B.M. and W.L. Fangman. 1992. A position effect on the time of replication origin activation in yeast. *Cell* **68**: 333–339.
 Ferguson, B.M., B.J. Brewer, A.E. Reynolds, and W.L. Fangman. 1991. A yeast origin of replication is activated late in S phase. *Cell* **65**: 507–515.
 Friedman, K.L. and B.J. Brewer. 1995. Analysis of replication intermediates by two-dimensional agarose gel electrophoresis. *Methods Enzymol.* **262**: 613–627.
 Friedman, K.L., J.D. Diller, B.M. Ferguson, S.V. Nyland, B.J. Brewer, and W.L. Fangman. 1996. Multiple determinants controlling activation of yeast replication origins late in S phase. *Genes & Dev.* **10**: 1595–1607.
 Gottschling, D.E., O.M. Aparicio, B.L. Billington, and V.A. Zakian. 1990. Position effect at *S. cerevisiae* telomeres: Reversible repression of Pol II transcription. *Cell* **63**: 751–762.
 Grunstein, M. 1998. Yeast heterochromatin: Regulation of its assembly and inheritance by histones. *Cell* **93**: 325–328.
 Hand, R. 1978. Eucaryotic DNA: Organization of the genome for replication. *Cell* **15**: 317–325.
 Hecht, A., S. Strahl-Bolisinger, and M. Grunstein. 1996. Spreading of transcriptional repressor SIR3 from telomeric heterochromatin. *Nature* **383**: 92–96.
 Ivy, J.M., A.J. Klar, and J.B. Hicks. 1986. Cloning and characterization of four *SIR* genes of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **6**: 688–702.
 John, B. 1988. The biology of heterochromatin. In *Heterochromatin: Molecular and structural aspects* (ed. R.S. Verma), pp. 1–67. Cambridge University Press, Cambridge, UK.
 Liang, C., J.D. Spitzer, H.S. Smith, and S.A. Gerbi. 1993. Replication initiates at a confined region during DNA amplification in *Sciara* DNA puff II/9A. *Genes & Dev.* **7**: 1072–1084.
 Louis, E.J. 1995. The chromosome ends of *Saccharomyces cerevisiae*. *Yeast* **11**: 1553–1573.
 Louis, E.J. and J.E. Haber. 1992. The structure and evolution of subtelomeric Y' repeats in *Saccharomyces cerevisiae*. *Genetics* **131**: 559–574.
 Lundblad, V. and E.H. Blackburn. 1993. An alternative pathway for yeast telomere maintenance rescues *est1*[−] senescence. *Cell* **73**: 347–360.
 Marahrens, Y. and B. Stillman. 1994. Replicator dominance in a eukaryotic chromosome. *EMBO J.* **13**: 3395–3400.
 McCarroll, R.M. and W.L. Fangman. 1988. Time of replication of yeast centromeres and telomeres. *Cell* **54**: 505–513.
 Newlon, C.S., I. Collins, A. Dershowitz, A.M. Deshpande, S.A. Greenfeder, L.Y. Ong, and J.F. Theis. 1993. Analysis of replication origin function on chromosome III of *Saccharomyces cerevisiae*. *Cold Spring Harbor Symp. Quant. Biol.* **58**: 415–423.
 Pasero, P. and S.M. Gasser. 1998. New systems for replicating DNA in vitro. *Curr. Opin. Cell Biol.* **10**: 304–310.
 Renauld, H., O.M. Aparicio, P.D. Zierath, B.L. Billington, S.K. Chhablani, and D.E. Gottschling. 1993. Silent domains are assembled continuously from the telomere and are defined by promoter distance and strength, and by SIR3 dosage. *Genes & Dev.* **7**: 1133–1145.
 Rivin, C.J. and W.L. Fangman. 1980. Replication fork rate and origin activation during the S phase of *Saccharomyces cerevisiae*. *J. Cell Biol.* **85**: 108–115.
 Santocanale, C. and J.F. Diffley. 1996. ORC- and Cdc6-dependent complexes at active and inactive chromosomal replication origins in *Saccharomyces cerevisiae*. *EMBO J.* **15**: 6671–6679.
 Wellinger, R.J. and V.A. Zakian. 1989. Lack of positional requirements for autonomously replicating sequence elements on artificial yeast chromosomes. *Proc. Natl. Acad. Sci.* **86**: 973–977.