Directional telomeric silencing and lack of canonical B1 elements in two silencer Autonomously Replicating Sequences in S. cerevisiae

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Abstract

Background: Autonomously Replicating Sequences (ARS) in S. cerevisiae serve as origins of DNA replication or as components of cis-acting silencers, which impose positional repression at the mating type loci and at the telomeres. Both types of ARS can act as replicators or silencers, however it is not clear how these quite diverse functions are executed. It is believed that all ARS contain a core module of an essential ARS Consensus Sequence (ACS) and a non-essential B1 element.

Results: We have tested how the B1 elements contribute to the silencer and replicator function of ARS. We report that the ACS-B1 orientation of ARS has a profound effect on the levels of gene silencing at telomeres. We also report that the destruction of the canonical B1 elements in two silencer ARS (ARS317 and ARS319) has no effect on their silencer and replicator activity.

Conclusions: The observed orientation effects on gene silencing suggest that ARSs can act as both proto-silencers and as insulator elements. In addition, the lack of B1 suggests that the ACS-B1 module could be different in silencer and replicator ARS.

Keywords: Autonomously replicating sequences, Telomere position effect, DNA replication, Gene silencing

Background

Origins of DNA replication in budding yeast are well defined DNA elements referred to as Autonomously Replicating Sequences (ARS). They consist of a core 11 base pair ACS (ARS Consensus Sequence, WTTTAYRTTTW) and three or four auxiliary B elements [1]. ACS is critical for the function of ARS [2-4]. It is the main site of binding of the Origin Recognition Complex (ORC), which nucleates the formation of pre-replicative complexes in G1 [5,6]. It has been shown that the flanking sequences of the core ACS can also contribute to the binding of ORC thus producing the 17 base pair extended ACS (EACS) [7,8]. Compared to ACS, the B elements are not so well characterized and their roles are poorly understood. B2 is believed to act as a site of DNA unwinding that allows for the initiation of replication [9,10]. B3 is a binding site for Abf1p [11]. While the significance of these elements for the activity of the origins is apparent, the mechanism of their action is unclear. The B1 element is positioned about 15 bases upstream of the core ACS. Using ARS1 as a model, it has been shown that B1 acts as a second binding site for ORC [5,6]. Earlier studies have proposed an AWnY consensus 14 bases upstream of ACS [12]. A more comprehensive analysis and alignment of multiple origins has shown better agreement for a WTW motif positioned 15–17 bases upstream of the core ACS [8]. Mutations in these WTW motifs have substantially reduced the replicator activity of most of the tested origins [8,13]. It has been proposed that the B1 element together with the extended ACS produce a variety of bi-partite sites that bind ORC with different affinities [7,12,14].

Besides their role in DNA replication, ARS and ORC play a central role in gene silencing at the constitutively repressed mating type loci HML and HMR and in the subtelomeric regions of the chromosomes [1]. The silencers, which flank HML and HMR and impose complete shut-off of the genes between them, are built up of various combinations of binding sites for Abf1p and Rap1p.
plus one of four ARSs (ARS301, ARS302, ARS317, ARS318) [1]. Mutations in the ACS of these ARSs substantially reduce gene repression and confer inability to mate [1]. Interestingly, at the HML-I and HMR-E silencers the orientation of the ACS-B1 elements of the ARSs has a directional effect on the levels of gene repression [15-17]. Multiple ARSs are also found in the core X and Y' subtelomeric regions [18,19]. At these positions they act as proto-silencers meaning that they relay and enhance the repression signals emitted by the telomeres [20]. It is not known if these ARSs have directional function. The ARSs at the subtelomeric and the mating type loci rarely fire at their native locations thus strengthening the notion that they have a silencing function independent of the initiation of DNA replication. However, when moved to a mini-chromosome or at different genomic position, these ARSs can fire as efficiently as any other ARS [21,22]. The basis of this dual function of ARSs is not fully understood [21].

Recent studies have shown that targeted mutations in the putative B1 (WTW) motifs of certain ARSs have little effect on their replicator activity [4,8]. It is unclear if the same mutations affect gene silencing. It remains possible that such ARSs contain a B1 at a different position. Ultimately, it is unclear if B1 plays a role in ARS-dependent gene silencing. In this study we have tested the role of B1 by parallel silencing and replicator assays. We have found that the putative B1 elements of two silencer ARSs (ARS317 and ARS319) are dispensable for both activities.

Results

Experimental strategy

To address the role of B1 in gene silencing, we isolated two silencer ARS (ARS317) from the mating type HMR locus and ARS319 from the UIR subtelomeric region) and two well-characterized replicator ARS (ARS305 and ARS605). These were inserted next to the VIII telomere in ACS-B1 and B1-ACS orientation (Figure 1) and the silencing of the adjacent URA3 was assessed. In addition, we performed scanning mutagenesis of the B1 elements of these ARSs and estimated the effects of the mutations on their silencer and replicator activity (Figure 2, Figure 3).

The silencer activity of all the ARSs was assessed by a routine TPE (Telomere Position Effect) assay [23]. The rationale of the assay is as follows: Telomeres recruit multiple Rap1 proteins, which in turn recruit Sir2/3/4 proteins [24,25]. The Sir proteins then spread over and de-acetylate the neighboring nucleosomes to establish a heterochromatin domain [1]. Depending on the scope of spreading, subtelomeric genes are either active or completely repressed and infrequently switch between the two states [23]. When URA3 is inserted at the VIII telomere (or at any other telomere) the proportion of repressed URA3 within a cell population is modulated by the strength of the subtelomeric proto-silencer elements [20]. This proportion is easily assessed as % FOA resistant cells ((FOA (5-fluoro-orotic acid) is converted to a toxin by the enzyme encoded by URA3)) [23]. Hence, in our assays the per cent FOA-resistant cells represents the proto-silencer strength of the engineered ARSs.

Parallel assays were conducted to test how the mutations in the B1 elements affect the replicator activity of the four ARSs and if these effects correlate to the decrease in ARS-driven silencing (Figure 3). To this end, all wild type and mutant ARS fragments were subcloned in mini-chromosomes containing CEN4 and URA3 (Figure 3) and mini-chromosome stability assays were performed. ARS/CEN mini-chromosomes are replicated once per cell cycle and are properly segregated during mitosis. Their normal loss rate is about 3-5% per generation [22,26]. In our analyses any increase in the loss rate is indicative of malfunctioning of the origin. This assay provides a highly sensitive measure of the activity of the origins and has been instrumental in the deciphering of the regulatory elements in many ARSs.

The orientation of ARSs determines the level of telomeric silencing

It has previously been shown that ARS317 exerts orientation-dependent silencing when the whole HMR-E
The silencer is moved to the HML locus [16]. The possibility of directional silencing prompted us to establish an orientation in which all analysed ARSs would produce similar levels of URA3 silencing. ARS305, ARS605, ARS317 and ARS319 were sub-cloned in both directions (ADH4-URA3-ACS-B1-tel and ADH4-URA3-B1-ACS-tel) and then inserted between ADH4 and the VII telomere of W303 cells as described previously [4,27,28]. Consistent with their established role as proto-silencers [20], in the URA3-ACS-B1-tel orientation all ARSs increased the proportion of FOA resistant (FOAR) cells to 67-81% relative to 36% in the construct with no ARS (Figure 1, upper part). No

Figure 2 Proto-silencer and replicator activity of ARS305 and ARS605. A. The ARSs and their mutant derivatives (shown on the left) were integrated in the VII telomere in URA3-ACS-B1-tel orientation. The WTW motifs are underlined. The mutations are depicted by small letters. The silencer activity of the constructs was assessed as % FOA<sup>+</sup> cells and plotted. Data is from Table 1. Statistical significance (p values) of the difference between the non-mutated construct and some of the mutants are shown next to each bar. The p values for the remaining constructs are significantly above 0.05 and are not shown. B. The ARSs and their mutant derivatives were cloned between CEN4 and URA3 to produce mini-chromosomes as indicated on the top of the figure. All mini-chromosomes were transformed in W303 cells and loss per generation was calculated. Data is from Table 1.

Figure 3 Proto-silencer and replicator activity of ARS317 and ARS319. A. The ARSs and their mutant derivatives (shown on the left) were integrated in the VII telomere in URA3-ACS-B1-tel direction. The putative WTW motifs are underlined. The mutations are depicted by small letters. The silencer activity of the constructs was assessed as % FOA<sup>+</sup> cells and plotted. Data is from Table 1. The p values for all constructs are above 0.05 and are not shown. B. The ARSs and their mutant derivatives were cloned between CEN4 and URA3 to produce mini-chromosomes. All mini-chromosomes were transformed in W303 cells and loss per generation was calculated and plotted. Data is from Table 1.
significant difference between silencer and replicator ARS was observed. In the opposite URA3-B1-ACS-tel orientation ARS305 produced FOA\textsuperscript{R} values comparable to the construct without any ARS (Figure 1, lower part). Remarkably, ARS317, ARS605 and especially ARS319 produced statistically significant decrease (p < 0.05) of silencing relative to the control thus clearly displaying anti-silencing properties. In summary, the orientation of ARS relative to the telomere has a major impact on the level of subtelomeric silencing. From a technical point of view, in the URA3-ACS-B1-tel orientation all ARSs showed similar proportions of FOA\textsuperscript{R} cells that allow for direct comparison of the role of their B1 elements in gene silencing.

The same B1 elements in ARS305 and ARS605 contribute to gene silencing and DNA replication

Next, we measured the contribution of the B1 elements to DNA replication and to gene silencing. We mutated the replicators (ARS305 and ARS605) and silencers (ARS317 and ARS319) in the region encompassing their putative B1 (WTW) motifs by replacing two bases at a time (Figure 2A, Figure 3A). All wild type and mutant ARSs were cloned in ADH4-URA3-ACS-B1-tel orientation, inserted in the \textit{VIIIL} telomere and subjected to the FOA-resistance assays. In parallel, the same ARSs were cloned in \textit{URA3/CEN4} mini-chromosomes and tested for their replicator activity. The substitutions in the WTW motifs (shown by the rectangle in Figure 2A) of ARS305 and ARS605 caused statistically significant decrease in the proportion of FOA\textsuperscript{R} cells relative to their non-mutated counterparts (Figure 2A). The flanking sequences in ARS305 had little effect, while in ARS605 they produced some minor reduction in silencer activity. In the mini-chromosome stability assay exactly the same mutations caused substantial increase of the loss per generation rates (Figure 2B). There is a good agreement in the magnitude of effects in the two assays with all mutants tested (Figure 2 A and B). These observations indicate that the same B1 elements in ARS305 and ARS605 contribute to their silencer and replicator activity.

Silencer ARSs lack functionally identifiable B1

Similar analyses of the two silencer ARSs (ARS317 and ARS319) showed that none of the two-base substitutions in the vicinity of the WTW element altered the levels of URA3 silencing (Figure 3A). To warrant for the existence of aberrantly positioned B1 we expanded the scanning substitutions as compared to ARS305 and ARS605, but no effects were observed. Similarly, the replicator activity of ARS317 and ARS319 remained largely unaffected by the mutations (Figure 3B). Only one of the ARS319 constructs showed modest increase in the loss rate of the mini-chromosome, but the mutation was outside of the canonical WTW element. Clearly, both assays failed to reveal a B1 element in ARS317 and ARS319.

Discussion

Lack of canonical B1 element

While the position and consensus of the B2, B3 and B4 auxiliary elements vary between different ARSs, the ACS-B1 module serves as a binary binding site for the association of ORC and seems highly conserved [2,8,11,29,30]. Indeed, recent studies have identified a strong WTW consensus 15–17 bases downstream of the core ACS [8,13]. Mutations in this B1 motif have caused significant loss of replicator activity in most ARSs [8,13], but its role in gene silencing has not been determined. Even more, mutations in the WTW consensus of one silencer ARS (ARS317) did not affect its replicator activity [8]. It seems conceivable that ARSs could utilize the WTW motif for replication and use an alternative B1 element for gene silencing [21]. It also seems possible that silencer and replicator ARSs have different B1 elements and different type of interaction with ORC [4,21]. Here we have addressed both possibilities. We have shown that exactly the same mutations impair the replicator and the silencing function of the tested replicator ARSs (Figure 2). Hence, the answer to the first question is negative: these two functions are not determined by alternative B1 elements.

Surprisingly, the analyses of the two silencer ARSs (ARS317 and ARS319) have revealed that neither the replicator nor the silencer function was affected by any of the two-base substitutions within the 10 base region of the putative B1 (Figure 3). Again, we failed to obtain any evidence in favor of optional usage of B1 in silencing and replication. However, it is apparent that ARS317 and ARS319 have a different B1 or do not possess one at all. In the core X and Y' subtelomeric elements there are more than 100 close matches to ARS319 with high levels of homology in their ACS-B1 module (Figure 4B). We suggest that all these plus ARS317 represent a subclass of ARS with a novel type of B1 element or with no B1 element.

ARS display considerable diversity of the sequences surrounding the core ACS [8,13,31]. To this diversity we add the extreme case of lack of a canonical B1. This is an intriguing issue. Being a secondary site for ORC binding, B1 is expected to be important if not essential. It is possible that we have identified no B1 element because in silencer ARS there is no second site of association for ORC. For example, a specialized extended 17 base pair EACS [7,8,12] could provide a single high-affinity site for the binding of ORC and minimize the significance of the B1 element. Alternatively, these ACS-B1 sites are bi-partite, but B1 is broader. If this is the
case, the two-base substitutions that normally destroy the WTW in replicator ARSs will not work on ARS319 and ARS317. Indeed, the alignment of ARS319 and other telomeric ARSs shows more than one potential WTW site in the area of B1 (Figure 4B). The same applies to ARS317 (not shown). However, the two ARS from the HML mating type locus do not show significant similarity to the putative B1 regions of ARS319 and ARS317 (not shown). Even more, the high overall homology of sub-telomeric ARSs (Figure 4B) precludes the recognition of a different B1 even if it existed. Hence, while a broader B1 in silencer ARS remains possible, a consensus is difficult to identify. Ultimately, it is possible that a combination of an extended ACS[12,13,31] and broader B1 provide alternatives for the binding of ORC that are hard to unveil by alignment algorithms.

Within eukaryotes, budding yeasts are the only known species with well defined sequences of origins and well defined binding sites for ORC. In all other species the origins are quite dissimilar and are recognizable only as A/T rich regions of DNA. This study together with other recent studies[8,12,13] suggests a certain level of diversity between different ARS in budding yeast. The significance of this diversity is yet to be determined.

**Directionality of silencing**

Silencers and proto-silencer in S. cerevisiae are cis-elements that serve as focal points for the recruitment of Sir proteins [1] and in most cases are thought to act in bi-directional fashion. However, while selecting the optimal conditions for our silencing assays we came across strong and reproducible orientation-dependent effects. All ARSs were potent proto-silencers only in the URA3-ACS-B1-tel orientation, the same orientation found in natural sub-telomeric ARS elements. In the opposite URA3-B1-ACS-tel orientation ARS319 displayed strong anti-silencing activity while ARS317 and ARS605 caused modest de-repression of URA3. These experiments have not been developed to establish if the ACS-B1 module or other unknown sites within the cloned fragments exert these directional effects. However, several arguments support the idea that ACS-B1 could play a central role. Earlier research has acquired evidence for directional silencing by HMR-E when it is inserted in the HML locus [15,17]. HMR-E consists of ARS317 and binding sites for Abf1p and Rap1p. Gene repression has been robust at the Abf1p binding site of HMR-E and weak at the ACS side [15]. A stably positioned nucleosome was found adjacent to the ACS site of ARS317 and

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*Figure 4 Alignment of replicator and silencer ARSs.* The ARSs listed below were aligned using WebLogo (weblogo.berkeley.edu). Diagram depicting the possible binding of ORC is shown on the right. **A.** Alignment of 25 replicator ARS as in [8]. The list of ARSs is available upon request. The WTW consensus of the B1 element is shown in the rectangle. **B.** Alignment of subtelomeric ARS. ARS319 was used to search for related sequences by BLAST. 144 matches were identified. The top 20 entries were all located in the subtelomeric regions. These were aligned at the ACS (WTTTAYRTTW) and analyzed by WebLogo.
not on the side containing the \( B1 \) element [15]. The binding sites for Abf1p and Rap1p also contribute to the directionality of \( HMR-E \). It is noteworthy that, excluding \( ARS317 \), none of our other constructs contains identifiable Abf1p or Rap1p binding sites thus leaving \( ACS-B1 \) as a likely candidate for the effects we have observed. In addition, \( ARS319 \) and not \( ARS317 \) displayed the strongest directional effects (Figure 1). If \( ACS-B1 \) is the key directional element, a stably positioned nucleosome next to \( ACS \) can stimulate the transfer of Sir proteins approaching from the telomere thus acting as a relay point. In the opposite orientation, a nucleosome-free DNA generated by the association of ORC [32] could prevent Sir protein spreading and act as a chromatin insulator [33]. However, why nucleosome-free DNA on one side of \( ARS \) would work as insulator in one orientation is yet to be established. Structural studies on ORC updates available in January 2010 of the amplified fragments are as follows: \( ARS305 \), \( III: 39392–39774; ARS605 \), \( VI: 135860–136202; ARS317 \), \( III: 292894–292369; ARS319 \), \( III: 315639–315989 \). The primer sequences are available upon request. The amplified fragments were sub-cloned in the BamH1 site of pUC41IV [27] between the telomeric TG1-3 repeats and the \( URA3 \) reporter. Our constructs do not contain any additional sub-telomeric elements. Two bases at a time were replaced in the vicinity of the \( B1 \) elements of the

**Materials and methods**

**Constructs**

\( ARS305 \), \( ARS605 \), \( ARS317 \) and \( ARS319 \) were amplified by PCR from the genomic DNA of \( W303 \) strain using Phusion polymerase (NEB) according to the instructions of the manufacturer. The genomic coordinates (as per the

| Constructs | FOA \(^a\) | STD | Loss/generation | STD |
|------------|-----------|-----|----------------|-----|
| no ARS    | 31%       | 6%  | n/a            |     |
| \( ARS305 \) | ACS…ATGTTTATTTTCCCTTTTTAA | 66% | 7%  | n/a            | 0%  |
|            | ACS…ATGTTTATTTTcGCTTTTAA | 69% | 7%  | n/a            | 0%  |
|            | ACS…ATGTTTATTTTcGCTTTTAA | 69% | 7%  | n/a            | 0%  |
|            | ACS…ATGTTTATTTTcGCTTTTAA | 38% | 7%  | n/a            | 0%  |
|            | ACS…ATGTTTATTTTcGCTTTTAA | 69% | 7%  | n/a            | 0%  |
| \( ARS605 \) | ACS…AGGAAAATTTCAAAAGCGT | 64% | 6%  | n/a            | 0%  |
|            | ACS…AGGAAAATTTCAAAAGCGT | 64% | 6%  | n/a            | 0%  |
|            | ACS…AGGAAAATTTCAAAAGCGT | 64% | 6%  | n/a            | 0%  |
|            | ACS…AGGAAAATTTCAAAAGCGT | 56% | 6%  | n/a            | 0%  |
|            | ACS…AGGAAAATTTCAAAAGCGT | 64% | 6%  | n/a            | 0%  |
| \( ARS319 \) | ACS…TAGTTTACTTTTTTTGTCA | 64% | 6%  | n/a            | 0%  |
|            | ACS…TAGTTTACTTTTTTTGTCA | 64% | 6%  | n/a            | 0%  |
|            | ACS…TAGTTTACTTTTTTTGTCA | 64% | 6%  | n/a            | 0%  |
|            | ACS…TAGTTTACTTTTTTTGTCA | 64% | 6%  | n/a            | 0%  |
|            | ACS…TAGTTTACTTTTTTTGTCA | 65% | 6%  | n/a            | 0%  |
| \( ARS317 \) | ACS…ATGTTTACTTTTTTTGTCA | 69% | 6%  | n/a            | 0%  |
|            | ACS…ATGTTTACTTTTTTTGTCA | 69% | 6%  | n/a            | 0%  |
|            | ACS…ATGTTTACTTTTTTTGTCA | 69% | 6%  | n/a            | 0%  |
|            | ACS…ATGTTTACTTTTTTTGTCA | 69% | 6%  | n/a            | 0%  |
|            | ACS…ATGTTTACTTTTTTTGTCA | 69% | 6%  | n/a            | 0%  |
|            | ACS…ATGTTTACTTTTTTTGTCA | 69% | 6%  | n/a            | 0%  |
|            | ACS…ATGTTTACTTTTTTTGTCA | 69% | 6%  | n/a            | 0%  |
|            | ACS…ATGTTTACTTTTTTTGTCA | 69% | 6%  | n/a            | 0%  |
|            | ACS…ATGTTTACTTTTTTTGTCA | 69% | 6%  | n/a            | 0%  |

**Table 1 Silencer and replicator activity of mutated ARSs**
ARs by site-directed mutagenesis. The mutated sequences are shown in Table 1. All mutations have been confirmed by DNA sequencing. The nucleotide sequences of the primers used to amplify genomic DNA and to mutate the cloned fragments are available upon request.

Growth media and conditions

W303 cells (ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3-1) were routinely grown on rich medium (YPD) at 23°C. Cells transformed with URA3 integrating fragments or URA3/CEN4/ARS mini-chromosomes were selected on Synthetic Complete (SC) medium without uracil. Cells with repressed URA3 were selected on SC medium supplemented with 1 g/l Fluoro-Orotic Acid (FOA) (Toronto Chemicals).

Telomere position effect (TPE) assays

Fragments containing ADH4, URA3, ARS and telomeric TG1,3 repeats were released by digestion of pUCAIV derivatives with Sall and EcoRI and used to transform W303 cells. This treatment efficiently integrates the constructs into ADH4 and the VIIIY telomere [27]. Telomeric integration was confirmed by PCR and variegated expression of URA3 [27]. To warrant the loss of un-integrated constructs (these are linear DNAs lacking CEN elements), transformants were re-streaked on SC-ura and SC/FOA plates and then an isolated colony from the SC-ura plate was grown for 20 generations in non-selective (YPD) medium. Serial 1:10 dilutions were prepared and 5 μl aliquots were spotted on SC and SC-ura plates. The % FOAR for each independent culture was calculated. Each mini-chromosome was independently transformed in W303 cells. Three colonies were isolated from SC-ura plates, re-streaked on SC-ura plates and suspended in non-selective (YPD) medium. The cultures were grown for 20 generations in non-selective YPD medium. Serial 1:10 dilutions of the cultures prior and after growth in non-selective medium were prepared and 5 μl aliquots were spotted on SC and SC-ura plates. The per cent of ura+ cells prior and after growth in non-selective medium were used to calculate the mini-chromosome loss per generation as in [22,26]. Each mini-chromosome was analysed in triplicate (three independent colonies per transformation) in three independent transformations. The average values and standard deviation were calculated in Microsoft Excel and are shown in Table 2.

**Table 2 Silencer activity of ARSs cloned in opposite orientations**

| Silencer activity (FOA-resistance assay) | FOA (%)  | STD (%) |
|-----------------------------------------|----------|---------|
| no ARS                                  | 36%      | 4%      |
| ...URA3-ACS319-B1-tel                   | 81% (n = 9) | 6%      |
| ...URA3-ACS317-B1-tel                   | 77% (n = 9) | 5%      |
| ...URA3-ACS305-B1-tel                   | 78% (n = 9) | 12%     |
| ...URA3-ACS605-B1-tel                   | 67% (n = 9) | 11%     |
| ...URA3-B1-ACS319-tel                   | 7% (n = 9)  | 7%      |
| ...URA3-B1-ACS317-tel                   | 21% (n = 9) | 11%     |
| ...URA3-B1-ACS305-tel                   | 47% (n = 9) | 7%      |
| ...URA3-B1-ACS605-tel                   | 26% (n = 9) | 8%      |

Mini-chromosome stability assay

The cloned and mutated ARSs were released from pUCAIV by digestion with BamH1 and sub-cloned in a pUC119 based mini-chromosome [2] containing URA3 and CEN4. Each mini-chromosome was independently transformed in W303 cells. Three colonies were isolated from SC-ura plates, re-streaked on SC-ura plates and suspended in non-selective (YPD) medium. The cultures were grown for 20 generations in non-selective YPD medium. Serial 1:10 dilutions of the cultures prior and after growth in non-selective medium were prepared and 5 μl aliquots were spotted on SC and SC-ura plates. The per cent of ura+ cells prior and after growth in non-selective medium were used to calculate the mini-chromosome loss per generation as in [22,26]. Each mini-chromosome was analysed in triplicate (three independent colonies per transformation) in three independent transformations. The average values and standard deviation were calculated in Microsoft Excel and are shown in Table 2.

**Abbreviations**

W: A/T; Y: C/T; R: A/C; ARS: Autonomously Replicating Sequences; ACS: ARS Consensus Sequence; ORC: Origin Recognition Complex; FOA: 5-fluoro-orotic acid; TPE: Telomere Position Effect.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

PC-R conceived and participated in the design of the study, cloned and mutagenized the analyzed ARSs, carried out the silencing and some of the replicator experiments and drafted the manuscript. SP and KS carried out the replicator assays and prepared figures. KY conceived and participated in the design of the study and wrote the manuscript. All authors have read and approved the manuscript.

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