The notion that telomeres are essential for chromosome linearity stems from the existence of two chief dangers: inappropriate DNA damage response (DDR) reactions that mistake natural chromosome ends for double-strand DNA breaks (DSBs), and the progressive loss of DNA from chromosomal termini due to the end replication problem. Telomeres avert the former peril by binding sequence-specific end-protection factors that control the access of DDR activities1–3. The latter threat is tackled by recruiting telomerase, a reverse transcriptase that uses an integral RNA subunit to template the addition of telomere repeats to chromosome ends.

Here we describe an alternative mode of linear chromosome maintenance in which canonical telomeres are superseded by blocks of heterochromatin. We show that in the absence of telomerase, Schizosaccharomyces pombe cells can survive telomere sequence loss by continually amplifying and rearranging heterochromatic sequences. Because the heterochromatin assembly machinery is required for this survival mode, we have termed it ‘HAATI’ (heterochromatin amplification-mediated and telomerase-independent).

HAATI uses the canonical end-protection protein Pot1 (ref. 4) and its interacting partner Ccq1 (ref. 5) to preserve chromosome linearity. The data suggest a model in which Ccq1 is recruited by the amplified heterochromatin and provides an anchor for Pot1, which accomplishes its end-protection function in the absence of its cognate DNA-binding sequence. HAATI resembles the chromosome end-maintenance strategy found in Drosophila melanogaster, which lacks specific telomere sequences but nonetheless assembles terminal heterochromatin structures that recruit end-protection factors. These findings reveal a previously unrecognized mode by which cancer cells might escape the requirement for telomerase activation, and offer a tool for studying genomes that sustain unusually high levels of heterochromatinization.

Ten to fifteen percent of cancer cells achieve unlimited proliferative potential without telomerase by using one or more method(s) termed alternative lengthening of telomeres (ALT). The modus operandi of ALT seems to involve recombination between residual telomere sequences6. Budding yeast survive without telomerase using a similar constellation of strategies in which either the telomeric repeats or sequences6. Budding yeast can also survive the absence of telomerase by recombining persisting telomere sequences, forming so-called ‘linear survivors’.

While investigating differences between linear and circular telomerase-minus survivors, we deleted the trt1 gene (which encodes the catalytic reverse transcriptase subunit of telomerase), isolated survivors and analysed their sensitivities to DSB-inducing agents. Whereas linear survivors display little or no sensitivity to these agents, circular survivors are exquisitely sensitive, with a susceptibility exceeding that of cells lacking the Rad3/ATR checkpoint regulator (Fig. 1a). Notably, a subset of trt1Δ survivors had an intermediate level of sensitivity; we named these HAATI survivors (Fig. 1a). Like circular survivors, HAATI cells are often elongated and have DAPI (4’,6-diamidino-2-phenylindole) staining patterns indicative of compromised chromosome segregation (Fig. 2). HAATI arise very infrequently when trt1Δ cells are propagated by repeatedly streaking single colonies on plates, a condition under which ~95% of survivors are circular. However, as HAATI survivors have a shorter doubling time than circulars, they have a competitive advantage and are enriched when trt1Δ cultures are grown in liquid media (Supplementary Table 1).

To determine whether the DSB resistance of HAATI survivors stems from chromosome linearity, we investigated their chromosome structure by pulsed-field gel electrophoresis (PFGE). Chromosomes of wild-type cells or linear survivors are readily resolved by PFGE, but circular chromosomes fail to enter the gel12 (Fig. 1b). HAATI genomes fail to enter gels, mirroring the behaviour of circular chromosomes (Fig. 1b). To further analyse the HAATI genome, we digested it with NotI, a rare-cutting restriction enzyme that releases twelve internal and four terminal fragments from chromosomes (Chr I and II (Chr III lacks NotI recognition sites). Ethidium bromide staining shows that the internal NotI fragments of HAATI chromosomes migrate as distinct bands whereas the terminal fragments are absent (Fig. 1d), again mirroring the behaviour of DNA from circular strains. However, Southern blot analysis reveals significant deviations from circular chromosomes. In NotI digests of circular chromosomes, the terminal fragments L, I, M and C are replaced by bands representing fused terminal fragments (L+I and C+M) (Fig. 1c). These bands are absent from blots of HAATI DNA, in which the majority of hybridization signal remains in the well. (Fig. 1d). PCR confirmed that the loss of L, I, M and C hybridization is not due to loss of the

---

HAATI survivors replace canonical telomeres with blocks of generic heterochromatin

Devanshi Jain1, Anna K. Hebden2, Toru M. Nakamura3, Kyle M. Miller4 & Julia Promisel Cooper1

©2010 Macmillan Publishers Limited. All rights reserved
fragments of HAATI chromosomes fail to enter gels. Left, ethidium bromide methanesulphonate. Were grown at 32°C for 2 days. 'O' denotes circular. MMS, methyl methanesulphonate. 

Both HAATIrDNA and HAATISTE (see below) with identical results. (see also Supplementary Fig. 3). All analyses in Fig. 1 were performed on both HAATrDNA and HAATISTE (see below) with identical results. Consider this ‘endedness’, we reintroduced telomerase into circular strains (discussed in legend to Supplementary Fig. 1). Thus, indicates a shared feature between HAATI chromosomes and Chr III (Supplementary Fig. 4). The inability to confer PFG entry to Chr III (see also Supplementary Fig. 5). Cells were analysed as in a. c, rDNA has spread to all three chromosomes in HAATrDNA cells, whereas HAATISTE cells contain rDNA only on Chr III (see also Supplementary Fig. 5). Cells were analysed as in a. d, NotI-digested HAATISTE chromosomes have amplified STE sequences. Analysis with (+T) and without Trt1 addition.

Figure 1 | Characterization of HAATI strains. a, HAATI are DSB resistant compared to circulars. Fivefold serial dilutions of cultures (10⁶ cells ml⁻¹) were grown at 32°C for 2 days. 'O' denotes circular. MMS, methyl methanesulphonate. b, HAATI whole chromosomes fail to enter pulsed-field gels. c, NotI digestion releases four terminal fragments, referred to as L, M, I and C, from the ends of Chr I and II; these are replaced by fusion fragments L+1 and C+M in circulars. d, Terminal, but not internal, fragments of HAATI chromosomes fail to enter gels. Left, ethidium bromide staining of NotI digest PFG. Right, Southern blotting of PFG. e, Cleavage at a single I-SceI site on Chr II fails to confer gel entry to HAATI chromosomes (see also Supplementary Fig. 3). All analyses in Fig. 1 were performed on both HAATrDNA and HAATISTE (see below) with identical results. 

probed sequences from the genome (data not shown). Thus, HAATI chromosomes fail to exhibit the fusion fragments diagnostic of circular strains. We further reasoned that if HAATI chromosomes were circular, they would be linearized by a single chromosome cleavage, allowing entry into gel. In contrast, if they harboured an alternative structure that prevented gel entry, for example, persistent unresolved recombination intermediates, cleavage might not lead to gel entry. To test this, we engineered a unique I-SceI site in Chr II of both HAATI and circular strains. As expected, I-SceI digestion of DNA from the engineered circular strain linearizes Chr II and confers its PFG entry (Fig. 1e and Supplementary Fig. 3). However, I-SceI digestion of the HAATI chromosome fails to confer gel entry (Fig. 1e). Similarly, the limited chromosome breakage induced by treatment with low-dose ionizing radiation fails to confer gel entry to HAATI chromosomes, whereas it does confer entry to Chr I and II of circular survivors (Supplementary Fig. 4). The inability to confer PFG entry to Chr III indicates a shared feature between HAATI chromosomes and Chr III of circular strains (discussed in legend to Supplementary Fig. 1). Thus, HAATI chromosomes are distinct from those of both linear and circular strains, most likely retaining linearity but sustaining a persistent secondary structure that prevents terminal fragments from migrating into gels.

If HAATI chromosomes are not circular, they must have ends. In considering this ‘endedness’, we reintroduced telomerase into circular and HAATI survivors by expressing plasmid-borne trt1+/+, referred to below as the ‘+T’ situation. Reintroduced Trt1 failed to affect Chr I or II of circular strains, as these chromosomes remained unable to enter PFG, whereas Chr III acquired telomeres along with the ability to enter PFG (Fig. 2a). In marked contrast, Trt1 reintroduction conferred telomere addition and PFG entry to all three HAATI survivor chromosomes (Fig. 2b, Supplementary Fig. 5).

A striking feature of HAATI+Trt1 strains is the marked alteration in chromosome size relative to wild type (Fig. 2b). These size variations occur continually before Trt1 reintroduction in HAATI cultures, and chromosome sizes are stabilized once Trt1 is expressed in HAATI+Trt1 cells (Supplementary Fig. 6). Thus, HAATI trt1Δ survivors undergo continual and marked size alterations.

Further analysis of HAATI+Trt1 cells revealed two subsets of HAATI, hereafter referred to as HAATrDNA and HAATISTE. The subtelomeric regions of wild-type Chr I and II comprise ~86 base pair imperfect repeats called sub-telomeric elements (STE), extending ~20 kilobases towards the chromosomal interior (Supplementary Fig. 7), whereas both telomeres of Chr III are bounded by the ribosomal DNA (rDNA) repeat regions, comprising ~10 kb repeats that span ~1 megabases (Supplementary Fig. 7). Remarkably, while the rDNA probe hybridizes only to Chr III in wild-type cells, it hybridizes to all three chromosomes in the HAATrDNA subset (Fig. 2c), indicating that the rDNA is amplified and spread in HAATrDNA survivors. Despite the excessive levels of rDNA hybridization in HAATrDNA+Trt1 chromosomes, the rDNA probe fails to hybridize with those NotI fragments of HAATISTE trt1Δ survivors that migrate upon PFG (Supplementary Fig. 8a); this suggests that the amplified rDNA is restricted to the terminal NotI fragments that fail to enter the gels. Accordingly, on Trt1 reintroduction, the NotI fragments containing rDNA acquire the ability to enter gels (Supplementary Fig. 8a).

The HAATISTE subclass of survivors exhibits a distinct pattern of hybridization. Like wild-type cells, HAATISTE+Trt1 cells have rDNA only on Chr III (Fig. 2c). However, they have markedly amplified and rearranged STE sequences. Whereas wild-type chromosomes have STE sequences only on their terminal NotI restriction fragments, the STE probe hybridizes strongly with every NotI restriction fragment derived from HAATISTE chromosomes, demonstrating that STE have spread to internal sites on Chr I and II (Fig. 2d). STE hybridization is also seen on Chr III of HAATISTE cells (Supplementary Fig. 8b). Like all HAATrDNA chromosomes, all HAATISTE chromosomes acquire telomeres and enter gels upon Trt1 reintroduction (Supplementary Fig. 8c). Dot blot analysis confirms the amplification of STE and rDNA sequences in HAATISTE and HAATrDNA, respectively (Supplementary Table 2 and Supplementary Fig. 9). Hence, although both classes of HAATI display DSB resistance, an inability of terminal chromosome fragments to enter gels, and
the acquisition of telomeres and chromosomal gel migration upon telomerase re-introduction, each has amplified a different class of repetitive sequence. In one case the rDNA has spread from the sub-terminal regions of Chr III to the termini of Chr I and II, whereas in the other case, STE sequences have spread from the terminal regions of Chr I and II to multiple sites in these chromosomes' interiors as well as onto Chr III. HAATI\(^{\text{STE}}\) survivors are exceedingly rare whereas HAATI\(^{\text{DNA}}\) survivors are frequent (Supplementary Table 1), most likely reflecting the drastic genomic disruption that accompanies HAATI\(^{\text{STE}}\) formation. Therefore, the analyses below were performed on HAATI\(^{\text{DNA}}\) except where indicated.

The rDNA and STE sequences of wild-type cells are packaged into heterochromatin characterized by histone H3-Lys 9 (H3K9) hyper-methylation\(^{16,17}\) and binding of Swi6, the fission yeast heterochromatin protein-1 (HP-1) orthologue. These hallmarks of the sequences amplified in HAATI raised the possibility that heterochromatin has a role in HAATI survival and indeed, the Swi6–green fluorescent protein (GFP) localization patterns of HAATI cells are consistent with expanded heterochromatin (Supplementary Fig. 10). Therefore, we investigated the requirement for the heterochromatin assembly machinery. Clr4, the single fission yeast orthologue of the Su(var.) 3-9 histone methyltransferase, is required for H3K9 methylation and heterochromatin formation\(^{18}\). trt1\(^{\text{Δclr4Δ}}\) cells were isolated from heterozygous diploids and propagated under competitive conditions alongside trt1\(^{\text{Δ}}\) single mutants. Whereas nearly all surviving trt1\(^{\text{Δ}}\)A cells exhibited HAATI survival (Supplementary Fig. 11a), most trt1\(^{\text{Δclr4Δ}}\) cell populations comprised circular survivors (Fig. 3a and Supplementary Fig. 11b). Moreover, deletion of clr4\(^{\text{Δ}}\) in an already-formed haploid HAATI survivor led to immediate circularization of the chromosomes in 25% of cases (Fig. 3b), directly linking Clr4 to maintenance of HAATI. In contrast, Clr4 is dispensable for the maintenance of linear or circular trt1\(^{\text{Δ}}\)A survivors (Fig. 3b). As seen for clr4\(^{\text{Δ}}\), deletion of swi6\(^{\text{Δ}}\) severely compromises HAATI formation (Supplementary Fig. 12). The residual occurrence of HAATI in clr4\(^{\text{Δ}}\)A or swi6\(^{\text{Δ}}\)A cells lacking Trt1 may reflect heterochromatin function carried over from the parental strain, or a rare ability of cells to retain downstream heterochromatin functions (see later) following loss of these genes. Collectively, these results indicate that the heterochromatic nature of the sequences amplified in HAATI chromosomes is crucial to their formation and maintenance.

The repeat amplification seen in HAATI indicates that the relevant sequences sustain vigorous levels of recombination. Indeed, deletion of rhp51\(^{+}\) (which encodes the fission yeast Rad51 orthologue) abolishes HAATI formation, as all trt1\(^{\text{Δrhp51Δ}}\) survivors raised in competitive conditions sustain circular chromosomes (Fig. 3b). Moreover, 9 out of 12 trt1\(^{\text{Δrad50Δ}}\) survivors grown in competitive conditions form circular survivors (Supplementary Fig. 13). Hence, not only heterochromatin factors, but also Rhp51 and Rad50, are important for HAATI survival.

Curiously, the circular trt1\(^{\text{Δ}}\) strains lacking Clr4, Swi6 or Rhp51 harbour di-chromosome circles (Fig. 3c and Supplementary Fig. 14). We speculate that dicentric circle formation reflects compromised centromere function in the absence of Clr4/Swi6 or Rhp51. Centromere inactivation, which would be necessary for propagation of dicentric chromosomes, may be frequent in the absence of Clr4/ Swi6 or Rhp51 and may confer an advantage to dicentricity. Indeed, Clr4-based heterochromatin formation is a known requirement for centromere function\(^{19}\) and Rhp51 was shown to suppress rearrangement of centromeric sequences\(^{20}\). Intriguingly, Rad50 does not seem to share a role in suppressing dicentric formation, as all trt1\(^{\text{Δrad50Δ}}\)A circulars isolated harbour mono-chromosomal circles (Supplementary Fig. 13).

The foregoing results suggest that HAATI chromosomes remain linear but fail to enter gels due to structures associated with the continual rearrangement of rDNA or STE sequences. The linearity of HAATI chromosomes made it crucial to ascertain whether they contain telomeric repeats; hence, we sought to release all terminal fragments for gel entry by digesting the genome with a cocktail of frequent-cutting restriction enzymes that do not digest telomere sequences. Digestion of wild-type or HAATI DNA with this cocktail reduces the majority of the genome to \(< 100 \text{ bp}\) fragments (Fig. 4a). Hybridization of digested wild-type DNA with a telomere probe reveals fragments of \(\sim 150–250 \text{ bp}\) (Fig. 4a). HAATI DNA was seen at 65 or 55°C, indicating that HAATI chromosomes lack stretches of telomeric DNA of greater than \(\sim 27 \text{ bp}\) (see Methods). Hybridization at 55°C, a temperature at which our probe should detect shorter telomere stretches, does yield a signal for DNA derived from HAATI\(^{\text{DNA}}\) survivors (Fig. 4a). This signal runs as a discrete band, indicating localization to an internal rather than a terminal (and therefore heterogeneously sized) region. The size of this band corresponds to that expected for a region within the rDNA that contains a 13-bp stretch of telomeric sequence\(^4\), indicating that this signal arises as a by-product of the rDNA amplification in HAATI\(^{\text{DNA}}\) survivors. Correspondingly, this short telomeric stretch resists extensive BAL-31 digestion (Fig. 4b), confirming its localization to the chromosomal interior rather than the terminus. No telomeric hybridization to HAATI\(^{\text{STE}}\) DNA was seen at 65 or 55°C (Fig. 4a). Hence, HAATI\(^{\text{DNA}}\) and HAATI\(^{\text{STE}}\) lack terminal telomeric repeat stretches.

The absence of telomere sequences at HAATI chromosome termini prompted us to investigate whether telomere proteins have a role in HAATI survival. Deletion of taz1\(^{-}\) has no effect on either HAATI subtype (Fig. 4c and Supplementary Fig. 15). Surprisingly, however, deletion of pot1\(^{+}\) from either HAATI\(^{\text{STE}}\) or HAATI\(^{\text{DNA}}\) cells leads to chromosome circularization (Fig. 4c and Supplementary Fig. 15). Hence, chromosome end protection in HAATI cells requires Pot1 despite the absence of its canonical binding sites.

As the heterochromatin assembly machinery is required along with Pot1 for HAATI survival, our attention turned to Ccq1 as a
potential Pot1 targeting factor. Ccq1 is a telomere-associated protein that forms a complex not only with Pot1, but also with the histone deacetylation machinery via the SHREC (Snf2/Hdac-containing repressor complex) heterochromatin complex. Thus, Ccq1 might serve as a link between the amplified heterochromatin and Pot1. Indeed, deletion of ccq1 in HAATI DNA leads to immediate chromosome circularization (Fig. 4c). This result is particularly striking as ccq1 deletion in wild-type cells promotes homologous recombination at telomeres, thereby opposing chromosome circularization. Therefore, Ccq1 provides a chromosome end-protection function in HAATI cells that it does not provide in wild-type cells.

The foregoing data indicate that Pot1, which is required for preventing circularization of HAATI chromosomes, is recruited to HAATI chromosome ends by non-telomeric heterochromatin. As rDNA forms the termini of all HAATI DNA chromosomes, we propose that Pot1 localizes to these rDNA termini. Our attempts to detect rDNA in Pot1 immunoprecipitates were unsuccessful (Supplementary Fig. 16). This result is perhaps not unexpected given that the rDNA is amplified in HAATI DNA cells, giving rise to ~6 Mb of rDNA, of which only a vanishingly small fraction would constitute the Pot1 binding region if it is restricted to the rDNA terminus. This restriction to the terminus could be explained by a mechanism in which not only heterochromatin, but also a 3' overhang, contributes to Pot1 recruitment. Such a single-stranded overhang could bind Pot1 directly, in a non-sequence-specific manner, or indirectly via recruitment of ssDNA binding proteins like RPA, which may in turn interact with Pot1. Indeed, native in-gel hybridization analysis reveals an Escherichia coli Exol-sensitive 3' single-stranded rDNA signal specifically in DNA from HAATI DNA strains; this rDNA overhang is abolished once bona fide telomeres have been added to the respective chromosome ends via Trt1 introduction (Supplementary Fig. 17). Hence, HAATI DNA chromosomes terminate with 3' overhangs of rDNA sequence.

Our data allow us to propose a model for the HAATI survival mode (Supplementary Fig. 1). The lack of high-affinity Pot1 binding sites (that is, telomeres) in HAATI cells enhances the availability of Pot1 for recruitment to non-telomeric heterochromatin by SHREC-Ccq1. Hence, the continual rearrangement and amplification of heterochromatic repeats insulates coding sequences from the end replication problem, while at the same time bringing Pot1 into the vicinity of the chromosome terminus. In addition, the presence of terminal 3' overhangs assists in concentrating Pot1 at the extreme chromosome end. Pot1 in turn provides an essential chromosome end-protection function. This function may be the restraint of 'runaway' 5' resection from the chromosomal terminus, as we have found that Pot1 inactivation leads to rampant telomeric C-strand loss. Inherent in this model is the idea that Pot1 serves an essential function without binding DNA directly, or by binding non-telomeric DNA sequences; we are currently probing which domains of Pot1 are essential for HAATI survival and whether they are separable from those required for canonical telomere maintenance.

The existence of HAATI shows that the telomere sequence per se is dispensable for chromosome linearity in fission yeast. Intriguing parallels can thus be drawn between HAATI survival and other instances in which 'generic' heterochromatin regulates chromosome dynamics in a manner usually associated with specific DNA sequences. Centromere identity is epigenetically determined in a number of organisms including fission yeast, in which centromere inactivation via excision of centromeric sequences can be bypassed by the assembly of kinetochore subtelomeric sites. Moreover, the HAATI strategy for linear chromosome maintenance is reminiscent of the approach taken by Drosophila and other genera of Dipterans. These organisms lack simple sequence repeats at their chromosomal termini, and although they often harbour terminal retrotransposons (HET or TART elements), the latter elements are dispensable for chromosome end maintenance. Crucially, regardless of the underlying sequences, Drosophila chromosome ends are packaged into heterochromatin, which is in turn associated with the recruitment of end-protection factors such as HOAP and HipHop. Our data indicate that the exclusively epigenetic strategy may be more conserved than initially thought and may represent a universal solution to the problem of linear chromosome maintenance. The shared ability to use HAATI to maintain chromosome linearity in such widely divergent organisms as flies and yeast argues that such mechanisms may be possible in human cells as well. The rearrangements associated with HAATI could both promote tumorigenesis and provide a telomere-independent means by which cancer cells could attain unlimited proliferative capacity.

**METHODS SUMMARY**

All experiments were performed using the *Schizosaccharomyces pombe* strains, genetic manipulations and selection protocols described in Methods. Pulsed-field gel electrophoresis, hybridization analysis and restriction digest procedures are explained in Methods.

Received 7 September 2009; accepted 21 July 2010.


Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank T. Cech for discussions and gratefully acknowledge that initial work by T.M.N. on reintroducing Trt1 to circular strains was performed in the Cech laboratory. We thank our current and former laboratory members for discussions and advice. This work was supported by Cancer Research UK.

Author Contributions D.J. performed the experiments in Figs 3 and 4, Supplementary Figs 6 and 9–17 and Supplementary Tables 1 and 2, and reproduced the initial work by T.M.N. on reintroducing Trt1 to circular strains. J.P.C. and D.J. generated the figures and wrote the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to J.P.C. (julie.cooper@cancer.org.uk).
METHODS

Strains and media. Strains used in this study are listed in Supplementary Information.

ttrt1Δ/trt1Δ, chrΔ4/trΔ4 chrΔ1/trΔ1, rhp51Δ/rhp51Δ trt1Δ/trt1Δ, swi6Δ/swi6Δ trt1Δ/trΔ1 and rad50Δ/rad50Δ trt1Δ/trΔ1 strains were made by one-step gene replacement in wild-type diploids.

tat1Δ HAATDNA, tat1Δ HAATSTE, pot1Δ HAATDNA, pot1Δ HAATSTE, ccq1Δ HAATDNA, chrΔ4 HAATDNA, chrΔ4 circular trt1Δ survivor and chrΔ4 linear trt1Δ survivor strains were constructed by one-step gene replacement in already-formed HAATI survivors, already-formed circular survivors and already-formed linear survivors, respectively. GFP, red fluorescence protein (RFP) and 6P K tags were integrated at the endogenous C termini of swi6Δ, hh11Δ and pot1Δ by one-step gene replacement in wild type or already-formed circular or HAATI survivor strains. Strains containing the I-SceI restriction site were constructed by inserting the I-SceI recognition sequence along with a KanMX cassette downstream of the his4 locus by one-step gene replacement in wild type, already-formed circular and already-formed HAATI survivors. The presence of the site was verified by Southern analysis. Strain 956 had an internal telomere at waf1 for use in experiments not described in this manuscript. Strain 8500 was derived from 956 by deletion of the internal telomere at waf1. None of the other strains contain this internal telomere stretch. +‘Trt1’ strains were constructed by transforming p-kanMX-trt1Δ–myc (ref. 31) into the indicated strains. Strains were grown at 32 °C in standard rich media (yeast extract media with supplements (YES), or yeast extract media with low adenine concentration (YE) for diploids as described32.

Cytological analysis. Cells were grown to log phase in rich media at 32 °C and visualized by light and fluorescence microscopy.

To visualize nuclear morphology, cells were fixed in 70% ethanol, rehydrated in water, stained with DAPI (Vector Laboratories) and imaged on a Zeiss Axioplan 2 microscope (Carl Zeiss MicroImaging) with an attached CCD camera (Hamamatsu). Images were captured and analysed using Velocity software (Improvision).

Swi6–GFP and Hht1–RFP were visualized as described previously33, with the following modifications: cells adhered to glass culture dishes were immersed in Edinburgh minimal media with required supplements. Images were captured with equal exposure for all cell types and images presented here are representative of 17 circular or HAATI survivor strains. None of the other strains contain this internal telomere stretch. +‘Trt1’ strains were constructed by transforming p-kanMX-trt1Δ–myc (ref. 31) into the indicated strains. Strains were grown at 32 °C in standard rich media (yeast extract media with supplements (YES), or yeast extract media with low adenine concentration (YE) for diploids as described32.

Pulsed-field gel electrophoresis of whole chromosomes. PFGE of whole chromosomes was performed as described previously34, with the following modifications: cells were spheroplasted with 0.6 mg ml−1 diethanolamine (pH 9.5), 0.001 M dithiothreitol and 0.02 mg ml−1 lysozyme overnight. Each plug was then preincubated on ice in 160 mM NotI buffer, 10 mM Nuclease ExoI digestion. The DNA was phenol-chloroform-extracted, ethanol-precipitated and an aliquot run on a 1% agarose gel to assess digestion via a decrease in size of the linear plasmid. Digested samples were then subjected to restriction enzyme cocktail digestion and Southern blot analysis as described above.

In-gel hybridization. Genomic DNA was digested with HindIII in NEBuffer 2 at 37 °C overnight. In-gel hybridization was performed as previously described37. The HindIII fragment of Ypl10.4 used as the rDNA probe36, either in its native condition or alkaline denatured, was used as control.

Nuclease Exol digestion. Genomic DNA was incubated with 40 units of Exol 1 in 1 μl Exol buffer (NEB) at overnight at 37 °C. DNA was phenol-chloroform-extracted and ethanol-precipitated. Treated samples were subjected to restriction enzyme digestion and in-gel hybridization as described above.

PFGE of restriction enzyme digested chromosomes. PFGE of restriction-enzyme digested chromosomes. For I-SceI restriction digestion, agarose plugs were preincubated at 4 °C for 18 h, incubated with RNase A at 37 °C for 1 h, then concentrated using a Qiagen QiAquick PCR Purification kit. The eluted DNA was transferred to a 96-well microtitre plate. WCE samples were serially diluted fivefold in TE. DNA was processed for Southern blotting as described below. The probes used were rDNA probe36 and telomere probe36.

Dot blot analysis. Genomic DNA (Supplementary Fig. 9) or DNA obtained from reverse crosslinked WCE and immunoprecipitation (Supplementary Fig. 16), diluted in Tris-Gly buffer, was precipitated with LiCl and hybridized with 10 μl of denaturant buffer (60 mg ml−1 NaOH, 3.125 mM NaCl) for 10 min. 31E2 32. Moreno, S., Klar, A. & Nurse, P. Molecular genetic analysis of fission yeast Schizosaccharomyces pombe. Methods Enzymol. 194, 795–823 (1991).

Figure S1. Model for the HAATI rDNA survival mode. HAATI chromosomes undergo constant expansion and contraction of the rDNA repeats, which spread to the termini of all 3 chromosomes. This continual sequence amplification insulates the genome from the end replication problem. Recombination intermediates associated with this amplification prevent terminal restriction fragments from entering gels. The amplified heterochromatic sequences bind Ccq1, which in turn contributes to Pot1 recruitment. Concurrently, the 3’ ss overhangs of rDNA contribute to Pot1 recruitment, resulting in restriction of Pot1 to chromosomal termini. Pot1 provides a crucial end-protection function, as its loss abolishes HAATI survival. Reintroduction of Trt1 results in telomere addition to all chromosome ends, preventing further local recombination and stabilizing chromosome size.
Several observations raise the possibility that Chr III utilizes the HAATI rDNA survival mode even in ‘circular’ trt1 Δ strains. Like all HAATI chromosomes, Chr III of circular strains undergoes telomere addition and migrates into gels upon Trt1 reintroduction (Fig 2a), fails to ‘open’ upon exposure to IR (Fig. S3), and fails to exhibit the expected fusion fragments upon treatment with a rare-cutting restriction enzyme (SfiI; data not shown). While these observations may stem from instability within the rDNA repeat region, they may hint that the disposition of rDNA at either end of Chr III facilitates HAATI processes even in cells harbouring circular Chr I and II.
Figure S2. HAATI cells are elongated and exhibit chromosome segregation defects.
Examples of DAPI stained cells, photographed as described in Methods. Cell elongation, anaphase bridges (examples marked with grey arrows) and asymmetric DNA distributions (examples marked with yellow arrows) are seen in both circular and HAATI cultures. Scale bars represent 7 μm.

---

Figure S3. Schematic of the expected patterns of migration for I-SceI digested chromosomes. Wt fission yeast lack I-SceI recognition sites. An I-SceI site was inserted at the his4 locus on Chr II. I-SceI digestion of engineered Chr II yields two bands of 3.6 and 1.0 Mbp. I-SceI digested circular chromosomes yield one band of 4.6 Mbp corresponding to the linearized whole chromosome. ‘Alternative’ structures may include persistent recombination intermediates or other branched configurations whose gel entry may not be resolved by I-SceI digestion. Maroon bars denote gel wells, light grey bars denote undigested chromosomes, yellow bars denote digested chromosomes, and dark grey diffuse bars denote degradation due to incubation in I-SceI buffer.
Figure S3. Schematic of the expected patterns of migration for I-SceI digested chromosomes. Wt fission yeast lack I-SceI recognition sites. An I-SceI site was inserted at the his4 locus on Chr II. I-SceI digestion of engineered Chr II yields two bands of 3.6 and 1.0 Mbp. I-SceI digested circular chromosomes yield one band of 4.6 Mbp corresponding to the linearized whole chromosome. ‘Alternative’ structures may include persistent recombination intermediates or other branched configurations whose gel entry may not be resolved by I-SceI digestion. Maroon bars denote gel wells, light grey bars denote undigested chromosomes, yellow bars denote digested chromosomes, and dark grey diffuse bars denote degradation due to incubation in I-SceI buffer.
Figure S4. Irradiated circular chromosomes enter gels, but HAATI chromosomes do not.

Agarose gel plugs (see Methods) containing circular or HAATI chromosomes were treated with increasing amounts of γ-radiation (0, 5, 10 and 15 Gy) and subjected to PFGE.
Fig. S5a

Figure S5. All three HAATI chromosomes enter gels upon Trt1 reintroduction. a, EtBr straining of the gel corresponding to Figs. 2b and 2c (and Figs. S7b and c below). b, Southern blot of the gel shown in (a), hybridized with probes specific to Chr I (cdc3) and Chr II (his4). Chr III is distinguishable by size from Chr I and II via EtBr straining (shown in the gel above); it also fails to hybridize with probes specific to Chr I and II.
Figure S6. HAATI chromosome size constantly changes but is stabilized upon Trt1 re-introduction. A single HAATI culture was propagated in liquid medium for 3 days. Aliquots were taken from this culture at days 1 (lanes 1 and 2) and 3 (lanes 3 and 4) and transformed with ptrt1+, to allow their chromosomes to acquire telomeres, enter gels and be analyzed for size. Genomic DNA was isolated from cultures derived from two transformants for each time point and subjected to PFGE, Southern blotting and hybridization to the rDNA probe. rDNA hybridizes to all three HAATI rDNA chromosomes (see below), and has therefore been used to visualise them. Lanes 5, 6, 7 & 8 represent aliquots taken from a single transformant of HAATI rDNA+Trt1 cells on consecutive days (lanes 5, 6, 7 and 8 represent days 1, 3, 5 and 7, respectively, after Trt1 re-introduction). Hence, lanes 1-4 represent snapshots of chromosome size over time in the absence of Trt1, while lanes 5-8 represent snapshots of chromosome size over time in its presence. The chromosome size alterations seen in lanes 1-4 indicate that size changes continually prior to Trt1 reintroduction, while the constancy of chromosome size in lanes 5-8 indicates that size is stabilized upon Trt1 reintroduction. HAATI strains with Trt1 addition are labelled as ‘H +T’.

Figure S7. Schematic depicting genomic localisation of STE and rDNA sequences in wt cells. Top panel: L, M, I and C fragments generated by NotI digest are depicted by light grey bars (with fragment sizes indicated below), STE with green arrows and rDNA with red bars. Bottom Panel: Expanded view of shaded yellow region from top panel. The C-probe hybridizes to the shown segment of the C fragment. STE regions (sometimes referred to as ‘TAS’) within it are demarcated by green bars.21,39

Figure S8. Rearrangements in HAATI chromosomes. a, The amplified rDNA in HAATI rDNA cells resides at chromosome termini. NotI digested chromosomes from wt, trt1− strains and...
Figure S7. Schematic depicting genomic localisation of STE and rDNA sequences in wt cells. Top panel: L, M, I and C fragments generated by NotI digest are depicted by light grey bars (with fragment sizes indicated below), STE with green arrows and rDNA with red bars. Bottom Panel: Expanded view of shaded yellow region from top panel. The C-probe hybridizes to the shown segment of the C fragment. STE regions (sometimes referred to as ‘TAS’) within it are demarcated by green bars.21,39.
**Figure S8.** Rearrangements in HAATI chromosomes. a, The amplified rDNA in HAATI^{DNA} cells resides at chromosome termini. NotI digested chromosomes from wt, trt1\(^{-}\) strains and
HAATI\textsuperscript{DNA+} Trt1 (marked as ‘H\textsuperscript{DNA+}T’) strains were subjected to PFGE, Southern blotting and hybridization with rDNA sequences. Internal NotI fragments of Chr I and II fragments enter the gel in all strains, as can be seen by EtBr staining. The rDNA hybridization to Chr I and II of HAATI\textsuperscript{DNA} strains remains in the well, rather than those internal fragments that migrate into the gel. However, upon Trt1 addition, new fragments containing rDNA migrate into the gel. b, HAATI\textsuperscript{STE} have amplified STE sequences onto Chr III. Southern blot of the gel shown in S4a, hybridized with STE probe. c, All three chromosomes in HAATI\textsuperscript{STE} acquire telomeres and enter gels upon Trt1 reintroduction. Southern blot of the gel shown in S4a, hybridized with a telomere probe. See figure S4 for comparison.
Figure S9. Quantitation of rDNA and STE amplification in HAATI survivors. Five-fold serial dilutions of DNA from wt and trt1Δ strains were transferred onto a membrane (see Methods), hybridized with an act1 probe, stripped and subsequently hybridized with either the rDNA (top panel) or STE probe (bottom panel). Quantitation (see Methods) of the resulting dot blots, represented relative to wt, is shown in Table S2. HAATIrDNA display a roughly 6-fold amplification of rDNA compared to wt cells, while HAATISTE have approx. 24-fold more STE hybridization than wt cells (Fig. S8 and Supplementary Table 2), consistent with our observation that the rDNA is restricted to the chromosome ends in the former while it spreads to multiple internal sites in the latter.
Figure S10. HAATI cells show expanded patterns of Swi6-GFP localisation.

Representative examples of cells harbouring endogenously GFP-tagged Swi6; one of the alleles encoding histone H3 (hht1+) is endogenously tagged with mRFP to allow chromatin visualization. Cells were photographed as described in Methods. A bright focus corresponding to the centromere cluster and 1-2 weak foci corresponding to telomeres are seen in wt cells24,40. In contrast, HAATI^{STE} cells contain not only a centromere focus but also distinct clouds of Swi6-GFP localisation, consistent with the amplified STE sequences, which have spread throughout the genome, assembling into Swi6-containing heterochromatin. HAATI^{DNA} cells contain distinct foci, similar to or in higher numbers than wt, despite lacking telomeric sequences at chromosome ends. This is consistent with localization of Swi6 to the amplified rDNA sequences, which are confined to chromosome ends.
Figure S11. *clr4* is involved in HAATI formation and maintenance. a, Cells deleted only for *trt1* form HAATI survivors under competitive conditions. Heterozygous *clr4Δ/clr4+/trt1Δ/trt1+* diploids were sporulated and 12 *trt1Δ/clr4+* progeny grown in competitive conditions, as described in the legend to Supplementary Table 1. Chromosomes from the resulting survivors were NotI digested and subjected to PFGE, Southern blotting and hybridization with L, M, I & C sequences. b, 5-fold serial dilutions of (10^7 cells/ml) cultures were plated and grown at 32°C for 2 days. The two *clr4Δ/trt1Δ* survivors that show a HAATI-type L, M, I & C hybridization patterns (asterisk) are MMS resistant relative to *trt1Δ* circulars, with one clone being significantly sicker under all conditions. *clr4Δ/trt1Δ* survivors with circular hybridization patterns (labelled as O *clr4Δ/trt1Δ* survivor) are MMS-sensitive, as are *trt1Δ* circular survivors.
**Fig. S12a**

**Expt. 1**

- For *swi6Δtrt1Δ* survivors:
  - LMIC probes
  - WT

- For *trt1Δ* survivors:
  - LMIC probes
  - WT

**Expt. 2**

- For *swi6Δtrt1Δ* survivors:
  - LMIC probes
  - WT

- For *trt1Δ* survivors:
  - LMIC probes
  - WT

**Fig. S12b**

- **0μg/ml TBZ**
  - wt
  - swi6Δ
  - trt1<sup>+</sup>/Δ
  - trt1<sup>+</sup>/Δ swi6<sup>+/A</sup>
  - trt1<sup>+</sup>/Δ swi6<sup>-/-</sup>

- **5μg/ml TBZ**
  - 0μg/ml
  - 5μg/ml
  - 10μg/ml
  - 15μg/ml

- **10μg/ml TBZ**
**Figure S12. HAATI survival requires Swi6.** a, The majority of swi6Δtrt1Δ survivors are circular. swi6Δ/swi6Δ trt1Δ/trt1Δ survivors were sporulated and progeny grown in competitive conditions, as described in the legend to Supplementary Table 1. Chromosomes from the resulting survivors were subjected to NotI digestion, PFGE Southern blotting and hybridization with L, M, I & C sequences. The top and bottom panels represent two individual experiments. Only 4 out of 20 total trt1Δswi6Δ survivors yielded the HAATI hybridization pattern. Notably, the trt1Δswi6Δ progeny of swi6Δ/swi6Δ trt1Δ/trt1Δ are often circular survivors, despite the fact that the vast majority of trt1Δswi6Δ progeny of trt1Δswi6Δ/+ diploids are HAATI (see Suppl. Table 1). We attribute this difference to haploinsufficiency of swi6Δ (see panel b), which, according to our results, would impede HAATI survival. b, Diploids heterozygous for swi6 deletion, but not clr4 deletion, are sensitive to the microtubule depolymerising agent thiabendazole (TBZ). 5-fold serial dilutions of 10^7 cells/ml cultures were plated on YE media containing the indicated quantities of TBZ and grown at 32°C for 2 days. All strains plated contain ade6 mutations that confer red colony colour for haploids and white colour for diploids on YE media. The TBZ sensitivity of diploids heterozygous for swi6 deletion suggests they are haploinsufficient for swi6 function.
Figure S13. Rad50 is involved in HAATI survival. *rad50Δ/trt1Δ* diploids were sporulated and *trt1Δrad50Δ* progeny were grown in competitive conditions, as described in the legend to Supplementary Table 1. Chromosomes from the resulting survivors were NotI digested and subjected to PFGE, Southern blotting and hybridization with L, M, I & C sequences. Only 3 out of 12 *trt1Δrad50Δ* survivors exhibit HAATI survival.
Figure S14. *trt1Δclr4Δ* and *trt1Δrhp51Δ* survivors harbor di-chromosome circles. a, The membranes shown in Figs. 3a and 3c were stripped and hybridized with probes specific to either end of Chr I (L and I probes alone), or one end of Chr II (the M probe alone). The circular fusion fragments in all *trt1Δclr4Δ* and a subset of *trt1Δrhp51Δ* circulars represent inter-chromosomal fusions between Chr I & II (also see Fig. 3c). Whether compromised centromere function also contributes to the reduced abilities of *rhp51Δtrt1Δ* and *clr4Δtrt1Δ* strains to engage in HAATI remains unknown; however, we disfavour this idea since it implies
Figure S15. **HAAT1**\textsuperscript{STE} chromosome maintenance requires Pot1 but not Taz1.

Chromosomes from the indicated strains were digested with NotI and subjected to PFGE, Southern blotting and hybridization with L, M, I & C sequences.
that centromere function is less important in circular strains than HAATi strains, a principle for which we can think of no rationalization.

Figure S15. HAATISTE chromosome maintenance requires Pot1 but not Taz1. Chromosomes from the indicated strains were digested with NotI and subjected to PFGE, Southern blotting and hybridization with L, M, I & C sequences.

Figure S16. rDNA enrichment cannot be detected via Pot1 ChIP in HAATiDNA extracts. Pot1 was tagged at its endogenous locus with PK in wt, circular trt1Δ and HAATiDNA strains. The cells were then subjected to ChIP using anti-PK antibodies (see Methods). Immunoprecipitated samples were spotted onto a membrane and probed for rDNA sequences (left panel). The membrane was rehybridized to telomere sequences (right panel) to confirm their enrichment in Pot1 immunoprecipitates of wt cells under these ChIP conditions. Numbers below rows indicate IP/WCE ratios.

Figure S17. HAATiDNA chromosomes terminate in 3' overhangs containing rDNA. DNA from log-phase cultures from the indicated strains was treated overnight with either E. coli Exonuclease I, or just Exonuclease I buffer (see Methods). The DNA was then digested with HindIII and subjected to native in-gel hybridization (see Methods); the native gels were hybridized with the rDNA probe. Following visualization, the gel was denatured and rehybridized with the rDNA probe under denaturing conditions (see Methods). The rDNA is prone to replication fork stalling and recombination. Hence, we expected that strains containing elevated levels of rDNA would exhibit ss rDNA hybridization; this ss hybridization would localize to internal chromosomal sites and would therefore be insensitive to treatment.
Figure S17. HAATTI\textsuperscript{DNA} chromosomes terminate in 3' overhangs containing rDNA. DNA from log-phase cultures from the indicated strains was treated overnight with either \textit{E. coli} Exonuclease I, or just Exonuclease I buffer (see Methods). The DNA was then digested with HindIII and subjected to native in-gel hybridization (see Methods); the native gels were hybridized with the rDNA probe. Following visualization, the gel was denatured and rehybridized with the rDNA probe under denaturing conditions (see Methods). The rDNA is prone to replication fork stalling and recombination. Hence, we expected that strains containing elevated levels of rDNA would exhibit ss rDNA hybridization; this ss hybridization would localize to internal chromosomal sites and would therefore be insensitive to treatment with ExoI. Such an ExoI-resistant signal is indeed apparent. However, an ExoI-sensitive ss rDNA fraction is clearly present uniquely in HAATTI\textsuperscript{DNA} cells.
### Table S1. Distribution of $\text{trt1}^\Delta$ survivor types.

Heterozygous $\text{trt1}^\Delta/\text{trt1}^+$ strains were sporulated and $\text{trt1}^\Delta$ progeny selected and grown in competitive conditions for 15-20 days (Expts. 1-4) or as single colonies for 5 streaks (Expt. 5). The resulting survivor populations were analysed for MMS sensitivity and chromosomal PFGE pattern. In experiments 1 (selection performed in liquid media) and 2 (selection performed in patches on solid media), 60% of survivors were HAATI$^{\text{DNA}}$ and 40% were linear survivors (i.e. they retained linear chromosomes whose terminal fragments exhibit proper gel migration and telomeric hybridization; these chromosomes also lack amplified heterochromatic sequences). In experiments 3 and 4 (selection performed in patches on solid media), 100% of survivors obtained were HAATI$^{\text{DNA}}$ survivors. Hence, HAATI$^{\text{DNA}}$ is the most frequent survival mode under competitive conditions, with $\text{trt1}^\Delta$ linear survivors arising with a variable frequency. We initially obtained one clone of HAATI$^{\text{STE}}$ by chance. In an attempt to re-isolate this survivor type (Expt. 5), 300 $\text{trt1}^\Delta$ single colonies were cultured by successively re-streaking on solid media and picking single colonies (i.e., continuous growth under non-competitive conditions). 95% of the resulting survivors were circular, 5% were HAATI$^{\text{DNA}}$ and 0.3% were HAATI$^{\text{STE}}$. While HAATI$^{\text{STE}}$ survivors grow more rapidly than circular survivors, they grow more slowly than HAATI$^{\text{DNA}}$ survivors, making them difficult to obtain in competitive conditions under which HAATI$^{\text{DNA}}$ predominate.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>n</th>
<th>linear $\text{trt1}^\Delta$ (%)</th>
<th>O $\text{trt1}^\Delta$ (%)</th>
<th>HAATI$^{\text{DNA}}$ $\text{trt1}^\Delta$ (%)</th>
<th>HAATI$^{\text{STE}}$ $\text{trt1}^\Delta$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt. 1</td>
<td>15</td>
<td>40</td>
<td>0</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Expt. 2</td>
<td>20</td>
<td>40</td>
<td>0</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Expt. 3</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Expt. 4</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Expt. 5 (single col.)</td>
<td>300</td>
<td>0</td>
<td>95</td>
<td>5</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Table S2. Quantitation of rDNA and STE amplification in survivors.

The rDNA, STE and act1 hybridization signals on the dot blot shown in Fig. S8 were quantified as described in
with ExoI. Such an ExoI-resistant signal is indeed apparent. However, an ExoI-sensitive ss rDNA fraction is clearly present uniquely in HAATIrDNA cells.

Table S1. Distribution of \( \text{trt1}^{\Delta} \) survivor types. Heterozygous \( \text{trt1}^{\Delta}/\text{trt1}^{+} \) strains were sporulated and \( \text{trt1}^{\Delta} \) progeny selected and grown in competitive conditions for 15-20 days (Expts. 1-4) or as single colonies for 5 streaks (Expt. 5). The resulting survivor populations were analysed for MMS sensitivity and chromosomal PFGE pattern. In experiments 1 (selection performed in liquid media) and 2 (selection performed in patches on solid media), 60% of survivors were HAATIrDNA and 40% were linear survivors (i.e. they retained linear chromosomes whose terminal fragments exhibit proper gel migration and telomeric hybridization; these chromosomes also lack amplified heterochromatic sequences). In experiments 3 and 4 (selection performed in patches on solid media), 100% of survivors obtained were HAATIrDNA survivors. Hence, HAATIrDNA is the most frequent survival mode under competitive conditions, with \( \text{trt1}^{\Delta} \) linear survivors arising with a variable frequency. We initially obtained one clone of HAATISTE by chance. In an attempt to re-isolate this survivor type (Expt. 5), 300 \( \text{trt1}^{\Delta} \) single colonies were cultured by successively re-streaking on solid media and picking single colonies (i.e., continuous growth under non-competitive conditions). 95% of the resulting survivors were circular, 5% were HAATIrDNA and 0.3% were HAATISTE. While HAATISTE survivors grow more rapidly than circular survivors, they grow more slowly than HAATIrDNA survivors, making them difficult to obtain in competitive conditions under which HAATIrDNA predominate.

Table S2. Quantitation of rDNA and STE amplification in survivors. The rDNA, STE and \( \text{act1} \) hybridization signals on the dot blot shown in Fig. S8 were quantified as described in Methods. Each value for rDNA or STE signal intensity was then normalised to the \( \text{act1} \) hybridization signal derived from the same sample.

<table>
<thead>
<tr>
<th></th>
<th>wt</th>
<th>O ( \text{trt1}^{\Delta} )</th>
<th>HAATI( \text{rDNA} )</th>
<th>HAATI( \text{STE} )</th>
<th>linear ( \text{trt1}^{\Delta} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>rDNA amplification relative to wt</td>
<td>1.0</td>
<td>2.8</td>
<td>6.3</td>
<td>3.2</td>
<td>1.3</td>
</tr>
<tr>
<td>STE1 amplification relative to wt</td>
<td>1.0</td>
<td>0.0</td>
<td>1.7</td>
<td>23.7</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Table S2. Quantitation of rDNA and STE amplification in survivors. The rDNA, STE and \( \text{act1} \) hybridization signals on the dot blot shown in Fig. S8 were quantified as described in Methods. Each value for rDNA or STE signal intensity was then normalised to the \( \text{act1} \) hybridization signal derived from the same sample.
### Strain Table

<table>
<thead>
<tr>
<th>Strain name</th>
<th>JCF number</th>
<th>genotype</th>
<th>mating type</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>108</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18</td>
<td>h-</td>
</tr>
<tr>
<td>wt</td>
<td>109</td>
<td>ade6-M216 his3-D1 leu1-32 ura4-D18</td>
<td>h+</td>
</tr>
<tr>
<td>rad3A</td>
<td>221</td>
<td>ade6-M210 leu1-32 ura4-D18 rad3::ura4</td>
<td>h-</td>
</tr>
<tr>
<td>‘linear’ trt1A</td>
<td>8525</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3</td>
<td>h+</td>
</tr>
<tr>
<td>‘circular’ trt1A</td>
<td>909</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3</td>
<td>h+</td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A</td>
<td>955</td>
<td>his3-D1 leu1-32 ura4::telo-LEU2 trt1::his3</td>
<td>h+</td>
</tr>
<tr>
<td>‘linear’ trt1A</td>
<td>8524</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3</td>
<td>h-</td>
</tr>
<tr>
<td>‘circular’ trt1A</td>
<td>956</td>
<td>his3-D1 leu1-32 ura4::telo-LEU2 trt1::his3</td>
<td>h+</td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A</td>
<td>8500</td>
<td>his3-D1 leu1-32 trt1::his3</td>
<td>h+</td>
</tr>
<tr>
<td>wt I-SceI 950</td>
<td></td>
<td>his4::I-SceI-kanMX</td>
<td>h-</td>
</tr>
<tr>
<td>‘circular’ trt1A 1-SceI</td>
<td>951</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3</td>
<td>h+</td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A 1-SceI</td>
<td>952</td>
<td>his3-D1 leu1-32 ura4::telo-LEU2 trt1::his3</td>
<td>h+</td>
</tr>
<tr>
<td>trt1Δtrt1′</td>
<td>1076</td>
<td>ade6-M210ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18 trt1::hygMX/trt1′</td>
<td>h?/h</td>
</tr>
<tr>
<td>trt1Δtrt1′ clr4Δclr4′</td>
<td>8526</td>
<td>ade6-M210ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18 trt1::hygMX/trt1′ clr4::kanMX/clr4′</td>
<td>h?/h</td>
</tr>
<tr>
<td>trt1Δtrt1′ rhp51A/rhp51A</td>
<td>8528</td>
<td>ade6-M210ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18 trt1::hygMX/trt1′ rhp51::kanMX/rhp51′</td>
<td>h?/h</td>
</tr>
<tr>
<td>trt1Δtrt1′ swi6Δswi6′</td>
<td>8565</td>
<td>ade6-M210ade6-M216 his3-D1/his3-D1 leu1-32/leu1-32 ura4-D18/ura4-D18 trt1::hygMX/trt1′ swi6::kanMX/swi6′</td>
<td>h?/h</td>
</tr>
<tr>
<td>trt1Δtrt1′ rad50A/rad50A</td>
<td>1060</td>
<td>ade6-M210ade6-M216 trt1::hygMX/trt1′ rad50A::mutMX/rad50A′</td>
<td>h?/h</td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A ucl1A</td>
<td>8520</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3 ucl1::hygMX; HAATI chr</td>
<td>h+</td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A po1A</td>
<td>1094</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3 po1::kanMX; circular chr</td>
<td>h+</td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A cco1A</td>
<td>1098</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3 cco1::hygMX; circular chr</td>
<td>h+</td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A ucl1A</td>
<td>8513</td>
<td>his3-D1 leu1-32 trt1::his3 ucl1::hygMX; HAATI chr</td>
<td>h+</td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A po1A</td>
<td>8502</td>
<td>his3-D1 leu1-32 trt1::his3 po1::kanMX; circular chr</td>
<td>h+</td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A clr4A</td>
<td>1071</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3 clr4::kanMX; HAATI chr</td>
<td>h+</td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A clr4A</td>
<td>1072</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3 clr4::kanMX; circular chr</td>
<td>h+</td>
</tr>
<tr>
<td>‘circular’ trt1A clr4A</td>
<td>1075</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3 clr4::kanMX; circular chr</td>
<td>h+</td>
</tr>
<tr>
<td>‘linear’ trt1A clr4A</td>
<td>8582</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3 clr4::kanMX; linear chr</td>
<td>h+</td>
</tr>
<tr>
<td>wt Pot1-PK 8566</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 pot1-6PK-kanMX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘circular’ trt1A Pot1-PK</td>
<td>8567</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3 pot1-6PK-kanMX; circular chr</td>
<td>h+</td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A Pot1-PK</td>
<td>8568</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3 pot1-6PK-kanMX; HAATI chr</td>
<td>h+</td>
</tr>
<tr>
<td>wt Swi6-GFP Hht1-RFP 1088</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 swi6-GFP-kanMX hht1-RFP-hygMX</td>
<td></td>
<td></td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A Swi6-GFP Hht1-RFP</td>
<td>8557</td>
<td>ade6-M210 his3-D1 leu1-32 ura4-D18 trt1::his3 swi6-GFP-kanMX hht1-RFP-hygMX; HAATI chr</td>
<td>h+</td>
</tr>
<tr>
<td>‘HAATIrDNA’ trt1A Swi6-GFP Hht1-RFP</td>
<td>8507</td>
<td>his3-D1 leu1-32 trt1::his3 swi6-GFP-kanMX hht1-RFP-hygMX; HAATI chr</td>
<td>h+</td>
</tr>
</tbody>
</table>