We thank Dr M. A. J. Williams and his associates, Mrs F. Dakin Williams, Dr D. Adamson, the late Dr W. H. Morton, and Dr Getaneh Assefa for geomorphic and stratigraphic interpretation; Professors G. H. Curtis and A. Cox for K/Ar and palaeomagnetic reversal dating; Dr M. Barber for NRM–TRM measurements; Drs R. Bonnefille and F. Gasse for palaeobotanical and limnological results; Mr D. Geraads and members of the team from Berkeley for faunal identifications and help with the excavations; Mrs B. C. Clark for editorial and secretarial assistance; Judith Ogden and Alison Galloway for the illustrations and the Ethiopian Government Antiquities Service and our Oromo workmen at Gadeb. The localities discussed here were excavated by an international team sponsored by the University of California, Berkeley and financed by the NSF.

Received 2 July; accepted 4 September 1979.

1. Williams, M. A. J., Williams, F. M., Gasse, F., Curtis, G. H. & Adamson, D. A. *Nature* (this issue).
2. Leakey, M. D. *Olduvai Gorge* Vol. 3, 225–234 (Cambridge University Press, 1971).
3. Walker, A. & Leakey, R. E. F. *Scient. Am.* **239**, 54–75 (1978).
4. Leakey, M. D. in *After the Australopithecines: Stratigraphy, Ecology and Culture Change in the Middle Pleistocene* (eds Butzer, K. W. & Isaac, G. L.) 477–493 (Mouton, The Hague, 1975).
5. Clark, J. D. & Kurashina, H. *Colloq. V, IXe Congr. Union int. Sci. Préhist. protohist.* (Preprint: 158–216, 1976).
6. Kurashina, H. thesis, Univ. California, Berkeley (1978).
7. Leakey, M. D. *Colloq. V, IXe Congr. Union int. Sci. prehist. protohist.* (Preprint: 24–41, 1976).
8. Chavaillon, J. & Chavaillon, N. in *Colloq. V, IXe Congr. Union int. Sci. prehist. protohist.* (Preprint: 43–69, 1976).
9. Isaac, G. L. in *After the Australopithecines Stratigraphy, Ecology and Culture Change in the Middle Pleistocene* (eds Butzer, K. W. & Isaac, G. L.) 496–542 (Mouton, The Hague, 1975).
10. Piperno, M. *Quaternaria* **18**, 347–382 (1976).

Isolation and characterisation of a yeast chromosomal replicator

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A yeast DNA sequence that behaves as a chromosomal replicator, ars*1 (autonomously replicating sequence), has been isolated. On transformation,* ars*1 allows autonomous replication of all co-linear DNA. The replicator can integrate into other replication units and can function in multimeric form. The 850-base pair* ars*1 element has no detectable homology to other yeast sequences. Such replicator-containing plasmids can be used for the isolation of DNA sequences in yeast cells as well as for the study of chromosomal DNA replication.*

THE replication of a cell's genome must be properly controlled to harmonise with the many other functions required for cell division. In bacteria, the rate of DNA replication is controlled at the onset of synthesis[1,2]. The initiation event occurs at a single site on the bacterial chromosome[3]. This initiation site or origin of replication has been genetically defined as the 'replicator' (ref. 4), an element that allows replication of all DNA attached to it. Several prokaryotic replicators have been isolated (see ref. 5 for examples) and their further biochemical and genetic analysis will help elucidate the molecular mechanism(s) that control the initiation of bacterial DNA synthesis.

How is DNA replication controlled in the larger genomes of eukaryotes? An abundance of physical data indicate that eukaryotic chromosomes are organised into tandem replication units (reviewed in refs 6–8). The rate of DNA chain elongation seems to be the same for all the replication units in a cell, whereas different units initiate DNA synthesis at different times. Furthermore, initiation is a non-random event. Replication units begin synthesis in a defined order and individual units demonstrate the same temporal specificity in successive divisions. However, the paucity of genetic and biochemical evidence means that it is impossible to determine whether such replication units are strictly analogous to prokaryotic chromosomes. For example, it has been proposed that initiation occurs not at a specific DNA sequence, but at a random site, determined solely by the spatial arrangement of the DNA within the highly structured chromosome[6,7].

During our studies on transformation of the yeast, *Saccharomyces cerevisiae*, we identified a segment of chromosomal DNA that could replicate without integrating into the yeast genome[9]. Here we further characterise this autonomously replicating segment. Its behaviour suggests that the DNA sequence contains a eukaryotic chromosomal replicator.

*ars*1: A chromosomal replicator

A 1.4-kilobase pair *Eco*RI endonuclease-generated DNA fragment, containing the centromere-linked *trp*1 gene was isolated (D.T.S. and R. W. Davis, in preparation). This fragment (Sc4101) was inserted into the *Eco*RI cleavage site of the bacterial plasmid, pBR322 (ref. 29) (Fig. 1). The resulting plasmid is termed YRp7. Covalently closed, supercoiled YRp7 DNA was used to transform a *trp*1$^-$ yeast strain. The efficiency with which these cells were transformed to Trp$^+$ was approximately 1,000-fold higher than the efficiency observed with other segments of yeast chromosomal DNA[9]. Furthermore, the high frequency of transformation was associated with autonomous replication. When DNA was isolated from these transformants and analysed by restriction endonuclease digestion and DNA–DNA hybridisation, the transforming sequences were detected as supercoiled, circular DNA and were not found integrated into the host genome. Presumably, the higher frequencies of transformation arise because autonomous replication allows heritable expression without requiring chromosomal integration via a less frequent recombination event. Thus, high transformation efficiency can be used as an indicator for autonomous replication of these hybrid molecules.

To test whether autonomous replication might be due to the particular sequences at the yeast/pBR322 junctions, the transformation efficiency of YRp7′, which contains the Sc4101 insert inverted relative to YRp7, was measured. Both YRp7 and YRp7′ gave rise to 2,000–4,000 Trp$^+$ colonies per μg DNA. As other yeast sequences (for example the *his*3, *leu*2, *ura*3 structural genes[9,11,12]) fail to replicate autonomously when inserted into pBR322, it is likely that the element that permits autonomous replication is located on Sc4101 itself. This was further investigated by inserting the Sc4101 DNA fragment into YIp1, a *his*3-containing yeast vector that transforms at low efficiency via integration into the host chromosome (Fig. 1). As expected, the frequency at which YRp7–Sc2605 and YRp7′–Sc2605 DNAs transformed *his*3$^-$ to His$^+$ was also high (2,000 His$^+$ colonies per μg DNA). Sc4101 has been joined to many

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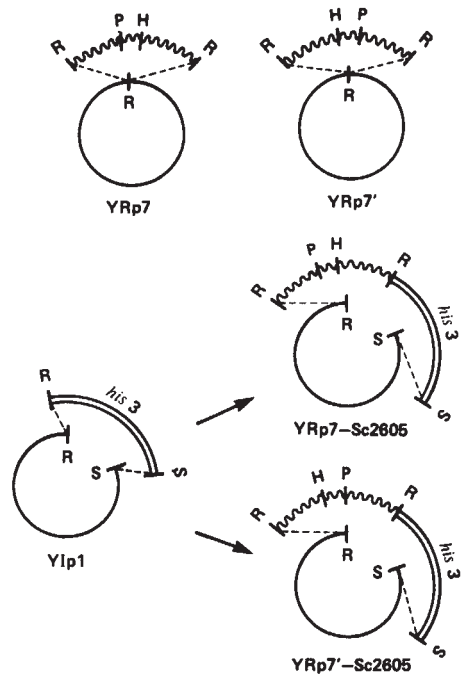

Fig. 1 Structure of several yeast replicator plasmids. Solid lines depict pBR322 plasmid sequences, wavy lines represent the *trp*1-containing 1.4-kilobase pair yeast fragment (Sc4101) and open bars a 6.0-kilobase pair yeast fragment (Sc2605) that contains the *his*3 structural gene. Not to scale. YRp7 and YRp7′ were constructed by the ligation of an *Eco*RI digest of λgt-Sc4104 and pBR322 as described previously[9]. YRp7–Sc2605 and YRp7′–Scα605 were similarly constructed by insertion of the Sc4101 fragment in either orientation into the *Eco*RI cleavage site of YIp1. *Eco*RI was the gift of Marjorie Thomas. Other restriction endonucleases and T4 DNA ligase were from New England Biolabs or Bethesda Research Laboratories and were used as directed. After ligation, the hybrid molecules were transformed[31] into trpC9830, an *E. coli* strain lacking *N*-(5′-phosphoribosyl)anthranilate isomerase. The presence of Sc4101 was then determined by testing for complementation of the bacterial lesion by the yeast *trp*1 gene (D.T.S. and R. W. Davis in preparation). The orientation and fidelity of the purified plasmid DNA were assessed by restriction endonuclease digestion and horizontal agarose gel electrophoresis[10]. Transformation of D13-1A (*a trp*1 *his*3-532 *gal*2 *SUC*2 *mal*$^-$) was carried out as described previously[9]. The transformation efficiencies for the YR plasmids cited in the text were determined on several separate occasions using other yeast vectors to control for experimental variation.

other yeast and bacterial sequences, producing hybrid molecules 4 to 50 kilobase pairs in total length. All these Sc4101-containing DNAs exhibited high frequency transformation and, concomitantly, replication in the absence of recombination with the host genome (S. Scherer, T. P. St. John, D.T.S. and K.S., unpublished observations). These data are consistent with the idea that Sc4101 contains, in addition to the structural sequence for the *trp*1 gene, a genetic element that allows autonomous replication, which we will call *ars*1 (for autonomously replicating sequence).

Yeast cells can be transformed simultaneously by two different DNA species at high frequency[13]. For example, when a mixture of YRp7–Sc2605 and YRp12 (YRp7′ with a 1 kilobase pair yeast DNA fragment carrying the *ura*3 gene inserted at the *Ava*I endonuclease cleavage site[12]) DNA was added to a *ura*3$^-$*his*3$^-$ strain, Ura$^+$His$^+$ co-transformants arose at the same frequency (2,000–5,000 colonies per μg) as Ura$^+$ or His$^+$ transformants. So, in these transformation conditions, DNA is saturating and each yeast cell is capable of receiving multiple DNA molecules. This observation can be used to design a *cis*/*trans* test for *ars*1 function. If *ars*1 functions in *trans*, co-transformation of a *his*3$^-$*trp*1$^-$ yeast strain by YIp1 and YRp7 DNAs should lead to a 1,000-fold increase in His$^+$ colonies compared with transformation by YIp1 alone. However, the mixture of YIp1 and YRp7 DNA gave rise to only 78 His$^+$ colonies per μg DNA. Concurrently, YRp7–Sc2605 DNA gave rise to 2,000 His$^+$ colonies per μg DNA. Thus Sc4101 allows high frequency transformation and autonomous replication of the *his*3 sequence only when located on the same molecule. As *ars*1 is linked to the yeast chromosomal *trp*1 locus and behaves as a *cis*-acting effector of DNA replication, we propose that *ars*1 is a yeast chromosomal replicator.

Autonomously replicating *ars*1 hybrid molecules are unstable

In a minimal medium supplemented with histidine and tryptophan, a *trp*1$^-$ *his*3$^-$ yeast strain will grow with a doubling time of 2.5 h. When transformed by YRp7–Sc2605 to His$^+$Trp$^+$, the strain doubles in 3.5–4 h in the absence of histidine and/or tryptophan. Similarly increased generation times are observed for all other *ars*1-containing transformants, regardless of the selective marker used. It is thus unlikely that the slow growth rate is due to a deficiency in expression of the transformed genes but is due rather to mitotic loss of the autonomously replicating hybrid DNA. In a logarithmically growing minimal medium culture of a *his*3$^-$ strain transformed by YRp7–Sc2605 DNA only 30–50% of the cells are phenotypically His$^+$. If this culture is diluted into a non-selective medium, the proportion of His$^+$ cells drops to 1–5% within 10 generations. The resulting *his*3$^-$ cells do not contain any hybrid DNA as determined by DNA–DNA hybridisation (data not shown).

There are several possible explanations for the mitotic instability of *ars*1-containing hybrid molecules. The isolated replicator may not contain all the information necessary for complete replication during each cell cycle. However, larger DNA fragments that contain additional DNA sequences adjacent to *ars*1 are still mitotically unstable (D.T.S. and R.W.D., in preparation). Alternatively, *ars*1-containing extrachromosomal elements may compete with the host chromosome for limited initiator(s). This is one proposed explanation for the instability of plasmids containing the *Escherichia coli* origin of replication[15,16]. Hybrids containing *ars*1 plasmids may replicate during each cell cycle but may fail to segregate properly during mitosis. In the last case, addition of other DNA sequences controlling chromosomal segregation would be expected to stabilise a replicator plasmid.

Intregation of *ars*1 hybrid molecules

The slow growth of strains containing *ars*1 hybrid molecules results in a strong selective pressure favouring any stably transformed cells. Such transformants can indeed be isolated. After many generations of growth in selective or non-selective conditions, cells were plated on non-selective medium. These colonies were tested for the stability of the transformed phenotype by replica plating to a selective medium. Stable transformants isolated in this way grew at the parental rate in minimal medium. The fate of the transforming DNA was investigated in four independently isolated stable strains (Fig. 2). The diagram at the bottom of Fig. 2 shows the digestion patterns expected for three possible conformations of the foreign sequences. The transforming DNA was identified by hybridisation and co-electrophoresed with the high molecular weight linear host DNA in all four cases (Fig. 2, lanes 1–4). The structure of the integrated DNA in one transformant was analysed by digestion with *Bam*HI endonuclease. The foreign pBR322 sequences in the stable transformant were detected as a single band (Fig. 2, lane 7) co-migrating with the *Bam*HI fragment from an unstable transformant (lane 6) and from the original plasmid DNA (lane 9). These data indicate that YRp7–Sc2605 is integrated at the *his*3 locus without major sequence alteration.

Given the appropriate genetic markers, stable transformants may be directly selected. Fragments containing non-complementing derivatives of the *his*3 gene (the missense mutations *his*3-38 and *his*3–532 (ref. 17), as well as an amber mutation[18] and two deletions[19]) were inserted into YRp7. These hybrid molecules were introduced into a *his*3-532*trp*1$^-$ strain by transformation to Trp$^+$. Although the transformants were initially His$^-$, His$^+$ recombinants could be selected at a frequency of 10^{-5}–10^{-6} per generation. The only exception to this result

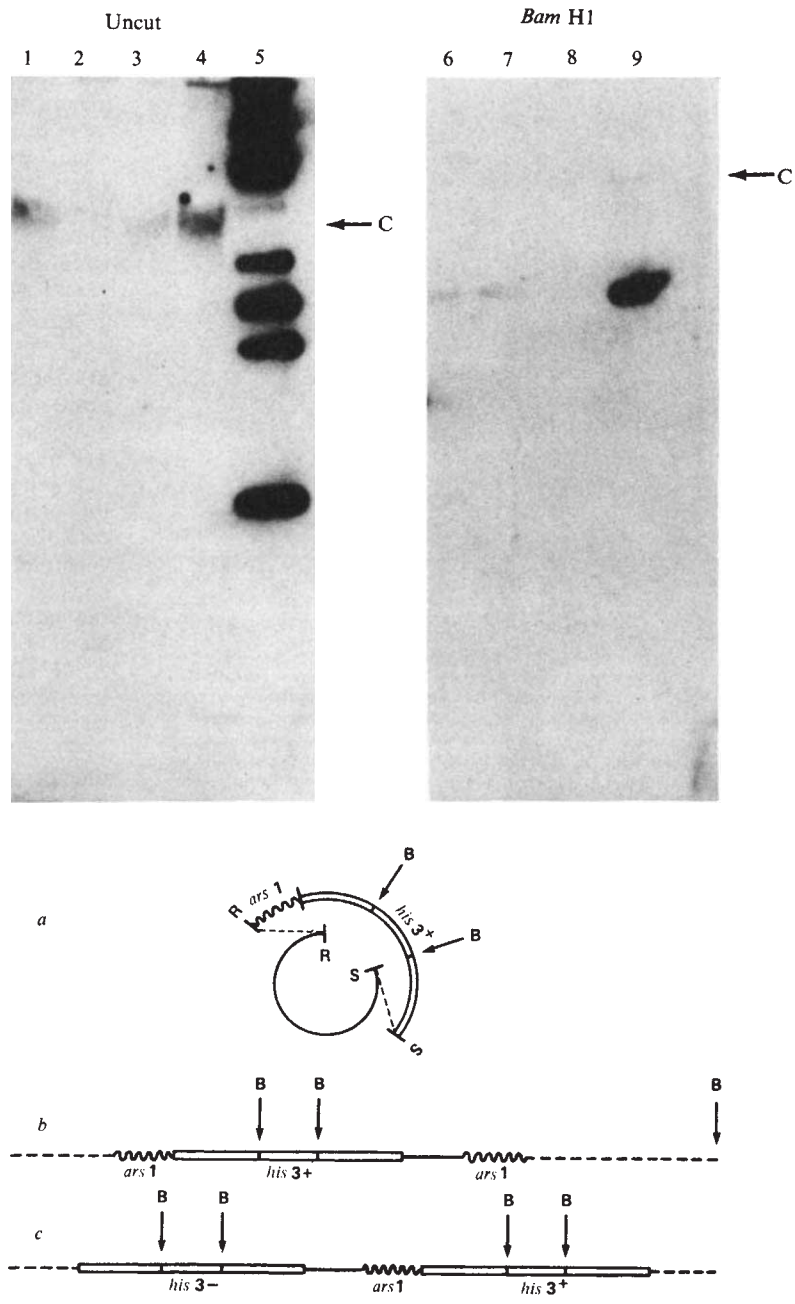

Fig. 2 Analysis of stable *ars*1 transformants. DNA was rapidly purified from transformants grown in minimal media (6.7 g l^{-1} Difco yeast nitrogen base, 2% glucose) as described previously[9]. In 'uncut', the DNA from four stable transformants described in the text was electrophoresed without endonuclease digestion in a 0.4% agarose gel (lanes 1–4). Lane 5 contains the original transforming DNA. The multiplicity of bands (several of which showed lower mobilities than the yeast chromosomal DNA) is apparently due to multimeric and nicked forms of the plasmid. In '*Bam*HI', DNA isolated from an unstable transformant (lane 6), a stable, integrated transformant (lane 7), the parental strain (lane 8), and the transforming plasmid DNA (lane 9) were digested with *Bam*HI restriction endonuclease and electrophoresed on a 0.4% agarose gel. The DNA in each gel was transferred to a sheet of nitrocellulose paper[25]. ^{32}P-labelled pBR322 DNA was prepared by nick-translation[26], heat denatured, and hybridised to the immobilised DNA spectra. These operations were carried out using modifications of the traditional procedures as described in ref. 27. Pictures of the two autoradiographs are presented here. Also depicted are three possible structures of transformed *ars*1 sequences: *a*, unintegrated and autonomously replicating; *b*, integrated by homologous recombination between the *ars*1 sequence and its chromosomal counterpart; and *c*, integrated by homologous recombination between *his*3 sequences. Letter and line designations are as in Fig. 1 with one addendum: B represents the predicted *Bam*HI endonuclease cleavage sites.

occurred when the transforming DNA contained the same *his*3 allele as the recipient yeast strain (*his*3–532), in which case no His^+ recombinants could be isolated. All the recombinants obtained were stable for both His^+ and Trp^+ and grew at the wild-type rate in minimal medium. Hybridisation analysis (as described above) indicated that the transforming DNA had integrated at the *his*3 locus without detectable sequence alteration (data not shown). The frequency of recombination between the *his*3–38 allele (on the transforming plasmid) and the *his*3-532 allele (in the recipient) was roughly the same as the frequency observed when the two alleles were present in a heterozygous diploid yeast strain. In all respects, the integration of *ars*1-containing hybrid molecules seems to be a simple consequence of mitotic recombination.

The organisation of DNA replication in *Saccharomyces cerevisiae* is similar to that of other eukaryotes. Fibre autoradiography and electron microscopy studies indicate that each chromosome is divided into several replication units and the initiation of DNA synthesis for each unit is rigidly controlled[20–23]. The ability of *ars*1 sequences to integrate has implications for this control. We have shown the DNA sequences containing a replicator can stably integrate at other loci. As *ars*1 is located near the centromere of chromosome IV and *his*3 maps on the left arm of chromosome XV, the two loci are probably in different replication units. The observation that *ars*1 can integrate at *his*3 indicates that either initiation of DNA replication at *ars*1 is suppressed in its new position or translocation of a second replicator simply creates a new functional replication unit.

Multimeric forms of *ars*1

Plasmids containing *ars*1 have also been integrated in the *trp*1–*ars*1 region near the centromere of chromosome IV (data not shown). As such a recombination event creates a tandem duplication, it suggests that multimers of a chromosomal replicator function normally. To investigate this further, we examined the *ars* phenotype of monomer, dimer and trimer YRp7.

When YRp7 is propagated in a recombination-proficient bacterial host, monomer, dimer and trimer forms of the plasmid arise. Each of the three forms of the plasmid DNA was purified

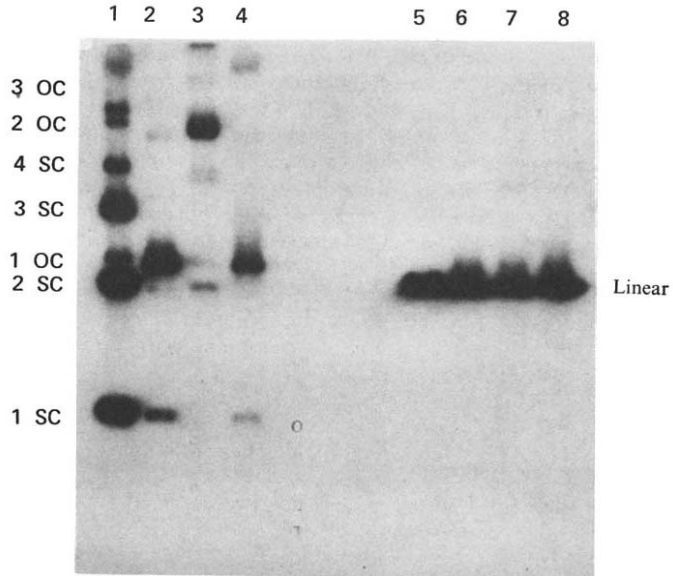

Fig. 3 Autonomous replication of monomer, dimer and trimer YRp7. Plasmid DNA purified from trpC9830 (Rec$^+$) carrying YRp7 was used to transform KM601, a *recA*$^-$*recB*$^-$ *E. coli* strain (gift of Kevin McEntee). Plasmid DNA was rapidly isolated from individual tetracycline-resistant transformants and its size was assessed by agarose gel electrophoresis. Transformants were then picked that contained predominantly monomer, dimer and trimer YRp7. The plasmid DNAs were purified and analysed by restriction endonuclease digestion and agarose gel electrophoresis. Each sample contained some larger multimers, but neither the dimer nor the trimer DNA preparations contained detectable amounts of monomer plasmid and all were free of any gross alterations of the *ars*1 sequences (data not shown). Monomer, dimer or trimer YRp7 DNA was used to transform D13-1A to Trp$^+$. DNA from the transformants was purified, electrophoresed in a 0.4% agarose gel, transferred to nitrocellulose and hybridised to ^{32}P-labelled pBR322 DNA as described in Fig. 2 legend. DNA in lanes 1–4 was uncut while the DNA samples in wells 5–8 were digested with *Bam*HI restriction enconuclease. Lanes 1 and 5 contained 1 μg of wild-type yeast DNA and 2 ng each of purified monomer, dimer and trimer YRp7 DNA. The various multimeric forms are designated by the numbers 1, 2, 3 and 4 (for monomer, dimer, trimer and tetramer) while SC and OC distinguish supercoiled from nicked, open circular DNA. Lanes 2 and 6 each contained 1 μg of DNA purified from D13-1A transformed to Trp$^+$ by the YRp7 monomer form. DNA samples purified from D13-1A transformed by dimer and trimer YRp7 were electrophoresed in lanes 3, 7 and 4, 8 respectively.

by clonal isolation in a *recA*$^-$*recB*$^-$ *E. coli* strain. Transformation of a recombination-proficient *trp*1$^-$ yeast strain to Trp$^+$ occurred at equal frequencies for monomer, dimer or trimer DNA (2,000–5,000 colonies per μg DNA). Strains transformed by each of the three DNAs had identical growth rates and were equally unstable mitotically. The structure of the transforming DNA in such strains was analysed (Fig. 3). Strains transformed with monomer and dimer YRp7 contained, respectively, monomer and dimer autonomously replicating supercoiled circles (lanes 2 and 3). Small amounts of other plasmid forms were detected (dimer in the monomer transformant as well as both monomer and tetramer in the strain carrying the dimer form). A strain transformed with trimer YRp7 DNA contained predominantly monomer plasmid (lane 4). It is unlikely that this isolate arose through transformation by contaminating monomer plasmid in the trimer DNA preparation (see Fig. 4 legend), so appearance of the monomer plasmid probably represents recombinational reduction of the multimeric form. The various circular forms in all three strains contained intact YRp7 sequences as judged by *Bam*HI endonuclease digestion which reduces all multimeric species to a single linear fragment (Fig. 2, lanes 5–8). Thus monomer and dimer YRp7 seem to be functionally equivalent. Some interconversion of multimeric forms occurs, probably by mitotic recombination in the yeast host cells.

A unique feature of *ars*1 is that it is tightly linked to the centromere of chromosome IV. In fact, we considered the possibility that the *ars* phenotype could be caused by a centromeric function. As described above, Sc4101 is capable of both stably integrating at other chromosomal loci and functioning in a dimeric state. If Sc4101 contained a centromere, both of these constructions would represent dicentric molecules which might be expected to undergo sequence alterations when propagated mitotically[30]. As we detected no such alterations, we conclude that Sc4101 is unlikely to have full centromeric function. However, these experiments suggest tests for segregational activity which may lead to the identification of other yeast fragments that contain centromeric sequences.

Delimitation of *ars*1

Sc4101 contains two functions: *trp*1 and *ars*1. To determine the position of the *ars*1 sequences we have constructed several deletions of the 1.4-kilobase pair *Eco*RI-generated fragment. The experimental approach is shown in Fig. 4. Unique restriction endonuclease sites in Sc4101 were fused to unique sites in the pBR322 sequence, deleting parts of both Sc4101 and pBR322. Utilising both YRp7 and YRp7′, it was possible to construct two pairs of complementary deletions; Yp411, Yp413 and Yp412, Yp414. Fragments containing the *ura*3 or *his*3 sequences (known to function when attached to an intact *ars*1 element) were inserted into each of the four deletions. Only Yp413/HIS3$^+$ and Yp412/URA3$^+$ DNAs were capable of transforming *his*3$^-$ and *ura*3$^-$ yeast strains, respectively. Both transformed at high efficiency (again ~2,000 colonies per μg DNA). Yp412/URA3$^+$ had a completely normal *ars*1 phenotype. Strains transformed with Yp413/HIS3$^+$, however, had a greatly reduced growth rate, with a generation time of about 11 h, and an enhanced mitotic instability. Assuming that juxtaposition of the *ura*3 or *his*3 sequences does not effect *ars*1 function, we conclude that the *ars*1 sequence is fully contained within the 850 base pairs between the *Eco*RI and *Hin*dIII cleavage sites and deletion of the 200 base pairs between the *Hin*dIII and *Pst*I sites is an *ars*1 mutation.

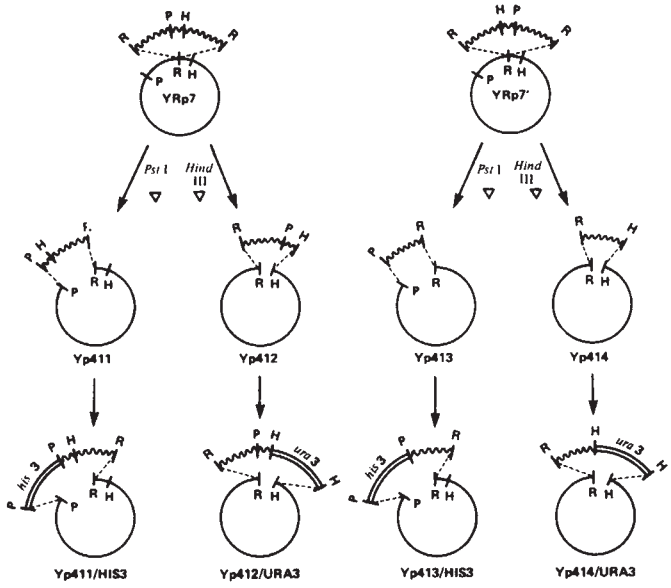

Fig. 4 Deletion analysis of Sc4101. Both *Pst*I and *Hin*dIII restriction endonucleases have unique cleavage sites in Sc4101 and pBR322. Digestion followed by circularisation and ligation (as described in Fig. 1) results in deletion of pBR322 and yeast sequences. The plasmids in the second line, derived in this manner from YRp7 and YRp7′, were identified by selecting the appropriate drug resistance after transformation of *E. coli*. Yeast structural genes were then inserted at the now unique *Pst*I or *Hin*dIII cleavage sites. The *his*3-containing yeast DNA fragment (Sc2710) was prepared by *Pst*I digestion of pGT2-Sc2605 (ref. 9) while the *ura*3 fragment (Sc2904) originated from *Hin*dIII cleavage of λ590–Sc2710 (ref. 12). The resulting plasmids shown in the second line were identified by selection for complementation of *his*B and *pyr*F lesions and for the appropriate drug resistances following transformation of the *E. coli* strains *his*B463 (ref. 28) and MB1000 (ref. 12). The structures of all of the molecules shown were confirmed by restriction endonuclease digestion and agarose gel electrophosesis. Line drawings and letterings are as described in Fig. 1 legend and text.

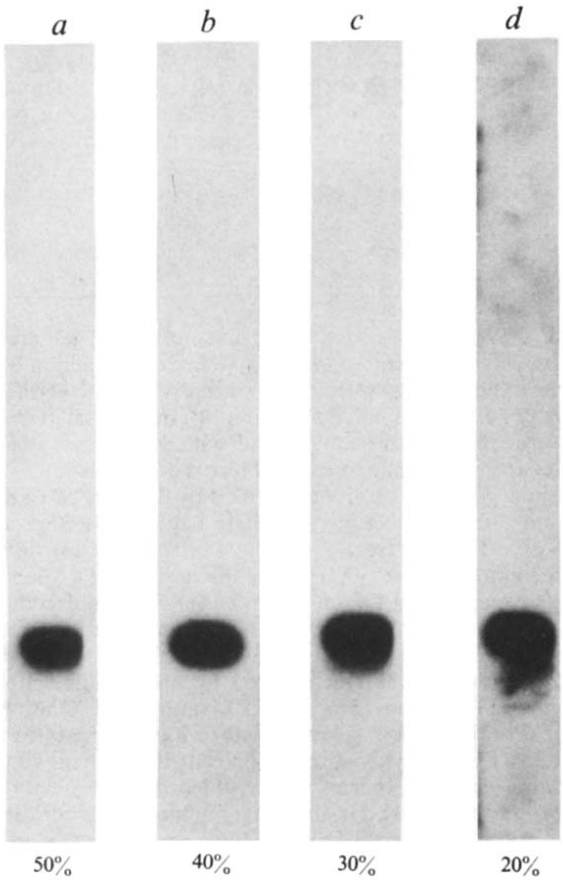

Fig. 5 Yeast sequences homologous to *ars*1. Yeast DNA was digested with *Eco*RI endonuclease and electrophoresed in a 0.6% agarose slab gel. The resulting *Eco*RI restriction spectra were transferred to nitrocellulose and then cut into four strips. After *Eco*RI digestion of YRp7 and gel electrophoresis, Sc4101 was purified by binding the DNA to a glass fibre filter (M. W. McDonnell and R.W.D., in preparation). The isolated fragment was then nick-translated. 10^6 c.p.m. of ^{32}P-labelled Sc4101 DNA was hybrised to each nitrocellulose strip in 5XSSPE (0.75 M NaCl, 50 mM $NaPO_4$, 5 mM EDTA), 0.2% SDS with 50% (v/v), 40%, 30% and 20% formamide (strips *a*, *b*, *c* and *d*, respectively). Each strip was washed in its own hybridisation conditions and autoradiographed. Long exposures were used to try to detect faint bands.

Are sequences homologous to *ars*1 found elsewhere in the yeast genome?

Using measured values for the DNA elongation rate and the total size of the yeast genome, it was estimated that a minimum of 70 initiations must occur if the cell is to replicate its DNA fully during the S phase of the cell cycle[22]. These other chromosomal replicators may have DNA sequences in common with *ars*1.

In an attempt to detect *ars*1 sequences present elsewhere in the yeast genome, ^{32}P-labelled Sc4101 DNA was hybridised to a restriction endonuclease spectrum of yeast DNA. The hybridisation conditions were varied to allow fragments with a significant proportion of mismatched base pairs to anneal. As shown in Fig. 5, even in the least stringent conditions (Fig. 5*d*), only the single homologous 1.4-kilobase pair fragment hybridised to Sc4101. The conditions used would have required at least 75% homology to *ars*1 (over a 500-base pair region) for hybridisation to occur. Alternatively, other yeast replicators probably have less than 20 sequential base pairs in common with *ars*1.

The usefulness of hybrid molecules containing *ars*1

We have previously discussed some of the uses of plasmids containing the Sc4101 fragment[9]. Results reported here point out additional applications. Vectors that contain *ars*1 (which we shall call 'YR vectors') can be maintained in yeast cells in two easily distinguishable states. Although autonomously replicating in selective conditions, YR transformants are mitotically unstable. In this state, the supercoiled YR DNA can be easily re-isolated (D.T.S., K.S. and M. Thomas, unpublished observations). In addition, stable transformants with integrated YR sequences can then be easily obtained at high frequency. In this second state, the location of the integrated DNA can be determined physically (ref. 9 and above) or by simple genetic analysis (see ref. 11). Such linkage analysis could determine the chromosomal location of a yeast DNA fragment that had been cloned in a YR vector. Integrated YR vectors can also be used for directed genetic manipulation, wherein a sequence is replaced by an altered but homologous counterpart through an integration–excision mechanism[9,12].

In addition to its uses as a vehicle for clonally isolating DNA in yeast, YRp7 will facilitate studies of yeast DNA replication in several ways. First, continuation of the preliminary genetic analysis described here will determine the sequences essential for the functioning of a eukaryotic chromosomal replicator. Second, other functions that control *ars*1 replication may be identifiable by genetic or biochemical techniques; for example, several mutants that are temperature-sensitive for the initiation of DNA synthesis have been characterised[24]; their effects on *ars*1 replication could be studied in detail. Third, the supercoiled YRp7 plasmid could be used (either with or without its chromatin protein complement) as a template for *in vitro* DNA replication systems.

The high frequency of transformation associated with *ars*1 suggests an easy method for the isolation of other chromosomal replicators. Random fragments of DNA can be inserted into a yeast vector that transforms only at low efficiency. Transformation of a yeast strain by the hybrid molecules will result in an enrichment for those fragments which allow autonomous replication. Studies of such *ars* loci isolated from yeast and other organisms are in progress.

The characterisation of a chromosomal replicator represents the first step towards the goal of constructing an operational eukaryotic chromosome. The isolation of other elements that control replication and proper segregation will not only elucidate the mechanisms of chromosome function but will provide the means for determining how chromosome structure affects gene expression.

Received 30 July; accepted 21 August 1979.

1. Maaløe, O. & Kjeldgaard, N. O. *Control of Macromolecular Synthesis* (W. A. Benjamin, New York, 1966).
2. Hemstetter, C., Cooper, S., Diorucci, O. & Revelas, F. *Cold Spring Harb. Symp. quant. Biol.* **33**, 809–822 (1968).
3. Carins, J. *J. molec. Biol.* **6**, 208–213 (1963).
4. Jacob, E. Brenner, B. & Cuzin, F. *Cold Spring Harb. Symp. quant. Biol.* **28**, 329–347 (1963).
5. *Cold Spring Harb. Symp. quant. Biol.* **43** (1978).
6. Hand, R. *Cell* **15**, 317–325 (1978).
7. Sheinin, R. & Humbert, J. *A. Rev. Biochem.* **47**, 277–316 (1978).
8. Pardee, A. B., Dubrow, R., Harmlin, J. L. & Kletzien, R. F. *A. Rev. Biochem.* **47**, 715–750 (1978).
9. Struhl, K., Stinchcomb, D. T., Scherer, S. & Davis, R. W. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1035–1039 (1979).
10. McDonnell, M. W., Simon, M. N., & Studier, F. W. *J. molec. Biol.* **110**, 119–146 (1977).
11. Hinnen, A., Hicks, J. B. & Fink, G. R. *Proc. natn. Acad. Sci. U.S.A.* **75**, 1929–1933 (1978).
12. Scherer, S. & Davis, R. W. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
13. Hicks, J. B., Hinnen, A. & Fink, G. R. *Cold Spring Harb. Symp. quant. Biol.* **43**, 1305–1313 (1978).
14. Wensink, P. C., Finnegan, D. J., Donelson, J. E. & Hogness, D. S. *Cell* **3**, 315–325 (1974).
15. Hiraga, S. *Proc. natn. Acad. Sci. U.S.A.* **73**, 198–202 (1976).
16. Yasuda, S. & Hirota, Y. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5458–5462 (1977).
17. Struhl, K. & Davis, R. W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5255–5259 (1977).
18. Struhl, K., Fink, G. R. & Davis R. W. *Nature* **279**, 78–79 (1979).
19. Struhl, K. & Davis, R. W. *J. molec. Biol.* (in the press).
20. Newlon, C. S., Petes, T. D., Hereford, L. M. & Fangman, W. K. *Nature* **247**, 32–35 (1974).
21. Petes, T. D., Newlon, C. S., Byers, B. & Fangman, W. L. *Cold Spring Harb. Symp. quant. Biol.* **38**, 9–16 (1973).
22. Petes, T. D. & Williamson, D. H. *Expl Cell Res.* **95**, 103–110 (1975).
23. Burke, W. & Fangman, W. L. *Cell* **5**, 263–269 (1975).
24. Hartwell, L. H. *Bact. Rev.* **38**, 164–198 (1974).
25. Southern, E. *J. molec. Biol.* **98**, 503–577 (1975).
26. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237–251 (1977).
27. Cameron, J. R., Loh, E. Y. & Davis, R. W. *Cell* **16**, 739–751 (1979).
28. Struhl, K., Cameron, J. R. & Davis, R. W. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1471–1475 (1976).
29. Bolivar, R., *et al. Gene* **2**, 95–113 (1977).
30. McClintock, B. *Proc. natn. Acad. Sci. U.S.A.* **18**, 677–681 (1932), **25**, 405–416 (1939).
31. Lis, J. T., Prestidge, L. & Hogness, D. S. *Cell* **14**, 901–917 (1978).