

**AUTONOMOUS REPLICATION SEQUENCES IN THE MAXICIRCLE
KINETOPLAST DNA OF *LEISHMANIA TARENTOLAE***

DALLAS HUGHES, LARRY SIMPSON*, PAUL S. KAYNE and NICOLAS NECKELMANN

Department of Biology and Molecular Biology Institute, University of California, 405 Hilgard Ave., Los Angeles, CA 90024, U.S.A.

(Received 8 May 1984; accepted 14 June 1984)

Four fragments from the maxicircle DNA of *Leishmania tarentolae* cloned into the selectable *Saccharomyces cerevisiae* shuttle vector, YIp5, exhibited autonomous replicating sequence (ars) activity. Two of the fragments (pSK120, pSK152) produced large yeast transformant colonies and two (pSK30, pSK150) produced small colonies. All yeast transformants contained extrachromosomal self replicating YIp5 hybrid plasmids as shown by mitotic instability in non selective medium and by the transformation of *Escherichia coli* with yeast minilysates and recovery of the plasmid from the transformed bacteria. The copy numbers of pSK30, pSK150 and pSK152 in the transformed yeast were approximately the same as that of the YRp12 control, which contains the yeast arsI element; the copy number of pSK120, however, was at least 10 fold lower. A 1.87 kb subfragment of the pSK120 fragment also showed strong ars activity. The entire DNA sequences of the pSK120, pSK152 and pSK150 fragments are known, and several yeast 11 mer consensus ars sequences are present within each fragment. In addition there is a sequence (Lt ars189) within the pSK152 subclone that has 78% similarity with a 189 nt sequence of an ars element from the *Crithidia fasciculata* maxicircle (Cf ars189), implying an evolutionary conservation of this putative origin of replication in at least two different kinetoplastid species. The relative positions of the Lt ars189 sequence in the *L. tarentolae* maxicircle map and the Cf ars189 sequence in the *C. fasciculata* map with respect to the 9 and 12S ribosomal genes are similar, implying an overall conservation of gene order in this portion of the transcribed regions of these two species and perhaps in all kinetoplastid species.

Key words: Origin of replication; Maxicircle DNA; Kinetoplast DNA; Autonomous replicating sequence

INTRODUCTION

The mitochondrial DNA of the kinetoplastid protozoa consists of two different molecular species, the minicircles and the maxicircles. These molecules are interlocked by catenation to form the single kinetoplast DNA (kDNA) network. The kDNA is located adjacent to the basal body of the flagellum within the matrix of the

* To whom correspondence should be addressed.

Abbreviations: ars, autonomous replicating sequence; kDNA, kinetoplast DNA; kb, kilobase pairs; nt, nucleotide pair.

single mitochondrion of the cell [1,2]. The maxicircle represents the informational DNA species; it contains the mitochondrial ribosomal RNA genes [3] and at least six structural genes, which have been identified by sequence analysis ([4] and de la Cruz, Neckelmann, Simpson and Simpson, unpublished data). The function of the minicircle DNA is unknown.

Replication of the maxicircle DNA seems to occur by a rolling circle-type model involving non catenated linearized intermediates [5]. One approach to the identification of the origin(s) of replication of the maxicircle is to determine the ability of DNA fragments to confer the property of autonomous replication to a selectable yeast plasmid, such as YIp5, that normally can only replicate by integration into chromosomal DNA [6]. DNA fragments from a variety of eukaryotic cells can act as autonomous replicating sequences (ars elements) in yeast [7]. There has been some correlation of ars activity in yeast with authentic origins of replication in the cases of the yeast 2 μ m plasmid [8], *Tetrahymena thermophila* ribosomal DNA [9], the yeast chromosomal ars1 and ars2 replicators [10], the *Xenopus laevis* mitochondrion heavy strand origin of replication [11] and yeast petite mitochondrial DNA replication origins [12], but the question remains open as to the generality of this correlation. The DNA sequences of the ars elements vary but Broach et al. [13] have identified within 10 yeast ars sequences an 11 mer consensus sequence. Fragments exhibiting ars activity have been isolated from the kinetoplast maxicircle DNA of *Trypanosoma brucei* [14] and *Crithidia fasciculata* [15]. A 2.1 kb *Crithidia* maxicircle fragment with ars activity has been subcloned to a 189 nt fragment (Cf ars189) that still exhibits strong ars activity [16]. In this paper we describe the isolation of four fragments from the maxicircle DNA of *Leishmania tarentolae* that exhibit ars activity in yeast, one of which shows a striking sequence similarity with the 189 nt ars sequence from *C. fasciculata*.

MATERIALS AND METHODS

Cells and strains. The derivation and culture of the clonal *L. tarentolae* strain was described previously [17]. *Escherichia coli* RR1 (F⁻, hsdS20, rec⁺ ara-14, proA2, lacY1, galK2, rpsL20, (Sm^r), xyl-5, mt1-1, supE44, λ^-) was used as the recipient for amplification of the yeast plasmids. *Saccharomyces cerevisiae* NNY-1 (met, trp1, ura3-52, his3-1, gal2, gal10, cir⁰) was used for the yeast transformations.

Plasmids and transformation procedures. YIp5 is a derivative of pBR322 which contains the ura3 gene as a selectable marker but lacks an autonomous replicating sequence [6]. YRp12 is a derivative of YIp5 which contains the yeast ars1 element [7]. pSK120, pSK150, pSK152 and pSK30 were constructed by ligation of gel isolated fragments with digested YIp5 DNA as shown in Fig. 1. The fragments were obtained by digestion of the plasmids, pLt120, pLt152, pLt150 and pLt30, which were obtained

as described elsewhere ([18] and de la Cruz et al, unpublished data). pDF10 contains the 1.87 kb LtS1 subfragment of the 120 insert and pDY5 contains a 0.9 kb subfragment of the 152 insert in YIp5. Plasmid DNA was isolated by CsCl-ethidium bromide gradient centrifugation of cleared lysates of chloramphenicol amplified *E. coli* cultures. Transformation of *E. coli* was by the CaCl₂ method [19] and the rapid boiling minilysate method [20] was used to obtain small amounts of plasmid DNA. Transformation of yeast was by the lithium acetate method [21]. Yeast minilysates were prepared by the method of Nasmyth and Reed [22]. Yeast plasmid copy number determination was by a modification of the method of Hyman [12]. Yeast culture media were as described by Hsiao and Carbon [23].

Blotting and sequence analysis. Digested DNAs and minilysate preparations were electrophoresed in 1% agarose submarine gels as described previously [18] and blotted either unidirectionally or bidirectionally [24] onto BA85 Schleicher and Schuell nitrocellulose filters. Hybridization and autoradiography were performed as described previously [18]. Densitometry was carried out using a Joyce-Loebell densitometer. Sequence analysis of the cloned maxicircle fragments was performed by the dideoxy chain termination method using fragments subcloned into M13 vectors (de la Cruz et al, unpublished data). For ease of discussion, we refer to the 6.56 kb pSK120 sequence as the '120' region of the maxicircle and the 6.96 kb F3-F6-F1 sequence as the 'F-3' region. Comparison and alignment of the *Cf ars189* sequence with the *L. tarentolae* maxicircle sequences was performed by the SEQFIT computer program of Staden [25] run on a VAX 11/780 computer.

RESULTS

***ars* activity of *L. tarentolae* maxicircle fragments.** The maxicircle DNA of *L. tarentolae* consists of an 18 kb transcribed region and a 12 kb nontranscribed region which we have termed the 'divergent region' due to its rapid rate of sequence change in nature. Several regions of the maxicircle have proved difficult to clone in *E. coli*; this includes 8.8 kb of the divergent region and two areas (4.4 kb) within the transcribed region, as shown in the restriction map in Fig. 1. Several of the maxicircle fragments previously cloned in pBR322, Lt120, Lt152, Lt150 and Lt30, were subcloned into YIp5 yielding the hybrid plasmids shown in the diagram in Fig. 2 and in the gels in Fig. 3. YIp5 is a pBR322 derivative that contains a yeast selectable marker (*ura3*) and lacks a eukaryotic origin of replication or *ars*; this plasmid can only replicate in yeast by integration into chromosomal DNA and therefore yields a low frequency of transformation of yeast to the *ura*⁺ phenotype (1–10 transformants per µg DNA). YRp12, which contains the yeast *arsI* element, was used as a positive control for high frequency transformation of yeast. As shown in Table I, all of the maxicircle hybrid plasmids transformed yeast from *ura*[−] to *ura*⁺ at a high frequency. The colony size of the transformed yeast

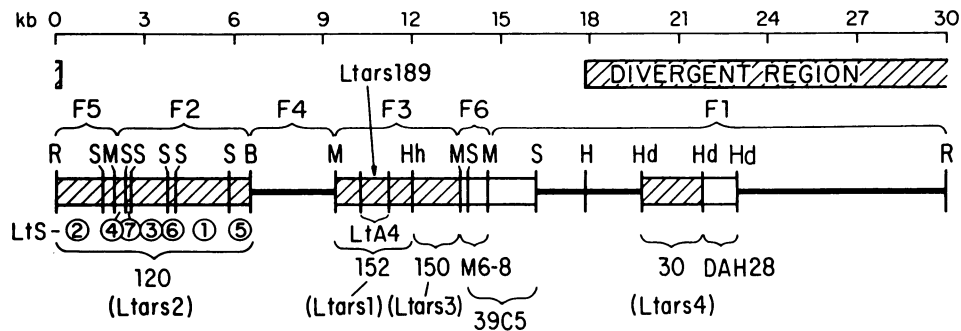


Fig. 1. Restriction map of the EcoRI-linearized maxicircle of *L. tarentolae* and the cloned subfragments. All cloned fragments are labeled and those tested for ars activity in YIp5 are indicated by crosshatching. The localization of the non-transcribed divergent region is also indicated. The Sau3A subfragments, LtS1-LtS7, of the 120 fragment and the LtA4 subfragment of the 152 fragment are shown. The position of the Ltars189 is indicated by an arrow. The MspI/BamHI subfragments of the EcoRI linearized maxicircle are labeled F1-F6. Restriction site symbols: R, EcoRI; S, Sau3A; M, MspI; B, BamHI; Hh, HhaI; H, HaeIII; Hd, HindIII.

varied; pSK120 and pSK152 produced large colonies comparable to the YRp12 control, whereas pSK150 and pSK30 produced small colonies. The log phase division times of the transformed yeast correlated well with colony size in that the pSK30 and pSK150 transformants divided more slowly than the pSK152 and pSK120 transformants (Table I).

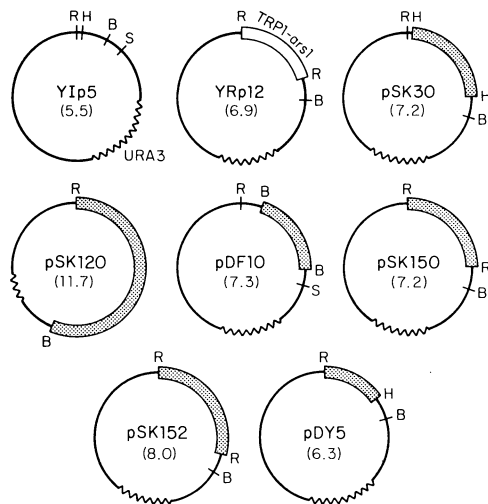


Fig. 2. Plasmids used to screen maxicircle fragments for ars activity. All plasmids are derivatives of YIp5, which contains the yeast *ura3* gene. The control plasmid, YRp12 [6], contains in addition the 1400 bp yeast *TRP1-ars1* sequence. The maxicircle fragments are indicated by stippled boxes.

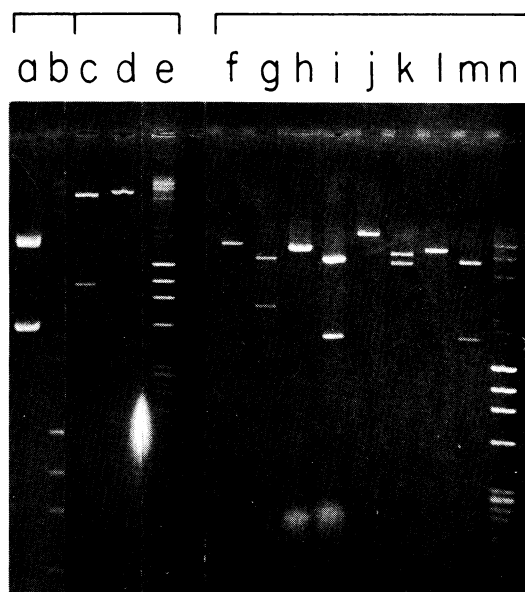


Fig. 3. Agarose gel electrophoresis of digests of hybrid plasmids. The lanes are bracketed to indicate separate gels. 1% agarose, TBE buffer. (a) pDF10 digested with EcoRI and SalI; (b), (e), and (n) λ DNA digested with HindIII mixed with ϕ X174RF DNA digested with HaeIII; (c) pDY5 digested with EcoRI and HindIII; (d) pDY5 digested with EcoRI; (f) pSK152 digested with BamHI; (g) pSK152 digested with EcoRI; (h) pSK150 digested with BamHI; (i) pSK150 digested with EcoRI; (j) pSK120 digested with EcoRI; (k) pSK120 digested with EcoRI and BamHI; (l) pSK30 digested with EcoRI; (m) pSK30 digested with HindIII.

TABLE I

Transformation of *ura⁻* yeast to *ura⁺* by hybrid plasmids

Plasmid	Number of transformants per μ g DNA	Colony size (mm)	Log phase division time (min)
YRp12	19.5–451 ^a	0.5–4.0	130
pSK30	268, 329, 328 ^b	0.5–1.0	480
pSK120	362	0.5–4.0	210
pDF10	213	0.5–4.0	210
pSK150	267, 310, 287 ^b	0.5–1.0	340
pSK152	642, 814 ^b	0.5–4.0	230
pDY5	861, 5458 ^b	0.5–4.0	190

^a Range of 5 experiments.

^b Each value is from a separate experiment.

In order to localize more precisely the *ars* activity within the 120 and 152 fragments, several YIp5 subclones of these fragments were tested for high frequency transformation of yeast. Three *Sau*3A subfragments of the 120 fragment (LtS1, LtS3, LtS5) were subcloned into YIp5. The only fragment with *ars* activity was the 1.87 kb LtS1 fragment, which is contained in the hybrid plasmid, pDF10 (Figs. 1, 2 and 3). In the case of the 152 fragment, a 0.9 kb subfragment (LtA4) was found to retain strong *ars* activity in the hybrid plasmid, pDY5 (Figs. 1,2 and 3); this was the only portion of the 152 fragment that was tested for *ars* activity. Both pDF10 and pDY5 transformed *ura*⁻ yeast to *ura*⁺ at a high frequency, producing large colonies (Table I). pDF10 plasmid could be recovered unchanged from bacteria transformed with yeast minilysates. In the case of pDY5, however, plasmid recovered from bacteria transformed with yeast minilysates had an insert that showed no homology with the LtA4 probe (results not shown). This implies that a yeast *ars* sequence had been substituted for the LtA4 sequence in the yeast host, but this was not studied further.

Evidence for extrachromosomal maintainance of hybrid DNA. Several lines of evidence indicated that the yeast hybrid plasmids were maintained extrachromosomally:

TABLE II

Mitotic stability and copy number of plasmids in yeast

Plasmid	Percentage of cells with plasmid ^a	Mitotic stability ^b	Copy number – per <i>ura</i> ⁺ cell				
			Densitometry ^c			Transformation of <i>E. coli</i> ^d	
			Exp. 1	2	3	Exp. 1	2
YRp12	8.5–16.3 ^e	0.013	1.0	1.0	1.0	1.0	–
pSK30	0.5–2.1 ^e	0.001	1.2	3.1	0.4	2.2	0.6
pSK120	8.4, 28.2, 23.4 ^f	0.03	0.04	–	0.001	–	0.0001
pDF10	4.1, 21.2 ^f	0.06	–	–	0.8	–	1.6
pSK150	0.2–2.9 ^e	0.02	1.6	2.2	0.4	5.7	0.7
pSK152	2.5–20.7 ^e	0.08	1.1	–	1.0	–	1.0
pDY5	18.0, 19.8 ^f	0.05	–	0.1	2.4	0.9	2.8

^a Relative plating efficiency (%) of cells spread on selective and non-selective media.

^b Relative plating efficiency (%) after 20 divisions in non-selective medium.

^c Plasmid DNA from identical numbers of cells was run in agarose, blotted, and probed with labeled pBR322 DNA. The autoradiographs were traced densitometrically and the peak areas compared relative to the YRp12 value taken as 1.0.

^d The plasmid DNA in c was also used to transform *E. coli* RRI to *amp*^R. The relative transformation efficiencies are given with the YRp12 values taken as 1.0.

^e Range of values from 5 experiments.

^f Each value is from a separate experiment.

(1) Mitotic instability of the *ura*⁺ phenotype in nonselective medium. All hybrid plasmids were lost from the cells after growth under nonselective conditions, and even under selective growth conditions only a portion of the cells retained plasmid, as shown in Table II.

(2) Recovery of plasmids from *E. coli* cells which were transformed with yeast minilysates. Gel profiles of digested plasmid DNAs isolated from *E. coli* transformants are shown in Fig. 4. pSK152, pDF10, pSK150 and pSK30 plasmids were isolated from transformed yeast clones and reintroduced into *E. coli* by transformation. The plasmids were reisolated from bacterial minilysates, digested to release the inserts and run in agarose. The pSK152 gel was dihybridized and probed with labeled pBR322 DNA and with labeled LtA4, a subfragment of Lt152 (lanes a and b). In all cases, fragments of the correct size were released from the bacterial plasmids, implying that no rearrangements had occurred in the yeast.

(3) Presence and copy number of hybrid plasmid DNAs in the yeast host by blot

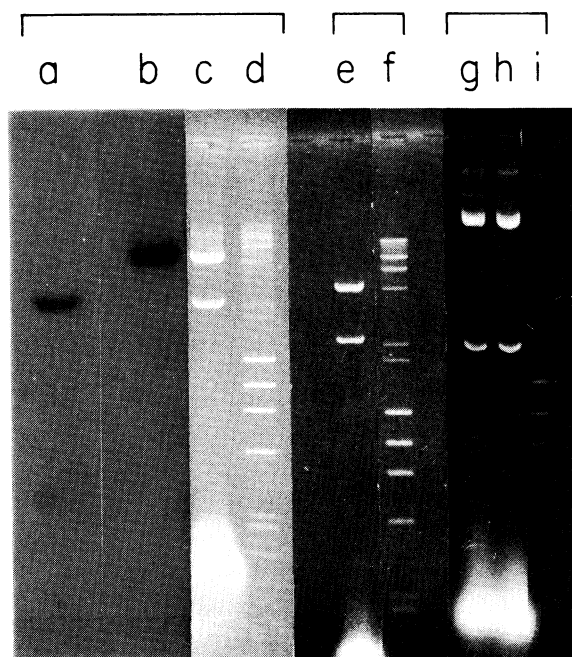


Fig. 4. Agarose gel electrophoresis of hybrid plasmids recovered from yeast transformants. The plasmids were isolated from yeast clones, reintroduced into bacteria by transformation, isolated from minipreps and digested with appropriate enzymes to release the maxicircle inserts. 1% agarose, TBE buffer. Lanes are bracketed to indicate separate gels. (c) pSK152 DNA digested with EcoRI. This lane was dihybridized and probed with nick-translated pBR322 (b) or LtA4 (a); (d), (f) and (i) molecular weight standards (see Fig. 3); (e) pDF10 digested with EcoRI and SaII; (g) pSK150 digested with EcoRI; (h) pSK30 digested with HindIII.

analysis. Plasmid DNAs were isolated from an equivalent number of cells of the pSK120, pSK30, pSK150, pSK152, pDY5 and pDF10 yeast transformants, electrophoresed in agarose and blotted onto nitrocellulose filters which were probed with labeled pBR322 DNA. Plasmid DNA from a YRp12 yeast transformant was used as a copy number control. As shown in Fig. 5, all preparations showed evidence of closed and open circular plasmid DNAs which hybridized with pBR322. The relative copy numbers of the hybrid plasmids in the yeast hosts were measured by quantitative densitometry of the autoradiographs and comparison to the value for YRp12, which was assumed to correspond to approximately 100 copies per cell [12] (Table II). Copy numbers were adjusted for the percentage of cells with plasmid (i.e. % of *ura*⁺ cells). These values were consistent with the number of *E. coli* transformants produced by equivalent amounts of yeast minilysates (Table II). All hybrid plasmids except pSK120 had approximately the same copy number as the YRp12 control; pSK120 appeared to have a lower copy number by at least one or two orders of magnitude.

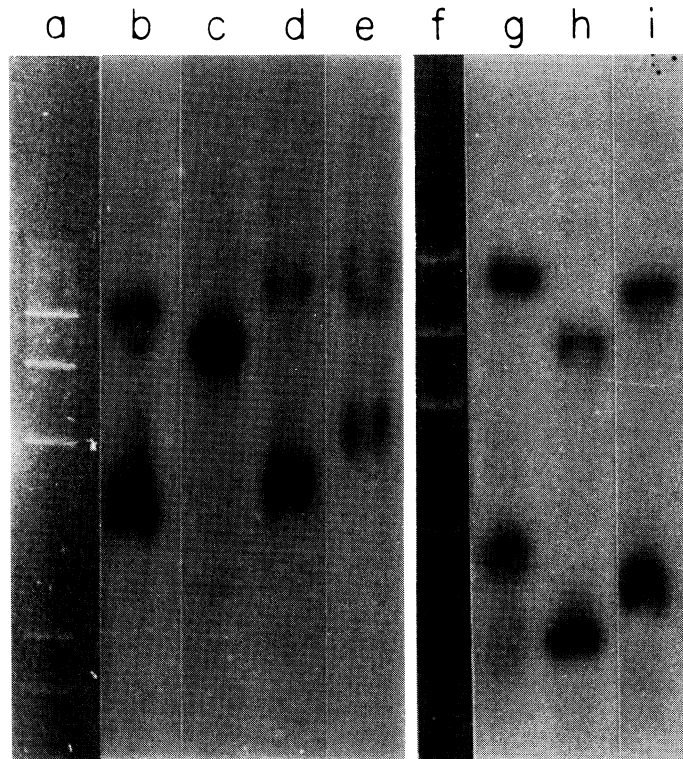


Fig. 5. Blot hybridization of undigested plasmid DNA isolated from yeast transformants with nick-translated pBR322 DNA. 1% agarose, TBE buffer. (a) and (f) λ DNA digested with HindIII; (b) YRp12; (c) pSK120; (d) pSK30; (e) pSK150; (g) pSK152; (h) pDY5; (i) pDF10. A lysate from the nontransformed host yeast strain NNY-1 was also run as a control with no detectable hybridization.

Presence of 11 mer yeast ars consensus sequences and a sequence homologous to the C. fasciculata 189 nt ars in the L. tarentolae maxicircle. The 11 mer yeast ars consensus sequence described by Broach et al. [13] is present 7 times in the 6.56 kb 120 sequence and 13 times in the 6.96 kb 'F-3' sequence (see arrows in Fig. 7) (de la Cruz et al., unpublished data). Comparison of the 189 nt *C. fasciculata* maxicircle ars sequence (Cf ars189) of Kim and Ray [16] with the entire 120 and 'F-3' *L. tarentolae* maxicircle sequences revealed a striking sequence similarity within a portion of the LtA4 subfragment of the Lt152 fragment (termed Lt arsl). As shown in Fig. 6, the overall sequence similarity is 78%, but the sequence similarity of the first 120 nt is 84%. The latter region contains one yeast ars consensus sequence common to both species and one conserved in *Crithidia* but differing by one nt in *Leishmania*. Consistent with this observation is the fact that the LtA4 fragment subcloned in the YIp5 hybrid, pDY5, exhibited strong ars activity in yeast, although as discussed above, this sequence was lost in the yeast host and was apparently substituted by a yeast ars sequence.

An alignment of the restriction maps of the *L. tarentolae*, *C. fasciculata* and the *T. brucei* maxicircles using the position of the 9 and 12 S genes is shown in Fig. 7. The putative divergent region of the *Crithidia* maxicircle is tentatively localized as the region which showed no hybridization to total labeled RNA [26]. It is interesting that the distance from the 9 and 12 S genes to the Cf ars189 in *Crithidia* is approximately equal to the distance from the 9 and 12 S genes to the Lt arsl sequence in *Leishmania*. It is also interesting that one of the two *T. brucei* maxicircle ars elements (Tb ars 2 in Fig. 1) [14] is roughly in the same relative position as the Cf 189 and the Lt arsl elements with respect to the 9 and 12 S genes. The Tb ars 1 corresponds to the Lt ars 2 element in

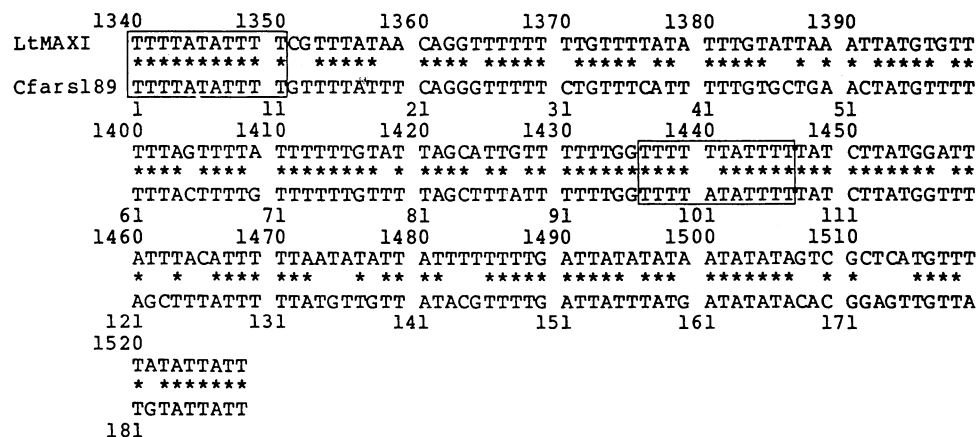


Fig. 6. Sequence similarity of the Cf 189ars with a portion of the *L. tarentolae* maxicircle sequence (Lt ars189). The *C. fasciculata* sequence is from Kim and Ray [16]. Identical nts are indicated by asterisks. The two yeast 11 mer consensus sequences are indicated by boxes. The numbering of the *L. tarentolae* sequence refers to the number of nts from the MspI site at the start of the Lt152 fragment. Overall similarity is 78%.

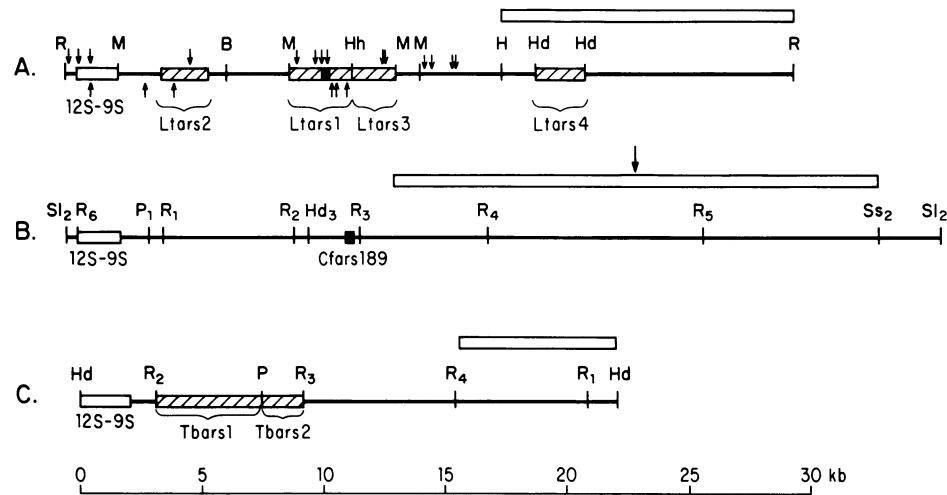


Fig. 7. Alignment of the restriction maps of the maxicircle DNAs from A, *L. tarentolae*, B, *C. fasciculata* and C, *T. brucei*. The maxicircles were arbitrarily linearized as shown and aligned using the positions of the 9 and 12 S ribosomal RNA genes. The positions of the non-transcribed divergent regions are indicated by open boxes above the lines. The *Crithidia* map is from Hoeijmakers et al. [26] and the *T. brucei* map from Muhich et al. [27]. In the *L. tarentolae* map the four fragments positive for ars activity (Lt ars1-4) are indicated by crosshatched boxes. The positions of 11 mer yeast consensus sequences are indicated by arrows with the strandedness given by the direction of the arrow. In the *Crithidia* and *L. tarentolae* maps, the positions of the Cf ars189 [16] and the Lt ars189 are indicated by dark boxes, and in the *T. brucei* map the positions of the Tb ars1 and Tb ars2 elements are indicated [15]. In the *Crithidia* map, the position of the putative origin of replication of the leading strand [5] is indicated by an arrow. Restriction site symbols in addition to those given in Fig. 1 legend: Ss, SstI; Sl, SalI; P, PstI.

relative localization. The Lt ars4 corresponds roughly in localization to the localization of the putative leading strand origin of replication in the *Crithidia* maxicircle, which also lies within the divergent region [5].

DISCUSSION

We have shown that at least four sequences exist in the maxicircle DNA of *L. tarentolae* which exhibit ars activity in a yeast plasmid. Two of the maxicircle sequences produced yeast transformants that divided at rates similar to the YRp12 control cells and two produced yeast transformants that divided more slowly. All hybrid plasmids replicated extrachromosomally in the yeast hosts. The pSK150, pSK152 and pSK30 hybrid plasmids were present at about 100 copies per yeast cell and the pSK120 plasmid at about 1–10 copies per cell.

The existence of multiple maxicircle fragments that exhibit yeast ars activity is consistent with the presence of multiple yeast 11 mer ars consensus sequences [13] in

the 80% AT maxicircle sequence. However the observed sequence conservation between the 189 nt *C. fasciculata* maxicircle ars (Cf ars189) of Kim and Ray [16] and the Lt ars1 subfragment of the LtA4 fragment is striking and possibly indicates a functional role for this sequence as a conserved kinetoplastid maxicircle DNA origin of replication. In addition, the presence of a yeast 11 mer ars consensus sequence within this conserved sequence is consistent with the strong ars activity of the 152 fragment in yeast. The Lt ars1 sequence in the *L. tarentolae* maxicircle lies within a 735 nt open reading frame termed ORF6 (de la Cruz et al, unpublished data). The translated amino acid sequence of ORF6 shows no sequence homology with any known mitochondrial genes. There is a low abundance transcript of approximately 1 000 nt as determined by probing Northern blots of kinetoplast RNA with nick-translated gel-purified LtA4 fragment (results not shown). The Lt ars2 element lies within the Lt S1 subfragment of the 120 region, which contains the open reading frame ORF4, the cytochrome oxidase subunit III gene and part of the cytochrome *b* gene (de la Cruz et al., unpublished data). Several yeast 11 mer ars consensus sequences are localized in this fragment (Fig. 1)

By pulse-labeling linear maxicircle replication intermediates Hajduk et al. [5] have identified a putative origin of replication of the leading strand in *C. fasciculata* and have noted that this origin does not correspond to the position of the Cf ars189 fragment [16]. They speculated that the Cf ars189 sequence may represent the origin of the lagging strand. From the alignment of the *Crithidia* and *L. tarentolae* maxicircle maps in Fig. 7, the origin identified by Hajduk et al. [5], which lies within the divergent region, corresponds roughly in position to the Lt ars4 element, which lies within the divergent region in the *L. tarentolae* maxicircle, and not to the Lt ars189 element.

The alignment of the Lt ars189 element and the Cf ars189 element in the *Crithidia* and *Leishmania* maxicircle maps suggests that the relative gene order in the transcribed region of the *Crithidia* maxicircle is similar to that in the *Leishmania* maxicircle and therefore to that in the *T. brucei* maxicircle [27]. The rough alignment of the two ars elements (Tb ars1, Tb ars2) in the *T. brucei* maxicircle map [15] with Lt ars1 and Lt ars2 in the *Leishmania* map is consistent with this interpretation. There are however some differences between the *Crithidia* and *Leishmania* maps, in that the non-transcribed or divergent region in *Crithidia* apparently begins approximately 3.5 kb before the beginning of the divergent region in *Leishmania* and there is a 2.5 kb transcribed region (fragment Ss2-S2) between the 9 and 12 S genes and the divergent region which is not present in *Leishmania* [26]. Further work is required to determine the detailed localization of structural genes in the maxicircle DNA of *Crithidia* as compared to that in the *L. tarentolae* maxicircle.

Further work is also required to determine if the ars189 sequence, which is strongly conserved between *Crithidia* and *L. tarentolae*, is a true origin of replication of the maxicircle.

ACKNOWLEDGEMENTS

We thank Agda Simpson for performing the Northern blot analysis and Vidal de la Cruz and Agda Simpson for discussions and assistance. We also thank Dan Ray for communication of unpublished results. This work was supported in part by USPHS Research Grants AI-09102 and 13027 to L.S. and by a Biomedical Science Support Grant. P.K. was supported by USPHS National Research Service Award GM-07104.

REFERENCES

- 1 Simpson, L. (1972) The kinetoplast of the hemoflagellates. *Int. Rev. Cytol.* 32, 139–207.
- 2 Englund, P., Hajduk, S. and Marini, J. (1982) The molecular biology of trypanosomes. *Annu. Rev. Biochem.* 51, 695–726.
- 3 Eperon, I., Janssen, J., Hoeijmakers, J. and Borst, P. (1983) The major transcripts of the kinetoplast DNA of *T. brucei* are very small ribosomal RNAs. *Nucleic Acids Res.* 11, 105–125.
- 4 Benne, R., DeVries, B., Van den Burg, J. and Klaver, B. (1983) The nucleotide sequence of a segment of *Trypanosoma brucei* mitochondrial maxicircle DNA that contains the gene for apocytochrome *b* and some unusual unassigned reading frames. *Nucl. Acids Res.* 11, 6925–6941.
- 5 Hajduk, S., Klein, V. and Englund, P. (1984) Replication of kinetoplast DNA maxicircles. *Cell* 36, 483–492.
- 6 Struhl, K., Stinchcomb, D.T., Scherer, S. and Davis, R.W. (1979) High frequency transformation of yeast: Autonomous replication of hybrid DNA molecules. *Proc. Natl. Acad. Sci. U.S.A.* 76, 1035–1039.
- 7 Stinchcomb, D.T., Thomas, M., Kelly, J., Selker, E. and Davis, R.W. (1980) Eukaryotic DNA segments capable of autonomous replication in yeast. *Proc. Natl. Acad. Sci. U.S.A.* 77, 4559–4563.
- 8 Broach, J.R. and Hicks, J.B. (1980) Replication and recombination functions associated with the yeast plasmid, 2 μ m circle. *Cell* 21, 501–508.
- 9 Kiss, G.B., Amin, A.A. and Pearlman, R.E. (1981) Two separate regions of the extrachromosomal ribosomal deoxyribonucleic acid of *Tetrahymena thermophila* enable autonomous replication of plasmids in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 1, 535–543.
- 10 Celniker, S.E. and Campbell, J.L. (1982) Yeast DNA replication in vitro: Initiation and elongation events mimic in vivo processes. *Cell* 31, 201–213.
- 11 Zakian, V.A. (1981) Origin of replication from *Xenopus laevis* mitochondrial DNA promotes high-frequency transformation of yeast. *Proc. Natl. Acad. Sci. U.S.A.* 78, 3128–3132.
- 12 Hyman, B.C., Cramer, J. and Rownd, R. (1982) Properties of a *Saccharomyces cerevisiae* mtDNA segment conferring high-frequency yeast transformation. *Proc. Natl. Acad. Sci. U.S.A.* 79, 1578–1582.
- 13 Broach, J.R., Li, Y.Y., Feldman, J., Jayaram, M., Abraham, J., Nasmyth, K.A. and Hicks, J.B. (1982) Localization and sequence analysis of yeast origins of replication. *Cold Spring Harbor Symp. Quant. Biol.* 47, 1165–1173.
- 14 Davison, J. and Thi, V.H. (1982) The *Trypanosoma brucei* maxicircle DNA contains ARS elements active in *Saccharomyces cerevisiae*. *Curr. Gen.* 6, 19–20.
- 15 Koduri, R. and Ray, D. (1984) Identification of autonomous replication sequences in genomic and mitochondrial DNA of *Crithidia fasciculata*. *Mol. Biochem. Parasitol.* 10, 151–169.
- 16 Kim, R. and Ray, D. (1984) A 189 base-pair fragment of *Crithidia fasciculata* maxicircle DNA confers autonomous replication in *Saccharomyces cerevisiae*. *Gene* (in press).

- 17 Simpson, L. and Braly, P. (1970) Synchronization of *Leishmania tarentolae* by hydroxyurea. J. Protozool. 17, 511-517.
- 18 Masuda, H., Simpson, L., Rosenblatt, H. and Simpson, A.M. (1979) Restriction map, partial cloning and localization of 9S and 12S RNA genes on the maxicircle component of the kinetoplast DNA of *Leishmania tarentolae*. Gene 6, 51-73.
- 19 Mandel, M. and Higa, A. (1970) Calcium-dependent bacteriophage DNA infection. J. Mol. Biol. 53, 159-162.
- 20 Holmes, D.S. and Quigley, M. (1981) A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. 114, 193-197.
- 21 Ito, H., Fukuda, Y., Muata, K. and Kimura A. (1983) Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. 153, 163-168.
- 22 Nasmyth, K. and Reed, S. (1980) Isolation of genes by complementation in yeast: Molecular cloning of a cell-cycle gene. Proc. Natl. Acad. Sci. U.S.A. 77, 2119-2123.
- 23 Hsiao, C. and Carbon, J. (1981) Characterization of a yeast replication origin, and construction of stable minichromosomes containing cloned yeast centromere DNA (CEN3). Gene 15, 157-166.
- 24 Smith, G. and Summers, M. (1980) The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzyloxymethyl paper. Anal. Biochem. 109, 123-129.
- 25 Staden, R. (1980) A new computer method for the storage and manipulation of DNA gel reading data. Nucleic Acids Res. 8, 3673-3694.
- 26 Hoeijmakers, J., Schoutsen, B. and Borst, P. (1982) Kinetoplast DNA in the insect trypanosomes *Crithidia luciliae* and *Crithidia fasciculata*. I. Sequence evolution and transcription of the maxicircle. Plasmid 7, 199-209.
- 27 Muhich, M., Simpson, L. and Simpson, A.M. (1983) Comparison of the maxicircle DNAs of *Leishmania tarentolae* and *Trypanosoma brucei*. Proc. Natl. Acad. Sci. U.S.A. 80, 4060-4064.

