

Homologous recombination between direct repeat sequences yields P-glycoprotein containing amplicons in arsenite resistant *Leishmania*

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ABSTRACT

The protozoan parasite *Leishmania* often responds to drug pressure by amplifying part of its genome. At least two loci derived from the same 800 kb chromosome were amplified either as extrachromosomal circles or linear fragments after sodium arsenite selection. A 50 kb linear amplicon was detected in six independent arsenite mutants and revertants grown in absence of arsenite rapidly lost the amplicon and part of their resistance. The circular extrachromosomal amplicons, all derived from the H locus of *Leishmania*, were characterized more extensively. In all cases, direct repeated sequences appeared to be involved in the formation of circular amplicons. Most amplicons were generated after homologous recombination between two linked P-glycoprotein genes. This recombination event was, in two cases, associated with the loss of one allele of the chromosomal copy. A novel rearrangement point was found in a mutant where the amplicon was created by recombination between two 541 bp direct repeats surrounding the P-glycoprotein gene present at the H locus. It is also at one of these repeats that an H circle with large inverted duplications was formed. We propose that the presence of repeated sequences in the H locus facilitates the amplification of the drug resistance genes concentrated in this locus.

INTRODUCTION

Gene amplification is a widespread phenomenon occurring in organisms spanning the phylogenetic tree. Amplified genes have been associated with drug resistance and malignant progression in mammalian cells (for review see 1). Amplification of DNA segments has been observed in wild type unselected stocks of the protozoan parasite *Leishmania* (2–5) and in several *Leishmania* species selected for drug resistance (6). Several

different loci of the *Leishmania* genome have been amplified after drug selection (7–12) the most extensively characterized being the H locus, a stretch of DNA of about 40 kb that is part of an 800 kb chromosome. At least three genes are encoded by the H locus; a P-glycoprotein gene homolog (13) associated with low level oxyanion resistance (14; B. Papadopoulou, et al., in preparation), a short-chain dehydrogenase involved in antifolate resistance (15, 16) and a homolog of argininosuccinate synthetase (unpublished results). Amplification of the H locus has been observed after selection with a number of unrelated drugs (17–19) and has been found to occur by more than one mechanism (19).

In mammalian cells, several mechanisms have been proposed for DNA amplification (1). One of these postulates the formation of amplicons from circular precursors called episomes (20, 21) or amplisomes (22). These circular precursors are smaller (120–1,400 kb) than the final amplicons and are constituted of either direct or inverted repeats (for review see 21). In drug resistant *Leishmania*, amplicons are generally extrachromosomal small circles (30–125 kb) that can have direct or inverted repeats (8, 19, 23). The small size of the amplicons found in *Leishmania*, as well as its small genome size (5×10^7 bp), make the visualization and characterization of amplicons easier than in mammalian cells. Moreover, the structural similarities between some amplicons found in cancer cells and those generated in *Leishmania* makes the use of the latter organism a useful and tractable model system to study gene amplification in drug resistant organisms.

In *Leishmania*, the few amplicons so far studied were always generated at the level of homologous sequences (19). To better understand the mechanism of gene amplification and to see how frequently *Leishmania* resort to homologous recombination to amplify its genome, we have induced gene amplification using arsenite selection and have characterized extensively the amplicons derived from the H locus.

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⁺ L07760, L07761 and M55402

MATERIALS AND METHODS

Cell lines and culture

The two parental cell lines *Leishmania tarentolae* TarII and TarVIa have been described previously (2). Cells were grown in SDM-79 medium at 29°C. In an attempt to induce the formation of H circles from the chromosomal H locus, individual lines of TarII WT were selected for resistance to sodium arsenite by stepwise increase from 400 nM to 20 or 50 μM. Two of these arsenite resistant mutants, TarIIAs20.3 and TarIIAs50.1 have been described elsewhere (19) and ten new arsenite resistant mutants are characterized in detail here.

DNA cloning

Total genomic DNA of *Leishmania* strains was prepared as described (13). The size of the *Hind*III restriction fragment containing the novel rearrangement point in TarIIAs50.1 was determined to be 4.2 kb by Southern blot analysis using probe 3, a 200 bp *Hind*III–*Eco*RV fragment (Figure 1). Total DNA of As50.1 was digested with *Hind*III and restriction fragments ranging from 4 to 5 kb were isolated from low melting point agarose, ligated to a pGEM3Zf bacterial vector (Promega) and transformed in *E. coli*. The transformants with the desired insert were picked up by colony hybridization using probe 3. Clones derived from the H locus representing the genomic non rearranged sequences were already available (2).

The linear amplicon in mutant As20.2 was isolated from agarose gel with the Sephaglas band prep kit according to the manufacturer's recommendations (Pharmacia). Cells were embedded in low melting agarose and lysed as described (24). Chromosomes were separated by TAFE electrophoresis (Beckman) using pulse of 40, 100 and 160 seconds for 12 hours each. The isolated linear amplicon DNA was cut with *Hind*III and fragments were cloned in pGEM3Zf. A clone with an insert of 500 bp was used as a specific probe for the detection of the linear amplicon.

PCR amplification

The novel junctions created after amplicon formation in the arsenite mutants were characterized by using the polymerase chain reaction (PCR). The *Taq* polymerase was from Perkin-Elmer Cetus. The oligonucleotide primers used in the PCRs had the following sequences: primer 1, 5'-GCATTGGCCGCACG-GTGCTC-3'; primer 2, 5'-GTGCAGCGCCATACACAGTC-3'; primer 3, 5'-GCTGGTAAGGAGCGAGTCAG-3' and primer 4, 5'-GTCTTTTGAACCACGGCTGC-3'. Primers were

incubated with *L. tarentolae* genomic DNA using a standard PCR protocol in a volume of 100 μl. The reaction mixture was incubated for 25 cycles at 94, 67 and 72°C for 1, 2, and 3 min., respectively.

DNA sequencing

Double-stranded plasmid DNAs, derived from pGEM3Zf vectors (Promega), were sequenced by the dideoxy chain termination method (25) using the Sequenase version 2.0 DNA sequencing kit from USB. Computer analysis of the nucleotide sequence was performed using the GCG software package (26). The nucleotide sequence reported here will appear in the EMBL/GenBank/DDBJ nucleotide sequence databases under the accession number L07760 (E repeat), L07761 (recombination between the B and E repeats) and M55402 (B repeat).

Southern hybridization

Genomic DNAs from *Leishmania* WT and arsenite resistant mutants were digested, resolved on 0.4–0.7% agarose gel and transferred to Hybond-N membranes (Amersham) by the method of Southern (27). Chromosome sized TAFE gels were transferred similarly. Hybond-N filters were prehybridized and hybridized with ³²P-radiolabelled specific probes as described (27). Washings were performed in 3×SSC-0.1% SDS and 0.1×SSC-0.1% SDS at 65°C. Different exposures of the hybridizing bands were quantitated using a gel-scan Compuset module on a Beckman DU-8B spectrophotometer, to estimate the copy number of the linear amplicon.

RESULTS

Selection for arsenite resistance promotes specific gene amplification in *Leishmania*

DNA amplification in *Leishmania*, following arsenite selection, was first reported by Detke et al. (28). More recently, we showed that parts of the H region of *Leishmania* were amplified *de novo* from the chromosomal copy in two arsenite resistant mutants. Preliminary evidences, as well as more recent mapping data, have suggested that the DNA sequences amplified in these two mutants were different (Figure 1, see also 19). The amplicon in mutant TarIIAs20.3 was extensively characterized, and we showed that it had been generated by homologous recombination between two linked P-glycoprotein genes (19). Since arsenite has already been shown to be a potent inducer of gene amplification in *Leishmania* (19, 28), we have selected ten more independent mutants in order

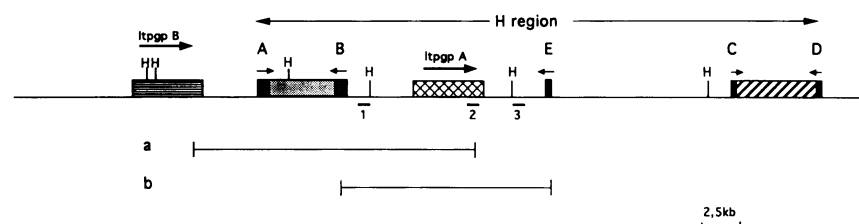


Figure 1. Map of the H locus and regions amplified in arsenite resistant mutants. The H region, as defined by White et al. (2) is indicated by an arrow above the map. The locus is delimited by two boxes that are both flanked by inverted repeats (A to D) (19). A sequence related to B is present within the H locus and is indicated with the letter E. The *Hind*III sites (H) of the locus are also indicated. The P-glycoprotein genes *ltpgpA* and *ltpgpB* (19, 29) are also indicated. The bars with numbers (1 to 3) below the map indicate DNA probes used in this study. Below the map, the plane lines with the letter a and b indicate the H locus-derived amplified regions in arsenite resistant mutants that are characterized in this study.

to study the mechanism of gene amplification in more detail. To monitor H region amplification in our arsenite resistant mutants, we hybridized a Southern blot of *Hind*III digested *Leishmania* genomic DNA with probe 2, a 850 bp *Sac*I fragment containing the second nucleotide binding site (nbs) of the *ltpgpA* gene (Figure 1). This probe recognizes five P-glycoprotein genes (29) in the wild type strain *L.tarentolae* Tar II (Figure 2, lane 1), a strain free of H circles (2). In the wild type strain *L.tarentolae* TarVIa, the 68 kb H circle is already present (2), thus explaining the amplified 9.5 kb *Hind*III restriction fragment hybridizing to

the nbs probe (Figure 2, lane 2). The copy number of the H circle in strain TarVIa increases once cells are under arsenite selection (Figure 2, lane 3).

We detected P-glycoprotein gene amplification in six out of twelve independent arsenite resistant cell lines derived from TarII (lanes 4 to 15 in Figure 2). Hybridization with gene specific probes for each of the five P-glycoprotein genes showed that all amplified fragments hybridized only to the probe specific for *ltpgpA*, the gene present on the H locus (results not shown). The amplified fragments in lanes 6, 8 and 13, comigrating with *ltpgpD* and *E*, are also hybridizing to the *ltpgpA* specific probe and might represent a fraction of the DNA refractory to digestion running in the compression zone of the gel. In most amplicons, the amplified *Hind*III restriction fragments had a size of 11 kb (Figure 2, lanes 6, 8, 10, 13, 15). Since in the WT cells the size of the *Hind*III fragment containing *ltpgpA* is 9.5 kb (Figure 2, lane 1), the 11 kb amplified *Hind*III restriction fragment is newly formed and should contain the rearrangement point, although we cannot formally exclude that this represent the product of a secondary rearrangement. Secondary rearrangements are generally not observed in *Leishmania* species (6). An 11 kb *Hind*III restriction fragment hybridizing with probe 2, was already noted before in mutant TarIIAs20.3 (19). This fragment was the result of an intrachromosomal recombination between the nbs consensus sequences of *ltpgpA* and *ltpgpB* (19, see also Figure 4A). In mutant As50.1 (Figure 2, lane 4) the amplified *Hind*III restriction fragment hybridizing to the nbs probe comigrates with the genomic fragment indicating that the rearrangement point is further away. All *ltpgpA* containing amplicons were migrating in a pulse independent manner (when compared to *Leishmania* chromosomes) in transverse alternating field gels (not shown), a characteristic of circular DNAs in pulse field gels (2, 30–32).

Examination of ethidium bromide stained TAFE gels indicated that an extra band, migrating more rapidly than the smallest

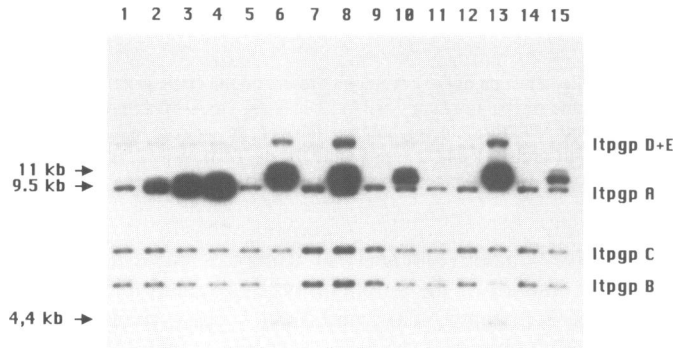


Figure 2. H region amplification in arsenite resistant mutants. Total DNA of *Leishmania tarentolae* wild type and mutant strains was digested with *Hind*III, electrophoresed in a 0.4% agarose gel for 36 hours at 1.2 V/cm in TEA buffer (27), blotted and hybridized to probe 2 (see Figure 1). This probe covers the second nucleotide binding site of *ltpgpA* and recognizes five P-glycoprotein genes *ltpgpA*, *B*, *C*, *D*, and *E* present in *Leishmania*. Lanes 1, *L.tarentolae* TarII wild type; 2, TarVIa wild type; 3, TarVIa As20; 4, TarIIAs50.1; 5, TarIIAs20.2; 6, TarIIAs20.3; 7, TarIIAs20.4; 8, TarIIAs50.5; 9, TarII As20.6; 10, TarIIAs20.7; 11, TarIIAs20.8; 12, TarIIAs20.9; 13, TarIIAs20.10; 14, TarIIAs20.11; 15, TarIIAs20.12. Mol. wts were estimated from the 1kb BRL ladder.

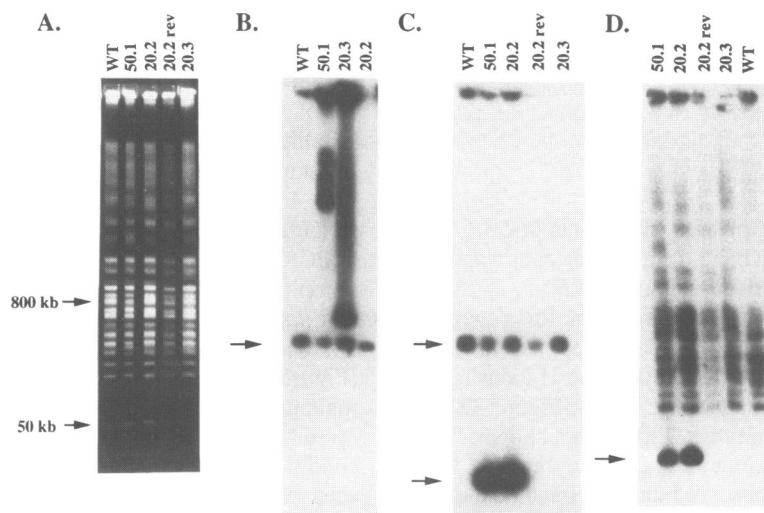


Figure 3. Characterization of a linear amplicon in arsenite resistant mutants. (A.) Chromosomes of *L.tarentolae* wild type TarII (WT) and mutants (As20.3, As50.1, As20.2 and As20.2rev for revertant) were separated by TAFE and stained with ethidium bromide. Molecular weights were determined using the yeast chromosomes and a lambda-PFGE markers (BioRad, Pharmacia). (B.) A chromosome sized-blot of *Leishmania* was hybridized with probe 2 (Figure 1) derived from *ltpgpA*. (C.) Chromosomes of *Leishmania* cells separated on the same gel as indicated in B were blotted and hybridized with a probe isolated from a plasmid bank made from the linear amplicon of mutant As20.2 (see Materials and methods). (D.) A chromosome sized blot of *Leishmania* wild type and mutants was hybridized with the insert of plasmid pT4 that contains the telomeric sequences of *T. brucei* (33). Arrows indicate the 800 kb H region containing chromosome and the 50 kb linear amplicon.

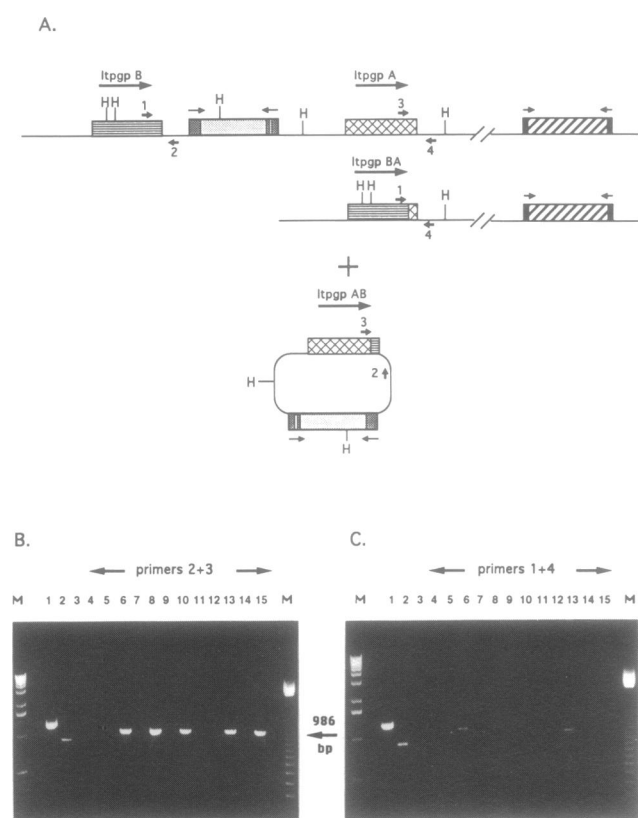


Figure 4. PCR analysis of rearrangement points of amplicons derived from the H region of *Leishmania*. (A) Schematic representation of the genomic rearrangement in mutants TarIIAs20.3 and TarIIAs20.10 (adapted from 19). Amplicons were formed by homologous recombination between *ltpgpA* and *ltpgpB* leaving one allele intact and the other one rearranged. Primers used for PCR analysis are indicated with numbers 1, 2, 3 and 4. (B) PCR amplification of the novel junction created after homologous recombination between *ltpgpA* and *B*; Lanes 1, TarII wild type (WT) using primers 1 and 2; 2, TarII WT with primers 3 and 4; 3, TarII WT; 4, TarIIAs50.1; 5, TarIIAs20.2; 6, TarIIAs20.3; 7, TarIIAs20.4; 8, TarIIAs50.5; 9, TarIIAs20.6; 10, TarIIAs20.7; 11, TarIIAs20.8; 12, TarIIAs20.9; 13, TarIIAs20.10; 14, TarIIAs20.11; 15, TarIIAs20.12. From lanes 3 to 15 the PCR reactions were done with primers 2 and 3; (C) PCR amplification of the novel chromosomal joint created between *ltpgpB* and *A* after a non conservative amplification. Same order than in part B but from numbers 3 to 15, primers 1 and 4 were used in PCR reactions. The mol. wt. markers are the 1 kb BRL ladder and the 100 bp Pharmacia ladder.

Leishmania chromosome, was present in mutants As50.1 and As20.2 (Figure 3A). This extra band, or a similar one, was also present in four other independent mutants, As50.5, As20.8, As20.9 and As20.10 (not shown), indicating that this region is under strong pressure when cells are selected for arsenite resistance. This DNA region is different than the H locus since it did not hybridize to an H locus specific probe (Figure 3B) or several other probes derived from the H locus (not shown). We have isolated the extra band in mutant As20.2, hybridized it to a chromosome sized blot, and observed that this band contained sequences that were repeated in several other chromosomes (not shown). A plasmid bank of the isolated fast migrating band was made (see Materials and methods) and fragments were isolated and tested in order to obtain a single copy probe. Hybridization with the single copy probe revealed that this extra band contained DNA that was amplified, and that this novel amplicon was also derived from the 800 kb chromosome from which the H locus amplicons were generated (Figure 3C and not shown). By

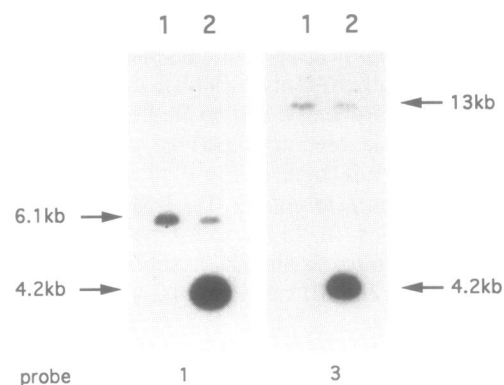


Figure 5. Localization of the novel joint present on the circle generated *de novo* in the arsenite resistant mutant TarIIAs50.1. Total DNAs of *L.taremolae* were digested with *HindIII*, electrophoresed through an agarose gel, blotted and hybridized to probes 1 and 3 (see Figure 1). 1, TarII WT; 2, TarIIAs 50.1. Fragments size was estimated from the 1 kb BRL ladder.

quantitative Southern blot analysis, using this single copy probe, the copy number of the amplicon was estimated to be 18 and 19 copies, in mutants As20.2 and As50.1, respectively. Growth of the As20.2 mutant in absence of arsenite to yield As20.2 rev, resulted in rapid loss of this amplicon (Figure 3A and 3C) which was associated with a 3 fold decrease in resistance to arsenite.

From several criterions, we conclude that this novel band is a linear amplicon of 50 kb. First, and more importantly, its migration, when compared to the linear chromosomes of *Leishmania*, was dependent on pulse, whereas the circular H region amplicons migrated in a pulse independent manner (not shown). Moreover, this linear amplicon hybridized to a probe containing telomeric sequences derived from *T. brucei* (33) under conditions where the amplified H circle present in mutants As50.1 and As20.3 failed to hybridize (Figure 3D, and not shown).

Conservative and nonconservative amplification of the H region in arsenite resistant mutants

We have characterized the novel junctions created after DNA amplification of the H region using PCR. With the appropriate set of PCR primers, it was possible to test whether the 11 kb amplified *HindIII* restriction fragment present in five arsenite resistant mutants (see Figure 2) has been created after homologous recombination between the *ltpgpA* and *B* genes like in mutant As20.3. With the set of primers 2 and 3 (Figure 4A) a PCR product of 986 bp should be generated only when homologous recombination between the nbs sequences of *ltpgpA* and *ltpgpB* had occurred. In mutants TarIIAs20.3, 50.5, 20.7, 20.10 and 20.12 a PCR product of the expected size was obtained (Figure 4B) strongly suggesting that the amplicons in these mutants were formed by homologous recombination between *ltpgpA* and *B* at the level of the nbs sequences. Under the conditions used no PCR products, using primers 2 and 3, were seen with wild type DNA or mutants having other types of amplicons (Figure 4B, lanes 3 and 4).

Although the amplicons in mutants TarIIAs20.3, 50.5, 20.7, 20.10 and 20.12 were all formed by homologous recombination between the nbs sequences of *ltpgpA* and *ltpgpB*, analysis of the Southern blot shown in Figure 2 suggested that two different mechanisms were involved, conservative and non conservative. The mutants in lanes 8, 10 and 15 showed the extra 11 kb band without alteration in the relative intensity of the band

have occurred by a slipped strand mispairing (reviewed in 34) during replication. A model representing how the amplicon was formed in mutant As50.1 is shown in Figure 7. Southern blot analysis (Figure 5 and not shown) indicated that the amplicon in As50.1 was generated by a conservative mechanism. This result, in addition to the results presented above, shows that several different types of sequences in *Leishmania* can be used for amplicon formation, provided that they are homologous and are arranged in direct repeats.

DISCUSSION

Arsenite resistance

Several amplicons generated after stepwise arsenite selection in *Leishmania* cells contained the P-glycoprotein gene *ltpgpA* (13). Although arsenite is not transported by mammalian P-glycoproteins, in bacteria a functional analogue of P-glycoprotein is responsible for oxyanion resistance (see 35 for review). The P-glycoprotein *ltpgpA* has been shown recently, by transfection experiments, to be involved in low level arsenite and antimony resistance (14; B.P. et al., in preparation). Since the mutants are highly resistant to arsenite, *ltpgpA* cannot be the sole determinant of resistance. The region common to all amplicons in arsenite mutants covers more than *ltpgpA* (see Figure 1) and it is possible that other H region derived gene products, or gene(s) present on the novel linear amplicon, can act in combination with *ltpgpA* to confer higher level of arsenite resistance. We are now testing these possibilities by transfection experiments.

Only three, out of twelve arsenite resistant mutants, As20.4, 20.6 and 20.11, lacked detectable DNA amplification and they must therefore resist arsenite by another mechanism. Arsenite is nevertheless a potent inducer of specific gene amplification in *Leishmania* (see also 28) and at least two distinct genomic regions present on the same chromosome are often amplified during arsenite selection. Sodium arsenite has also been found to be an inducer of gene amplification in mammalian cells (36).

Characterization of amplicons

All *ltpgpA* containing amplicons were extrachromosomal circles formed by homologous recombination between direct repeats. At least three different events were associated with the formation of circular amplicons. First, three amplicons were generated by a conservative homologous recombination between the nbs sequences of *ltpgpA* and *B* which share an identical stretch of 750 bp (19). Second, two amplicons were generated by homologous recombination between the two P-glycoprotein genes but with the loss of one chromosomal allele (Figure 4A). Finally, one amplicon was formed by a conservative homologous recombination between two, apparently non coding, direct repeats of 541 bp (*B* and *E* in Figure 7). The prevalence of one type of amplicon over the other (five versus one) might be explained by the longest stretch of perfect identity available for homologous recombination. *Leishmania* is very efficient, however, at using repeated sequences to create circles. The sequence length required could be as short as 8 bp (37), and it is likely that 541 bp would be sufficient. Since in most of the arsenite resistant mutants the amplicons were generated by homologous recombination between the two P-glycoprotein genes *ltpgpA* and *ltpgpB*, this might indicate that the fusion protein is capable of conferring higher level of drug resistance. Transfection experiments with a fusion gene has failed to show higher level of resistance, however (in preparation). Finally, it is possible that sequences present in the

prevalent amplicon and absent in the amplicon of mutant As50.1, confer a higher level of resistance or confer more stability to the amplicon.

We have previously shown that the sequence indicated by the letter A in Figure 1 is an inverted repeat of sequence B. We have proposed that H circles could be formed by annealing of A and B during DNA replication (2, 19, 38). We now report here that the H locus contains an extra copy of part of sequence B, designated E in Figure 1, and that homologous recombination between B and E provides another mechanism for *ltpgpA* amplification. The presence of several copies of similar sequences in direct or inverted repeats that facilitates, under selective pressure, the amplification of several drug resistance genes of the H locus is in line with our hypothesis that the H region of *Leishmania* has evolved as a defense mechanism against cytotoxic drugs (15, 19, 38).

Amplicons in *Leishmania* are generally circular (reviewed in 6, 23) but karyotypic analysis of different *Leishmania* species has revealed the presence of small chromosomes that could occur in multiple copies (39, 40). These linear chromosomes either appeared *de novo* during serial passages (39) or after nutrient stress or subcloning (40). Recently, a 280 kb linear amplicon containing the amplified IMP dehydrogenase gene in a mycophenolic acid-resistant *L. donovani* (10) and a 140 kb linear amplicon in a α -difluoromethylornithine resistant *L. donovani* (11) have been described. In six of our arsenite resistant mutants, we observed a 50 kb linear amplicon that was generated *de novo* from an 800 kb chromosome. In contrast to the amplicon found in the mycophenolic resistant strain (10), but similarly to the linear amplicon found in the α -difluoromethylornithine resistant *L. donovani* (11), the linear amplicon found in our arsenite resistant mutants was unstable since it was lost rapidly when selective pressure was removed. Although a partial loss in resistance was associated with the loss of the linear amplicon, it remains to be seen whether a gene present on this amplicon is associated with arsenite resistance. Interestingly, the linear amplicon was generated from the same chromosome from which the H region amplicons were derived. At present it is not known whether this physical linkage is fortuitous or has a role to play in arsenite resistance. In three mutants, As50.1 (Figure 3B, 3C), As50.5 and As20.10 (Figure 2 and not shown) both the linear 50 kb amplicon and the circular *ltpgpA* containing amplicon were present. The presence of multiple amplicons in the same *Leishmania* cell is not without precedent since two amplicons have been noted in a methotrexate resistant *Leishmania* (8) or in an α -difluoromethylornithine resistant *L. donovani* (11).

Formation of circular amplicons in *Leishmania*

All the circular amplicons characterized in *Leishmania* were created by homologous recombination between low copy number directly repeated sequences. The same genomic region could be amplified either in a conservative or nonconservative fashion. These results are better accommodated by the 'deletion plus episome' model (21) where excision of the chromosomal copy occurs at the level of homologous sequences. If excision preferentially occurs during normal replication, the daughter with the circle has equal chance of getting the deleted or intact chromosomes depending on segregation. In five mutants which have the same region amplified, two amplicons were found in a cell with a deleted allele of *ltpgpA* and three with both alleles intact. Although the numbers are small, they agree with the proposed model of deletion of a chromosomal copy across a

standard replication loop. A model has been recently proposed for DNA amplification in cancer cells, where the initiating event involves chromosome breakage within a replication bubble (41). Even if the structure of episomes found in cancer cells is similar to those found in *Leishmania* (6, 21), the exact mechanism of their formation seems to differ since in the few instances where the rearrangement points were mapped, circular amplicons found in cancer cells were not formed at the level of homologous sequences (1). In *Leishmania* however, all amplified circles so far characterized were generated by excision of a chromosomal copy mediated by homologous recombination (19, 37, this work). Recently, DNA recombinational events at the level of directly repeated promoter sequences have also been proposed to be one way of controlling the expression of the variant cell surface glycoprotein in the related parasite *Trypanosoma brucei* (42). This is not so surprising since contrarily to cancer cells, homologous recombination is the preferred route of integration of exogenous DNA in *Leishmania* and *Trypanosoma* (43–45). It will be interesting to determine how linear amplicons are generated and whether homologous sequences are also involved during excision.

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REFERENCES

- Stark, G.R., Debatisse, M., Giulotto, E., and Wahl, G.M. (1989). *Cell* **57**, 901–908.
- White, T.C., Fase-Fowler, F., van Luenen, H., Calafat, J., and Borst, P. (1988). *J. Biol. Chem.* **263**, 16977–16983.
- Petrillo-Peixoto, M.L., and Beverley, S.M. (1988). *Mol. Cell. Biol.* **8**, 5188–5199.
- Hightower, R.C., Ruiz-Perez, L.M., Lie Wong, M., and Santi, D.V. (1988). *J. Biol. Chem.* **263**, 16970–16976.
- Tripp, C.A., Wisdom, W.A., Myler, P.J., and Stuart, K.D. (1992). *Mol. Biochem. Parasitol.* **55**, 39–50.
- Beverley, S.M. (1991). *Ann. Rev. Microbiol.* **45**, 417–444.
- Coderre, J.A., Beverley, S.M., Schimke, R.T., and Santi, D.V. (1983). *Proc. Natl. Acad. Sci. USA* **80**, 2132–2136.
- Beverley, S.M., Coderre, J.A., Santi D.V., and Schimke R.T. (1984). *Cell* **38**, 431–439.
- Detke, S., Chaudhuri, G., Kink, J.A., and Chang, K-P. (1988). *J. Biol. Chem.* **263**, 3418–3424.
- Wilson, K., Beverley, S.M., and Ullman, B. (1992). *Mol. Biochem. Parasitol.* **55**, 197–206.
- Hanson, S., Beverley, S.M., Wagner, W., and Ullman, B. (1992). *Mol. Cell. Biol.* **12**, 5499–5507.
- Henderson, D.M., Sifri, C.D., Rodgers, M., Wirth, D.F., Hendrickson, N., and Ullman, B. (1992). *Mol. Cell. Biol.* **12**, 2855–2865.
- Ouellette, M., Fase-Fowler, F., and Borst, P. (1990). *EMBO J.* **9**, 1027–1033.
- Callahan, H.L. and Beverley, S.M. (1991). *J. Biol. Chem.* **266**, 18427–18430.
- Papadopoulou, B., Roy, G., and Ouellette M., (1992). *EMBO J.*, **11**, 3601–3608.
- Callahan, H.L. and Beverley, S.M. (1992). *J. Biol. Chem.* **267**, 24165–24168.
- Ellenberger, T.E., and Beverley, S.M. (1989). *J. Biol. Chem.* **264**, 15094–15103.
- Katakura, K., and Chang, K.-P. (1989). *Mol. Biochem. Parasitol.* **34**, 189–192.
- Ouellette, M., Hetteima, E., Wust, D., Fase-Fowler, F., and Borst P. (1991). *EMBO J.* **10**, 1009–1016.
- Carroll, S.M., DeRose, M.L., Gaudray, P., Moore, C.M., Needham-Vandevanter, D.R., VonHoff, D.D., and Wahl, G.M. (1988). *Mol. Cell. Biol.* **8**, 1525–1533.
- Wahl, G.M. (1989). *Cancer Res.* **49**, 1333–1340.
- Pauletti, G., Lai, E., and Attardi, G. (1990). *Proc. Natl. Acad. Sci. USA* **87**, 2955–2959.
- Stuart, K.D. (1991). *Parasitol. Today* **7**, 158–159.
- Van der Ploeg L.H.T., Schwartz, D.C., Cantor, C.R., and Borst, P. (1984). *Cell* **37**, 77–84.
- Sanger, F., Niclen, S., and Coulson, A.R. (1977). *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
- Devereux, J., Haerberli, P., and Smithies, O. (1984). *Nucleic Acids Res.* **12**, 387–395.
- Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). *Molecular cloning; a laboratory manual*. Cold Spring Harbor Laboratory Press.
- Detke, S., Katakura K., and Chang K.-P. (1989). *Exp. Cell Res.* **180**, 161–170.
- Borst, P., Baas, F., Lincke, C.R., Ouellette M., Schinkel, A.H., and Smit, J.J.M. (1992). In: *Proceedings Bristol-Myers Squibb Symposium on Cancer Research, 'Drug resistance as a biochemical target in cancer chemotherapy'*. Tsuruo, T., Ogawa, M., Carter S.K., eds. **14**, 11–26. Academic Press, NewYork, NY.
- Garvey, E.P., and Santi, D.V. (1986). *Science* **233**, 535–540.
- Hightower, R.C., Metge, D.W., and Santi, D.V. (1987). *Nucleic Acid Res.* **15**, 8387–8398.
- Beverley, S.M. (1988). *Nucleic Acid Res.* **16**, 925–939.
- Van der Ploeg, L.H.T., Liu, A.Y.C., and Borst, P. (1984). *Cell* **36**, 459–468.
- Meyer, T.F., Gibbs, C.P., and Haas, R. (1990). *Annu. Rev. microbiol.* **44**, 451–477.
- Kaur, P., and Rosen, B.P. (1992). *Plasmid* **27**:29–40.
- Lee, T.-C., Tanaka, N., Lamb, P.W., Gilmer, T.M., and Barrett, J.C. (1988). *Science* **241**, 79–81.
- Liu, J., Salinas G., Gajendran, N., Muthui, D., Muylidermans, S., and Hamers R. (1992). *Mol. Biochem. Parasitol.* **50**, 351–354.
- Ouellette M., and Borst P. (1991). *Res. Microbiol.* **142**, 737–746.
- Beverley, S.M. and Coburn, C.M. (1990). *Mol. Biochem. Parasitol.* **42**, 133–142.
- Rovai, L., Tripp, C., Stuart, K., and Simpson, L. (1992). *Mol. Biochem. Parasitol.* **50**, 115–126.
- Windle, B., Draper, B.W., Yin, Y., O'Gorman, S., and Wahl, G. (1991). *Genes Develop.* **5**:160–174.
- Gottesdiener, K.M., Goriparthi, L., Masucci, J.P., and Van der Ploeg, L.H.T. (1992). *Mol. Cell. Biol.* **12**:4784–4795.
- Cruz, A. & Beverley, S.M. (1990). *Nature* **348**, 171–173.
- Lee, M.G.-S. and van der Ploeg, L.H.T. 1990. *Science* **250**, 1583–1586.
- Ten Asbroek, A.L.M.A., Ouellette, M., & Borst, P. (1990). *Nature* **348**, 174–175.