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Recurrent polymorphisms in small chromosomes of *Leishmania tarentolae* after nutrient stress or subcloning

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Molecular karyotypes of the UC, LEM87 and LEM115 *Leishmania tarentolae* strains were obtained. All strains had 24–28 chromosomal bands which varied in size between 300 kb and 2.9 Mb. Several recurrent chromosomal polymorphisms occurred in LEM115 after nutrient shock or subcloning. One type of polymorphism involves the truncation of a 365-kb chromosome which contains the miniexon genes. This specific chromosome breakage appears to be induced by the nutrient shock or subcloning process and also occurs spontaneously during routine passage. Another polymorphism is the appearance of a 90-kb minichromosome (115-SNA1) after severe nutrient shock. This appears to be selection of a pre-existing cell type from a mosaic population. The 115-SNA1 minichromosome has sequence homology with a minichromosome in LEM87 cells but shows no homology with any chromosomes in 115wt or other strains. The copy number of 115-SNA1 varies with culture conditions, suggesting a relaxed centromeric control. The nature and origin of this minichromosome is not known.

Key words: *Leishmania tarentolae;* Polymorphism; Nutrient stress; Subcloning

Introduction

The development of pulsed field gel technology [1,2] has allowed the resolution of multiple uncondensed chromosomes in kinetoplastid protozoa [3] which exhibit a high degree of inter- and intra-specific polymorphism. Several mammalian *Leishmania* species have been shown to possess 22–28 chromosomal bands ranging in size from 200–2200 kb [4–7]. The appearance of small multicopy chromosome bands in some strains has been explained as selection from a mosaic population or mobilization from a genomic reservoir [8,9]. In the case of the mammalian *Leishmania*, both intra- and inter-specific chromosomal polymorphisms have been reported [5,6,8–10]. A mobilization of closed circular DNA molecules encoding specific nuclear DNA sequences in response to drug pressure has been observed in cultured *Leishmania tropica* [11–15]. Spontaneous amplification of the mini-exon array and changes in chromosome size have been shown to occur in cultures of *Leishmania major* [16]. Chromosomal rearrangements in *Leishmania infantum* have been shown to occur during the cloning process and a suggestion was made that strains possess a 'mosaic' structure with different cells possessing homologous chromosomes of different sizes due to frequent DNA amplification/deletion events [9]. Another phenomenon that has been observed in several species of mammalian leishmania is the appearance of small linear chromosomes, which in some cases exhibit inter-specific cross-hybridization. For example, the LD-1 element occurs in several species of *Leishmania* as a small linear chromosome and in some strains as a circular extrachromosomal DNA

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Abbreviations: SNA, small nucleic acid; CHEF, contour-clamped homogeneous electric field; OFAGE, orthogonal field alternating gel electrophoresis.

(CD-1) [17]. In *L. major* a class of small linear chromosomes appeared under varying culture conditions and it was suggested that these minichromosomes may arise from a conserved chromosomal reservoir [18].

The lizard *Leishmania* spp. represent a subgroup of the genus *Leishmania* [19], and *L. tarentolae* has been used extensively as a model system for the analysis of mitochondrial genetic expression [20]. Two subgroups of *L. tarentolae* have been defined based on kinetoplast DNA sequences: subgroup A consists of the UC, T and K strains, which were originally derived from an Algerian gecko isolate; subgroup B consists of the LEM87, 115, 124, 125, and 306 strains, which were isolated from geckos in southern France [19].

In this paper we describe the molecular karyotypes of several of these strains of *L. tarentolae* and we describe specific chromosomal polymorphisms which occur after nutrient shock or subcloning in the LEM115 strain.

Materials and Methods

Cell lines. Cells were cultured in Difco brain heart infusion medium (BHI) at 27°C as described previously [19]. The *L. tarentolae* strains used include the UC strain from subgroup A and the LEM87 and LEM115 strains from subgroup B [19]. The medium was supplemented with 10% inactivated fetal bovine serum and 10 μg ml^{-1} tryptophan for LEM115 and LEM87 strains. Strain LEM115wt represented an uncloned stock. Strain LEM115A represents cells which grew out from a LEM115wt culture after a nutritional shock in which most of the cells had died after 15 days at 27°C without change of medium. Strain LEM115A1 was derived without any apparent selection from a single culture of LEM115wt cells during serial passage.

Several clonal lines were derived from LEM115wt, 115A and 115A1 cell populations either by plating on 0.8% agar prepared with BHI-hemin and 20% fetal bovine serum or by selection of single cells in microdrops of culture medium. Several of the clonal lines derived from the wt stock culture had the 115A1 karyotype and therefore were labeled 115A1.

Other cell lines used include the *L. tarentolae* Subgroup A T strain, the *L. tarentolae* subgroup B LEM124, LEM125, and LEM306 strains and *Leishmania hertigi* [19].

Preparation of chromosomal DNA. Cells were embedded in low melting agarose blocks and processed as described previously for pulsed field gel electrophoresis [19]. Electrophoresis was performed at 12°C, with voltage, agarose concentration and switch time varying according to the experimental schedule. Both OFAGE and CHEF gels were employed. Chromosomes from *Schizosaccharomyces pombe* strain 972h and *Saccharomyces cervisiae* strain YNN295 (BioRad Laboratories) and lambda phage multimers (Clontech) were used as size markers. DNA from individual bands was recovered by electroelution in dialysis tubing.

Hybridization probes. All probes were gel-purified plasmid insert DNAs radioactively labeled with [^{32}P]dATP by nick translation as described [21], with the exception of the telomeric sequence oligomer, which was 5′ end-labeled with gamma-[^{32}P]ATP by T4 kinase. The LR1 and LD1/CD1 probes were obtained from Ken Stuart and the p7R50-p19 probe from Steve Beverley. The telomere probe was a 24-mer synthetic oligomer containing the repetitive sequence CCCTAA [22]. The mini-exon probe was a cloned 417-bp mini-exon repeat from *L. tarentolae*, obtained from D. Campbell. The α-tubulin gene from *L. enrietti*, pLT1, contains a 2-kb insert that contains the gene repeat unit [23,24]. The pP121/3 plasmid from *L. major*, contains a 850 bp insert with the β-tubulin repeat unit [5]. The ribosomal RNA gene probe is from a cosmid clone (cost10) of *T. brucei* genomic DNA [25]. The pLTU1 plasmid contains a 220 bp fragment from the ubiquitin gene of *L. tarentolae* (J. Fleishmann and D. Campbell, personal communication). The P100/11E plasmid contains 1.2 kb from the coding region of aldolase

reductase from *L. major* [26]. pTR15 has a 15 kb fragment of amplified extrachromosomal DNA from tunicamycin-resistant *Leishmania mexicana amazonensis* that contains the *N*-acetylglucosamine-1-phosphotransferase gene [27]. The calmodulin probe is a 500-bp cDNA from *T. brucei* in pUC8 (Wong and Campbell, personal communication). The pPDI plasmid contains a 500-bp fragment from the protein disulphide isomerase gene from *T. brucei* strain 425 [28].

Cloning of 115-SNA1. Five *Hin*dIII fragments and one *Eco*RI/*Bam*HI fragment from the gel-isolated 115-SNA1 band were cloned into pGEM7 (Promega) and recombinants selected. Plasmid minipreps were by the method of Holmes and Quigley [29]. Restriction maps were constructed for all of the recombinant clones (data not shown); all proved to be unique and non-overlapping.

Hybridization conditions. Agarose gels were blotted by diffusion onto BA83 filters as described [21]. Hybridization was carried out for 18 h at 65°C in Blotto using 1×10^6 cpm ml^{-1} of probe DNA. Filters were washed for 30 min at room temperature in 2 × SSC/0.1% SDS, followed by 2 washes in 0.1 × SSC/0.1% SDS, 45°C, and then subjected to autoradiography.

Results

Karyotypes of five strains of L. tarentolae. Pulsed field gel karyotypes of the UC, LEM115 and LEM 87 strains of *L. tarentolae* are presented in diagrammatic form in Fig. 1. The LEM115A and LEM115A1 substrains will be discussed below. These patterns were obtained by running gel electrophoretic separations under both CHEF and OFAGE conditions, using multiple pulse times either in different runs or in the same run. Compression bands which contained multiple chromosomes of different sizes as well as non-specifically trapped DNA were observed under all pulse time conditions in different regions of the gels and represented the major artifact of this technique. Resolution of chromosomes trapped in compression bands was accomplished by electrophoresis under different pulse time conditions or by combining several pulse time plateaus in one separation.

All three strains contained at least 24 distinct bands of varying fluorescent intensities after staining with ethidium bromide. The apparent non-stoichiometric intensities of several of the bands suggests either comigration of additional chromosomes of the same size or aneuploidy. All chromosome bands appear to be linear since the apparent molecular sizes did not change with pulse frequency. The wells retained variable amounts of DNA, which probably consisted of molecules which were not released by the lysis conditions employed or which possessed structural features or molecular associations that prevented migration into the gel.

Strain LEM87 contains several minichromosomes. In addition to the higher molecular weight bands, strain LEM87 contained four small bands, labeled 87-SNA1-4 (SNA = 'small nucleic acid') (Fig. 2, lane 1). The 230-kb 87-SNA1 band appeared to be multicopy from the staining intensity.

Chromosomal localization of several specific genes and gene families. The results of hybridization of heterologous gene-specific probes to blots of pulsed field gels (data not shown) are presented in the diagram in Fig. 1. The localization of alpha- and beta-tubulin, aldolase reductase, ubiquitin, calmodulin, *N*-acetylglucosamine 1-P transferase, rRNA and protein-disulfide isomerase gene probes to specific bands are shown.

Shortening of chromosome 4 and appearance of a minichromosome in substrains of LEM115. Substrain LEM115A1 represents a karyotypic variant (see Fig. 1 diagram) that appeared without apparent selection during serial passage of log phase LEM115wt cells and overgrew the wt cells in several cultures. As the cells were subcultured, the staining intensity of the

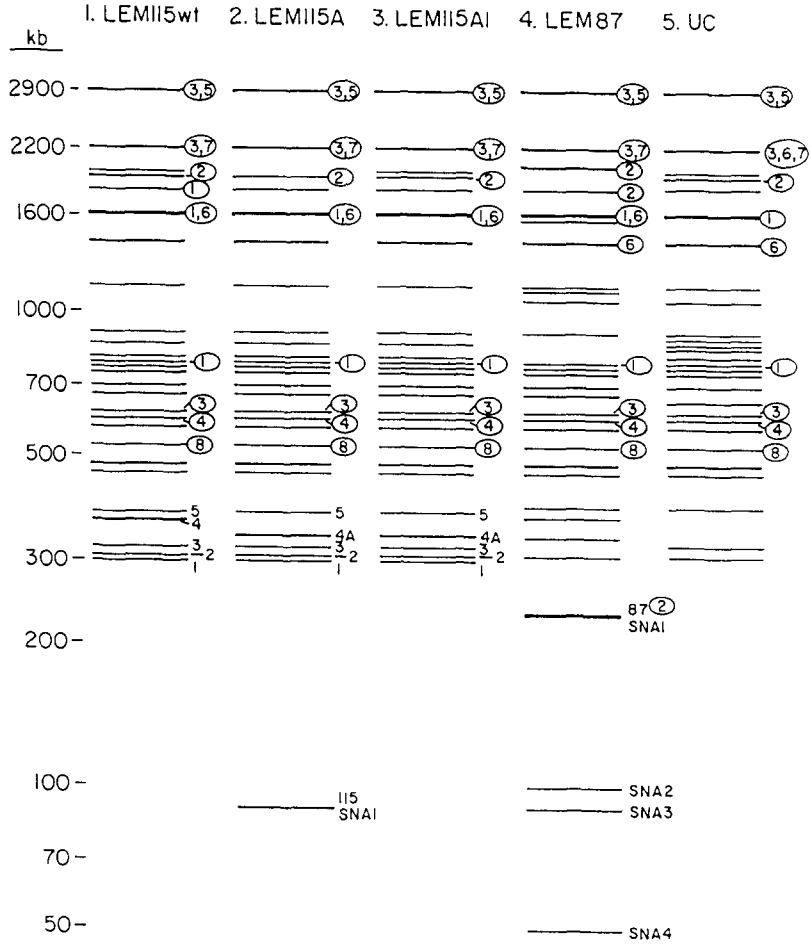

Fig. 1. Molecular karyotype and genetic linkage map of *L. tarentolae*. Each lane represents the chromosome bands visualized by OFAGE and/or CHEF. Approximate sizes were calculated using size markers as indicated in Materials and Methods. Chromosomes 1-4 and 1-4A are indicated, as are the minichromosomes 115-SNA1 and 87-SNA1-4. Chromosomal localizations of specific genes and gene families are indicated by numbers in circles adjacent to the bands. Genes: (1) β-tubulin; (2) aldolase reductase; (3) ubiquitin; (4) calmodulin; (5) *N*-acetylglucosamine-1-phosphotransferase; (6) rRNAs; (7) protein disulfide isomerase; (8) α-tubulin.

365-kb band 4 gradually decreased and finally disappeared, and the 340-kb band 4A appeared and correspondingly increased in staining intensity. The 115A1 karyotype also appeared in several clonal lines derived from 115wt cells (which were an uncloned stock): out of 20 clonal lines, 14 exhibited the wt karyotype, 6 the A1 pattern (loss of chromosome 4 and appearance of chromosome 4A), and 1 had both chromosomes 4 and 4A. These latter cells were recloned, and out of 30 subclones, all exhibited the A1 karyotype.

Another chromosomal polymorphism was observed when LEM115wt cells were subjected to a severe nutritional shock which resulted in death of most of the cells. The survivors which grew out after subculture in fresh medium showed a distinct karyotype and were labeled substrain LEM115A. LEM115A cells showed two polymorphisms: band 4 disappeared and 4A appeared (Fig. 2, lane 3), as in the 115A1 cells described above; in addition, a new high copy number 90-kb minichromosome band appeared. This minichromosome was labeled 115-SNA1. LEM115A cells lacked a 2-kb chromosome which was present in both

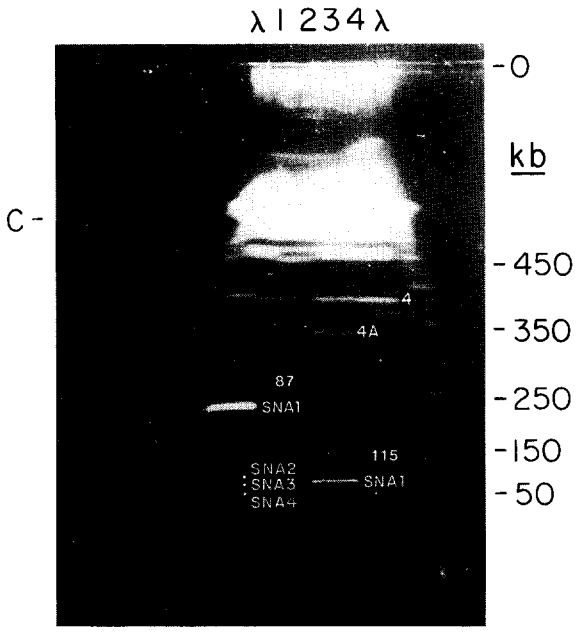

Fig. 2. Separation of small chromosomes of LEM115 strains by OFAGE. The 'O' marks the origin and 'C' the position of the compression band. Lane 1, LEM87; 2, UC; 3, LEM115A; 4, LEM115wt. A λ multimer size marker is shown on the far left and right lanes. OFAGE gel was run for 40 h at 250 V with pulse time of 35 s. Sizes of LEM87 small chromosome bands: 87-SNA4, 50 kb; 87-SNA3, 90 kb; 87-SNA2, 100 kb; 87-SNA1, 230 kb; band 1, 300 kb; band 2, 335 kb; band 3, 370 kb; band 4, 390 kb. Sizes of UC small chromosomes: bands 1 and 2, 320 kb; band 3, 380 kb.

115wt and 115A1 cells. This polymorphic marker indicates that 115A1 cells are derived from the major clonal line in the 115wt stock and that 115A cells are derived from a minor cell type in that mosaic population.

Evidence for breakage of chromosome 4 in 115A and 115A1 cells. DNA gel-isolated from band 4 of LEM115wt cells showed strong hybridization only to itself in 115wt chromosomal blots and to band 4A in 115A and 115A1 chromosomal blots (data not shown). DNA gel-isolated from band 4A of LEM115A1 cells showed hybridization to itself in 115A and 115A1 cells, and to chromosome 4 in wt cells (Fig. 3). The additional faint lower band in lanes 2 and 4 represents hybridization to chromosome band 3, due to unavoidable cross contamination of the gel-isolated 4A probe with band 3 DNA. These data indicate that band 4A represents a truncated chromosome 4. The expected 5-kb cognate fragment has not yet been identified.

Chromosome 4 contains the mini-exon array. A mini-exon hybridization probe identified only chromosome 4 in 115wt cells and chromosome 4A in 115A and 115A1 cells (Fig. 4). This probe also hybridized with a large chromosome in all three strains (data not shown). These results confirm that chromosome 4A represents a truncated version of chromosome 4, and suggest that mini-exon expansion/deletion phenomena may contribute to this polymorphism.

Sequence homologies of 115A-SNA1 minichromosome. Gel-isolated 115-SNA1 DNA from LEM115A cells hybridized with another minichromosome band of 90 kb from LEM87 (band 87-SNA3), but did not hybridize with a minichromosome band from *L. hertigi* (Fig. 5). The blot in Fig. 6 shows that 115-SNA1 DNA did not hybridize with any small or large chromosomes from 115wt, 115A or 115A1 cells. Hybridization was observed to DNA in the well and, in this gel, to DNA in the artifactual compression band running at approximately 1600 kb under this specific combination of pulse time conditions. This compression band was resolved into several large chromosomes (see Fig. 1) in separate gels using different pulse times and no hybridization of the 115-SNA1 probe was detected (data not shown). In addition, cloned DNA fragments from 115-SNA1 showed no hybridization to any chromosome other than itself (data not shown). The genetic origin of the 115-SNA1 DNA remains an open question. The possibility exists that it is mobilized from a high molecular weight DNA trapped in the well. It is also possible that it is derived from an extrachromosomal genetic element.

The high copy number 230-kb minichromosome in LEM87 DNA (band 87-SNA1) was gel-isolated and the DNA used as a hybridization probe. As shown in Fig. 7, the probe hybridized with itself and showed a faint hybridization to 115-SNA1. Since the probe

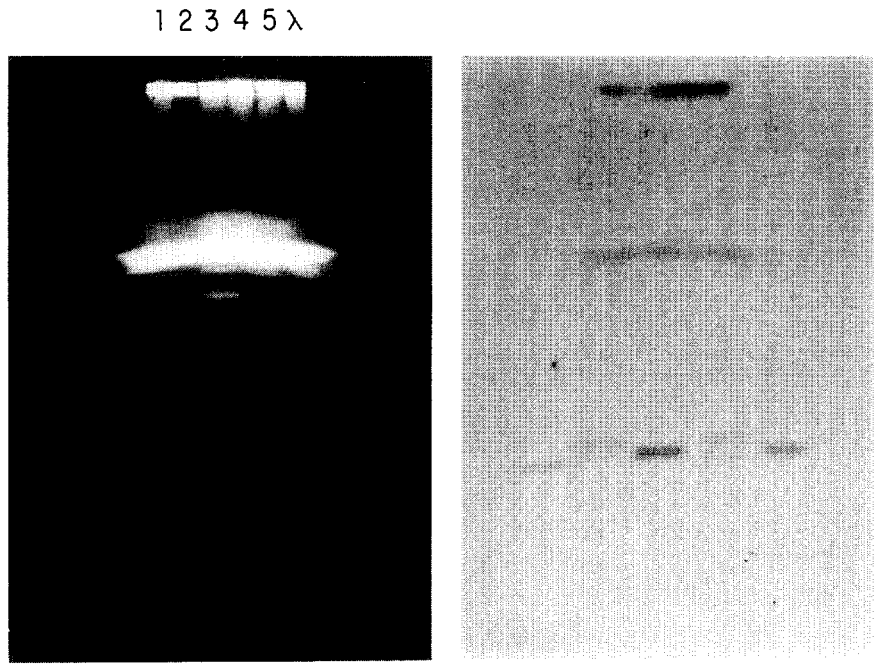

Fig. 3. Chromosome band 4 DNA from LEM115wt has sequence homology with chromosome band 4A DNA from LEM115A and LEM115A1. Gel-isolated band 4A DNA from LEM115A was used as a hybridization probe. Lane 1, UC; 2, LEM87; 3, LEM115A1 clone 20; 4, LEM115wt clone 1; 5, LEM115A clone 8. OFAGE gel was run for 24 h with a pulse time of 35 s.

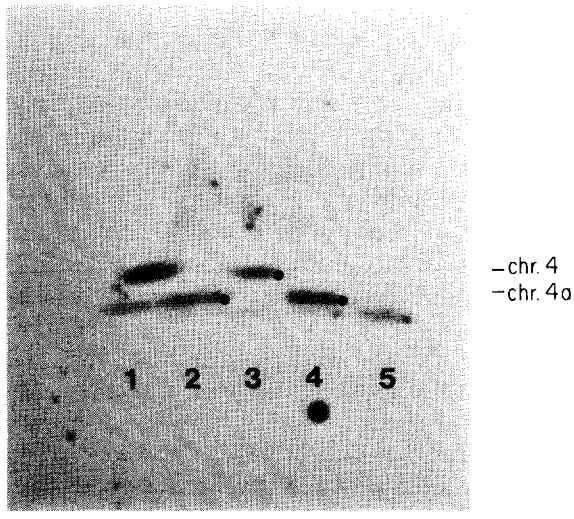

Fig. 4. Mini-exon genes are localized on chromosome band 4 from LEM115wt and chromosome band 4A from LEM115A and LEM115A1. Hybridization probe was the cloned *Crithidia fasciculate* mini-exon repeat. Lane 1, LEM87. 2, LEM115A1. 3, LEM115wt. 4, LEM115A. 5, UC. OFAGE gel was run for 48 h at 200 V with pulse time of 35 s. The positions of chromosomes 4 and 4a in LEM115wt and 115A1 are shown on the right.

was gel-isolated and probably was unavoidably contaminated with the closely migrating 87-SNA3 band, we attribute the hybridization to 115-SNA1 to this cross contamination and conclude that there is probably no sequence homology with 115-SNA1. There was some hybridization with a few large chromosomes in LEM115wt, LEM115A and LEM87. This latter localization is consistent with our finding (Fig. 1) that aldolase reductase genes are localized on the 87-SNA1 minichromosome and on two large chromosomes in this strain.

115-SNA1 from LEM115A did not show sequence homology with a cDNA probe from the LR1 RNA virus from *L. braziliensis guyaniensis* [30] (data not shown), nor with the LD1/CD1 circular extrachromosomal DNA from *L. donovani* ITMAP-263 [17] (data not shown), nor or with the p7R50-P19 plasmid containing a fragment from a mini-chromosome (SLD 715) from *L. major* clone 7-R50 [31] (data not shown). The SLD-715

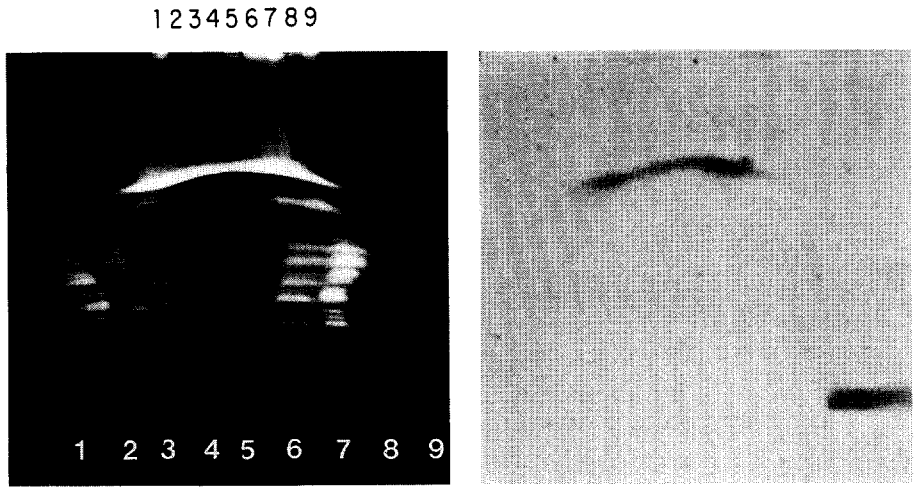

Fig. 5. The 115-SNA1 minichromosome shares sequence homology with the 87-SNA3 minichromosome. The hybridization probe was gel-isolated 115-SNA1 DNA. Lanes 1, UC; 2, *L. hertigi*; 3, *L. tarentolae* T strain; 4, LEM306; 5, LEM124; 6, LEM125; 7, LEM115wt; 8, LEM115A; 9, LEM87. OFAGE gel was run at 200 V for 40 h with a pulse time of 70 s. The *L. hertigi* minichromosome is not visible in the stained gel.

probe, however, did hybridize with the 87-SNA1 minichromosome band from LEM87 (data not shown).

The SNA1 minichromosome from LEM115A contains linear DNA and has telomeres. The presence of repetitive CCCTAA telomeric sequences was examined by hybridization with an oligomeric probe. All chromosomes contained telomeric sequences, including the minichromosomes 115-SNA1, 87-SNA1, 87-SNA2 and 87-SNA3 (data not shown).

Irradiation of agarose blocks with up to 32

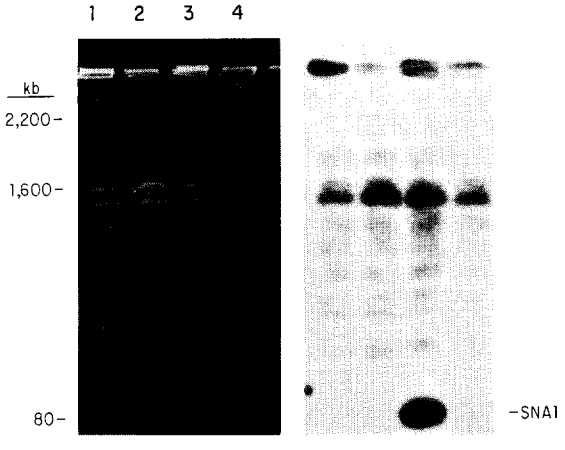

Fig. 6. Lack of hybridization of 115-SNA1 DNA with chromosomes of LEM115wt or LEM115A1. The hybridization probe was gel-isolated 115-SNA1 DNA. Lanes 1, LEM115wt clone 1; 2, LEM115A1 clone 20; 3, LEM115A clone 8; 4, LEM115wt clone 1. The CHEF gel was run at 130 V for 24 h using a pulse time of 70 s, followed by 24 h using a pulse time of 270 s. The strong hybridization at 1600 kb is a compression band artifact caused by these pulse-time conditions.

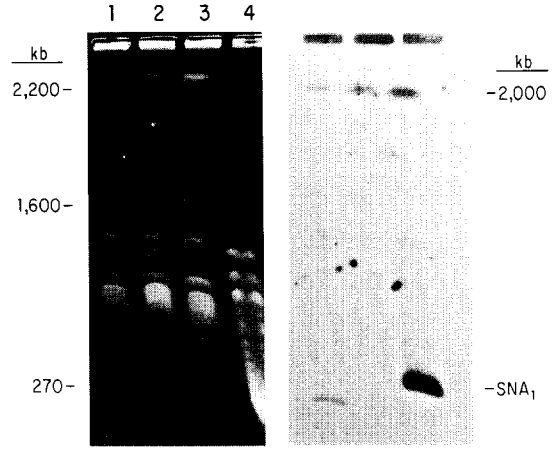

Fig. 7. 87-SNA1 DNA does not hybridize with 115-SNA1 DNA. Lane 1, LEM 115A clone 8. 2, LEM 115 wt clone 1. 3, LEM 87. Yeast, *S. cerevisiae* marker. The 87-SNA1 DNA was gel-isolated and labeled in vitro for use as a hybridization probe. The faint cross-hybridization to the 115-SNA1 band is due to contamination of the probe with 87-SNA3 DNA, which is known to hybridize with 115-SNA1 DNA. The CHEF gel was run at 120 V for 48 h using a pulse time of 270 s, followed by 4 h using a pulse time of 200 s.

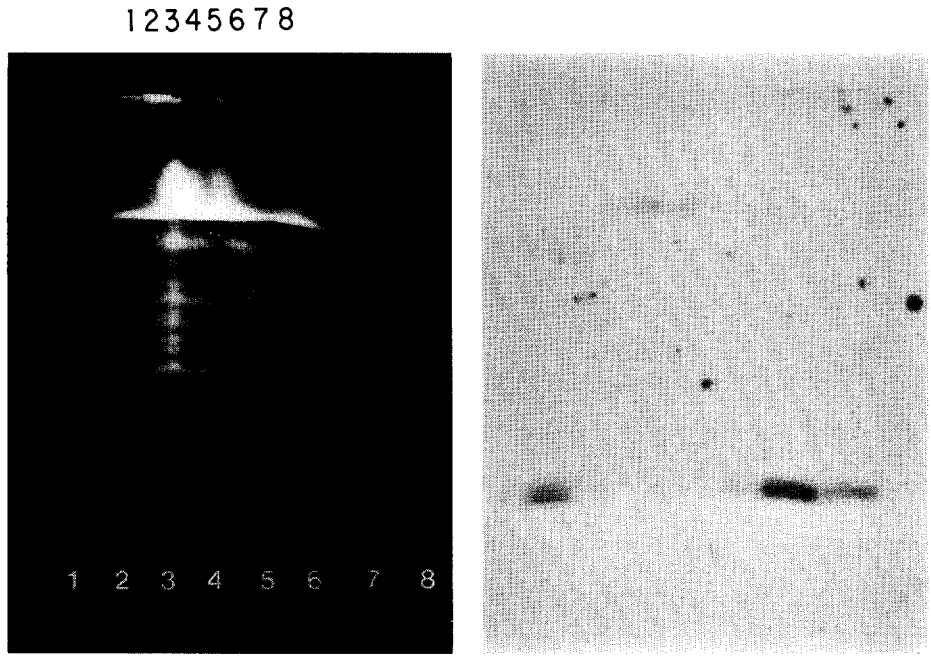

Fig. 8. Effect of serum concentration and growth conditions on copy number of the 115-SNA1 minichromosome band. Lanes 1, LEM115A, 20% serum, 16 days in stationary phase; 2, LEM115A, 20% serum, 13 days in exponential phase followed by three days in stationary phase; 3, LEM115A, 20% serum, 47 days in log phase; 4, LEM115A, 20% serum, 38 days in log phase; 5, LEM115A, 20% serum, 28 days in log phase; 6, LEM115A, no serum, 45 days in log phase; 7, LEM115A, with 10% serum; 8, LEM 115wt.

kRad gamma radiation had no effect on the gel mobility of the 115-SNA1 minichromosome band, suggesting that 115-SNA1 contains linear DNA (data not shown). In line with these results, the SNA1 band was also susceptible to digestion with exonuclease III and BAL 31 nuclease (data not shown).

The copy number of the SNA1 minichromosome from LEM115A varies with culture conditions. During subculture of LEM115A cells for 45 days in BHI medium without serum, the copy number of the 115-SNA1 minichromosome increased several fold relative to that from cells grown for the same time in 10% serum (Fig. 8, lane 6). Subculture of log phase 115A cells for 28 days in medium with 20% serum resulted in a 50% decrease in the 115-SNA1 copy number (lane 5). After ten days (lane 4) or 18 days (lane 3) further subculture in 20% serum, the 115-SNA1 band was no longer visible. Cell division in the serum-containing medium was required to bring about the loss of the SNA1 band, probably by selective inhibition of replication and subsequent dilution. This was shown by maintaining 115A cells in stationary phase for 28 days in 20% serum and observing no decrease in SNA1 copy number (lane 1). However, stationary phase by itself did not cause the recovery of the SNA1 band, as shown by maintaining cells in 20% serum in log phase for 13 days and then in stationary phase for 3 days and observing a significant decrease in SNA1 copy number (lane 2).

We also observed that several clonal lines of LEM115A cells, obtained by plating on agar, also exhibited a decrease in 115-SNA1 copy number when cultured in medium with 20% serum (data not shown). Subsequent subculture of these clonal lines in serum-free medium caused a gradual increase in the 115-SNA1 copy number (data not shown). These results indicate that the change in copy number of the SNA1 minichromosome by growth under different culture conditions is not the result

of selection of a subpopulation from the original uncloned stock.

Discussion

The distribution of chromosomes from *L. tarentolae* is similar to that from the mammalian leishmania and quite dissimilar to that from the African trypanosomes, which have approximately 100 minichromosomes in the 50–150 kb range, five chromosomes in the 200–430 kb range and nine chromosomes in the 680 kb to 3 Mb range [32]. This karyotypic similarity agrees with the taxonomic localization of this lizard *Leishmania* as a subgroup in the genus *Leishmania*. The extent of intraspecific chromosomal polymorphisms in the various *L. tarentolae* strains is also consistent with the extent of polymorphisms observed to occur in several mammalian *Leishmania* species. We estimated a minimum number of 25 chromosomes for LEM115A and LEM87, 26 for LEM115wt and 28 for UC.

Genes for α- and β-tubulin, ubiquitin, calmodulin and protein-disulfide isomerase reside on chromosomes of similar sizes in all five strains examined, indicating that these chromosomes are indeed homologous. The aldolase reductase genes are localized on a single chromosome in the LEM115 and UC strains, but on 3 chromosomes of different sizes in the LEM87 strain. In some cases, specific genes are localized on chromosomes of different sizes in different strains, even though chromosomes of the same size exist in the different strains. For example, all of the lines have 1800 kb, 1600 kb and 1400 kb chromosomes, but the rRNA genes are not consistently localized in a band of the same size. Another example is the dispersion of β-tubulin genes to an additional 1800 kb chromosome in LEM115wt. The α-tubulin genes in all strains are located on a 525 kb DNA chromosome and are unlinked to the β-tubulin genes. These latter results are consistent with previous observations [5] in *L. major*, which showed a unitary α-tubulin gene localization and multiple β-tubulin localizations.

Most of the gene localizations determined in this study are to the large and intermediate-sized chromosomes, several of which exhibit an apparent heterogeneity in copy number as determined by relative intensity of staining with ethidium bromide. The question of chromosomal ploidy in kinetoplastids is still far from settled. Diploidy has been suggested for the *T. brucei* nucleus as a whole by comparison of DNA single copy complexity with DNA measurements [33], for *T. brucei* housekeeping genes [32,34] and the *L. major* dihydrofolate reductase-thymidylate synthetase gene (chromosome V) and mini-exon locus (chromosome II) by molecular cytogenetic analysis [16], and for the *L. donovani* adenine phosphoribosyl transferase locus by biochemical genetic analysis [35]. However, several workers have noted that many bands are often not stoichiometric in staining intensity and have suggested the possibility of aneuploidy or differences in sizes of homologous chromosomes for some chromosomes to explain these observations [7–9,32]. This remains an unsettled question.

We have also analyzed two karyotypic changes that occurred in the LEM115 strain of *L. tarentolae* during subculture. One change, which occurred both during routine subculture and after single cell cloning, involves a breakage of chromosome 4, producing a truncated chromosome 4A lacking approximately 25 kb. Since the mini-exon array is localized to this chromosome, and since expansion of the mini-exon array has been observed to occur in *L. major* in the apparent absence of selection [16], we suggest that miniexon expansion/deletion phenomena may contribute to this polymorphism.

The second chromosomal change involved the appearance of a high copy number 90-kb minichromosome, 115-SNA1. This was shown, however, to be the result of the selection of a pre-existing variant from the original stock population after a nutrient shock. The original genomic origin of 115-SNA1 is unknown since homologous sequences were apparently not present in any of the larger chromosomes in 115wt cells. However, substantial sequence

homology was observed with another 90-kb minichromosome found in LEM87 cells (87-SNA3). It is possible that this genetic element is derived by mobilization of a genomic sequence from a chromosome trapped in the well, but it is equally possible that it is derived from an extrachromosomal viral-like entity which somehow has acquired telomeres. The loose copy number control of the 115-SNA1 minichromosome is consistent with this interpretation, but further work is required to establish the genomic origin and the information content of this minichromosome.

The appearance of small linear chromosomes in the absence of any obvious selective pressure has previously been observed in *L. major* [18]. However, the SDL-715 probe from *L. major* did not hybridize with 115-SNA1, but it did hybridize with a high copy number minichromosome from LEM87 87-SNA1. Both the *L. major* SDL-715 and the *L. tarentolae* LEM87 87-SNA1 show sequence homologies with genomic sequences in large chromosomes, unlike the LEM115 115-SNA1.

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