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Electrophoretic Karyotype and Chromosome Assignments for a Pathogenic and a Nonpathogenic Strain of *Entamoeba histolytica*

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The electrophoretic karyotypes of a pathogenic and a nonpathogenic strain of *Entamoeba histolytica* were determined by pulsed-field gel electrophoresis. A number of previously isolated genes were assigned to specific chromosomal bands. Significant differences between the chromosomal patterns of these strains as well as in the assignment of most genes were found.

Morphologically indistinguishable pathogenic (P) and nonpathogenic (NP) strains of *Entamoeba histolytica* can be differentiated by a number of biochemical and molecular criteria (4, 11, 32), but whether these represent two different species is still unclear (21, 22, 26, 34, 35). The genome of *E. histolytica* and that of related amoebae are unusual in that the rRNA genes (rDNA) are present exclusively as extrachromosomal, apparently self-replicating circles (3, 13, 16). In order to increase our knowledge about the genomic organization of P and NP strains, we have used pulsed-field gel electrophoresis (PFGE) to separate the chromosomes of these strains and to assign several genes to them.

Two strains were investigated in this study. The *E. histolytica* P strain, HM-1:IMSS (clone 6), was grown axenically in TYI-S-33 medium (6). The NP strain, SAW760RR clA, originally cloned by P. G. Sargeaunt, and its P derivative, obtained as described previously (23), were grown together with their associated bacterial floras in the above medium. Freshly harvested trophozoites (10^7 cells per ml) were inserted into agarose blocks and incubated for 7 days at 50°C in a solution containing EDTA (0.5 M, pH 9), *N*-lauryl-Sarkosyl (1%), and proteinase K (2 mg/ml; Boehringer, Mannheim, Germany). Agarose blocks were stored at 4°C in 0.5 M EDTA (pH 9). The bacterial flora associated with strain SAW760RR clA was grown separately in TYI-S-33, and blocks containing bacterial DNA were prepared similarly.

The hexagonal PFGE apparatus used in this study (Pharmacia LKB, Uppsala, Sweden) gave relatively good resolution, although the separation of smaller molecules (<800 kb) was often incomplete. PFGE was performed on 1.2% agarose (IBI, New Haven, Conn.) in 0.5× TBE buffer (30) at 10°C with a constant field strength of 5.3 V/cm. Two sets of conditions were used: (i) P_1 (first pulse) = 5 min, D_1 (first duration) = 20 h; P_2 = 10 min, D_2 = 36 h; P_3 = 20 min, D_3 = 40 h; and (ii) P_1 preceded by a period in which P'_1 = 75 s and D'_1 = 20 h. Molecular size markers prepared from *Saccharomyces cerevisiae* and *Candida albicans* (Clone Tech, Palo Alto, Calif.) were used.

Several probes derived from previously isolated genes were used for Southern hybridization. Labeling was usually done with the Random Primed DNA Labeling Kit (Boehringer) (8, 9). Probes N1 and N2 (untranslated regions of the actin genes; see below) were end labeled by polynucleotide kinase (USB, Cleveland, Ohio). The *E. histolytica* paramyosin gene (1) was from Nancy Guillen (Pasteur Institute, Paris, France). The galactose-specific lectin gene of NP *E. histolytica* (20) was from B. Mann, University of Virginia, Charlottesville. The DNA transfer to Magna membrane (MSI, Westborough, Mass.) was performed under neutral conditions. High-stringency hybridizations and washing conditions were as described previously (14).

A schematic representation which summarizes the data on electrophoretic karyotypes of *E. histolytica* strains obtained from various PFGE gels stained with ethidium bromide or hybridized with radioactive probes is presented in Fig. 1. The resolution achieved with different ranges in molecular size varied with the electrophoretic conditions used. Up to 16 bands could be observed after ethidium bromide staining (Fig. 2). These karyotypes somewhat resemble the electrophoretic karyotypes previously described (26, 37). Several additional bands were observed only after hybridization with radiolabeled probes (Fig. 1). The nonstoichiometric intensities of several bands (Fig. 2) observed under all conditions used suggest that they may contain multiple heterologous chromosomes of the same size or that certain molecules may have higher ploidies. As has been previously noted for several other protozoans (12, 33, 38), some DNA appears to remain trapped in the wells, indicating that a full separation was not accomplished. Furthermore, a diffuse wide band was seen in the upper part of the lanes containing the P and NP forms of strain SAW760RR clA (Fig. 2). This band, which in separate gels was shown to contain all of the bacterial DNA (data not shown), is absent from the lane containing the axenically grown strain HM-1:IMSS (Fig. 2).

Significant differences between the band patterns of the strains tested can be seen. For example, a band (2,600 kb) found in the P form of SAW760RR clA is absent from the NP form, while another band (1,820 kb) is unique to the NP strain (Fig. 2). The reason for these differences between the P and NP forms of this *E. histolytica* strain is not yet clear.

The mobilities of relatively small circular DNA molecules in PFGE are known to differ from those of linear molecules

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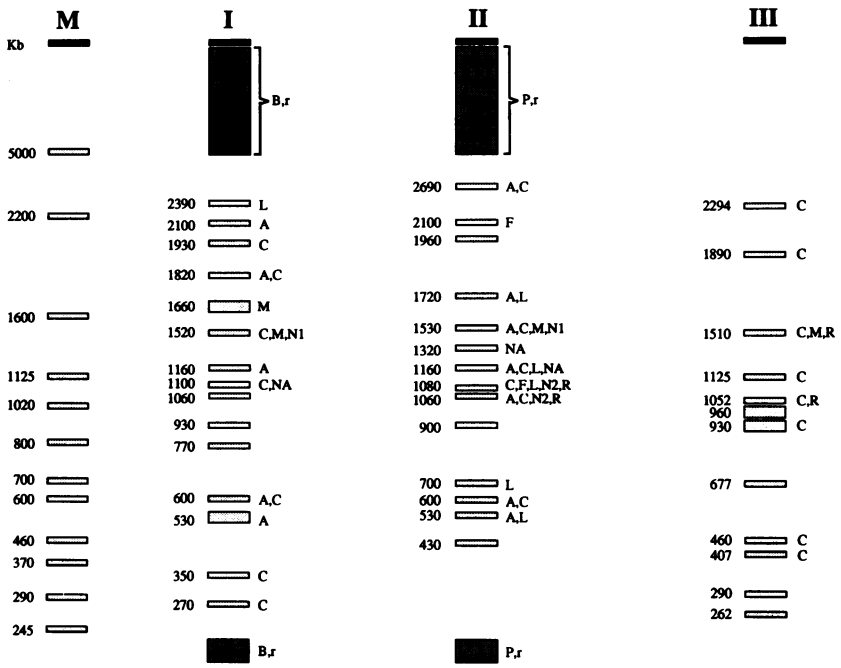

FIG. 1. Diagrammatic representation of electrophoretic karyotype of *E. histolytica* NP SAW760RR clA (I), P SAW760RR clA (II), and HM-1:IMSS cl6 P (III). The molecular sizes of the bands marked (in kilobases) were calculated from the estimated sizes of *S. cerevisiae* and *C. albicans* chromosomes which were used as standards for each PFGE gel. A number of bands included in the diagram were detected by Southern hybridization. The width of the chromosomal bands is representative of the relative ethidium bromide fluorescence or radioactive labeling observed. The chromosomal assignment of the genes tested is summarized. C, actin conserved coding region probe (450 bp) (14); N1, actin 5′ untranslated region of 1,000 bp (5′) (14); N2, actin 5′ untranslated region of 141 bp (7); A, 70-kDa P-specific antigen isolated by monoclonal antibody (MAb) E8.5 (1,400 bp) (25); L, *N*-acetylgalactosamine-specific lectin from NP strain SAW760RR clA (20); M, paramyosin probe (2,200 bp) (1); NA, probe of the 60-kDa surface antigen specific for NP strains isolated by MAb 318-28 (600 bp) (24); F, ferredoxin gene (cDNA probe) (190 bp) (15); R, ribosomal protein L21 (cDNA probe) (600 bp) (27); P, tandemly repeated DNA segments specific to P strains (145 bp) (11); B, tandemly repeated DNA segments specific to NP strains (133 bp) (11); r, structural region of 25S extrachromosomal element containing rDNA (2,200 bp) (16).

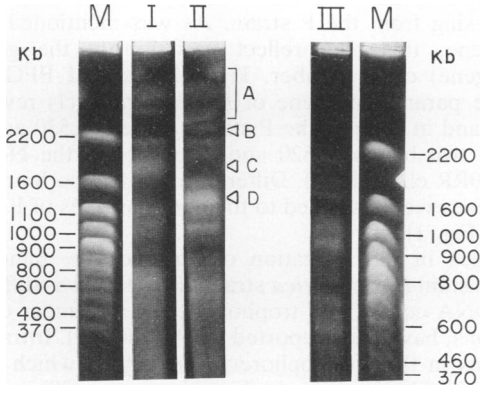

FIG. 2. Ethidium bromide staining of PFGE gels. Lanes: M, molecular size markers; I, NP SAW760RR clA; II, P SAW760RR clA; III, HM-1:IMSS cl6 (P). Molecular sizes (in kilobases) are marked beside each chromosomal band. Bands: A, diffuse zone where the bacterial DNA and the circular rDNA molecules migrate; B, 2,600-kb band found in the P form of SAW760RR clA; C, 1,820-kb band most likely containing multiples of chromosomes, unique to the NP form of SAW760RR clA; D, band suspected to contain multiple chromosomes in P SAW760RR clA.

(2, 31). Most of the extrachromosomal circular rDNA molecules of *E. histolytica* (16) migrated in the diffuse zone at the top of the gels (Fig. 2). Hybridization with the rDNA probe B22, which consists of structural rDNA sequence (25S), as well as probes P145 and B133, which are derived from tandem repeats that are exclusively amplified in P and NP strains, respectively (11), resulted in three labeled domains (Fig. 3). Hybridization was detected at the origin of the gel, in the diffuse zone, and in a fast-migrating band at <200 kb. A similar hybridization pattern, which is indicative of the different topological forms of the circular molecule, was obtained when the H16 plasmid containing a 16-kb fragment of the rDNA molecule (16) was run under the same conditions (data not shown). None of the above amoebic rDNA probes cross-hybridized with the bacterial DNA when run separately on PFGE gels (data not shown).

Two copies of genes in the *E. histolytica* actin gene family have been fully characterized (7, 14). The two genes were found to be almost identical in their coding regions, but they differ significantly in their 5′ and 3′ untranslated flanking regions. Hybridizations with probe C, the coding region probe, enabled us to locate this gene on six and seven bands in the P and NP forms of strain SAW760RR clA, respectively (Fig. 4). This resembles the findings on *Acanthamoeba castellanii* (28) and *Naegleria gruberi* (5), in which actin genes are spread on six to nine distinct chromosomes. Hybridization with probe N1, consisting of the 5′ noncoding region of the actin gene isolated in our laboratory (14), gave

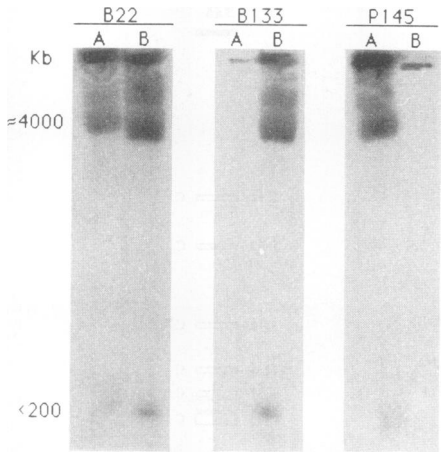

FIG. 3. Southern hybridization of PFGE blots with probes P145 and B133, derived from tandemly repetitive elements of the extrachromosomal circular molecules of P or NP *E. histolytica* (11, 16), and probe B22, consisting of common rDNA structural region of this molecule (16). The filters were blotted as described previously, hybridized, washed under high-stringency conditions, and exposed to X-ray film for 45 min at room temperature. Lanes: A, NP SAW760RR clA; B, P SAW760RR clA. Molecular sizes (in kilobases) are at the left.

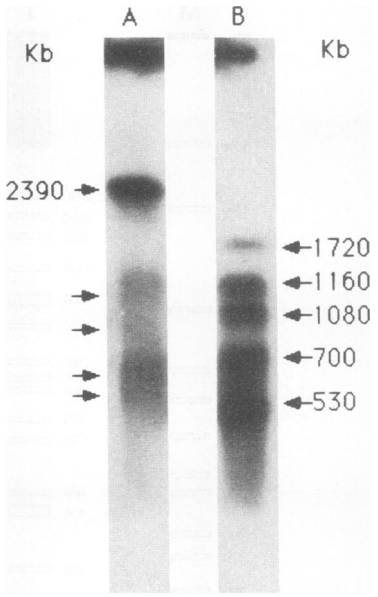

FIG. 5. Southern hybridization of PFGE blots with Gal-specific lectin probe (20) isolated from NP strain SAW760RR clA. Lanes: A, NP SAW760RR clA; B, P SAW760RR clA. The blot was hybridized and washed under high-stringency conditions as described previously and exposed for 20 h at −70°C with an intensifying screen. Molecular sizes (in kilobases) are indicated by arrows.

only one band in either P or NP strains coinciding with the actin band at 1,530 kb (Fig. 4). Hybridization with probe N2, containing the 5′ flanking domain of the second actin gene (7), gave bands which coincided with two other actin bands (1,080 and 1,060 kb, Fig. 1). These two bands also hybridized with the ribosomal protein L21 probe (RP-L21), confirming our previous findings that this actin copy is physically linked to an RP-L21 gene (27). Large differences in the molecular sizes and the intensities of the actin-containing chromosomes were observed (Fig. 4). This may reflect (i) the presence of several actin copies on certain chromosomes, (ii) the existence of pseudogenes possessing interrupted or partial sequences of actin, or (iii) the higher ploidy of a specific chromosome (29).

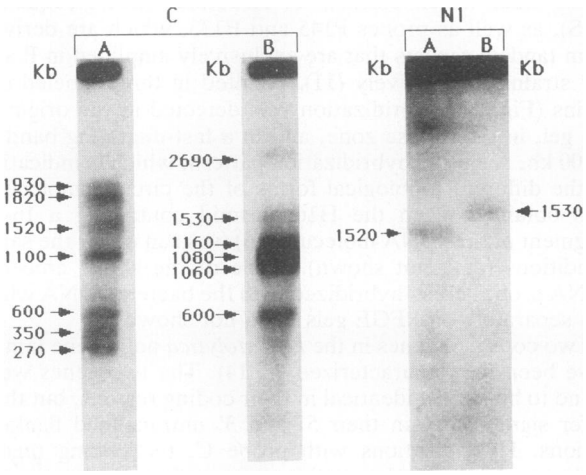

FIG. 4. Southern hybridization of PFGE blots with probe C, containing 450 bp of actin coding region, and probe N1, containing specific 5′ untranslated domain of copy isolated in our laboratory (14). Lanes: A, NP SAW760RR clA; B, P SAW760RR clA. The filters were hybridized and washed under high-stringency conditions and exposed to X-ray film for 24 h at −70°C. Molecular sizes (in kilobases) are indicated by arrows.

Hybridization with the gene coding for the galactose-specific lectin isolated from the NP strain whose sequence has considerable homology to that found in the P strain (20) showed one band with a strong signal at 2,390 kb and a number of less resolved, weaker bands between 1,100 and 500 kb in the NP strain (Fig. 5). Five bands (1,720, 1,160, 1,080, 700, and 530 kb) with similar intensities were obtained for the P SAW760RR clA strain (Fig. 5). The high-molecular-weight band (with a size of 2,390 kb) present in the NP strain was missing from the P strain. As was mentioned for the actin gene, this may reflect variation in the gene (or pseudogene) copy number. Hybridization of PFGE blots with the paramyosin gene of *E. histolytica* (1) revealed a single band in each of the P strains tested (1,530 and 1,510 kb) and two bands (1,520 and 1,660 kb) in the NP strain SAW760RR clA (Fig. 1). Differences were also found when other genes were assigned to the chromosomes of P and NP strains (Fig. 1).

Changes in the migration of chromosome bands of a drug-resistant *E. histolytica* strain (26), as well as differences in the DNA contents of trophozoites grown under different conditions, have been reported previously (19). Intraspecies variations in the electrophoretic karyotype, which involve breakage and healing of subtelomeric regions (29), crossover events (36), and amplifications (10), are a well-documented phenomenon for several other protozoans and have been previously used to distinguish the provenance of isolates (17, 18). Selective pressures caused by drugs or variations in culture conditions have also been previously shown to induce chromosome size polymorphism in *Plasmodium falciparum* (10), *Giardia* species (17), and *Leishmania* species (29). The effects of such factors on the electrophoretic

patterns of *E. histolytica* are currently being investigated. Moreover, the karyotypes of additional P and NP strains have to be examined before a definite classification of the two types of amoeba can be established. Such information could be helpful for understanding the genetic differences that underlie the pathogenicity as well as the plasticity of the parasite genome.

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