Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5241–5245, June 1992
Genetics

Restriction mapping of the genome of the protozoan parasite *Theileria parva*

(pulsed-field gel electrophoresis/chromosomes/*Sfi* I linking clones)

SUBHASH P. MORZARIA*† AND JOHN R. YOUNG*‡

*International Laboratory for Research on Animal Diseases, P.O. Box 30709, Nairobi, Kenya

Communicated by Don W. Fawcett, January 2, 1992

ABSTRACT **We have used a modified linking clone strategy and pulsed-field gel electrophoresis to derive a map of the 29 *Sfi* I and 4 *Not* I sites in the 10 million base pair genome of the protozoan parasite *Theileria parva*. This was achieved in the absence of classical genetic information. The map reveals four chromosomes. Several genes, including those for parasite antigens, have been located on the map, as has the single locus carrying the major repetitive sequence in this organism. The map forms the basis for a study of sexual recombination in *T. parva*, which will be of importance in the application of present methods of immunization. Aspects of the mapping strategy may be useful in the study of other small eukaryotic genomes.**

The protozoan parasite *Theileria parva* is the causative agent of an economically important tropical disease of cattle, East Coast fever. It is transmitted by the tick *Rhipicephalus appendiculatus*, in which, according to morphological evidence, a sexual cycle may occur (1). The developmental stages of the parasite in cattle occur within lymphocytes and erythrocytes and are considered haploid (2).

Cell-mediated strain-specific immunity can be achieved by infection and simultaneous treatment with long-acting tetracycline (3, 4). This implies antigenic polymorphism in the parasite and means that sexual recombination may be important in generating antigenic diversity in the field. Since no means are available for classical genetic analysis of this parasite, we have initiated studies of sexual recombination by the construction of a physical map of large restriction enzyme fragments. Our previous study confirmed an earlier estimate of 10 million base pairs (Mb) for the size of the haploid genome of *T. parva* and revealed extensive polymorphism in *Sfi* I restriction fragment lengths, mostly within telomeric regions (5). Here we describe the completion of a map of 29 *Sfi* I restriction sites and 4 *Not* I sites in the genome of the Muguga stock of *T. parva*. The locations of all available cloned sequences have been determined, including polymorphic sequences that can be used for studies of sexual recombination.

MATERIALS AND METHODS

Parasite DNA. DNA from six *T. parva* stocks—Muguga [International Laboratory for Research on Animal Diseases (ILRAD) 2949 and 3087], Mariakani (ILRAD 3029), Marikebuni (ILRAD 3014), Kibarani (ILRAD 2448), and Uganda (ILRAD 3066)—was prepared either in solution (5, 6) or in low-melting-point agarose as described (7).

Enzymes and Radioisotopes. Restriction enzymes (New England Biolabs) and T4 DNA ligase, the Klenow fragment of DNA polymerase I, and radioisotopically labeled nucleotides (Amersham) were used as specified by the suppliers.

Pulsed-Field Gel Electrophoresis (PFGE). Digestion of agarose-embedded DNA and separation of *Sfi* I fragments by clamped homogeneous electric field (CHEF) electrophoresis were carried out as described (7, 8). *Not* I fragments and intact chromosomes were separated by PFGE using the Pulsaphor system with point and hexagonal array electrodes (Pharmacia). Concatemers of bacteriophage λ *c*I857$_S$7 [48.5 kilobases (kb)] and chromosomes of *Saccharomyces cerevisiae* and *Pichia pinus* (gift from Cassandra Smith, University of California, Berkeley), were used as size markers.

Southern Blots. DNA from pulsed-field gels was transferred to nitrocellulose paper after partial depurination (6) or ultraviolet nicking (7). Filters were preincubated in 6× SSC/5× Denhardt's solution (5) containing sonicated denatured salmon sperm DNA at 100 μg/ml for at least 5 hr at 65°C and then with radiolabeled probe for 16 hr in the same conditions. Filters were washed twice in 0.2× SSC/0.1% SDS at 65°C before autoradiographic exposure. Linking clones were radiolabeled with [^{32}P]dCTP by nick translation (6). Hybridization with a *Plasmodium berghei* telomeric repeat oligonucleotide $(CCCTGAACCCTAAA)_2$ (9, 10) and partial-digest mapping were performed as described (11). For the allocation of hybridization signals to specific PFGE-separated bands, autoradiographs were superimposed with an exact 1:1 reproduction of photographs of ethidium bromide-stained gels.

Production of *Sfi* I Linking Clones. The isolation of *Sfi* I linking clones has been described elsewhere (12). Briefly, 1 μg of DNA from two libraries of 70,000 clones in *Escherichia coli*, carrying 4- to 6-kb *Sau*3AI inserts of *T. parva* Muguga genomic DNA into Bluescript SKM13+ plasmid vector (Stratagene), was treated with 10 units of *Sfi* I for 4 hr. Linearized molecules, recovered by entrapment of the circular molecules in SeaPlaque agarose (FMC) (12), were recircularized and reintroduced into *E. coli*. Small-scale plasmid preparations (13) from the transformants obtained were analyzed by agarose gel electrophoresis, and those containing inserts of appropriate length were further characterized by electrophoretic analysis of *Sau*3AI and *Sau*3AI + *Sfi* I digests. Agarose gels were stained with ethidium bromide to reveal the larger *Sau*3AI fragments. For resolution of smaller fragments on acrylamide gels [0.4 mm thick, 5% acrylamide/0.25% bisacrylamide in 1× TBE (5)], the *Sau*3AI staggered ends were labeled with deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate in the presence of dCTP, dGTP, dTTP, and the Klenow fragment of DNA polymerase I. After electrophoresis, gels were fixed in 10% methanol/10% acetic acid/80% water (vol/vol), dried, and exposed to x-ray film.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Mb, million base pairs; PFGE, pulsed-field gel electrophoresis; CHEF, clamped homogeneous electric field.

†To whom reprint requests should be addressed.
‡Present address: Agricultural and Food Research Council, Institute for Animal Health, Compton, RG16 ONN, U.K.

RESULTS

***Sfi* I Fragments, Chromosomes, and Telomeres.** Fig. 1 shows the resolution of *Sfi* I fragments of *T. parva* DNA by CHEF electrophoresis. Most bands form a series whose staining intensity is consistent with the presence of one fragment per genome. Two regions containing more strongly staining bands (m) were resolved into separate bands by using different pulse regimes (Fig. 1*B*). Such gels were used in addition to the standard gels when identification of hybridizing fragments in Southern blots was ambiguous. We have numbered 33 *Sfi* I fragments (Fig. 1*A*); each one carries a prefix S in the text below. Some bands appeared consistently fainter than expected for their size (f), perhaps due to partial digestion or to genomic heterogeneity within the parasite population (5). Hybridization of a *P. berghei* telomeric repeat oligonucleotide, which detects telomeric sequences in *T. parva* (5), to *Sfi* I blots identified 7 fragments as telomeric (Fig. 1*C*). Higher-resolution gels showed an additional telomeric fragment 108 kb long. The probe also recognizes 8 *Eco*RI fragments that are susceptible to *Bal*-31 digestion (R. Bishop, personal communication). The faintly hybridizing bands in Fig. 1 may be internal repeats or partial digestion products (5). This analysis and PFGE confirmed that there are four chromosomes, approximately 3.2, 2.3, 2.2, and 2.2 Mb (Fig. 1*D*). The *T. parva* genome size is thus approximately 10 Mb, which agrees with the previous estimates (5, 14).

Isolation of Linking Clones. Statistical considerations suggested that it would be necessary to analyze 200 random linking clones to have >95% probability of obtaining clones spanning each of 29 *Sfi* I sites. We characterized 355 clones obtained from the plasmid library, and only 199 of these contained the expected sizes of insert, 4–6 kb. Shorter inserts may have been due to nonspecific cleavage by the large excess of *Sfi* I used to digest the library DNA, which was considered appropriate, as observations of PFGE gels of *Sfi* I-digested *T. parva* DNA had shown substantial variation in the cutting rate at different *Sfi* I sites. *Sau*3AI/*Sfi* I digests of the 199 clones were examined to identify groups of clones spanning the same *Sfi* I site. The sizes of *Sau*3AI fragments cut and/or the double-digest fragments produced were used to assemble such groups. A sample gel is shown in Fig. 2. This gel revealed seven clones containing the same *T. parva* *Sau*3AI fragment (350 bp) cut identically by *Sfi* I (to 330 bp). Analysis of 199 putative linking clones sorted 123 of them into 21 groups, each representing a single *Sfi* I site. Four groups contained only one clone, and one *Sfi* I site was isolated 19 times. The remaining clones either were not linking clones or had *Sfi* I sites so close to *Sau*3AI sites (50 bp) as to escape detection by this method. Two *Sfi* I sites in the same *Sau*3AI fragment would be unlikely to have compatible cohesive ends and would not have been recovered by our isolation procedure. Because the failure to obtain clones containing the remaining *Sfi* I sites was likely due to factors other than chance, no further clones were analyzed.

Application of Linking Clones. Linking clones were used in three kinds of analysis. Hybridization to blots of *Sfi* I fragments was used to establish linkage between *Sfi* I fragments directly. The clones were then used as specific markers to assign *Sfi* I fragments to separated chromosomes and *Not* I fragments. Finally, two clones were used for partial digestion ladders.

Fig. 3 shows the hybridization of six linking clones to blots of *Sfi* I-digested *T. parva* Muguga DNA. While some clones hybridized to only two *Sfi* I fragments, establishing the link between them, others hybridized to more than two bands. There are three possible explanations for these: presence of reiterated sequences within linking clones, genomic heterogeneity within the parasite population, and partial digestion at some *Sfi* I sites.

In some cases the presence of partial digest fragments (indicated by the prefix P) helped to confirm the mapping.

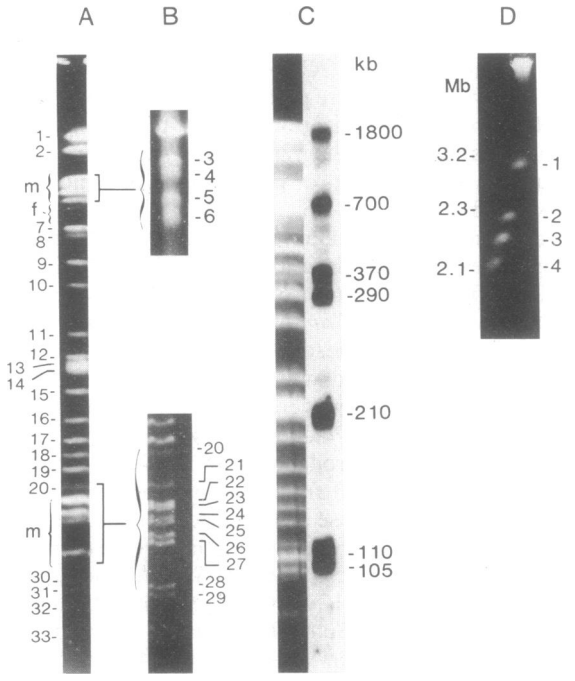

FIG. 1. Electrophoretic separation of *T. parva* Muguga DNA. (*A*) Ethidium bromide-stained *Sfi* I fragments separated by CHEF electrophoresis using pulses of 10 s for 16 hr followed by 40 s for 3 hr, at 10 V/cm (5). Fragment numbers are shown on the left. Regions with comigrating fragments (m) and consistently faint bands (f) are indicated. (*B*) Increased resolution of selected regions using (*Upper*) pulses of 40 s for 21 hr and (*Lower*) 3 s for 21 hr. (*C*) Hybridization of *Sfi* I fragments (*Left*) with telomeric repeat probe (*Right*). (*D*) Separation of chromosomes (numbered 1–4) by using the Pulsaphor apparatus with point electrodes and pulses of 900 s for 24 hr, 600 s for 24 hr, 480 s for 24 hr, and 400 s for 24 hr in 1% agarose/1× TBE at 3 V/cm.

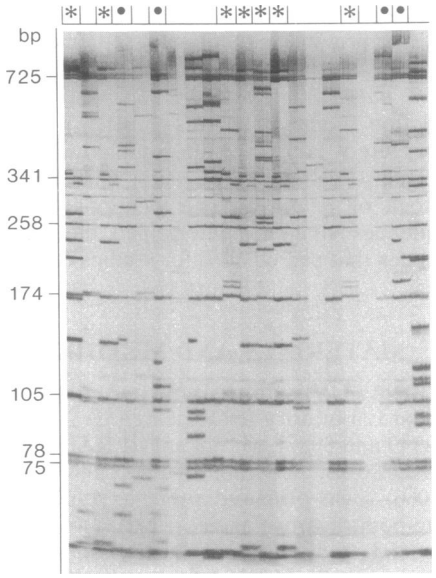

FIG. 2. Characterization of linking clones. Plasmids digested with *Sau*3AI and with both *Sau*3AI and *Sfi* I were end-labeled and analyzed in two adjacent tracks, left and right, respectively, of each pair. Seven clones carrying inserts with the same *T. parva* *Sfi* I site are shown by asterisks. Different cloned *Sfi* I sites are indicated by dots. bp, Base pairs.

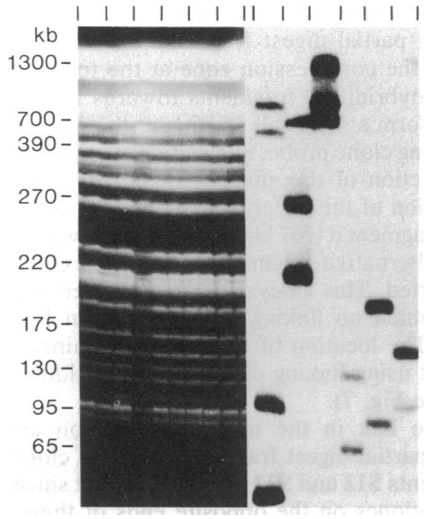

FIG. 3. Hybridization of linking clones to *Sfi* I fragments of *T. parva* DNA. (*Left*) ethidium bromide-stained gel. (*Right*) Result of hybridization of strips of a blot of the same gel with six different linking clones.

Fig. 4 illustrates this kind of analysis. The linking clone L hybridizes with S20, S21, and P14 (Fig. 4*A*). The latter fragment (P14) comigrates with S14, which is linked to S10 (see Fig. 7). Both P14 and S20 hybridize with the telomere probe (not shown) and show exactly the same increase in length in *T. parva* Mariakani DNA (Fig. 4*A*, lane 3), suggesting that they are derived from the same telomere. The length of P14 (218 kb) is the sum of the lengths of S20 and S21, which is consistent with its being produced as the result of partial digestion of the *Sfi* I site spanned by clone L. At first sight, clone M appears to contradict the above interpretation, since it hybridizes strongly with S21 and P14. However, clone M also hybridizes weakly with S2 (Fig. 4*C*), and another clone, M^1, which has the same *Sfi* I site as clone M, hybridizes

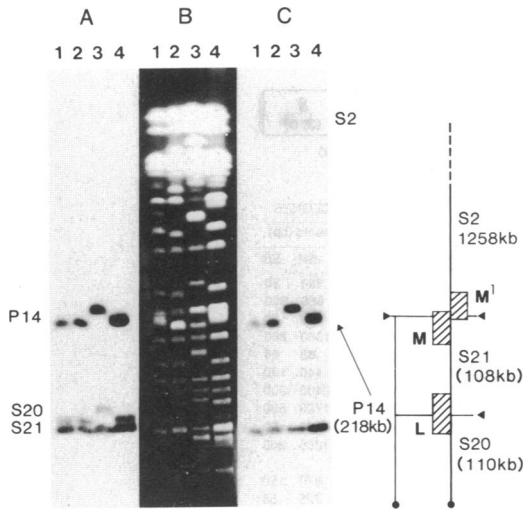

FIG. 4. Hybridization of linking clones L, M, and M^1 to *Sfi* I fragments of different *T. parva* stocks. Tracks from left to right (lanes 1–4) contained DNA from stocks Marikebuni, Kibarani, Mariakani, and Muguga, respectively. Two sets of tracks, one of which is shown stained with ethidium bromide (*B*) were blotted from the same gel and hybridized with the linking clones L (*A*) and M (*C*). The map (not to scale) on the right shows the deduced arrangement of *Sfi* I fragments detected by these probes. The telomere repeats are indicated by closed circles, the position of linking clones by hatched boxes, and *Sfi* I sites by arrowheads. The fragment P14 produced by partial digestion of one SfiI site is shown to the left.

strongly with S2 and weakly with P14 and S21 (not shown). The weak hybridizations are due to minimal overlap of M and M^1. These results are consistent with clone L linking S20 and S21 and clone M linking S21 and S2 (Fig. 4). This illustrates the importance of recognizing partial digestion, the exploitation of interstock polymorphisms, and the usefulness of alternative linking clones spanning the same *Sfi* I site when interpreting Southern blot data.

Physical Grouping of *Sfi* I Fragments. Southern blot analyses established 21 links between *Sfi* I fragments. There remained ambiguities in the positions of eight *Sfi* I fragments, mainly due to missing linking clones and multiple hybridizing bands. Most of this ambiguity could be resolved by independently determined allocation of *Sfi* I fragments to contiguous groups. The *Sfi* I fragments composing the largest chromosome were determined by digestion with *Sfi* I after electrophoretic separation of this chromosome (Fig. 5*A*).

Four recognition sites for *Not* I were detected by double digestion with *Not* I and *Sfi* I. Their locations within *Sfi* I fragments were determined by hybridization of *Not* I + *Sfi* I digests with linking clones including the ends of the cut *Sfi* I fragments (not shown). All linking clones and the gel-purified fragments S16 and S4, for which no linking clones were identified, were hybridized to blots of separated chromosomes of *T. parva* Muguga (e.g., Fig. 5*C*) and to blots of *Not* I fragments of *T. parva* Marikebuni DNA (e.g., Fig. 5*B*). The latter stock was used because *Not* I fragments were better resolved, while its *Sfi* I fragments are almost identical with those of *T. parva* Muguga (5). The allocation of linking clones, and thus the *Sfi* I fragments they linked, to separate regions of the genome in this way resolved most of the remaining ambiguity.

Partial Digestion. Fragments produced by partial cutting of *Sfi* I sites were examined, both those in deliberately produced partial digests and those occurring in existing experiments.

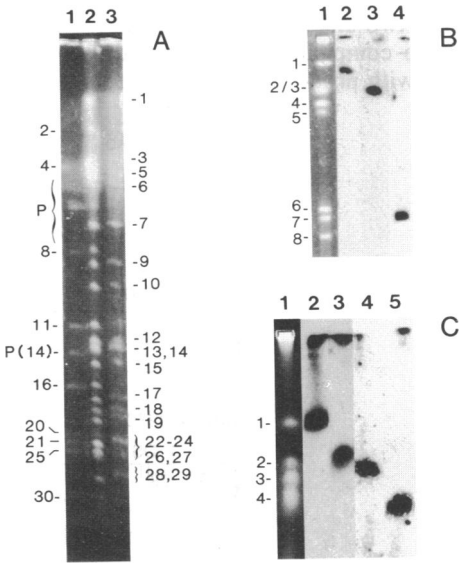

FIG. 5. (*A*) Ethidium bromide-stained *Sfi* I fragments of electrophoretically separated chromosome 1 (lane 1), of the total genome (lane 2), and comigrating chromosomes 2, 3, and 4 (lane 3) of *T. parva* Muguga. The chromosomes were separated in Incert agarose (FMC) and digested in the gel with *Sfi* I, and the fragments were resolved as described for Fig. 1. (*B*) Hybridization of three linking clones (lanes 2–4) to *Not* I fragments (lane 1) of *T. parva* Marikebuni DNA. Fragments were separated by using a hexagonal array of electrodes in the Pulsaphor apparatus with pulses of 250 s for 18 hr, 180 s for 18 hr, and 120 s for 12 hr at 160 V. (*C*) Hybridization of four linking clones (lanes 2–5) to separated chromosomes of *T. parva* Muguga (lane 1). Chromosomes were separated as described in the legend of Fig. 1.

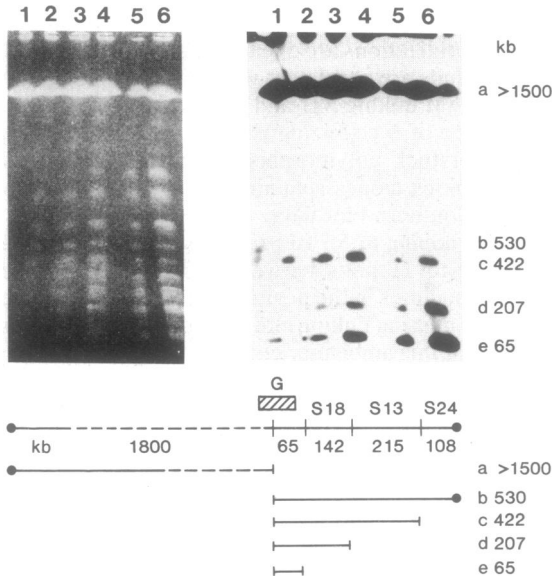

FIG. 6. Partial digest mapping of *Sfi* I sites at the end of chromosome 2. Hybridization of linking clone G (hatched box) to a series of samples of *T. parva* Muguga DNA digested with increasing amounts of *Sfi* I and resolved by using point electrodes in the Pulsaphor apparatus with pulses of 100 s for 48 hr at 10 V/cm. Ethidium bromide-stained fragments are shown on the left, hybridized fragments on the right. The deduced map and the derivation of detected partial fragments from it (a–e), with their sizes determined by λ phage DNA markers, are indicated at the bottom.

Deliberately produced partial *Sfi* I digests were used to resolve ambiguities at two locations. In each case the probe chosen hybridized to one large *Sfi* I fragment. For example, on chromosome 2 (see Fig. 7) the order of fragments 18 and 13 was determined by using the linking clone G, which hybridized to two discrete fragments, S1 (1800 kb) and S29 (65 kb) of the completely *Sfi* I-digested *T. parva* Muguga DNA. Thus, with probe G and the separation conditions of *Sfi* I partially digested DNA as described for Fig. 6, any hybridizing partial digest fragment including S1 would be trapped in the compression zone at the top of the gel. The remaining hybridizing fragments towards the bottom of the gel would form a series all starting at the *Sfi* I site, spanned by the linking clone probe, and ending at successive *Sfi* I sites in the direction of the smaller linked *Sfi* I fragment. The determination of the order of S18 and S13 depended on the length of fragment d (207 kb, Fig. 6), which was distinguished from the alternative fragment of 280 kb predicted with the order inverted. This analysis also confirmed the location of S24, for which no linking clone had been found, on this telomere. The location of S16 was determined in a similar experiment using linking clone V in the middle of chromosome 1 (see Fig. 7).

Only one link in the map depended on analysis of a persistent partial digest fragment. Linking clone B showed that fragments S12 and S32 were linked, but since there were no linking clones on the opposite ends of these fragments, their order was undefined (see map in Fig. 7). The linking clones B and H both hybridized weakly to a fragment staining weakly with ethidium bromide, whose length was the sum of S12 and S7, suggesting that these fragments were linked. This partial digest fragment did not hybridize with linking clones W or P, so it included neither fragment S15 nor fragment S26. If the order of S12 and S32 were inverted, the partial digest fragment would have included the three fragments S12, S32, and S7, and sites on both ends of S32 would necessarily have been partially digested. Linking clones B and H would then also have detected smaller partial-digestion fragments, S12 + S32 (B only) and S32 + S7 (B and H). Neither of these was detected. Thus the order of S12 and S32 and the link between S12 and S7 were established (half-shaded circle, Fig. 7).

These analyses left only one possible arrangement of fragments on each chromosome, the remaining links being unambiguously implied (circled dots, Fig. 7). After completion of the map, we were able to account for all but one case of hybridization of linking clones to more than two *Sfi* I fragments by partial-digest fragments consistent with the derived map.

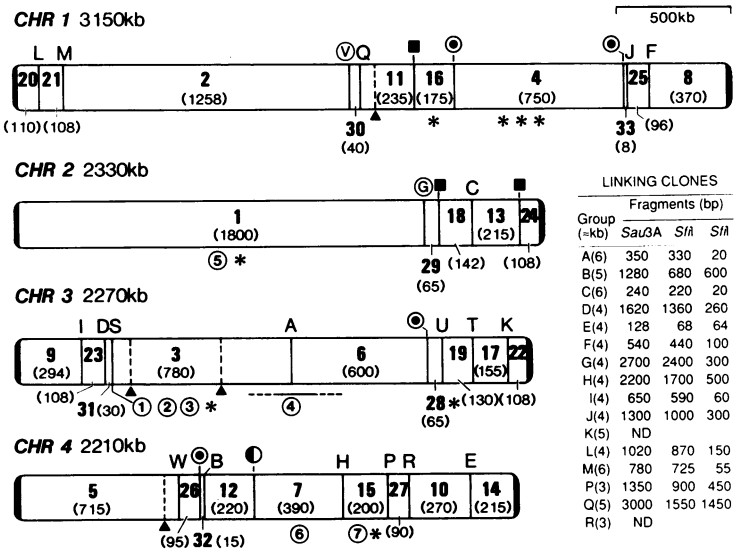

FIG. 7. Map of the genome of *T. parva* Muguga, showing sizes of the four chromosomes, with rounded ends as telomeres. *Sfi* I fragments are numbered 1–33 and their sizes in kb are given in parentheses. *Sfi* I sites are shown by vertical lines and *Not* I sites by closed triangles and broken vertical lines. Linking clones spanning the *Sfi* I sites are indicated by a circled or uncircled capital letter. Solid squares indicate sites confirmed by partial mapping. Circled letters indicate the linking clones used in this analysis. Half-shaded circle and circled dots indicate partial digestion and implicit matching, respectively. Circled numbers 1–7 represent the locations on the map of 47-kDa, 67-kDa, and 50-kDa antigen genes, *T. parva* repetitive sequence, heat-shock gene, 105-kDa antigen gene, and casein kinase II α subunit gene, respectively. Asterisks show the locations of uncharacterized cloned cDNAs. Approximate sizes of the linking clones and the *Sau*3AI fragments bearing *Sfi* I sites are shown in the table. ND, not done.

The one exceptional case may be due to reiteration of a sequence in the linking clone elsewhere in the genome.

Final Map. Fig. 7 shows the complete map of *Sfi* I and *Not* I sites in the genome of *T. parva* Muguga. Twenty-one of the *Sfi* I sites are represented in linking clones. Of the remaining eight links, four were established by analysis of partial digests and four were implied because no alternative arrangements of fragments were possible. Because no linking clones were obtained for these sites, some may consist of more than one *Sfi* I site close together. Indeed, this may explain for the failure to obtain linking clones. Since we can easily detect the 8-kb fragment S33 (chromosome 1) on overloaded normal agarose gels (not shown), the distances between any such sites must be less than 8 kb. The presence of these sites would make little difference to the overall map. Of the 33 *Sfi* I fragments, the orientation of 31 is known in that at least one end is identified by a linking clone or a telomere. We have located several cloned genes of *T. parva*, including a major repetitive sequence (14, 15) on the map (Fig. 7).

DISCUSSION

We have constructed a map of the genome of *T. parva* in the absence of any genetic information. Our strategy consisted of three steps. First, the statistically required number of linking clones was obtained and the clones were analyzed. This failed to yield all *Sfi* I sites and left many possible arrangements of fragments. These ambiguities were reduced by allocating *Sfi* I fragments to genomic subregions by digestion of separated chromosomes and by hybridization of the linking clones to chromosomes and *Not* I fragments. The few ambiguities that remained were readily resolved by analysis of partial digests. The search for more linking clones could have been extended, but the return for effort expended would have progressively decreased. In principle, the entire genome could have been mapped by partial-digest analysis, but the complexity of interpretation without a restricted set of alternative configurations would be daunting. The hybrid approach allowed rapid exploitation of easily obtained linking clones and completion of the map with a small number of easily interpreted partial-digest experiments.

Choice of a method to construct restriction maps of small eukaryotic genomes depends crucially on the properties of the genome, the number of chromosomes, and the frequency of cutting by available enzymes. When there are many easily resolved chromosomes, a map may be constructed simply with a few enzymes each having a small number of sites (16). Of the many enzymes we used, only *Not* I had fewer sites than *Sfi* I. A *Not* I map would have had little resolving power. The simple pattern obtained on digestion of *T. parva* DNA with *Sfi* I, where most individual fragments could be resolved, greatly simplified the linking clone approach and obviated the need for jumping clones used in mapping more complex genomes (17). Had this not been so, ambiguity of linking clone blots would have been much greater and jumping clones might have been required.

More problems occurred with extensive partial digestion of several *Sfi* I sites than we expected. This complicated initial interpretation of Southern blot data with linking clones. Some *Sfi* I sites remained partially digested even with large amounts of enzyme. If this is characteristic of *Sfi* I sites, it substantially militates against the choice of this enzyme for linking clone studies if alternatives are available.

Despite the choice of short fragments to make the linking library, we had expected problems with repetitive sequences in the linking clones. However, only one linking clone was affected by this problem. One linking clone was situated between arrays of a major repetitive sequence (TpR) present in the *T. parva* genome (④ in Fig. 7) but did not itself contain the repetitive sequence. The TpR sequence is confined to a central region of chromosome 3 and is present in the form of repeats interspersed with short regions of unique sequence (18). Whether the linking clone represents an unusually long segment of unique sequence in this array, or whether there are two widely separated arrays of repeats, we cannot judge.

Our eventual aim is to gain understanding of the genetics of *T. parva*. We have used a physical mapping approach because no experimental system exists for genetic studies. The map is being used to study the basis of polymorphism among strains (5, 14, 15) and to locate markers for detecting and analyzing sexual recombination in this parasite. Of particular interest will be the extent of recombination affecting genes encoding epitopes involved in immune protection.

We thank Ms. T. Batavia and Mr. T. Ndolo for technical assistance and Prof. C. Cantor and C. Smith and their colleagues Dr. T. Yang and Ms. J. Nikolik for helpful advice and provision of size markers. Various genes and cDNA clones of *T. parva* were provided by Drs. K. Iams, V. Nene, P. Toye, O. ole-MoiYoi, J. Gerhards, and R. Williams. This is International Laboratory for Research on Animal Diseases publication no. 992.

1. Melhorn, H. & Schein, E. (1984) *Adv. Parasitol.* **23,** 37–103.
2. Irvin, A. D. & Boarer, C. D. H. (1980) *Parasitology* **80,** 571–579.
3. Radley, D. E. (1981) in *Advances in the Control of Theileriosis*, eds. Irvin, A. D., Cunningham, M. P. & Young, A. S. (Nijhoff, Boston), pp. 227–237.
4. Goddeeris, B. M., Morrison, W. I., Teale, A. J., Bensaid, A. R. & Baldwin, C. L. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5238–5242.
5. Morzaria, S. P., Spooner, P. R., Bishop, R. B., Musoke, A. J. & Young, J. R. (1990) *Mol. Biochem. Parasitol.* **40,** 203–212.
6. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
7. Smith, C. L., Klco, S. R. & Cantor, C. R. (1988) in *Genome Analysis: A Practical Approach*, ed. Davies, K. E. (IRL, Oxford), pp. 41–72.
8. Chu, G., Vollrath, D. & Davis, R. W. (1986) *Science* **234,** 1582–1585.
9. Ponzi, M., Pace, T., Dore, E. & Frontali, C. (1985) *EMBO J.* **4,** 2991–2995.
10. Berger, S. L. & Kimmel, A. R. (1987) in *Guide to Molecular Cloning Techniques* (Academic, New York), pp. 438–439.
11. Smith, C. L. & Condemine, G. (1990) *J. Bacteriol.* **172,** 1167–1172.
12. Young, J. R. & Morzaria, S. P. (1991) *Genet. Anal. Tech. Appl.* **8,** 148–150.
13. Holmes, D. S. & Quigley, M. (1981) *Anal. Biochem.* **114,** 193–197.
14. Allsopp, B. A. & Allsopp, M. T. E. P. (1988) *Mol. Biochem. Parasitol.* **28,** 77–84.
15. Conrad, P. A., Iams, K. P., Brown, W. C., Sohanpal, B. & ole-MoiYoi, O. K. (1987) *Mol. Biochem. Parasitol.* **25,** 213–226.
16. Corcoran, L. M., Thompson, J. K., Walliker, D. & Kemp, D. J. (1988) *Cell* **53,** 807–813.
17. Poustka, A. & Lehrach, H. (1986) *Trends Genet.* **2,** 174–179.
18. Bishop, R., Baylis, H., Allsopp, B., Morzaria, S. P., Musoke, A. J. & Dolan, T. T. (1990) in *Annual Scientific Report* (ILRAD, Nairobi), p. 6.