Nucleic Acids Research, Vol. 20, No. 3 581–586

Chromosome translocation in *Plasmodium berghei*

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Received September 2, 1991; Revised and Accepted December 27, 1991

ABSTRACT

We decribe a chromosome translocation in a karyotype mutant of the rodent malarial parasite *Plasmodium berghei*. In this mutant (named EP) a small chromosome (chromosome 7), which has exhibited a size range between 0.9 and 1.4 Mb in other clones of *P. berghei*, is translocated to chromosome 13 or 14 with a size of about 3 Mb. By comparison of Apa-I restriction fragments of the chromosomes from mutant EP and from a reference clone (named HP) of *P. berghei*, we found evidence for a junction of subtelomeric chromosome 7 sequences and internal chromosome 13/14 sequences. In addition, a new chromosome of 1.4 Mb (named EP7) is present in mutant EP, which is (mainly) composed of sequences of chromosome 13/14. EP7 contains one telomeric region derived from chromosome 13/14. We found evidence that internal sequences of chromosome 13/14 are joined to telomeric sequences in the other telomeric region of EP7. The karyotype of mutant EP was stable during asexual and sexual multiplication and we found no indications for phenotypic changes.

INTRODUCTION

The size of chromosomes of malaria parasites is highly variable. In many studies size differences between homologous chromosomes have been detected in parasites isolated from field isolates and from laboratory cultures. In *P. falciparum* size differences arise during meiosis and during asexual multiplication in *in vitro* cultures. During meiosis unequal crossing over between homologous chromosomes appear to play a role in the generation of novel sized chromosomes (1–5). In culture size polymorphisms can arise as a result of deletion or amplification of DNA sequences (6–9). Changes in size are often due to rearrangements in the subtelomeric region of a chromosome. In *P. falciparum* the subtelomeric regions containing 21 bp repeats are especially subject to rearrangements (1,10), although the possible function of such changes remains unknown. In contrast to the telomeric regions, the internal regions of the chromosomes appear to be stable and translocation of coding sequences has never been detected.

We studied the generation of chromosome size variation in the rodent parasite *P. berghei*. In *P. berghei* mutants with novel sized chromosomes arise frequently during mitotic multiplication in vivo (11). As with *P. falciparum*, rearrangements also occur mainly in the subtelomeric regions. In mutants which were studied in detail, size variation could almost exclusively explained by the loss or addition of subtelomeric 2.3 kb repeat sequences (12, 13). These repeats can be present in more than 300 copies per genome and are directly joined to the real telomeric repeats (14). We found that chromosomes which originally lack 2.3 kb repeats can acquire these repeats, presumably from non-homologous chromosomes (13). It has been suggested that this sequence plays a role in recombinational events between homologous sequences on non-homologous chromosomes or act as a transposable element like the Y′ repeats in *S. cerevisiae* in yeast (14). As with *P. falciparum*, the internal regions of the chromosomes of *P. berghei* appeared to be conserved. However, we recently found a karyotype mutant in which size variation could not be explained by subtelomeric rearrangements. In this paper we show that in this mutant a chromosome of about 1.4 Mb is translocated to a large chromosome of about 3 Mb. In addition, a new chromosome of 1.4 Mb is present, composed of a part of the large chromosome. We discuss the possible mechanism by which this mutant is produced.

MATERIALS AND METHODS

Parasites

Mutant EP is obtained by limiting dilution from the EP line of the ANKA strain of *P. berghei*. The EP line was kindly provided by Dr. W. Eling, Nijmegen, The Netherlands. The other clones and lines from the ANKA and K173 strain of *P. berghei* have been described before (11, 12).

Separation of chromosomes

The method of Field Inversion Gel Electrophoresis (FIGE) was used to separate the chromosomes of *P. berghei* (11). Electrophoretic conditions are specified in the legends to the figures. *S. cerevisiae* chromosomes were used as molecular weight markers.

Restriction digests of separated chromosomes

Individual chromosomes were excised as small agarose blocks from FIGE gels after ethidium bromide staining of the gel. The blocks were rinsed once for 15 min with 10 mM Tris.HCl, 1 mM EDTA, pH 8, once with double distilled water and three times for 15 min in fresh restriction buffer. Thereafter blocks were incubated for 24 hour in 200 µl restriction buffer containing 100 µg/ml of bovine serum albumin (BSA; Promega) and 10 U

of the restriction enzyme. Restriction fragments were separated using FIGE. Electrophoretic conditions are specified in the legends to the figures.

Blotting, labelling and hybridization

Agarose gels were blotted to Hybond-N plus (Amersham) membranes. DNA probes were radiolabelled by random priming. Hybridisation was performed under standard conditions at 60°C. Blots were washed at 60°C as follows: 3×15 min in 3×SSC/0.5% SDS, 3×15 min in 1×SSC/0.5% SDS. A further 30 min wash in 0.1×SSC/0.5% SDS at 60°C was performed when telomeric (pTB4.1) probe was used.

Isolation and characterization of probes

The following chromosome specific probes were used: Six anonymous probes specific for chromosome 7 (3.1, 3.2, 3.3, 3.8, 3.18, 3.50) and two anonymous probes specific for chromosome 13/14 (13.33, 13.45), which were selected from EcoRI genomic libraries of *P. berghei* (ANKA strain) and described in (12); an 1 kb PCR fragment of the dihydrofolate reductase/thymidylate synthase (DHFR) gene of *P. chabaudi*, amplified using primers based on the published sequence of this gene (15); a probe (pPb SL7.8) hybridising with the four small-subunit ribosomal RNA genes of *P. berghei* (described in (16) and obtained from Dr. A. Waters, N.I.H., Bethesda); an 1.1 kb probe corresponding to the *P. berghei* Actin I gene (unpublished results), isolated by hybridization with the equivalent gene of *P. falciparum* (17); a probe (pTB4.1) recognizing the telomeres of *P. berghei* (18) and a 2.3 kb subtelomeric repeat probe, which is also specific for *P. berghei* (14, 19). In addition to these probes, we generated a number of clones specific for certain Apa-I restriction fragments of individual chromosomes. The Apa-I restriction fragments were excised as small agarose blocks after ethidium bromide staining of the gel. The DNA in the blocks was digested with HindIII following the procedure as described for chromosome digestions. The digested DNA was collected by electrophoresis using a Biotrap (Schleicher and Schuell) and purified using standard methods. The purified DNA was digested with HindIII/EcoRI and cloned in pUC19. More details of these probes are specified in Table 1.

Table 1. Probes specific for Apa-I fragments of chromosomes 7 (HP7) and 13/14 (HP13/14) of *Plasmodium bergei*. See figure 4 for the numbering (A-J) of the Apa-I fragments.

Apa-I fragment	estimated size (kb)	probe name	probe size (kb)
Probes recognizing Apa-I fragments of both HP7 (HP clone) and EP13/14			
A	630 in HP7	pPb SL7.8**	7.8
	900 in EP13/14	3.50*	3.7
		pTB 4.1* (in HP7)	0.5
		2.3 kb*	2.3
B	270 kb	3.1*	1.7
		3.3*	2.3
		DHFRpc***	1.0
C	140 kb	3.2*	2.0
		3.18*	2.8
D	50 kb	3.1	1.7
Probes recognizing Apa-I fragments of both HP13/14 and EP7 (HP clone)			
E	330 kb	EP7II.6	1.2
		Actin I	1.1
F	300 kb in EP7	EP7II.5	0.5
	625 kb in HP13/14	EP7II.10	1.2
		pTB4.1*(in EP7)	0.5
G	200 kb	EP7.6	1.8
H	140 kb	EP7.4	0.3
		pTB4.1	0.5
I	80 kb	EP7II.6	1.2
J	25 kb	HPI.9	0.4
Probes recognizing Apa-I fragments of both HP13/14 and EP13/14 (HP clone)			
K	950 in EP13/14	HPII.6a	1.7
	625 in HP13/14	HPII.28	1.8
		HPII.31	2.3
L	800 kb	HPI.6	1.0
		HPI.9	0.4
		HPI.12	2.1
M	450	HPI.10	0.4
N	350	13.33*	4.5
O	340	13.45*	3.2

* Probes are described in reference 12.
**Genomic fragment of ribosomal RNA operon of *P. berghei* (16).
***An 1 kb PCR-amplified fragment of the DHFR gene of *P. chabaudi*. The fragment was amplified using primers derived from the published sequence (15).

RESULTS

The karyotype of clones of *P. berghei*

Parasites of the ANKA and K173 strain of *P. berghei* have 14 chromosomes (12), which can be separated by Field Inversion Gel Electrophoresis (FIGE). For the identification of the individual chromosomes we use the HP clone (=clone 8417) of the ANKA strain as a reference clone (Fig. 1) and the homologous chromosomes in other clones are determined with chromosome specific probes as described (12).

Chromosome 7 of *P. berghei*

Chromosome 7 of *P. berghei* is recognized by a number of anonymous probes (12) and by probes recognising the dihydrofolate-reductase gene (DHFR) and the small-subunit

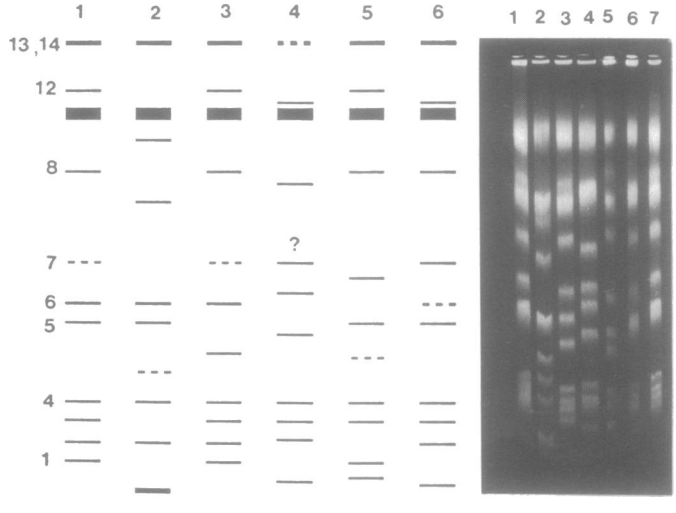

Figure 1. Comparison of the karyotype of mutant EP of *P. berghei* (ANKA strain) with the karyotype of several clones from the ANKA and K173 strain. The HP clone (clone 8417; lane 1) is used as a reference clone for chromosome numbering. Chromosomes which hybridize to chromosome 7 specific sequences (see Fig. 2) are shown as a dotted line. (1,7) HP clone; (2) clone 1 (K173 strain); (3) HP53m (this clone was isolated after 53 mechanical passages of the HP clone; Janse *et al.*, in press); (4) mutant EP; (5) clone 8458 (ANKA strain); (6) clone 233 (ANKA strain; this clone was obtained from Dr. R.E. Sinden, London). Chromosomes were separated using FIGE (95 hour, 4.5 V/cm, linearly increasing pulse time from 30–360s and forward/backward ratio of 3/1).

rRNA gene (Figs. 2 and 3; table 1). The size is variable between clones and ranges between 0.9 and 1.4 Mb (Figs. 1 and 2). Digestion of chromosome 7 with Apa-I yields eight fragments in different clones from different strains (Fig. 4). Two of these are recognized by the telomeric (pTB4.1) and the subtelomeric 2.3 kb repeat probe (Fig. 5, lanes 1 and 4). Only the telomeric fragments are variable in size between different clones and strains of *P. berghei*, due to differences in the number of 2.3 kb repeats (12; unpublished results). The largest telomeric Apa-I fragment in the HP clone is 630 kb and contains a large number of 2.3 kb repeats (Fig. 5).

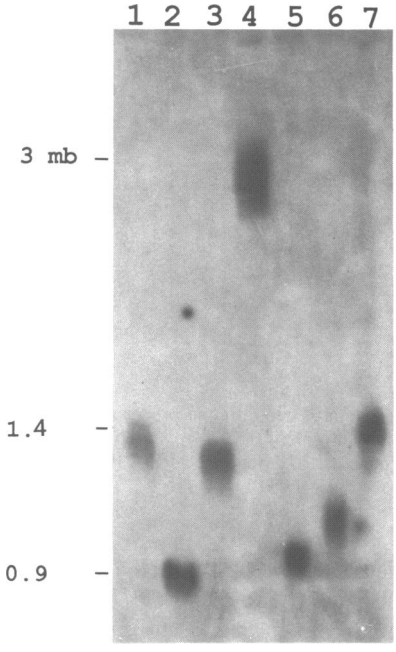

Figure 2. Hybridization of a chromosome 7 specific probe (3.50; see table 1) to chromosomes of mutant PE and with chromosomes of other clones of *P. berghei*. See figure 1 for details of the FIGE separation of the chromosomes.

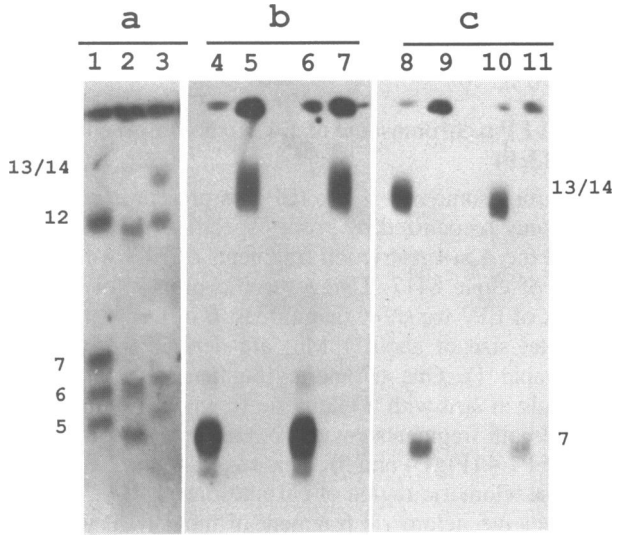

Figure 3. Hybridization of chromosome specific probes to chromosomes of mutant EP and to chromosomes of other clones of *P. berghei*. a) Hybridization with pPb SL7.8, a probe specific for the four RNA genes of *P. berghei*. These are located on chromosome 5, 6, 7 and 12. Lane 1: clone HP53m of the ANKA strain; lane 2: clone 1 of the K173 strain (chromosome 5 and 6 comigrate; see fig. 1); lane 3: mutant EP of the ANKA strain. b) Hybridization with two probes specific for chromosome 7 (probe 3.50: lane 4,5 and probe 3.3: lane 6,7). Lane 4 and 6: clone HP; lane 5 and 7: mutant EP. c) Hybridization with two probes specific for chromosome 13/14 (probe EP7.6: lane 8,9; probe EP7.4: lane 10,11). Lane 8 and 10: clone HP; lane 9 and 11: mutant EP.

Chromosomes 13 and 14 of *P. berghei*

Chromosomes 13 and 14 comigrate during pulsed field electrophoresis in all clones and strains of *P. berghei* and their size was estimated at 3−3.5 Mb (12). They are recognized by a number of specific probes (table 1). The gene coding for actin I is located on one of these chromosomes (in an earlier report (12) actin I was wrongly assigned to the group of chromosomes 9,10 and 11). Digestion of these chromosomes with Apa-I yields 25 to 27 fragments with a total size of 5.2 to 5.6 Mb (Fig. 4). Four Apa-I fragments are recognized by the telomeric probe (Fig. 5, lanes 5 and 6), which have a different size in the different clones (unpublished results).

Karyotype of mutant EP

After ethidium bromide staining of FIGE-separated chromosomes, the karyotype pattern of mutant EP seems not to be significantly different from that of other clones of the ANKA strain (Fig. 1). Probes specific for chromosomes 1−6 and 8−12 hybridise to chromosomes with a size comparable to their homologous chromosomes of other clones of *P. berghei*. However, all probes specific for chromosome 7 of *P. berghei* hybridize to chromosome 13/14 of mutant EP (=EP13/14)(Figs. 2 and 3a,b; table 1). In addition, a 'new' chromosome of about 1.4 Mb (=EP7), is present. This chromosome is only recognized by probes which are specific for chromosome 13/14 of *P. berghei* (Figs. 3c; table 1).

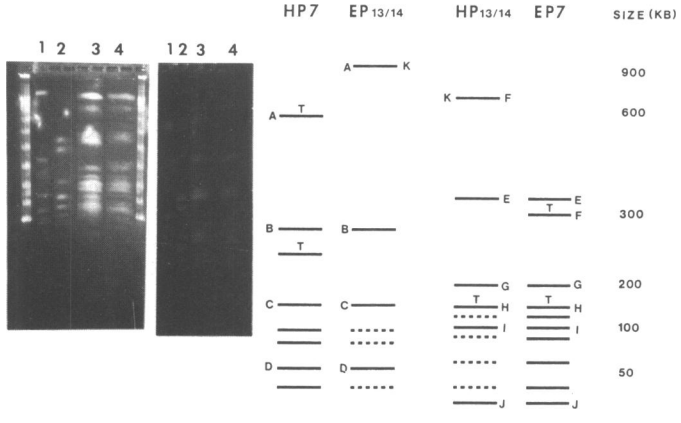

Figure 4. Comparison of Apa I fragments of chromosomes HP7 and EP13/14 and of chromosomes HP13/14 and EP7. Two FIGE separations (a,b) are shown with different pulse times for optimal separation of the small or large fragments). In the schematic representation all Apa I fragments from HP7 and EP7 are shown and the telomeric fragments (T) are indicated. From EP13/14 and HP13/14 only those fragments are shown which were identified by EP7 and HP7 specific probes (A-J; see for details of the probes table 1). In addition fragments with an identical size to fragments of EP7 or HP7 are indicated by dotted lines. FIGE conditions were as followed: a) a first run of 23 hour with increasing pulse time from 10−42 s, followed by a second run of 4.5 hour with increasing pulse time from 9−14 s, 4.5V/cm and forward/backward ratio of 3/1. b) a first run of 24 hour with increasing pulse time from 12−50 s, followed by a second run of 5 hour with increasing pulse time from 10−17 s.

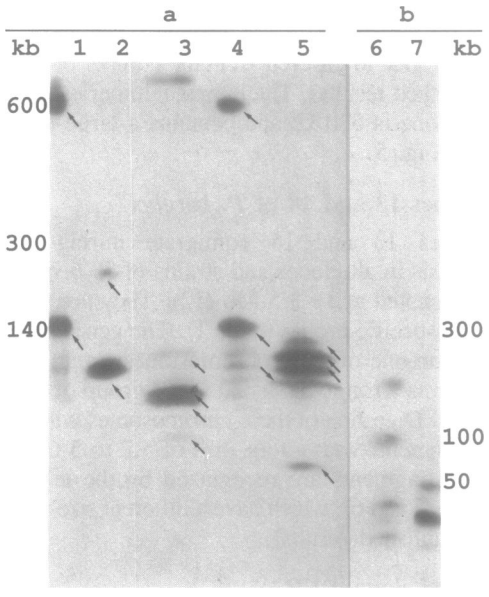

Figure 5. Hybridization of a probe (pTB 4.1) specific for the telomeres of *P. berghei* with Apa-I (a) or Sac-I (b) fragments of chromosome EP7 (lane 2) and EP13/14 (lane 5,7) of mutant PE and of chromosome HP7 (lane 1,4) and HP13/14 (lane 3,6) of clone HP. / telomeric fragments; / contamination of telomeric fragments of other chromosomes. We often found some contamination with smaller chromosomes in FIGE separated chromosomes as was shown by digestion of and hybridization with (sub)telomeric probes to all individual chromosomes (results not shown). FIGE separation of the restriction fragments was performed as described for Fig. 4a. a) Hybridization after Apa-I digestion. Hybridised blots were washed as follows: 3×15 min in 3×SSC/0.5% sds and 3×15 min in 1×SSC/0.5% SDS. Under these conditions pTB 4.1 hybridises to the telomere and to the 2.3 kb subtelomere repeats, due to the presence of short stretches of a telomere related sequence within the 2.3 kb repeat unit (19). As a result the telomeric fragments containing 2.3 kb repeats show a more intense signal. The large (900kb) fragment of EP13/14 (lane 3) is not positive to probe pTB 4.1 under stringent conditions, but only hybridizes to the 2.3 kb probe (results not shown). b) Hybridization after Sac-I digestion. Blots were washed as described above followed by a further 30 min. wash in 0.1×SSC/0.5% SDS.

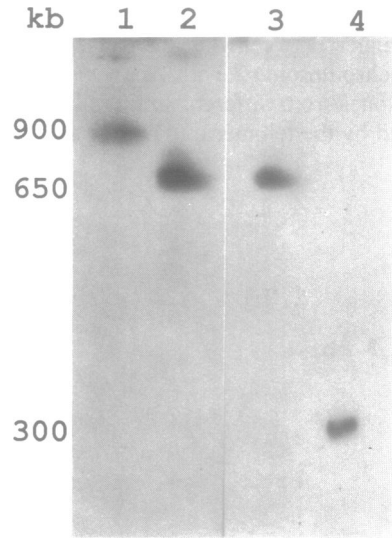

Figure 6. Hybridization of two different probes specific for the 650 kb Apa-I fragment of chromosome 13/14 of *P. berghei* (HPII28: lane 1,2; EP7II.5: lane 3,4) with Apa-I fragments of chromosomes EP13/14 (lane 1) and EP7 (lane 4). Apa-I fragments were separated by FIGE.

In mutant EP chromosome 7 sequences are translocated to chromosome 13/14

Hybridization of chromosome 7 specific probes to chromosome EP13/14 suggests that sequences of chromosome 7 are translocated to chromosome 13 or to chromosome 14 in mutant EP. To study this possibility we compared chromosomes 7 and 13/14 from mutant EP (EP7 and EP13/14, respectively) with chromosomes 7 and 13/14 from our reference clone of *P. berghei* (HP7 and HP13/14, respectively). The possibility existed that in mutant EP a chromosome, containing HP7 specific sequences, was present which had the same size as chromosome 13/14 and thus cannot be separated from these chromosomes by FIGE. However, the total size of Apa-I fragments of EP13/14 (5.0−5.4 Mb) corresponds to the size of HP13/14 precluding the presence of an extra chromosome. In addition, only four Apa-I fragments are recognized by the telomeric probe (Fig. 5), which shows that only two chromosomes, 13 and 14, comigrate during FIGE separation of the chromosomes.

To study the presence of chromosome 7 specific sequences in EP13/14 we compared Apa-I restriction fragments of HP7 with those of EP13/14. In EP 13/14 Apa-I fragments are present, showing the same size as the HP7 Apa-I fragments (Fig. 4). Using specific probes for several Apa-I fragments of HP7, we found that at least four of the eight HP7 fragments, with a total size of about 1 Mb, are present in EP13/14 (Fig. 4). A 900 kb fragment of EP 13/14 hybridises to probes specific for a 630 kb telomeric fragment of HP7 (Fig. 4, table 1). This fragment hybridises also to probes which are specific for HP13/14 (Fig. 6). This result show that in mutant EP a junction between sequences of chromosome 7 and sequences of chromosome 13/14 is located on a 900 kb fragment and proves that chromosome 7 sequences are translocated in mutant EP. The 900 kb fragment do not hybridize to the telomeric probe under stringent washing conditions (results not shown), but weakly hybridizes to the 2.3 kb probe (Fig. 5).

In mutant EP a chromosome of 1.4 Mb is composed of a large part HP13/14

A new chromosome of 1.4 Mb (EP7) is present in mutant EP, which is only recognized by probes specific for HP13/14. We compared the Apa-I restriction fragments of EP7 with those of HP13/14 of clone 8417. Using specific probes for the Apa-I fragments of EP7 we show that at least 6 of the 10 fragments, with a total size of about 1 Mb, are derived from HP13/14 (Fig. 4, table 1). One telomeric fragment (140 kb) of EP7 corresponds in size with a telomeric fragment of chromosome 13/14 and both fragments contain 2.3 kb repeats and hybridize to probe EP7.4 (Figs 4 and 5). This suggests that EP7 contains at least one telomeric region of chromosome 13/14. The other, 2.3 kb negative, telomeric fragment of mutant EP with a size of 300 kb, hybridises with probes which recognize an internal fragment of 650 kb of HP13/14 (Fig. 6). This suggests that EP7 arose by breakage of chromosome 13 or 14 followed by addition of telomeric sequences at the site of breakage and that this site is located on the 650 kb Apa-I fragment of HP13/14.

Asexual and sexual multiplication of mutant EP

Mutant EP was cloned by the method of limiting dilution of the EP line. The EP line had been derived from the ANKA strain by mechanical passage of parasites of the ANKA strain in mice.

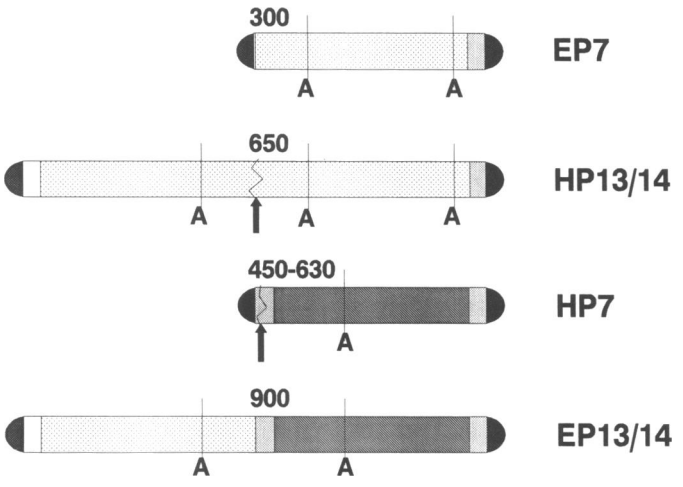

Figure 7. Schematic representation of chromosomes 7 and 13/14 from the HP clone of *P. berghei* and of the recombined chromosomes EP7 and EP13/14 from mutant EP. The size of several Apa-I (A) restriction fragments is shown. The arrows and crooked lines indicate the supposed breakage sites, which underly the translocation event. Regions containing islands of the 2.3 kb repeat are indicated by the closely dotted pattern and the main body of the two HP chromosomes are shaded differently to emphasise the exchange that has occurred in mutant EP.

Parasites from the original ANKA strain show a karyotype comparable to clone 8458 (see fig. 1), while the parasites from the uncloned EP line have the same karyotype as mutant EP. This suggests that mutant EP arose during asexual multiplication of the parasites of the ANKA strain and overgrew the parental line. We recently demonstrated that karyotype mutants arise frequently during asexual multiplication of *P. berghei* in mice which are able to rapidly replace the original parasites (20). However, we cannot exclude the possibility that mutant EP was already present in the original ANKA strain and arose during the sexual cycle, which takes place during mosquito passage. To see whether the chromosome translocation influenced phenotypic characters of the parasite, we compared several growth characteristics of mutant EP with those of other clones of *P. berghei*. The light-microscopic morphology of asexual and sexual stages was not different from the morphology of parasites of clone 8417. In synchronized infections the duration of the asexual cycle was 22−24 hours and the gametocyte production 15−20%, comparable to high-gametocyte producer clones of the ANKA strain (21, 22). The karyotype remained stable during at least 10 weeks of asexual multiplication. After mosquito passage of mutant EP through *Anopheles stephensi* the karyotype was unaltered (two experiments; results not shown), indicating that the karyotype is also stable during meiosis, which takes place in the mosquito midgut, just after fertilization (23).

DISCUSSION

Our results demonstrate a translocation, in which two non-homologous chromosomes are involved, resulting in two rearranged chromosomes in a mutant of the ANKA strain of *P. berghei*. This is the first time that a chromosome translocation and translocation of coding sequences (DHFR, ribosomal and actin genes) to non-homologous chromosomes has been found in *Plasmodium*. We show that (a large part of) chromosome 7 of *P. berghei* is translocated to chromosome 13 or 14 in the mutant. In addition, a new chromosome is present, consisting (mainly) of chromosome 13/14 sequences, including one telomeric region (Fig. 7). Therefore, in addition to rearrangements in the subtelomeric regions, chromosomes can vary in size due to translocation events and new linkage groups can be generated in *Plasmodium* by joining of two fragments of non-homologous chromosomes. We found, however, no evidence for phenotypic changes as a result of the translocation.

The molecular events leading to chromosomal translocation in cells of higher eukaryotes, for example in B-cell and T-cell lymphomas and leukemias, are at present largely unknown (24). A translocation event requires breakage and rejoining of the DNA molecules involved. These processes could take place at random sites by illegitimate, interchromosomal recombination or certain sites in the DNA may be predisposed to recombine in certain cells under certain conditions. In *P. falciparum* there is evidence of the introduction of double-stranded breaks in the DNA at specific sites in the chromosome. Healing of the chromosomes can occur by the enzymatic addition of telomere repeats (7, 8). This mechanism appears to have played a role in the translocation event described here. Breakage of chromosome 13 or 14 in two parts, followed by telomere addition at the site of breakage may have resulted in the formation of the new chromosome of 1.4 Mb (Fig. 7). Indeed, our results strongly suggest that the new chromosome comprises only chromosome 13/14 sequences, containing one telomere derived from chromosome 13/14, while at the other end of the chromosome the telomeric sequences are directly joined to sequences which are in the normal situation internally located on chromosome 13/14.

We showed that the site of breakage in chromosome 13 or 14 lies on a 650 kb Apa-I fragment. Probes recognizing this fragment, either hybridize to the 300 kb telomeric fragment of the new chromosome or hybridize to a 900 kb fragment of chromosome 13 or 14 of the mutant (Fig. 6). Interestingly this 900 kb fragment is also recognized by probes specific for the large telomeric fragment of chromosome 7, indicating that sequences of chromosome 7 are directly joined to chromosome 13/14 at the site of breakage. If this is the case, the 900 kb Apa-I fragment should than comprise 350 kb (650 minus 300) of sequences derived from chromosome 13/14 and 550 kb (900 minus 350) of sequences derived from chromosome 7. Indeed, the size of the large telomeric Apa-I fragment of chromosome 7 lies between 450 and 630 kb, the exact size dependent on the number of 2.3 kb repeats (12). The 900 kb fragment is not recognized by the telomeric probe but hybridizes weakly to the 2.3 kb probe indicating that a small number of 2.3 kb repeats is present. These results may suggest that the 2.3 kb repeats of the large telomeric fragment of chromosome 7 are directly joined to chromosome 13/14 sequences, which may imply that these repeats have played a role in the recombination event underlying the translocation. Evidence for the involvement of the 2.3 kb repeats in recombinational events has been presented before (12, 13). However, sequence information on the junction between sequences from chromosome 7 and from chromosome 13/14 is needed for an insight into the possible mechanism by which the translocation occurred.

Translocation, deletion, duplication and many other rearrangements in chromosomes of eukaryotes appear often to be produced by illegitimate recombination events during mitosis (24). Despite the extensive genetic characterization of many kinds of recombination events, few specific functions have been ascribed to mitotic recombination. Some rearrangements in

eukaryotes, however, have obvious biological implications, for example immunoglobulin and T-cell receptor rearrangements (25) and rearrangements resulting in mating type switches in yeast (26) and antigenic variation of trypanosomes (27). In malaria parasites the possible function of rearrangements in which subtelomeric repeat sequences are involved, is unknown. In *P. berghei* the presence of telomere-related, 160 bp-stretches within subtelomeric 2.3 kb repeats, regularly spaced in an organization similar to that described for $(C_{1-3}A)_n$ repeats in *S. cerevisae*, suggested that these sequences might play a role in recombinational events which lead to the maintenance and dispersal of subtelomeric repeated structures (14). Our observation that 2.3 kb negative chromosomes can aquire these repeats during mitosis support this hypothesis (13). Subtelomeric rearrangements in *P. falciparum* involve breakage of chromosomes and addition of telomere repeats to 'heal' the broken ends (7). It has been suggested that this mechanism is involved in repositioning genes in the vicinity of chromosome ends and may be functioning in gene activation in *P. falciparum* (28). Evidence for this latter hypothesis is, however, lacking. In addition to these subtelomeric rearrangements, we show here that chromosome translocations occur in malaria parasites. Since it is the first time that such an event is detected in *Plasmodium*, it seems that translocation does not occur frequently with a specific biological function. Understanding of the mechanisms underlying the translocation event, however, might be helpful for the development of methods for targeted integration of DNA sequences in the genome of *Plasmodium*.

ACKNOWLEDGEMENTS

This work was carried out under EEC contract TS2-0126-I(NL) in the framework of the Program 'Science and Technology for Development'. We acknowledge the gift of the EP line from Dr. W. Eling (Nijmegen).

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