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Interchromosomal exchange of a large subtelomeric segment in a *Plasmodium falciparum* cross

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Duplications and interchromosomal transpositions of chromosome segments are implicated in the genetic variability of *Plasmodium falciparum* malaria parasites. One parasite clone, HB3, was shown to lack a subtelomeric region of chromosome 13 that normally carries a *PfHRPIII* gene. We show here that the chromosome 13 segment carrying *PfHRPIII* was replaced in HB3 by a duplicated terminal segment from chromosome 11. Mapping results indicate that the segment includes at least 100–200 kb of subtelomeric DNA and contains duplicated copies of the *Pf332* and *RESA-2* genes. We followed inheritance of this duplication in a genetic cross between the HB3 and another *P.falciparum* clone, Dd2, that is euploid for the *Pf332*, *RESA-2* and *PfHRPIII* genes. Three types of progeny from the cross showed expected inheritance forms: a Dd2 euploid parent type, an HB3 aneuploid parent type, and a recombinant euploid type that carried *PfHRPIII* from Dd2 chromosome 13 and *Pf332* from HB3 chromosome 11. However, a fourth euploid progeny type was also observed, in which the chromosome 13 segment from HB3 was transposed back to replace the terminus of chromosome 11. Three of 14 individual progeny were of this type. These findings suggest a mechanism of recombination from subtelomeric pairing and exchange between non-homologous chromosomes in meiosis.

Key words: duplicative transposition/genetic cross/malaria/*Pf332* and *RESA-2* genes/subtelomeric recombination

Introduction

There is evidence that heterologous chromosomes can align and undergo transpositions of large segments of DNA. Non-homologous chromosome pairing was observed by electron microscopy and provided cytogenetic evidence for physical association near telomeres during meiosis both in human fetal oocyte cells (Speed, 1988) and in *Saccharomyces cerevisiae* (Loidl *et al.*, 1994). Dancis and Holmquist (1979) presented evidence for telomeric association during mitosis. Brown *et al.* (1990) hypothesized that chromosome mispairing may be facilitated by subsets of similar sequences or repeat families near the chromosome ends, and presented evidence for suborganization of human telomere-associated DNA (also Wilkie *et al.*, 1991). But direct evidence of exchanges of large subtelomeric regions between heterologous chromosomes has not been reported. For heterologous chromosomes that do pair, Carpenter (1987) suggested the specificities would be immediately checked by heteroduplex formation so that without homology the complex would dissociate. This model predicts the occurrence of recombination between the terminal regions of heterologous chromosomes when such regions share homologous sequences.

Large polymorphic variations in chromosome size have been mapped to the subtelomeric regions of organisms with smaller genomes such as *S.cerevisiae* (Horowitz *et al.*, 1984; Zakian and Blanton, 1988; Louis *et al.*, 1994), *Plasmodium* species (Corcoran *et al.*, 1988; Sinnis and Wellems, 1988; Ponzi *et al.*, 1990) and *Giardia* (Adam *et al.*, 1991). Various mechanisms may produce these size polymorphisms in *Plasmodium falciparum*. Chromosome breakage and healing during mitotic expansion of parasite populations is one mechanism for loss of subtelomeric sequences and genes from *P.falciparum* chromosomes (Pologe and Ravetch, 1988; Cappai *et al.*, 1989; Scherf *et al.*, 1992). Expansion of large tandemly repeated units ('amplicons') within individual chromosomes has been shown to be another means of generating large chromosome size polymorphisms (Triglia *et al.*, 1991). Homologous recombination among subtelomeric repetitive elements has been postulated as a third mechanism of polymorphic variation (Corcoran *et al.*, 1988). Homologous chromosomes of different sizes readily undergo meiotic recombination after zygote formation to produce new size variants (Walliker *et al.*, 1987; Sinnis and Wellems, 1988).

Since many large deletions and loss of genes from these regions allow the complicated parasite life cycle to remain intact through mosquitoes and chimpanzees (Walliker *et al.*, 1987; Wellems *et al.*, 1990), the importance of subtelomeric regions in *P.falciparum* has been unclear. Some genes localized in subtelomeric regions are presently being tested as vaccine candidates, underscoring the need to understand the dispensable nature of these regions and their role in genetic diversity. Here we report genetic events in a *P.falciparum* cross that, without serious adverse effect on viability of certain progeny, led to transpositions of subtelomeric segments between chromosomes 13 and 11. The findings suggest a mechanism of subtelomeric pairing and recombination between heterologous chromosomes during meiosis.

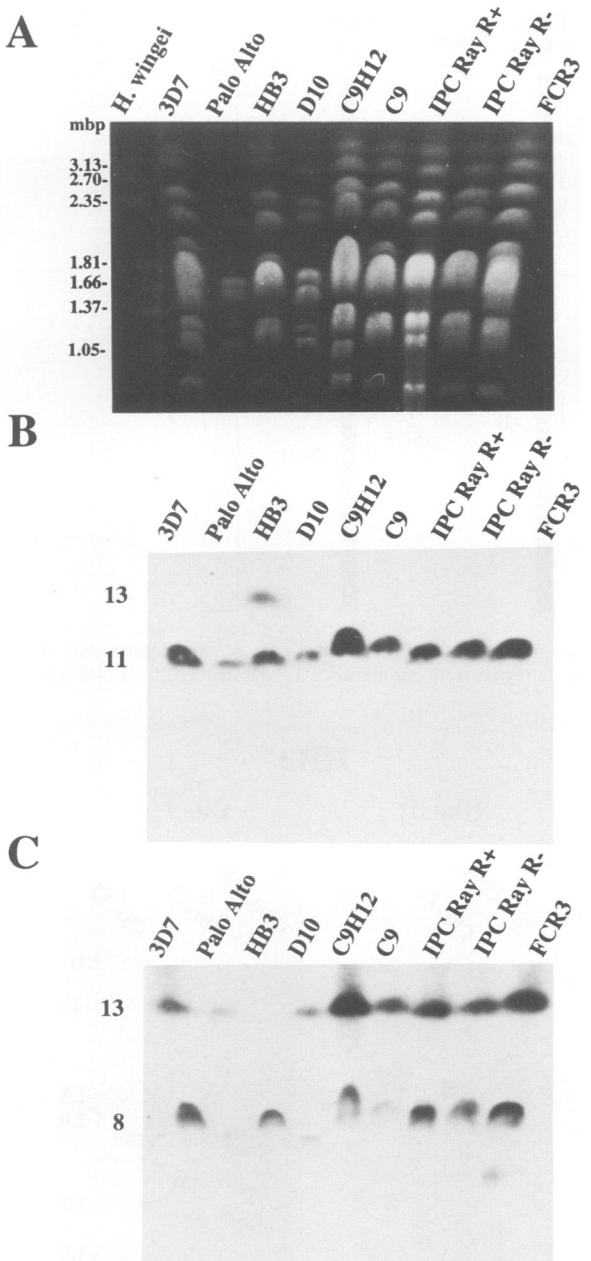

Fig. 1. PFGE and hybridization patterns of nine *P.falciparum* lines. (**A**) PFGE separations revealed by ethidium bromide staining. *Hansenula wingei* chromosome size markers are indicated (mbp = megabase pairs). (**B**) Southern hybridization patterns from the *Pf332* probe after washing at high stringency (0.1 × SSC/0.1% SDS at 65°C). (**C**) Hybridization patterns of the *PfHRPIII* probe after washing at moderate stringency (2 × SSC/0.1% SDS at 65°C) which allows cross-hybridization to the homologous *PfHRPII* gene on chromosome 8. *PfHRPIII* is absent from the HB3 *P.falciparum* clone.

Results

Duplication and transposition of a subtelomeric region from chromosome 11 to chromosome 13 in the P.falciparum HB3 clone

P.falciparum chromosomes 11 and 13 are heterologous and are respectively 2400 and 3200 kb long as determined from their physical maps and sets of unique restriction fragment length polymorphism (RFLP) markers (Walker-Jonah *et al.*, 1992). *Pf332*, which encodes a megadalton antigen in blood-stage parasites, is located in the subtelomeric region of chromosome 11 (Mattei and Scherf, 1992). *RESA-2* (Cappai *et al.*, 1992) is also located in this subtelomeric region (this study). In a karyotype study of nine different parasite lines we found that the *P.falciparum* HB3 clone yielded two chromosome hybridization signals with each of these gene probes. Figure 1A and B shows the hybridization with a *Pf332*-specific probe. Mapping with the chromosome 11- and 13-specific markers *pC11.1* and *pSL5* (Walker-Jonah *et al.*, 1992) indicated that a subtelomeric segment containing *Pf332* and *RESA-2* was duplicated on chromosome 13 in the *P.falciparum* HB3 clone.

A region at one end of chromosome 13 of clone HB3 has also been found to be involved in deletion of the *PfHRPIII* gene (Wellems *et al.*, 1987). Figure 1C shows that the deletion of *PfHRPIII* from the *P.falciparum* HB3 clone correlates with the duplication of *Pf332* and *RESA-2*. The HB3 clone is therefore aneuploid for these genes.

To study the chromosome rearrangements underlying the aneuploid state of the HB3 clone we isolated agarose-embedded chromosome 11 and 13 DNAs from *P.falciparum* clones HB3 and Dd2 and compared their long-range restriction maps. Figure 2 presents the terminal 1000 kb of restriction maps from the HB3 and Dd2 clones. On HB3 chromosome 11 *Pf332*, *RESA-2* and *pC11.1* hybridization signals were localized to a 150 kb *Bgl*I fragment 50 kb internal to the *rep20* repeats and the telomere. Signals from the Dd2 chromosome 11 restriction fragments mapped similarly except that the terminal *Bgl*I site was absent. On HB3 chromosome 13 the telomeric repeats, *rep20*, *Pf332* and *RESA-2* all mapped to a terminal 200 kb *Sma*I restriction fragment while *pC11.1* was not detected, indicating that the breakpoint of the transposition lies within this 200 kb *Sma*I restriction fragment. The Dd2 chromosome 13 terminus yielded no hybridization signals when probed with *rep20*, *Pf332* or *RESA-2* but showed the expected subtelomeric 200 kb *Sma*I fragment containing *PfHRPIII*.

Distinguishable Pf332 allelic forms on HB3 chromosomes 11 and 13

The *Pf332* gene is composed largely of tandem repeats, has an open reading frame of ~25 kb, and exists in many polymorphic forms (Mattei and Scherf, 1992). Total HB3 genomic DNA digested with *Hinc*II or *Dde*I and probed with the cloned *Pf332* sequence (pG9) suggested that the duplicated *Pf332* genes on HB3 chromosome 13 contained distinguishable RFLPs (Figure 3). To confirm this we purified the HB3 chromosomes 11 and 13 DNAs, digested them with *Hinc*II and *Dde*I and analyzed the resulting fragments by Southern blot analysis. Figure 3 shows that the chromosome DNAs yield distinguishable patterns of hybridization which together account for the combined pattern from total genomic DNA. Two different alleles (which we term *Pf332*A and *Pf332*B) are present on HB3 chromosomes 11 and 13, respectively. Restriction digestion experiments with *Dra*I were also performed and indicated different alleles on the two chromosomes (data not shown).

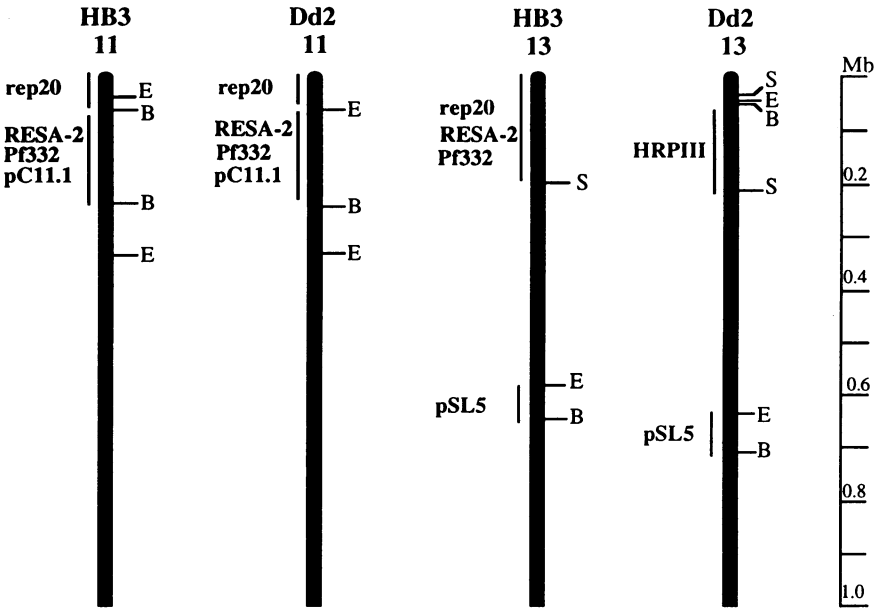

Fig. 2. Long range restriction maps of the terminal regions of HB3 and Dd2 chromosomes 11 and 13. Locations of the genetic markers are indicated at the left of each map. HB3 chromosome 13 contains a large transposed subtelomeric region from chromosome 11 that includes the *Pf332* and *RESA-2* genes. Restriction endonuclease sites: S, *Sma*I; B, *Bgl*I; E, *Eag*I.

Stable inheritance of Pf332 and PfHRPIII aneuploidy in a P.falciparum cross

A *P.falciparum* cross between the HB3 and Dd2 parasite clones (Wellems *et al.*, 1990) was analyzed for the inheritance of *Pf332* parental forms. For this work we examined 14 progeny clones that were independent recombinants from the HB3 × Dd2 cross (Walker-Jonah *et al.*, 1992). Figure 4A presents results from a pulsed field gel electrophoresis (PFGE) separation of the progeny chromosome DNAs probed for *Pf332*. Two progeny, TC-05 and 3B-D5, showed the parental HB3 pattern of *Pf332* hybridization signals on both chromosomes 11 and 13. The same pattern was obtained with the *RESA-2* probe (data not shown) while the *PfHRPIII* probe yielded no signals from progeny TC-05 and 3B-D5 (Walker-Jonah *et al.*, 1992). Table I summarizes the data from these karyotyping studies.

Recombination among allelic forms and transposition of chromosome termini

The copy from the Dd2 parent was also distinguishable in *Hinc*II digests as a third allele designated *Pf332*C (Figure 4B). Examination of restriction digests of progeny DNA shows that some progeny had inherited the *Pf332*C allele from the Dd2 parent (Figure 4B, right: QC-23, B1-SD, QC-13, QC-01, 3B-A6). Others (Figure 4B, left) had inherited the *Pf332*A allele from HB3 chromosome 11 (1B-B5, SC-01, QC-34, GC-06) or had both copies *Pf332*A and *Pf332*B from the HB3 chromosomes as described above (TC-05, 3B-D5). An unexpected finding, however, was that the HB3 chromosome 13 *Pf332*B allele occurred alone in three progeny (Figure 4B, left: GC-03, B4-R3, TC-08; cf. Figure 3) and that this allele was found in these progeny on chromosome 11 (Figure 4A). These inheritance patterns were confirmed by additional restriction digestion analysis with *Dra*I and *Dde*I (data not shown). Table II summarizes these results.

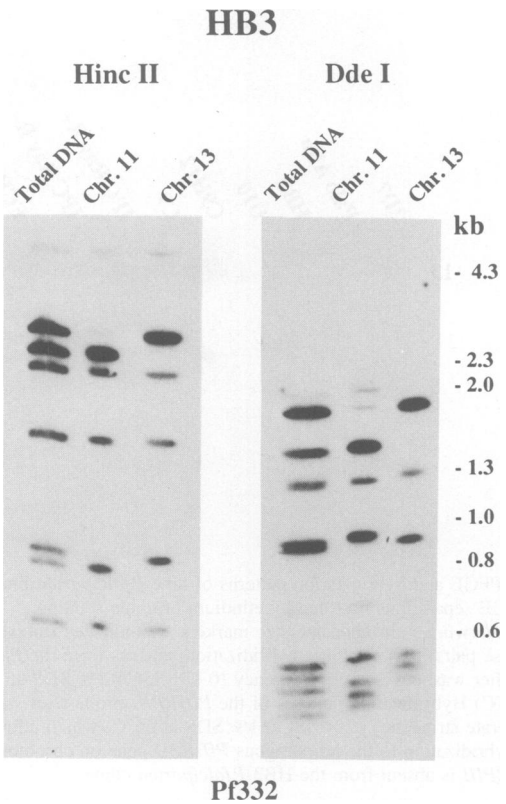

Fig. 3. Restriction enzyme patterns of genomic DNA, chromosome 11 and chromosome 13 from the *P.falciparum* HB3 clone. DNA from chromosomes 11 and 13 was isolated by preparative PFGE. The left and right panels show the patterns from *Hinc*II and *Dde*I, respectively. Superposition of the bands observed with the *Pf332* probe on chromosomes 11 and 13 gives the same pattern as seen with total genomic DNA.

Although the transposition of the *Pf332* allele from chromosome 13 to 11 most likely involved a large subtelomeric segment, a local event such as gene conversion

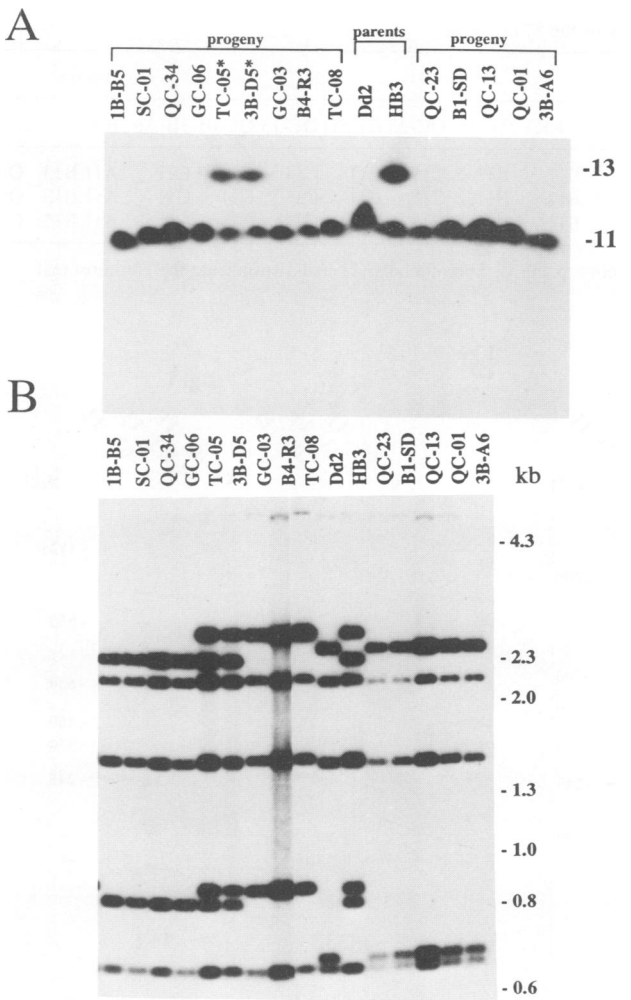

Fig. 4. *Pf332* hybridization patterns of PFGE separations and restriction digested genomic DNAs from the *P.falciparum* HB3 × Dd2 cross. (**A**) Results of hybridization to PFGE separations of chromosomes from the parents and progeny. Two progeny clones, TC-05 and 3B-D5 (asterisked), carry a copy of the *Pf332* gene on both chromosomes 11 and 13. (**B**) Southern blot analysis of genomic DNA of the cross digested with *Hin*cII. The parent HB3 and Dd2 clones yield distinct *Pf332* hybridization patterns. Progeny patterns to the right of the parent lanes show the Dd2 allele, whereas progeny patterns to the left of the parent lanes show bands only from the HB3 parent. Clones TC-05 and 3B-D5 carry copies of *Pf332* on both chromosomes 11 and 13 and have a pattern identical to that of HB3. Three clones (GC-03, B4-R3 and TC-08) have the *Pf332* RFLP from HB3 chromosome 13 but carry this *Pf332* allele on chromosome 11.

remained a possible explanation. To verify that a large segment at the chromosome terminus had indeed been exchanged, *Eag*I and *Bgl*I restriction fragments of chromosome 11 from the TC-08, B4-R3 and GC-03 progeny were compared with those from Dd2 chromosome 11 and HB3 chromosomes 11 and 13 (Figure 5). Figure 5A shows that similar *Eag*I fragments were detected by the *Pf332* probe in each of the three progeny. Subsequent probing of the blots with the telomeric probe and *rep20* detected these same restriction fragments (Figure 5A and data not shown). Thus the TC-08, B4-R3 and GC-03 chromosome 11 termini all lack an *Eag*I site separating *Pf332* from the telomere, a feature that is characteristic of the HB3 chromosome 13 terminus but not of the HB3 and Dd2 chromosome 11 termini (Figures 5A and 2). Figure 5B shows that TC-08, B4-R3 and GC-03 chromosomes 11 all lack a *Bgl*I site near the terminus, again consistent with transposition of a large subtelomeric segment from the HB3 chromosome 13 terminus carrying the *Pf332*B allele. Analysis of these data and yeast artificial chromosome (YAC) mapping experiments (unpublished) have indicated that the size of the transposed segment is between 100 and 200 kb. A summary of the genetic analysis of the progeny is diagrammed in Figure 6.

Discussion

Except for a brief diploid phase after mating in the midgut of the mosquito, the *P.falciparum* malaria parasite is haploid throughout the life cycle (Walliker *et al.*, 1987). The parasite contains 14 nuclear chromosomes which have a conventional linear structure bounded by telomeric repeats (reviewed by Triglia *et al.*, 1992). Internal to these telomeric repeats are large subtelomeric regions (~50–250 kb) that contain various families of repetitive DNA and relatively few genes (Corcoran *et al.*, 1988; Vernick and McCutchan, 1988; Lanzer *et al.*, 1993; Dolan *et al.*, 1993a; de Bruin *et al.*, 1994). The function of these subtelomeric regions is unclear, but the fact that the genes they harbor are frequently cloned in serological screens indicates that these regions are important in the antigenic definition of the parasite and in the modulation of host–parasite interactions.

Deletions of genes are described from a number of different chromosome termini. Chromosome breaking and healing events have been demonstrated near or in such subtelomeric genes as *PfHRPI*, *PfHRPII*, *RESA*, *Pf11-1*

Table I. Localization of the *Pf332*, *RESA-2* and *PfHRPIII* genes and anonymous DNA probes on chromosomes 11 and 13 in the *P.falciparum* HB3 × Dd2 cross

	Progeny														Parents	
Chromosome localization	1B-B5	SC-01	QC-34	GC-06	TC-05	3B-D5	GC-03	B4-R3	TC-08	QC-23	B1-SD	QC-13	QC-01	3B-A6	HB3	Dd2
Pf332	11	11	11	11	13,11	13,11	11	11	11	11	11	11	11	11	13,11	11
PfHRPIII	13	13	13	13	neg.	neg.	13	13	13	13	13	13	13	13	neg.	13
RESA-2	11	11	11	11	13,11	13,11	11	11	11	11	11	11	11	11	13,11	11
YAC *Pf332*-AV	11	11	11	11	13,11	13,11	11	11	11	11	11	11	11	11	13,11	11
YAC *Pf332*-BV	11	11	11	11	13,11	13,11	11	11	11	11	11	11	11	11	13,11	11

DNA probes *Pf332*-AV and *Pf332*-BV were derived from the ends of a 70 kb YAC insert from the subtelomeric region of chromosome 11 containing the entire *Pf332* gene (D.Mattei, J.Ravetch and A.Scherf, manuscript in preparation). neg. = negative.

Table II. Inheritance of the three different types of parental restriction patterns of the *Pf332* gene

	Progeny														Parents	
RFLP type of *Pf332*	1B-B5	SC-01	QC-34	GC-06	TC-05	3B-D5	GC-03	B4-R3	TC-08	QC-23	B1-SD	QC-13	QC-01	3B-A6	HB3	Dd2
*Dra*I	A11	A11	A11	A11	A11,B13	A11,B13	B11	B11	B11	C11	C11	C11	C11	C11	A11,B13	C11
*Dde*I	A11	A11	A11	A11	A11,B13	A11,B13	B11	B11	B11	C11	C11	C11	C11	C11	A11,B13	C11
*Hinc*II	A11	A11	A11	A11	A11,B13	A11,B13	B11	B11	B11	C11	C11	C11	C11	C11	A11,B13	C11

A, B and C represent the different *Pf332* alleles distinguishable by their restriction patterns. The numbers (11 and 13) indicate the chromosomal location of the *Pf332* gene in progeny and parental clones.

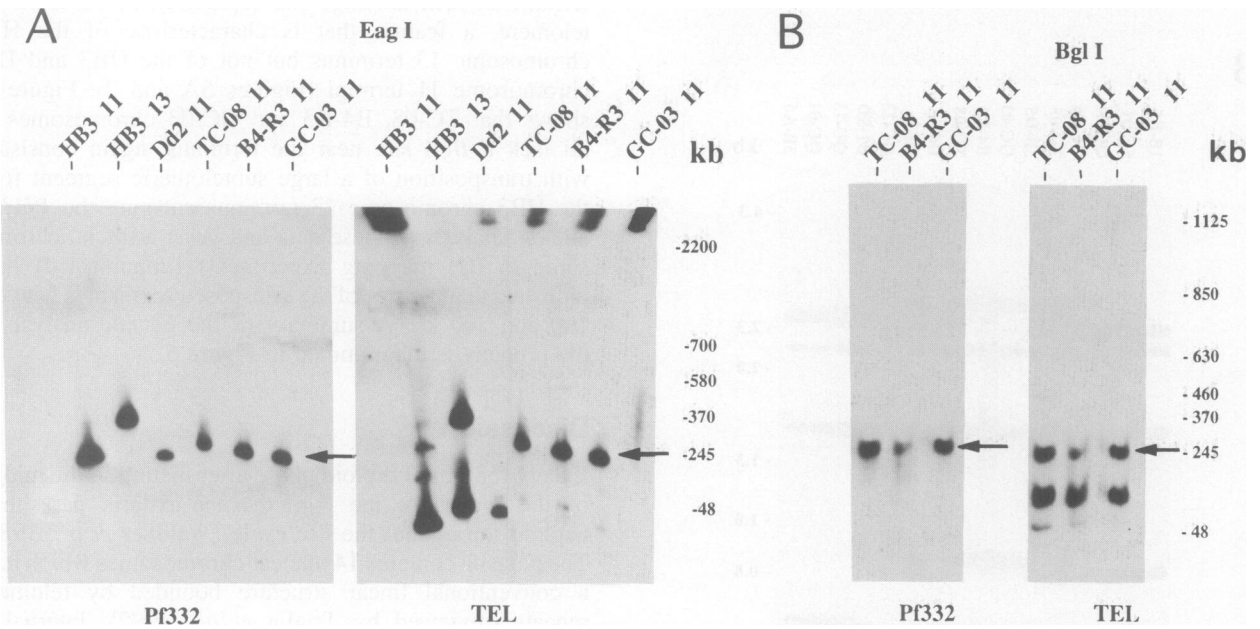

Fig. 5. Restriction analysis of chromosome 11 from progeny carrying a subtelomeric exchange from chromosome 13 on that chromosome. Panels **A** and **B** present *Eag*I and *Bgl*I restriction fragments of chromosome 11 from the progeny TC-08, B4-R3 and GC-03 and the parents Dd2 and HB3 plus chromosome 13 from HB3. The *Pf332* gene in the progeny co-localizes with the telomere repeats as found on chromosome 13 of HB3 (indicated by arrows; see also Figure 2). The TC-08, B4-R3 and GC-03 chromosome 11 termini all lack an *Eag*I site separating *Pf332* from the telomere, a feature that is characteristic of the HB3 chromosome 13 terminus but not of the HB3 and Dd2 chromosome 11 termini.

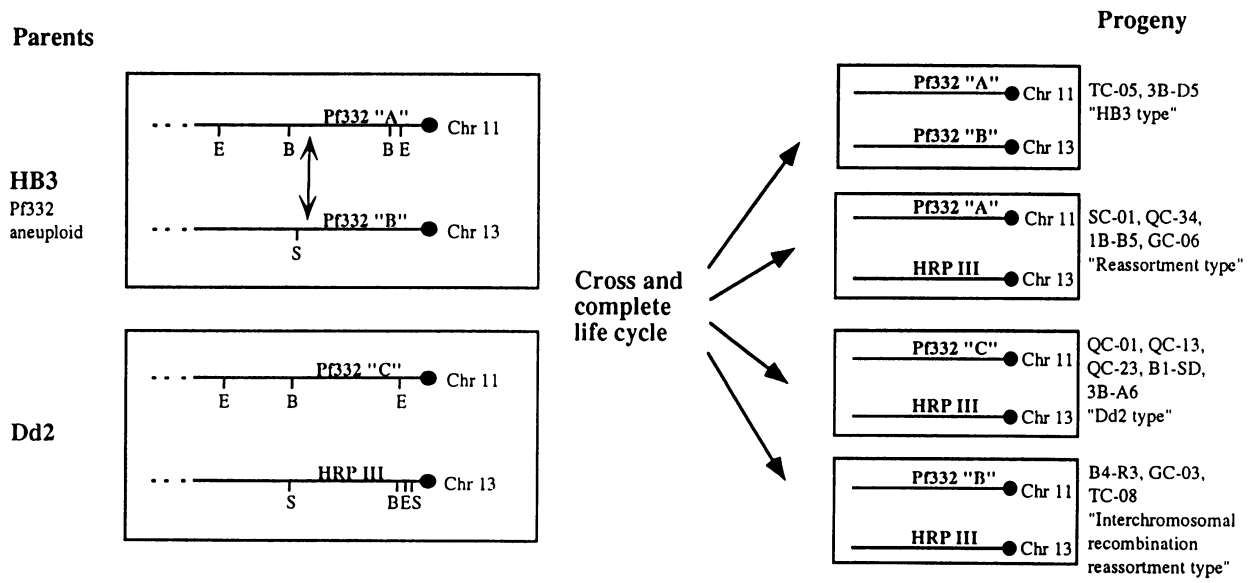

Fig. 6. Schematic illustration of inheritance of the chromosome 11 and 13 subtelomeric regions in the HB3 × Dd2 cross. Restriction enzymes: E, *Eag*I; B, *Bgl*I; S, *Sma*I. The approximate breakpoint in the interchromosomal recombination and reassortment type is indicated by an arrow.

and *Pf332* (Pologe and Ravetch, 1988; Cappai *et al.*, 1989; Scherf and Mattei, 1992; Scherf *et al.*, 1992; Mattei and Scherf, 1994). Some chromosome breakages occur frequently: the chromosome terminus containing the *Pf11-1* gene, for example, is estimated to undergo breakage in 0.03–0.15% of parasites during each erythrocytic stage cycle in several *P.falciparum* lines (Scherf and Mattei, 1992). Maintenance of these genes in wild-type parasites is evidence for selection pressures and the importance of the gene products *in vivo*.

Double-stranded DNA breaks create 'reactive' ends which can be repaired by the addition of telomere repeats (for review see Blackburn, 1991). The broken chromosome end may be lost or may translocate to a heterologous chromosome. In *Plasmodium*, very large chromosome segments may be involved. For example, Janse and Mons (1992) have reported the fusion of two heterologous chromosome regions into a large hybrid chromosome in a mouse malaria parasite, *P.berghei*. Depending upon how the fused chromosomes are carried through the mitotic process and the viability of daughter cells, such translocation events may produce aneuploid parasites which become fixed in the population. Subsequent divergence of the duplicated regions would lead to variant forms and new genes. The results of several studies support this hypothesis. Recognizably related genes such as *PfHRPII* and *PfHRPIII* which are located, respectively, on chromosomes 8 and 13 (Wellems and Howard, 1986; Wellems *et al.*, 1987), *RESA* and *RESA-2* on chromosomes 1 and 11 (Corcoran *et al.*, 1988; Cappai *et al.*, 1992), *GBP* and *GBP-H* genes on chromosomes 10 and 14 (Nolte *et al.*, 1991), and *Pf11-1* and *Pf332* on chromosomes 10 and 11 (Scherf *et al.*, 1988, 1992; Mattei and Scherf, 1992) support the idea of frequent duplication and divergence of antigenically important genes from common ancestral sequences. All of these genes map to subtelomeric regions, indicating that the gene pairs were generated by duplications of chromosome termini. Further evidence for transposition of telomeric genes comes from a recent karyotype analysis of *P.falciparum* wild isolates from West Africa. Eight different isolates were found to carry the *RESA-2* gene on chromosome 11 and a cross-hybridizing copy on chromosome 14 (K.Hinterberg and A.Scherf, manuscript in preparation).

Pf332 and *RESA-2* aneuploidy in the *P.falciparum* HB3 clone evidently arose by duplicative interchromosomal transposition of the chromosome 11 terminus to chromosome 13. This rearrangement was not detectable in nine other laboratory isolates that were studied nor was it detected in 20 field isolates from South America and West Africa (unpublished). Thus the duplication may represent a recent event than the more widespread *PfHRPII/PfHRPIII*, *GBP/GBP-H*, *RESA/RESA-2* and *Pf332/Pf11-1* gene pairs. Another hypothesis is that the duplication arose and was fixed in a parasite population that is genetically isolated or may be at an early stage of species divergence from *P.falciparum*. Studies of cytoplasmic inheritance have recently suggested such a possibility for the HB3 clone, which derives from Honduras (Vaidya *et al.*, 1993). To address this possibility it will be necessary to compare the cytoplasmic genome types and karyotypes of additional isolates from Central America.

The 14 progeny of the HB3 × Dd2 cross analyzed in this work have been shown to be the genetically distinct products of independent meiotic events (Walker-Jonah *et al.*, 1992). Observation in three of the 14 progeny of a transposition from chromosome 13 to chromosome 11 indicates a relatively high rate of recombination between the respective subtelomeric regions. Transposition must have occurred after zygote formation and before completion of meiosis because all progeny possessing the *Pf332*B allele on chromosome 11 have the *PfHRPIII* gene from the Dd2 parent on chromosome 13. Long range restriction mapping indicates that the exchanged segments of DNA are large (>100 kb) and are not explained by gene conversion events. The 'B' allelic form of *Pf332* that moved from chromosome 13 to 11 produces functional protein as judged by immunofluorescence analysis of asexual blood-stage parasites using anti-*Pf332*-specific antibodies (data not shown).

The absence of other aneuploid progeny such as for example the Pf332C-11/Pf332B-13 reassortment type (Figure 6) may reflect low numbers of aneuploid forms coming through the cross which are too few to give a distribution of all possible genotypes. After all there were only two 'HB3-type' *Pf332*A-11/*Pf332*B-13. We speculate, however, that aneuploid progeny carrying the *Pf332*A allele on chromosome 13 could be found in an HB3 self-fertilization experiment. An interesting question also is whether there is low frequency exchange between the homologous ends of chromosomes during asexual (mitotic) reproduction. In yeast and other eukaryotes genetic recombination occurs 3–4 orders of magnitude less frequently in mitosis than in meiosis (Esposito and Wagstaff, 1983; reviewed by Petes and Hill, 1988).

Antigens at the surface of *P.falciparum*-infected erythrocytes undergo clonal variation at a rate of 2% per generation (Roberts *et al.*, 1992, 1993). Although much work remains to be done before the underlying genetic mechanisms of antigenic variation are understood, divergence of subtelomeric gene families appears to be one important means of producing variation in a large number of alleles in the parasite population. The nature of the events described here appears to differ from the programmed shifts in surface glycoproteins that arise by duplication and transpositions of gene cassettes in African trypanosomes, expressed copies of which are usually also subtelomeric in location (reviewed by Van der Ploeg *et al.*, 1992). It appears instead that many variant genes in *Plasmodium* may develop not by such programmed shifts but by occasional duplications and transpositions of very large subtelomeric regions from one chromosome to another. After recombination in the zygote, these duplications become established in the population and diverge. Over the long term this is a powerful adaptive mechanism by which *P.falciparum* parasites generate new diversity in their antigenic profiles.

Materials and methods

P.falciparum lines and in vitro cultivation

P.falciparum lines were maintained in culture as described by Trager and Jensen (1976). The parents and 14 independent recombinant progeny from the HB3 × Dd2 genetic cross have been described (Wellems *et al.*, 1990; Walker-Jonah *et al.*, 1992). Other *P.falciparum* lines included 3D7 (Walliker *et al.*, 1987), Palo Alto Uganda (Gysin *et al.*, 1982), D10, C9H12, C9 (Scherf *et al.*, 1992); IPCRay R+, IPCRay R− (Tourneur *et al.*, 1992) and FCR-3 (Trager and Jensen, 1976).

Agarose gel electrophoresis and Southern blotting

P.falciparum DNA was digested with restriction enzymes, separated on 1% agarose gels, blotted under alkaline conditions onto nylon filters (Hybond N+, Amersham) and hybridized to radiolabeled probes by standard procedures (Sambrook *et al.*, 1989). Between hybridizations probes were stripped from blots by washing in 0.4 M NaOH for 45 min and in 0.1 × SSC, 0.1% SDS, 0.2 M Tris−HCl pH 7.5 for 15 min.

DNA probes

The pG9 probe containing *Pf332* repetitive sequences (Mattei and Scherf, 1992), the *PfHRPIII* gene probe (Wellems *et al.*, 1987), the subtelomere *rep20* probe (Patarapotikul and Langsley, 1988), the probe containing *P.berghei* telomere repeats (Ponzi *et al.*, 1985) and the anonymous pC11.1 and pSL5 probes (Walker-Jonah *et al.*, 1992) were prepared by standard protocols and labeled by random priming (Amersham Corp., Arlington Heights, IL). The *Pf332*-AV and *Pf332*-BV DNA probes were derived from each end of the 70 kb insert from a YAC clone (de Bruin *et al.*, 1992) carrying the chromosome 11 subtelomeric region that includes the *Pf332* and *RESA-2* genes (D.Mattei, J.Ravetch and A.Scherf, manuscript in preparation). An oligonucleotide (5′-TTGAATAAGTATGAAACAACA-3′) corresponding to the 5′ end of the *P.falciparum RESA-2* (Cappai *et al.*, 1992) gene was ^{32}P-labeled using T4 kinase.

Pulsed field gradient gel electrophoresis and chromosome mapping

PFGE was performed in a CHEF apparatus at 18°C using 0.5 × TBE as running buffer. *P.falciparum* chromosome blocks were prepared according to Kemp *et al.* (1985). *Hansenula wingei* chromosomes (Bio-Rad; strain YB-4662-VIA) were used as size markers. Chromosomes (500–3500 kb) were fractionated in 0.7% agarose (chromosomal grade, Bio-Rad) with switching time ramped from 100 to 300 s for 24 h at 90 V followed by switching time ramped from 300 to 720 s for 24 h at 80 V. These conditions allowed separation of the large chromosomes within 48 h.

Isolation and restriction digests of individual chromosomes for chromosome mapping were performed as described by Dolan *et al.* (1993b). Chromosome fragments (50–2500 kb) were separated in 1.2% agarose gels with the switching time ramped from 50 to 90 s at 200 V for 20 h. *Saccharomyces cerevisiae* chromosomes (Bio-Rad) and bacteriophage lambda concatemers (Bio-Rad) were used as size markers. After electrophoresis, gels were stained with ethidium bromide, photographed, exposed to UV light (260 nm) for 5 min and blotted onto nylon filters (Hybond N+, Amersham) as described above.

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