Subtelomeric Structure of *Plasmodium falciparum* Chromosomes

R Scotti, T Pace, L Roca, C Frontali

Lab.Cell Biology, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

Previous studies of subtelomeric regions in Plasmodium berghei *led to the identification of subtelomeric repeats (2.3kb long) present in a variable number at many chromosomal ends. Both loss and increase in 2.3kb-repeat copy number are involved in chromosome-size polymorphisms.*

Subtelomeric losses leading to chromosome-size polymorphisms have been described by several authors in P.falciparum *where the structure of subtelomeric regions is not known in detail. We therefore undertook their characterisation, by means of chromosome walking and jumping techniques, starting from the telomere-flanking sequence present in pPftel.1, the* P.falciparum *telomeric clone described by Vernick and McCutchan (1988).*

The results indicate that at least 20 (out of 28) chromosomal ends in P.falciparum *3D7 chromosomes share a subtelomeric region, about 40kb long, covering (but not limited to) the Rep20 region. Non repetitive, AT-rich portions flanking the Rep20 region on both sides are also conserved at most chromosomal ends.*

Key words: *Plasmodium falciparum* - subtelomeric structure - repetitive DNA

Subtelomeric regions are known to be preferential sites for chromosomal rearrangements and deletions in many lower eukaryotes. In the genus *Plasmodium* these events have been shown to be responsible for observable chromosome-size polymorphisms. In *P.falciparum* several cases have been described where distal genes involved in terminal deletions are interrupted, and the interested chromosome is healed by direct growth of telomeres from within the gene. These events are generally well detailed at the level of the gene sequence, while the subtelomeric regions subject to such instabilities are not well characterised.

Low resolution maps are available for some *P. falciparum* chromosomes. A distinctive feature in these maps is the presence of a conserved ApaI site (Corcoran et al. 1988), which sets the distal limit of a fragment containing the Rep20 region. The latter consists of tandemly arranged, 21bp, imperfect repeats, but it is not clear how this region is connected to the telomere.

We have previously reported (Ponzi et al. 1990) the characterisation of immediately subtelomeric regions in *Plasmodium berghei*. These regions present a periodic structure in which telomere-related sequences regularly alternate with a 2.1kb repeat. The resulting 2.3kb repetitive elements were shown to be present in variable copy number at several (but not all) chromosomal extremities, and to be endowed with mobility, being able to be transferred to chromosomal extremities formerly devoid of them (Pace et al. 1990). A very similar structure has been described for the subtelomeric organisation in the yeast *Saccharomyces cerevisiae*.

From preliminary results (Scotti et al. 1993), a quite different subtelomeric organisation appears to be present at most *P. falciparum* chromosomal extremities, which share a PstI terminal fragment about 40kb long, encompassing (but not limited to) the Rep20 region. The present study aims at a more detailed description of this conserved region, through walking and jumping techniques.

MATERIALS AND METHODS

Strain - *P.falciparum* 3D7 was the source of genomic DNA used to construct a Sau3A partial library in lambda-ZAP (Stratagene).

Probes - Clone 26, described by Scotti et al. (1993), was used to start chromosome walking from the telomere inward. Clone pTB4.1 from *P.berghei*, described by Ponzi et al. (1990), was used as a purely telomeric probe.

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Chromosome jumping - The following strategy was used. High molecular weight genomic DNA from *P. falciparum* 3D7 was trimmed with Bal31, then digested with PstI and ligated to SmaI+PstI linearised pUC8. The ligation products were circularised at high dilution, then digested with AccI, and recircularised. This procedure eliminates a large part of the fragment of plasmodial origin, leaving in the final circle only its outer portions. Selection with ampicillin resistance and hybridisation to the telomeric probe pTB4.1 allows identification of recombinant clones originating from terminal PstI restriction fragments and containing their proximal PstI-AccI subfragment.

RESULTS

Clone 26 (Scotti et al. 1993) was used as the starting probe to walk along the region common to 20 out of 28 chromosomal extremities in *P. falciparum* 3D7. Clone 26 overlaps the proximal part of the telomeric clone pPftel.1 described by Vernick and Mc Cutchan (1988). Its sequence (Scotti et al. 1993) does not reveal any periodicity. Two clones were selected, using clone 26 as a labelled probe, from a Sau3A partial genomic library. Partial sequencing allowed their correct orientation. The longest one, clone 3 (about 6kb long) was then used to select from the same library four clones, which could be correctly oriented by partial sequencing and shown to overlap on one side the proximal part of clone 3 and to conform to the Rep20 consensus at the other end. The clones most probably derive from different chromosomes, as shown by small differences in their sequenced extremities. In particular the sequences available for the region of overlap with clone 3 differ only for point mutations. The region is moderately (61%) AT-rich, it does not contain repetitive structures, and exhibits two conserved ApaI sites, 124bp apart, most probably to be identified with the ApaI site indicated by Corcoran et al. (1988). The shorter of the four clones (clone 140, 4kb long) was subcloned to obtain TaqI subfragments covering its proximal portion.

By sequencing the TaqI subfragments of clone 140, we were able to locate the distal border of the Rep20 region at 12.5kb from the telomere end. It could also be shown that a non-degenerate 12bp repeat, related to the 21bp consensus, is present in at least 14 copies at the distal border of Rep20.

Since walking through the Rep20 region is an almost imposible endeavour, we resorted to chromosome jumping to be able to explore its proximal flanking region. It was thus possible to obtain a clone located beyond Rep20, which was then used for further walking in both directions. While physical mapping of the proximal region is presently under way, we are in position to estimate in about 24kb the length covered by Rep20. Several clones completely embedded in the Rep20 region were also partially sequenced. The only exception to the 21bp consensus was found in a 23bp repeat present in at least 9 non-degenerate tandem copies.

A few telomeric repeats are irregularly scattered in the unique region proximal to Rep20. They are in reverse orientation with respect to the actual telomere, so that they cannot be easily correlated with the direct telomeric sequences regularly organised in *P. berghei* subtelomeres.

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