

Isolation of a Distally Located Gene Possibly Correlated with Gametocyte Production Ability

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Previous studies were focussed on the attempt to correlate observable variations in the size of Plasmodium berghei chromosomes with the loss of ability to produce viable gametocytes. A temporal coincidence between the appearance of a subtelomeric deletion on P. berghei chromosome 5 and the loss of the ability to produce viable gametocytes was observed in a clone (HPE) directly derived from the high gametocyte-producer clone 8417 during mechanical passages. Interestingly enough, three P. berghei sexual-specific genes have already been mapped on internal fragments of this chromosome. A novel gene, clone 150, isolated from a genomic library of clone 8417 using a probe enriched for sexual-specific transcripts, maps on chromosome 5 within 100kb from the telomere. Subtelomeric deletions of chromosome 5 affecting two non-producer clones involve part of the transcribed region of this gene.

Key words: chromosome-size polymorphism - sexual-specific genes - gametocyte differentiation

During mitotic multiplication in the vertebrate host or *in vitro* cultures, a percentage of *Plasmodium* parasites takes the decision to differentiate in male and female gametocytes. The complex differentiative program occurring in the haploid committed cell, most probably involves the switching-on, and possibly the silencing, of many genes. In effect, several gametocyte-specific genes have been isolated, mainly in *P. falciparum* (reviewed by Alano 1991). The characterization of their 5' and 3' untranslated regions would be of great help in the attempt to define structural elements and/or factors involved in the regulation of their expression. So far, however, molecular events which regulate the initial commitment and the progression leading to mature sexual cells, are completely unknown.

A reduction in gametocyte conversion rate has been often observed in parasite populations forced to propagate only through mitotic division. One would expect that mutations conferring the gametocyteless phenotype might occur at several steps along the pathway which leads to the formation of viable gametocytes. They might arise either from large rearrangements which can be revealed by Pulse Field Gel Electrophoresis (PFGE) analysis, or from point mutations, or from limited rearrangements which remain undetected.

A significant correlation between the loss of ability to produce viable gametocytes and the appearance of a large deletion on chromosome 5 was observed (Janse et al. 1992) in a *P. berghei* clone (HPE) derived from the high producer clone 8417 through mechanical passages.

In the present study we describe the characterization of a gene located on chromosome 5, which is actively transcribed in the 8417 gametocyte-producer clone and is silent in two non producer clones. Its inactivation is probably due to a subtelomeric deletion whose starting point maps within the transcribed region of the gene.

MATERIALS AND METHODS

Strains - *P. berghei* ANKA clone 8417 (high gametocyte-producer), and clone HPE (non-producer) derived from 8417 by mechanical passages were used, along with *P. berghei* K173 clone 1 (Janse et al. 1992).

Subtraction strategy - A genomic DNA library was constructed from clone 8417 DNA partially digested with *Sau3A* restriction enzyme, using lambda ZAP (Stratagene) as a vector. Plaques were screened with a subtracted probe obtained as follows: labelled cDNA was synthesized on polyA+ RNA extracted from asynchronously growing 8417 parasites and hybridized to a 10-fold excess of cold polyA+ RNA prepared from asynchronously growing non-producer parasites (clone 1 derived from

K173 strain). Messages common to both clones were thus subtracted from the pool (Invitrogen, Subtractor I kit). The single stranded labelled cDNA in excess constitutes a probe enriched for sequences expressed exclusively in the producer clone.

PFGE analysis and digestion of purified chromosomes - P. berghei chromosomes were separated in an LKB Pulsaphor apparatus equipped with a hexagonal electrode array (CHEF) as described by Ponzi et al. (1990). Separated chromosomes were excised as agarose blocks, rinsed in digestion buffer before adding the appropriate restriction enzyme.

RESULTS

CHARACTERIZATION OF CLONE 150

Clone 150 was selected from the high-producer genomic library using the subtracted cDNA as a probe (see Materials and Methods).

The cloned fragment, 2.9kb in size, highlights a hybridization band of about 1.8kb on total RNA extracted from 8417 producer clone; no signal was detected on RNA from clone 1 derived from K173 non-producer strain. The cloned fragment comprises 900bp of the 3' end of the gene and a non-transcribed region. Part of the coding sequence and the 5' end of the gene are missing.

CHROMOSOME MAPPING

When clone 150 is used as a probe on PFGE separated chromosome it hybridizes to chromosome 5 of the reference clone 8417.

P. berghei chromosome 5 is not subject to frequent rearrangements during mitotic multiplication of the parasites. This fact is probably due to the absence in subtelomeric position of the highly recombinogenic 2.3kb repeats tandemly arranged over 10-100kb at several chromosomal extremities (Ponzi et al. 1990, Dore et al. 1990). We have recently cloned two independent fragments (clone 46 and 47) located in proximity of chromosome 5 telomeres. Both recognize the two ends of the chromosome, indicating the presence of a terminal conserved region.

Three sexual-specific genes map on internal fragments of this chromosome. These are: B9 (cloned by us but still not characterized), Pbs21 (Paton et al. 1993), and C-type small ribosomal subunit RNA gene (Waters, personal communication). In order to determine whether clone 150 is located in internal or subtelomeric position, we digested pu-

rified chromosome 5 with different restriction enzymes and probed the digests with telomeric and subtelomeric sequences; we were thus able to demonstrate that the gene under study is located within 100kb from the chromosome end.

A similar analysis was carried out on K173 clone 1, in which chromosome 5 and 6 comigrate. Since it is impossible to purify them separately, 4 bands are lit up by a telomeric probe after restriction enzyme digestion. Chromosome 5 telomeric bands were identified using the specific subtelomeric probe (clone 47). These bands differ in size from those of 8417 chromosome 5, indicating that some rearrangement occurred in subtelomeric position. The use of internally located probes shows that the internal organization of this chromosome is conserved in the two clones.

By using different subfragments of clone 150 as probes it was possible to show that in K173 clone 1 a deletion in chromosome 5 involves the entire flanking region and part of the transcribed sequence of clone 150. This most probably accounts for the absence of the specific transcript.

In order to confirm the relevance of the above described rearrangement in gametocytogenesis, we characterized the chromosome 5 deletion occurring in the gametocyteless clone HPE. This clone was derived from the 8417 producer-clone by prolonged mechanical passages, during which it progressively overgrew the original producer clone (Janse et al. 1992). Also in this case a deletion involves part of clone 150 transcribed sequence and the complete flanking region. The breakage point maps approximately to the same region as in K173 clone 1.

At the moment we cannot state whether the gene product of clone 150 is directly involved in gametocytogenesis or whether it marks a deletion affecting uncharacterized sequences which have a role in gametocyte differentiation.

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