Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1769 (2007) 506 – 513
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbaexp

An enhancer-like region regulates *hrp*3 promoter stage-specific gene expression in the human malaria parasite *Plasmodium falciparum*

Carlos López-Estraño [a,*], Anusha M. Gopalakrishnan [a], Jean-Philippe Semblat [b,c,1], M. Ross Fergus [a], Dominique Mazier [c], Kasturi Haldar [b]

[a] *Department of Biology, Life Sciences Bldg. Room 409B, The University of Memphis, 3774 Walker Ave. Memphis, Tennessee 38152, USA*
[b] *Departments of Pathology and Microbiology-Immunology, Northwestern University, The Feinberg School of Medicine, Chicago, IL 60611, USA*
[c] *Immunobiologie Cellulaire et Moleculaire des Infections Parasitaires, Centre Hospitalier-Universitaire Pitie-Salpetriere, Universite Pierre et Marie Curie, Paris, France*

Received 30 January 2007; received in revised form 25 April 2007; accepted 26 April 2007
Available online 8 May 2007

Abstract

The asexual blood stage of *Plasmodium falciparum* is comprised of morphologically distinct ring, trophozoite and schizont stages. Each of these developmental stages possesses a distinct pattern of gene expression. Regulation of *P. falciparum* gene expression is thought to occur, at least in part, at the promoter level. Previously, we have found that although the *hrp*3 mRNA is only seen in ring-stage parasites, deletion of a specific sequence in the 5′ end of the promoter region decreased ring-stage expression of *hrp3* and enabled detection of its transcripts in trophozoite-stage parasites. In order to investigate this stage specific regulation of gene expression, we employed a series of nested deletions of the 1.7-kb *hrp*3 promoter. Firefly luciferase gene was used as a reporter to evaluate the role of promoter sequences in gene regulation. Using this approach, we identified a ring-stage specific regulatory region on the *hrp*3 promoter located between −1.7 kb and −1.1 kb from the ATG initiation codon. Small 100–150 bp truncations on this enhancer-like region failed to uncover discrete regulatory sequences, suggesting the multipartite nature of this element. The data presented in this study demonstrate that stage specific promoter activity of the *hrp*3 gene in *P. falciparum* blood stage parasites is supported, at least in-part, by a small promoter region that can function in the absence of a larger chromosomal context.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Plasmodium; transcription; regulation; histidine-rich protein 3; stage specificity; expression

1. Introduction

Plasmodium falciparum infection remains a major disease burden in the developing world [1,2]. Therefore, it is critically important to understand the details of regulation of gene expression during the parasite development. Infection in the human host results in a series of morphologically distinct stages that include ring, trophozoite, schizont and merozoite, during the parasite development within the mature erythrocyte. Transit through developmental stages is linked to strict regulation of distinct stage specific patterns of gene expression. Even housekeeping genes thought to be expressed throughout the cell cycle exhibit complex patterns of developmental expression [3–7].

In the parasite, regulation of gene expression seems to occur at the transcriptional and post-transcriptional level. While trans-

Abbreviations: dhfr-ts, dihydrofolate reductase-thymidylate synthase; bp, base pair; nt, nucleotides; *cam*, calmodulin; *msp*1, merozoite surface protein 1; *hrp*3, histidine-rich protein 3; *hrp*2, histidine-rich protein 2; PfEMP1, *Plasmodium falciparum* erythrocyte membrane protein 1; *hsp*86, heat shock protein 86; *pfs*16, *Plasmodium falciparum* sexual-stage antigen 16; *pfs*25, *Plasmodium falciparum* sexual-stage antigen 25; RNA, ribonucleic acid; rRNA, ribosomal ribonucleic acid; CAT, chloramphenicol acetyl transferase; PCR, polymerase chain reaction; hpi, hours post-invasion; mRNA, messenger ribonucleic acid; *rif*, repetitively Interspersed Family; *stevor*, subtelomeric variable open reading frame; HDGFP, human dihydrofolate reductase fused to *Aequorea victoria* Green fluorescent protein

* Corresponding author. Tel.: +1 901 678 2245; fax: +1 901 678 4457.
E-mail address: cestrano@memphis.edu (C. López-Estraño).
[1] Present address: ICAPB, Ashworth Laboratories, King's Building, Edinburgh EH9 3JT, UK.

0167-4781/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbaexp.2007.04.009

criptome and proteome analyses indicate that some genes are regulated post-transcriptionally [8,9], other genes are regulated at the level of transcription. For example, analyses of 5′ end upstream regions of asexual and sexual stages have been shown by transfection studies that they function as promoters by supporting reporter gene expression [10–16]. Moreover, functional and bioinformatic studies of sexual and asexual *Plasmodium* promoters have uncovered putative *cis*-acting regulatory elements possibly involved in gene regulation [17–22]. In addition, it has been recently shown that the expression of parasite variant protein PfEMP1 is regulated at the level of transcription initiation [23]. Taken together, these results indicate the presence of transcriptional gene regulation in the parasite. Although, basal and stage specific *cis*-acting elements in the parasite promoters remain poorly described owing to the high A+T content of its genome.

During our studies on promoter recombination using *hrp*3 promoter nested deletions, we identified a region between −1.7 kb and −1.1 kb, from the ATG initiation codon, involved in ring stage specificity [24]. Unexpectedly, further promoter deletion switched the steady state accumulation of the reporter gene mRNA from ring to trophozoite stage of the parasite development. These results suggest the presence of stage specific *cis*-acting regulatory elements on the *hrp*3 promoter.

P. falciparum histidine-rich protein-2 and -3 (*hrp*2, 3) as well as the Knob associated histidine-rich protein (*kahrp* or *hrp1*) comprise the histidine-rich protein family. The members of this family are expressed at the early stage of the parasite development [25,26]. We took advantage of the previously reported *hrp*3 promoter nested deletions [24] to further characterize the putative ring specific regulatory element. The *hrp*3 promoter region between −1.7 kb and −1.1 kb, from the ATG initiation codon, is involved in ring stage specific gene expression [24]. This region when placed upstream of the calmodulin (*cam*) promoter; it specifically increased the reporter activity in ring-stage parasites but had little effect on trophozoite parasites, suggesting that this enhancer-like region contains stage specific regulatory elements. This presumptive element did not show homology to other *Plasmodium* promoter regions, suggesting that its function may be regulated through other mechanisms such as transcription initiation or nuclear localization rather than being sequence specific. Although, sequence-dependent regulation cannot be rule out. Here, we identified in *P. falciparum* an enhancer-like region, carrying stage-specific *hrp*3 promoter elements. Our findings contribute to the understanding of the regulation of gene expression in the malaria parasite.

2. Experimental procedures

2.1. Plasmid constructs

The *hrp*3 promoter deletions were made using Erase-a-base® (Promega) and pHRPCAT [10] containing the *hrp*3 promoter driving the chloramphenicol acetyl transferase (CAT) gene. *Kpn*I–*Dra* III-digested pHRPCAT was incubated with exonuclease III (exo III) at predetermined times to obtain specific-sized promoters of 1.1 kb and 0.6 kb. The firefly luciferase (FFL) gene was amplified by PCR from the pVLH plasmid [15] using oligonucleotides 5′-GAC**ATG-CAT**GAAGACGCCAAAAACATAAAG-3′ and 5′-GAC**AAGCTT**GCTTA-CAATTTGGACTTTCCG-3′, (boldfaced nucleotides represent *Nsi*I and *Hin*dIII restriction sites, respectively). The FFL was then used to replace the CAT gene in exoIII-digested pHRPCAT to generate pH1.7FL, pH1.1FL and pH0.6FL. Short *hrp*3 promoter deletions were generated by PCR using the specific oligonucleotides (Table S1, supplementary information).

All pcamSluc-derived constructs were engineered by first creating a *Sal*I site at the 5′ end of the *cam* promoter in the pcamGFP plasmid [27]. Secondly, the firefly luciferase gene substituted the GFP gene to generate pcamSluc. Subsequently, the *hrp*3 promoter region from −1.7 kb to −1.4 kb (fragment A) was amplified by PCR using the oligonucleotides HAF (5′-ACGC**GTC-GAC**CGCCCAATCATTATTTTATG-3′) and HAR (5′-ACGC**GTCGAC**CCCA-TAAAATATAAAAATAATTTG-3′). In addition, the region A was divided using PCR into fragment A1 from −1.7 kb to −1.1 kb with the oligonucleotides HAF and HA1R (5′-ACGC**GTCGAC**CATTTATATTTATATTAAGAG-3′), and fragment A2 from −1.4 kb to −1.1 kb using the HA2F (5′-ACGC**GTCGAC-**GAATATATTCATAATTATAATATTG-3′) and HAR oligonucleotides, boldfaced nucleotides in all primers represent the *Sal*I restriction site. These fragments were placed into pcamSluc to generate pAhrpcamSluc, pA1hrpcamSluc and pA2hrpcamSluc. We then amplified the *hrp*3 promoter region from −1.1 kb to −0.6 kb (fragment B) by PCR using the oligonucleotides HBF (5′-ATC**GTCGAC**TATGTATATGTATGTATTTTAAAATATAATAAAATG-3′) and HBR (5′-ATC**GTCGAC**GTATGGATAGATTTTATTTTTAAAAAAATAA-TAAATTTTATTATATTC-3′). Subsequently, fragment B was divided by PCR into fragment B1 −1.1 kb to −0.8 kb using HBF and HB1R (5′-ATC**GTCG-AC**ATTATGAATATAAGAATATTCCATCTATCTTATG-3′) oligonucleotides; and fragment B2 from −0.8 kb to −0.6 kb using HB2F (5′-ATC**GTCGAC**AA-TATTCAAAAAATAACAGATTTAAACCCTCAAAAATATAG-3′) and HBR. A *Sal*I restriction site (boldfaced nucleotides) was engineered at the 5′ and 3′ end on each PCR product. Fragments B, B1 and B2 were then inserted into *Sal*I-digested pcamSluc to generate pBhrpcamSluc, pB1hrpcamSluc and pB2hrpcamSluc respectively. A *Renilla* luciferase plasmid was constructed as reported by Militello and Wirth [28]. The *Renilla reniformis* luciferase gene was amplified by PCR using the plasmid pRL-null (Promega) as template and forward oligonucleotide 5′-TTA**ATGCAT**ATGCTTCGAAAGTTTATGATC-3′ and reverse oligonucleotide 5′-TTC**AAGCTT**ATTGTTCATTTTTGAGAACTCGC-3′ containing engineered restriction enzyme sites (*Nsi*I and *Hin*dIII, boldfaced

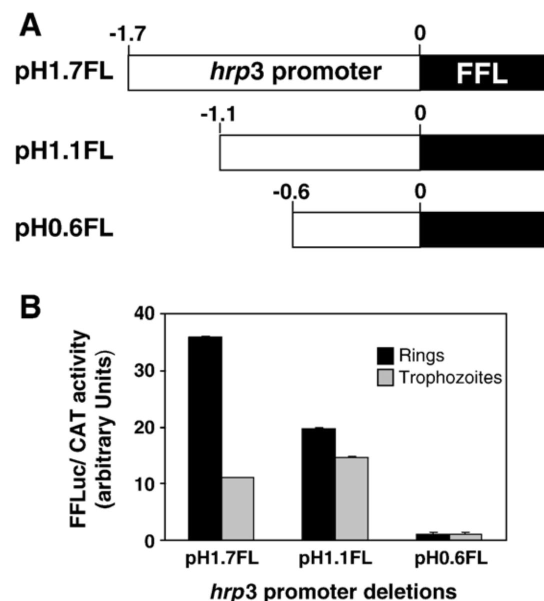

Fig. 1. Deletion of *hrp*3 promoter reduced the ring parasite firefly luciferase activity. (A) Schematic representation of *hrp*3 promoter truncations driving firefly luciferase (FFL). (B) Transient expression of luciferase gene in ring and trophozoite parasites. FFL activity was normalized by cotransfection using Chloramphenicol acetyltransferase gene (CAT) as described in Experimental procedures. Bars are the mean of four independent experiments±SD.

Download English Version:

https://daneshyari.com/en/article/1947182

Download Persian Version:

https://daneshyari.com/article/1947182

Daneshyari.com