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Short communication

A novel DEAD box helicase Has1p from *Plasmodium falciparum*: N-terminal is essential for activity

Krishna Prakash, Renu Tuteja *

Malaria Group, International Centre for Genetic Engineering and Biotechnology, P. O. Box 10504, Aruna Asaf Ali Marg, New Delhi-110067, India

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ABSTRACT

Helicases catalyze the opening of nucleic acid duplexes and are implicated in many nucleic acid metabolic cellular processes that require single stranded DNA or reorganization of RNA structure. Previously we have reported that *Plasmodium falciparum* genome contains a number of DEAD box helicases. In the present study we report the cloning, expression and characterization of one of the novel members of DEAD box family from *P. falciparum*. Our results indicate that it is a homologue of Has1p from yeast and it contains DNA and RNA unwinding, nucleic acid-dependent ATPase and RNA binding activities. This enzyme can utilize all the nucleosidetriphosphates (NTPs) and deoxy nucleosidetriphosphates (dNTPs) for its unwinding activity. Using a truncated derivative of this protein we further report that the N-terminal region of the protein is essentially required for its activity. These studies suggest that besides the conserved helicase domain the highly variable N-terminal region also contributes in the activity of the protein.

Helicases are responsible for the unwinding of nucleic acid duplexes in an ATP-dependent manner [1,2]. The energy for this unwinding is provided by the intrinsic nucleic acid-dependent NTPase activity of helicases. Depending on the type of substrate the helicases are classified as DNA or RNA helicases. RNA helicases are implicated in many cellular processes that require reorganization of RNA structure, such as transcription, mRNA splicing, translation initiation, RNA editing, export, and degradation. These enzymes are identified by the presence of nine different conserved motifs [3]. Due to the presence of sequence DEAD in one of the motifs, these are commonly known as DEAD box helicases [4]. Members of this family are present in almost all the organisms and are involved in many different biological processes including DNA repair, transcription, pre-rRNA processing, ribosome biogenesis and splicing [5].

Plasmodium falciparum is a parasite, which causes the most lethal form of malaria [6]. A malaria vaccine would be the ultimate weapon to fight this deadly disease but unfortunately despite encouraging advances a vaccine against malaria is not available yet. Moreover the parasite and the mosquito vector have developed drug resistance gradually therefore controlling this disease is a daunting task [7]. The rational development of novel and affordable anti-malarial drugs for the treatment of malaria and the identification of new drug targets is an important goal. The recent completion of malaria genome project and availability of new technologies for genome wide comparison of genomes is helpful in identifying key targets in biochemical pathways that are parasite specific and can be interrupted without deleterious consequences for the host. One of the promising targets could be helicases, which are key enzymes and required for almost all the nucleic acid transactions in malaria parasite [8]. Previously we have reported that *P. falciparum* genome contains a number of helicases and at least 22 DEAD box helicases [9,10]. In the present study we report the cloning, purification and characterization of one of the helicases of DEAD box family from the malaria parasite *P. falciparum*. Our studies reveal that this enzyme is homologous to Has1p, contains DNA and RNA helicases, ssDNA and RNA-dependent ATPase and RNA binding activities. We also report that the N-terminal region of the protein is essentially required for all its activities.

Using bioinformatics analysis it has been reported previously that *P. falciparum* genome contains a number of putative DEAD box helicase genes [9–11]. We reported that a putative RNA helicase with PlasmoDB number PFF1500c contains all the characteristic features of DEAD box family [9,10]. Therefore this helicase was selected for cloning and characterization. *P. falciparum* (strain 3D7) was cultured as described earlier [12]. Total RNA was isolated and was used for the preparation of cDNA using a cDNA synthesis kit (Superscript first-strand synthesis system from Invitrogen, Carlsbad, CA, USA). This cDNA was used as a template because the genomic DNA contains one intron. The sequence was obtained from PlasmoDB (http://PlasmoDB.org) [13] and the complete open reading frame (1806 base pair) of helicase gene of *P. falciparum* was PCR amplified using the forward primer PfH69F (5′-G*GGATCC*ATGATGGATGATGATAAT-3′) and the reverse primer PfH69R (5′-C*CTCGAG*TTATTTAAATTTTTTTTTTTG-3′). The restriction sites are written in italics. The PCR product of ~1.8 kb was gel purified and cloned into the pGEM-T vector from Promega

* Corresponding author. Tel.: +91 11 26741358; fax: +91 11 26742316.
E-mail addresses: renu@icgeb.res.in, renututeja@gmail.com (R. Tuteja).

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(Madison, WI, USA) and the positive clones were sequenced by automated DNA sequencing. The nucleotide sequence was submitted to the GenBank and the accession number is FJ641053. The nested forward primer PfH69F1 (5′-G*GGATCC*ATGTTTGAAGAATTAAATA-TATGTG-3′) and the reverse primer PfH69R were used for the amplification of the nested gene (1359 base pair), which lacked first 149 amino acids. The DNA bands were excised using BamHI and XhoI enzymes (New England Biolabs, Beverly, MA, USA) and gel purified for subcloning into the protein expression vector pET-28a (Novagen, Madison, WI, USA).

The sequence analysis indicated that this gene encodes a polypeptide of 601 amino acid residues with a predicted molecular

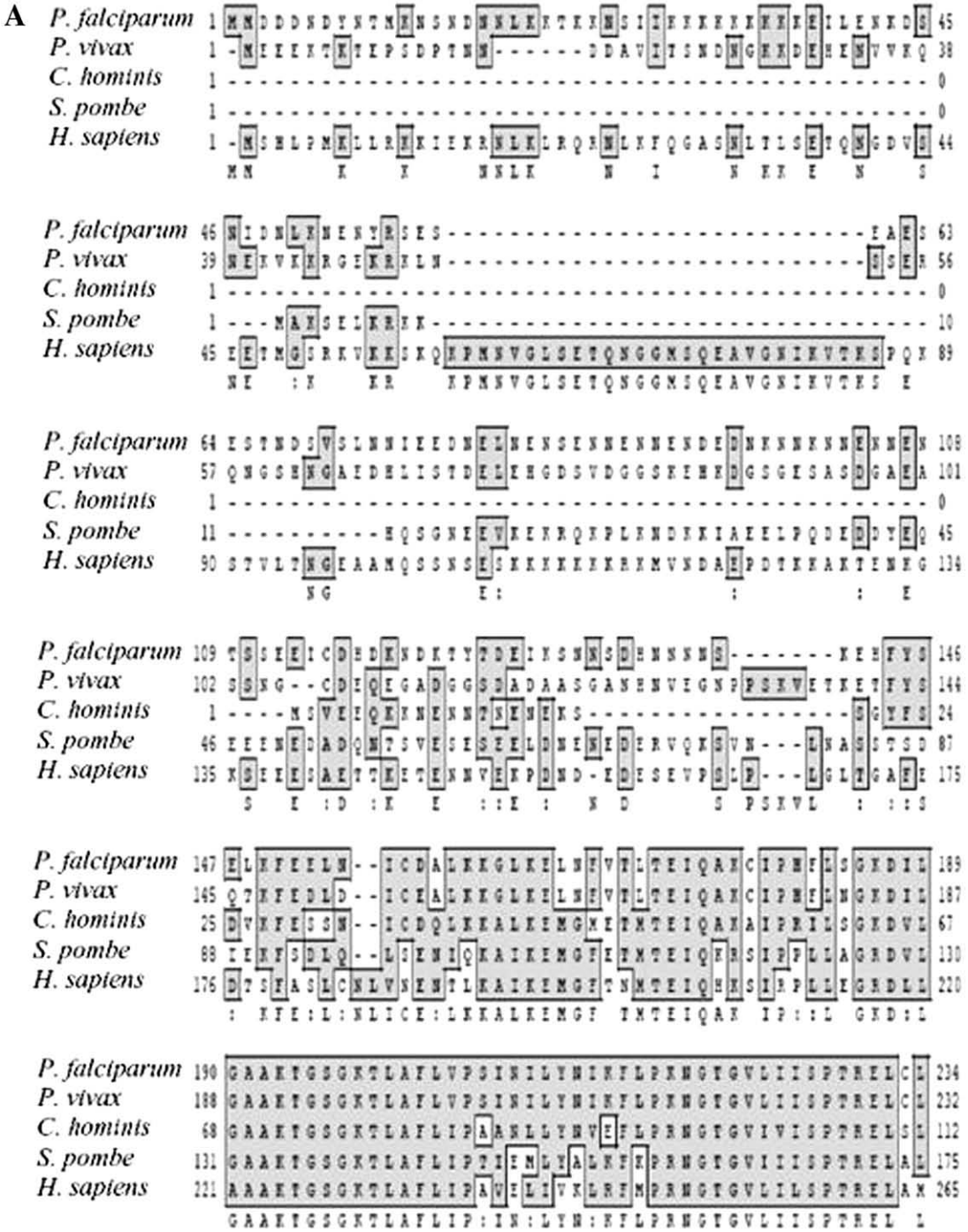

Fig. 1. A. Comparison of amino acid sequence. The sequence of *P. falciparum* homologue (PfH69) was compared with different proteins from *Plasmodium vivax* (XP_001616317), *Schizosaccharomyces pombe* (NP_594488), *Cryptosporidium hominis* (XP_666241) and *Homo sapiens* (NP_006764). Multiple alignments were done using ClustalW program. The accession numbers of the aligned sequences are written in brackets. B. Alignment of the N-terminal region of Has1p homologues. C. Domain wise comparison of PfH69 of *P. falciparum* (i) with its human homologue (ii). The conserved sequences of each domain are written inside the boxes. The numbers refer to the amino acids separating the various domains and the length of N- and C-terminal extensions. This figure is not drawn to scale. Lower part of each panel shows the amino acid position and structure of each domain. The domain analysis was done by using 'Scan Prosite' at http://expasy.org/tools/scanprosite/. The text in bracket is the name of the domain and the numbers are position of respective domains in the protein. D. Structure modeling. PfH69 sequence was submitted to Swissmodel server and the structure was obtained. The molecular graphic images were produced using the UCSF Chimera package from the resource for Biocomputing, Visualization, and Informatics (http://www.cgl.ucsf.edu/chimera) at the University of California, San Francisco (supported by NIH P41 RR-01081). a. PfH69; b. template H1V8 and c. superimposed image.