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Mini-review

Helicases involved in splicing from malaria parasite *Plasmodium falciparum*

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ABSTRACT

An interesting element of eukaryotic genomes is the large quantity of non-coding intervening sequences commonly known as introns, which regularly interrupt functional genes and therefore must be removed prior to the use of genetic information by the cell. After splicing, the mature RNA is exported from the nucleus to the cytoplasm. Any error in the process of recognition and removal of introns, or splicing, would lead to change in genetic message and thus has potentially catastrophic consequences. Thus splicing is a highly complex essential step in eukaryotic gene expression. It takes place in spliceosome, which is a dynamic RNA–protein complex made of snRNPs and non-snRNP proteins. The splicing process consists of following sequential steps: spliceosome formation, the first transesterification and second transesterification reactions, release of the mature mRNA and recycling of the snRNPs. The spliceosomal components produce a complex network of RNA–RNA, RNA–protein and protein–protein interactions and spliceosome experience remodeling during each splicing cycle. Helicases are essentially required at almost each step for resolution of RNA–RNA and/or RNA–protein interactions. RNA helicases share a highly conserved helicase domain which includes the motif DExD/H in the single letter amino acid code. This article will focus on members of the DExD/H-box proteins involved specially in splicing in the malaria parasite *Plasmodium falciparum*.

Contents

1. Introduction

The transcripts contain introns that are usually removed from pre-messenger RNA (pre-mRNA) in the process of pre-mRNA splicing. The mechanism of splicing is common to almost all the eukaryotes and it requires a complex trans-acting apparatus known as spliceosome. Spliceosome is a complex structure composed of RNA and proteins and is assembled de novo for the splicing of introns from nuclear pre-mRNAs. It is well established that the excision of the intron in the form of a lariat and ligation of the exons are ATP dependent. The spliceosome is composed of five small nuclear ribonucleoprotein particles (snRNPs), designated U1, U2, U4, U5, and U6 and a number of proteins of different types such as small G proteins, zinc-finger proteins and ATP-dependent RNA helicases. Helicases are DExD/H-box enzymes ubiquitously present in all organisms from bacteria to humans and also in viruses [1]. This is a large protein family and its members share a ~300 amino acid domain with eight highly conserved sequence motifs. The various motifs have been named as Q, I, Ia, Ib, II, III, IV, V and VI and based on the mutational analysis and structural data in a variety of other systems, the specific roles for a

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number of the conserved motifs have been suggested [2]. Studies over the years have suggested that helicases rarely exhibit functional complementarity despite exhibiting significant sequence and biochemical relatedness, implying that each helicase performs a unique function(s) in cellular physiology.

The studies on the role of the spliceosomal helicases/ATPases in assembly suggest that each ATP-dependent step most likely is mediated by a single family member and these RNA-dependent ATPases contain weak in vitro RNA unwinding activity [3]. The mechanism of nuclear splicing has been comprehensively studied in *Saccharomyces cerevisiae* by the isolation of a number of mutants which affect pre-mRNA splicing. It has been suggested that eight DExD/H-box proteins are essentially required for splicing in yeast and these are Prp5p (*p*re-*R*NA *p*rocessing), Sub2p, Prp28p, Brr2p (*B*ad *R*esponse to *R*efrigeration), Prp2p, Prp16p, Prp22p and Prp43p [4,5]. The formation of an early intermediate, the pre-spliceosome in the pathway, which contains only the U1 and U2 snRNPs, requires Prp5p and the successive steps at the end of the pathway; activation of the spliceosome, activation of the second step and release of the products require Prp2p, Prp16p, and Prp22p respectively in order [3,5].

Plasmodium falciparum is the malaria parasite, which causes the most lethal form of the disease [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 yet available. 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 [8]. Therefore it is important to study the basic biology of this parasite in detail. Toward this goal using bioinformatics approach we have reported the genome wide analysis of helicases from *P. falciparum* [9,10]. Here we report the detailed computational analysis of helicases involved in splicing from this parasite and their comparison with yeast homologues.

2. Prp5p

This gene was cloned from *S. cerevisiae* (ScPrp5p) by complementation of a temperature-sensitive mutation and it codes for a ~96 kDa protein [11] (Fig. 1, 1a, Table 1). This is a DEAD box protein and it was shown that it contains RNA-dependent ATPase activity [12]. Its homologue was also identified in *Schizosaccharomyces pombe* (SpPrp5p) [13]. The mutational analysis of ATPase motifs I and II suggested that ATP binding and hydrolysis by Prp5p are essential for pre-spliceosome complex A formation and these data also demonstrated that Prp5p provides a bridge between U1 and U2 snRNPs at the time of pre-spliceosome formation [13]. ScPrp5p utilizes the energy released by ATP hydrolysis to rearrange local RNA–RNA or RNA–protein interactions in order to allow U2 snRNP to join the complex.

Blast analysis of PlasmoDB (www.Plasmodb.org) identified the gene with PlasmoDB number PFE0430w as the homologue of Prp5p. This gene encodes a protein of ~176 kDa, which is larger in size as compared to its human and yeast homologues (Table 1). This protein contains a long N-terminal lysine and asparagine rich region of 749 amino acids as compared to its yeast counterpart, which has 282 amino acids in the N-terminal (Fig. 1, 1b). The importance of various amino acid rich regions is not well known but in most of the *P. falciparum* proteins these are present [14,15]. It has been suggested that these regions are looped out and most probably they do not play any significant role in the activity of most of the proteins [14,15]. The PfPrp5p contains a few RS (4) and RD/RE (4/3) dipeptide repeats, which are characteristic signature motif of a number of proteins involved in pre-mRNA splicing. In addition it also contains a large number of KE (25), KD (12) and KS dipeptide repeats. It is interesting to note that PfPrp5p contains three nuclear localization signals (NLS) from amino acids 126–140, 176–191 and 226–240 as opposed to ScPrp5p which contains only one NLS from amino acids 79–95 (Fig. 1, 1b).

Table 1
Helicases involved in splicing.

Name	Yeast homologue	Mol. wt. (kDa)	PlasmoDB no.	Mol. wt. (kDa)	Human homologue	Mol. wt. (kDa)
Prp5p	YBR237W	96	PFE_0430w	176	DDX46	117
Sub2p	YDL084W	50	PFB_0445c	52	DDX39	49
Prp28p	YDR243C	67	PFE0925c	131	DDX23	96
Brr2p	YER172C	246	PFD1060w	337	SNRNP200	200
Prp2p	YNR011C	100	PFL1525c	137	DHX16	119
Prp16p	YKR086W	121	MAL13P1.322	135	DHX38	141
Prp22p	YER013W	130	PF10_0294	150	DHX8	139
Prp43p	YGL120C	88	PFI0860c	95	DHX15	90

Column 2 indicates the gene number at *Saccharomyces* genome database (http://www.yeastgenome.org/).

3. Sub2p

Sub2p was identified by sequence similarity with UAP56 and by genetic complementation in both *S. cerevisiae* and *S. pombe* [16,17]. Sub2p is involved in genome instability and may also play a role in transcription, splicing and export, through its association with different co-factors [18,19]. ScSub2p is required at multiple steps in splicing, including formation of the branch point-dependent commitment complex and U2 snRNP addition to the branch point. Sub2p/UAP56 remains associated with the spliced mRNA as part of the exon junction complex (EJC), together with yet another DEAD-box protein, eIF4AIII [20] where it plays a role in nuclear export. After the U1 and U2 snRNPs bind the pre-mRNA, the tri-particle U4–U6/U5 snRNP joins and subsequently U1 and U4 snRNAs are released from the active spliceosome.

Sub2p/UAP56, a ~50 kDa DECD helicase, is also required in the early steps of RNA transport, and it associates with spliced mRNAs carrying the EJC (Fig. 1, 2a). Sub2p is also required for the export of intron-less mRNAs. In yeast, the ATPase activity of Sub2p is required for its release from mRNA and its replacement by the mRNA-transport factor Mex67 [21]. In yeast, expression of a mutant Sub2p or overexpression of Sub2p leads to defects in mRNA export and accumulation of poly(A) RNA in the nucleus [21].

Blast analysis of PlasmoDB (www.Plasmodb.org) identified the gene with PlasmoDB number PFB0445c as the homologue of ScSub2p (Fig. 1, 2b) [9,10]. We have cloned and characterized PFB0445c homologue in detail [22]. This homologue known as PfU52 is a ~52 kDa protein (Table 1) and it contains nucleic acid-dependent ATPase, RNA binding and RNA helicase activities [22]. PfU52 is involved in splicing and using site directed mutagenesis studies we have shown that amino acid residues outside the helicase motifs are essentially required for all of its activities [22].

4. Prp28p

ScPrp28p is a ~67 kDa DEAD-box protein (Fig. 1, 3a) required for the first step of mRNA splicing and it is part of the penta-snRNP complex which is composed of U1, U2 and U4/U6–U5 snRNPs. It is necessary for the ATP-dependent destabilization of U1 snRNA and its replacement by U6 snRNA at the splice site [23,24]. The purified ScPrp28p had no helicase activity on an artificial duplex RNA substrate, suggesting that a cofactor is required for its activity [23]. It is possible that only a low level of helicase activity is required in vivo for its action [23]. Previous studies have suggested that regions of