UAP56- a key player with surprisingly diverse roles in pre-mRNA splicing and nuclear export

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INTRODUCTION

UAP56 is a member of DEAD box family of ATP-dependent RNA helicases. UAP56 has both RNA-stimulated ATP binding/hydrolysis activity and ATP-dependent RNA unwinding activity (1-3). UAP56 was originally identified in a two-hybrid screen for proteins that interact with an essential human splicing factor-U2AF65 (1). Studies in different organisms indicate that UAP56 protein is not only essential in pre-mRNA splicing (1-4) but is also important in mRNA nuclear export (5-7) and cytoplasmic mRNA localization (8) (Table 1).

Structure, biochemical function and cellular localization of UAP56

hUAP56 (human UAP56) is a 56-kDa, 428 amino acid open reading frame encoding a DEAD box RNA helicase. A variety of metazoan UAP56 contain a DECD motif instead of a canonical DEAD/H motif (1). The crystal structure of UAP56 indicates that the overall fold of N-and C-terminal domains is highly similar to the corresponding domains of a prototypic DExD/H-box protein eIF4A (10, 11) with differences at the loops and termini. A citrate ion, which mimics the phosphates of NTP and retains the P loop in the open conformation, occupies the NTP binding pocket (Fig. 1) (9).

Like other DEAD-box family RNA helicases, purified human UAP56 is an active RNA-stimulated ATPase that can only hydrolyze ATP (3, 9, 10). UAP56 is also an ATP-dependent RNA helicase that can unwind 5' or 3' overhangs or blunt end RNA duplexes in vitro. The mutations in the conserved helicase motifs I, II, and III cause the loss of ATPase or helicase activity of UAP56 (Fig. 1) (9).

As with most splicing factors, UAP56 is localized in the nuclear speckle. Here, UAP56 is co-localized with a splicing and EJC protein-SRm160 (Fig. 1) (12).

UAP56 and pre-mRNA splicing

UAP56 regulates pre-mRNA splicing in various ways from yeast to human. In pre-mRNA splicing, introns are removed to convert pre-mRNA to mature RNA. Pre-mRNA splicing occurs in a large RNA-protein complex called the spliceosome (12-15). Spliceosome formation is a complicated stepwise process. Complex E (early complex) is formed by the binding of U1 snRNP to the 5' splice site. Pre-spliceosome (complex A) is then formed by the binding of U2 snRNP to the branch point. Complex B is formed by the binding of U4/U5/U6 tri-snRNP to the pre-spliceosome. Catalytically active complex C is formed by the structural rearrangement of complex B (16-19). The chemistry of pre-mRNA splicing is a two-step procedure. In the first step, the 5' splice site is cleaved to produce linear exon1 and lariat configured intron/exon2. In the second step, the 3' splice site is cleaved and the two exons are ligated to produce mature RNA (20, 21).

Pre-mRNA splicing requires ATP hydrolysis and RNA unwinding (22-26). These processes are performed by a series of splicing factors that are members of the DExD/H-box protein family (27-30). Several DExD/H-box splicing factors possess an ATP-dependent RNA unwinding/helicase activity and use ATP hydrolysis as a driving force to modulate specific RNA structural rearrangements during spliceosome assembly (31, 32). UAP56 is one of the DExD/H-box family proteins with those functions (1, 2, 4).

Human UAP56 was cloned as an essential splicing factor-U2AF65 associated protein (1). UAP56 is an essential splic-
UAP56 function in splicing and nuclear export
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**Table 1. Enzyme activity and cellular function of UAP56 in different organisms**

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<th>Organism</th>
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**Fig. 1.** Structure, biochemical activity and cellular localization of UAP56.

**Fig. 2.** Function of UAP56 in pre-mRNA splicing.

...ing factor required for the U2 snRNP-branchpoint interaction (1). The first of the ATP-dependent pre-mRNA splicing processes is the stable binding of U2 snRNP at the pre-mRNA branchpoint. The basis of ATP use for the interaction between U2 snRNP and the branchpoint was unclear until the discovery of UAP56. In particular, none of the known mammalian factors required for this step were found to hydrolyze ATP. The interaction between the essential splicing factor U2AF65 and the pre-mRNA polypyrimidine tract is essential for the entry of U2 snRNP into the spliceosome. UAP56 hydrolyzes ATP to facilitate the U2 snRNP-branchpoint interaction (1). Yeast UAP56 homolog-sub2p has a similar function in pre-mRNA splicing, which is required for the formation of the commitment complex. Therefore, at least two DExD/H box proteins, Prp5p and sub2p, mediate the U2 snRNP-branchpoint interaction (33). Deletion of MUD2 (the yeast homolog of U2AF65) abrogates the functional requirement of sub2 (4). The function of UAP56 in pre-mRNA splicing was highlighted recently in a study (2) of a series of human UAP56 mutants in ATP-binding. ATP hydrolysis or dsRNA unwindase/helicase activity identified the functions of these domains in pre-mRNA splicing. UAP56 is involved in a serious of pre-mRNA splicing steps. In the prespliceosome assembly, ATP-binding and ATPase activities of UAP56 are required for its interaction with U2AF65, and subsequent recruitment into the prespliceosome. In the mature spliceosome formation, ATP-binding, ATPase activity and dsRNA unwindase/helicase activity of UAP56 are required. In this process, hUAP56 contacts U4 and U6 snRNA to promote the unwinding of the U4/U6 duplex to facilitate...
nucleus, consequently inhibiting protein synthesis (40, 43, 44). Hel is required for bulk mRNA export from the nurse cell nucleus that supply most of the material to growing Drosophila oocytes and the organization of chromatin in the oocyte nucleus (48). UAP is required for the patterning, localization of gurken, bocoid and oskar mRNA (Fig. 3) (48).

In Caenorhabditis elegans, depletion of UAP56 results in strong suppression of green fluorescent protein (GFP) transgene because of the nuclear retention of its mRNA. Overexpression of UAP56 causes a rapid loss of GFP expression and lethality at all stages of development (45).

Because UAP56 associates with spliced mRNAs with exon junction complex (EJC), UAP56 might provide a functional link between splicing and nuclear export (49). Sub2p is also associated with a heterotetrameric THO complex, which functions in transcription in yeast (42). This implies a role of UAP56 in the coupling of transcription to mRNA export.

CONCLUSION

UAP56 provides an excellent example that pre-mRNA splicing and mRNA nuclear export is linked. UAP56 itself plays various roles in pre-mRNA spliceosome formation and mRNA nuclear export. The study of the UAP56 complex will unearth new splicing and nuclear export factors, and likely a new mechanism of pre-mRNA splicing and mRNA nuclear export.

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