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A DEAD Protein that Activates Intron Self-Splicing without Unwinding RNA

Short Article

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Summary

The group II intron ai5γ from *S. cerevisiae* requires high temperature and salt to self-splice in vitro, but it is assisted by the protein Mss116 in vivo. Here we show that Mss116 can stimulate splicing of ai5γ under near-physiological conditions in vitro, which represents one of the first cases in which a DExH/D protein is shown to act on its natural target. Importantly, we demonstrate that a small subset of DEAD-box proteins can also stimulate ai5γ splicing in vitro and may represent a distinct subfamily of DEAD-box proteins that functions in RNA tertiary structure assembly. Mutational analysis shows that while ATPase activity is required for stimulation of splicing by Mss116, helicase activity is not. This finding indicates that Mss116 is unlikely to promote intron splicing through the unwinding of kinetic traps. Rather, we propose that Mss116 promotes the ordered assembly of large RNA molecules through stabilization of on-pathway intermediates.

Introduction

Group II introns are large catalytic RNAs that self-splice independently in vitro in the presence of high metal ion concentrations and elevated temperatures (Lehmann and Schmidt, 2003). Under these conditions, the catalytic core of intron ai5γ folds slowly and directly to the native state through a rate-limiting obligate intermediate within intron domain 1 (Su et al., 2005). In a cellular environment, ai5γ is likely to adopt an alternative strategy for stabilizing this folding intermediate and reaching the native state. RNA tertiary folding in vivo is often navigated through the action of proteins, which can destabilize misfolded intermediates, stabilize transient intermediates, or stabilize weaker but correctly folded domains (Schroeder et al., 2004).

Protein cofactors such as Mss116 are required for group II intron splicing in vivo. Mss116 was originally identified in a genetic screen for nuclear genes that affect yeast mitochondrial function (Faye and Simon, 1983), and it was later identified as a DEAD-box protein (Seraphin et al., 1989). In *S. cerevisiae,* splicing of all mitochondrial introns, including ai5γ, is facilitated by Mss116 (Huang et al., 2005). Mss116 deletion mutants can be rescued by overexpression of Cyt-19, which is a related DEAD-box protein from *N. crassa* (Huang et al., 2005). Cyt-19 also stimulates in vitro splicing of noncognate group II introns under near-physiological conditions (Mohr et al., 2006).

Mss116 and Cyt-19 belong to the DExH/D family of proteins, which is a ubiquitous class of nucleic acid remodeling motors that is essential for RNA and DNA metabolism (Linder, 2000). Despite their central role in biology, we know little about what these proteins actually do. Since their original description, they have been considered "helicases" and are classified as a subset of helicase superfamily 2 (SF2) based on conserved signature motifs (Gorbalenya and Koonin, 1993). While the ATPase, helicase, nucleic acid binding, and remodeling activities of several family members have been characterized (Cordin et al., 2006), only one member (DbpA) has been studied in the context of a biologically relevant RNA substrate (Diges and Uhlenbeck, 2001). Given that ai5γ is a natural RNA substrate for Mss116, an in vitro system recapitulating their interrelated activities would be extremely significant and valuable.

Newly discovered DExH/D proteins are usually presumed to function through duplex unwinding, but the information that has accumulated thus far suggests that activities of this family are more varied than ever anticipated. While some unwind RNA duplexes processively, such as Nph-II and NS3 (Jankowsky et al., 2000; Serebrov and Pyle, 2004), these same proteins can have other functions, such as protein displacement (Fairman et al., 2004; Jankowsky et al., 2001) or single-strand translocation (Kawaoka et al., 2004). Another family member, Ded1, displays both RNA unwinding and annealing activity (Yang and Jankowsky, 2005). Many of these proteins can only unwind very short duplexes (Cordin et al., 2006). In fact, eIF4-AIII cannot unwind RNA but serves as a "clamp" to anchor the exon-junction complex (EJC) (Shibuya et al., 2004). One unifying property that these proteins share is that they are ATP-dependent RNA-binding proteins. Most of them hydrolyze ATP in the presence of nucleic acids, resulting in protein conformational changes that can be coupled to a diversity of mechanical processes, including duplex unwinding, rearrangement of nearby proteins, and annealing of RNA strands. The specific mechanism by which Mss116 promotes group II intron function has remained unclear, primarily because an in vitro system for studying the interplay between Mss116 and the ai5γ intron was lacking.

Here we show that Mss116 stimulates splicing of ai5γ under near-physiological conditions in vitro. Importantly, we find that this activity is generalizable and that it is observed for a subset of functionally unrelated DEAD proteins that share an appended, arginine-rich C-terminal domain. We find that Mss116 behaves like a typical DEAD-box protein: it cleaves ATP upon binding RNA, and it unwinds short duplexes with a 3′ single-stranded (ss) overhang, 5′ss overhang, or a blunt end. However, by creating Mss116 mutants and studying their ATPase, unwinding, and splicing activities, we find that helicase activity is not required for Mss116-stimulated splicing of ai5γ. Thus, we have shown that Mss116 catalyzes RNA folding through an unconventional

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mechanism and defines a specific subclass of DEAD proteins, thereby broadening the spectrum of activities attributable to DEAD proteins.

Results

Mss116 Stimulates Group II Intron Self-Splicing

Self-splicing of the yeast intron ai5γ was tested under a variety of conditions in the presence and absence of full-length, untagged Mss116 protein. At the normal growth temperature for yeast (30°C), ai5γ displays only trace amounts of self-splicing activity. However, in the presence of Mss116 protein and ATP, ai5γ splices efficiently at 30°C under near-physiological ionic conditions (8 mM $MgCl_2$, 100 mM KCl, and 40 mM MOPS [pH 7.5]) (Figure 1A). A minimum of 6 mM Mg^{2+} is required for this reaction, and activity is observable up to 35 mM Mg^{2+} (data not shown). ATP is required for Mss116-stimulated splicing, and it cannot be substituted by ADP, the nonhydrolyzable analogs AMPPNP and β,γ-methylene-ATP, or the transition state analog ADP-orthovanadate (Figure 1B and see Figure S1 in the Supplemental Data available with this article online). Importantly, Mss116-facilitated splicing generates the same reaction products as observed previously for ai5γ self-splicing under high salt and temperature conditions: lariat intron, linear intron, ligated exon, free 5′ exon, and free 3′ exon.

Multiple Proteins Stimulate Splicing

Given that Mss116 and Cyt-19 both stimulate splicing of ai5γ, it was important to determine whether this property is a generalizable feature of DExH/D proteins. To test this, we examined ai5γ reactivity in the presence of diverse DEAD and DExH proteins, including those that have never been implicated in RNA processing and those that are not localized to the mitochondrion. For example, we examined the effects of NS3, a processive DNA and RNA helicase from the hepatitis C virus (Pang et al., 2002); Dbp8, a DEAD-box protein implicated in 18S RNA synthesis (Granneman et al., 2006a); Dbp8 in complex with Esf2, a cofactor that stimulates Dbp8 ATPase activity in vitro (Granneman et al., 2006b); and Ded1, a yeast protein that is implicated in translation initiation (Chuang et al., 1997). Neither the DExH protein NS3 nor the DEAD proteins Dbp8 or Dbp8/Esf2 stimulate splicing of ai5γ (Figure 1C). Remarkably, however, the translation initiation factor Ded1 stimulates ai5γ splicing under the same conditions used for Mss116 and Cyt-19 (Figure 1A).

Mss116 Is an RNA-Dependent ATPase and Helicase

To provide a biochemical foundation for understanding Mss116-stimulated splicing, we examined ATPase and unwinding activities of Mss116 (Figures 2B, 4A, and 4B). We find that Mss116 is an RNA-stimulated ATPase that cleaves ATP with a specific activity of 56.7 ± 13 pmol ATP/pmol protein·min, which is a value typical for DEAD-box proteins (Cordin et al., 2004). In the absence of nucleic acid, the specific activity for ATP hydrolysis is reduced to 14.5 ± 11 pmol ATP/pmol protein·min (Figure S2).

Helicase assays reveal that Mss116 unwinds short duplexes without any apparent directionality, since substrates containing both 3′ss or 5′ss overhangs are unwound (Figure 4A). Unlike the processive DExH helicases NS3 and Nph-II, Mss116 readily unwinds blunt-ended duplexes and therefore does not require prior loading on ssRNA. The fact that Mss116 cannot unwind longer duplexes (Figure 4A) suggests that Mss116, like other DEAD-box proteins (Cordin et al., 2006), is nonprocessive.

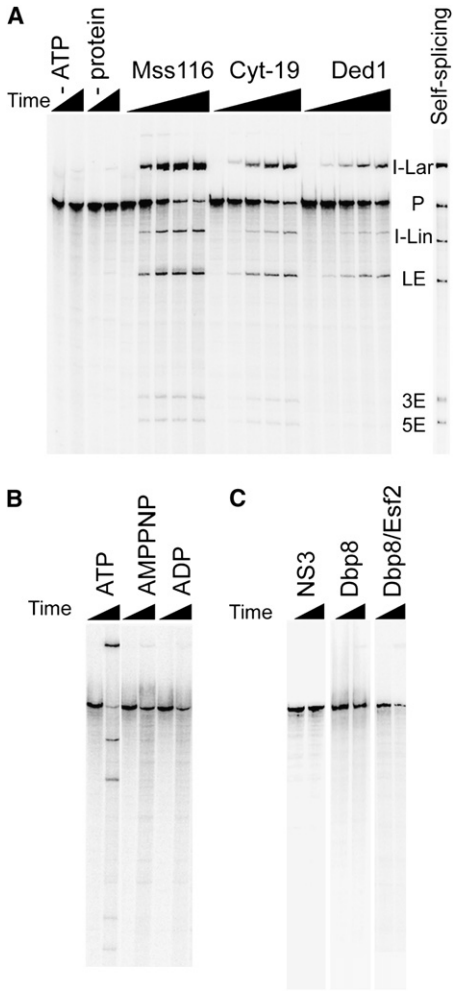

Figure 1. Protein-Mediated Splicing

(A) Mss116-, Cyt-19-, and Ded1-promoted splicing of ai5γ. Reactions were performed with 600 nM protein, 1 mM ATP, and 8 mM $MgCl_2$ at 30°C; time points, 0, 15, 30, 60, and 120 min. Control-lane time points, 0 and 120 min. Self-splicing products are provided as a marker. I-Lar, intron lariat; P, precursor; I-Lin, a population containing linear and/or broken lariat intron RNA; LE, ligated exons; 3E, 3′ exon; and 5E, 5′ exon.

(B) Splicing with 600 nM Mss116 in the presence of ATP, AMPPNP, and ADP. Time points, 0 and 120 min.

(C) 600 nM NS3, Dbp8, and the Dbp8/Esf2 complex cannot facilitate splicing. Time points, 0 and 120 min.

Mss116-Stimulated Splicing Does Not Require Helicase Activity

To understand the mechanistic basis for Mss116-stimulated splicing, we mutagenized two of the most highly conserved regions of the protein, both of which are required for helicase activity by DExH/D proteins. First, we mutagenized the SAT sequence of motif III (SAT→AAA, Figure 2A), which prevents coupling between ATPase