Protein-Facilitated Ribozyme Folding and Catalysis

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ABSTRACT

In vivo, large RNAs rely on proteins to fold to their native conformation. In the case of the S. cerevisiae group II intron ai5γ, the DEAD-box protein Mss116 has been shown to promote the formation of the catalytically active structure. However, it is a matter of debate whether it does this by stabilizing on-pathway intermediates or by disrupting misfolded structures. Here we present the available experimental evidence to distinguish between those mechanisms and discuss the possible interpretations.

INTRODUCTION

Group II introns are large ribozymes (400-1000 nt) that are able to self-splice from their host mRNA. Although their overall sequence is not conserved, all group II introns share a similar secondary structure which consists of six distinct domains that are arranged around a central wheel (Fig. 1) (1).

![Fig. 1 Nucleotide sequence and secondary structure of the S. cerevisiae group II intron ai5γ. The domains are numbered with roman numerals, known tertiary interactions are indicated by greek letters. EBS: exon binding site, IBS: intron binding site.](image)

These six domains form a compact tertiary structure (2) that can catalyze the two consecutive transesterification steps required for splicing (Fig. 2).

The yeast ribozyme ai5γ, one of the most thoroughly characterized group II introns, requires unphysiologically high salt concentrations in vitro to adopt its native, catalytically active structure (3). In vivo, proteins are thought to assist ribozyme splicing by helping the large RNA to fold. The two major ways for proteins to do this is by promoting the formation of the native fold, or by disrupting stable misfolded structures called kinetic traps.

The DEAD-box protein Mss116 from S. cerevisiae has recently been found to promote ai5γ-splicing in vivo and in vitro (4-6). This protein belongs to the helicase superfamily 2 (7) whose members often have ATPase, RNA binding, RNA unwinding and remodeling activities (8). However, the exact mechanism of how Mss116 can stimulate group II intron splicing has not been elucidated yet.

RESULTS AND DISCUSSION

In order to investigate the role of Mss116 in group II intron splicing, we created several mutants that compromise its ATP-binding, ATP hydrolysis and unwinding activities. K158R, a mutant that lost its ability to bind ATP, could not stimulate ai5γ splicing (6). K158A, which retained only residual ATPase activity, was able to promote splicing on a very low level (6). A SAT/AAA mutation had previously been shown to uncouple ATP hydrolysis from unwinding in related DEAD-box proteins (8). We found that in Mss116, this mutation decreased the ATPase activity to ~20% of the wild-type activity and abrogated its ability to unwind an RNA duplex of 12 bp (Fig. 3A). Unexpectedly, the SAT mutant is still able to promote splicing to a considerable degree (observed splicing rate constant ~ 5-fold slower than wild-type Mss116, Fig. 3B). This lead us to the conclusion that ATP hydrolysis is required to promote ai5γ-splicing, but unwinding is not.
Therefore we propose a role for Mss116 as an ATP-dependent conformational switch that promotes formation of the native RNA structure.

CONCLUSION

The ai5γ intron and its host-encoded splicing factor Mss116 are excellent model systems for studying the effects of DEAD-box proteins on folding of large RNAs. Using various mutants of Mss116, we have begun to lay the groundwork for identifying these complex interactions.

Our mutational analysis revealed that the difference in splicing rate constants between wild-type and mutant Mss116 proteins correlates very well with their difference in ATPase activity, strongly suggesting that ATP hydrolysis is required for protein-mediated splicing. However, making a similar connection about unwinding activity proved more complicated because unwinding efficiency varies strongly with the tested model substrate (9). Unwinding and splicing can therefore not be directly compared until the structure on which the protein acts is identified and characterized. In the meantime, it is premature to strongly support any of the proposed mechanisms for protein-assisted splicing.

Since Mss116 and related DEAD-box proteins can facilitate splicing of different group I and group II introns, it is likely that these proteins perform a range of different functions in order to assist the folding of a wide variety of large RNAs. New approaches will be necessary to dissect these roles.

REFERENCES


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