Structural Basis for RNA Unwinding Caused by DEAD-box Protein *Drosophila* Vasa

RNA helicases catalyze the alteration of higher-order RNA structures (secondary structure melting, strand separation, and RNA-protein dissociation) coupled with ATP hydrolysis. By doing so, RNA helicases regulate various cellular processes involving RNA. DEAD-box proteins constitute the largest RNA helicase family, and are widely found, from bacteria to humans. They are named after a strictly conserved sequence, Asp-Glu-Ala-Asp (D-E-A-D), and have two RecA-like domains (the N- and C-terminal domains) as a catalytic core.

To elucidate the RNA unwinding mechanism of such proteins, we determined the structure of the core of the *Drosophila* DEAD-box protein Vasa bound to a single-stranded RNA molecule and AMPPNP, an ATP analogue (Fig. 1), at a resolution of 2.2-Å using **BL41XU** beamline [1,2]. AMPPNP strongly interacts with both of the N- and C-terminal domains, thereby bringing them into the closed form. We also identified one water molecule as the putative attacking water for ATP hydrolysis. The water molecule was ideally located for a nucleophilic in-line attack, and interacted with conserved residues (Fig. 2(a)). These results indicate that the present structure is the functional ATP-bound form in the catalytic cycle. The bound RNA runs perpendicular to the cleft and is sharply bent, avoiding a clash with a conserved α-helix (the "wedge" helix) in the N-terminal domain (Fig. 2(b)). Clearly, such a bend would be incompatible with a continuous RNA duplex. The U6 phosphate atom, located at the bending point, is recognized by the conserved residues (Fig. 2(c)). Many of the

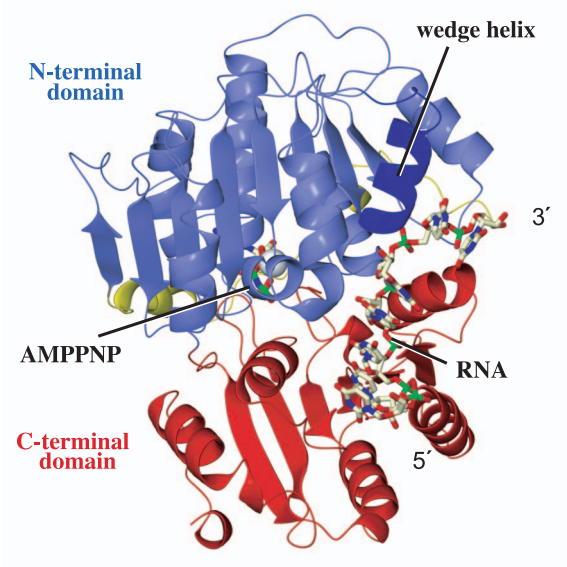

Fig. 1 Overall structure of Vasa•RNA•AMPPNP complex.

conserved residues form an extensive network of interdomain interactions (interactions between the amino- and carboxy-terminal domains) near the RNA and ATP binding sites (Fig. 2(a) and data not shown).

To examine the roles of the conserved residues, we developed mutant proteins and analyzed their RNA binding, ATPase, and RNA unwinding activities (Fig. 3). When RNA-binding residues such as R328, R378, R528 and T546 were mutated, all three of these activities were affected. This is consistent with the fact that Vasa requires RNA for efficient ATPase activity. Interestingly, when residues involved in

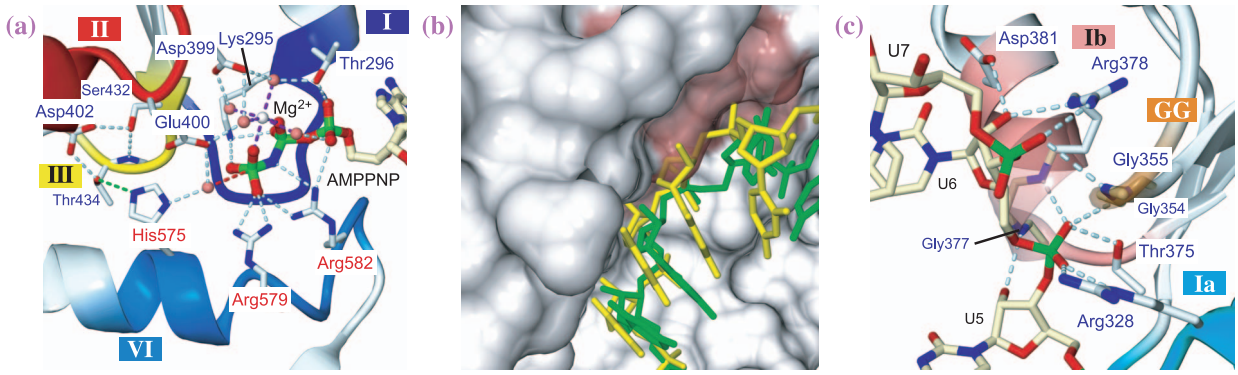

Fig. 2. RNA and ATP bindings. Motifs I, Ia, GG, Ib, II, III, and VI are marked. **(a)** ATPase site. Blue and red characters indicate residues from the N- and C-terminal domains, respectively. Water molecules are shown as pink spheres. Purple and green broken lines indicate coordinated bonds and interdomain interactions, respectively. The red broken line connects the putative attacking water and the γ-phosphorus atom. **(b)** Steric hindrance induced by wedge helix. When a modeled straight RNA (yellow) is superposed on the bound RNA in the present structure (green), it clashes with the wedge helix (pink). **(c)** RNA binding near the bend.

Mutation	Interaction	RNA Xlink (%)	ATPase (%)	unwinding
WT	-	100	100	+++
R328A	RNA	39	25	++
E329A	Inter-domain	150	72	++
Q333A	Inter-domain	47	45	+
R378A	RNA	28	17	+
D381A	RNA	309	118	+++
Q525A	Inter-domain	61	70	-
R528A	RNA	29	7	-
T546A	RNA	52	0	-
R551A	Inter-domain	55	95	+
D554A	ATP / Inter-domain	110	50	-

Fig. 3. Summary of mutational analyses.

interdomain interactions (e.g., E329, Q333, Q525, R551, and D554) were replaced, only RNA unwinding activity was greatly affected without serious decreases in RNA binding or ATPase activities. In other words, the ATPase and RNA unwinding activities are uncoupled in these mutants.

On the basis of these results, we propose a possible mechanism of the unwinding caused by DEAD-box proteins (Fig. 4). The protein can cooperatively bind ATP and the duplex region of substrate RNA. On binding, the protein induces a sharp bend in one strand by providing steric hindrance with the wedge helix. As a result, some base pairs nearby are disrupted and the duplex is destabilized. Then, ATP is hydrolyzed, and the complex dissociates to release the destabilized RNA and recycle the protein. In the uncoupled mutants, the protein can bind two substrates but cannot precisely locate the wedge helix relative to the bound RNA, which results in abortive ATP hydrolysis.

This mechanism is different from those for other helicases, and we propose that this mechanistic difference is correlated with the cellular functions of these proteins. Higher-order RNA structures are fundamental to their functions, and RNA helicases modulate them in a very specific manner. A possible advantage of the present mechanism is that the protein can directly interact with the target regions and minimize the unfavorable modulation near them. This unique mechanism may enable the DEAD-box proteins to be involved in specific, yet ubiquitous, roles in RNA metabolism and regulation.

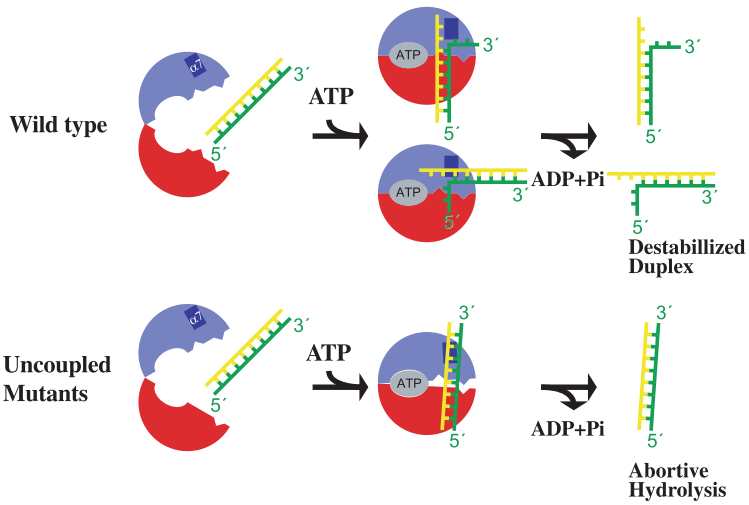

Fig. 4. Proposed mechanisms of RNA unwinding caused by the DEAD-box protein and abortive ATP hydrolysis of the uncoupled mutants.

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