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Journal

Structure, 22(7)

ISSN

1359-0278

Authors

Avanzino, Brian C

Fraser, Christopher S

Publication Date

2014-07-01

DOI

10.1016/j.str.2014.06.005

Peer reviewed

Published in final edited form as:
Structure. 2014 July 8; 22(7): 931–932. doi:10.1016/j.str.2014.06.005.

DEAD Box Unwinding Caught in the Act

Brian C. Avanzino and **Christopher S. Fraser***
Department of Molecular and Cell Biology, College of Biological Sciences, University of California, Davis, CA 95616

SUMMARY

In this issue of *Structure*, Sun and colleagues describe the link between the dynamic conformational cycle and RNA unwinding activities of the DEAD box helicase, eIF4AI.

Virtually all aspects of RNA metabolism including transcription, splicing, translation and RNA decay involve restructuring of duplexed RNA by helicases. Unlike canonical helicases, RNA helicases in the DEAD box family do not unwind duplex regions by translocation along the RNA. Instead, these helicases use ATP to bind directly to a short duplex region and unwind it by a mechanism of local strand separation (Linder and Jankowsky, 2011). All DEAD box proteins possess two RecA-like domains that form a “dumbbell” structure that can adopt both open and closed conformations (Figure 1). Capturing structures of DEAD box helicasesin different stages of their unwinding cycle has provided a framework to explain their mechanism of duplex unwinding. In the absence of ATP, the two RecA domains move apart into an open conformation that results in a weak affinity for RNA (Linder and Jankowsky, 2011). Binding of ATP and RNA promotes a closed conformation of the RecA domains that induces a bending of the RNA backbone that is not compatible with duplex formation (Mallam et al., 2012). It is expected that rapid cycling between these two conformations in an ATP dependent manner will result in productive duplex unwinding. However, observing the relationship between these conformational changes together with the timing of duplex unwinding has not been previously undertaken.

In this issue of *Structure*, Sun and colleagues use a single molecule FRET (smFRET) assay to precisely monitor the conformational cycle of a DEAD box helicase during unwinding of a RNA hairpin in real time (Sun et al., 2014).The DEAD box helicase used in this study is eukaryotic initiation factor 4AI (eIF4AI), which unwinds mRNA 5′ UTR secondary structure to promote ribosome recruitment and translation initiation (Parsyan et al., 2011). Although eIF4AI possesses weak helicase, ATPase, and RNA binding activities, these can be greatly stimulated by the addition of at least three accessory proteins, including eIF4G, eIF4E and either eIF4B or eIF4H (Feoktistova et al., 2013; Ozes et al., 2011;Rogers et al., 2001). To monitor the conformational changes of eIF4AI, a donor fluorophore is attached to

*Correspondence should be addressed to C.S.F (csfraser@ucdavis.edu), Phone: (530) 752-1716, Fax: (530) 752-3085.

one RecA-like domain of eIF4AI and an acceptor is attached to the other RecA-like domain. This generates a low FRET state upon opening and a high FRET state upon closing of eIF4AI (Figure 1).In order to observe eIF4AI conformational changes in real time, the authors encapsulate a RNA hairpin, the double labeled eIF4AI and the accessory protein eIF4H in lipid vesicles. These vesicles are immobilized to a surface by a biotin moiety to enable monitoring by total internal reflection fluorescence (TIRF) microscopy. Using this approach, the authors find that ATP binding induces a transition from the open conformation of eIF4AI to a closed conformation that is bound to RNA. Hydrolysis of ATP and release of inorganic phosphate then results in the return of eIF4AI to its open conformation. By comparing the dwell times of the closed and open conformations of eIF4AI tothe“waiting” and “unwinding” times of a labeled RNA hairpin undergoing eIF4AI helicase action (Sun et al., 2012),the authors make the surprising finding that the opening of the eIF4AI conformation corresponds with the RNA unwinding step (Figure 1). This is in contrast to structural models and gel shift assays that have generally indicated that closing of the helicase destabilizes the RNA duplex, while ATP hydrolysis and opening facilitates helicase recycling (Linder and Jankowsky, 2011; Mallam et al., 2012).However, since eIF4AI alone does not result in duplex unwinding in the smFRET assay, it is not clear if this model will apply to all DEAD box helicases or if it reflects an important function of eIF4H in unwinding. Adapting this technique to observe eIF4AI conformation and RNA unwinding simultaneously in the same system with the additional stimulatory factors eIF4G, eIF4E and eIF4B is indispensable for generating a complete understanding of eIF4AI dynamics.

In this study the authors also utilizesm FRET to characterize the mechanism of action of hippuristanol, a potent and highly specific eIF4AI inhibitor that prevents RNA binding to eIF4AI (Bordeleau et al., 2006).Here, the authors find that hippuristanol locks eIF4AI in the closed conformation to inhibit RNA unwinding (Sun et al., 2014).However, in contrast to bulk assays, the smFRET data indicate that hippuristanol does not appear to inhibit RNA binding to eIF4AI/eIF4H complexes (Bordeleau et al., 2006). The reason for this discrepancy is not clear, but it may be due to the ability of eIF4H to bind RNA loops and stabilize eIF4AI on the RNA substrate. Since eIF4AI is an attractive therapeutic target for inhibiting translation initiation, it will be interesting to use this approach to determine if other small molecule inhibitors can be found that target other steps in the helicase cycle.

Overall, this study elegantly explains the mechanism of eIF4AI conformational changes during RNA unwinding using a single molecule approach. The role of additional physiologically relevant eIF4AI stimulating proteins including eIF4G, eIF4E, and eIF4B remains to be determined. Interestingly, an independent study just published elsewhere used a similar method to examine how eIF4G, eIF4B and different RNAs modulate the conformational cycle of yeast eIF4AI (Harms et al., 2014). The addition of eIF4G and eIF4B in those experiments accelerates the cycling of eIF4AI conformations, which is consistent with the observations that eIF4G and eIF4B cooperatively activate human eIF4AI duplex unwinding in bulk assays (Ozes et al., 2011; Parsyan et al., 2011). It will now be important to determine the dynamics of eIF4AI helicase activity in combination with the small ribosomal subunit during scanning through structured 5′ UTRs. Although the general ATPase conformational cycle is likely to be universal among DEAD box proteins, it is desirable to use the approach described by Sun et al. (2014) to explore the relationship

between the conformational cycle and duplex unwinding for different DEAD box proteins given their different accessory factor requirements.

Acknowledgments

We thank John Hershey for his critical reading of the manuscript. This work was supported by the US National Institutes of Health (NIH) through an NIHR01 grant (R01GM092927; to C.S.F) and an American Heart Association and Myocarditis Foundation predoctoral fellowship (B.C.A.).

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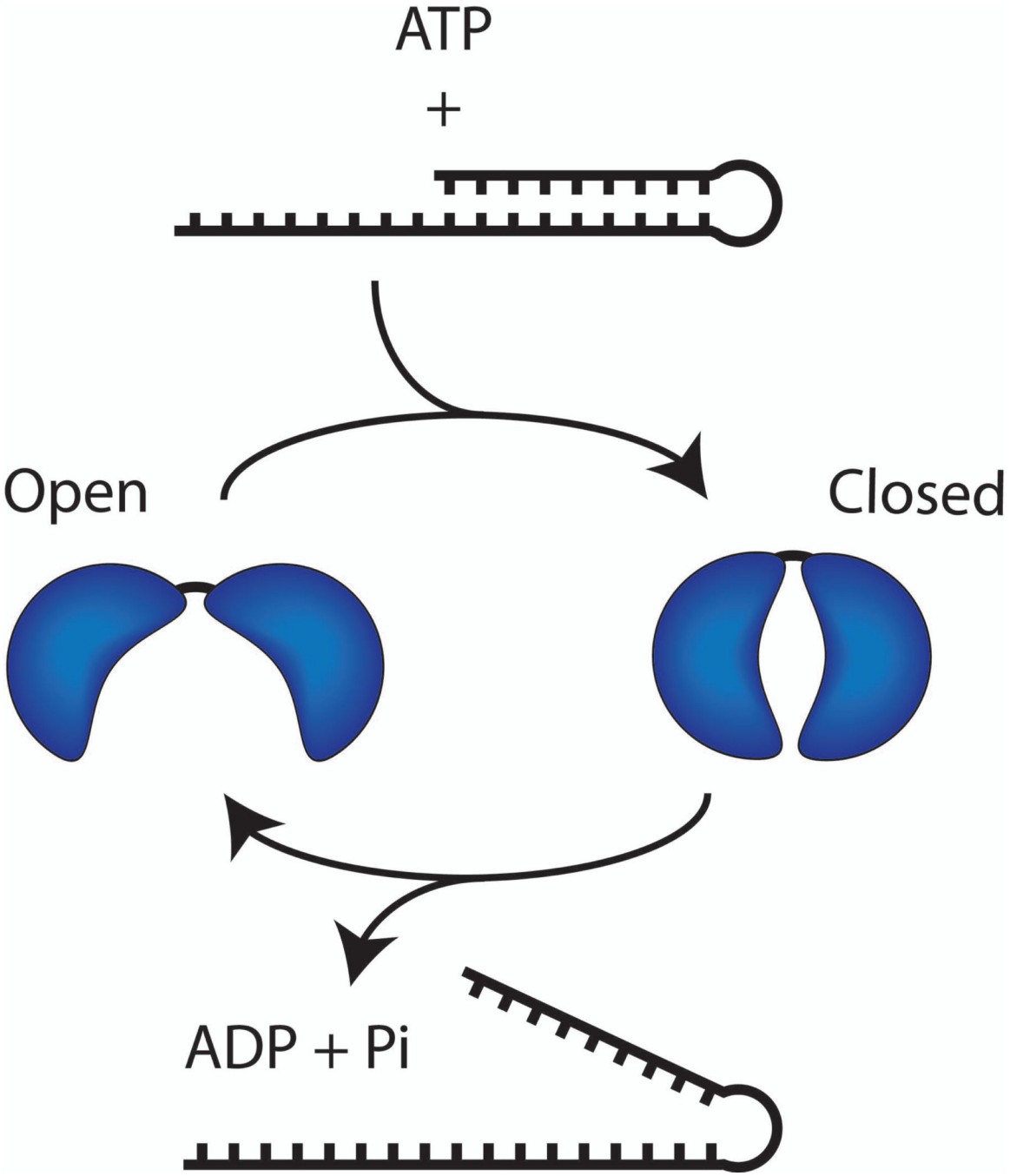

Figure 1. Schematic Diagram of the Proposed eIF4AI Catalytic Cycle

Free eIF4AI exists in an open conformation. Binding of ATP, a RNA hairpin and eIF4H (not shown for clarity), results in eIF4AI adopting a closed conformation. Upon ATP hydrolysis and subsequent ADP and inorganic phosphate release, eIF4AI returns to the open conformation resulting in unwinding of the RNA hairpin.