Review of: "Structural Basis for Dimerization and Activation of UvrD-family Helicases"

Hala Chamieh[1]

1 Faculty of Science, Lebanese University, Lebanon

Potential competing interests: No potential competing interests to declare.

The manuscript presented by Chadda et al. describes the structural basis of UvrD1 activation from*Mycobacterium tuberculosis* . The work is very interesting as researchers propose for the first time from EM reconstruction images how the 2B domain of UvrD potentially regulates its unwinding activity. They suggest this domain is an auto-inhibitory domain acting as a regulatory element for allosteric activation of the unwinding activity. By constructing a homology model with the conserved UvrD from *E.coli,* they propose that the observed mechanism is conserved among all UvrD family members.

- The UvrD dimer was shown to occur in two different states. Did the researchers digest their sample with DNase prior to image reconstruction? Some proteins are purified in a bound form to DNA/nucleotides. Can they exclude that it is not an already nucleotide/DNA bound state? Can you provide specific examples of how DNase treatment could clarify the states of the UvrD dimer?
- Are there any cysteines in the protein other than in the 2B domain that can contribute to the observed conformational change? What is the observed conformation in EM for the cysteine mutants?
- The GIG motif in the 2B domain seems to be important for DNA binding. How does it affect DNA binding, and what would be the protein conformation mutant in EM?
- Jia et al. previously reported rotation of the 2B domain coupled to nucleotide and DNA binding that was salt-dependent (Jia et al., 2011). Did the researchers observe a conformational change related to salt concentration as the one observed for EcUvrD, or was it solely related to the ox-red state?
- The researchers state that the observed mechanism is conserved among all UvrD family members. Could you suggest additional experiments/explanations for the conservation of this mechanism in other members where the 2B domain is less conserved?

Minor comments:

- The position of conserved residues in the 2B domain with Cys representation should be shown in Fig. 1 along with the primary structure and domain organization, as the reader has to refer each time to the manuscript published in PNAS by the same authors to access information related to the motif and conservation among other UvrD members.