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Characterization of the ATPase Activity of CasDinG

Christian Cahoon
Utah State University, christiancahoon12@gmail.com

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Christian D. Cahoon, Hannah Domgaard, Matt J. Armbrust Ryan Jackson
Department of Chemistry & Biochemistry, Utah State University, Logan, UT

Abstract & Background

The battle between bacteria and phage has been ongoing for eons. This battle has generated the evolutionary pressure necessary for the development of microbial immune systems. Characterization of these systems has led to the discovery of molecular tools such CRISPR-Cas systems. This system uses a genetic memory of past viral infections coupled with associated proteins to form ribonucleoprotein complexes which seek out and destroy foreign genetic elements. These systems have been repurposed by scientists to create powerful gene editing tools. With such powerful molecular tools being discovered, we have continued the characterization of the Type IV-A CRISPR Cas system. Our work centers on the Type IV-A CRISPR Cas system from the organism *Pseudomonas aeruginosa 83.* Previous work has shown that the Type IV-A CRISPR-associated proteins form a ribonucleoprotein complex, and that an ancillary gene *casdinG* is required for immune function. Phylogenetic analysis shows that Type IV-A CRISPR associated DinG (CasDinG) is distinct from chromosomally associated DinG proteins involved in recombination and repair, suggesting that CasDinG may play a CRISPR-specific role in type IV immunity. The research in this presentation shows the characterization of the ATPase activity of CasDinG, an XPD family helicase. We show that ATP hydrolysis is substantially increased in the presence of nucleic acid

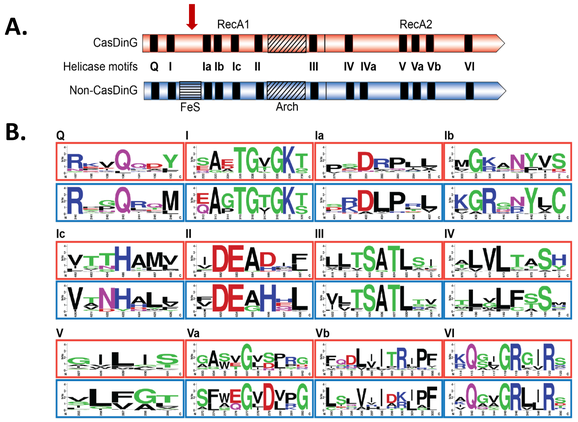


Figure 1. A. A comparison of CasDinG gene organization to Non-CasDinG genes. The common helicase motifs are noted. CasDinG lacks a FeS cluster, typically composed of four cysteine residues upstream of the RecA1 domain. The FeS cluster is necessary for the helicase activity of many DinG enzymes. **B.** Web logos showing the amino acid sequence consensus for the helicase motifs.[2,3]

How does ATP Hydrolysis Occur?

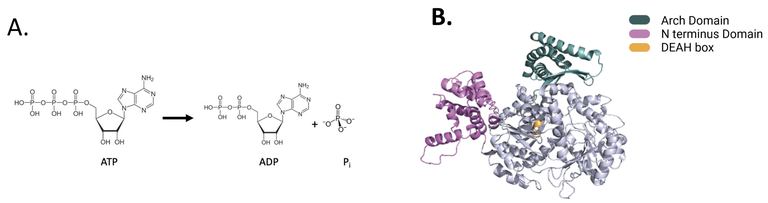


Figure 2. A. A general schema of how ATP is hydrolyzed into ADP and inorganic phosphate (P_i). The third or gamma phosphate is coordinated by amino acids, Mg^{2+} and water to allow for the cleavage of the phosphate from the phosphate backbone. This cleavage and relief of the strong and unfavorable negative charge of the third phosphate leads to the release of free energy which allows for structural changed in the protein to occur. These changes are correlated with specific enzymatic activities. In the case of CasDinG these hypothesized activities are helicase activities or nuclease activities. **B.** This panel shows a predicted structure of CasDinG. Highlighted in gold we see the DEAH box which is used to coordinate the ATP and allow for more rapid hydrolysis of the ATP and the release of the free energy stored in ATP. The other two domains, are potential regions where other putative activities such as nuclease and helicase activities could be found in the protein structure.

How is ATP hydrolysis assayed?

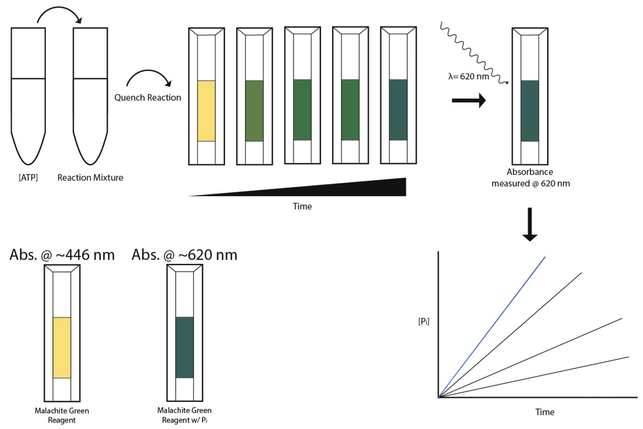
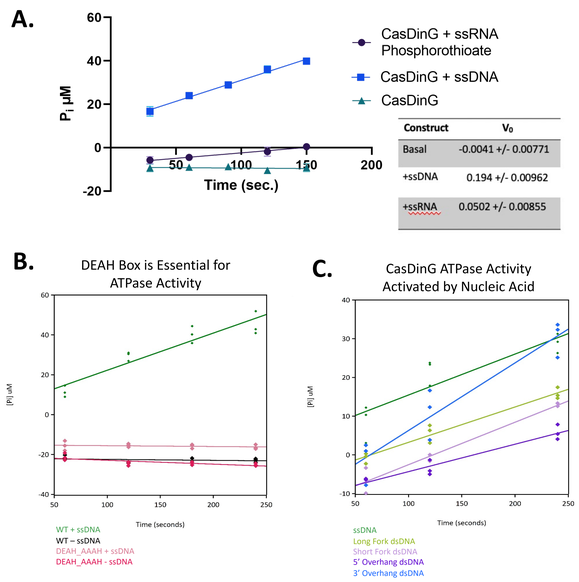


Figure 3. This figure shows the methodology of detecting the rate of enzymatic ATP hydrolysis. A concentration of ATP is added to a reaction. At set time points a sample is draw and quenched in malachite green reagent. The quenched reaction is developed over 30 minutes. The concentration of inorganic phosphate (P_i) is assayed by a spectrophotometer which analyses the absorption of light at a specific wavelength, 620 nm. The absorption is converted to concentration using a standard curve and is plotted as a function of time. The slope of these lines in the initial velocity or V0 of the enzyme at that concentration of ATP.

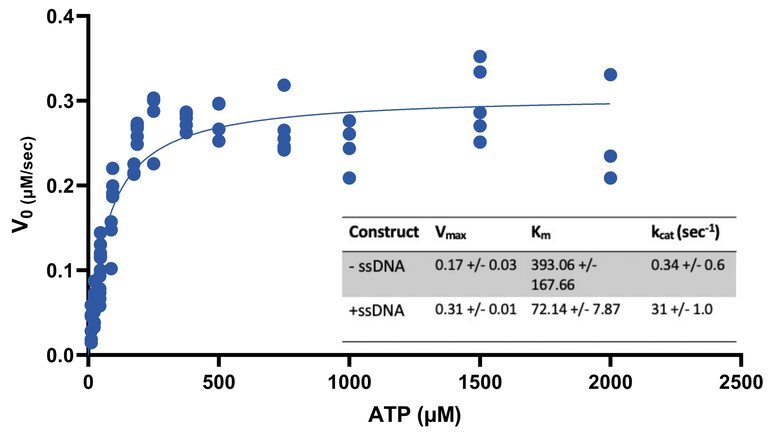
ATP Hydrolysis is activated in the presence of Nucleic Acid



Construct	V_0
Basal	-0.0041 +/- 0.00771
+ssDNA	0.194 +/- 0.00962
+ssRNA	0.0502 +/- 0.00855

Figure 4. A. These are initial velocity (V_0) plots of ATPase activity in the with and without single stranded nucleic acid. We can see that without nucleic acid the ATPase activity is ~ 0. In the presence of single stranded DNA (ssDNA) we see significant activation of ATP hydrolysis. We also see activation in the presence of single stranded RNA. The initial velocities are tabulated on the right of the plots. **B.** V_0 plot showing the ATPase activity of WT CasDinG and the DEAH_AAAH mutant. Only WT CasDinG in the presence of ssDNA shows ATPase activity showing that the DEAH box is necessary for ATPase activity. **C.** V_0 plot showing the differential ATPase activity of WT CasDinG in the presence of nucleic acid substrates. The highest initial rates of ATPase activity are seen with ssDNA and the 3' overhang dsDNA substrate. [5]

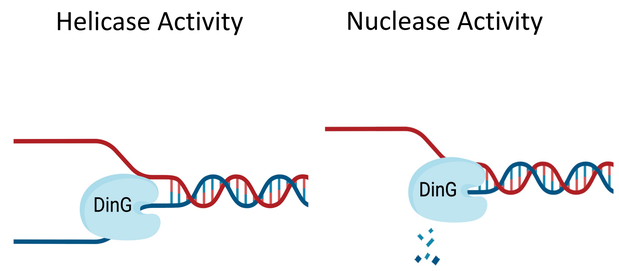
Michaelis-Menten analysis of ATPase activity



Construct	V_{max}	K_m	k_{cat} (sec^{-1})
- ssDNA	0.17 +/- 0.03	393.06 +/- 167.66	0.34 +/- 0.6
+ssDNA	0.31 +/- 0.01	72.14 +/- 7.87	31 +/- 1.0

Figure 5. Michaelis-Menten Analysis of CasDinG ATP hydrolysis in the presence of ssDNA. These values were obtained at 10 nM DinG with 60 nM ssDNA. Basal rates were obtained at 500 nM CasDinG. We see ~91-fold increase in k_{cat}. CasDinG is significantly activated in the presence of single stranded DNA.

Conclusion & Future Directions



P. *aeruginosa 83* CasDinG is phylogenetically distinct from non-CRISPR associated DinG genes and is essential for Type IV-A interference of a target plasmid. We have found that CasDinG ATPase activity is stimulated by nucleic acid substrates, with the highest rates of ATP hydrolysis being accompanied by ssDNA. Future ATPase analysis will be performed to understand the kinetic parameters of CasDinG in the presence of single stranded RNA and other substrates such as plasmid DNA. We are interested in understanding the role of CasDinG in the presence of the Type IV-A ribonucleoprotein complex Work is currently being done to determine binding substrate preferences as well as whether other DNA/RNA duplexes can be unwound. We are working towards understanding if and how CasDinG interacts with the Type IV-A complex through *in vitro* methods and structural studies. Understanding the molecular details of CasDinG operates will provide pivotal details on how the Type IV-A system interacts with nucleic acid.[2,3]

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