Understanding the Functions and Implications of FadR in *Vibrio cholerae*

The 2015-2016 Pingry SMART Team Project

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Abstract

Cholera is a bacterial disease caused by ingestion of the bacterium *Vibrio cholerae* through contaminated food or water. The main symptom of cholera is diarrhea, which leads to approximately 142,000 deaths from fatal dehydration annually. The transcription factor, FadR, is the master regulator of fatty acid metabolism and plays a key role in *V. cholerae* virulence. The level of fatty acids inside the bacterium regulate the production of cholera toxicity factors and also influences the activity of FadR. Using X-ray crystallography, the structure of FadR was solved in both the presence and absence of DNA and the fatty acid ligand, oleolyl-CoA. In the absence of a ligand, the FadR dimer adopts a conformation capable of binding DNA. In the ligand bound structure of *V. cholerae* FadR, two fatty acid binding sites were identified. The second fatty acid binding site was discovered to be made up of a 40 amino acid insertion in the protein that is absent in *E. coli* FadR. In the presence of a second ligand, FadR undergoes a dramatic conformational change causing the protein to release from DNA. The additional fatty acid binding site in the *V. cholerae* version of FadR may improve transcriptional regulation and efficiency compared to its *E. coli* counterpart.

Background on Cholera

Cholera is a bacterial disease caused by the bacterium *Vibrio cholerae* that is contracted after ingestion of contaminated water or food. Cholera infects approximately 4.3 million people every year and causes up to 142,000 deaths annually (WHO). The disease's largest presences are in Asia, Africa, and South and Central America. The most common symptom is severe diarrhea that can lead to fatal dehydration. However, up to 80% of cases can be treated with oral rehydration therapy.

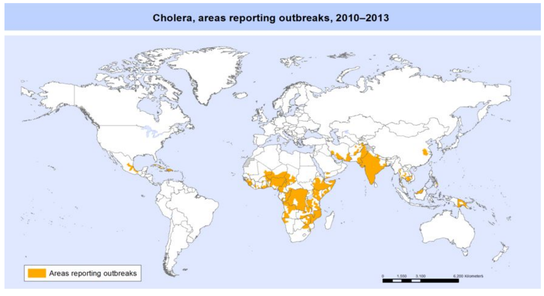
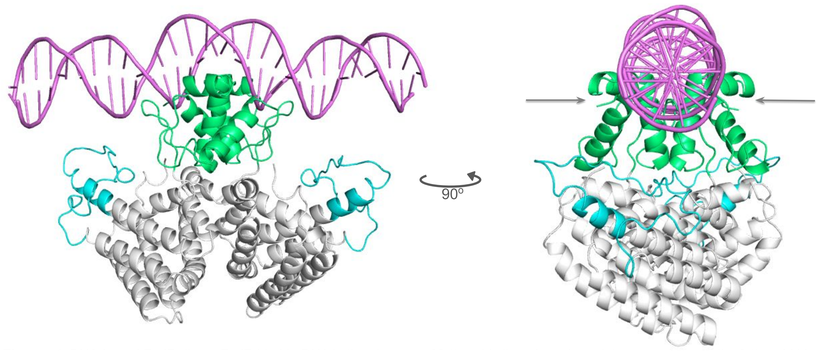


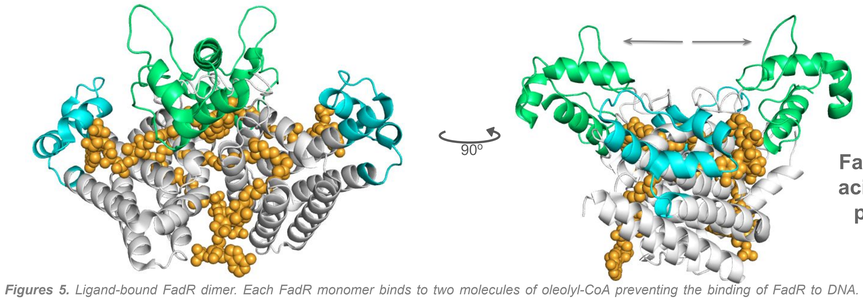
Figure 1. *Cholera, areas reporting outbreaks, 2010-2013.* Courtesy of the *World Health Organization*.

Structure of FadR



FadR dimer binds DNA in the absence of fatty acid ligands

Figures 4. *DNA-bound FadR dimer. FadR bind to DNA in the absence of fatty acids ligands and regulates expression of the fatty acid biosynthesis machinery. The DNA strands are purple; the DNA binding domains are green; and the ligand binding domains are white with the 40 amino acid insertion in cyan. (PDB ID 4P9U)*



FadR dimer binds two fatty acid molecules per subunit preventing DNA binding

Figures 5. *Ligand-bound FadR dimer. Each FadR monomer binds to two molecules of oleolyl-CoA preventing the binding of FadR to DNA. The ligands are orange; the DNA binding domains are green; and the ligand binding domains are white with the 40 amino acid insertion in cyan. (PDB ID 4PDK)*

FadR functions inside the cell as a homodimer. *V. cholerae* FadR is highly similar in structure and function to the *E. coli* version of FadR. Both proteins have a similar DNA binding domain at the N-terminus of the protein (amino acids 2-72 in *V. cholerae* FadR). The DNA-binding domains undergo a hinge-like conformational change at amino acid residues 72 and 75 that allow for FadR's binding to DNA. In the DNA-bound conformation, FadR is unable to bind to fatty acid ligands. A 40-residue insertion (residues 138-177) accounts for the major structural difference between *V. cholerae* FadR and *E. coli* FadR. This insertion stabilizes the wing of the protein and acts as a second ligand-binding site for FadR's long chain fatty acid ligand, oleolyl-CoA.

V. cholerae FadR vs *E. coli* FadR

Both *E. coli* FadR and *V. cholerae* FadR function as fatty acid regulators within the bacterium. However, *V. cholerae* FadR has one additional ligand binding site which allows this protein to function more efficiently. *V. cholerae* FadR's second binding site is composed of a 40 amino acid insertion. The similarities between FadR in these two bacteria can be largely attributed to their 52% amino acid sequence identity and high structural similarity.

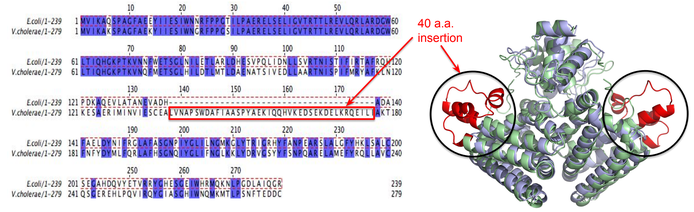
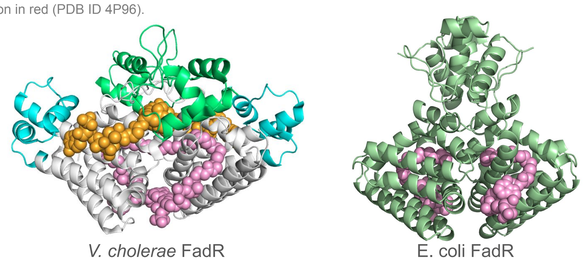


Figure 6. Sequence (left) and structural (right) alignments of apo FadR from *E. coli* and *V. cholerae*. The highlighted purple sections indicate sequence identity. The insertion in *V. cholerae* FadR is boxed in red on the sequence alignment while the corresponding amino acids are colored red in the structure alignment. For the structural alignment, *E. coli* FadR is blue (PDB ID 1E2X) and *V. cholerae* FadR is green with the insertion in red (PDB ID 4P96).



V. cholerae FadR
binds two oleoyl-CoA (C-18 monounsaturated)

E. coli FadR
binds one myristoyl-CoA (C-14 saturated)

Figure 7. Structures of *V. cholerae* FadR (left, 4PDK) and *E. coli* FadR (right, 1E2X) bound to their respective fatty acid ligands. Both proteins form dimers in solution while each subunit of V. cholerae FadR binds two oleoyl-CoA molecules (orange and pink spheres) and E. coli FadR binds one myristoyl-CoA (pink spheres).

Future Implications

The identification of the second ligand binding site in *V. cholerae* marks the discovery of a more efficient regulatory mechanism that explains the effectiveness of FadR as a master regulator of fatty acid synthesis. This may lead to advancements in current treatments for cholera and further investigation of FadR's role in the virulence of other pathogens.

Function of FadR

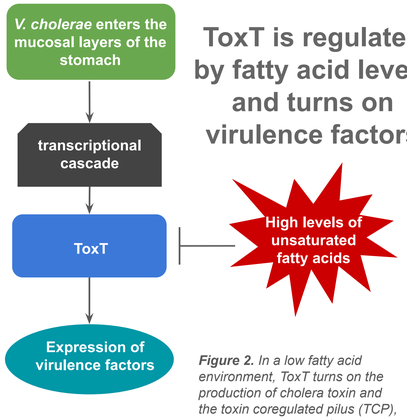


Figure 2. *In a low fatty acid environment, ToxT turns on the production of cholera toxin and the toxin coregulated pilus (TCP), the two key virulence factors in the V. cholerae toxicity pathway.*

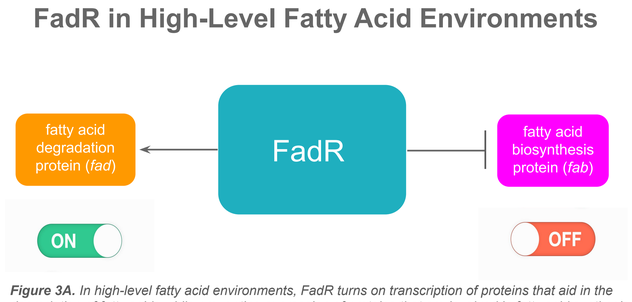


Figure 3A. *In high-level fatty acid environments, FadR turns on transcription of proteins that aid in the degradation of fatty acids while preventing expression of proteins that are involved in fatty acid synthesis.*

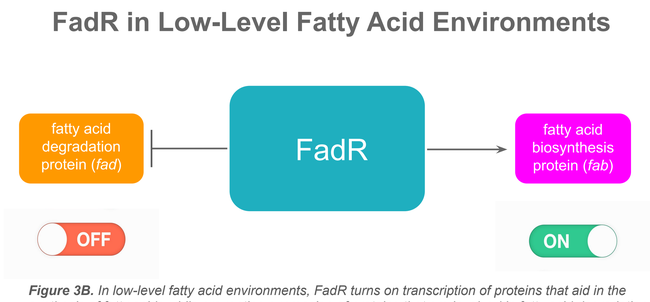


Figure 3B. *In low-level fatty acid environments, FadR turns on transcription of proteins that aid in the synthesis of fatty acids while preventing expression of proteins that are involved in fatty acid degradation.*

FadR regulates fatty acid levels within the bacterium *V. cholera* thus regulating ToxT, the master regulator of virulence factors that cause cholera. FadR's regulation of fatty acids is vital to cholera's virulence. Elevated levels of fatty acids inhibit the expression of ToxT and *V. cholerae's* virulence factors by binding to ToxT and preventing its interaction with DNA.

Citations/Acknowledgements

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A special thank you to…
Dr. Jon Kull Dr. Tim Herman Dr. Diane Munzenmaier Mark Hoelzer
Dr. Colleen Kirkhart Deirdre O'Mara
Center for Biomolecular Modeling 3D Molecular Design