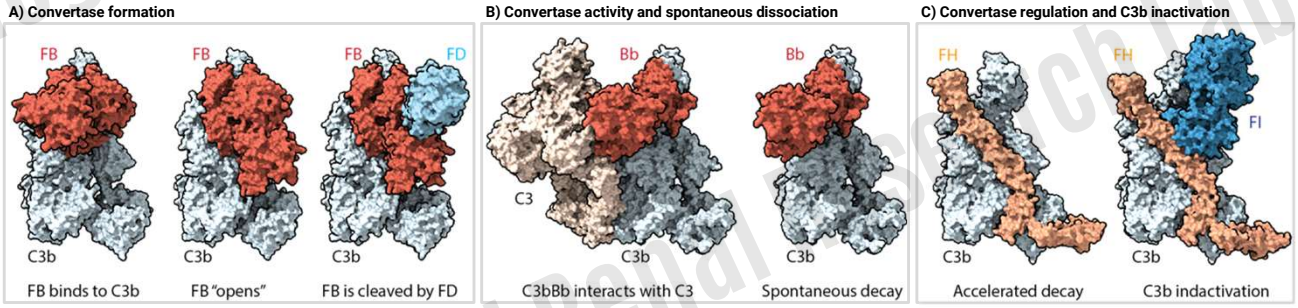
Functional characterization of complement gene variants recapitulates and supports AP C3-convertase molecular dynamics

Hector Martin Merinero, Yuzhou Zhang and Richard J.H. Smith

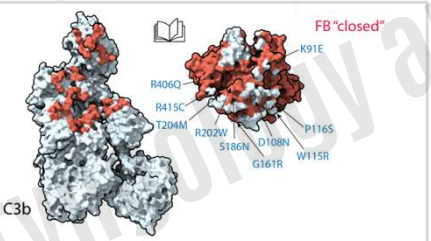
Molecular Otolaryngology and Renal Research Laboratories, Carver College of Medicine
University of Iowa, Iowa City, Iowa, United States

Abstract

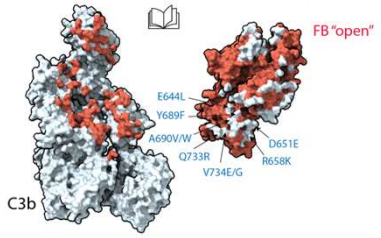
Knowledge about the alternative pathway C3-convertase (C3bBb) structure, function and molecular dynamics is supported by several multidisciplinary inputs, including structural data (X-ray crystallography, electron microscopy, cryo-electron microscopy), research tools (antibodies, nanobodies, mutants, animal models), complement inhibitors (humanized antibodies, small molecules) and the functional characterization of disease-associated complement gene variants. In this work we review more than 100 variants functionally characterized in C3, FB, FD, FH and FI that inform the C3bBb molecular dynamics, support the current model, and reveal the robustness of years of research.



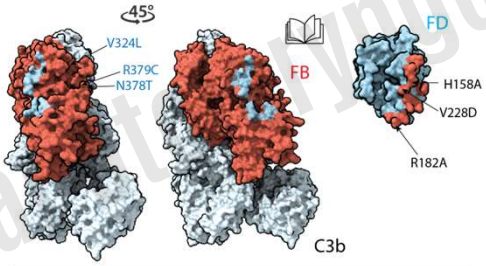
A) Convertase formation



Most *CFB* variants that impair C3b-binding cluster on the exposed surface of the Ba fragment that directly contacts with C3b[1]. No variants in C3b have been described to specifically affect FB binding.

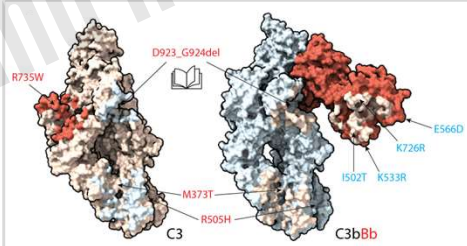


The remaining variants that impair C3b-binding cluster on an exposed surface of the serine protease (SP) domain[1]. This surface is exposed upon the 'opening' conformational change adopted by FB; before this transition, it does not contact C3b.

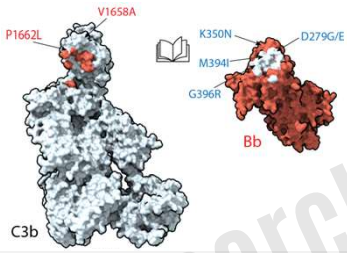


Very few variants specifically impair FD-mediated cleavage of FB into Ba and Bb. Notably, in-lab-designed FD variants cluster on the FD-FB interaction surface[2], whereas variants in FB are located far from the interaction site, suggesting that they affect the exposure of the scissile loop to FD-mediated cleavage[1].

B) Convertase activity and spontaneous dissociation

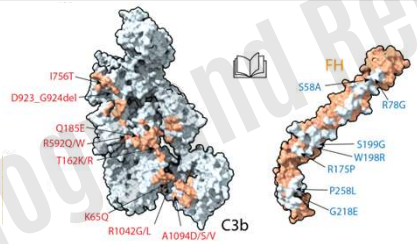


C3bBb is a labile complex, and its interaction with C3 substrate results in rapid proteolytic cleavage, making it very difficult to analyze. The few variants informing about this interaction cluster on a surface of the MG-ring of C3/C3b[3-5], and around the catalytic triad of the FB SP domain[1]. All these variants have been found to alter the activation rate of C3 by C3bBb.

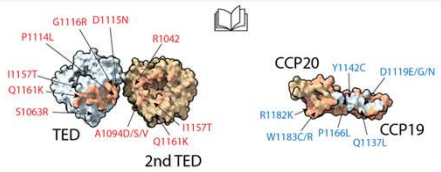


Variants that affect C3bBb stability are located specifically at the C-terminal end of the C345c domain of C3b[6] and in the MIDAS of FB vWA domain[1,7-9]. Together, these regions constitute the only interaction surface between the two proteins.

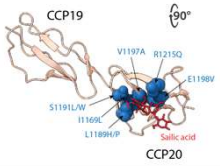
C) Convertase regulation and C3b inactivation



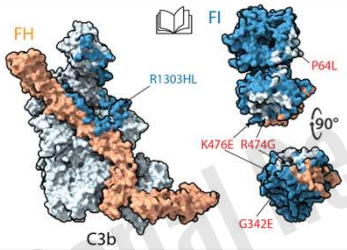
C3bBb natural decay can be accelerated by the regulators of complement activation (RCAs). To do so they must bind to the convertase. Many variants in C3b and FH, the RCA with the largest number of functionally characterized variants, inform about this interaction. Collectively, these variants map the interaction surface[5,10-11].



The C-terminus of FH binds specifically to the TED domain of C3b. This interaction can occur through two distinct surfaces that are supported by variants that impair this interaction[10-11].

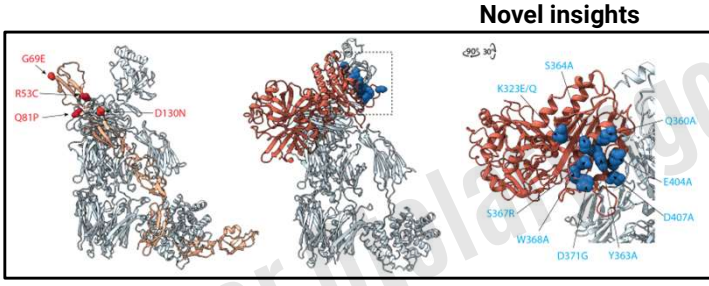


Other C-terminal FH variants impair FH function without affecting C3b binding. They cluster in the SCR 20 and delineate the sialic acid-binding pocket[12].



Inactivation of C3b to iC3b by FI, with an RCA as a cofactor, prevents the formation of new convertases. Variants in FI that disrupt this regulatory process cluster on the surfaces that interact with C345c and CUB domains of C3b[12-13]. In addition, two variants have been identified that specifically alter the FI cleavage site on C3bfound[14].

Novel insights



Some functionally impaired variants in FH[11] and FB[1,15] cannot be accommodated within the different steps reviewed in this poster. They impair the RCA-mediated accelerated decay of C3bBb, and cluster on two specific surfaces.

Although FH-Bb competitive replacement, steric hindrance, and electrostatic repulsion have been proposed as mechanisms underlying RCA-mediated decay acceleration, FB variants cluster away from the putative FH repulsion surface. This supports a direct FH-Bb interaction and suggests that convertase decay acceleration is more complex than originally proposed[16].

Acknowledgements

This work was supported in part by the NIH R01 DK110023; we thank those patients who have made this research possible.

References (PMIDs)

1. Unpublished
2. 21205667
3. 25712219
4. 41383613
5. 20852386
6. 22250080
7. 17182750
8. 24652797
9. 32540405
10. 25608561
11. 34189567
12. Unpublished
13. 17597211
14. 26895476
15. 10072540
16. 17182573