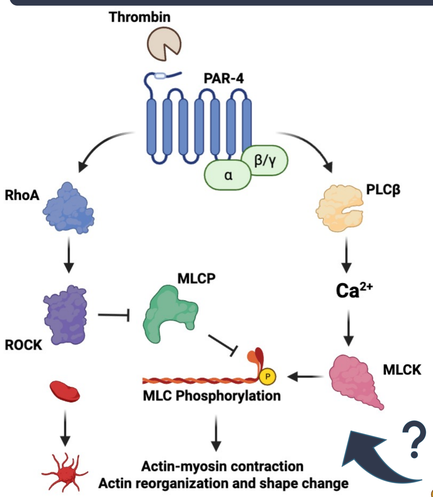
University of British Columbia/Department of Biochemistry and Molecular Biology

The Role of Filamin A in the Platelet Shape Change Reaction

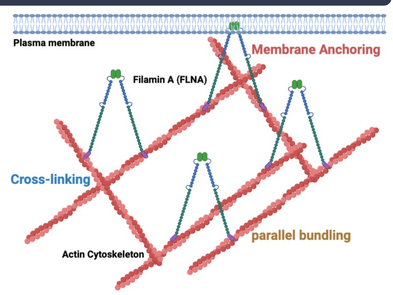
Felix Hong[1,2], Molly Y. Mollica[4,5,6], Nathan J. Sniadecki[6,7], José A. López[4,5], Hugh Kim[1,2,3]

[1]Centre for Blood Research, University of British Columbia, Vancouver, BC, Canada., [2]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada. [3]Department of Oral Biological and Medical Sciences, University of British Columbia, Vancouver, BC, Canada. [4]Bloodworks Research Institute, Seattle, WA, USA. [5]Department of Medicine, University of Washington, Seattle, WA, USA. [6]Department of Mechanical Engineering, University of Washington, Seattle, WA. USA. [7]Department of Bioengineering, University of Washington, Seattle, WA, USA.

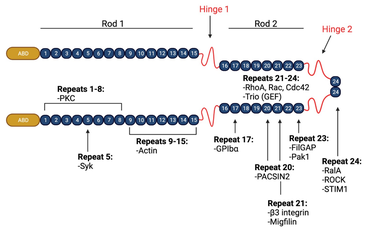
Introduction



Platelets are cellular fragments produced by megakaryocytes in the bone marrow and are important regulators of vascular hemostasis, wound healing, and inflammation. Platelet function involves shape change following their activation. The shape change reaction is largely controlled by the phosphorylation of its myosin light chain (MLC), which leads to activation of myosin and contraction of the actin cytoskeleton.



Filamin A (FLNA) is a large actin-binding protein that crosslinks actin filaments into orthogonal networks, which provide structural integrity to the cell.

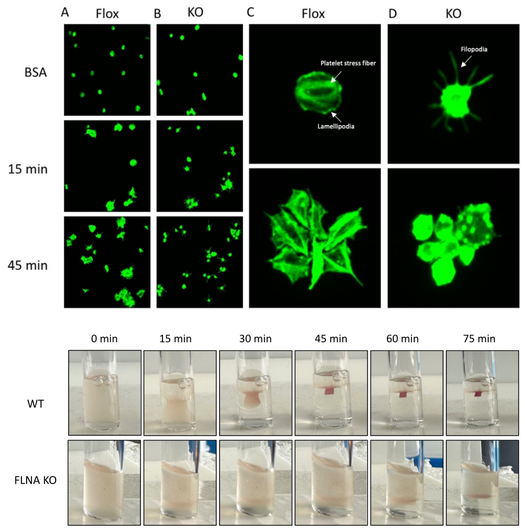


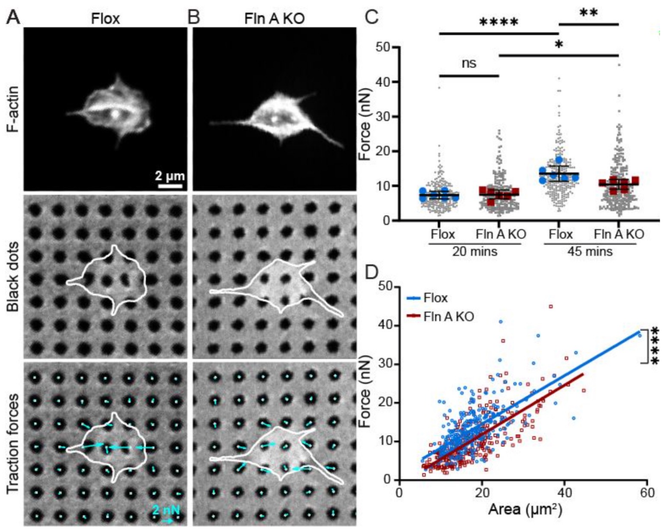
FLNA can bind over 50 proteins including cell surface receptors and signaling effectors. Therefore, FLNA serves as a major signaling scaffold regulating diverse signaling events.

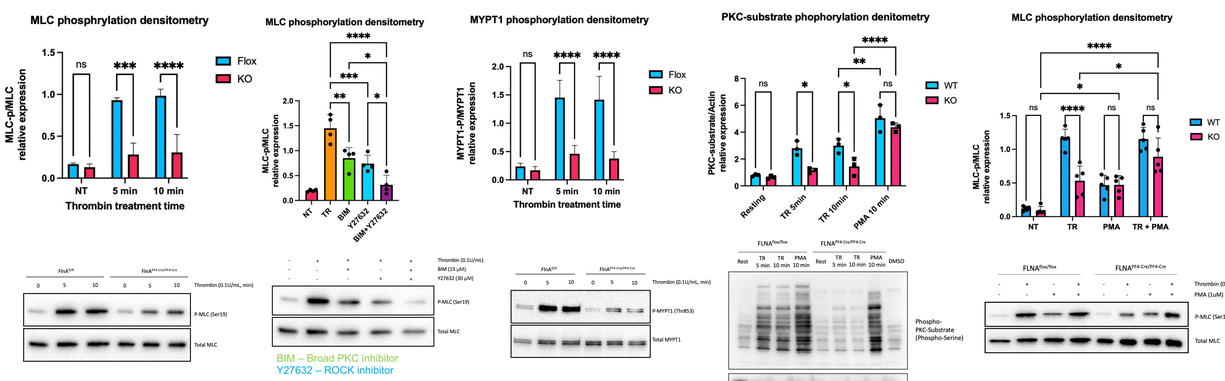
Aim

To investigate the role of filamin A in the regulation of myosin light chain phosphorylation in platelets. The research approach required the use of mice with a megakaryocyte/platelet-specific deletion of filamin A.

Results





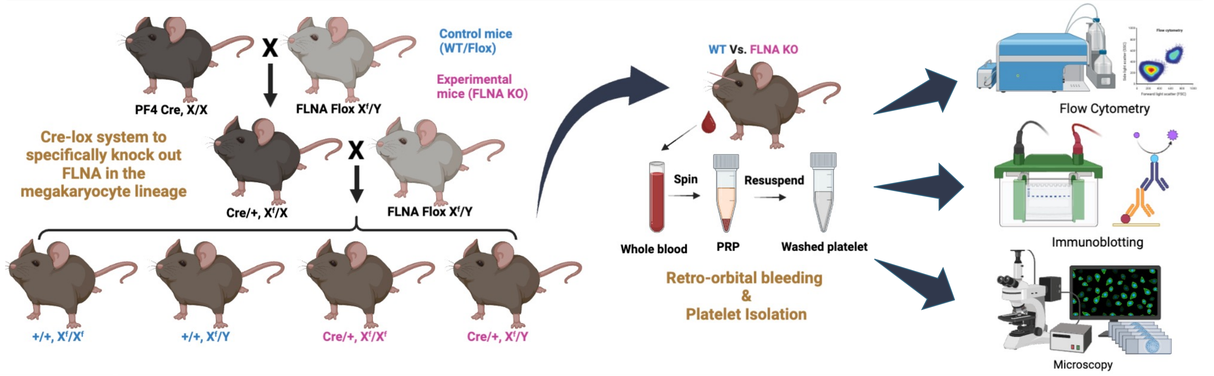


FLNA-KO platelets phenotypes

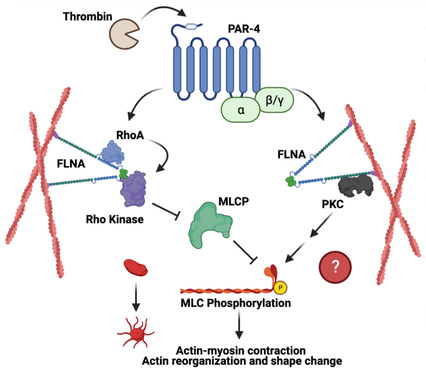
- Have spreading morphological defects on fibrinogen-covered glass
- Are unable to fully retract a fibrin clot with thrombin stimulation
- Have lower contraction force on fibrinogen-covered surface

- FLNA-KO platelets have lower p-MLC upon thrombin activation
- ROCK and PKC independently regulate MLC phosphorylation
- FLNA-KO platelets have lower phosphorylation in both MLCP & PKC downstream effectors
- PMA (PKC activator) and thrombin rescued p-MLC

Methods



Conclusions



Our data indicate that FLNA is an essential regulator of platelet shape change and contraction in response to thrombin signaling. FLNA likely regulates this signaling event by acting as an upstream scaffold to bridge the signaling gap between the plasma membrane and downstream effectors including Rho, ROCK, and PKC, which regulate MLC and initiate actomyosin contraction.

Acknowledgements

Supported by a Canadian Institutes of Health Research (CIHR) Project Grant and a Michael Smith Health Research BC Scholar Award (to HK). FH acknowledges graduate student support from CANVECTOR, and the UBC Centre for Blood Research (CBR) Graduate Award program. The authors report no conflicts of interest

THE UNIVERSITY OF BRITISH COLUMBIA

THE CENTRE FOR BLOOD RESEARCH

Contact

Felix Hong

Email: felix04@mail.ubc.ca

Department of Biochemistry and Molecular Biology, Faculty of Medicine