

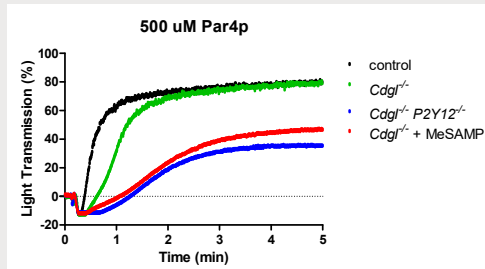
IMPLICATION OF RAPGEF2 IN A NOVEL PKC-MEDIATED PATHWAY OF PLATELET RAP1 ACTIVATION

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Introduction

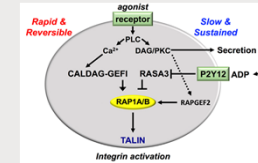
Platelets are small nucleate blood cells that are critical for hemostatic clot formation following injury of a blood vessel. Adhesion of platelets to the extracellular matrix and platelet-platelet aggregation are highly dependent on the inside-out activation of the membrane-embedded integrin α IIb β 3 by the cytoskeletal protein TALIN^{1,2}. Recruitment and activation of TALIN is mediated by the small GTPase RAP1, a 'molecular switch' that cycles between a GDP-bound inactive state and a GTP-bound active state^{1,3}. Activation of GTPases via GTP-loading is catalyzed by proteins called guanine nucleotide exchange factors (GEFs); the inactivation of GTPases is mediated by GTPase activating proteins (GAPs). The Bergmeier Lab has previously proposed a two-pathway model of platelet integrin activation in which the calcium-binding GEF CalDAG-GEFI is responsible for a rapid and reversible activation of RAP1, and engagement of the P2Y12 G-protein coupled receptor (GPCR) results in inhibition of the GAP RASA3, permitting sustained activation of RAP1^{1,2,3,4,5}. However, further studies have shown that high doses of platelet agonists are capable of inducing aggregation via a PKC-mediated pathway in platelets deficient in both CalDAG-GEFI and P2Y12, suggesting the existence of an alternative signaling cascade. Recent proteomics studies⁴ in platelets have shown significant expression of RAPGEF2, another GEF with RAP specificity. As such, this work investigates the involvement of RAPGEF2 in a third pathway of platelet RAP1 activation.

Genetic and Pharmacological Inhibition of CalDAG-GEFI- and P2Y12-Mediated Rap1 Activation

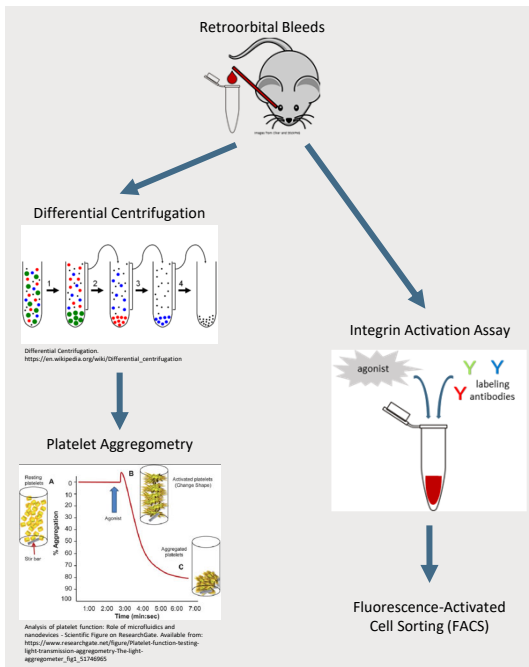


Discussion

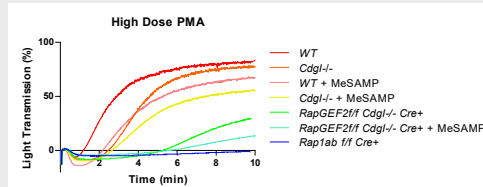
Platelets deficient in both RAPGEF2 and CalDAG-GEFI were found to have impaired aggregation and integrin activation in comparison to platelets lacking only CalDAG-GEFI. Furthermore, P2Y12-inhibited platelets deficient in CalDAG-GEFI were capable of significant integrin activation and aggregation. These findings implicate RAPGEF2 as a GEF in an alternative PKC-mediated pathway of activation of the small GTPase RAP1 (and subsequent integrin-dependent platelet-platelet aggregation). It is also noted that platelets deficient in both GEFs still exhibit higher levels of aggregation and integrin activation than platelets lacking RAP1, suggesting that there may be yet another RAP1-specific GEF in platelets. The previously described proteomics studies also identified RAPGEF6 as another GEF with RAP specificity expressed in platelets⁴; therefore, future studies will investigate a potential role for RAPGEF6 in RAP1 activation.



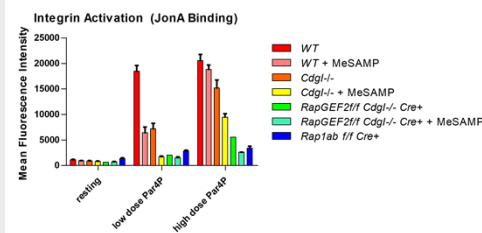
Materials and Methods



Platelets Deficient in RAPGEF2 Exhibit Impaired Aggregation in Response to PKC Agonists



Platelets Deficient in RAPGEF2 Exhibit Lower Levels of Integrin Activation



References and Acknowledgements

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