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PLATELETS & THROMBOPOIESIS

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Applying the brakes to platelet activation

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In this issue of *Blood*, Gegenbauer and colleagues provide a new insight into the regulatory mechanisms that allow platelets to produce an optimal response to vascular injury.[1] "Optimal responsiveness" in the context of hemostasis means preventing circulating platelets from activating needlessly, allowing them to respond quickly when necessary and limiting platelet activation to avoid excessive platelet accumulation. How all of this is achieved is still not fully understood, but the new study nicely pulls together older work on the classic restrainers of platelet activation (endothelium-derived PGI_2 and NO) with emerging ideas about the role of RGS proteins (regulators of G protein signaling).

To put all of this in context, it is worth recalling that with the exception of collagen, most platelet agonists activate G protein–coupled receptors (GPCRs). This includes thrombin, ADP, thromboxane A_2 (TxA_2), and epinephrine. GPCRs signal primarily by activating heterotrimeric ($\alpha\beta\gamma$) GTP-binding proteins. G protein α subunits (G_α) bind GDP in their resting state. Receptor activation forces an exchange of GTP for GDP, partially dissociating the heterotrimer and allowing signaling downstream of both Gα and $G_{\beta\gamma}$. G protein–dependent signaling terminates when G_α hydrolyzes bound GTP back to GDP.

RGS proteins enter this story because of their ability to hasten the termination of G protein signaling by accelerating the hydrolysis of GTP bound to G_α. There are at least 37 RGS family members in the human genome. Although uncertainty exists about the number expressed in platelets, two (RGS10 and RGS18) are readily detectable on Western blots[1,2] and by quantitative transcript analysis.[3] They are relatively small proteins, consisting primarily of a core "RGS" domain with short N- and C-terminal extensions.

The best evidence that RGS proteins act as regulators in platelets comes from experiments with mice carrying a serine to glycine substitution at position 184 in the α subunit of G_{i2}, the G protein that couples to platelet $P2Y_{12}$ receptors for ADP.[4] $P2Y_{12}$ receptors are best known in the clinical setting for being the target of antiplatelet agents such as clopidogrel and prasugrel. The $G_{i2\alpha}$(G184S) substitution blocks interactions with RGS proteins as a class without affecting the ability of G_{i2} to couple receptors to signaling pathways. $G_{i2\alpha}$(+/G184S) platelets show increased responsiveness to agonists in vitro and increased accumulation after vascular injury in vivo. In other words, loss of $G_{i2\alpha}$:RGS interactions produces a gain of function, suggesting that the normal role of RGS proteins is to limit platelet activation.

Conceptually, RGS proteins provide both a solution to questions about managing platelet responsiveness and the following conundrum. At a molecular level, how can RGS proteins limit platelet activation and still allow it to occur when needed? Part of the answer comes from combining the elegant work by Gegenbauer and colleagues with observations from our laboratory.[2] Building on an earlier study by Garcia and colleagues,[5] Gegenbauer et al show that RGS18 in platelets is phosphorylated on Serine 49 and Serine 218, and that phosphorylation of Ser49 (and possibly Ser218) increases with platelet activation. Neither serine is within the RGS18 RGS domain. However, phosphorylation allows RGS18 to bind to 14-3-3γ, one of a family of phosphoserine-binding scaffold proteins expressed in platelets. Serine phosphorylation of RGS proteins and the subsequent binding of 14-3-3 proteins inhibits their ability to turn off G protein–dependent signaling.[6] Tying RGS18 phosphorylation to the inhibition of platelets by PGI_2 and NO, Gegenbauer et al show that PGI_2 (by raising cAMP levels and activating protein kinase A) and NO (by raising cGMP levels and activating protein kinase G) cause phosphorylation of Ser216 in RGS18, an event that displaces 14-3-3γ from RGS18 and increases its ability to terminate G protein–dependent signaling (see figure for PGI_2 and G_q-dependent signaling).[1,7] The kinase that phosphorylates RGS18 on Ser49 and Ser218 has yet to be identified, although one candidate is protein kinase C, which is activated in platelets downstream of thrombin, TxA_2 and ADP receptors coupled to G_q.[8]

As it turns out, 14-3-3γ is not the only scaffold protein in platelets that can bind RGS proteins. Platelets express the neuronal protein spinophilin (SPL), which in resting platelets forms a complex with the tyrosine phosphatase, SHP-1, that can bind to either RGS18 or RGS10, forming a heterotrimer.[2] Within this complex, SPL is constitutively phosphorylated on two tyrosine residues, one of which (Y398) supports the binding of SHP-1 (see figure). Platelet activation by thrombin or TxA_2 (but not ADP) activates SHP-1 by causing phosphorylation of Y536, triggering dephosphorylation of SPL and dissociation of the SPL/RGS/SHP-1 complex. Knocking out the gene encoding spinophilin in mice (which should increase the amount of free RGS protein) causes a loss of function, while blocking activation of SHP-1 (thereby locking up RGS proteins in the SPL/RGS/SHP-1 complex) causes a gain of function. This suggests that binding to spinophilin inhibits RGS10 and RGS18 activity.[2]

Placed in the context of "optimal platelet activation," the model shown in the figure proposes that, rather than competing for RGS proteins, spinophilin and 14-3-3γ engage in a handoff during platelet activation that restricts the amount of free RGS18. Specifically, the model suggests that in quiescent platelets circulating in an environment in which they are regularly exposed to PGI_2 and NO, some of the RGS18 is bound to spinophilin at any given time and held in an essentially inactive state (box 1 in figure), while some is free and potentially phosphorylated on Ser216. Phosphorylation of RGS18 on Ser216 prevents binding to 14-3-3γ and preserves (or even promotes) RGS18 activity of the free protein (box 2 in figure). By being available to inactivate G_q or G_i, this provides a buffer against unwarranted platelet activation.

Once platelets encounter an agonist, gradual dissociation of the SPL/RGS/SHP-1 complex occurs, taking 30 to 45 seconds to reach completion.[2] As the complex decays, additional RGS18 presumably becomes available for suppressing G protein–dependent signaling, but its ability to do so is limited by subsequent S49/S218 phosphorylation and binding to 14-3-3γ (box 3 in figure). If this model is correct, then the reports that we have summarized here (all of which have appeared

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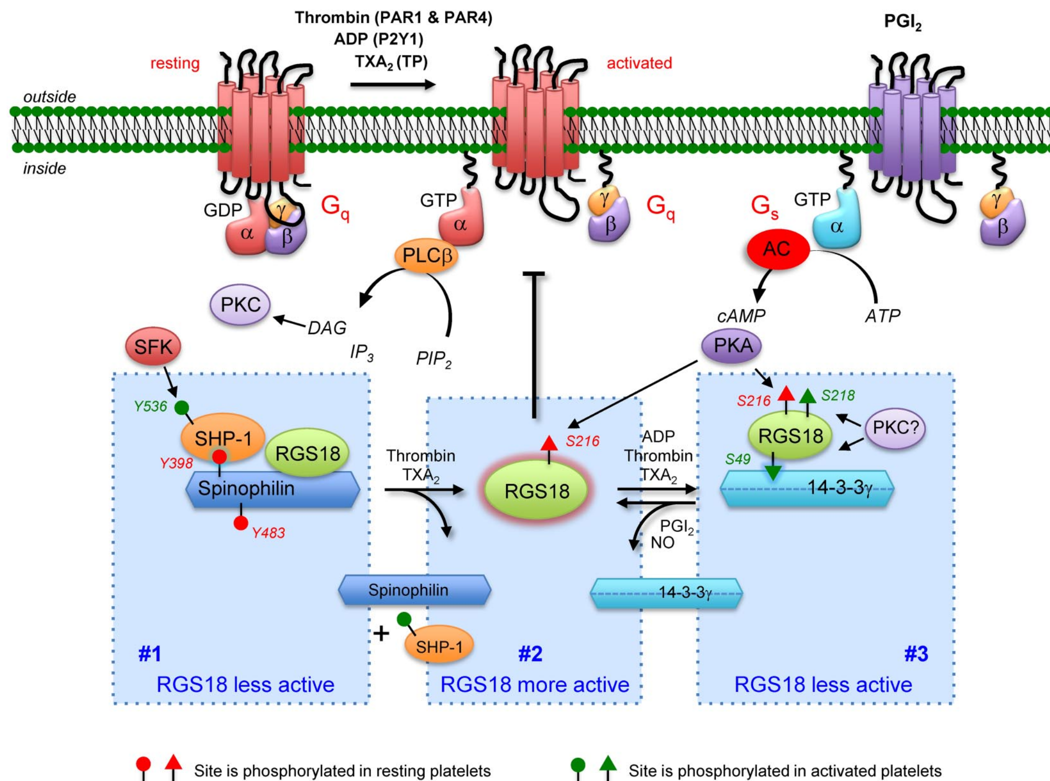

The RGS handoff in platelets. RGS proteins serve to limit platelet activation by limiting the duration of G protein–dependent signaling, illustrated here for G_q-mediated activation of phospholipase C leading to the production of IP_3 and diacylglycerol from phosphatidylinositol-4,5-bisphosphate (PIP_2), the second messengers that increase the Ca^{++} concentration in platelets and activate protein kinase C. This model suggests that the amount of free RGS18 (and by implication RGS10) in resting platelets is determined in part by binding to spinophilin and by protein kinase A (PKA)– or protein kinase G (PKG, not shown)–mediated phosphorylation of Ser216. In activated platelets, release of RGS18 from spinophilin/SHP-1 complex promotes inhibition of signaling, but subsequent phosphorylation of Ser49 and Ser218 allows sequestration of the RGS protein by a 14-3-3γ unless Ser216 phosphorylation is present. Not all aspects of this model have been established, but it provides testable hypotheses for tightly regulating the availability of free RGS proteins in platelets. AC indicates adenylyl cyclase; NO, nitric oxide; PGI_2, prostaglandin I_2; SFK, Src family kinase; PKC, protein kinase C; and TxA_2, thromboxane A_2. The authors thank Dr Timothy J. Stalker (Perelman School of Medicine, University of Pennsylvania) for assistance with the figure.

in *Blood*) suggest that free RGS18 availability is closely controlled in both resting and activated platelets.

The model shown in the figure remains to be fully tested and may break in the process. Unanswered questions include (1) the applicability to RGS10 of some observations that are currently limited to RGS18, (2) the impact of Ser49, Ser216 and Ser218 phosphorylation on the binding of RGS18 to spinophilin, and (3) the determination of whether spinophilin and 14-3-3γ are segregated within the cells so that local concentrations of free RGS proteins vary spatially as well as temporally. What can be said at this point with some degree of assurance is that if RGS proteins are part of the braking system that limits platelet activation, then spinophilin and 14-3-3γ working in tandem keep the brakes from locking up.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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Comment on Sorvillo et al, page 3828

ADAMTS13 meets MR, then what?

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In this issue of *Blood*, Sorvillo and colleagues demonstrate that a macrophage mannose receptor (MR) on antigen-presenting cells facilitates uptake of ADAMTS13 antigen.[1] The findings provide the first hint on how ADAMTS13 antigen may be endocytosed, processed, and presented to immune T cells.

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