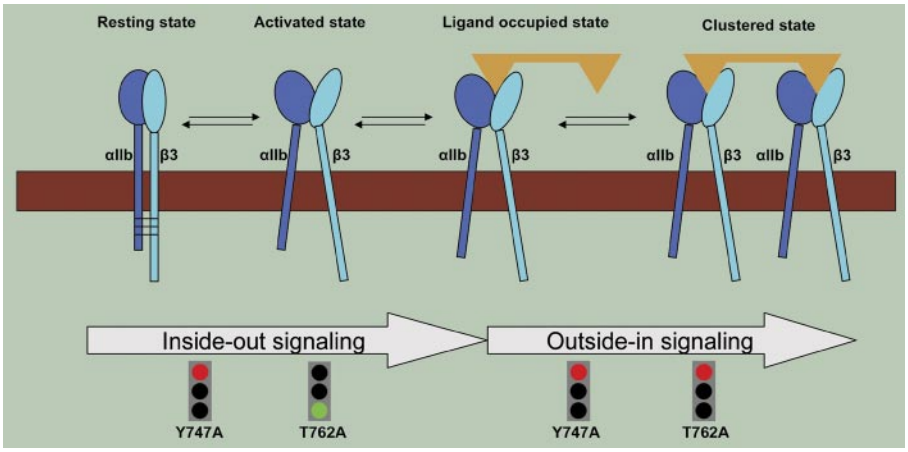

Distinction between inside-out and outside-in signaling across integrin αIIbβ3.

stabilizes the aggregate and supports responses, including platelet spreading and clot retraction. Hence, inside-out and outside-in signaling constitute the 2 elements of the bidirectional signaling across αIIbβ3 (see figure) and represent potential antithrombotic therapeutic targets, and their dissection is indispensable to our understanding of platelet biology.

The inside-out and outside-in signaling pathways ultimately trace to cytoplasmic tails (CTs) of αIIbβ3, which serve as receivers and transmitters of bidirectional signaling within the receptor. While the CTs of the αIIb and β3 subunits are short, they are structurally complex and interact with numerous binding partners. Ordinarily, the contribution of specific structural elements or individual amino acids can be dissected by straightforward mutagenesis. However, platelets are anucleated; and alternative approaches have had to be invoked to examine inside-out and outside-in signaling. These approaches have included the following: (1) expression of αIIbβ3 in heterologous cells; (2) transfection of the primary megakaryocytes; (3) introduction of membrane-permeable peptides into platelets; and (4) generation of knock-in mice expressing mutant αIIbβ3. Each of these approaches has been used successfully to probe integrin signaling, but each approach has inherent limitations.

By infecting fetal liver cells from β3-deficient mice with retrovirus engineered to encode wild-type β3-subunit and transplanting these cells into irradiated recipients, Zou and colleagues show that these cells can successfully express αIIbβ3 and rescue both inside-out and outside-in signaling in blood platelets. This strategy is then used to introduce specific mutations into the mouse platelets. Inside-out signaling is evaluated by the capacity of the platelets to bind soluble fibrinogen, and outside-in signaling is assessed by platelet spreading. A single point mutation in the midregion of the β3 CT, β3Y747A, prevents restoration of both inside-out and outside-in signaling, while a point mutation at the extreme C-terminus of β3, β3T762A, results in selective loss of outside-in signaling. These results are generally consistent with those derived from the other approaches we outlined and fortify the conclusion that different sites within the β3 CT can mediate different aspects of the bidirectional signaling.

The experimental approach for in vivo expression in platelets described by Zou et al here and by others[1] allows analyses of αIIbβ3 activation in platelets in vivo and has high throughput potential. Fetal liver transplantation could undoubtedly be applied to other platelet proteins provided that a deficient mouse background is available. Low, variable, and unstable expression of the platelet protein may set limitations on this approach. Nonetheless, the Zou et al paper sets the precedent for a new strategy to dissect inside-out and outside-in signaling across αIIbβ3 in vivo.

The authors declare no competing financial interests. ■

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HEMATOPOIESIS

Comment on Raslova et al, page 3225, and comment on Macaulay et al, page 3260

MEGAprofiles provide big insights into platelet function

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This issue of *Blood* contains reports from 2 groups that have used microarray profiling of $CD34^+$-differentiated hematopoietic stem cells to dissect molecular mechanisms regulating megakaryocytopoiesis and/or for identification of functionally novel platelet receptors. Both reports provide new insights into proplatelet formation and platelet function.

Hematologists are intrigued by megakaryocytes (MKs), hematopoietic cells readily distinguishable by their abundant cytoplasm and large size (~ 35-160 μm), nuclear polyploidy (up to 64N), and relative paucity (0.02%-0.05% of total nucleated bone marrow cells). While discovery of the existence and hemostatic functions of blood platelets is generally assigned to Bizzozero based on pioneering observations made during the 1880s, linking megakaryocytes to platelet biogenesis is credited to James Wright in 1906, who made conclusions based on histomorphometric similarities of granular and cytoplasmic content between both cell types.[1] In the subsequent century, considerable insight has been achieved in identifying receptors and signaling pathways that regulate platelet and megakaryocyte biology. Nonetheless, the genetic machinery controlling megakaryocyte transition into the distinctive endomitotic switch that precedes the epiphany of cytoplasmic maturation, membrane demarcation, proplatelet formation, and platelet release remains incompletely

understood. Similarly, the application of genomic technologies for identification of novel, functionally-important platelet genes and proteins remains a high priority.[2]

Raslova and colleagues describe cellular mRNA profiling to specifically dissect genetic changes occurring during an in vitro model of megakaryocyte differentiation and ploidization. Cells were flow-sorted by modal ploidy, and gene changes were compared between 2 cellular subsets: aggregate 2N+4N MKs versus aggregate 8N+16N MKs. Of interest, transcript changes were limited to approximately 350 genes across all the subsets, 106 of which were consistently down-regulated and 248 of which were consistently up-regulated between the 2 groups. Further analysis highlighted additional differences between the up-regulated and down-regulated subsets; specifically, members of the latter subset not infrequently (24/105) corresponded to genes involved in DNA replication (arrest) and recombination repair, while a majority of the former corresponded to genes important to platelet biogenesis, viability, and function (ie, actin and microtubule cytoskeleton, glycoproteins, and signaling/transport proteins). It is important to note, however, that when the gene subsets are carefully analyzed by gene ontology functional classification, considerable overlap exists between these 2 groups, confounding detailed interpretations. Nonetheless, the data do support a role of ploidization in modulating gene expression, although a direct, regulatory role in platelet biogenesis remains speculative.

The study by Macaulay and colleagues adapts a nearly-identical in vitro strategy of MK differentiation, coupled with a bioinformatic strategy to specifically identify novel transmembrane domain–containing MK receptor proteins. An initial gene list of 151 transcripts was assembled using paired, comparative expression profiling with $CD34^+$-differentiated erythroblasts, and the list further pared using strict criteria to identify putative, functionally-relevant platelet proteins. Five of 8 highly-selected genes were characterized by transcript and protein expression studies, 3 of which were shown to be platelet restricted *(G6b, G6f,* and *LRRC32),* and another of which *(SUCNR1)* encoded the G protein–coupled succinate receptor.[3] More detailed functional studies established that succinate (a key component of the citric acid cycle) exhibited costimulatory effects on platelet aggregation induced by various platelet agonists (adenosine diphosphate, thrombin receptor activating peptide, and a glycoprotein VI–specific collagen peptide). The latter functional data are especially insightful in that they identify a novel, cocoupling signal transduction pathway in platelets, while opening new avenues of research linked to platelet hyperreactivity.[4]

While both study designs overlap in their initial in vitro differentiation strategies using megakaryocytes, the conclusions, future directions, and ability to compare data sets are distinct and limited. One restriction inherent in cross-experimental microarray data-sharing is the disparate platforms used among investigators, well exemplified in these studies, that used nonoverlapping oligonucleotide or cDNA probe sets for their analyses.[5,6] This limitation does not minimize results, although it emphasizes the importance of validation strategies of transcript differences initially identified by microarray. The identification of a costimulatory succinate receptor on platelets represents a discrete end product of integrated MK transcriptomic studies, coupled with a concrete hypothesis and sophisticated experimental design to characterize novel functional receptors. Likewise, the application of microarray technology to dissect MK ploidization is highly novel, and although the results are less focused in scope, they are likely to yield broader implications in the foreseeable future. Finally, such unique data sets open up exciting opportunities for sophisticated data mining likely to provide unexpected insights into molecular mechanisms of MK and platelet function.[7]

The author declares no competing financial interests. ■

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IMMUNOBIOLOGY

Comment on León-Ponte et al, page 3139

Serotonin: a real blast for T cells

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Mouse T lymphocytes unexpectedly produce the classic neurotransmitter serotonin, which—upon binding the constitutively expressed 5-HT_7 G_s-coupled receptor-subtype—signals ERK1/2 phosphorylation and NFκB activation to boost their stimulation.

Serotonin/5-hydroxytryptamine (5-HT), often perceived as the brain's "happy chemical," is—increasingly—assuming a prominent role in immune regulation. In fact, this "neurotransmitter" is primarily a product of the periphery, with gut enterochromaffin cells the principal factories. Intestinal lymphocytes could certainly be exposed directly to their output, though platelets are typically proffered as the source of the monoamine within the immune system: these 5-HT–loaded reservoirs delivering their potent cargo at sights of inflammation and immunologic reactivity.[1,2]

In this issue of *Blood,* León-Ponte and colleagues not only consolidate serotonin's importance to T-cell activation but also tender a paradigm whereby the monoamine is provided by the immune cell itself. Critical to any outcome from 5-HT exposure—whether the source be autocrine or paracrine—is a capacity for target cells to sense the monoamine. This could be via the serotonin transporter (SERT) or one or more of 14 receptor subtypes (13 in