The mechanism by which plasmin contributes to fibrin removal in vivo is intriguing. Clearly, initial extracellular production of fibrin degradation products by plasmin is necessary. In addition, plasmin activity promotes phagocytosis by both macrophages[4] and dendritic cells.[5] This involves both recognition of prey and direct effects on engulfment of prey, events which are regulated by plasmin-mediated activation of intracellular signaling pathways that regulate expression of major genes involved in phagocytosis.[4] There may be an analogous mechanism for cellular fibrin uptake. It is also possible that plasminogen may bind to fibrin and bridge fibrin to the cell surface via cellular plasminogen receptors. Future studies to examine fibrin endocytosis in plasminogen receptor-deficient mice would be informative.[6] In addition, mice deficient in thrombin-activatable fibrinolysis inhibitor (that removes plasmin[ogen] recognition sites from fibrin and from cell surfaces) may show enhanced fibrin uptake and could also shed light on these issues.

The results of Motley and colleagues provide a strong rationale for future studies to investigate the effect of deletion of CCR2-positive macrophages in disease models with a prominent extravascular fibrin deposition component. At the same time, the role of CCR2-positive macrophage endocytosis in intravascular fibrin clearance should be evaluated as this may have a profound effect on disease progression. For example, intravascular fibrin accumulation as well as extravascular fibrin accumulation are contributing features of Alzheimer disease[7] and impaired recruitment of the CCR2-positive macrophage population is reported in Alzheimer disease patients (reviewed in Naert and Rivest[8]).

Thus, therapeutically increasing the number and/or activity of CCR2-positive macrophages may be an attractive therapeutic strategy for a variety of disease processes involving both extravascular and intravascular fibrin deposition.

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

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● ● ● ● **PLATELETS AND THROMBOPOIESIS**

Comment on Alhasan et al, page e1

Platelets enrich their transcriptome circle

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In this issue of *Blood*, Alhasan et al[1] report the existence of circular RNAs (circRNAs) in circulating human platelets, thereby revealing yet another facet of their already diverse transcriptome.

Platelets are short-lived (~8-11 days), translationally competent, and capable of premature messenger RNA (pre-mRNA) splicing into mature mRNAs.[2] They do this using a process expected to generate lariats, which are formed of normally spliced out, circularized introns. Alhasan et al[1] now report that platelet mRNAs may also undergo exon back-splicing to generate circRNAs (see figure). To detect circRNAs, the authors analyzed RNA sequencing datasets from human platelets and searched for "back-splice" exon junctions, inconsistent with the underlying genomic DNA, by using
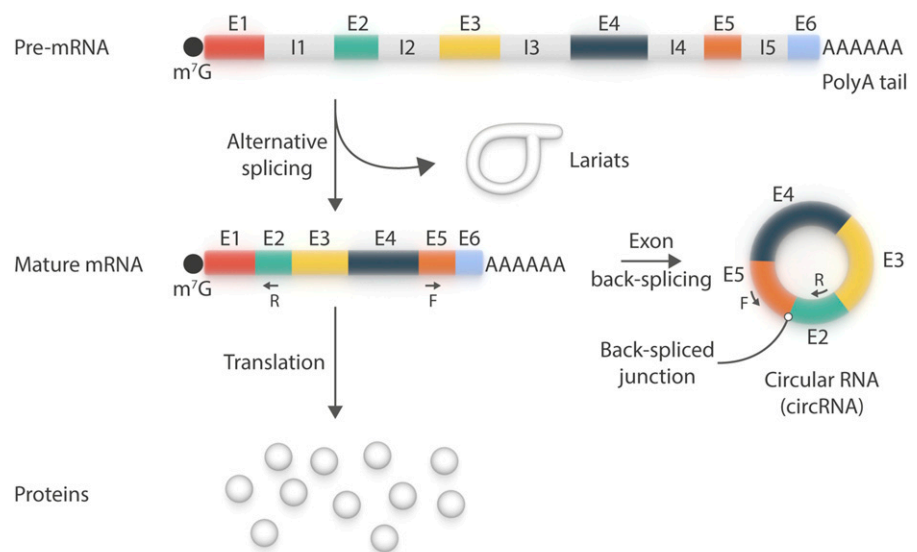
circRNA is a new member of the platelet transcriptome. circRNA species primarily use canonical splice sites and may be amplified by reverse transcriptase polymerase chain reaction using pairs of oligonucleotides (F+R) designed to be divergent on the linear messenger RNA (mRNA) species, but convergent on circRNAs. The existence of lariats in platelets remains to be confirmed. E, exon; F, forward; I, intron; m7G, 7-methylguanylate cap; R, reverse. Professional illustration by Somersault18:24.

specialized bioinformatic tools. They then validated some of the circular reads by reverse transcriptase polymerase chain reaction. Next, they enriched the circRNA content of their samples by selectively digesting linear RNA with ribonuclease R, to which circRNAs are resistant and that has been used to define them.

Using this approach, the authors observed marked circRNA enrichment in platelets (and erythrocytes) relative to nucleated cells, mainly as a result of differential decay of linear RNAs. Conversely, circRNA enrichment in platelets vs cultured megakaryocytes, in the absence of de novo gene transcription, is consistent with circRNAs being produced in platelets rather than being inherited from their precursor cells. Two circRNA junctions (E4-E2 and E5-E2; E, exon) of one gene, *MAN1A2*, are even more abundant in platelets (and erythrocytes) than the linear mRNA. Based on their findings, the authors proposed that mRNA translation in platelets occurs against the backdrop of a rapidly degrading transcriptome, which would explain the relatively weak association between the human platelet transcriptome and proteome.[3]

At the interface of the platelet transcriptome and proteome is an unusually abundant and diverse array of microRNAs (miRNAs),[4] which are key regulators of gene expression. Considering that (1) platelet mRNAs harbor 3′ untranslated regions (3′UTRs) twice as long as those of nucleated cells (1047 vs 492 nucleotide),[5] (2) 3′UTRs are known to contain microRNA (miRNA) binding sites, and (3) 2 circRNAs from nucleated cells act as miRNA sponges,[6] it would be interesting to determine whether platelet circRNAs are enriched in 3′UTR exons and miRNA binding sites, because this could explain the relative abundance of circRNAs and miRNAs in platelets.

To gain further insights into the biology of circRNAs in platelets, we may want to know if platelets contain RNA binding protein Quaking, an alternative splicing factor regulating the production of more than one-third of abundant circRNAs during human epithelial-mesenchymal transition.[7] The biological relevance of platelet circRNAs, if any, remains unclear: do they play a role inside of platelets, or are they simply byproducts of platelet mRNA degradation? The latter assumption does not necessarily preclude them

from mediating biological effects outside of platelets. Indeed, circRNAs may still play a role in other cells, as shown previously for mRNAs and miRNAs,[8] provided that they are expelled within exosomes or larger microparticles, under basal conditions or upon activation, which remains to be seen. Because exon-intron circRNAs regulate transcription in nucleated cells,[9] it is tempting to speculate that circRNAs that accumulate in platelets may end up regulating gene expression and function of other recipient cells of the circulatory system.

The possible clinical implications of platelet circRNAs may soon be studied and revealed, because we will want to know if circRNA abundance and/or sequences vary depending on the race, gender, age, or health status of the subjects. Are circRNAs present and conserved in the platelets of small animal models, such as the mouse? If so, very informative in vivo studies could be performed. The suggestion that platelet lifespan may be determined by translational competence support a possible relationship between circRNA levels and platelet half-life or aging. Could circRNAs be used as biomarkers of platelet-related diseases, or signal shorter platelet lifespan or premature platelet aging? Could these conditions be unveiled simply by measuring the level of specific circRNAs by quantitative PCR?

Produced upon cytoplasmic fragmentation of bone marrow megakaryocytes, circulating platelets contain an impressive array of RNA species (messenger RNA, transfer RNA, microRNA, long noncoding RNA)[10] that now includes circRNAs. These recently described RNA species were identified by Alhasan et al[1] among gigabytes of platelet RNA sequencing data simply by using publicly available

bioinformatic tools, as one would experience by looking at the full moon with a telescope instead of binoculars. Has anyone thought that, by analyzing the transcriptome of an anucleate element of the blood, we would go round (and round) in circles?

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● **THROMBOSIS AND HEMOSTASIS**

The heads and the tails of malaria and VWF

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In this issue of *Blood*, O'Regan et al have extended our understanding of von Willebrand factor (VWF) in the pathogenesis of malaria.[1] According to the World Health Organization (http://www.who.int/gho/malaria/en/), malaria affects 3.2 billion people in 97 countries with 198 million cases having occurred in 2013,