for inhibition of apoptosis. *Blood*. 2015;125(25):3896-3904.

6. Hills AG, Forsham PH, Finch CA. Changes in circulating leukocytes induced by the administration of pituitary adrenocorticotrophic hormone in man. *Blood*. 1948;3(7):755-768.

7. Nagase H, Miyamasu M, Yamaguchi M, et al. Glucocorticoids preferentially upregulate functional CXCR4 expression in eosinophils. *J Allergy Clin Immunol*. 2000;106(6):1132-1139.

8. Cronstein BN, Kimmel SC, Levin RI, Martiniuk F, Weissmann G. A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. *Proc Natl Acad Sci USA*. 1992;89(21):9991-9995.

9. Khoury P, Stokes K, Gadkari M, et al. Glucocorticoid-induced eosinopenia in humans can be linked to early transcriptional events. *Allergy*. 2018;73(10):2076-2079.

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PLATELETS AND THROMBOPOIESIS

Comment on Liang et al, page 2679

Breaking barriers: Quebec platelet disorder

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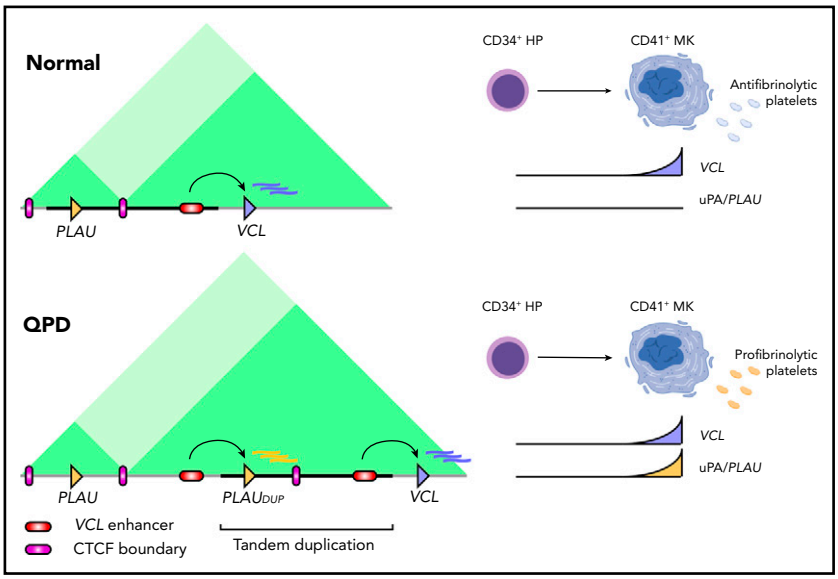
In this issue of *Blood*, Liang et al illustrate the functional mechanism that determines a megakaryocyte-specific increase in PLAU expression and consequent platelet-dependent fibrinolysis in Quebec platelet disorder (QPD).[1]

A decade after the discovery that QPD is caused by a tandem duplication,[2] Liang et al demonstrate that this causes the additional copy of PLAU to be on the opposite side of a CTCF genomic boundary. As a consequence, the extra copy is not insulated from the effects of one of the transcriptional enhancers of the VCL gene, resulting in a high level of transcription (see figure). This phenomenon is known as "enhancer hijacking" and was observed in Burkitt lymphoma almost 40 years ago.[3] In the intervening years, other examples have been discovered, but our ability to diagnose the consequences of new occurrences remains limited and time consuming.



Schematic representation of the interactions across the PLAU locus in nonaffected individuals (normal, top) and in individuals affected by Quebec platelet disorder (QPD, bottom). The different genomic compartments are represented by the pink (sub-TAD$_{PLAU}$) and green box (sub-TAD$_{VCL}$). The duplicated segments are represented in blue and orange. TAD, topologically associating domain. See Figure 6 in the article by Liang et al that begins on page 2679.

The advent of next-generation sequencing allowed for parallel testing of known causative variants in protein coding genes via gene panels or whole-exome sequencing,[3,4] with a diagnostic rate of ~50% in selected cases of platelet and bleeding disorders. Whole-genome sequencing has begun to show the role of the noncoding portion of the genome in rare diseases,[5] but it has also put our as yet incomplete understanding of the regulatory grammar in the human genome under the spotlight. Moreover, the exponentially increasing amount of available whole-genome sequencing has brought to light a large numbers of rare variants of unknown significance, including insertions, deletions, and rearrangements.[5]

Efforts to catalog the regulatory elements of blood cells[6,7] have been largely successful in determining which elements in the genome are marked as active and in which cell type. These findings, coupled with DNA long-range interaction maps,[8] have allowed the creation of references for each gene and its regulatory landscape. These have been extremely useful for pinpointing causal genetic variants,[5] but it is still difficult to draw general conclusions that can be applied each time a genetic variant is suspected to be responsible for a disease. Our understanding of buffering and compensatory mechanisms between regulatory sequences of gene expression thresholds and of genome compartmentalization is still at a stage at which multiple lines of evidence are required to unequivocally assign causality to noncoding genetic variants and to determine the mechanism of action. Liang et al used a robust study design involving a combination of recall by genotype, publicly available data, an animal model, and ex vivo molecular biology experiments. Altogether, the evidence demonstrated that the tandem duplication previously associated with QPD places the additional copy of PLAU in front of a strong transcriptional enhancer, which under normal conditions, drives the expression of the VCL gene to high levels but is restricted from accessing PLAU because of the DNA local 3-dimensional conformation and the boundary between the two. The VCL enhancer is also part of the duplication; therefore, VCL expression remains unaffected and the additional CTCF site present does not create

additional DNA loops. In summary, this study nicely illustrates the necessary steps to ascertain the causal role of noncoding genetic variants and their mechanism of action in the setting of hematologic rare diseases when there are only a handful of affected individuals worldwide.

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REFERENCES

1. Liang M, Soomro A, Tasneem S, et al. Enhancer-gene rewiring in the pathogenesis of Quebec platelet disorder. *Blood*. 2020;136(23):2679-2690.
2. Johnson B, Lowe GC, Futterer J, et al; UK GAPP Study Group. Whole exome sequencing identifies genetic variants in inherited thrombocytopenia with secondary qualitative function defects. *Haematologica*. 2016;101(10):1170-1179.
3. Dalla-Favera R, Bregni M, Erikson J, Patterson D, Gallo RC, Croce CM. Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc Natl Acad Sci U S A*. 1982;79(24):7824-7827.
4. Downes K, Megy K, Duarte D, et al; NIHR BioResource. Diagnostic high-throughput sequencing of 2396 patients with bleeding, thrombotic, and platelet disorders. *Blood*. 2019;134(23):2082-2091.
5. Turro E, Astle WJ, Megy K, et al; NIHR BioResource for the 100,000 Genomes Project. Whole-genome sequencing of patients with rare diseases in a national health system. *Nature*. 2020;583(7814):96-102.
6. Stunnenberg HG, Hirst M; International Human Epigenome Consortium. The International Human Epigenome Consortium: A Blueprint for Scientific Collaboration and Discovery. *Cell*. 2016;167(5):1145-1149.
7. Kundaje A, Meuleman W, et al; Roadmap Epigenomics Consortium. Integrative analysis of 111 reference human epigenomes. *Nature*. 2015;518(7539):317-330.
8. Javierre BM, Burren OS, Wilder SP, et al. Lineage-specific genome architecture links enhancers and non-coding disease variants to target gene promoters. *Cell*. 2016;167(5):1369-1384.e19.

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Li et al, page 2691

Iron on the move: mobilizing liver iron via NCOA4

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In this issue of *Blood*, Li et al[1] have demonstrated that NCOA4, the autophagic receptor for ferritin, is necessary for the mobilization of liver iron stores during stress erythropoiesis in mice.

Acute blood loss, whether through hemorrhage, hemolysis, or phlebotomy, triggers erythropoiesis. Expansion of developing erythroid precursors necessitates mobilization of stored iron to accommodate increased synthesis of heme in the erythron. Where does all this iron come from? The short answer is ferritin. Although dietary iron absorption is increased during stress erythropoiesis, the most rapidly available source of iron is in this ubiquitous iron storage protein. Iron deposition into ferritin and mobilization out of ferritin are tightly controlled in mammals, and multiple modes of regulation affect both the "ins" and the "outs." In 2014, nuclear receptor coactivator 4 (NCOA4) was identified as an autophagic receptor for ferritin, with a capacity to direct ferritin into autophagosomes destined for destruction in the lysosome.[2,3] Here, Li and others show that NCOA4 is required to mobilize the iron stored in hepatocyte ferritin so that it may be used for stress erythropoiesis. Their work also suggests a new mode of iron-dependent transcriptional regulation for NCOA4 that involves the hypoxia-inducible factors 1 and 2.

In mammals, cytosolic ferritin is a symmetrical oligomeric complex, composed of 24 subunits of H- and L-chains, that form a hollow sphere into which iron is deposited.[4] In cells, the amount of iron deposited into ferritin is largely proportional to the level of ferritin protein synthesized in the cytosol. The major means of controlling synthesis is through translational repression via the iron-regulatory proteins 1 and 2.[5] When cells are iron depleted, the iron-regulatory proteins accumulate in their RNA-binding form, bind to the 5′ end of ferritin transcripts, and block translation of the ferritin messenger RNA (mRNA). Under conditions of iron excess, these same regulatory proteins lose their RNA-binding activity and ferritin mRNAs are actively translated. Thus, cells can expand their iron storage in ferritin when intracellular iron is abundant.

Cells also express cytosolic iron-binding proteins, termed iron chaperones, that deliver iron to ferritin through an iron-mediated protein–protein interaction.[6] The major iron chaperone in mammalian cells is poly(rC)-binding protein 1 (PCBP1). PCBP1 is a multifunctional adapter protein that can bind both iron–glutathione complexes[7] and cytosine-rich nucleic acids at separate sites on the protein. In developing erythroid cells, PCBP1 promotes α- and β-globin transcript stability through its RNA-binding activity. It also facilitates efficient iron delivery to ferritin through its iron chaperone activity. In both in vitro and in vivo models of red blood cell development in mice, transient, PCBP1-mediated, storage of iron in ferritin was necessary for efficient heme and hemoglobin synthesis.[8]

Iron is released from ferritin primarily through its degradation in the lysosome. This process occurs continuously at basal levels under conditions of iron balance and is upregulated when cells become iron starved.[4] In cultured cells, ferritin degradation is controlled through the activity of NCOA4. NCOA4 binds to ferritin H-chain and recruits the complex to autophagic membranes. These membranes form autophagosomes that fuse with lysosomal membranes and release their contents into the lysosome for degradation. Lysosomal iron released from ferritin can be transported into the cytosol or mitochondria for use in iron cofactor synthesis or be exported from the cell via ferroportin, the sole iron efflux pump in mammals.

The major tissue for iron storage in mammals is the liver, where hepatocytes accumulate iron within ferritin. In order for ferritin iron within the liver to be used for erythropoiesis, the iron must be mobilized to leave the liver. Li and colleagues have addressed the question of whether NCOA4 in hepatocytes is needed to mobilize liver iron stores for use in erythropoiesis.[1] Using hepatocyte-specific depletion