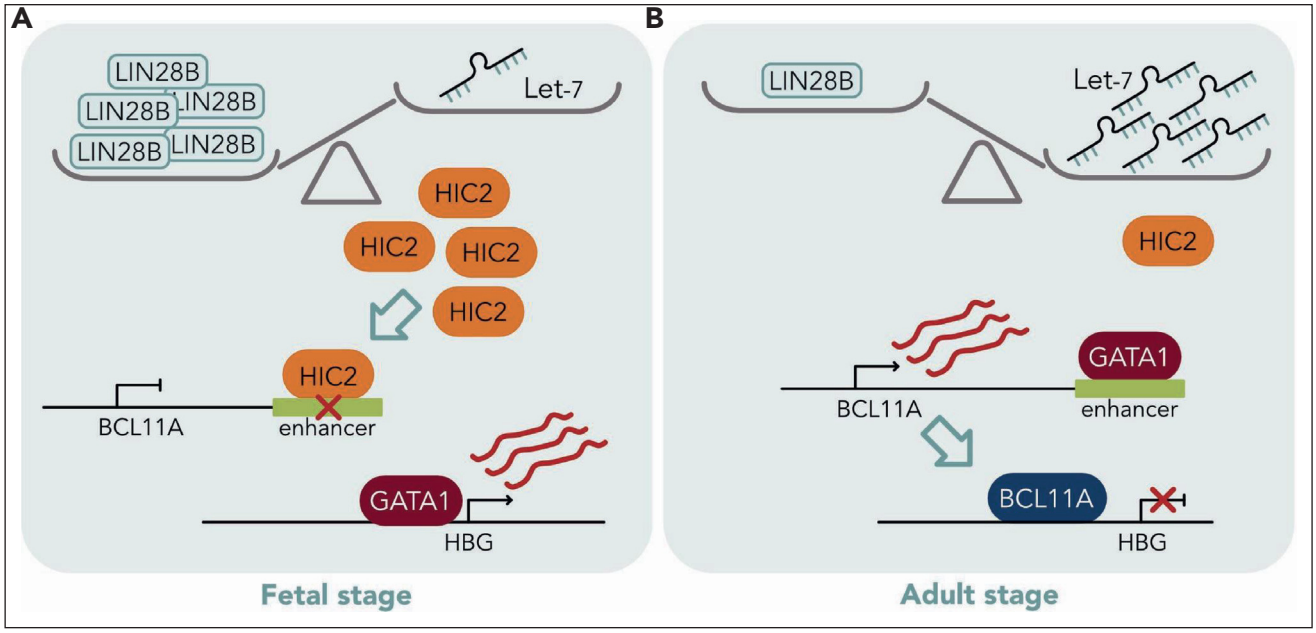

HIC2 links LIN28B/*let-7* to HBG transcription. (A) The balance depicts abundance of RNA binding protein LIN28B in fetal cells resulting in blocked *let-7* miRNA biogenesis. HIC2 can then inactivate the *BCL11A* enhancer, allowing transcription of *HBG* and HbF production. (B) In adult cells, LIN28B is low and *let-7* is abundant, binding to the 3′ UTR of *HIC2* and leading to its degradation. Now, GATA1 can occupy the *BCL11A* enhancer, *BCL11A* is activated and then the protein silences *HBG*. Professional illustration by Somersault18:24.

BCL11A and *HBG* transcriptional regulation (see figure).

Is HIC2 the only relevant *let-7a/f* target in the pathway to *HBG* transcriptional control? Chromatin structure analyses are supportive of this hypothesis. In agreement with the few gene expression changes in the let-7 inhibition model, there were relatively few changes observed using assay for transposase-accessible chromatin (ATAC)-sequencing. Interestingly, HIC2 and GATA binding motifs were enriched among the peaks that lost accessibility, consistent with common targets. Globally, the sites that lost accessibility correlated with increased HIC2 signal and decreased GATA1 signal. The *BCL11A* enhancer exemplifies this exchange. Increased HIC2 signal and decreased GATA1 signal at the enhancer correlated with decreased long-range interactions with the *BCL11A* promoter and decreased transcription.

In a final experiment to clinch that HIC2 critically connects *let-7* to *HBG*, Huang et al depleted HIC2 in *let7a/f*-inhibited HUDEP2 cells. BCL11A was restored to levels in *let-7a/f*-inhibited cells alone and *HBG* was resilenced. The present report connects the dots between *let-7* miRNA and *HBG*, showing that HIC2 is targeted for degradation by *let-7*, and supplies a satisfying explanation to the proposal of the Miller group that small RNAs have a central role in globin gene switching. The miRNA pathway provides a so far underexplored regulatory option for augmenting fetal hemoglobin.

Conflict-of-interest disclosure: A.D. declares no competing financial interests. ■

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THROMBOSIS AND HEMOSTASIS

Comment on Arce et al, page 1992

Taking AIM

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In this issue of *Blood*, Arce and colleagues present an elegant study showing that the autoinhibitory module (AIM) of von Willebrand factor (VWF) may be targeted to either be activated or inhibited.[1] The VWF A1 domain contains the binding site for platelet glycoprotein Ib-alpha (GPIb) and this represents

the initial port of interaction for circulating platelets and VWF at sites of injury.[2] Recent work has demonstrated that the 2 proline and O-linked glycan-rich regions that exist on either side of the VWF A1 domain cooperate to control access of GPIb to its binding site.[3-5] These have been termed the N-terminal and C-terminal AIM (N-AIM and C-AIM, respectively).

In their study, Arce et al generated nanobodies against a VWF A1 protein containing both AIM sequences. Next, they selectively sorted the nanobodies to isolate specific binders to the 2 AIM portions. Three nanobodies, 6C4, 6C11, and 6D12, induced platelet activation and binding to GPIb similar to ristocetin, indicating activation of VWF. These 3 nanobodies all bound to the N-AIM with high (nanomolar) affinity and, very interestingly, 6D12 recognized an epitope in the N-AIM that includes the crucial O-linked glycans.[6,7] Finally, for this part of the study, the authors used hydrogen-deuterium exchange to demonstrate that 6D12 binds to the N-AIM and increases accessibility of the GPIb binding site. In addition to providing useful information on how the N-AIM functions, these activating nanobodies may well prove to be valuable research tools. Because they behave similarly to ristocetin, these nanobodies may be useful in diagnostic testing for VWF by overcoming some of the pitfalls associated with ristocetin, such as the P1467S mutation that prevents ristocetin binding.[8]

Arce and colleagues also investigated nanobodies against the C-AIM and identified 2 clones: Nd4 and Nd6. In contrast to the N-AIM nanobodies, Nd4 and Nd6 both inhibited platelet aggregation and, moreover, inhibited VWF-mediated platelet capture under arterial shear stress. The authors then solve the crystal structure of the VWF-A1 domain in complex with Nd6, with some stunning results. The Nd6 clone interacted with the C-AIM regions with some physical distance away from the A1 domain; furthermore, for the first time, the structure was found to resolve one of the O-linked N-acetylgalactosamine residues at position 1468. Most strikingly, it may be first time this structure demonstrated strong evidence of an interaction between N-AIM and C-AIM. From the resolved residues, both AIM modules pointed away from the A1 domain and cooperated to form the entire AIM module that controlled access to the GPIb binding site.

This important study sheds further light on how the VWF A1 domain engages with GPIb and the intricate molecular process that prevents aberrant platelet binding while allowing the VWF molecule to respond to shear forces and promote platelet binding. Although targeting the VWF A1 domain for antithrombotic therapy is not new, understanding how we can selectively target this region without drastic compromise of function may lead to novel and improved anti-VWF targeting agents. This is certainly something to AIM for.

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

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THROMBOSIS AND HEMOSTASIS

Comment on Kumar et al, page 2005

Prothrombin forced into an awkward position

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In this issue of *Blood*, Kumar et al use a model antibody to show that a subpopulation of antiprothrombin antibodies found in people with antiphospholipid syndrome act as anticoagulants by stabilizing prothrombin into a conformation that limits its activation by the prothrombinase complex.[1]

Diagnosis of the rare autoimmune disorder antiphospholipid syndrome requires the persistent presence of antiphospholipid antibodies in people with thrombosis or pregnancy-associated morbidity. Thrombotic risk is estimated with antibody profiles based on 3 different antiphospholipid antibodies: antibodies that cause prolongation of clotting time in sensitive clotting assays, so-called lupus anticoagulant, antibodies against the plasma protein β2-glycoprotein I, and antibodies against the phospholipid cardiolipin.[2] Of these 3 antiphospholipid antibodies, only lupus anticoagulant is independently associated with increased risk of thrombosis or pregnancy morbidity. Thrombotic risk is highest in people who are triple positive (ie, positive for anti–β2-glycoprotein I and anti-cardiolipin antibodies as well as lupus anticoagulant).[2]