

regulation of lytic granule trafficking and fusion by distinct receptors. Thus, further experiments are needed to address how dynein and kinesin motor proteins are recruited to regulate the tug-of-war facilitating granule movement along microtubules.

In conclusion, a critical molecular complex for cytotoxic lymphocyte granule movement and exocytosis has been identified. Besides Rab27a, mutations in the gene encoding Slp3, *SYTL3*, could be associated with immunodeficiencies. Further work will hopefully address how different motor proteins for lymphocyte cytotoxicity are regulated spatially and temporally.

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● ● ● PHAGOCYTES & GRANULOCYTES

Comment on Zardo et al, page 4034

MicroRNAs function on a new level

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In this issue of *Blood*, Zardo et al describe an emerging mechanism by which microRNAs can regulate gene expression in blood cells via transcriptional gene silencing (TGS).¹ The authors provide evidence that miR-223 binds to specific sites within the promoter of its target gene *Nfia* and represses transcription by influencing epigenetic events. These observations support the notion that multiple mechanisms of miRNA-mediated gene repression exist and should be considered when investigating gene targets of a given miRNA of interest.

MicroRNAs (miRNAs) clearly regulate gene expression at the posttranscriptional level by recruiting the RNA-induced silencing complex to the 3' untranslated regions (UTRs) of target mRNAs, and this has been shown to be a major mechanism underlying their function in mammalian cells.² However, the current study demonstrates a role for miRNAs in the direct regulation of gene transcription in mammalian cells, and is among only a few reports having made this connection to date.^{3,4}

The article focuses on the epigenetic silencing of the gene encoding the transcription factor

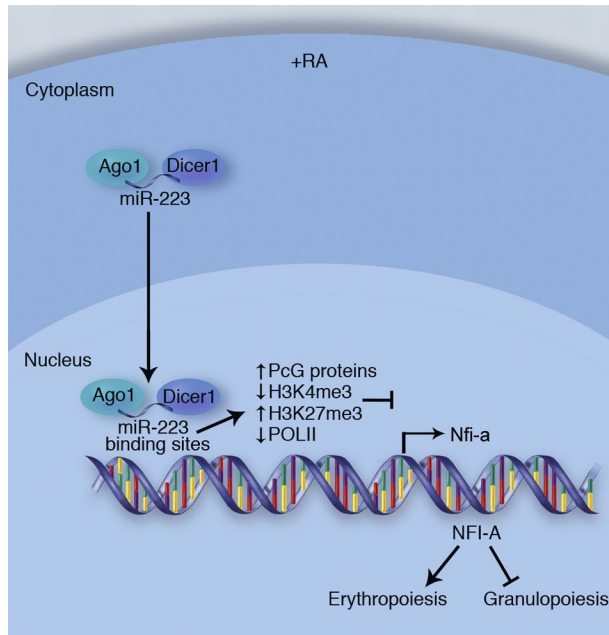
NFI-A by miR-223 (see figure). NFI-A is an established repressor of granulopoiesis and a previously identified posttranscriptional target of miR-223.⁵ Experiments found that miR-223, which localized to the nucleus after the induction of granulopoiesis by all-*trans*-retinoic acid, directly interacted with complementary binding sites in the promoter of *Nfia* and served as a guide for the localization of specific protein complexes that mediated epigenetic silencing of the *Nfia* gene. Chromatin immunoprecipitation (ChIP) experiments identified increased H3K27me3 and decreased H3K4me3 and Pol-II promoter binding after induction of granulopoiesis.

Polycomb group proteins (PcGs) and components of the RNAi machinery, including Dicer1 and Ago1, also interacted with the *Nfia* promoter region under these conditions. Importantly, these events were shown to occur through a mechanism involving miR-223. While the experimental system used here demonstrates that this regulatory mechanism influences granulocyte development, these findings imply that other blood cell types might use a similar miRNA-dependent approach to accomplishing TGS during lineage fate decisions that occur during hematopoiesis.

Along with these new and exciting observations that miRNAs can directly regulate transcription in mammalian blood cells come many questions about the process. For instance, how widespread is this mechanism? miRNA transcriptional regulation could be specific to certain types of genes, like *Nfia*, or might be a common regulatory mechanism used to modulate a majority of genes. It would be interesting to know if miRNAs commonly target the same gene at both the transcriptional and posttranscriptional levels to ensure repression as appears to be the case for *Nfia*. In addition, while the authors indicate some degree of evolutionary conservation of the miR-223 binding site sequences in the promoter of *Nfia*, it remains to be determined whether sequence conservation of miRNA promoter binding sites are common like they are in 3' UTRs. Other questions include how Ago1 and Dicer1 function to impact histone marks and other epigenetic events that control gene transcription after their recruitment by miRNAs, and whether or not miRNAs can activate gene transcription in certain contexts. This is clearly a new frontier in miRNA biology that warrants further investigation.

A variety of different types of noncoding RNAs (ncRNAs) are now known to modulate target gene transcription. Examples include long intergenic ncRNAs (lincRNAs),⁶ promoter associated RNAs⁷ and exogenously delivered small interfering RNAs (siRNAs) designed to target gene promoters.⁸ Endogenously expressed miRNAs can now be added to this group of transcription-regulating ncRNAs, which can take advantage of their Watson-Crick base pairing with promoter DNA sequences to recruit regulatory protein complexes to specific gene promoters to repress transcription.

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



In response to all-*trans*-retinoic acid (RA), a signal that promotes granulocyte development, miR-223 translocates into the nucleus along with Dicer1 and Ago1. Once in the nucleus, miR-223-RNAi complexes bind to miR-223 targets sites located in the 5' regulatory region of the *Nfi-a* gene. This triggers a number of epigenetic changes leading to transcriptional silencing of *Nfi-a*. Reduced expression of NFI-A redirects cellular development away from erythropoiesis and toward granulopoiesis. Professional illustration by Marie Dauenheimer.

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● ● ● PLATELETS & THROMBOPOIESIS

Comment on Psaila et al, page 4066

Platelets and eltrombopag: a not-so-sticky situation

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In this issue of *Blood*, Psaila and colleagues examine the in vivo effects of eltrombopag, a thrombopoietin receptor agonist (TPO-RA), on platelet function and reactivity.¹

Immune thrombocytopenia (ITP) is an autoimmune disease associated with increased platelet destruction and ineffective thrombo-

poiesis that presents with variable amounts of bleeding. Thrombopoietin (TPO) is a hematopoietic growth factor that stimulates

megakaryopoiesis and platelet production by activating the cell through the TPO-receptor (TPO-R, c-MPL). In 2008, two small molecule TPO-R agonists (TPO-RAs), eltrombopag and romiplostim, were approved for use in adult patients with chronic (> 12 months) ITP and low platelet counts. TPO-R is present not only on hematopoietic stem cells and megakaryocytes but also on platelets, and stimulation of platelets with TPO potentiates platelet activation to numerous agonists.² Clinical trials examining the efficacy and safety of both eltrombopag and romiplostim have reported adverse events of thrombosis at a rate of 2% to 4% although the overall rates of thrombosis are similar between control and treated patients.³ Several case reports have also been published describing significant thrombosis in patients treated with TPO-RAs, prompting concerns about the potential for platelet activation by TPO-RAs.

Eltrombopag is a small molecule, oral TPO-RA that interacts with the transmembrane domain of TPO-R leading to JAK/STAT pathway signaling in platelets and megakaryocytes.⁴ A previous in vitro study suggested that eltrombopag, in contrast to TPO, did not alter reactivity of platelets⁴ and the authors suggested that a study examining the in vivo effects of eltrombopag is needed to better understand the potential for platelet activation in the target population.⁴ Psaila et al have answered the call and examined the in vivo effect of eltrombopag on platelets.¹

Studies of platelet function in patients with thrombocytopenia are difficult as low platelet counts may significantly affect the results of standard platelet aggregometry studies. Many studies have examined the utility of flow cytometry to interrogate platelet function.⁵ Psaila et al use flow cytometry to study the reactivity of platelets even at very low platelet counts⁶ and compare flow cytometry results in both control subjects with normal platelet aggregation studies and in thrombocytopenic patients with ITP. In control subjects, there was a direct correlation between platelet aggregation by light transmission aggregometry and flow cytometric platelet function. They used glycoprotein (GP) Ib (CD42) surface expression, which is probably most correlated with mean platelet volume/surface area of the platelets and is expected to decrease due to proteolysis on platelet activation, activated GPIIb/IIIa (CD41) by PAC-1 binding,¹ and