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## RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Ma et al, page 2302

# A master erythroid regulator gets its own GPS

Lily Jun-shen Huang<sup>1</sup> and James J. Bieker<sup>2</sup> | <sup>1</sup>University of Texas Southwestern Medical Center; <sup>2</sup>Icahn School of Medicine at Mount Sinai

**In this issue of *Blood*, Ma et al discuss their discovery of a novel function of G protein pathway suppressor 2 (GPS2) in promoting erythroid differentiation through stabilizing the erythroid master regulator erythroid Krüppel-like factor (EKLf, also known as KLF1).<sup>1</sup>**

GPS2 is a protein with diverse functions. It was originally identified during a screen for suppressors of Ras activation in the yeast pheromone response pathway, but it plays a role in many processes, including inflammation, lipid metabolism, and B-cell development.<sup>2,3</sup> GPS2 is an integral subunit of the nuclear receptor co-repressor (NCoR) complex, thereby contributing to transcriptional repression. It also serves as a transcriptional co-activator, and it modulates chromatin remodeling. In the cytosol, GPS2 restricts activation of signaling proteins downstream of tumor necrosis factor, insulin, B-cell antigen receptor, and Toll-like receptor. Many GPS2 functions have been attributed to its ability to directly inhibit the enzymatic activity of Ubc13, an E2 ubiquitin-conjugating enzyme, which works with many E3 ubiquitin ligases to mediate synthesis of extended

lysine 63 (K63) ubiquitin chains on target proteins.<sup>3</sup>

By using GPS2 knockout mice and GPS2 knockdown CD34<sup>+</sup> cell cultures, Ma et al identified a novel function of GPS2 in promoting terminal erythroid differentiation by interacting with and protecting EKLf from proteasomal degradation. EKLf plays a critical role in cell differentiation because of its global regulation of erythropoiesis.<sup>4</sup> EKLf protein levels are known to fluctuate during this process, and cellular control of EKLf protein levels has been previously suggested.<sup>5</sup> First, EKLf is ubiquitinated, and its degradation is controlled by the proteasome. Second, it contains 2 PEST sequences that are involved in the process, possibly via its interaction with protein phosphatase PPM1B. Third, noncovalent interactions with ubiquitin via its transcriptional

activation domain (TAD) are also implicated in maintenance of steady-state levels.<sup>6</sup> Interestingly, Ma et al found that neither GPS2-mediated Ubc13 inhibition nor ubiquitination is involved in EKLf stabilization by GPS2. These findings are novel in several ways. First, they identify a Ubc13-independent function of GPS2 in erythropoiesis. Second, EKLf stability is regulated by GPS2 and depends upon an EKLf region that is different from either its PEST or TAD domains. The stabilization of EKLf protein by GPS2 thus enables the normal progression of erythropoiesis to occur as aligned with the known role of EKLf in this process, particularly at late stages.<sup>7</sup>

What remains to be uncovered is how GPS2 inhibits EKLf recognition and/or degradation by the proteasome. Because the authors found that a substantial amount of EKLf is ubiquitinated and that GPS2 expression does not affect EKLf ubiquitination, they were able to speculate that GPS2 prevents EKLf from entering into the proteasome. Alternatively, GPS2 may participate in ubiquitin-independent proteasomal degradation, which has been shown to contribute to the turnover of proteins with intrinsic disordered regions that give them flexibility to interact with multiple partners.<sup>8</sup> Although the EKLf zinc finger region is highly structured, at least some parts of the molecule seem to be disordered,<sup>9</sup> and it interacts with a plethora of partners.<sup>5</sup> It will also be interesting to determine whether there is crosstalk between GPS2-mediated EKLf stability and other EKLf modifications such as phosphorylation and sumoylation.

Fine tuning of the proteasomal control mechanism of GPS2 for EKLf is critical for optimal regulation of red cell protein levels and function because a haplo-insufficient quantity of EKLf leads to altered expression of downstream targets (eg, BCL11a,  $\gamma$ -globin, Lu, CD44) that, although benign, can yield clinically beneficial effects.<sup>4</sup> In this context, it is of interest that 1 of these mutations (Q211R), identified in a patient with increased fetal hemoglobin, is located in the highly conserved region of interaction with GPS2. The mutation weakens this association and is less stable, which helps explain the effects of at least some of the nontruncating mutations that lie outside the zinc finger region of EKLf.

These connections also suggest that small molecules or small interfering RNA approaches directed at decreasing GPS2 function or level in the red cell may provide a novel means for hemoglobin F induction. It is important to note, however, that EKLF-independent GPS2 functions, shown by the early embryonic lethality of GPS2 knockout mice, should be taken into account in the exploration of such approaches. A corollary of this idea would be that direct control of EKLF protein levels, perhaps following a cereblon-based design,<sup>10</sup> is also a feasible approach.

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

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