The erythroid/myeloid lineage fate paradigm takes a new player

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The erythroid versus myeloid lineage fate decision controlled by cross-antagonism between GATA1 and PU.1 serves as a mechanistic paradigm for transcription factor interplay during lineage specification. In this issue of *The EMBO Journal*, Monteiro *et al* (2011) examined this paradigm in blood populations in zebrafish embryos, and demonstrated that, unexpectedly, the regulatory interactions between GATA1 and PU.1 lead to different outcomes depending on the cellular contexts and the development stages. GATA1/PU.1 cross-antagonism is also modulated by a third factor transcriptional intermediate factor 1 γ (TIF1 γ), a chromatin-associated haematopoietic regulator.

Vertebrate haematopoiesis is a highly orchestrated process coordinated by a complex regulatory network of lineage-restricted transcription factors (Orkin and Zon, 2008). The cross-antagonism between GATA1 and PU.1 during erythroid–myeloid lineage bifurcation represents one of the earliest decisions during haematopoietic development and serves as a paradigm for the transcription factor interplay underlying cell fate switching and lineage reprogramming (Graf and Enver, 2009). GATA1 is a critical factor for erythroid and megakaryocytic differentiation and controls the expression of genes specific for these lineages. In contrast, PU.1 is required for myeloid cell differentiation and mediates the

Figure 1 GATA1/PU.1 cross-antagonistic paradigm takes different forms during haematopoiesis in zebrafish embryos. A scheme of developmental time windows for haematopoiesis sites in the zebrafish is shown on the top. A schematic representation of the classical GATA1/PU.1 cross-antagonistic paradigm is shown in the middle panel. In the ICM, TIF1 γ is required for the expression of both GATA1 and PU.1 (left panel). Here, GATA1 antagonizes PU.1, but PU.1 does not repress GATA1 expression. In the CHT, TIF1 γ favours the expression of GATA1 over PU.1, while GATA1 and PU.1 antagonize each other's activity (right panel). Note that while PU.1 positively autoregulates in all the haematopoietic progenitors, the autoregulatory status of GATA1 varies from stimulatory (in ICM) to repressive (in CHT). The interplay among GATA1, PU.1 and TIF1 γ affects erythroid/myeloid lineage output at different haematopoietic sites in zebrafish embryos.

induction of myelomonocytic genes. From overexpression studies in both cell lines and primary cells, it is evident that GATA1 and PU.1 can specify erythroid and myeloid lineages by antagonizing each other's transcriptional activity (Graf, 2002). GATA1 and PU.1 also control their own expression by forming autoregulatory loops. Genetic studies have validated the mutual antagonism between GATA1 and PU.1 by loss-of-function studies in zebrafish embryos (Galloway *et al*, 2005; Rhodes *et al*, 2005) and by using GATA1 and PU.1 transcriptional reporters in mice (Arinobu *et al*, 2007).

The cross-antagonistic model between GATA1 and PU.1 provides a simplified scheme for lineage commitment for the erythroid/myeloid branch point during haematopoietic development. However, the presence of additional binding partners for both proteins may create a far more complex regulatory network that includes additional inputs (Chickarmane *et al*, 2009). The study by Monteiro *et al* (2011) has demonstrated that one such factor, TIF1 γ , is involved in erythroid/myeloid lineage fate decision by way of modulating GATA1 and PU.1 expression.

The findings build on a careful analysis of *moonshine* (*mon^{tg234}*), a mutant zebrafish with defects in erythroid maturation due to loss of TIF1 γ (Ransom *et al*, 2004). In the caudal haematopoietic tissue (CHT, analogous to fetal liver in mammals), deficiency in TIF1 γ leads to loss of definitive erythroid cells accompanied by a dramatic increase in myeloid cells (Monteiro *et al*, 2011). As loss of TIF1 γ affects erythroid versus myeloid outcomes from haematopoietic progenitors in CHT, the researchers investigated the epistatic relationships between TIF1 γ , GATA1 and PU.1. By morpholino-mediated knockdown followed by gene expression analysis, Monteiro *et al* demonstrated that there is a deviation from the GATA1/PU.1 cross-antagonistic paradigm in this population of blood precursors. Specifically, PU.1 antagonizes GATA1 activity and regulates itself positively, while GATA1 antagonizes PU.1 expression, consistent with the paradigm. However, GATA1 regulates itself negatively, in contrary to the paradigm (Figure 1). Moreover, TIF1 γ modulates the GATA1/PU.1 interplay by favouring GATA1 expression over PU.1 expression, consistent with the expansion of myeloid output in the TIF1 γ -deficient *moonshine* embryos. These findings indicate that the GATA1/PU.1 regulatory interplay may be cell context-dependent and is modulated by TIF1 γ .

Having established TIF1 γ as an important modifier of the GATA1/PU.1 interplay, Monteiro *et al* next examined this regulatory triad in other blood populations within the zebrafish embryos. Surprisingly, in the primitive haematopoietic tissue intermediate cell mass (ICM) and pro-definitive erythro-myeloid progenitors (EMPs), the autoregulatory status of GATA1 varies from stimulatory (in ICM) to no autoregulation (in EMP). Therefore, there is an unappreciated complexity of the GATA1/PU.1 cross-antagonistic paradigm in haematopoietic progenitors *in vivo*. Although the model takes different forms in different blood-producing populations, a unified theme is still apparent. Particularly, PU.1 always antagonizes GATA1 along with stimulating its own expression wherever myelopoiesis occurs. Similarly, GATA1 always antagonizes PU.1 expression wherever erythropoiesis occurs. However, the autoregulatory status of GATA1 may vary from autostimulation to autorepression depending on the cellular context. Importantly, the erythroid/myeloid output is modulated by a third factor TIF1 γ , which controls the differential expression of

GATA1 and PU.1 in the definitive CHT. Regulation through TIF1 γ differs in primitive erythroid and pro-definitive EMPs, in which it positively regulates both GATA1 and PU.1 (Figure 1). Thus, the work of Monteiro *et al* adds to the existing literature an important example of how overly simplified paradigms may need to be modified according to cellular context.

A better understanding of the erythroid versus myeloid lineage fate decision necessitates a more comprehensive analysis of the molecular mechanisms underlying this sophisticated transcription factor interplay. Earlier studies from forced expression experiments demonstrated that GATA1 and PU.1 functionally cross-antagonize each other through direct physical interaction (Graf and Enver, 2009). Specifically, ectopically expressed GATA1 inhibits PU.1 activity by displacing c-Jun, an important cofactor of PU.1 for the myelomonocytic program. Conversely, PU.1 expressed in erythroid precursors interacts with GATA1 and recruits a repressive complex consisting of the RB protein and the histone methyltransferase SUV39H. The precise role of TIF1 γ in transcriptional regulation is less defined. TIF1 γ encodes an E3 ubiquitin-protein ligase composed of multiple conserved domains implicated in protein-protein interaction and chromatin association, including the RBCC domain, PHD finger and bromodomain. Recent work by Bai *et al* (2010) has demonstrated that TIF1 γ controls erythroid gene expression by regulating transcription elongation. By a genetic suppressor screen, Bai *et al* initially demonstrated that loss of function of Pol II-associated factors PAF or DSIF rescued erythroid gene transcription in TIF1 γ -deficient *moonshine* mutant. Further biochemical and genetic analysis established physical interactions among TIF1 γ , erythroid-specific SCL complexes, and the positive elongation factors p-TEFb and FACT. Therefore, TIF1 γ couples the blood-specific transcriptional complex with Pol II elongation machinery to promote the transcription elongation of erythroid-selective genes (Bai *et al*, 2010). It remains to be determined how TIF1 γ mediates gene repression. TIF1 γ may directly repress its target genes through yet-to-be-defined mechanisms. Alternatively, it may activate a set of intermediate repressor proteins. It will also need to be clarified, in future studies, whether GATA1 and PU.1 are involved in TIF1 γ 's activity, and if so, how. It is formally possible that all three factors can function through physical interactions within a chromatin environment. Thus, future genome-wide chromatin occupancy studies should provide some clues into the molecular understanding of this critical lineage bifurcation process. Regardless of the exact mechanisms, Monteiro *et al*'s study reveals sophisticated transcription factor interplay during haematopoiesis. The interactions among key lineage-specifying regulators can be modulated depending on the cellular contexts and the development stages.

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Conflict of interest

The authors declare that they have no conflict of interest.

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