

Creating Cellular Diversity through Transcription Factor Competition

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Abstract

The development of blood cells has long served as a model system to study the generation of diverse mature cells from multipotent progenitors. The article by Org et al (2015, this issue) reveals how transcription factor competition on primed DNA templates may contribute to embryonic blood cell specification during the early stages of mesoderm development. The study not only provides new insights into the functionality of the key haematopoietic transcription factor Scl/Tal1, but also provides a potentially widely applicable framework for transcription factor-mediated cell fate specification.

Main Text

The development of cells with distinct tissue identities from multipotent embryonic progenitor cells represents a hallmark of all multicellular organisms. Since different tissue identities are to a large extent mediated by tissue specific gene expression patterns, transcription factor (TF) proteins responsible for establishing tissue-specific expression profiles have long been recognised as key regulators of early tissue development.

Nevertheless, much remains to be learned about the molecular mechanisms by which a given TF can promote one fate over others within multipotent progenitor cells, and thus determine which mature tissue a given cell will differentiate into.

Org et al (Org et al, 2015) in this issue of the EMBO Journal investigated this question by studying the basic helix-loop-helix TF Scl, also known as Tal1. The Scl gene was originally discovered through its involvement in recurrent chromosome translocations in T-cell acute lymphoblastic leukaemia. Translocations cause ectopic expression of Scl in T-cells, which

was subsequently shown in mouse models to constitute a leukaemia initiating event (for review see (Begley & Green, 1999)). Scl's reputation as the master regulator of blood cell development was established when a series of papers reported that Scl is essential for the development of all blood lineages during early development (Porcher et al, 1996; Robb et al, 1996; Robb et al, 1995; Shivdasani et al, 1995). Developmental haematopoiesis occurs in multiple waves, first producing primitive red blood cells, followed by multipotent blood progenitor and finally pluripotent haematopoietic stem cells. With the exception of the first wave of primitive erythropoiesis where acquisition of blood fate by early mesodermal cells may occur very rapidly (Padrón-Barthe et al, 2014), fate restriction of early mesodermal cells towards a haematopoietic identity for all subsequent waves is thought to occur in a stepwise fashion, where cells progressively lose the potential to form cardiac muscle, smooth muscle and endothelium (Ciau-Uitz et al, 2014). During this process, cells transit through intermediate haemangioblast and hemogenic endothelial cell stages, and Scl has been shown to be required for the transition to hemogenic endothelium (Figure 1A) (Lancrin et al, 2009). Since Scl is required for the development of blood cells produced by all waves of early haematopoiesis, Scl stands out as being critically important for the acquisition of a haematopoietic fate by any early mesodermal cell.

Following up on initial observations in Scl^{-/-} embryonic stem cells (ESCs) (Ismailoglu et al, 2008), the Mikkola group showed in 2012 that deletion of Scl not only abrogates all haematopoietic development, but also increases the numbers of cells that will acquire alternative early mesodermal fates, in particular cardiac muscle (Van Handel et al, 2012). The new study by Org et al builds on these observations, by addressing two key questions about Scl function during early mesodermal fate specification: (i) how does Scl promote haematopoietic fates, and (ii) how does Scl inhibit cardiomyocyte differentiation. Using a

combination of genome-scale approaches, Org et al (Org et al, 2015) integrated genome-wide maps of Scl binding in ESC-derived mesodermal cells with gene expression profiling, genome-wide profiles of histone modification status, genome-wide maps of the bHLH TF Hand1 known to promote cardiac development, and also genome-wide maps for members of the GATA family of TFs known to be involved in either blood or cardiac development. Importantly, analysis of wild-type cells was complemented with key experiments performed in cells derived from TF knock-out ESCs. The comprehensive new datasets reported by Org et al (Org et al, 2015) represent a very valuable new resource for the wider haematopoiesis research community. Moreover, the comparative and integrated analysis performed by the authors provides a number of important new insights, which include: (a) Scl appears to influence mesoderm diversification largely through binding to both haematopoietic and cardiac enhancers, (b) repression of the cardiac fate by Scl appears to be the consequence of blocking the binding of cardiac-promoting TFs to the same sites, with decommissioning of cardiac enhancers during the ensuing blood differentiation being accompanied by the removal of activating histone marks, (c) Scl does not appear to act as a pioneer factor that opens up chromatin, but instead exploits a pre-configured epigenetic landscape, and (d) haematopoietic Gata TFs can bind together with Scl to both cardiac and haematopoietic enhancers, and critically contribute to activating the haematopoietic elements whereas they are apparently dispensable for the repression of the cardiac enhancers.

While the set of conclusions listed above provides important new insights into cell fate control during early mesodermal development (see Figure 1B), what makes the paper by Org et al (Org et al, 2015) even more exciting is that it poses a number of intriguing questions for future investigation. For example, if Scl does not function as a pioneer factor, what is the identity of the TFs that open up the chromatin template for subsequent Scl binding? Related

to this point, Porcher et al (Porcher et al, 1999) showed in 1999 that the DNA binding domain of Scl is not required for its early role in blood specification, but then becomes important later during development for the maturation of red blood cells. This observation could be interpreted to mean that Scl may function as a pioneer factor during these later stages of red blood cell development, since binding to DNA seems to require Scl's own DNA binding domain rather than being mediated by collaborating TFs. Org et al (Org et al, 2015) also reinforce the notion that Scl binding profiles differ greatly when analysed in different cellular contexts (Beck et al, 2013; Kassouf et al, 2010; Palii et al, 2011; Tijssen et al, 2011; Wilson et al, 2009; Wu et al, 2014). Importantly, previous studies identified TFs that may play a role in establishing cell-type specific binding profiles of Scl (Calero-Nieto et al, 2014; Lichtinger et al, 2012). To fully understand Scl's earliest function during mesoderm diversification, it will be critical to identify such TFs for these early stages. It may turn out that these are the very same TFs mentioned above when discussing the need to identify the factors that establish the chromatin template for Scl binding.

Lastly, with Scl blocking Hand1 binding to cardiac enhancers and thereby inhibiting mesoderm differentiation into cardiac cells, is there a reciprocal inhibition of blood development by Hand1 (or some other bHLH factor), by means of blocking the binding of Scl to haematopoietic enhancers? And if such reciprocal blocking were to be a widespread phenomenon in early multipotent cells, what are the specific mechanisms that will cause resolution of cellular fates down just one of the alternative outcomes? A complex interplay of external (signalling) and internal (TF network execution) processes is likely to be responsible, and it is difficult to see how some of the key questions will be resolved without taking molecular analysis down to the single cell level. Single cell profiling of both ESC and embryo-derived early blood-fated mesodermal cells has recently been reported (Moignard et al, 2015; Wilkinson et al, 2014). Such approaches have the potential to identify and

characterise the nature of those, potentially rare, cells that co-express lineage-determining TFs such as Scl and Hand1 during early development. Time-lapse imaging approaches at single cell resolution represent another key tool that will likely be essential to resolve the mechanisms underlying fate resolution of early multipotential cells. However, their adaptation from previous ESC based experiments (Eilken et al, 2009) to whole embryo analysis is likely to require overcoming significant technical challenges, both in terms of embryo in vitro culture protocols and image analysis of dynamic 3-dimensional objects at single cell resolution. Importantly, in addition to advancing our understanding of fundamental biological processes involved in cell fate control, a better understanding of blood and cardiac development has substantial “translational” implications, in the short term probably most likely in relation to the generation of more accurate in vitro models of human disease, but in the longer term also in relation to the development of cell-based therapies for regenerative medicine applications.

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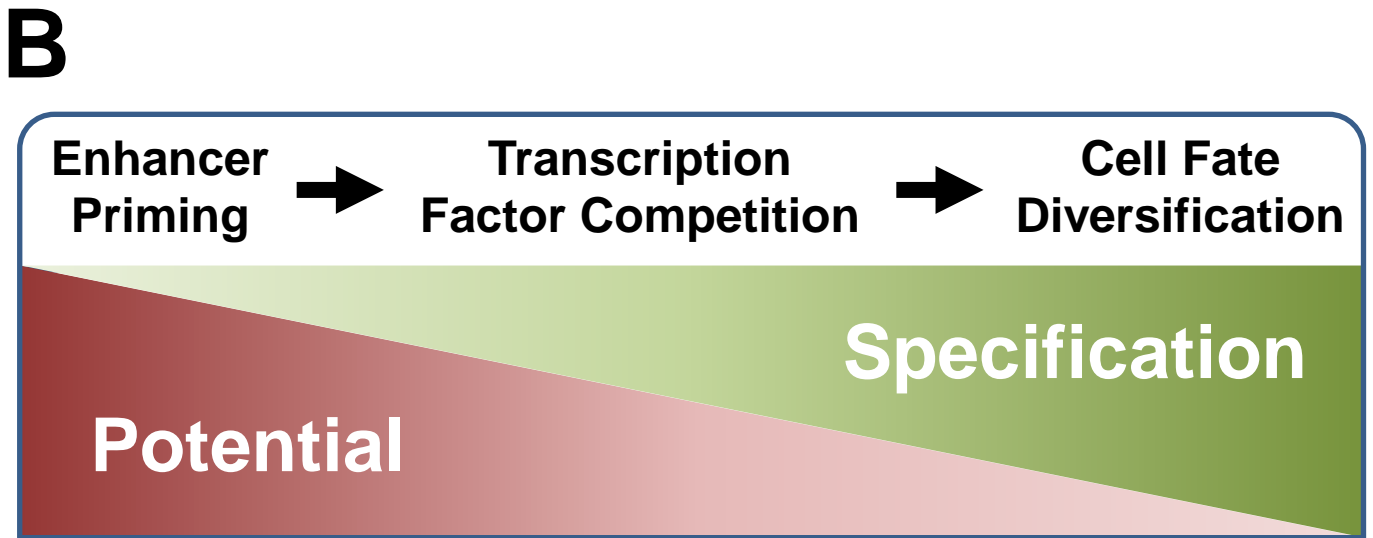
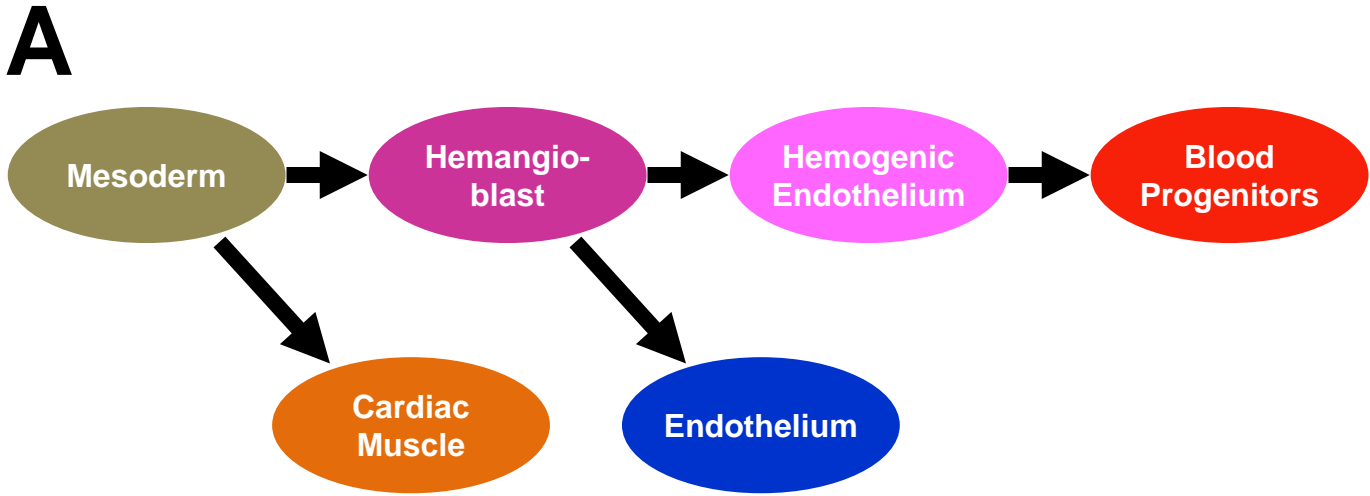


Figure 1: A) Development of blood cells from early mesoderm. Shown are the lineage relationships between early cardiac, endothelial and blood development, together with haemangioblast and hemogenic endothelial intermediate stages. **B)** Model illustrating the generation of diverse cell fates through transcription factor competition on enhancer elements. Enhancers are opened up (primed), which is followed by competitive binding of transcription factors promoting alternative fates. During this process, multipotent cells gradually lose their potential, and become increasingly specified to a particular lineage.