EXPERIMENTAL CELL RESEARCH 329 (2014) 255–264

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/yexcr

Review Article

# Transcriptional network control of normal and leukaemic haematopoiesis

*Jonathan I. Sive, Berthold Göttgens**

*Department of Haematology, Cambridge Institute for Medical Research and Wellcome Trust and MRC Cambridge Stem Cell Institute, University of Cambridge, Cambridge, UK*

ARTICLE INFORMATION

*Article Chronology:*
Received 29 May 2014
Received in revised form
26 June 2014
Accepted 28 June 2014
Available online 8 July 2014

*Keywords:*
Haematopoiesis
Transcriptional regulation
Transcription factor

ABSTRACT

Transcription factors (TFs) play a key role in determining the gene expression profiles of stem/progenitor cells, and defining their potential to differentiate into mature cell lineages. TF interactions within gene-regulatory networks are vital to these processes, and dysregulation of these networks by TF overexpression, deletion or abnormal gene fusions have been shown to cause malignancy. While investigation of these processes remains a challenge, advances in genome-wide technologies and growing interactions between laboratory and computational science are starting to produce increasingly accurate network models. The haematopoietic system provides an attractive experimental system to elucidate gene regulatory mechanisms, and allows experimental investigation of both normal and dysregulated networks. In this review we examine the principles of TF-controlled gene regulatory networks and the key experimental techniques used to investigate them. We look in detail at examples of how these approaches can be used to dissect out the regulatory mechanisms controlling normal haematopoiesis, as well as the dysregulated networks associated with haematological malignancies.



## Contents



*Corresponding author.
E-mail addresses: jis30@cam.ac.uk (J.I. Sive), bg200@cam.ac.uk (B. Göttgens).

http://dx.doi.org/10.1016/j.yexcr.2014.06.021
0014-4827/

## Roles of transcription factors in cell differentiation

The haematopoietic system has long been at the forefront of research into the processes of normal and abnormal cell differentiation, both in foetal development and in the ongoing production of mature cells in adult life [1,2]. Analysis of surface expression markers and isolation of distinct populations by flow cytometry has enabled the precise immunophenotypic characterisation of both developmental and mature forms of blood cells. Despite ongoing refinement, the "haematopoietic tree" outlining the major haematopoietic developmental pathways is well-defined in general terms with at least 14 mature cell types present in the normal adult, and a significant progress has been made into understanding the processes regulating these progressions [2,3].

The key role of transcription factors (TFs) in regulating haematopoietic cell fate has long been recognised. Many TFs were originally identified following the observation of novel transcripts resulting from chromosomal translocations in haematological malignancies, for example Runx1 in the Runx1 (AML1)–ETO fusion protein of t(8;21) acute myeloid leukaemia (AML) [4], Scl(Tal1) in t(1;14) T-cell acute lymphoblastic leukaemia (T-ALL) [5] and Lmo2 in t(11;14) T-ALL [6]. More recently, cellular reprogramming across haematopoietic lineages using combinations of TFs [7] and induction of pluripotency [8] has reinforced the key roles TFs play in determining cell fate.

An early attempt to provide a conceptual framework for cell fate decisions was made by Waddington, who conceived of an irreversible process described in terms of balls rolling downhill through bifurcating valleys which represented distinct maturing lineages [9]. The ability to manipulate these processes, with cells able to move between lineages under appropriate stimulation [10,11], has suggested that a more plastic model more accurately represents reality in vivo. Using concepts from the network theory, Waddington's valleys can be replaced by "attractor states" – relatively stable conditions representing the totality of expressed genes at a given point, through which cells transit during differentiation [12]. Control of gene expression occurs through the combination of TFs, epigenetic regulators, and the cis-regulatory elements within the genome with which they interact (promoters, enhancers etc.) [13]. The totality of these components may be considered a "gene regulatory network", and the inputs and outputs modelled as network motifs. Alon has described these in detail, and shown that motifs such as coherent and incoherent feed forward loops have demonstrable biological counterparts [14]. An example from haematopoiesis is the positive feed-forward loop formed by the Scl complex and Myb controlling the gene expression programme of T-ALL cells, as described below [15].

Just as normal haematopoiesis is determined by the effects of gene regulatory networks on gene expression, disturbances of these networks by mutations, deletions or oncogenic fusions of TFs can lead to alternative attractor states, representing malignant transformation. In the review below, we discuss experimental and analytical techniques for investigating normal and dysregulated gene regulatory networks in haematopoietic tissue, and go on to describe examples of these from the recent literature.

## Key experimental techniques in establishing TF networks

Investigation into the roles of TFs in gene regulatory networks requires the identification of candidate TFs and their regulatory elements within a given system, and then confirmation of DNA binding and its effect on gene expression and cell phenotype. Interactions between multiple TFs may be suggested by the analysis of parallel DNA-binding experiments, and biophysical experiments can then identify individual components of TF-complexes and the interactions between them. Final integration of all the components and interactions increasingly requires bioinformatic analyses and computational network modelling; this can then be used iteratively to suggest new hypotheses for subsequent experimental validation. A schema illustrating this approach is shown in Fig. 1.

## Identification of transcription factors

As noted above, initial identification of many TFs resulted from observed gene-fusions produced from chromosomal translocations in haematological malignancies [4–6]. Knockout studies such as those on Scl in foetal development, can then determine the relevance of a given TF in haematopoiesis [16,17].

Following the completion of the human genome project, it has been possible to identify putative TFs by sequence analysis for known DNA-binding motifs in coding regions, and subsequently assess the expression of these transcript sequences in different cell types. Dedicated online resources for the blood system, including gene expression compendia such as Gene Expression Commons [18], have further improved this process.