Identification of Target Genes of an Erythroid Transcription Factor Complex Containing SCL (TAL1)

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Thesis submitted for the degree of

Doctor of Philosophy

University of Cambridge

2008

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Abstract

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Haematopoiesis is the process whereby haematopoietic stem cells give rise to mature blood cell lineages. The SCL (TAL1) gene encodes a key transcription factor (TF) which is expressed in various blood lineages and is essential for haematopoietic development. It has been shown that the SCL protein forms a multi-protein complex during erythroid development with other TFs (GATA1, E2A, LDB1, and LMO2) which binds to a sequence-specific motif to regulate its target genes. Two complementary approaches were used here to identify novel target genes regulated by this TF complex during erythroid development.

In the first approach, short interfering RNAs (siRNAs) were transfected into the K562 cell line to knockdown transiently each of five TFs found in this complex. For these five TFs, a knockdown efficiency of at least 70% was confirmed at the mRNA and protein level within 48 hours after transfection. The biological consequences of these knockdowns were studied using Affymetrix GeneChips in order to identify gene expression changes of downstream targets. In the second approach, chromatin immunoprecipitation (ChIP) was performed for the five TFs of the complex in the K562 cell line and the resultant ChIP material was hybridised to a human transcription factor promoter microarray. A number of novel target genes for the SCL erythroid complex were identified and verified independently using both approaches. The data presented in this thesis revealed that members of the SCL-containing erythroid complex are involved in auto-regulation and regulate genes which have key roles in haematopoiesis or control chromatin structure and function. These findings demonstrate that the expression of this TF complex is tightly controlled and point to an important role for it in orchestrating fundamental biological processes which have profound effects on gene expression in erythroid development.