Transcriptional regulation in mouse macrophages: the role of enhancers in macrophage activation and infection

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Abstract

Macrophages are sentinel cells essential for tissue homeostasis and host defence. Owing to their plasticity, macrophages acquire a range of functional phenotypes in response to microenvironmental stimuli. Of those, M(IFN-γ) and M(IL-4/IL-13) macrophage activation states are well known for their opposing pro- and anti-inflammatory roles. Imbalance in these populations of macrophages has been implicated in progression of various diseases. Macrophages also comprise the first line of an organism's defence against *Mycobacterium tuberculosis*, the causative agent of tuberculosis; interactions between the bacteria and host macrophages define the infection outcome.

The area of mammalian transcriptional regulation progressed remarkably with recent advances in high-throughput technologies. Enhancers emerged as crucial regulatory DNA elements capable of activating transcription of target genes at distance in an orientation-independent manner. A recent discovery revealed that enhancers can be transcribed themselves into enhancer RNAs, or eRNAs. Enhancers were shown to be pervasive, yet the associated regulatory patterns remain largely unknown and require further research.

In this thesis, we investigated *in silico* transcribed enhancers in mouse tissues and cell lines, with a particular focus on macrophages. We have performed a large-scale study to identify transcribed enhancers across multiple tissues and to characterise their

properties. In macrophages, we have established the most accurate, to our knowledge, genome-wide catalogue of transcribed enhancers and enhancer-gene regulatory interactions. We have inferred enhancers that might drive transcriptional responses of protein-coding genes upon M(IFN-γ) and M(IL-4/IL-13) macrophage activation, and demonstrated stimuli specificity of regulatory associations. We have conducted the first to our knowledge study of the role of transcribed enhancers in macrophage response to *Mycobacterium tuberculosis* infection. Taken together, the present work provides new insights into genome-wide enhancer-mediated transcriptional control of macrophage protein-coding genes in different conditions. Given the increasing promise for enhancer- and chromatin-directed therapy, this work paves the way for further studies towards host-directed therapies and novel treatments for tuberculosis and immune diseases associated with macrophage dysfunction.

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List of publications

The results presented in Chapter 3 of this thesis were published as:

- Denisenko, E., Guler, R., Mhlanga, M. M., Suzuki, H., Brombacher, F., Schmeier, S. (2017). Genome-wide profiling of transcribed enhancers during macrophage activation. *Epigenetics & Chromatin*, 10(1), 50.

In addition to the work presented in this thesis, the author has made significant contributions to the following publications:

- Denisenko, E., Ho, D., Tamgue, O., Ozturk, M., Suzuki, H., Brombacher, F., Guler R., Schmeier, S. (2016). IRNdb: the database of immunologically relevant non-coding RNAs. *Database*, 2016, baw138.
- Hon, C. C., Ramilowski, J. A., Harshbarger, J., Bertin, N., Rackham, O. J., Gough, J., Denisenko, E., Schmeier, S., . . ., Forrest, A. R. (2017). An atlas of human long non-coding RNAs with accurate 5' ends. *Nature,* 543(7644), 199-204.
- Hoeppner, M. P., Denisenko, E., Gardner, P. P., Schmeier, S., Poole, A. M. (2018). An evaluation of function of multicopy non-coding RNAs in mammals using ENCODE/FANTOM data and comparative genomics. *Molecular Biology and Evolution*, 35(6), 1451-62.

Table of abbreviations

Abbreviation	Meaning
BMDM	Bone marrow-derived macrophages
bp	Base pair
CAGE	Cap analysis of gene expression
ChIP-seq	Chromatin immunoprecipitation followed by sequencing
DEG	Differentially expressed gene
DNA	Deoxyribonucleic acid
eRNA	Enhancer RNA
E-P	Enhancer-promoter
FDR	False discovery rate
GRO-seq	Global nuclear run-on sequencing
GSEA	Gene set enrichment analysis
H3K27ac	Acetylation of histone H3 at lysine 27
H3K4me1	Monomethylation of histone H3 at lysine 4
IFN-γ	Interferon gamma
IL-13	Interleukin-13
IL-4	Interleukin-4
kb	Kilobase
lncRNA	Long-noncoding RNA
LPS	Lipopolysaccharide

Abbreviation	Meaning
M.tb	*Mycobacterium tuberculosis*
Mb	Megabase
mRNA	Messenger RNA
PRR	Pattern recognition receptor
RNA	Ribonucleic acid
RNAPII	RNA polymerase II
RNA-seq	RNA sequencing (whole transcriptome shotgun sequencing)
SE	Super (stretch) enhancer
SEM	Standard error of the mean
TAD	Topologically associating domain
TB	Tuberculosis
TF	Transcription factor
TFBS	Transcription factor binding site
TLR	Toll-like receptor
TPM	Tags per million
TSS	Transcription start site
TT-seq	Transient transcriptome sequencing

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