Abstract

During embryonic development, pluripotent stem cells (PSCs) acquire defined identities culminating in the generation of terminally differentiated cell types. This process is orchestrated by the precise spatiotemporal regulation of genomic output, especially in the context of transcription and translation. However, the current understanding of the identity and function of transcriptional and translational regulators programming cell fate decisions is far from complete, specifically in the context of humans.

Using a human PSC-based cardiac differentiation model, a class of divergent long non-coding RNAs (lncRNAs), termed yin yang lncRNAs (yylncRNAs), was defined. yylncRNAs are divergent lncRNAs expressed from genomic loci encoding developmental regulators, mirroring each other's cell-type specific expression pattern. In a case study, *yylncT*, accompanying the key mesoderm regulator *BRACHYURY* (*T*), was characterized. *yylncT* localizes specifically to the active *T* locus during mesoderm commitment. Mechanistically, *yylncT* directly binds *de novo* DNA methyl-transferase 3 (DNMT3B), locally inhibiting its activity at the *T* locus during mesoderm differentiation. Consistent with a transcript-specific function, depletion of *yylncT* disrupts the activation of the *T* locus, thereby abolishing mesoderm commitment of hPSCs.

In an effort to identify currently unknown regulators of translation relevant in early embryonic development, in the second part of the thesis translation state-specific ribosome-associated proteins (TS-RAPs) were globally identified in hPSCs by polysome profiling followed by mass spectrometry. This led to the discovery of 1326 TS-RAPs, including RBPMS, a poorly characterized RBP, whose role in translation is currently unknown. Orthogonal approaches including gSTED and IP-mass spec revealed that RBPMS directly interacts with the translation machinery in hPSCs. Enhanced cross-linking followed by sequencing (eCLIP-seq) elucidated that RBPMS preferentially binds to the 3' UTRs of mRNAs coding for distinct pluripotency regulators, translational factors and secreted signaling proteins. Depletion of RBPMS in hPSCs led to global attenuation of translation including its direct targets. This led to severe defects in mesoderm induction, subsequently hampering cardiac differentiation, highlighting the pivotal role of RBPMS in the exit of pluripotency and mesoderm formation.

Collectively, this thesis revealed hitherto unknown regulators of transcription and translation that ensure embryonic cell-fate transitions in humans.