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Dissecting gene expression regulation in mouse embryonic stem cells

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av medicine doktorsexamen framläggs till offentligt försvar i Major Groove Department of Molecular Biology, University hospital area, building 6L. Fridag den 12 May, kl. 09:00.

Avhandlingen kommer att försvaras på engelska.

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Dissecting the gene expression regulation in mouse embryonic stem cells.

Abstract
Every cell within an organism is derived from a single fertilized egg that undergoes cellular differentiation and development to generate mature specialized cells. Mouse embryonic stem cells (ESCs) derived from the inner cell mass (ICM) of the pre-implantation blastocyst have proven to be a model to study gene expression during differentiation and development. In this thesis, we integrate different layers of gene expression programs, from epigenetics to post-translational regulation, to unravel the intricate network of pluripotency and differentiation in ESCs.

We show that Lysine-specific histone demethylase 1 (LSD1), an epigenetic regulator that removes mono- and di-methyl groups from lysine 4 of histone H3 (H3K4), is not essential for ESC self-renewal. However, the enzymatic activity of LSD1 is indispensable for differentiation. We observe a gain of H3K4me1 in the regulatory regions of pluripotency genes in *Lsd1* knockout (KO) ESCs that do not alter gene expression programs related to the ESC state. Additionally, we uncover that independently of its catalytic activity, LSD1 stabilizes the DNA maintenance methylation machinery, such as DNMT1 and UHRF1 proteins, through interaction with ubiquitin-specific peptidase 7 (USP7), which ultimately maintains the DNA methylation equilibrium in the ESC state.

Furthermore, we identify chromodomain-helicase-DNA binding protein 7 (CHD7) as a novel interacting partner of LSD1 in ESCs. CHD7 is an ATP-dependent chromatin remodeler that regulates cell type-specific gene expression, specifically during neurogenesis. Herein, we show that *Chd7/Lsd1* double KO ESCs showed a severe defect in differentiation, whereas *Chd7* KO ESCs differentiated with mild dysregulation of ectodermal markers. This data suggests that there is a crosstalk between epigenetic regulators which mediate a distinct set of gene expression programs during lineage-specific commitment.

Besides the core pluripotency factors OCT4, SOX2, and NANOG, a cascade of co-transcriptional events such as alternative splicing (AS) and regulation by RNA binding proteins (RBP) also play a critical role in self-renewal and cell-fate decisions. Indeed, we identify Zinc Finger Protein 207 (ZFP207) as a novel RBP, essential to maintain ESC identity *in vitro*. In addition to impaired neuroectodermal differentiation, we also find abnormal AS events that lead to a differentiated cell-like pattern upon depletion of ZFP207 in ESCs.

Altogether, the work of this thesis illustrates the complexity of gene expression regulation that modulates pluripotency and differentiation.

Keywords
ESCs, LSD1, CHD7, ZFP207, Alternative splicing, DNMT1, UHRF1

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