Mechanisms of embryonic stem cell division and differentiation

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Thesis Summary

The regulatory mechanisms governing dramatic proliferative changes during early mouse development are not well understood. This thesis aims to address this question using *in vitro* model systems of mouse embryogenesis. In particular, this thesis aimed to assess the function of the elevated, constitutive levels of cyclin dependent kinase 2 (CDK2) activity in embryonic stem (ES) and early primitive ectoderm-like (EPL) cells and the changes associated with differentiation into EPL embryoid bodies, *in vitro* equivalent of differentiation primarily to a mesodermal fate. It was determined that active CDK2 complexes associate with an increased proportion of substrates in pluripotent ES and EPL cells compared to EPL embryoid bodies. In addition, this thesis assessed the presence of other G1 CDK activity, determining that ES cells have high levels of constitutive CDK6 activity, which is refractory to inhibition by p16. Lineage specific decreases in CDK6 activity highlighted the complexities regulating cell proliferation during differentiation. Due to the reported constitutive E2F target gene expression in ES cells, this thesis also aimed to further analyse the regulation and activity of E2F transcription factors and pocket proteins in ES cells. It was demonstrated that constitutive phosphorylation of p107 and increased E2F-4 stability in ES cells contributes to increased levels of free E2F-4, that binds E2F target gene promoters *in vivo*. The importance of CDK regulation of p107 in ES cells was demonstrated by analysis of ectopic expression of phosphorylation-resistant mutant p107. The increased sensitivity of EPL cells to ectopic p107 highlighted differences in pluripotent cell populations. In addition, it was determined that differential regulation of p107 during differentiation was associated with increased p107 binding to E2F target gene promoters and decreased E2F target gene expression. Differentiation associated changes in regulation and activity of cell cycle regulators demonstrated in this thesis are important for understanding the pre-gastrulating mouse embryo and to enable regulation of pluripotent cell differentiation for therapeutic use.

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