Cell cycle regulation of gene expression at single-cell level

Forskningsleder Kedar Natarajan

Gruppens kerneforskningsområder

We all arise from a single fertilised cell that ultimately undergoes cell cycle (i.e. growth, duplication and division) and gives rise to all the ~37 trillion cells in our body. The evolutionarily conserved and tightly regulated cell cycle process not only scales up cellular numbers for making tissues, organs but also critically coordinates cell state and fate decisions. The self-renewing and pluripotent embryonic stem cells (ESCs) are precursor for specification of all lineages and ultimately all tissues and organs (Figure 1A). Not surprisingly, a dysregulated cell cycle progression is the hallmark of cancers and leads to developmental, genetic (Down syndrome), neurodegenerative and auto-immune disorders. Our research group uses a interdisciplinary systems biology approach to dissect the interplay between cell-cycle and gene expression regulation at single-cell resolution. We aim to identify new cell cycle regulators of gene expression and cell fate, which are masked and missed in population level approaches. Towards this, we use transgenic ex vivo self-renewing, pluripotent embryonic stem cells (ESCs) and in vivo mouse model systems that express fluorescence cell cycle reporters and genome editing Cas9 protein (CRISPR-Cas9) (Figure 1B). We apply and integrate a wide variety
of experimental approaches with computational methods and state-of-art functional technologies including single-cell imaging, single-cell genomics/transcriptomics/multi-omics and high-throughput sequencing. We develop computational methods for characterise cellular dynamics methods and also functionally characterise candidate roles in ESCs and in-vivo perturbation experiments.

Er du interesseret i at skrive projekt i gruppen, så kontakt:
knn@bmb.sdu.dk,
+45 65 50 89 29

Figure 1

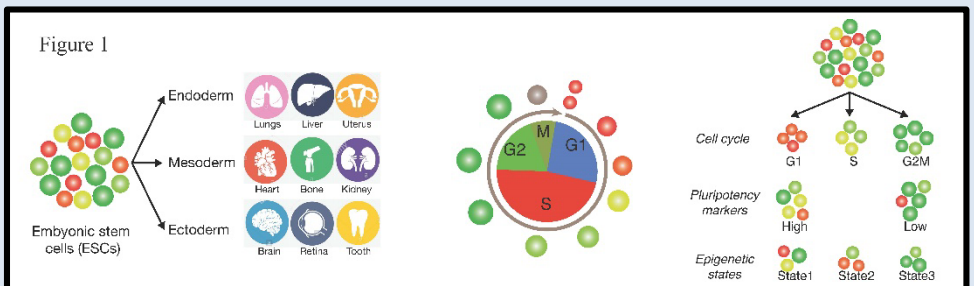


Figure 2

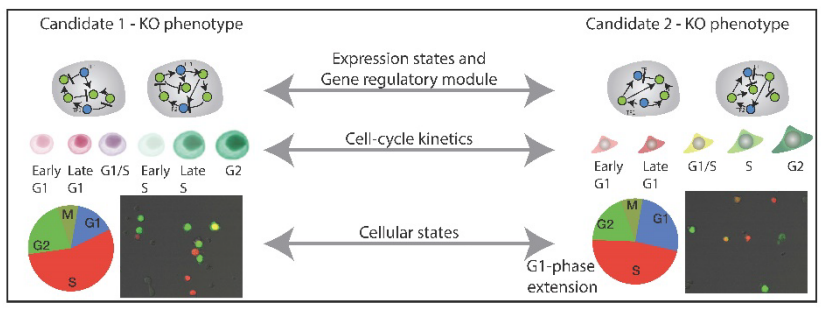
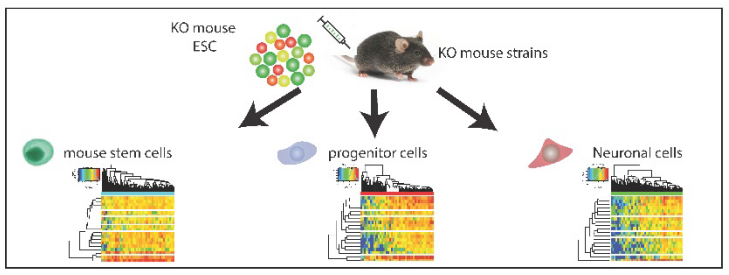


Figure 3



Projekter	Beskrivelse
Characterising single-cell cell cycle dynamics across different mouse tissues	Throughout development from 1-cell to 2-cell, 4-cell to morula, blastomeres to ICM and ESCs, gastrulation to lineage commitment, cell cycle and its dynamics coordinate gene expression and direct cell state, fate specification. Here, we will characterise cell cycle dynamics (doubling time, phase proportions, durations and kinetics) using cell cycle sensors in ESCs, their differentiation and across different mouse tissues at both single-cell and bulk level. Further characterise cells from different tissues using FACS, functional genomics methods and next-generation sequencing, to understand global heterogeneity, cell-to-cell variation, single-cell and bulk population kinetics and dynamics; and predict cell fate and responses (Figure 2A).
Computational prediction of biological process modules and networks from singlecell RNA-sequencing data	There is a wealth of publicly available high-throughput bulk and single-cell ESC data from stem cell atlas's, repositories and large consortiums. In this project, we will perform integrative computational analysis to mine and combine public data to make new predictions on factors regulating gene expression and cell state. Specifically, we will focus on known and new genes, modules and regulatory networks involved in cell cycle and gene expression regulation (Figure 2A). We will extend this computational prediction to include other biological processes as well as build new methods.
Identification and validation of new cell cycle regulators of stem cell fate using integrative single-cell approaches	In this project, we will perturb the the putative cell cycle regulators (from computational analysis) in ESCs using CRISPR-Cas9 system. We will validate perturbation using time-lapse imaging to assess bulk and single-cell phenotype, followed by genotyping and detailed characterization. This validation will help assess candidate roles in cell cycle regulation of ESCs.