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Studying the Cell Cycle using Systems Biology and High Content Characterization of the Ubiquitin Proteasome System

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ABSTRACT

The cell cycle is the process through which our cells grow and divide in a carefully orchestrated and controlled manner. An uncontrolled cell cycle is a basic hallmark of cancer, in which excessive cell growth leads to adverse effects on the tissue- and organ-level. The Ubiquitin Proteasome System (UPS) is responsible for the destruction of unwanted proteins in the cell, but has additionally been identified to inhabit regulatory roles in a myriad of cellular processes. This thesis aimed to study the cell cycle and the ubiquitin proteasome system using high-throughput and high-content approaches.

The first approach aimed at observing the endogenous fluctuations of mRNA, proteins, phosphorylations, and intra-cellular compartmentalization over the cell cycle, and how these systems are regulated and coordinated throughout the cell cycle, described in Study I and II. Aside from characterizing cell cycle oscillation patterns of transcripts, proteins, phosphorylation events and subcellular localization changes, the dynamics between transcriptional and proteomic regulation was further investigated by comparing oscillation patterns of corresponding mRNA and protein pairs.

The second approach aimed to investigate how one of the largest enzyme families in the human proteome, the UPS, affects the cell cycle and responses to external and intrinsic DNA damage. This was done through a phenotypical characterization after silencing the genes comprising the family in a high-content imaging study, Study III. The results revealed many novel UPS genes as essential for proper progression through the cell cycle and maintenance of DNA integrity. By combining multiple reporter systems in one high-content study, correlations between cell cycle, viability and DNA Damage Response phenotypes could be performed. This revealed an increased tendency for G1/S-phase cell cycle arrests after signs of spontaneous DNA damage, and an enrichment for G2 cell cycle arrests after failure of 53bp1 recruitment to double-strand breaks.

Aside from providing data resources and system biology results regarding the interaction between different cellular process, the studies also identified specific genes and proteins in novel roles regarding these basic cellular processes. In Study II, the methyl-transferase protein MAT2A was discovered to change subcellular localization in synchronization with the cell cycle, possible to provide the higher source of methyl groups needed during S-phase and G2-phase. In Study IV, the E3 ubiquitin ligases ARIH1 and ARIH2 were investigated for effects on proliferation and growth of glioblastoma multiforme. The high-content Study III identified many novel UPS genes with a myriad of cell cycle and DNA damage phenotypes, among them the E3 ubiquitin adapter BTBD1. Silencing of BTBD1 incurred dramatic phenotypes on the cell cycle and DNA damage responses, and BTBD1 was further characterized and identified to be essential for proper function of the DNA topoisomerase TOP1.

Throughout these projects, in order to validate novel findings, methods to control specific gene expression levels was developed, utilizing shRNA and CRISPR/Cas9-mediated silencing as well as a flexible method of overexpression. These systems are described in Study IV.

The presented studies combine high-content and high-throughput approaches with novel visualization and analysis methods to distill information from complex data, both to summarize interactions between mRNA, proteins, and function, but also to identify novel regulators of basal cellular processes.