ENGLISH SUMMARY

Driving Mitosis by Cdk1 and Greatwall

Cell proliferation relies on the existence of a so-called cell division cycle. The genetic information in our DNA controls many, if not all, processes that are needed to direct this cycle. The human genome is stored on 23 chromosome pairs, of which only 1 pair is sex-determining. The genome harbours roughly 19,000 genes of which each is designated to encode for a specific protein. Whereas not all proteins may be essential, many of them are given a particular function that makes them important for certain biological processes. These processes may include energy production, which is necessary to build proteins but is also of vital importance for cell cycle continuation. Protein synthesis in G1 phase of the cycle precedes the duplication of the genomic DNA in S phase. Since the genome is essential for cell viability, its duplication ensures that two of its exact copies are presented in the newly formed daughter cells, once the cell cycle is completed. During G2, another phase where cells continue to grow, additional proteins are synthesized that enables the onset of the final stage, mitosis. Here, the duplicated genome is disjointed and distributed over the two cells that are formed. Mechanical forces physically separate the daughter cells, and so ensure both copies of the DNA are equally divided (see Figure 1, **Chapter 1**).

The major driving forces behind the cell cycle are enzymes known as the Cdks, in complex with their respective cyclin counterpart. Together, these cyclin-Cdk complexes may modify a broad range of substrates by a process called protein phosphorylation. These modifications are notorious for their regulation of protein function and are needed to drive certain cellular processes. The major Cdk that promotes cell division is Cdk1. This kinase partners with either cyclin A or cyclin B, of which both are able to direct the substrate specificity of Cdk1. The substrates phosphorylated by cyclin A-Cdk1 and cyclin B-Cdk1 may overlap to a certain extent. The phosphorylation status of these Cdk1 targets may either have a positive or negative regulatory effect on the respective substrate. In any case, the 'phosphoregulation' of Cdk1 targets is essential to drive mitosis.

Mitosis is driven by numerous protein kinases that take orders from their royal leader, Cdk1. In **Chapter 2** we have investigated the requirement of a kinase called MASTL in the commitment to mitosis. We demonstrate that MASTL functions as the human orthologue of Greatwall kinase, and that it specifically acts by suppressing rebellious PP2A-B55. PP2A is a phosphatase that counteracts Cdk1 by erasing the phosphorylation marks that it puts onto substrates. Activation of Cdk1 alone is insufficient to allow for its complete substrate phosphorylation; the inhibition of PP2A-B55 signifies an additional layer of regulation. Our data reveals that MASTL itself is highly phosphorylated in mitosis, most likely by cyclin B-Cdk1. We show that transient depletion of *MASTL* prevents the inhibition of PP2A-B55 at the decisive G2-to-mitosis transition point. While cells are still able to activate cyclin B-Cdk1, its peak level of substrate phosphorylation is clearly reduced. We provide evidence that this reduction in cyclin B-Cdk1 substrate phosphorylation may have deleterious

consequences for daughter cell formation. We show that cells are able to complete mitosis with high PP2A-B55 activity, but remain stuck at the final stage of abscission. The presence of so-called DNA bridges between anaphase chromosomes maintains connectivity between the daughter cells (see also Figure 3, **Chapter 5**). These DNA bridges are resolved in the successive cell cycle, but depending on their severity, may be a potential source of genome instability.

This work was further extended in **Chapter 2** (**Addendum**), where we investigated the relative contribution of the putative MASTL substrates Arpp19 and Ensa in the ability to suppress PP2A activity. We show that the evolutionary conserved Greatwall phosphorylation site in both proteins is essential to convert them into potent PP2A inhibitors. In addition, we elaborate on the specifics of DNA bridge establishment (**Chapter 3**). Our data demonstrates that depletion of *MASTL* blocks the destruction of cyclin B1, a mechanism that normally ensures the inactivation of mitotic Cdk1. These findings expand our current understanding of cyclin B1 proteolysis by the APC/C, a machine that is designed to destroy mitotic cyclins. We show that MASTL enhances the cyclin B1-Cdk1–dependent phosphorylation of the APC/C. Consequently, cyclin B1-Cdk1 directs its own inactivation mechanism by targeting cyclin B1 for degradation. We suggest that excess cyclin B1, present in anaphase, may still have an inhibitory role towards separase, the protease that directs the physical separation of each chromosome pair. As a result, some sister chromatids may remain connected during mitotic exit, forming the DNA bridges that are visible in G1 phase. This stresses the importance of the timely cyclin B1 destruction that occurs before anaphase.

In **Chapter 4** we set out to identify the target proteins that are negatively regulated by cyclin B-Cdk1. Our approach makes use of a small-molecule that specifically inhibits Cdk1 activity. We demonstrate that partial inhibition of Cdk1 activity allows cells to enter mitosis, but causes a severe problem in prometaphase. Mitotic cells with suboptimal Cdk1 activity fail to align their chromosomes and delay significantly in mitosis. Once these cells exit from mitosis, they often produce non-identical daughter cells due to imbalanced chromosome segregation in anaphase. As a result, many of the generated cells are incompatible with life and enter a death state known as apoptosis. We describe an elegant genome-wide drug sensitivity screen and reveal splice variant of PRC1 (PRC1-1) and its binding partner, the motor protein KIF4, as key inducers of fatal mitosis in response to Cdk1 inhibitory strategies. Our data suggest that cyclin B1-Cdk1 has an essential function in preventing the onset of late mitotic events which requires the inhibition of both PRC1-1 and KIF4.

Finally, in **Chapter 5** we discuss the consequences of Cdk1 suppression by multiple approaches and how this affects cell cycle progression. We elaborate on the cyclin B-Cdk1 activity thresholds that dictate whether a cell commits to mitosis, or, alternatively, keeps cells in G2 phase. In addition, we comment on the reversal of cyclin B1-Cdk1 phosphorylations during mitotic exit and how mitotic phosphatases promote their own activation once cyclin B1 is eliminated. We conclude by highlighting the importance of proper cyclin B1-Cdk1 activation

in the execution of mitosis. Any defects in its activation may promote genomic instability, a major driving force for tumorigenesis.