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Transcriptional Regulation at the G2/M Transition in the Budding Yeast, *Saccharomyces cerevisiae*

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Abstract

The eukaryotic cell cycle is a highly ordered and tightly regulated process through which a cell replicates its genetic material, segregates its sister chromatids and divides into two genetically identical cells. Regulation and ordering of the cell cycle is through cyclin dependent kinases (cdk). The cdk is a protein complex that is comprised of a catalytic subunit (cdk) and a regulatory subunit (cyclin) which, as their name indicates, oscillate throughout the cell cycle giving specificity to the cdk for different phases of cell division. This oscillation is controlled, at least in part, through gene regulation.

Microarray analysis performed on the budding yeast, *Saccharomyces cerevisiae*, revealed several waves or "clusters" of transcriptional activity associated with cell cycle progression. One of these "clusters", the "CLB2 cluster", is comprised of 35 genes that are important for the G2/M transition and mitotic progression. Previous work on the upstream activating sequences of two "CLB2 cluster" members, *CLB2* and *SWI5*, revealed the binding of an Mcm1p homodimer and, until recently, an unidentified activity SFF (*SWI5* Factor). Recently in our laboratory an activity that followed SFF binding *in vitro* was purified and identified as Fkh2p.

In this thesis the biochemical and genetic characterisation of Fkh2p identifies it as a major component of SFF. Firstly, Fkh2p has been shown to bind DNA in an Mcm1p

dependent manner and that the Fkh2p DNA binding domain is essential for this interaction. Furthermore, the protein interaction domain of Mcm1p has been demonstrated to be essential for ternary complex formation. Through deletion studies, Fkh2p, along with a functionally redundant protein Fkh1p, have been shown to control the periodic expression of the "CLB2 cluster" genes. Furthermore, the functional characterisation of Fkh2p domains reveals an important role for both the Forkhead associated domain and the C-terminus. Finally, Ndd1p, another protein important for mitotic progression, is shown to be important for "CLB2 cluster" regulation by de-repressing Fkh2p and activating gene expression. In addition, the role of cdk activity is shown to act through the "CLB2 cluster" upstream activating sequences, possibly through Ndd1p.