Chapter 1

INTRODUCTION

Cell cycle in eukaryotes is a very vital process which requires tight and precise regulation. Any deregulation in the process may lead to genomic instability resulting in various diseases in human including cancer. The dynamicity of cellular proteome with respect to relative abundances and functions of its components plays a major role in regulation of eukaryotic cell cycle progression. In addition to the regulation at transcription level, the post-transcriptional processes including mRNA turnover, epigenetic mechanisms and post-translational modifications contribute critically to regulate cell cycle progression. Among various post-translational modifications such as phosphorylation, acetylation, methylation, glycosylation and ubiquitination, which are critical for cellular functions, reversible phosphorylation is the major driving force of eukaryotic cell cycle, including the faithful replication of genomic DNA. In fact, all the phases of eukaryotic cell cycle are strictly monitored by the master regulators cyclin dependent kinases (Cdks) that phosphorylate serine/threonine residues of various target proteins contributing crucially to the diverse activities of the regulatory process. The most important job of the eukaryotic cell cycle machinery is to ensure just one round of precise and accurate replication of the vast genome in a particular cell division cycle. Considering the fact that replication initiates from thousands of origins in eukaryotes to complete the process in a reasonable duration of time, a strong licensing mechanism is active to allow firing of an origin only once during particular cycle. Phosphorylation events by cyclin-Cdks play an important role in regulating the replication licensing in eukaryotes.
The replication in eukaryotic cells takes place in synthesis or S-phase and the replicated DNAs are segregated into daughter cells in mitosis or M-phase. After the completion of one cycle at M-phase, the subsequent S-phase initiates after a gap or G1-phase. Similarly, another gap or G2-phase exists between S- and M-phases. The cell cycle begins at the end of G1-phase with the activation of a series of cyclin-Cdks affecting the initiation of DNA replication at S-phase. The cyclin-Cdk activity remains high during S, G2 and M-phases and cells exit mitosis with the decline of the kinase activity. Therefore, the cell cycle can broadly be divided into two periods based on the presence or absence of Cdk activity – low Cdk activity period during most of G1-phase and high kinase activity period during S, G2 and M-phases. Intriguingly, such a periodic variation of low and high Cdk activity stages drives the replication licensing to ensure one round of replication per cycle. A licensed pre-replication complex consisting primarily of the origin recognition complex (ORC), Cdc6, Cdt1, and Mcm2-7 is formed only during the low Cdk activity stage in G1-phase. In S-phase, when cyclin-Cdks become active, it phosphorylates various components of pre-replication complex affecting the firing of the licensed origin and initiation of replication. Interestingly, the phosphorylation of the components of pre-replication complex either leads to ubiquitination of the proteins followed by proteasome mediated degradation or their export out of the nucleus. Thus, during S to M-phases, the high Cdk activity prevents the assembly of pre-replication complex at the origins which have already fired. A licensed pre-replication complex can only be formed again at the same origin once the cell completes the mitosis and proceeds to G1-phase of the next cycle having low Cdk activity. The regulatory process is a very complex one involving dynamic interaction between many factors, in addition to the major pre-replication components as mentioned above, modulated by post-translational modifications, most importantly by reversible phosphorylation. More recently, the involvement of DNA repair protein Ku in the assembly of pre-replication complex has been established, though the mechanism of regulation of its periodic activity during cell cycle progression remains unknown. Interestingly, many potential Cdk target motifs are present in Ku70 subunit of the heterodimeric protein and some of the cyclin-Cdks have been shown to phosphorylate it. However, the functional significance of such phosphorylation events remains to be established.

Therefore, in the present study, this lacuna in the knowledge regarding Cdk phosphorylation dependent modulation of the activity of Ku protein has been addressed in
order to elucidate the mechanism of its replication related function during cell cycle progression. Before reporting the experimental result, an in-depth and critical review of the current scientific literature on post-translational modification, regulation of eukaryotic cell cycle particularly by cyclin dependent kinases, replication licensing and cellular functions of multifunctional Ku protein is presented in the next chapter of the thesis. Then the aims and objectives of the study are explained in the chapter after that. Next, the information on the materials used and the experimental techniques are explained in detail in Chapter 4. Most of the details about the source of the chemicals and reagents, buffers, vectors and strains, peptides, primers and oligonucleotides are presented in tabular forms for easy reference. The detailed experimental results are described with illustrated figures and graphs in Chapter 5. Briefly, the results described in the Chapter provide an insight how the phosphorylation of Ku70 subunit by cyclin-Cdks regulates its replication related activity during cell cycle progression. Due to the formation of the complexes of Ku70 with cyclin-Cdks and its phosphorylation, the Ku dimer is disrupted. This results in the decrease of the amount of functional Ku dimer available for binding to replication origins during the high Cdk activity phases, thus preventing premature assembly of origin recognition complex till the end of mitosis. After the degradation of cyclin B1 at the end of M-phase, Ku70 can be converted to dephosphorylated state and is able to form heterodimer with Ku80 for taking part in origin licensing during G1-phase. Finally, in Chapter 6, the importance of the findings of the present study is discussed in the context of available knowledge in the relevant field of research and concluding remarks including the future prospects are made. The detailed references of all the articles consulted during the whole study are listed in the Bibliography section. The work has been published in a peer-reviewed journal and presented in national conferences whose detailed information is provided at the end of the thesis. A copy of the published article is also attached.