Introduction

During successive cell division cycles, identical genetic information must be precisely transmitted from the mother cell to the two daughter cells. This is achieved by duplicating the cell's genomic DNA in S-phase and distributing the two copies equally to the daughter cells at M-phase. Complete and accurate DNA replication is integral to the maintenance of the genetic integrity of all organisms. Sequential assembly and reorganization of complex arrays of proteins are crucial for the coordinated execution of initiation, elongation, and termination processes of DNA replication (Hayashi and Masukata, 2010). In metazoans, the rate of DNA replication is regulated at the initiation step by the judicious activation of an appropriate number of initiation events proportional to the rate of cell division. In order to duplicate relatively large genomes of eukaryotic cells rapidly within S phase, DNA replication initiates from multiple distinct sites called origins which are distributed fairly regularly along chromosomes (Lei and Tye, 2001).

By separating replication initiation into two steps, cells insure that each origin undergoes only one initiation event in each S phase and that the genome is duplicated precisely once in each cell cycle (Arias and Walter, 2007). During the first step called "licensing", which occurs in early G1, the pre-replicative complexes assemble at origins via the sequential binding of the ORC, Cdc6, Cdt1, and the MCM2-7 helicase. In the second step, which occurs at the G1/S transition, S phase-specific kinases co-operate with numerous factors including Cdc45 to activate the MCM2-7 helicase, leading to origin unwinding and replisome assembly. These events are tightly controlled during cell cycle by separation of pre-RC formation and replication initiation in to two mutually exclusive phases (Lebofsky and Walter, 2007).

The accessibility of origin to the proteins that initiate replication is influenced by chromatin structure. This is not only critical for selection of origins but also determines the replication timing of the selected origins. Generally, the replication origins located in a relatively open chromatin domain are considered to be more efficient. Indeed, chromatin modifications mediated by histone acetyl transferases (HATs) and histone deacetylases (HDACs) impact the activation of replication origins. In addition to histone modifiers, the ATP-dependent chromatin remodelers also have been shown to be required for replication initiation. Thus, cell cycle control of chromatin remodeling and epigenetic modifications at origins seems to be key determinants of replication initiation. However, the contribution of chromatin structure and remodeling in the regulation of mammalian origins remain obscure (Hayashi and Masukata, 2010).

The human lamin B2 replicator is one among the few well characterized mammalian replication origins that has confined initiation sites (Paixao *et al.,* 2004). It is one of the early replicating origins and overlaps with the 3' end of the lamin B2 gene and the promoter of the TIMM 13 gene (Falaschi *et al.,* 2007). The knowledge of protein-DNA interactions along the topological structure in the origin activation/deactivation cycle, prioritize lamin B2 as the only human replicator studied so far in terms of the molecular and structural transactions. The topology of DNA alone do not specify the functional modulation of origin, rather, the interrelated chromatin structure is also indispensible. Nevertheless the specialized chromatin dynamics of human lamin B2 replicon and its role in replication initiation remains enigmatic.

Another important question in understanding the regulation of DNA replication is how transcription and replication are co-ordinated at the chromatin level to avoid polymerase collisions (Aladjem, 2007). Central to this, the chromosomal traffic control regulated by both replication and transcription machineries at lamin B2 replicon is perplexing ever since of its mapping between two transcribing genes. Further, the role of

transcription factors are not only limited to transcription as evident from its role on activation of replication origins by assisting the binding of replication initiation proteins (Kohzaki and Murakami, 2005). However, the molecular cross-talk of transcription factors in the initiation of replication from lamin B2 replicon remains elusive albeit of its co-localization with the active promoter of TIMM 13 gene.

In order to puzzle out these issues, the present doctoral thesis is focused on the following objectives:

1. Elucidation of chromatin structure of lamin B2 replicon.
2. Exploring the molecular underpinnings of chromatin dynamics and replication initiation at lamin B2 replicon.
3. Analyzing the plausible role of transactivators in the regulation of replication initiation at lamin B2 replicon.