Chapter I
Introduction

The maintenance of the genetic material DNA in every dividing cell is crucial as it transfers genetic information from one generation to the next. Cells duplicate their DNA by a complex but highly regulated process, called DNA replication. To maintain the genomic integrity the fidelity of this process must be very high.

In prokaryotes, according to ‘replicon model’ proposed by Jacob et al. (1963), initiation of DNA replication is governed by two major components- replicator and initiator. These both are critically important for the initiation of DNA replication. Replicator is a cis-acting genetic element on chromosomes to which trans-acting proteins, i.e., initiators bind and initiate DNA replication. In circular prokaryotic chromosomes, replication starts at a single site and the replication forks moving away from each other finally merge resulting in to complete duplication of the genome. Thus the prokaryotic chromosomes act as a ‘single replicon’ (Jacob et al. 1963).

On the other hand, large linear chromosomal DNA molecules in eukaryotes contain multiple initiation sites (origins), distributed along the length of the chromosomes at intervals ranging from 10 kb to >300 kb (Huberman and Riggs 1968; Yurov and Liapunova 1977; Dubey and Raman 1987). Among eukaryotes origins are best characterized in the yeasts, Saccharomyces cerevisiae and Schizosaccharomyces pombe where short specific DNA fragments function as origins. These short DNA fragments are called Autonomously Replicating Sequence (ARS) elements because plasmids bearing them can replicate autonomously in yeast cells and transform yeast cells at a high frequency (Stinchcomb et al. 1979; Struhl et al. 1979; Hsiao and Carbon 1979; Reviewed in Campbell and Newlon 1991). Different origin mapping techniques have demonstrated that ARS elements can function as replication origins in plasmids as well as in chromosomes (Brewer and Fangman 1987; Huberman et al. 1987; Friedman et al. 1997; Yamashita et al. 1997).

In S. cerevisiae, ARS elements are 100-150 bp long and have unique characteristic 11 bp conserved nucleotide sequence, the ARS consensus sequence (ACS), which is essential for origin activity (Campbell & Newlon 1991; Newlon et al. 1993). Single base pair deletion or mutation
in this consensus sequence results into loss of origin activity. Majority of these origins function efficiently on chromosomes as well as in plasmids firing once in almost every cell cycle.

Replication origins of *S. pombe*, unlike *S. cerevisiae* are larger, 600-1200 bp, and there is no consensus sequence ([Maundrell et al. 1988](#)). *S. pombe* origins are highly A-T rich, located in intergenic regions where the stretches of asymmetric As and Ts are present throughout their length ([Zhu et al. 1994; Clyne and Kelly 1995; Dubey et al. 1996; Kim and Huberman 1998; Okuno et al. 1999](#)). Origins in *S. pombe* show greater variation in their activity as compared to *S. cerevisiae*. Generally, fission yeast cells appear to contain a much larger proportion of weak replication origins than do budding yeast cells. The replication origins in mammalian cells, due to lack of any signature sequence are not defined well ([Leonard and Méchali 2013](#)).

It is important to note that each segment of genome in eukaryotes replicates at a precise time during S phase of the cell cycle. The replication timing control, in which a replication origin fires at a prefixed time during S phase, is common in eukaryotic chromosomes from yeasts to higher eukaryotes ([MacAlpine and Bell 2005; Gilbert et al. 2010](#)). It is suggested that the replication timing program in mammalian cells is established during early G1-phase of the cell cycle, called timing decision point (TDP) ([Dimitrova and Gilbert 1999](#)). Earlier, replication studies on mammalian chromosomes have shown that the Giemsa light R bands replicate early while the dark G bands replicate late in S-phase. Surprisingly, the G-bands have higher A-T content than the R-bands ([Latt 1975; Holmquist et al. 1982](#)). In yeasts, especially *S. cerevisiae* and *S. pombe*, origin licensing takes place at all the defined sites, but only the specific sets of origins fire in early S phase ([Raghuraman et al. 2001; Wyrick et al. 2001; Hayashi et al. 2007](#)).

The temporal replication pattern in eukaryotes is governed by several factors including the chromatin structure, chromosomal position, nuclear localization and various *cis* and *trans*-acting regulators. Replication origins present in euchromatic region generally fire early and those in heterochromatic regions fire late ([MacAlpine et al. 2004; Hiratani and Gilbert 2009](#)). In heterochromatin, origins present in telomere or subtelomere regions fire late while those in centromere or silent mating type (mat) locus replicate early in *S. pombe* ([Kim et al. 2003; Hayashi et al. 2009](#)). Epigenetic marks like, increased histone acetylation leads to an early activation of the late origins ([Vogelauer et al. 2002](#)). Various studies have shown the role of nuclear positioning in replication timing control. Early replicating regions are generally present
in inner nuclear spaces while the late replicating regions are associated with insoluble nuclear matrix (reviewed by Aparicio 2013 and Renard-Guillet et al. 2014).

Recently, in *S. cerevisiae* and *S. pombe*, the global replication timing regulators have been identified (Knott et al. 2012; Tazumi et al. 2012; Hayano et al. 2012). In *S. cerevisiae*, the transcription factors Fkh1 and Fkh2 were found active in replication timing regulation of early origins. These bind with fkh1/2 binding sites, present near early replicating origins, and facilitate their early activation (Knott et al. 2012; Lõoke et al. 2013). In *S. pombe*, Taz1 and Rif1, the two telomere binding proteins control replication timing of late replicating origins. Taz1 binds with a 50-bp fragment containing two sets of telomeric repeat motifs, present near a subset of late origins and regulate timing of those origin in a Rif1 dependent manner (Tazumi et al. 2012). Rif1 mediated replication timing control is conserved from yeasts to mammalian cells (Hayano et al. 2012; Cornacchia et al. 2012; Yamazaki et al. 2012; Peace et al. 2014). In rif1Δ *S. pombe* cells, the replication timing pattern of early and late origins is disturbed. A set of late origins start firing early while the early origin’s activation is delayed (Hayano et al. 2012). Recently, the Rif1-binding sites have been discovered which are present in close vicinity of a subset of late origins (Kanoh et al. 2015).

Binding of Fkh1/2, Taz1 and Rif1 to *cis*-acting genetic elements has suggested the possible role of such elements in replication timing regulation. In *S. pombe*, a 200-bp stretch containing a cluster of three 10-bp G rich consensus sequence, called late consensus sequence (LCS), regulates the timing of associated origins in plasmid context. The 200-bp late replication enforcing element is capable of delaying the replication timing of *ars727* in a mono-ARS plasmid and the timing of an early replicating r-DNA origin, *ars3001*, in a bi-ARS plasmid, in orientation dependent manner (Yompakdee and Huberman 2004). This was the first high resolution characterization of any *cis*-acting element which controls the replication timing of associated origins in plasmids. The replication properties of *S. pombe* origins vary between plasmids and chromosomes, e.g., *ars727* is chromosomally inactive, but fires late in plasmids (Kim and Huberman 2001; Yompakdee and Huberman 2004; reviewed in Masukata 2004). While studying the replication timing of a 75-kb region of chromosome II, Dubey et al. (2010) have found a shift from early-to-late replication within 10-kb of *ars727* which indicates the involvement of LRE in replication timing transitions in that locus.
To characterize the functionality of LRE in replication timing regulation in chromosomes the present study was planned with the following objectives-

1. To confirm the role of LCS in determining chromosomal replication timing.

2. To find out the effect of LCS on other replication origins.