Chapter - 1

General Introduction & Objectives
1.1 Nuclear architecture of a eukaryotic cell

The organization of chromosomal domains in the cell nucleus is a key contributor to genome function. Chromosomes are non-randomly positioned in the nucleus and occupy spatially distinct and well-defined sub-compartments of the nucleus referred to as the chromosomal territory (Cremer and Cremer, 2001; Parada and Misteli, 2002). In addition to chromosomal territories, specialized sub-compartments can be discerned in the nucleus. For instance, the nucleolus is the site for ribosomal DNA transcription and processing (Olson et al., 2000), or Splicing factor compartment that stores components required for processing of pre mRNA and provides them to the site of transcription in its vicinity (Misteli, 2000; Spector, 1990; Spector et al., 1991) and PML bodies (Dundr and Misteli, 2001). Using cytological techniques like fluorescence in-situ hybridization (FISH) and chromosomal painting along with live cell imaging microscopy and more advanced spinning-disc confocal imaging, chromosome organization has been studied. This organization has implications in genome stability and gene regulation (Meaburn and Misteli, 2007; Parada and Misteli, 2002; Parada et al., 2004).

Nuclear processes like transcription, RNA processing, DNA replication and repair occur in spatially constrained discrete regions within the nucleus. These “compartments” are membraneless sub-organelles characterized by a distinct set of resident proteins. The structural basis of this organization is not very well understood. Macromolecular self assembly and interaction with the nuclear skeleton or matrix have been proposed to play a role this compartmentalization (Misteli, 2001). Any disturbance in the architectural framework of nuclear compartments leads to diseased state of the cell. For example, mutations in \textit{EMD} or \textit{LMNA} genes encoding nuclear envelope structural proteins and causes a heritable degenerative disease called Emery–Dreyfuss muscular dystrophy (EDMD), that leads to muscle wasting and cardiomyopathy (Capell and Collins, 2006). The search for molecular mechanisms have shown that mutation in \textit{EMD} or \textit{LMNA} cause disturbance in the architecture of cell due to defect in nuclear architecture. The nuclear envelope links the nucleus to the rest of the cell, not only by harbouring nuclear pores that regulate the molecular transport, but also interconnecting
the nucleus to the cytoplasm through direct interaction with cytoskeleton elements, including cytoplasmic actin and microtubules. This shows that proper nuclear organization is essential for normal functioning of the cell, but however, the molecular mechanisms that act as driving forces for spatial distribution and maintenance of nuclear compartments are still largely unclear. For example, it remains unclear whether the symptoms of EDMD result directly from structural defect in the nuclei of muscle cells or a histone methylation patterns in lamin A mutants (Bank et al., 2011). Even though a lot of cell biological data has been accumulated based on tracking of chromosomes and the proteins associated with them, a systematic analysis of the structural and molecular basis of chromosome organization in the context of nuclear architecture has not been done.

1.2 Spatial organization of sub-nuclear compartments

Two conceptually distinct models were proposed to describe the positioning of chromosomal domains in separate sub-nuclear compartment of cell nucleus. First, genome organization may be determined by the distinct structural and physical properties of transcriptionally active and silenced regions of the genome. Gene rich and transcriptionally active regions are generally considered decondensed whereas silenced genome regions are generally in a more condensed state. It is possible that structurally similar regions might have occupied a distinct sub-nuclear compartment (Iborra and Cook, 2002). Self -organization model proposes that, the silenced regions of several chromosomes may associate with each other in three-dimensional space at the nuclear periphery and active regions are physically segregated from silenced regions and occupy the interior region of the nucleus (Cremer and Cremer, 2001).

The most prominent example for self-organization of sub-nuclear compartment is nucleolus. Clustering of transcriptionally inactive regions of several chromosomes occurs in the nucleolus where the tandem repeats of ribosomal genes from several chromosomes congregate. The nucleolus disintegrates during cell division when ribosomal DNA is repressed and reappears during late telophase when ribosomal DNA
transcription resumes (Dundr et al., 2000; Olson et al., 2000). Disappearance of nucleolus by inhibition of ribosomal RNA synthesis and de novo formation of micronucleoli by introduction of extra copies of rDNA provides an experimental evidence for self-organization of sub-nuclear compartments (Oakes et al., 1998; Oakes et al., 1993). The clustering effect might be due to the interaction of heterochromatin and euchromatin specific proteins.

Self-organisation of genomic domains is also observed by regulatory interactions between multiple chromosomes with shared transcription factors (Misteli, 2009). For example, in humans, activation of \( IFN-\beta \) gene located on chromosome 9 is proximal to its regulatory enhancer regions on chromosome 4 and 18 showing that functionally co-regulated regions on different chromosomes coalesce by transient interactions of regulatory and transcriptions factors (Apostolou and Thanos, 2008). Similarly, clustering of all \( tRNA \) genes located on distinct chromosomes in three-dimensional space near the nucleolus in \( Saccharomyces cerevisiae \) also suggests that regions in different chromosomes coalesce for efficient transcription and processing (Thompson et al., 2003). Taken together, these data support that self-organization is the most convincing model to bring about stable spatial genome organization because it accounts for several prominent features of chromosome and gene positioning patterns.

An alternative to a self-organization model suggests that spatial genome organization is based on the interaction of chromatin regions with specific architectural elements of the cell nucleus such as components of the nuclear matrix or other as yet unidentified protein(s). For example, SATB1 is a thymocyte specific architectural protein that appears to form a nuclear network and specific chromatin regions of thymocyte specific genes associated with it, in an activity dependent manner (Parada et al., 2004).

A further likely contributors to spatial genome organization are centromeres and telomeres. Centromeres are chromosomal elements that are found in all eukaryotes and contain repetitive sequences. In many organisms including yeast and mammals, centromeres are clustered together into multiple groups within the nucleus (Jin et al., 2000; Martou and De Boni, 2000). In other organisms, including \( Drosophila \) and plants,
centromeres are anchored to the nuclear periphery (Abranches et al., 1998; Hochstrasser et al., 1986). These observations suggest that centromeres might contribute to genome organization by bringing chromatin from several chromosomes together and maintaining heterochromatic foci. Because centromeric heterochromatin regions can act as silencers in trans, their silencing activity might also constrain the ability of chromatin regions from multiple chromosomes to dissociate from each other (Csink and Henikoff, 1998; Dernburg et al., 1996).

1.3 Heterochromatin and euchromatin compartments in eukaryotic nucleus

Several experimental models support that gene poor and transcriptionally inactive heterochromatin are preferentially associated or found at nuclear periphery and gene rich and highly transcribed regions at centre of the nucleus (Kozubek et al., 2002; Lukasova et al., 2002; Tanabe et al., 2002). This pattern of spatial organization of sub-nuclear compartments is an intriguing and attractive mechanism to bring about the chromosomal domains and genes is largely driven by the sum of all interactions acting on a given genomic region by regulatory elements associated with one chromosome or another tethering to the nuclear periphery and nucleating a target gene or clustering of active genes more interior of the nucleus. This pattern of spatial organization varies in different cell types and even in same cell type, re-organization of nuclear compartments takes place during cell division and in response to external stimuli (Cremer et al., 2006; Kim et al., 2004; Misteli, 2005; Walter et al., 2003). All these observations suggest that chromosomes or target genes occupy spatially distinct sub-compartments thereby reducing its degrees of spatial freedom and thus contributing toward the formation of a global localization pattern. However, this spatial organization cannot apply to all genes, and probably not even majority of genes. The same correlation has been made for single genes in numerous experimental evidences where inactive regions move from nuclear periphery to the centre of the cell nucleus.

Taken together, these data support that genome functions occur in the context of nuclear architecture. However, the mechanisms involved in the organization of sub-
nuclear compartments within the three dimensional space of the nucleus are not known much. Therefore, elucidation of the molecular mechanisms underlying in the non-random organization of eukaryotic nucleus may help to better understand its genome function.

1.4 Organization of transcriptional silent domains in \textit{S. cerevisiae}

A striking example of the spatial organization of chromosomes is seen at the HM loci, telomere regions and rDNA locus that are refractory to transcription (silenced) in budding yeast, \textit{Saccharomyces cerevisiae} (Fox and McConnell, 2005; Lustig, 1998; Rusche et al., 2003). Telomeres are specialized nucleoprotein structures with tandem repeats of TG\textsubscript{(1-3)n}, which serve as protective “caps” on chromosome ends. In yeast, telomere and subtelomeric regions are highly compacted like the heterochromatin of higher eukaryotes and are inaccessible to RNA polymerase II (Gottschling et al., 1990). These highly ordered telomeres are clustered together into 3-8 foci at the nuclear periphery (Hediger et al., 2002; Loayza and de Lange, 2004). These clusters also contain the cryptic mating-type loci \textit{HML} and \textit{HMR} on either arms of chromosome III. \textit{HML} and \textit{HMR} contain the genes encoding for \textit{\alpha} mating type and \textit{a} mating type respectively. These two copies of mating information are transcriptionally repressed and serve as donors for the active mating type locus, \textit{MAT} (Brand et al., 1985; Rine and Herskowitz, 1987). Both telomeres, along with the sub-telomeric regions, and the HML/R loci are heterochromatinized (Rusche et al., 2003). \textit{rDNA} locus contains about 100-200 copies of 9.1kb array of genes encoding 35S and 5S ribosomal RNA separated by nontranscribed spacer regions NTS1 and NTS2 on chromosome XII and maintain as silenced domain (Bryk et al., 1997; Smith and Boeke, 1997).

Immunofluorescence studies showed that all 64 telomeres of the 32 chromosomes in a diploid \textit{Saccharomyces cerevisiae} are clustered together into 3-8 foci during interphase near the nuclear periphery (Gotta et al., 1996). Telomeres are co-localized with \textit{HM} loci forming a sub-nuclear heterochromatin compartment at the nuclear periphery (Laroche et al., 2000). When subtelomere regions were observed in a population of living cells, they were consistently found to be at nuclear periphery.
Telomeres are known to be clustered at nuclear periphery by two redundant pathways. Telomere end binding heterodimer protein Yku70/80p anchors telomeres to the nuclear periphery through interaction with unidentified membrane proteins of nuclear envelope and/or the nuclear pore complex (Hediger et al., 2002). In the absence of any one of the heterodimeric subunits of Yku, some telomeres lose their association with nuclear periphery and delocalize more into the nuclear interior while others remain anchored to nuclear periphery by a Sir4-dependent mechanism (Laroche et al., 1998). The interaction between the C-terminal domain of Sir4 and the perinuclear protein, Esc1p, is sufficient which in turn is bound to telomeres and anchor them to the nuclear periphery (Andrulis et al., 2002; Hediger et al., 2002; Taddei et al., 2004). Mps3p, a SUN (Sad1-UNC-84) domain protein of inner nuclear membrane that is enriched at the SPB also interacts with Sir4p through its N-terminal acidic domain and tethers the telomeres to the nuclear periphery (Bupp et al., 2007).

1.4.1 Sub-nuclear organization favours gene silencing

The highly ordered organization of silent chromatin consisting of the telomeres and \( HM \) loci, at nuclear periphery maintains higher concentration of Sir (silent information regulator) proteins in its vicinity, which is essential for efficient repression of silent chromatin (Gotta et al., 1996). The tandem repetitive DNA at the telomeres provides sequence specific binding sites for Repressor and Activator protein (Rap1p) and pools this protein near telomeres (Gilson et al., 1993; Longtine et al., 1989). This Rap1p along with DNA repair/telomere binding protein Yku70p sequesters silent information regulators at the telomeres (Moretti et al., 1994; Tsukamoto et al., 1997).

More importantly, several lines of experimental evidences suggest that the subnuclear clustering of the two heterochromatin parts of the chromosome at the nuclear periphery favours gene silencing. Firstly, \( yku70 \) mutations that disrupt the clustering of telomeres and Sir proteins, disrupt silencing (Laroche et al., 1998). Secondly, ectopic loci that are flanked by silencers are able to establish silencing when placed proximal to telomeres or silencer flanked ectopic regions are normally active in transcription, but
can be silenced on overexpression of Sir3 or Sir4 proteins or dispersion of Sir proteins throughout the nucleus by disrupting telomere organization (Maillet et al., 1996; Marcand et al., 1996). Thirdly, silencing can be established by artificially tethering ectopic loci to the nuclear periphery (Andrulis et al., 1998). This was elegantly demonstrated by artificially targeting proteins of the endoplasmic reticulum to a silencer defective HMR locus via Gal4 DNA binding domain leading to the establishment of silencing (Andrulis et al., 1998; Brand et al., 1987). These proteins promote repression by tethering the HMR locus to telomeric pools of Sirp, found at the nuclear periphery. Therefore, from the above experimental lines of evidence, it is clear that sub-nuclear organization of telomeres at the nuclear periphery is crucial for establishing and maintaining silencing. Recent experiments have further demonstrated at least three proteins are important for this peripheral localization of telomeres. Esc1, an inner nuclear membrane, Sir4p and Yku70/80p, the telomere end binding protein that has essential roles in DNA repair by non-homologous end joining, all are required for this peripheral anchoring (Gartenberg et al., 2004). However, the structural basis, like the underlying nuclear architecture component that these proteins anchor the telomeres to and the basis of clustering of specific telomeres into separate groups per se is not understood.

1.4.2 Silencing at HM loci, telomeres and rDNA loci

Heterochromatin is established in yeast in a multi-step pathway. Specific DNA sequences, termed as silencer elements E and I, flank the silenced region of the HM loci. These sequences recruit DNA binding proteins: Rap1, Abf1 and Orc1. These silencer bound proteins in turn, recruit the Silent Information Regulators (Sir1p, Sir2p, Sir3p, and Sir4p). Sir2p, an evolutionarily conserved NAD-dependent histone deacetylase, begins to deacetylate specific sites on histones H3 and H4 in the nucleosomes. These deacetylated nucleosomes serve as high affinity binding sites for Sir3p and Sir4p. The Sirp bound nucleosomes are refractory to transcription, and thus such regions are silenced or heterochromatinized. The mechanism of heterochromatin establishment at the telomeres is very similar; however, instead of 3 different silencer elements recruiting
three different proteins, the telomere repeat sequences themselves serve as multiple binding sites for Rap1. The C-terminal domain of Rap1 binds Sir4, an interaction antagonized by two other Rap1 binding proteins Rif1 and Rif2 (Mishra and Shore, 1999; Moretti et al., 1994; Rusche et al., 2002). This Rif-mediated antagonism is also

![Diagram of gene silencing](Image)

**Figure 1: Schematic representation of gene silencing:** Silent information regulator Sir4 recruits to the telomere binding protein Rap1 and thereby recruits other Sir proteins and hypoacetylated histones forming a compact structure that is inaccessible to RNA polymerase II. Mating locus recruits Sir1-4 and shows stable repression. At rDNA locus, Sir2 hypoacetylates nucleosomes and maintains silencing.

counteracted by the DNA end-binding heterodimer complex Yku70/Yku80 which anchors the telomeres to the nuclear envelope via Sir4 binding (Bertuch and Lundblad,
2003; Laroche et al., 1998; Mishra and Shore, 1999). These proteins recruit other Sir proteins to form a holo-Sir complex. Sir proteins spread from sites of nucleation and deacetylate the histones H3 and H4 and then bind more stably to the deacetylated nucleosome (Moazed et al., 2004). Silencing at rDNA locus is different from the HM loci and telomeres only Sir2, protein involves to establishing and maintaining silencing (Smith and Boeke, 1997).

Although Sir proteins are essential for repression of RNA polymerase II dependent transcription (Aparicio et al., 1991), the mechanism by which Sir proteins block transcription is still unclear. It has been proposed that Sir proteins mediate silencing by compacting chromatin into a more condensed state making it inaccessible to RNA polymerase II (Rusche et al., 2003). Experimental evidence for this model comes from studies that show that restriction enzymes and DNA methylases, which were ectopically expressed in the cell, bind less frequently to the HM loci and telomeres than active regions (Chen and Widom, 2005; Gottschling, 1992). However, Sir protein binding is not enough for gene silencing. For instance, Sir proteins have been shown to bind from HML to the telomere but reporter genes inserted into this intervening region are not silenced (Bi, 2002). The current understanding is that silencing requires an unknown but crucial step in establishment of gene silencing other than binding of Sirp to nucleosomes.

### 1.5 Chromosome organization and Genome stability

Accurate transmission of genetic material from mother to daughter cell requires proper segregation of chromosomes during cell division. The generation and survival of all organisms depends on numerous processes that need to be tightly coordinated to ensure the genome integrity and to promote genome propagation. A key event in the cell cycle is the efficient and error-free DNA replication and faithful segregation of every pair of duplicated chromosomes to daughter cells. A complete understanding of the regulation and control over cell division is critical to elucidate the mechanisms that govern self-renewal, proliferation and development of the organism. Defects in chromosome segregation lead to aneuploidy, where entire chromosomes are gained or
lost. Aneuploidy is a hallmark of most tumor cells and has been postulated to be major factor in the evolution of cancer; this could be due to mis-segregation during cell division. Cancer is generally considered an age-associated disease where somatic cells accumulate spontaneous miscarriages (DePinho, 2000).

One of the early steps in cancer progression is accumulation of higher mutation rate than normal. However, on the basis of rates of spontaneous missegregation observed in human cells, the steady accumulation of mutation does not account for the number of genetic changes that are present in most tumors (Bielas et al., 2006; Lengauer et al., 1998; Loeb et al., 2003). Several cancerous cells show genome instability, in the form of mutations and chromosome rearrangements. For example, common mutations found in colon cancer cells increase genome instability (Grady, 2004). Two types of elements have a key role in chromosome rearrangements; those that act as trans acting factors to prevent mutation and DNA rearrangements, among them are replication, repair and checkpoint proteins whereas cis acting chromosomal hotspots of instability such as fragile sites and highly transcribed DNA sequences. Although such events can be harmful for the cell and the organism, they also drive evolution at the molecular level and generate genetic variation. Genetic instability can also have a specialized role in the generation of variability in developmentally regulated processes, such as immunoglobulin diversification (Maizels, 2005). Notably, there is a well established principle that cancer risk increases with age in mammals; similarly, increase in genome stability that is linked to ageing in budding yeast is also seen. This constitutes a rational explanation for the association of high cancer risk with age in mammals.

Genetic instability is further classified into different classes according to the type of events stimated. Micro and minisatellite instability (MIN) leads to repetitive-DNA expansion or contractions and can occur by mismatch repair (MMR) impairment or by homologous recombination (HR). Gross chromosomal rearrangements within two genetically linked DNA fragment by HR or end joining between non-homologous DNA fragments leads to translocation, duplication, inversion or deletion events. Genetic instability is also caused by failures in either mitotic chromosome transmission or the spindle checkpoint leading to abnormality in chromosome number. This phenomenon is referred to as chromosomal instability (Draviam et al., 2004).
1.6 Kinetochore architecture in budding yeast

For the past two decades, proteomics, microscopic studies and other biochemical approaches have allowed the isolation of the kinetochore sub-complexes. Several features of these kinetochores in yeast provide advantages for experimental analysis. Budding yeast is a good experimental model organism to address the role of kinetochore in chromosome segregation during mitosis and meiosis. In budding yeast, kinetochore assembles at the centromeric DNA in a sequence specific manner. The centromere is a ~125bp sequence, and is comprised of three elements (CDEI, II and III) which help in assembly of kinetochores. However, in humans and fission yeast, centromeric sequences are extended over several kilobases. Although these centromeres contain certain repeated sequences, the position of the centromeres is thought to be specified by epigenetic mechanisms rather than sequence specific binding events (Blower et al., 2002; Cheeseman et al., 2004). From enhanced microscopy and tomography experiments, another unique feature of budding yeast kinetochore has emerged: a single microtubule attaches to each kinetochore, whereas in other organisms multiple microtubules attached to a single kinetochore (Dorn et al., 2005).

Kinetochores act as molecular motors which connect centromeres to the microtubules. The kinetochore could be composed of more than 65 proteins; majority of them are organized into 14 subcomplexes according to their relative position in the centromeric DNA and microtubules. Kinetochore are categorized into three distinct regions: a) inner kinetochore protein complex, capable of binding to centromere DNA sequences and providing a DNA-protein platform upon which other kinetochore protein complexes assemble; b) central kinetochore protein complex bridges the inner kinetochore to the microtubule-binding outer kinetochore protein complex and c) outer kinetochore proteins connect the microtubules and other microtubule associated proteins (MAPs), to the kinetochore. Members of all these kinetochore proteins have vertebrate homologs, suggesting that the overall kinetochore structure has been conserved from yeast to mammals (Tytell and Sorger, 2006).
Figure 2: Chromosome segregation and kinetochore assembly: Accurate transmission of genetic material from mother cell to daughter requires proper assembly of kinetochores and stable interaction with microtubules. Panel B shows the structure of kinetochores that are assembled at centromere DNA region. Panel C shows the current predicted molecular organization of kinetochore complex.

1.7 Spindle assembly and checkpoint activation in defective kinetochores

Kinetochores are essential components for proper segregation of chromosomes during mitosis. The conserved mitotic checkpoint regulators monitor the formation of bipolar kinetochore-microtubule attachments and in the event of error, spindle checkpoint proteins arrest a cell in mitotic metaphase. The primary signal for activation of spindle checkpoint is the lack of attachment between the centromere and spindles, implicating kinetochores as the source of the signal. In fact, strains with deletions of kinetochore proteins Ndc10 and Spc25 are defective for the mitotic checkpoint,
indicating either that an intact kinetochore is required for checkpoint function or that these proteins signal to the mitotic checkpoint regulators (McCleland et al., 2003).

How kinetochores change through the cell cycle is not clear. In *Saccharomyces cerevisiae*, centromeres are clustered together and form a rosette-like structure and are positioned near the spindle pole body (which is embedded in the nuclear envelope) via microtubules throughout the cell cycle (Bystricky et al., 2004; Guacci et al., 1997; Jin et al., 2000) Centromeric clustering was recently confirmed by using more advanced technique of chromosome conformation capture (3C) coupled with massive sequencing performed on the yeast genome (Duan et al., 2010). The 3C technique relies on the capture by mild cross-linking of interaction chromatin segment can be elucidated by further intrachromosomal ligation, inverse PCR and so on (Dekker et al., 2002; Lieberman-Aiden et al., 2009; Simonis et al., 2006). This 3C technique confirmed centromere clustering in a rosette-like structure, with majority of interchromosomal contacts being concentrated near centomere DNA sequences (Duan et al., 2010; Rodley et al., 2009). Each centromere connects to the plus stand of microtubule through molecular motors called kinetochore (Joglekar et al., 2009). During interphase, the 16 assembled microtubules on kinetochores maintain the attachment between centromere and the spindle pole body SPB (Furuyama and Biggins, 2007). Although the microtubules grow and shrink continuously, the distance between centromere and SPB has been never closer than ~200nm during G1 phase. For a short period of time, centromeres are detached from the spindle pole body (SPB) in S-phase of the cell cycle. Centromere replication takes place in the late S-phase of cell cycle. The new SPB is formed *de novo* in the vicinity of the old SPB during early S-phase (Adams and Kilmartin, 2000; Lim et al., 1996; Pereira et al., 2001) and the new SPB might be too immature to generate microtubules when centromeres are to be recaptured. The old SPB usually organizes the microtubules for centromere recapture. It is hypothesized that, centromeres are forced to replicate in late S-phase of cell cycle, as by this time, the new SPB is matured and ready to recapture the centromere. All these controls and regulations suggest that the disruption of the kinetochore-microtubule architecture leads to a perturbation of the centromere clustering (Jin et al., 2000). One possible influence on chromosome organization in yeast thus appears to be the microtubule-dependent
anchorage of centromeres to the SPB that is embedded in the nuclear envelope throughout the cell cycle.

Through genome-wide protein interaction studies, it is known that Nkp2 colocalizes with the spindle pole body (Huh et al., 2003) and is a component of Ctf19. Other components of the Ctf19 complex, Iml3 and Mcm17, are involved in accurate segregation of sister chromatids and prevent non-disjunction of sister chromatids during meiosis II. These proteins are also involved in proper localization of Shugoshin protein (Sgo1) to the heterochromatin free ~50kb pericentromeric domains thereby protecting it from separase-dependent cleavage. These pericentromeric regions are bound by the cohesins which helps in accurate chromosome segregation during cell division. Ctf19p is required for the increased binding of cohesins at the pericentromere regions, but whether this function is shared with other components of the Ctf19 complex is not known (Eckert et al., 2007; Ghosh et al., 2004; Marston et al., 2004).

One of the key questions in kinetochore biology is the molecular architecture of the kinetochore itself. Interactions between components have been identified through several approaches including synthetic lethal genetic analyses, immunoprecipitation experiments followed by proteomics, two hybrid analyses etc. However, as shown in Figure 2, several interactions remain to be mapped. Also the importance of individual components in maintaining the fidelity of the chromosome segregation and their sub groups and sub functions are not clear.

In this work we report that NKP2 encodes an important component of the kinetochore. Although our screen was designed to discover components involved in nuclear organization, the chromosome loss induced by NKP2 dosage was scored as loss in silencing owing to is stochastic behaviour. As very little is known about the role of Nkp2 in the kinetochore, we pursued our investigations into the importance of Nkp2 function. We found that overexpression of kinetochore protein Nkp2, leads to loss of chromosomes at a low rate. However, loss of nkp2 leads to increased genome instability via loss of chromosomes. We also carried out synthetic genetic interactions between multiple components of the kinetochore and nkp2Δ and find that some of those components cooperate with NKP2 to promote chromosome transmission fidelity.
1.8 Objectives of the study

From the detailed overview presented above, it is evident that the spatial organization of genes and chromosomes plays an important role in nuclear functions. New imaging and biochemical techniques, studies of yeast nucleus have led to significant insight into chromosome arrangement and dynamics. Yeast telomeres provide a model system to investigate the molecular principles involved in chromosome–chromosome interactions and the assembly of chromosome based compartments. They also provide us with a tool to study the chromosome-nuclear envelope interactions. Keeping in view these observations, the present study has been undertaken with the objectives to investigate the molecular principles and understand many aspects of three dimensional chromosomal architecture, chromosome dynamics and functional compartmentalization. Given the implications for fundamental genome functions, a major objective to identify or better understand the principles that drive chromosomal organization.

To address this issue we performed

- Genetic screen for factors that disrupt nuclear envelope anchoring dependent silencing.
- Identification and characterization of components indentified in the screen.