Chapter-1

Introduction

Eukaryotic cells effectively package their long DNA inside a micron sized nuclei in the form of chromatin, the fundamental unit of which is a nucleosome - an octameric unit wound around by 146 base pairs of DNA. The octamer contains two copies each of the four conserved core histones - H2A, H2B, H3 and H4 **[1-5]**. The nucleosomal units are separated by a region of linker DNA, which is associated with a less conserved histone usually referred to as the linker histone H1. The linker histones clamp the entry and exit sites of DNA around the core histone octamer and contribute to the formation of a condensed higher order chromatin structure. The tight packaging of the nucleosome complex constitutes barriers to the regulatory machinery to access DNA. Therefore it requires the chromatin to be continually remodeled and the histone-DNA interactions in the nucleosome be relaxed to allow polymerases and other proteins to access the DNA template **[6]**.

The organization of chromatin within live cells is dynamic. The core histones within the nucleosome are in dynamic equilibrium even under mitotic conditions where the chromatin is condensed **[7]** and the levels of exchange vary with the different stages of cell cycle. Chromatin dynamics at the global level during interphase has been studied using GFP-fused core histones and other nucleosomal components. Single cell photobleaching experiments reveal that H2B-GFP exchanged more rapidly than H3-GFP and H4-GFP **[8]**. Radio labeled histone exchange data indicate that H2A and H2B exchange more rapidly than H3 and H4, and some of this exchange appeared to depend on continuing transcription **[9]**.

In living cells, the linker histone H1 is mobile and interacts transiently with a particular nucleosome. H1 molecules are continuously exchanged within the chromatin binding sites in a ‘stop-and go’ process in which H1 stays on a binding site for a certain time. Once dissociated, it rapidly and randomly moves to other binding sites **[10]**. Post translational modifications on the histone tails such as acetylation, methylation, phosphorylation, ubiquitination, etc., alter local

chromatin structure and influence histone-DNA interactions **[11]**. Cell death or stress has also been shown to cause dramatic changes in chromatin structure **[12]**.

Cell cycle–related chromatin decondensation and H1-phosphorylation are associated with changes in the mobility and interaction of the linker histones. Phosphorylated linker histones have a lower affinity of interaction with the chromatin fiber in living cells. Mutation of amino acid residues for the phosphorylation targets decreases the mobility of H1 **[13].** The mobility of H1 is also modulated by its interaction with other nuclear proteins such as HP1 through post-translational modification of H1 **[14]**. The temporary dissociation of H1 from its binding sites enables the binding of other proteins to the vacant sites. The competition between linker histones and HP1 to occupy a binding site can be site-specific or non–site-specific. HMG (High mobility group) proteins being one of the major chromatin binding proteins transiently interact with the chromatin fiber and hence affect the interaction of H1 with nucleosomes **[15, 16]**. The HMG group of proteins has a site-specific target inside the cell nucleus. During the transient dissociation of H1 from its binding site, the rapidly moving HMGs occupy the vacant site and rapidly modify the chromatin structure. The HMG-H1 competition takes a vital role in linker histone mobility and chromosomal compaction

A dynamic chromatin organization is critical for many cellular processes including transcription, replication, repair, recombination and chromosome segregation. Posttranslational modifications of histones play crucial roles in the regulation of chromatin structure and assembly, epigenetic status of chromatin, DNA repair, and transcription as well as in as development, differentiation and reprogramming **[17]**. Modifications, such as H3-acetylation on lysine-9 or H3-tri-methylation on lysine-4, were shown to be highly associated with transcriptional activity; while H3-K9-tri-methylation is correlated with inactive chromatin, and has broad roles in transcriptional repression, gene silencing, maintenance of heterochromatin, and epigenetic inheritance of heterochromatin. **[18]**. HDAC (Histone De-Acetylase) inhibitors block the action of histone deacetylases and spread the euchromatin pattern in the cell nucleus. This HDAC inhibitor globally

alters the chromosomal pattern by chemically modifying the core and linker histones. But very little is known about the mobility of the core and linker histones and the possible interactions with the chromatin in the cells treated with HDAC (Histone-De-Acetylase) inhibitor.

The histone variant deposition controls gene regulation and other nuclear processes. While the bulk histones are deposited over S phase during replication in a replication dependent chromatin assembly pathway, the histone variants undergo a replication independent chromatin assembly. Variants of H2A and H3 are regionally distributed in the nucleus and have distinct functions in epigenetic silencing and gene expression.

It is shown that the mammalian histone variant plays a critical role in early development **[19, 20]**. H2A.Z is important for the transcription regulation of Tetrahymena and *Drosophila* genes. CAF-1 mediates the deposition of H3.1 in the UV-damaged sites and promotes the in vitro chromatin assembly in a DNA synthesis-coupled manner on replicating and newly repaired DNA **[21]**.

Variants of linker histones are found to be involved in regulation as well as in the developmental process. Different linker histone variants show significantly different binding properties *in vivo*. Histones H1.1 and H1.2, which have shortest C-terminal tails, rapidly undergo binding and dissociation events while H1 variants with longer C-terminal domains exchange much more slowly. H1.3, H1.4, and H1.5, take longer time to equilibrate after the initial photobleaching event. Different histone H1 subtypes span a broad range of recovery times. Both histone H1.1 and histone H1.2 bind to chromatin with the lowest affinity, and this affinity increases sequentially with increasing C-terminal tail length of histones of the linker increases H1.3, H1.4 and H1.5. FRAP experiments on SK-N-SH neuroblastoma cells show that among the different subtypes of EGFP tagged linker histones, H1.1-H1.5 fluorescence recovery decrease substantially as C terminal tail length increases. As the C-terminal domain is swapped between loosely bound histone H1.1 and histones H1.4 or H1.5 (which have highest binding affinity) the

recovery decreases significantly. In a similar experiment in the H1.4-H1.1 hybrid, which consists of the N terminus and globular domains of histone H1.4 fused to the C-terminal domain of histone H1.1, the interaction with the chromatin fiber decreases and the recovery curve of H1.4 –H1.1 follows the H1.1 recovery pattern. It is observed that H1.4-H1.1 hybrid with N-terminal and globular domain of H1.4 and C terminal tail domain of H1.1 interaction is much weaker compared to H1.1-H1.4 hybrid where N-terminal and globular domain of H1.4 is fused with the C terminal tail domain of H1.1 **[22]**.

Cellular differentiation involves changes in gene expression and nuclear architecture as well as tissue specific cellular-morphological changes. It is expected that the chromatin structure in embryonic stem-cells must be plastic enough to accommodate such rapid and substantial changes. Differentiation of mouse ES cells is accompanied by global changes in histone modifications, like, an increase in H3-triMeK9 **[23, 24]** or a decrease in H3-triMeK4 **[25-27]**. It is observed that in hippocampus neural progenitor cells, class II HDACs are up regulated during neuronal differentiation **[28]**, but in contrary, HDAC inhibition promoted neuronal differentiation of similar cultured progenitor cells **[29]**.

H3.3 displays a dynamic turnover throughout germ cell development of *C.elegans* and is shown to be deposited at the active transcription sites in various somatic cells **[30]**. It is found that the H3.3 incorporates during the first germ-line stem cell division and continues throughout meiosis, and ends up in sperm and eggs. It is observed that, H3.3 becomes depleted of primordial germ cells, and the meiotically silenced X chromosome is deficient in H3.3 **[31]**.

Linker histone variants play an important role in defining the stem cell differentiation to a particular lineage. It is observed that, H1-0 accumulates in a period restricted to neuronal terminal differentiation **[32]**, indicating the role of this variant in the alteration in chromatin structure during neuronal differentiation.

A recent work has established that a global modulation of chromatin dynamics mediated by dephosphorylation of linker histone H1 is necessary for erythroid differentiation. The chromatin binding properties of H1-GFP variants are different in untreated and differentiated MEL cells. FRAP recovery data indicates an increase in half live ($t_{1/2}$) of the fluorescence recovery timescale in the differentiated cells compared to the undifferentiated cells **[33]**. This establishes a direct connection between the changes in H10–chromatin interactions in a cell to its commitment to a specific cell type. It is observed that the maternally expressed linker histone B4 is replaced by somatic linker histones (H1a) during early *Xenopus* embryogenesis **[34]**. According to the proposed idea, due to the 'loose' binding of the maternally expressed linker histones (B4) the chromosomal structure becomes more fluidic, facilitating the binding of chromatin remodeling factors, while in the later stage, the chromosome becomes condensed due to the tight binding of somatic linker histones.

Fluorescence based spectroscopic methods and genetic tagging of different proteins of interest has enabled one to measure the diffusion properties of different proteins inside the live cell nucleus. FRAP (Fluorescence Recovery After Photobleaching), FCS (Fluorescence Correlation Spectroscopy) enable us to understand the diffusion mechanism of different core and linker histones and its interaction with the chromatin fiber in living cells. Real-time experiments on histone dynamics give us a unique opportunity to understand the dependence of histone dynamics on different cellular and developmental context. Recent experiments and computer simulations show that bigger size inert particles follow sub-diffusive behavior inside a live cell nucleus as well as in the cytoplasm. While a smaller particle like EGFP, diffuses normally. The autocorrelation function for EGFP indicates that the viscosity inside the cytoplasm and nucleus are very similar in normal physiological conditions. Using fluorescence correlation spectroscopy experiments in the cell cytoplasm the diffusion of passive particles (like TMR-dextrans) with different molecular size distributions are measured **[35]**. The correlation curve indicates that as the particle size increases the sub-diffusive

parameter (β) decreases from unity. Using computer simulations, the experimental findings are also validated **[35]**. These experimental findings indicate the degree of anomalous diffusion of tracer particles in highly concentrated dextrans solutions.

Experiments have been done to understand the diffusion of nucleosomal core particles either in free solution as a function of the salt concentration or in a DNA solution, as a function of the salt and DNA concentration. In aqueous solution, the NCPs follow Brownian diffusion behavior with a weak salt dependence. Increasing salt concentration leads to an increase in the diffusion coefficient, related to a decrease of the hydrodynamic radius. Inside a heterogeneous DNA mesh structure the NCP shows sub-diffusive transport.

High resolution crystal structures of the mono-nucleosome and of a tetra-nucleosome highlight the structural components of the chromatin fiber in detail, and biochemical experiments over the past decades have provided valuable information on the histone modifications and the interacting domains. Yet the dynamics of the core and linker histone and their interactions with the chromatin fiber to maintain the differential compaction state remain to be understood.

Though recent biophysical experiments describe the mobility of core histones inside live cell nuclei **[36]** the diffusive mechanism of the core and the linker histones in the nuclei and their site specific interactions have not been established. Hence a combinatorial approach involving spectroscopic methods as well as numerical simulation is required for a complete understanding of the biophysical mechanism of the core and linker histone diffusion inside a live cell nucleus.

This thesis work aims to decipher the diffusion mechanisms of the core and linker histones inside live cell nuclei and their influence on chromatin assembly. I have tried to understand the diffusion mechanism of the core and linker histones inside the interphase cell nucleus in different cellular context. FCS experiments are performed to understand the state of the freely diffusing core histones in the cell nucleus, as well as to quantify the interaction timescale of the linker histones in

live cells. The experimental results are also validated through numerical simulations. To understand the functional implication of histone dynamics, biophysical experiments are done in the early embryo of fruit-fly (*Drosophila melanogester*). Experiments are performed to understand the role of histone dynamics to understand the plasticity in chromatin assembly in the nuclei of early stages of *Drosophila* embryo.

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