Chapter-4

Conclusion

This thesis work attempts to explain alterations in chromatin dynamics in living cells due to chemical modifications on histones and during early embryogenesis. Fluorescence based spectroscopic techniques and live cell imaging have been employed to understand the diffusive mobility of the core and the linker histones inside a live cell nucleus and their transient interactions with the chromatin fiber.

My work has served to establish that the core histones inside live cell nuclei are in multimeric form and play a vital role in maintaining the epigenetic state of the chromatin fiber whereas in an over-expressed cytoplasm they are in monomeric form. The diffusion of multimeric core histones is sensitive to the architecture of the chromatin assembly and is reminiscent of a dynamic polymer matrix characterized by a mesh size, leading to a sub-diffusive transport of the freely diffusing core histones inside the cell nucleus. The multimeric form of the freely diffusing core histones in the cell nucleus is ATP dependent illustrating the existence of a dynamic equilibrium between the chromatin-bound and free fraction of core histones that is tuned by energy-dependent processes. The core histone mobility is invariant across organisms, (mammalian cell lines or polytene chromosomes), suggesting an evolutionarily conserved local chromatin architecture in these two systems. In living cells, the diffusion of linker histones is significantly distinct from that of the core histones. H1 molecules are continuously exchanged among chromatin binding sites in a 'stop-and go' process. H1 stays on a binding site for a limited time, then dissociates and diffuse to other binding sites. The movement of H1 in the nucleus is governed mostly by its interactions with chromatin. The interaction timescales of the linker histones with the chromatin fiber point to a dynamic compaction state of the chromatin fiber at the level of a single nucleosome. The interaction timescale of the linker histones are independent of ATP concentration in the cell nucleus. The mobility of the linker histones is strongly defined by their interactions with chromatin. Indeed the mobility of the linker histones in the cytoplasm confirmed that the second timescale in the diffusion behavior arises due to the interactions with the chromatin assembly. The various

subtypes of linker histones are found to undergo similar diffusive processes where the minor variations in the interaction timescales could possibly correspond to differential tail lengths of the histones. This interaction timescale ~30 ms, measured as the mean correlation timescale, may suggest a mechanism to introduce dynamic local conformational fluctuations in chromatin assembly. The diffusion mobility of the core and linker histones is strongly dependent upon the state of the cell. Staurosporine induced cell death leads to complete loss of core histone mobility whereas the linker histone mobility is partially reduced. The core and the linker histones are seen to colocalize perfectly in interphase HeLa cells while the different subtypes of linker histones show partial co localization. The experimentally observed sub diffusive behavior of multimeric core histones and distinct interaction timescales of the linker histones have been validated using numerical simulations.

Towards understanding alteration in histone dynamics due to chemical modification, we used Trichostatin-A (TSA), a histone deacetylase inhibitor, which increases the acetylation levels of core histones resulting in euchromatin spreading in live cells. Experimental data indicate an increase in the interaction timescale of the freely diffusing linker histone due to altered chromosomal structure and possible chemical modification in TSA induced decondensed nuclei. Even under a decondensed state of the chromatin, the multimeric form of the freely diffusing core histones is maintained in TSA treated cell nuclei.

Our experiments indicate a change in chromosomal organization in the earlier part of Drosophila embryo development. Fluorescence recovery data for both the core and the linker histones indicate chromosomal plasticity in early development even after cellularization. After ~5 hrs from cellularization, the chromosomal structure becomes well defined and acquires a differentially compacted structure inside the cell nucleus. The phenomenon is observed both in the euchromatin regions and heterochromatin regions after 5 hrs from 13^{th} nuclear division. Our result indicates that before cellularization, a higher exchange of the maternally expressed core and the linker histones between the yolk and the nucleus is important to maintain the epigenetic state of the cell nucleus. This indicates that the maternally expressed multimeric cores and the linker histones play a vital role in

epigenetic maintenance leading to homogeneity in the chromatin organization before cellularization.

Our results indicate that the core and linker histone dynamics is a key player in the structure and function of the chromatin fiber inside the cell nucleus. The dynamic compaction state of the chromatin fiber due to the differential interaction of the core and linker histones with the chromatin fiber is vital in diverse developmental context.