# Probing histone dynamics and its functional implications within living cells

**by**
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# Synopsis of thesis

**Introduction:**

DNA in eukaryotic cells is packaged within the nucleus in a structural hierarchy, the basic unit of which is a nucleosome. This octameric complex formed by two sets of the core histones H2A, H2B, H3 and H4 is wound around by 146 base pairs of DNA. Linker histones (H1/H5) compact the chromatin into higher order structures, restricting access of regulatory factors and nucleosome remodeling complexes to their chromatin binding sites. The post-translational modifications on the histone tails and the binding-unbinding kinetics of the core and linker histones play an important role in chromosomal compaction as well as in the maintenance of epigenetic state of the chromatin fiber. Histone mobility is an important factor for transcription competence and gene expression is tightly coupled to the modulation of chromatin through the dynamic nucleosomal structure.

Though experimental evidence supports that histone dynamics is crucial for coupling chromatin to gene expression, the biophysical mechanisms underlying the dynamics is poorly understood. In my thesis, I have attempted to explore the mechanisms of histone diffusion inside the cell nucleus and their coupling to the chromatin assembly within live cells. The diffusion mechanisms have been studied in interphase cells under different cellular conditions and by causing functional perturbations on histones. The role of histone dynamics during early embryogenesis has also been addressed.

**Approaches:**

To address these questions, we have used fluorescence based techniques like Fluorescence Recovery After Photo bleaching (FRAP), Fluorescence Correlation Spectroscopy (FCS) and live cell imaging on HeLa cells and Drosophila salivary gland cells. The core and the linker histones used in the experiments were tagged either with EGFP or mRFP.

FRAP experiments were done on live cell nuclei to estimate the fraction of the bound and the unbound histones in a specific location inside the cell nucleus and to determine the exchange rate of these proteins in real time. FCS was employed to

understand the diffusion mechanism of the core and linker histones inside the live cell nucleus, in a complex and heterogeneous cellular environment. Numerical simulations of histone diffusion were used to validate the experimental data. Confocal imaging enabled the mapping of the functional localization of the different histones inside the live cell nuclei.

**Experiments**

The initial phases of my work were aimed at understanding the diffusive mobility of the core and linker histones in interphase cells. Our photo bleaching experiments on HeLa cells and on the polytene chromosomes of salivary glands of Drosophila melanogaster are consistent with earlier FRAP studies and show that, the rate of exchange of the bound and free core histones is significantly slower than that of the linker histones which recover in hundreds of seconds. But linker histones recover much slower compared to freely diffusing proteins such as EGFP (Enhanced Green Fluorescent Protein).

FCS experiments done to understand the diffusion mechanism of the core histones (H2B EGFP and H4 EGFP) within the cell nucleus indicate that the core histones are in multimeric form inside the cell nucleus whereas in an over expressing HeLa cytoplasm, they are in a monomeric form. It is also observed that this multimeric form of the core histones is conserved in mammalian HeLa cells and in Drosophila salivary gland cells. The autocorrelation function for the diffusion of linker histones is significantly different from that of the core histones and indicates a distinct interaction dependent timescale in addition to the purely diffusive correlation timescale. This interaction time scale vanishes completely upon deletion of the C and N terminal tail domains of the linker histones. The FRAP recovery curves follow a normal diffusive recovery supporting FCS experiment. It is also found that in the over expressing HeLa cytoplasm, the additional timescale vanishes completely, indicating that this additional timescale of the linker histones is due to the interaction of these proteins with the chromatin fiber. Various subtypes of the linker histone also show that this interaction timescale correlates directly with the length of the C-terminal tail of these different subtypes (H1.1, H1.2, H1.4 and H1.5). We also observed that the multimeric form of the core histones is ATP dependent, while the

interaction timescale of the linker histones is independent of the ATP concentration in the cell nucleus.

Since the diffusion mechanism of the core and the linker histones might also depend upon the state of the cell, we studied histone diffusion with induction of cell death using staurosporine. The core histone diffusion stops completely while the linker histone diffusion is significantly reduced on induction of apoptosis. 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.

Numerical simulations done to understand the diffusion mechanism of the core and the linker histones, show that the particles exhibit sub-diffusion as the size of the pores in the mesh structure decreases. To understand the origins of the extra timescale in the linker histone diffusion, the 'diffusion-with-interaction' model is used, where a few random binding sites were numerically generated in 3D space. At the time of diffusion as the particle reaches any one of the binding sites, it pauses there for a certain timescale, obtained from our experimental data, and once it is released from the site it starts to diffuse normally without memory. We found here that the simulated autocorrelation function of the particle gives rise to an additional interaction timescale, validating our experimental data.

The next phase of my thesis was focused on exploring how chemical modifications of the core and linker histone affect their mobility and interaction with the chromatin fiber. Towards this, we used Trichostatin-A (TSA), a histone deacetylase inhibitor, which increases the acetylation levels of core histones resulting in euchromatin spreading in live cells. We find that the interaction timescale of the freely diffusing linker histone increases significantly in TSA induced decondensed nuclei. The multimeric form of the freely diffusing core histones are also maintained in TSA treated cell nuclei in spite of significant chromosomal decondensation. Upon decondensation of chromatin fiber, the interaction timescales of the linker histones increase significantly, though the pool of multimeric form of the freely diffusing core histones remains unaltered.

The final phase of my work was centered on understanding how histone dynamics influences chromatin organization during embryonic development. Using transgenic fly

embryos where H2B is genetically tagged to EGFP and by microinjecting fluorescently labeled linker histones into the Drosophila embryo, we have studied the diffusion behavior of core and linker histones, the exchange of these proteins with the chromatin fiber and the transport of these proteins through the nuclear pore before and after cellularization in a developing fruit fly embryo. Experimental data indicate a high degree of exchange of the multimeric core histones through the nuclear pore before cellularization. As the embryo proceeds from $11^{th}$ to $13^{th}$ nuclear division, the exchange rate also decreases significantly and vanishes completely after cellularization. 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. It is found that early cells inside the Drosophila embryo show a loosely wound nucleosome, indicating a direct connection between the state of differentiation of the cells and the structure of the nucleosome.

**Conclusion:**

In my thesis work, I have tried to establish the coupling between histone dynamics and the functional state of the chromatin fiber as well as its role in early embryogenesis. We have observed that the core histones are in multimeric form and play a vital role in maintaining the epigenetic state of the chromatin fiber. 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 dependence of the interacting timescales with the length of the tail domains, as well as the chemical modifications of the chromatin fiber in a differentiated cell line, is also established. Our experiments suggest that chromatin structure is plastic in the earlier part of drosophila embryo development and that 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, before cellularization.

## Publications

**1.** EGFP-Tagged Core and Linker Histones Diffuse via Distinct Mechanisms within Living Cells.
**Dipanjan Bhattacharya,** Aprotim Mazumder, S. Annie Miriam, and G. V. Shivashankar. ***Biophysical Journal***. (2006). 91: 2326–2336.

**2.** Chromatin Structure Exhibits Spatio-Temporal Heterogeneity within the Cell Nucleus**.**
Bidisha Banerjee, **Dipanjan Bhattacharya,** and G.V. Shivashankar. ***Biophysical Journal***. (2006). 91: 2297–2303.

**3.** Development of single molecule tracking confocal fluorescence microscope for gene expression analysis.
Deepak Sinha, **Dipanjan Bhattacharya**, Bidisha Banerjee, Feroz Meeran & G.V.Shivashankar : ***Current Science*** *(2004), 87, 239-244*
.
**4.** Spatio-temporal heterogeneity in chromatin organization and histone dynamics within a developing drosophila embryo.
**Dipanjan Bhattacharya,** Aprotim Mazumder, G.V Shivashankar (Preprint:07)

**5.** Role of HDAC inhibitor on histone dynamics and its expression within living cells**.**
Jyothsna Rao, **Dipanjan Bhattacharya,** Apurva Sarin and G. V. Shivashankar.
**(Preprint:2007)**

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