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**Supporting Material**

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Dipanjan Bhattacharya, Shefali Talwar, Aprotim Mazumder, and G.V.Shivashankar

**Supplementary materials file**

# Spatio-temporal plasticity in chromatin organization in mouse cell differentiation and during *Drosophila* embryogenesis

Dipanjan Bhattacharya[1], Shefali Talwar[2], Aprotim Mazumder[2] & G.V.Shivashankar[1, 2, #]

[1] Raman Research Institute, Bangalore - 560080, India
[2] National Centre for Biological Sciences, TIFR, Bangalore - 560065, India

**Supplementary material:**

**Epigenetic Modifications in maintaining chromatin plasticity using Immunofluorescence:**

Cells ($10^5$ cells/dish) were grown on coverslip dishes and washed with M1 Buffer (NaCl 150 mM, HEPES 20 mM, Kcl 5 mM, $CaCl_2$ 1 mM, MgCl2 1mM and pH 7.4) and fixed with 4% PFA for 20 min, washed twice with M1 Buffer, permeabilized with 0.2% NP-40 for 10 mins and again washed twice with M1 Buffer. The permeabilized cells were blocked with 10 mg/ml BSA in M1 Buffer for 1 hr at room temperature. The primary antibody diluted in block was incubated for 2 hours at room temperature and washed twice with block. The secondary antibody was incubated for 1 hour and washed off with block solution. The following antibodies were used: H1 (1:100 dilution mouse monoclonal, Upstate, 05-457), acetylated H3 (1:200 dilution rabbit polyclonal, Upstate, 06-599), H3K27 tri methylated (1:100 dilution rabbit polyclonal, Upstate, 07-449), RNA pol II phosphorylated subunit (1:200 dilution rabbit polyclonal, Abcam, ab5131-50), Lamin B1 (1:200 dilution rabbit polyclonal, Abcam, ab16048-100). The secondary antibodies were anti rabbit (Upstate, 12-510) and anti mouse (Upstate, 12-509) Rhodamine labelled (1:200 dilution). To visualize DNA Hoechst 33342 (Sigma) was used. To quantify levels of histone modifications, the images were analyzed using custom programs written using LabVIEW and ImageJ. The region of interest of antibody fluorescence images were thresholded using the corresponding DNA Hoechst 33342 fluorescence. The thresholded pixel values were binned and all the intensity values lower than half-maximum past the mean was discarded as background noise. The remaining intensely stained pixel values were used to calculate the mean.

**Plasmids:** The H2B gene was digested from pBOS-H2B-EGFP using KpnI and BamHI restriction sites and cloned into pEGFP N1 vector (Clontech, Palo Alto, CA). The H1.5 EGFP N1 plasmid has been described previously (20). Mouse HP1α-EGFP N1 plasmid was made as

# Corresponding author: shiva@ncbs.res.in

follows: the gene was first amplified from mouse cDNA using primers (NM_007626, HP1Fwd-5'GGGGTACCACCGCCATGGGAAAGAAGAC3' and HP1 Rev-5'CGGGATCCCGGCTCTTCGCGCTTTC3'), was digested and ligated into pEGFP N1 plasmid, using KpnI and BamHI restriction sites. The pEGFP-LaminB1 C1 plasmid was sourced from Ellenberg lab at EMBL.

**Supplementary figure captions:**

**S1.** To quantify the fluctuation of the nuclear boundary, time-lapse imaging of EGFP-LaminB1 protein was done in ES cells and PMEF. Image-J was used for noise reduction, and to convert these images to binary images. A LabVIEW program was used for edge detection and to find the nuclear radii (mean) at different angles in $1^o$ intervals (from $0^o$ to $359^o$) from its center of mass. The mean square changes of the nuclear radius over all the angles, in different time points were used for quantifying the fluctuation of the lamin boundary. The change in the nuclear radii $(\delta r_i)$ at any time point (t) at an angle (i= $0^o$, $1^o$, $2^o$,…..$359^o$) compared to the radii at t=0 at the respective angles, were numerically calculated. The mean square fluctuation $[\langle(\delta r)^2\rangle = \Sigma(\delta r_i)^2 / N]$ in the nuclear radii over all angles was plotted at different time points to compute the membrane fluctuation timeseries.

**S2A.** Fluorescent images of PMEF nuclei transiently expressing H2B-EGFP. Shown are images - prebleach (0 sec), immediately after bleach (10 sec) and after 325 sec. PMEF nuclei, show negligible recovery in this time interval. The scale bar is 2μm.

**S2B.** Fluorescent images of PMEF nuclei transiently expressing HP1α-EGFP are shown prebleach (0 sec), immediately after bleach (7 sec) and after 35 sec. The scale bar is 2μm. **S2C.** Representative correlation curves for the core histone H2B-EGFP in both ES cells and in PMEFs. The inset shows the mean and standard deviation of the correlation times for ES cells and PMEFs.

**S2D.** Representative autocorrelation curves of H1.5-EGFP, obtained by FCS measurements, in ES cells and PMEFs.

**S3A. & C.** ES cells and PMEFs were stained with antibodies against H1, acetylated H3 and H3K27 trimethylation, RNApol-IIp and Lamin B1, and detected with rhodamine-labeled secondary antibodies. Representative images are shown in different rows (green). The merge

with the corresponding DNA images as visualized by staining with Hoechst 33342 (red) are also presented. The scale bar is 5μm.

S3**B. & D.** Mean Pixel intensity of the stated stains in both ES cells and PMEFs (n=30, each) are shown. Imaging was done with identical settings.

**S4A.** Fluorescent images of nuclei in the *Drosophila* embryo after 13$^{th}$ nuclear division (before cellularization), before (0sec), and after (57 secs, 439 secs) long-strip photobleaching of different marked regions. The scale bar is 20 μm.

**S4B.** Fluorescence intensity at the marked regions indicates that the exchange mechanism of H2B-EGFP is independent of position and flows in the yolk.

**S4C.**The panel of images shows recovery in whole nucleus FRAP of linker histones (labeled with Alexa-488) at the indicated time points. The scalebar is 5μm. The recovery is quantified in the normalized fluorescence intensity plot, for a nucleus at 0 hrs from 13$^{th}$ nuclear division.

**S5A.** Representative autocorrelation curves for core histone H2B-EGFP in a *Drosophila* embryo. The correlation curves indicate that the core histones have subdiffusive transport inside the cell nucleus, both at 0hrs and at 5 hrs from the 13$^{th}$ nuclear division. Typical correlation timescales indicate the core histones to be in a multimeric state inside the cell nucleus as well as in the yolk before cellularization. The mean and the standard deviation of the correlation timescales for H2B-EGFP (0hrs after 13$^{th}$ nuclear division, 5 hrs after 13$^{th}$ nuclear division, and in the yolk at 0hrs from 13$^{th}$ nuclear division) are shown in the inset. (n=30).

**S5B.** Confocal fluorescent images of the nuclei before photobleaching both in the euchromatin and heterochromatin regions are shown, with the bleach regions indicated. The FRAP curves show negligible recovery in the both these regions at 5 hrs from 13$^{th}$ nuclear division.

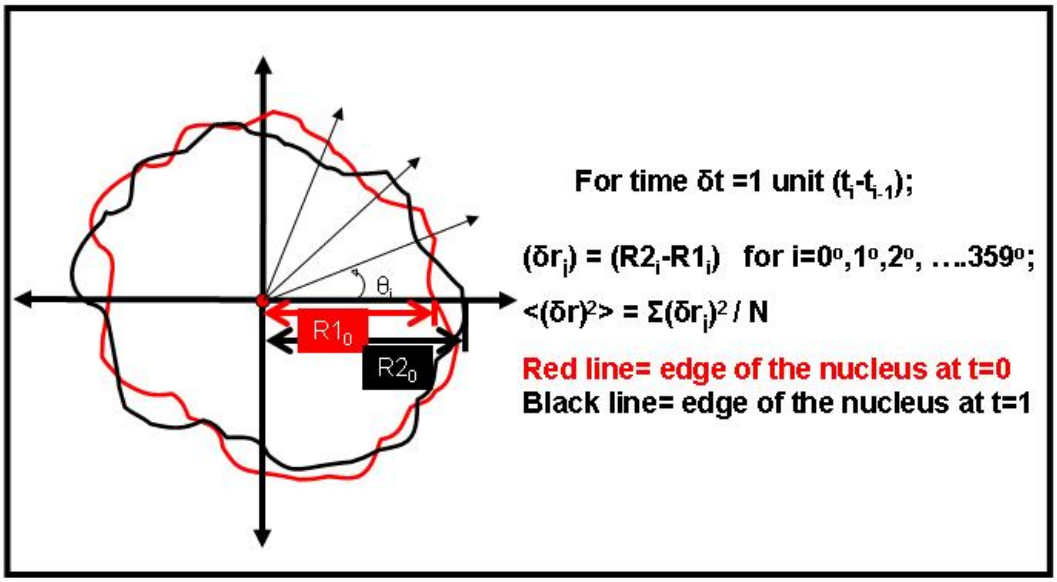
**S6A.** Alexa-488 labeled linker histones show fast fluorescence recovery in the *Drosophila* embryo nuclei even after cellularization. **Inset:** Confocal fluorescence images of linker histones indicate proper nuclear localization after cellularization (1hour from 13$^{th}$ nuclear division). The bleach region is marked.

**S6B.** Probability of different correlation timescales in the FCS curves using MEMFCS software for Alexa-488 labeled linker histones in an embryo (after cellularization) or in HeLa nuclei. Note the complete absence of interaction dependent timescales in the nuclei of the early *Drosophila* embryo. Maximum entropy method (MEMFCS) is a routine to identify distinct correlation

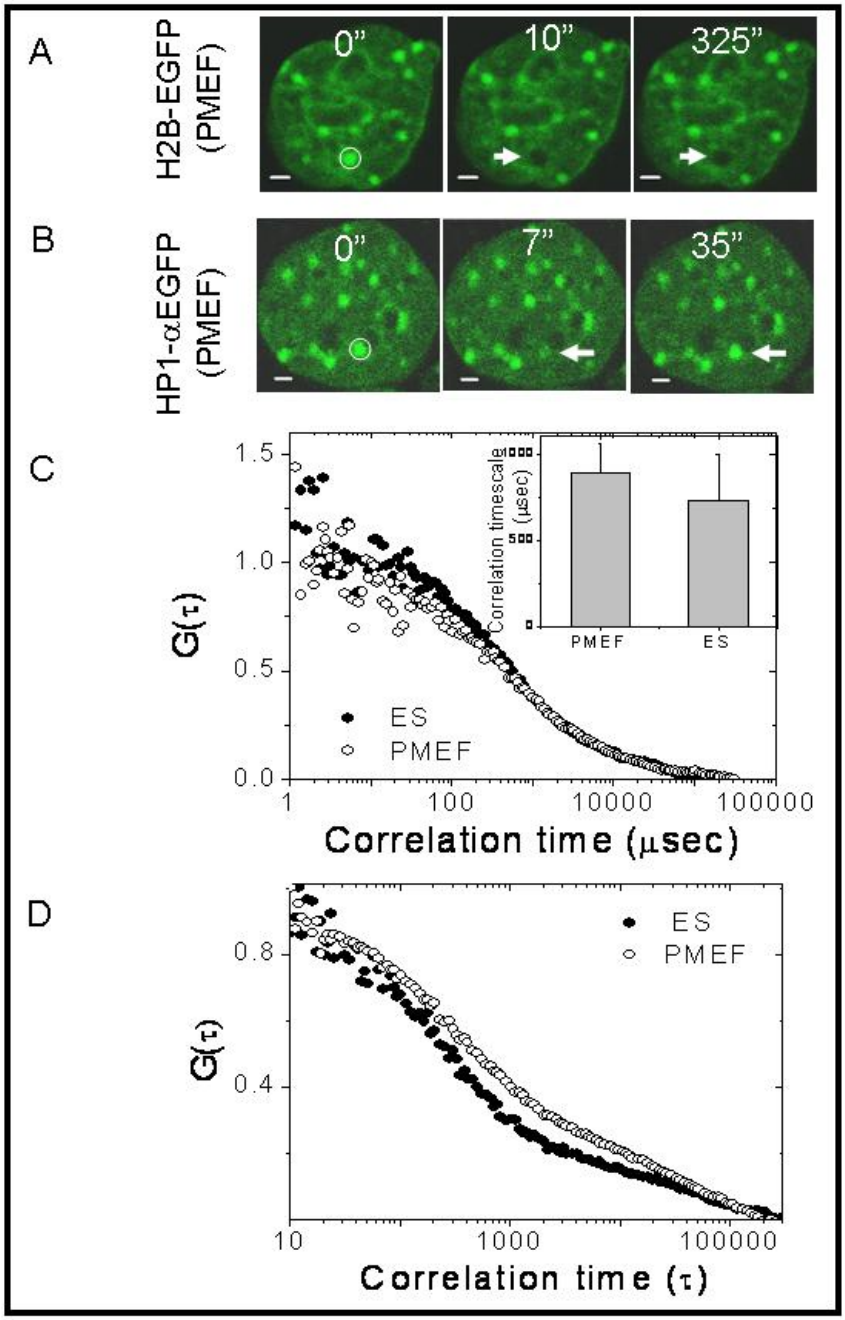
timescale and species in an FCS autocorrelation curve (Periasamy, N. & Verkman, A. S. (1998) *Biophys J* **75,** 557-67). We have used this routine to probe the interaction timescale of linker histone binding to chromatin assembly.

**Movies:**

1. M1: Lamin B1 in ES cells 808 sec
2. M2: Lamin B1 in PMEF cells 808 sec
3. M3: Core histone: H2B-EGFP in ES cells 882 sec
4. M4: Core histone: H2B-EGFP in PMEF cells 882 sec
5. M5: HP1α in ES cells 114 sec
6. M6: HP1α in PMEF cells 114 sec.
7. M7: Drosophila embryo: Whole nucleus H2B-EGFP after $12^{th}$ nuclear division 788 sec
8. M8: Drosophila embryo: Whole nucleus FRAP H2B-EGFP after 0hrs from $13^{th}$ nuclear division 796 sec
9. M9: Drosophila embryo: Whole nucleus FRAP H2B-EGFP after 1 hrs from $13^{th}$ nuclear division 796 sec
10. M10: Drosophila embryo: Long strip FLIP after 1 hrs from $13^{th}$ nuclear division.
11. M11: Drosophila embryo: Small region FRAP after 0hrs: small FRAP core: 1.5 micron bleach 167 sec timescale
12. M12 Drosophila embryo: Small region FRAP after 5hrs: small FRAP core: 1.5 micron bleach 168 sec timescale.




Supp. Figure -1: Bhattacharya et.al.

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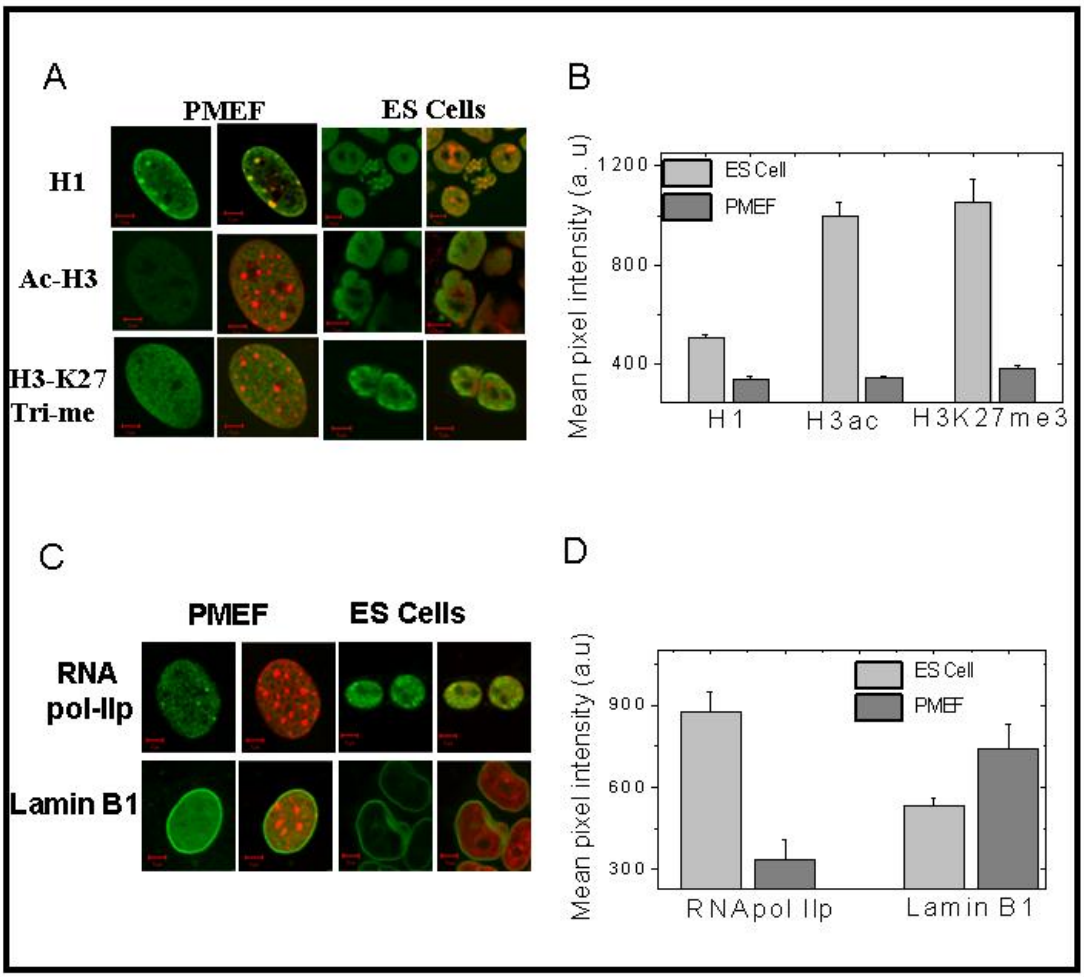


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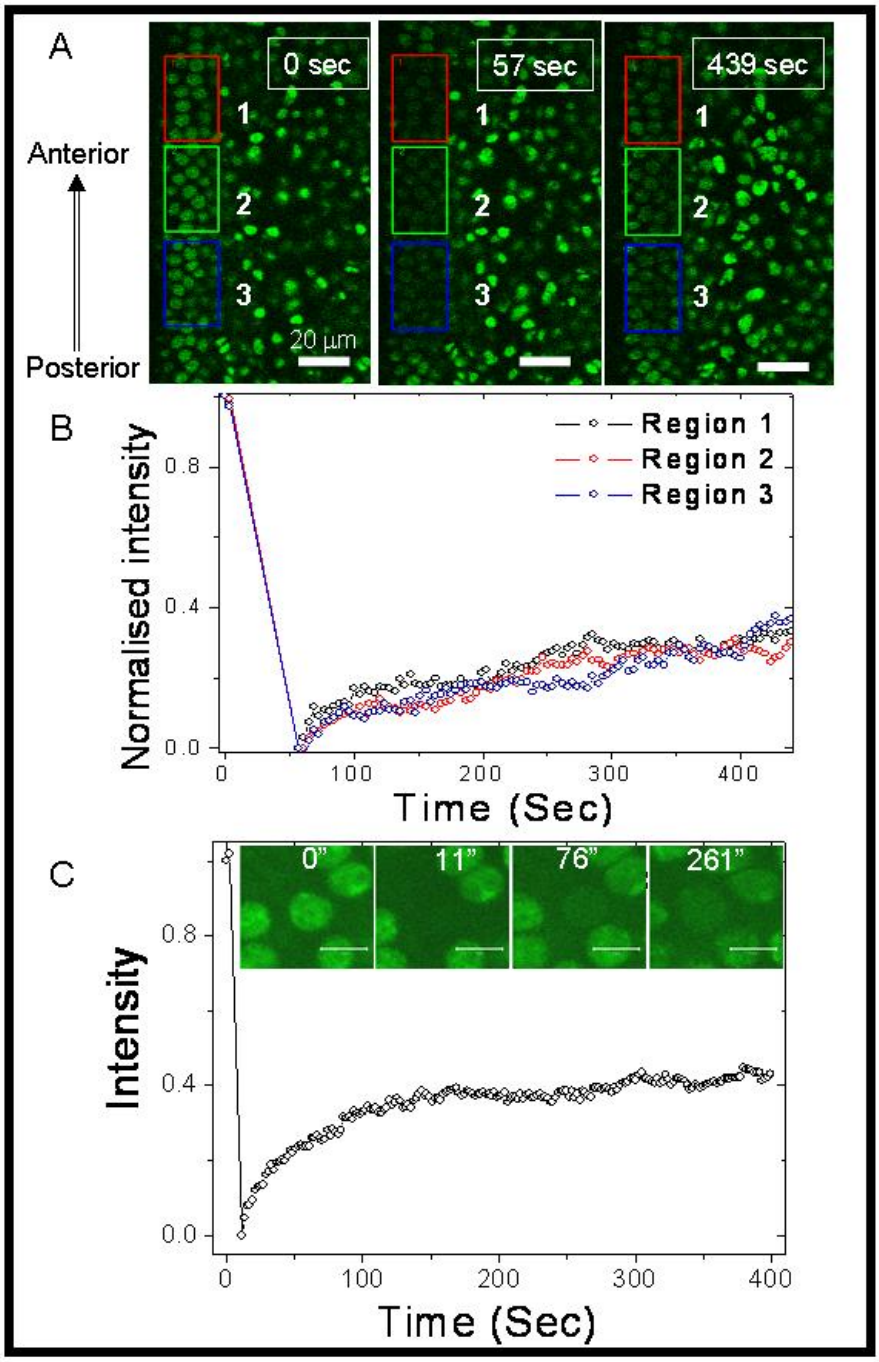


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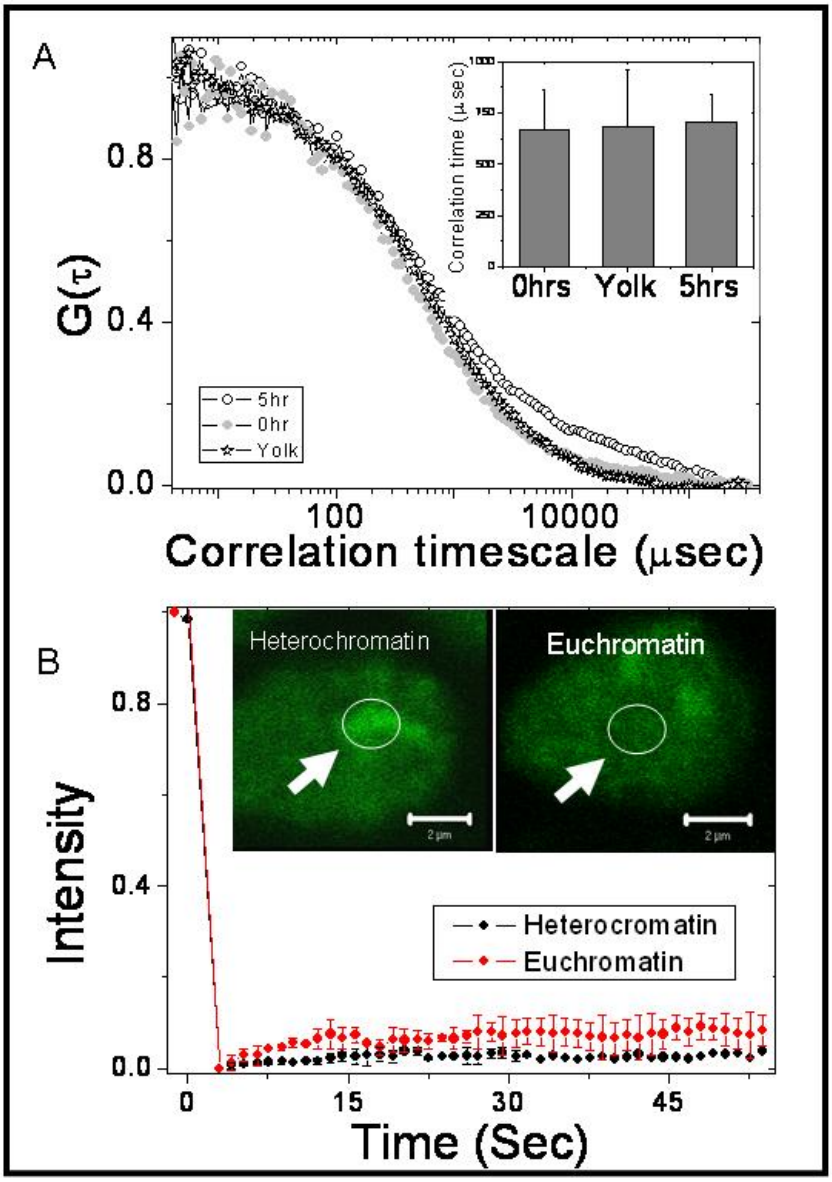


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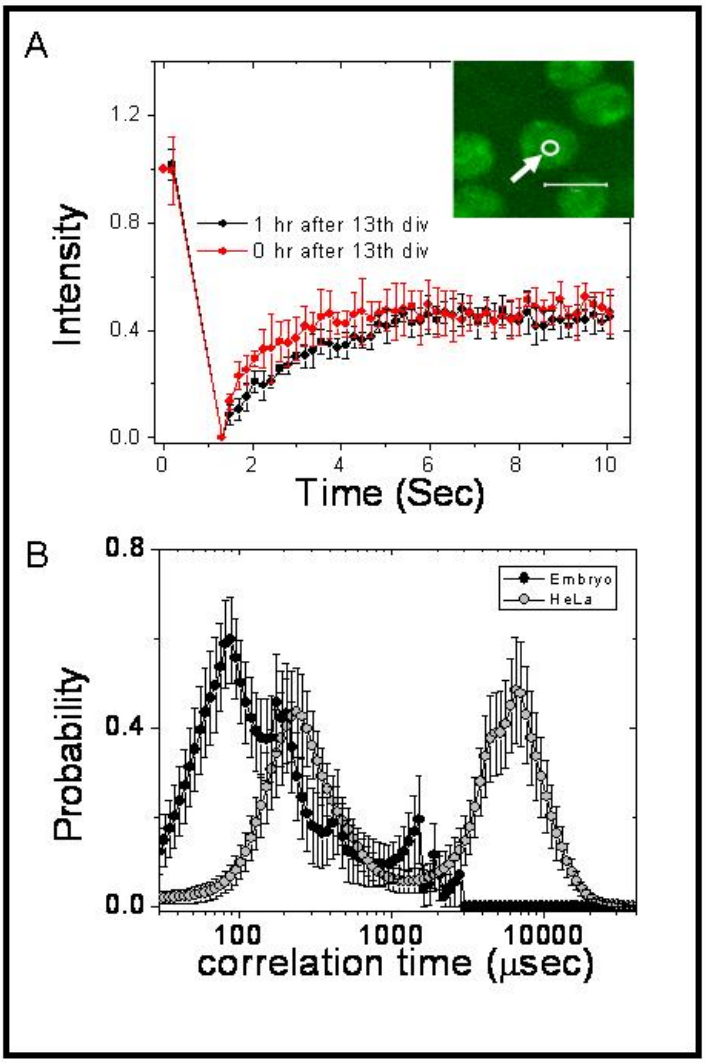


Supp. Figure -6: Bhattacharya et.al.