Probing the dynamic organization of transcription compartments and gene loci within the nucleus of living cells

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Supplementary material:

Figure S1: We demonstrate in **Figure S1a** the nuclear localization of TC by imaging multiple z-planes of the HeLa nucleus with H2B-EGFP and TR-UTP. One of the z-sections of fluorescent images of TC (red) and chromatin (green) is shown in **Figure S1a**. The top and right section of the image shows the XZ and YZ section of the cell nucleus along the green and red line. The TC can be seen well inside the cell nucleus in XZ and YZ sections. In addition to TC inside the nucleus there are TR-UTP enriched sites in the cytoplasm which are also dynamic but do not respond to the perturbations done to the chromatin architecture or transcription state of the cell. Figure S1b shows that the cytoplasmic enrichment of Cy5-UTP (red) co-localizes with mitochondrial marker MitoTracker Red (green).

Figure S2: The parameters determining the mean squared displacement $MSD(\tau) \approx D \times \tau^{\alpha}$, where D-the diffusion constant and α are then used to characterize the nature of TC dynamics. Mean α values was used to classify the mobility of TC that diffuse freely (normal diffusion, 0.8<α<1.2), in physical constrained environment (sub-diffusion α<0.8) or in presence of external forces (super-diffusion α>1.2). Using trajectories $\vec{x}(t)$ obtained previously, the mean square displacement for each TC is calculated using equation 1. $MSD(\tau) = \left\langle \{\vec{x}(t+\tau) - \vec{x}(\tau)\}^2 \right\rangle_t$ (1)

Where, $\vec{x}(t)$ represents position of particle at time t, and τ is arbitrary interval of time. **Figure S2a** shows typical MSD plots for the three classes of diffusion. The nature of diffusion or mobility can be analyzed by plotting the MSD against τ. To find the nature of mobility (sub-diffusion, super-diffusion or normal diffusion) we fitted the MSD computed from Eq.1 to the following equation. $MSD(\tau) \approx D \times \tau^{\alpha}$ (2)

For particles undergoing normal diffusion MSD depends linearly on τ i.e. $\alpha \cong 1$, however if the movement of diffusing particle is obstructed because of confinement or assisted

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with external force, then the diffusion is called anomalous and the value of α is less than 1 for diffusion in confinement and greater than 1 in case of assisted diffusion. In cases where nature of mobility of the particle is time dependent i.e. when the normally diffusing particle gets confined or pulled by a molecular motor, the MSD does not follow a single exponent α for all time τ, rather we find a τ dependent exponent. To analyze the anomalous and time dependent behavior of the TC and gene locus better, we plotted $\log\left(\frac{MSD}{\tau}\right)$ versus $\log\tau$ (**Figure S2b**). In log-log plot the particles undergoing anomalous diffusion gives positive slope for super diffusion and negative slope for sub-diffusion, and normal diffusion causes zero slopes. In **Figure S2b** we show MSD/time verses time in log-log plot for a TC, it is evident from the plot that this TC has regimes of super diffusion till log (τ) = 1.6 and for subsequent time it exhibits sub diffusion. Inset to **Figure S2b** shows the trajectory for which the MSD is computed. We fit equation 2 to first 15 second data of the MSD to get α and D. The size dependence of TC mobility is checked by plotting mean α values against the area of TC. **Figure S2c** shows the velocity distribution of TC and the fraction of super, sub and normal diffusion of TC is plotted in the inset. **Figure S2d** depicts a size-independent behavior of TC diffusion.

Figure S3a depicts the change in super-diffusive motion of TC when treated with TSA for 12 or 24 hours. Chromatin de-condensation directly affects the localization and movement of TC. Further, by arresting cells at the early S phase where progress of replication is stalled by aphidicolin, we find a larger fraction of TC undergoing super-diffusion (**Figure S3a**). **Figures S3b**, **S3c** demonstrates that variation in TC mobility caused by treatment of cells with TSA and Aphidicolin - due to perturbations in the nuclear environment affect the mean and standard deviation of the TC velocities as evident from the distributions. The velocity distributions are 20 point average of raw data and the solid line is a spline fit. **Figure S3d** illustrates the differential changes observed between the cytoplasmic and nuclear fractions of TC upon addition of inhibitors.

Figure S4a displays the scheme for marking the gene loci and controlling its transcription state. **Figure S4b (i)** shows the nuclear localization of the labeled gene loci where the left and lower panels are the XZ and YZ cross-sections. **Figure S4b (ii)** is an image of the gene loci labeled cell with H2B-EGFP marking the chromatin. A very low EGFP expressing cell was used where the H2B-EGFP expression was much higher. **Figure S4b (iii)** is a plot of the line scan across the gene locus. While due to the upstream labeling with mRFP-LacI-NLS, its signal maximizes at the gene locus, H2B-EGFP signal remains similar throughout. This shows that the transfected gene locus is physically proximal to the genomic chromatin. The mobility of gene loci when assayed with the TC labeled with Cy5-UTP has been shown in **Figure S4c** and **S4d**. **Figure S4c** shows TC from the same cell show different ranges of mobility, while in **Figure S4d** a TC co-localizing with the gene locus and moving together is shown.

Table ST1: lists for the TC the mean velocities and α under various conditions and for the gene loci mean α under various conditions.

Supplementary Movies:

Made at 30 Frames Per Second (FPS)

Name	**Binning**	**Total time (sec)**
SM1_WT	1.59 sec	370
SM2_TSA	1.59 sec	470
SM3_Aphi-arrest	1.59 sec	470
SM4_Geneloci_TC (gene loci in red, Cy5-UTP in green)	4.30 sec	222

*(displayed every 8 frames)

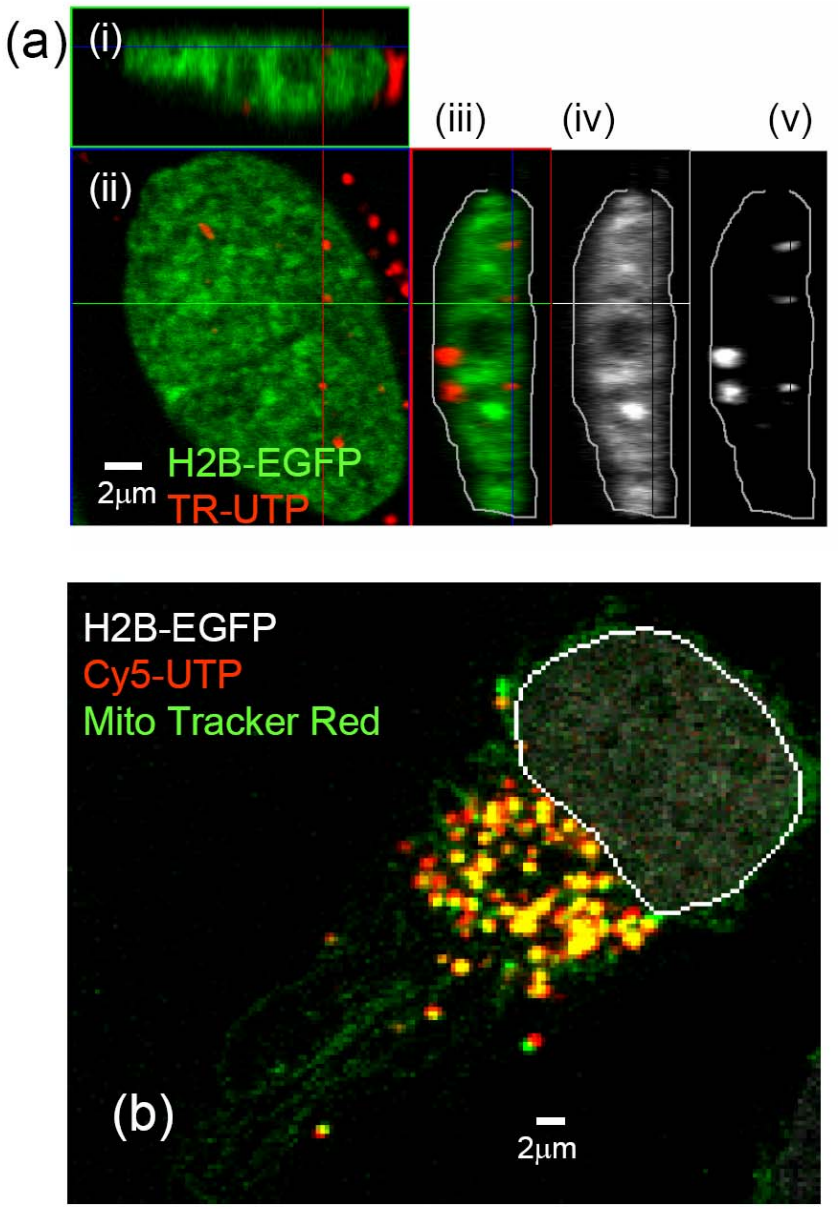

Figure S1: Sinha et al

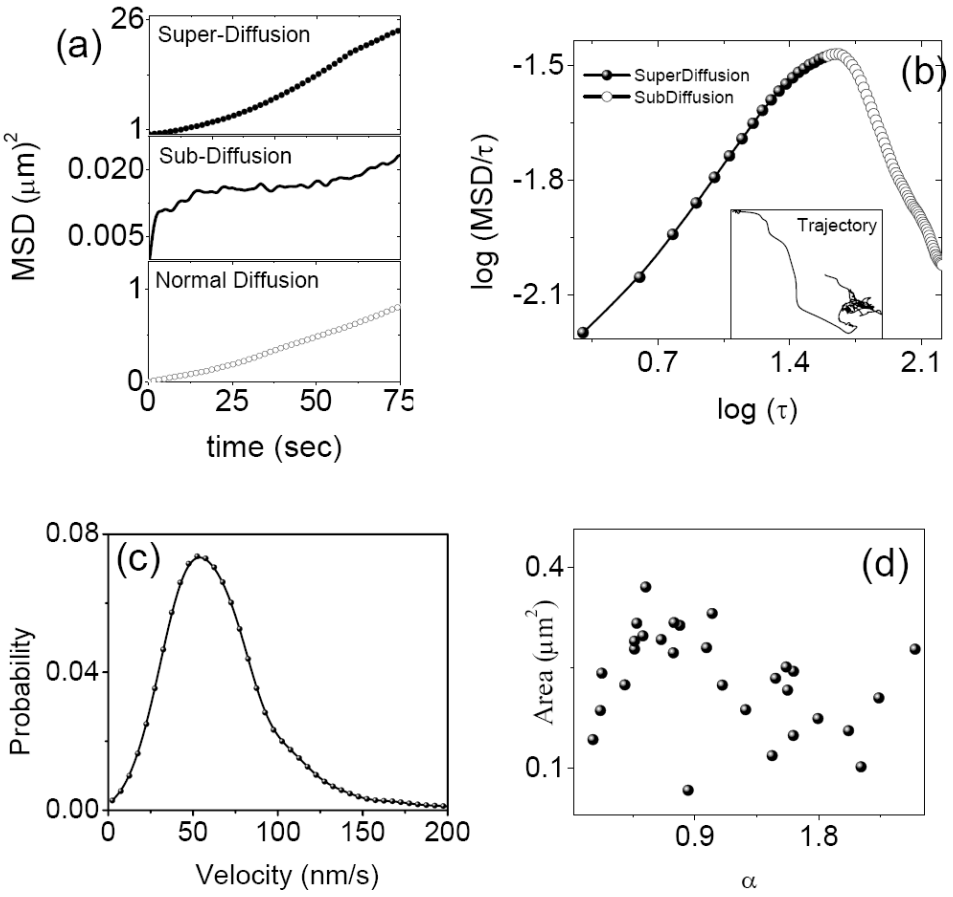

Figure S2: Sinha et al

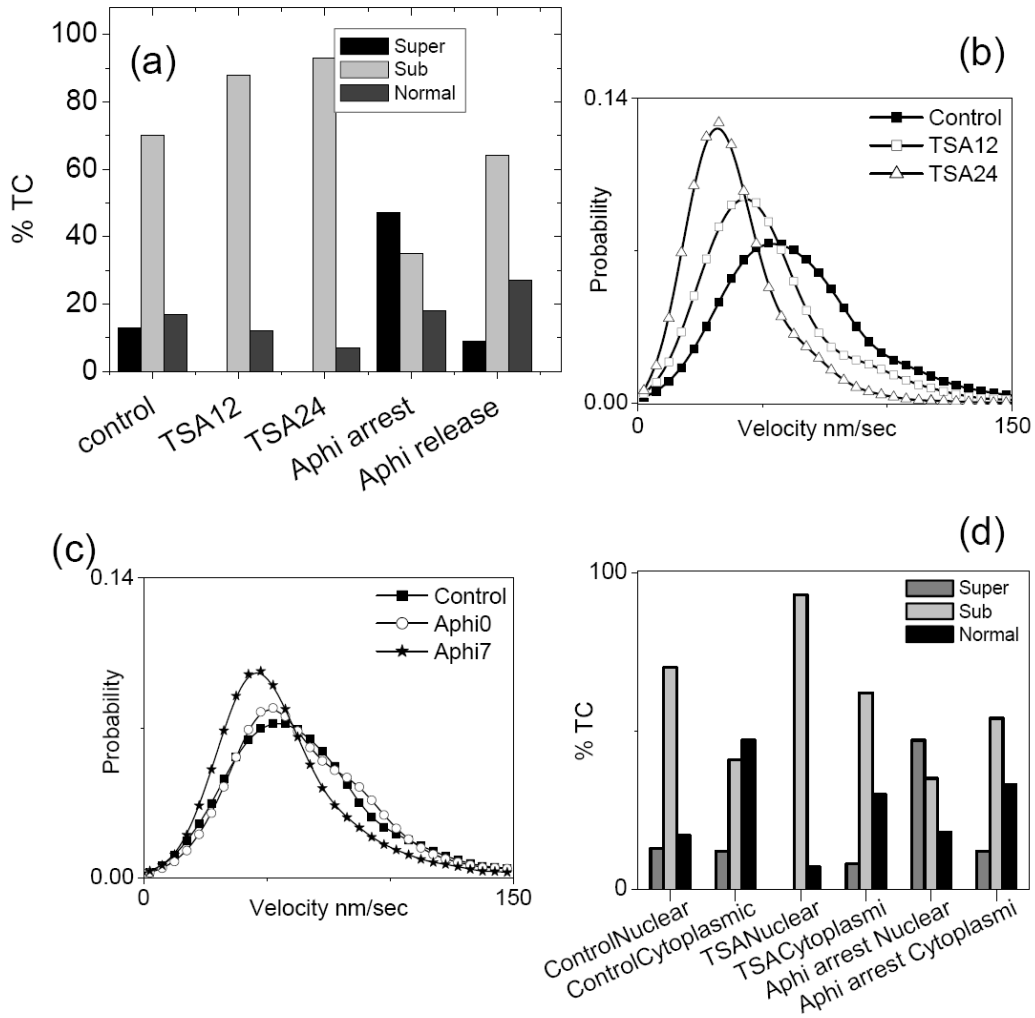

Figure S3: Sinha et al

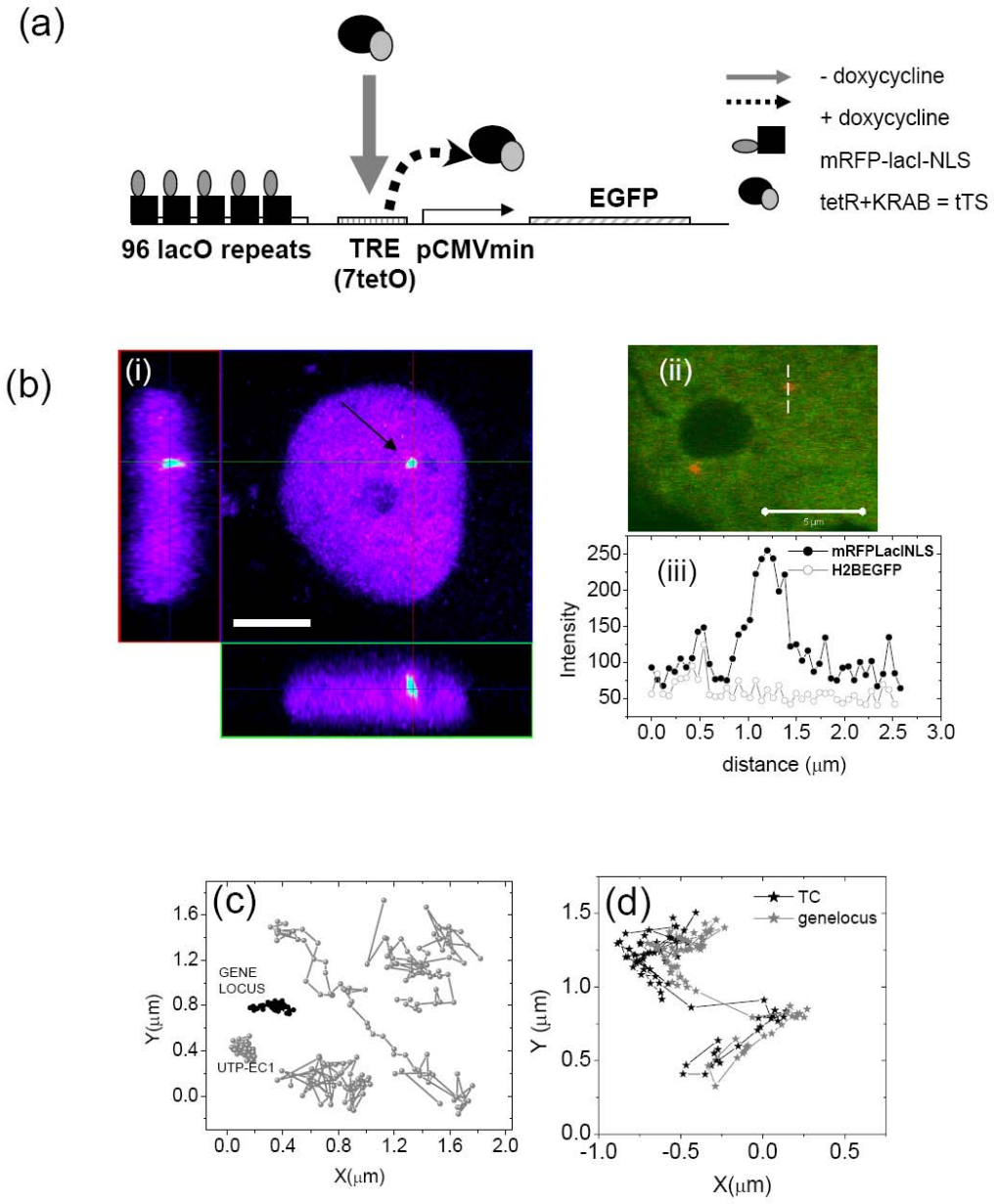

Figure S4: Sinha et al

TC	Velocity ± se * (nm/sec)	α ± se *
Control	71 ± 0.3	0.58 ± 0.08
TSA12	60 ± 0.2	0.42 ± 0.05
TSA24	40 ± 0.1	0.39 ± 0.04
Aphi arrest	80 ± 0.5	1.03 ± 0.08
Aphi release	60 ± 0.6	0.69 ± 0.05
LOCI		
Repressed	-	0.23 ± 0.04
Expressing	-	0.59 ± 0.04
+DRB	-	0.20 ± 0.03
DRBremoved	-	0.43 ± 0.06
Repressed+TSA	-	0.26 ± 0.05
Expressing+TSA	-	0.51 ±0.08

* se: standard error of the mean

Table ST1: Sinha et al