

CHROMATIN DYNAMICS IN THE DROSOPHILA NUCLEUS

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The extent to which structure influences gene function is one of the most important problems in nuclear architecture research. In *Drosophila* homologous chromosomes are usually paired in diploid nuclei and this pairing can occur even after translocation of one allele to another chromosome. To investigate the extent and importance of chromatin mobility in nuclear function we have established two experimental approaches to measure mobility in living *Drosophila*.

In one system small arrays of lac operator sequences (lacO arrays) are inserted into the genome and followed by the confocal imaging of the bound GFP lac repressor (GFP-LacI) in live whole animals and tissues. The lacO arrays or lacO arrays combined with host sequences that bind specific repressor or insulator proteins allow us to assess the influences of higher-order chromatin complexes on the mobility.

In the other system we constructed fly lines expressing a photoactivatable histone2AvD-paGFP fusion protein that becomes stably incorporated into nucleosomes. Two-photon photoconversion at 820 nm in a CLSM generated fluorescent loci that could be tracked in time and space while preserving viability of live *Drosophila* embryos and larval tissues. Single photon activation at 408 nm or other two-photon wavelengths were toxic [1].

In both systems fluorescent loci, which ranged in size from 0.2-0.8 μm^2 , were tracked using specialized routines written in DIPImage and mean square displacement (MSD) plots for each locus were generated. From the curve of the averaged MSD for these loci we found that the movements are subject to restricted diffusion. The average diffusion rate constants were calculated from the initial slope of the MSD plots and compared for different stages of development, different locations in the nuclear volume and, in the case of the LacO arrays, for presence or absence of associated sequences. The data show rates of diffusion that are more comparable to values found in yeast than for mammalian nuclei although the restricted area is much larger than that of yeast nuclei.

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[1] J.N. Post, K.A. Lidke, B. Rieger, and D.J. Arndt-Jovin. "One- and two-photon photoactivation of a paGFP-fusion protein, a phototoxicity study in live *Drosophila* embryos." *FEBS Letters*. **579**:325-330 (2005) (This report constitutes the first demonstration of two-photon activation of paGFP and the use of a paGFP-fusion protein in investigations of whole organisms.)