

The mechanisms that establish higher-order chromosome structures during metazoan development and the roles these structures play in gene regulation have been elusive. The Meyer lab explores the interplay between chromosome structure, chromatin modification, and long-distance regulation of gene expression in the context of *Caenorhabditis elegans* X-chromosome dosage compensation to understand the mechanisms that repress X gene expression in XX hermaphrodites by half to balance it with X expression from XO males. We have built an integrated experimental platform that will enable us to synthesize information obtained from dynamic molecular imaging of components of the dosage compensation complex (DCC) inside cells of living *C. elegans* embryos with a nanoscopic structural view of the DCC and chromosome conformation inside the same cells and with the cellular transcriptome.

Previously, we showed that five of ten subunits of the dosage compensation complex (DCC) are homologous to components of condensin, a complex that restructures and resolves mitotic and meiotic chromosomes in preparation for their segregation during cell division. The DCC drives the remodeling of X-chromosome topology in hermaphrodites during dosage compensation by compacting X chromosomes and by creating topologically associating domains (TADs) through binding to its highest affinity binding sites on X. The TAD architecture regulates *C. elegans* lifespan but not dosage compensation, while the X compaction plausibly facilitates dosage compensation by an unknown mechanism.

To decipher the mechanisms driving nematode dosage compensation, we examine dosage compensation from the perspective of changes in (1) Transcription kinetics of X-chromosome genes using genome integrated MS2/PP7 transcriptional reporter cassettes; (2) Kinetics of the interaction of transcription factors, chromatin remodelers, RNA polymerases, and epigenetic modifiers to the X chromosome at high spatiotemporal resolution; (3) Chromatin dynamics and compaction of the X chromosome at local and global scales using dCas9-ArrayG/N based locus tracking and a combination of Oligopaint FISH probes and multispectral imaging; and (4) distribution of DCC components and RNA polymerase on X chromosomes and autosomes using multiwavelength STORM (stochastic optical reconstruction microscopy).

While each of the approaches provides unique information about specific aspects of the dosage compensation process, we have now built a single imaging platform that will allow us to seamlessly integrate dynamic molecular imaging of DCC subunits inside living *C. elegans* embryonic cells, high-resolution imaging of DCC components on X chromosomes, precision mapping of cell-specific chromosome-wide chromatin conformation, and multiplexed RNA FISH-based imaging of the cellular transcriptome.

Some of the outstanding questions in *C. elegans* X-chromosome dosage compensation we are addressing include the following: (1) How do DCC proteins nucleate and spread on the X chromosome; (2) How does chromatin structure respond to and/or regulate the DCC nucleation and spreading process; and (3) How is DCC nucleation and spreading on the X chromosome functionally connected to transcriptional attenuation in hermaphrodites? To generate high-resolution maps of DCC proteins and RNA polymerase inside embryonic nuclei, we will carry out stochastic optical reconstruction microscopy (STORM) of the different proteins, and analyze their relative distribution in space. When combined with oligopaint-STORM we will delineate the distribution of DCC proteins and RNA polymerase on specific chromosomal territories. This

approach is crucial to our understanding of how changes in the distribution of different DCC proteins and RNA polymerase may regulate X chromosome dosage compensation.