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Large-scale Chromatin Structure and Dynamics: a Combined Structural and Molecular Approach

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As a means of visualizing large-scale chromatin structure and dynamics our laboratory has used engineered chromosome regions tagged with lac operator repeats [1]. Recent progress in our laboratory focuses on analyzing multi-copy transgene arrays constructed from bacterial artificial chromosomes carrying 100-250 kb insertions of Drosophila or mammalian genomic DNA. These BAC transgene arrays not only recapitulate transcription of model gene loci to within several fold of the endogenous locus but also recapitulate locus specific patterns of large-scale chromatin compaction and intranuclear positioning.

Using this BAC transgene array approach we have been asking what cis and trans factors control large-scale chromatin structure and dynamics. We have identified cis elements within a genomic region surrounding the human HBB locus that confers autonomous targeting of integrated HBB BAC to the nuclear periphery [2]. These "PTRs", or peripheral targeting regions, also confer increased H3K9me3 modification over the HBB BAC that is required for peripheral targeting. Our working hypothesis is that they act as epigenetic modifying sequences that nucleate a distinct epigenetic state over the HBB BAC transgenes, modifying both nuclear localization and chromatin compaction. Similarly, we have identified targeting of Hsp70 BAC transgenes to nuclear speckles, tracing this targeting activity to the Hsp70 promoter [3, 4]. Using RNAi we have begun to use these same transgene arrays to identify trans factors involved in large-scale chromatin structure. We have carried out a Drosophila S2 RNAi candidate screen examining ~200 chromatin modifying trans factors, identifying a number which result in either decreased or increased decondensation of a MT BAC transgene array after transcriptional activation by copper.

Simultaneously, we are trying to examine the changes in large-scale chromatin structure caused by manipulating these cis and trans regions using a combination of wide-field light microscopy, super-resolution light microscopy, and electron microscopy.

A surprising result from our work is that these BAC arrays from linear, large-scale chromatin "fibers" which remain condensed through both transcription and replication at compaction ratios 5-10 fold higher than that estimated for a 30 nm chromatin fiber even for "decondensed", transcriptionally active structures [3]. Thus both transcription and replication occur on a globally condensed template. A major question then is how these condensed structures can serve as a DNA template for transcription and replication and whether the polymerases move within these structures along the DNA or whether the DNA is mobile and moves to the polymerases.

A second surprising result from our work is that although these BAC transgene arrays, Mbps in size, form linear, fiber-like structures, there appears to be looping of DNA within these structures, as inferred by the number of GFP-lac repressor foci relative to the number of BAC copies [5]. This has led us to propose a "dynamic plasticity" model for large-scale chromatin structure in which long-range looping

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interactions co-exist with a global folding of chromatin into large-scale chromatin fibers [1]. Experiments now in progress support this dynamic plasticity model of large-scale chromatin organization within endogenous chromosomes during DNA replication.

A major goal of our work moving forward is to integrate these structural investigations of large-scale chromatin folding with global, genomic analysis. We are working on developing new genomic methods that bear directly on structure as directly measured by microscopic methods. Initial work has used mapping genome- nuclear speckle association as a model system for developing such methods.

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