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# Heterochromatin Domains: Uncoupling Epigenetic Modifications and Chromatin Structural Parameters

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The mammalian genome is organized into silenced and active domains, which are constituted by chromatin fibres. The fibres are comprised of nucleosome subunits in which nearly two turns of DNA wrap around a histone protein core. This first order of DNA structure is known as the 10 nm chromatin fibre. Almost since the discovery of the nucleosome our widely accepted paradigm of genome regulation has included higher orders of chromatin organization based on the coiling or folding of the 10 nm chromatin fibre into the 30 nm chromatin fibre. These 30 nm chromatin fibres could occlude activating factors, thereby preventing transcription of genomic domains. Thus, the transition between the euchromatin "open" 10 nm and the heterochromatin "closed" 30 nm chromatin fibre would ultimately dictate genome activation and silencing. Additionally, these 30 nm fibres were thought to be further condensed into large structural heterochromatin domains defined biochemically by specific post-translational modifications of their nucleosomes' core histones.

To investigate the relationship between epigenetic modifications and chromatin structure *in vivo* we took advantage of energy-loss transmission electron microscopy, specifically Electron Spectroscopic Imaging (ESI). ESI enables the visualization of phosphorus-rich chromatin fibres in relation to protein-rich nuclear domains at nucleosome resolution without the use of contrast enhancing agents. ESI is able to distinguish individual nucleosomes and even DNA linker sequences *in situ,* ESI, however, suffers from the same limitation of conventional transmission electron microscopy that biological material overlapping in the z-dimension cannot be distinguished and is projected onto a single image plane. To address how higher order chromatin fibres are organized *in vivo* in three dimensions (3D), we combined ESI with electron tomography (ESI-tomography). The resulting tomograms give us the first glimpse of chromatin fibre 3D organization *in situ* at high spatial resolution. Surprisingly, when we analyzed even the most compact heterochromatin domains by ESI-tomography within the mammalian mouse genome, we detected exclusively 10 nm chromatin fibres [1].

This finding raises a fundamental question about the structural role of chromatin organization in genome regulation; what is ultimately responsible for generating the distinct structural and functionally distinct domains present within the mammalian genome if it does not involve transitions between 10 and 30 nm higher order chromatin fibre assemblies?

We were thus motivated to combine ESI-tomography with correlative immunofluorescence microscopy [2] to investigate the structural parameters of chromatin fibres that define heterochromatin domains. We used tri-methylated histone H3 lysine 9 antibodies directed against constitutive heterochromatin domains in mouse embryonic fibroblast cells to identify the compact chromocentres in these cells. We imaged these heterochromatin domains and the surrounding euchromatin regions by ESI-tomography and calculated the distance between nucleosomes, the degree of chromatin fibre bending and the distance between adjacent chromatin fibres within these two distinct chromatin compartments (Figure 1). Surprisingly, our preliminary analysis reveals no significant differences between nucleosome spacing

in "open" versus "closed" chromatin domains. However, two other factors, fibre bending and inter-chromatin fibre spacing, are significantly different, arguing these may be sufficient to account for the "open" versus "closed" differences. In addition, the relationship between protein structures and chromatin fibre assemblies can now be described in 3D. We have noted an intimate association with protein-structures and individual chromatin fibres. The combination of ESI with tomography and correlative fluorescence imaging provides a powerful platform to investigate the relationship between chromatin structure and function.

References:
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[2] E. Fussner, *et al,* EMBO J **4;30(9)** (2011) p. 1778-89.
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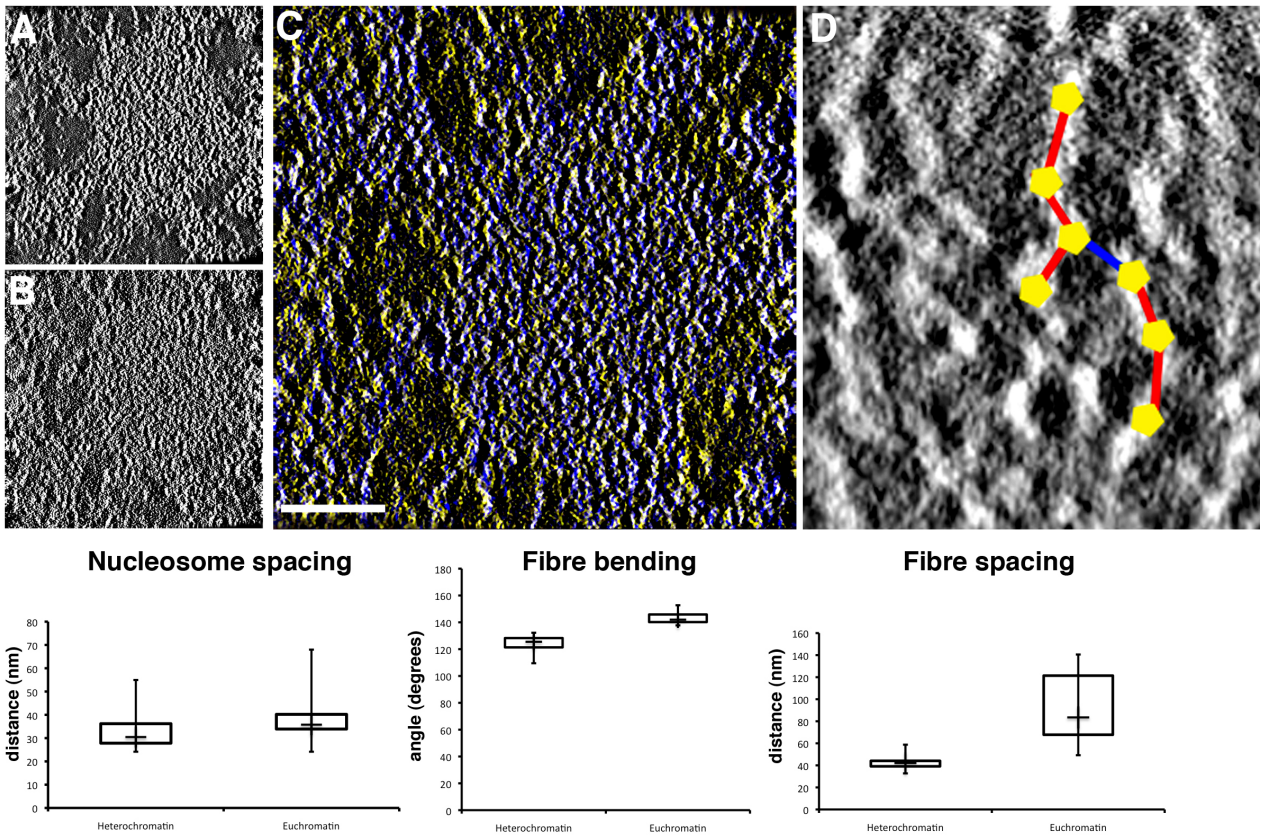


**Figure 1.** Central slabs of phosphorus (A) and nitrogen (B) ESI-tomograms can be combined (C) to illustrate the relationship between chromatin fibres (purple) and protein-based structures (yellow). The three-dimensional reconstructions can be analyzed to determine the distance between nucleosomes (yellow) within continues fibres (red) and between adjacent fibres (blue) to assess nucleosome and fibre spacing and fibre bending. Scale bar represents 0.2 μm.

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