

"Epichromatin: Nucleosomes at the Surface"

In eukaryotic cells, interphase chromatin is bounded by the nuclear envelope (NE) with intimate contacts to the inner nuclear membrane. During the late 1960's, ultrastructural studies of fixed cells demonstrated a layer of chromatin adjacent to the NE. Recently, we discovered two specific monoclonal antibodies (PL2-6 and 1H6) that highlight this surface layer (denoted by us "epichromatin") using immunostaining methods. These antibodies give identical staining patterns in diverse cell types, spanning from humans to plants. Thus, it is clear that the epichromatin epitope cannot be DNA sequence specific, but must reflect conserved features of the nucleosome. Furthermore, we demonstrate that these antibodies can interact with "all" mononucleosomes and with "all" chromatin exploded from a cell. We present cryoEM evidence that the epichromatin region in a cell nucleus exhibits ~30nm thick chromatin fibers layered adjacent to the nuclear envelope, which are reduced to ~15 nm thickness following fixation and plastic embedding.

In order to analyze the DNA composition of epichromatin, we developed an immunoprecipitation method to study its properties in a differentiating human cell system (HL-60/S4). We observed that, in the undifferentiated and differentiated cell forms, a similar ~5% of the genome is represented in epichromatin, which is enriched in retrotransposon Alu, but impoverished in both transcription permissive and repressive histone markers. By employing monovalent Fab fragments from PL2-6, we show that there are two binding sites for the antibody on each nucleosome. The peptide sequence of the antibody binding site resembles that of known nucleosome "acidic patch" binding proteins (e.g., LANA and CENP-C). We suggest that the generality of binding PL2-6 to epichromatin derives from the evolutionary conservation of the nucleosome acidic patch. The intense immunostaining at the periphery may be a consequence of increased amounts of chromatin and more accessible acidic patches facing the nuclear envelope.