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EPIGENETICS

Silence of the nucleosomes

Tumour-suppressor genes are often epigenetically silenced in cancer cells, a phenomenon that is associated with increased cytosine methylation. A new study shows that for one promoter region this methylation is accompanied by the presence of nucleosomes that are largely absent in this region in normal cells.

As previously shown, CpG islands have a chromatin structure that is different from bulk chromatin in normal cells, and they are often found at the promoters of cancer-associated genes. When associated with actively transcribed genes, they have low levels of cytosine methylation and histone H1 presence, but high levels of acetylation on those histones that are present, such as H3; this is reversed when the gene is silenced in cancer. In normal cells, CpG island promoters are also DNase I hypersensitive, which is why the authors decided to investigate whether changes in nucleosome occupancy were associated with silencing. They looked at the *MLH1* promoter, which they showed to be bidirectional for two transcripts of *MLH1* and one transcript of *EPM2AIP1*.

IMAGE SOURCE

Having confirmed the expected methylation and DNase I hypersensitivity patterns of both expressing and silenced cell lines, the authors separated specific nucleosomes from both cell lines using a sucrose gradient, and then used PCR to identify the DNA that was bound to each one. In the silenced lines nucleosomes were positioned all along the *MLH1* promoter, but in the expressing lines the region was largely unoccupied, containing just a single nucleosome immediately after the transcription start site. In support of this, chromatin immunoprecipitation showed that these same regions that lack nucleosomes in expressing cells have low histone H3 occupancy. Furthermore, a methylase-based single-promoter assay revealed regions of inaccessibility to methylase in the silenced lines that correlate with the proposed positions of nucleosomes.

Having demonstrated that nucleosome presence correlates with silencing of the promoter, the authors looked for changes in nucleosome occupancy upon activation of silenced lines through demethylation with 5-aza-2′-deoxycytidine. By using the same assays as before, they showed that a subset of promoters undergoes nucleosome eviction upon reactivation. They propose that these are the promoters that cease being silenced, as the expression levels correlate with the proportion of promoters at which nucleosomes have been evicted.

These results suggest a model in which silencing is directly due to the presence of nucleosomes, and that gain and loss of nucleosomes is triggered by changes in cytosine methylation. It will be interesting to find out how nucleosome positioning is inherited in cancer cells and whether changes in nucleosome positioning could be responsible for the use of alternative transcription start sites within the promoter.

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ORIGINAL RESEARCH PAPER Lin, J. C. *et al.* Role of nucleosomal occupancy in the epigenetic silencing of the *MLH1* CpG island. *Cancer Cell* **12**, 432–444 (2007)

FURTHER READING Esteller, M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nature Rev. Genet.* **8**, 286–298 (2007)