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Following the Modulation of Transcriptional Activity of Genes by Chromatin Modifications during the Cell Cycle in Living Cells

Authors : Sharon Yunger, Liat Altman, Yuval Garini, Yaron Shav-Tal

Abstract : Understanding the dynamics of transcription in living cells has improved since the development of quantitative fluorescence-based imaging techniques. We established a method for following transcription from a single copy gene in living cells. A gene tagged with MS2 repeats, used for mRNA tagging, in its 3' UTR was integrated into a single genomic locus. The actively transcribing gene was detected and analyzed by fluorescence in situ hybridization (FISH) and live-cell imaging. Several cell clones were created that differed in the promoter regulating the gene. Thus, comparative analysis could be obtained without the risk of different position effects at each integration site. Cells in S/G2 phases could be detected exhibiting two adjacent transcription sites on sister chromatids. A sharp reduction in the transcription levels was observed as cells progressed along the cell cycle. We hypothesized that a change in chromatin structure acts as a general mechanism during the cell cycle leading to down-regulation in the activity of some genes. We addressed this question by treating the cells with chromatin decondensing agents. Quantifying and imaging the treated cells suggests that chromatin structure plays a role both in regulating transcriptional levels along the cell cycle, as well as in limiting an active gene from reaching its maximum transcription potential at any given time. These results contribute to understanding the role of chromatin as a regulator of gene expression.

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