

Mechanisms of Irreversible Gene Silencing During Thymocyte Development.

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Reduced nuclease accessibility, deacetylation and methylation of core histones, CpG methylation, and localization to foci of pericentromeric heterochromatin have all been shown to contribute to the establishment of silent chromatin structures. However, the sequence of events leading to the heritable silencing of developmentally regulated genes in mammalian cells has not been established. We have studied the sequence of events leading to the silencing of the murine terminal transferase (TdT) gene during the maturation of double-positive thymocytes. Previous studies showed that this gene, like the RAG1 and RAG2 genes, is irreversibly silenced and repositioned to foci of pericentromeric heterochromatin during this developmental step. By comparison, maturation of the VL3-3M2 thymocyte line is accompanied by reversible inactivation of TdT and RAG transcription, with no pericentromeric repositioning. The goal of our analysis was therefore to determine the sequence of events contributing to heritable silencing by analyzing in primary thymocytes the kinetics of CpG methylation, histone deacetylation and methylation, pericentromeric repositioning, and reduced restriction enzyme accessibility. By comparing the events occurring in primary thymocytes to those occurring in VL3-3M2 cells, the events that specifically correlate with heritable silencing as opposed to transient inactivation have also been defined.

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