DEDICATED SITES OF GENE EXPRESSION IN THE NUCLEI OF MAMMALIAN CELLS

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In multi-cellular eukaryotes, patterns of gene expression are determined by the availability of transcription factors and reflect the developmental history of different cells. However, while genetic information ensures that genes are expressed appropriately from their natural chromosomal position many lines of evidence suggest that complex epigenetic factors influence expression from ectopic sites. In particular, the expression of ectopic reporter genes in transgenic animals demonstrates that the developmental history of cells and availability of particular transcription factors are not sufficient to guarantee desired levels of gene expression in every case.

We are attempting to establish how epigentic features influence gene expression in higher eukaryotes. As a first step towards this goal, we have described the organization of active sites of gene expression. In the first instance, HeLa cells were used as a model system to show that active genes are transcribed in dedicated transcription 'factories' that contain all the components needed for the synthesis and maturation of RNA. The number of engaged polymerases and active genes in transcription sites occupied by RNA polymerases I, II and III have been determined. In addition, we have monitored the distribution of critical components of the transcription machinery to establish the number of functional molecules present in a cell at any moment. For general transcription factors, about 20,000 molecules are bound to DNA; interestingly, this is between 5 and 60% of molecules in the cell for different factors. The TATA binding protein appears to bind to promoters even when other factors are not present – about 40,000 molecules of TBP are present in nuclease sensitive chromatin. This is very different, however, to the situation found with the largest subunit of RNA polymerase II – about 65,000 molecules are engaged with DNA but these cannot be removed by nuclease digestion. These data imply that while active polymerase complexes maintain a close association with the nucleoskeleton, gene promoters are only transiently associated with this structure during their transcription cycle.