Genome wide determination of RNA Polymerase binding profiles in Yeast

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The expression profiles of a particular gene give a clue to its biological role, while gene expression patterns in a cell can provide information about its state. Virtually all the differences in cell type or states are correlated with the changes in the mRNA levels of the genes. DNA microarrays provide a practical tool for studying gene expression on a genome-wide scale. Genome-wide expression monitoring technology describes the level of each mRNA species in a population, but this information alone is not always sufficient. Since the level of mRNA accounts for its synthesis as well as degradation, our knowledge of genome-wide transcriptional regulation is incomplete. Recent findings on the involvement of different isoforms of RNA polymerase II during different stages of transcription suggest that a more precise method can be devised to study transcriptional regulation.

Eukaryotic transcription occurs by a complex mechanism in which the carboxy-terminal domain (CTD) of the largest subunit of RNA polymerase II plays a vital role. The CTD consists of a heptapeptide repeat Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7 that is most often phosphorylated at the serine-2 and serine-5 positions. Phosphorylation of this enzyme has proven to be critical to the process of transcription: phosphorylation at the serine-5 position occurs first at or near the promoter, but the isoform phosphorylated at the serine-2 position has been cross-linked to the open reading frame, indicating that the DNA associated with this isoform is expected to complete transcription. We have exploited this unique mechanism employed by RNA polymerase II to differentially isolate chromatin fragments that are enriched with different phosphorylated forms of RNA polymerase II. Subsequently, we have hybridized the enriched chromatin fraction to a DNA microarray, thus revealing more precise information about the emergence of genome-wide expression signatures.