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RNA促进小鼠重组染色质白蛋白基因DNaseⅠ消化敏感性

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摘要　同样的基因在不同的分化细胞中表达不同，基因的选择性表达问题涉及分化和衰老的本质。转录基因对DNaseⅠ(DNA酶Ⅰ)消化敏感，本文研究了RNA对小鼠重组染色质白蛋白基因DNaseⅠ消化敏感性的影响。分离BALB/c小鼠脑细胞核，加入终浓度为2mol/L的NaCl破坏核小体结构，加入不同量、不同来源的RNA，装透析袋，逐渐降低离子强度进行染色质重组。重组染色质中加入DNaseⅠ消化DNA，PCR扩增白蛋白基因的外显子1到外显子2约1200bp区段，PAGE电泳后，用银染色观察不同来源RNA促进DNaseⅠ对白蛋白基因的消化作用。不同组织来源（肝、肺、肾、脑）RNA对小鼠重组染色质中白蛋白基因DNaseⅠ消化敏感性均有促进作用，其中肝和肺RNA促进消化作用较强；酵母tRNA无显著促进消化作用；消化促进作用与RNA剂量有关。RNA能增加DNaseⅠ对白蛋白基因的消化敏感性且有组织（细胞）来源特异性。又委托丹麦Chemical R D 实验室合成2条与白蛋白基因互补的各23核苷酸的RNA，用其进行重组试验。结果表明,重组混合物中含有低至0.2μg/mL的RNA，即可以发挥显著的DNase Ⅰ消化促进作用。

关键词　基因敏感性　RNA　染色质重组　PCR　衰老

分类号

RNA Responsible for Conferring a DNase I Sensitive Structure on Albumin Gene in Assembled Chromatin

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Abstract

Although the set of genes is virtually the same in all tissues,differential gene expression is appeared in cells of different kinds.Differentiation and ageing are associated with regulation of gene expression that is a fundamental mechanism in eukaryotic development and survival.The sensitivity to DNase I of actively transcribed genes seems to be a general phenomenon.The purpose of the study is to test whether RNAs obtained from different organs or cells can enhance susceptibility of albumin gene to DNase I digestion in BALB/c mouse brain chromatin assembled.RNAs extracted from rat liver,lung,kidney,brain,tRNA from yeast and synthesized RNAs (23nt completed with mouse alb gene) were added to a system of chromatin reconstitution that was achieved by dialysis from high ionic strength solution.Assembled chromatin was digested with DNaseⅠ(12.5μg/mL) at 20 ℃ for 1 min,then PCR assay was used to detect the level of albumin gene digested.PCR products (1200 bp) were run on a 6% polyacylamide gel and analyzed by silver stain assay.RNAs from different organs and synthesized RNAs all increased the sensitivity of albumin gene to DNaseⅠ attack in mouse assembled chromatin.The effect was more obvious in liver and lung RNAs than in kidney and brain ones.tRNA from yeast did not enhance the sensitivity of albumin gene to DNaseⅠ digestion.RNA increased albumin gene sensitivity to DNaseⅠ in a dose-dependent manner.We report here for the first time that RNAs can enhance susceptibility of albumin gene to DNaseⅠ digestion.The effect is associated with RNA sources or sequences.It is generally agreed that the formation of gene sensitivity to DNaseⅠ,by unfolding of a tightly packed chromatin fiber,is the first step in gene activation,then RNAs that recognize complementary DNA sequences may be the specific factors that affect DNA supercoiling and determine the sensitivity of gene to DNaseⅠ digestion.Here we describes "RNA Population Gene Activating Model" that gives a logical interpretation of events leading to expression of specific genes during normal development and differentiation,in the same time,explains ageing and oncogenesis.Gene expression in eukaryotic cells requires two level regulations.The first may be controlled by RNAs that locate complementary regions within the genomes and make these regions loosened

potentially,and the second is mainly involved in sequence specific and nonspecific proteins by which genomic regions bound by RNAs are unfolded.In eukaryotic cells,RNA fragments cleaved from all transcripts mix together to form "RNA populations" in which the majority is intron RNA.Every type of RNA fragments and its homologous sequences act as a group to form certain concentration in which repetitive sequences are more effective.If it is considered that there are many groups of RNA fragments in a particular cell,then different groups of RNA fragments are presented in dissimilar cell types of differentiation.Between DNA replication and nucleosome formation,RNA fragments in nuclear liquid will compete with DNA for binding to complement regions,then the chromatin regions bound to RNA can not be wrapped to form typical nucleosomes.After DNA doubles and is divided into 2 cells,these regions containing atypical nucleosomes become loose by function of non-histone.Transcriptionally active regions of chromatin are loose conformation but loosened regions are not always transcriptionally active.In every division,cells surfer in the described procedure that genes express RNAs,then RNAs recognize and imprint DNA.There are different RNA populations in different cells so that they imprint different genes,which is the primary mechanism by which same genes have expression distinctness.Since loosened genes are similar to bacterial operator system,factors in environment around cells play roles in inducing different gene expression to form different RNA population,which is the primary reason of cell differentiation.RNA population produced by certain impressions in genome can not imprint to form the same ones,otherwise immortal cells will be emerged,so that this program also controls ageing and oncogenesis.

Key words gene sensitivity RNA chromatin assembly PCR ageing

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