A conserved thyroid-specific enhancer in the human and mouse Pax8 locus.

ID - 154

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Motivation

In the thyroid bud at mouse embryonic day E13.5, the expression of the transcription factors known to be involved in the differentiation process of this tissue starts to be detected. Among these factors there is Pax8, which belongs to the Pax family of genes and is also expressed in kidney and in developing excretory system.

During the past years, the role of Pax8 in the fully developed thyroid gland was studied in deep and it was undoubtedly established that Pax8 plays a key role in thyroid development and differentiation. Although the function as well as the downstream target of Pax8 were well studied, very little is known about its transcriptional regulation.

Our aim was to unravel the molecular mechanisms by which the expression of the transcription factor Pax8 is regulated in the thyroid. To this end we analyzed the genomic region flanking the Pax8 gene.

Methods

We used the 'comparative genomics' approach between Homo sapiens and Mus musculus on regions surrounding the human and mouse Pax8 locus for about 275 Kb. We used the UCSC genome browser to survey for the mouse and human Pax8 locus, and by using the Vista program we performed a detailed comparison between 275 kb of the orthologous regions of the human and mouse Pax8 gene searching for conserved non-coding elements. During the analysis we considered sequences as conserved if they aligned without a gap for >100 bp in the VISTA alignment, and have >70% nucleotide identity. We used in parallel two alignment programs, a global comparison program (VISTA) and a local comparison program (PIPMaker) and they result completely overlapping, retrieving the same conserved sequences.

Afterwards, to investigate the potential biological activity of the identified conserved regions, we subcloned and tested each of them in a cellular system for the ability to stimulate the expression of a reporter gene. DnaseI footprinting and EMSA assays were used to characterize the most interesting regions.

Results

A total of 91 conserved elements fitting the up-cited criteria were identified. We discard 11 of them because of their overlap with coding sequences inside the Pax8 gene (exons and UTRs). The remaining 80 were defined as non-coding conserved elements (CNS). Of these 80 CNS, 25% were overlapping Pax8 intronic regions, 26% were at the 3 end of the gene and 44% (32 CNS) were located at the 5 end of Pax8 start of

transcription, more than 30kb away from any known gene. The level of conservation ranged between 73.5% and 89.5%.

The main goal of this comparative analysis was to highlight conserved regions that might play a role in regulating Pax8 expression, so we focused on CNS in the 5UTR until about 100 kb upstream. Among these, one sequence located 80 Kb upstream Pax8 coding region was able to induce transcription in a thyroid-specific manner. Thus, we concentrated our studies on this region (named H87) and we have characterized it in details. By DnaseI footprinting assays we have determined the presence of several binding sites within this region, and we used a computational analysis to predict the putative candidates that could bind to the identified binding sites. Subsequently, we have validated the predictions by EMSA assays and we have studied the functional role of the experimentally confirmed binding sites by generating deletion mutants of the H87 region that were tested for the ability to activate transcription.

In conclusion, we have isolated a thyroid-specific regulatory element located 80kb upstream the Pax8 gene that likely plays a role in the regulation of the expression of this gene.

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