Ultra Conserved Elements: twofold functions ?

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Motivation

A new paradigm has emerged in biology in which RNA molecules are active participants in regulating, catalysing and controlling many reactions that define fundamental processes in cells. In general, RNAs that have such regulatory functions do not encode a protein and are therefore referred to as non-coding RNAs (ncRNAs), (Goodrich J.A., 2006, Nature). Moreover, in recent years, there have been increasing reports of functional non-protein-coding RNAs (ncRNAs) that are involved or implicated in developmental, tissue-specific, and disease processes (Pang et al. 2005, Nucleic Acids Res.) In a paper published in Science in 2004 the authors identified 481 segments longer than 200 base pairs (bp) that are absolutely conserved (100% identity with no insertions or deletions) between orthologous regions of the human, rat, and mouse genomes. These ultra-conserved elements (UCEs) are present in all chromosomes, except 21 and Y, and were proposed to act as expression control elements (Bejerano G., et al. 2004, Science). We hypothesized that at least some of these elements belong to the non-coding RNA family, which includes microRNA, siRNA, snoRNA. Lately these elements have been tested as enhancer by Rubin and coworkers. Some of these seem to act as enhancers while others do not. These data give fuel to the hypothesis that these elements can have twofold function - they can be transcribed or can work like enhancer. This fascinating hypothesis has prompted us to examine the actual expression of UCEs and to determine whether or not they can be part of regulatory RNAs.

Methods

To identify RNA secondary structures we used bioinformatics tools as Mfold and Evofold which are available online (http://www.bioinfo.rpi.edu/applications/mfold ; http://www.soe.ucsc.edu/~jsp/EvoFold/). EvoFold is a comparative method for identifying functional RNA structures in multiple-sequence alignments. It is based on a probabilistic model-construction called a phylo-SCFG. Each prediction consists of a specific secondary structure and a folding potential score. (Pedersen, J.S., et al. 2006, Plos Computat. Biol.). Mfold describes a number of closely related software applications for the prediction of the secondary structure of single stranded nucleic acids. (M. Zuker., 2003, Nucleic Acids Res.). RT-PCR experiment using total RNA extracted from mouse embryos at 14,5 day and different cell types as murine embryonic stem cell (ES), NIH3T3, P19 were performed to assess the expression of the selected UCEs.

Results

Our main aim is to discover, using the mouse as an animal model, if these ultra conserved elements can be transcribed into RNAs that form secondary structures like hairpins or stem-loops. Therefore, we already mapped the human UCEs on the mouse genome and created a database to perform statistical analysis of UCEs. A total of 71% of the 481 UCEs were intergenic and non-annotated segments. We found that 255 UCEs out of 481, analyzed with Evofold and Mfold, show secondary structure and 44 of these seem also to function as enhancers as shown by Rubins group. We performed RT-PCR experiments in which we demonstrated the expressions of 11 selected UCEs in total RNA from mouse embryos at 14.5 day (E14.5) and in citoplasmatic and nuclear RNA from murine embryonic stem cell (ES). We analyzed 10 intergenic UCEs interspersed in the genome and we observed by RT-PCR that six of these elements seem to be transcribed. In addition, upstream the PAX2 gene there is an example of a cluster of UCEs. Therefore, we performed also RT-PCR experiments in cell lines (NIH3T3, P19) and in adult murine tissues. We noticed that the UCEs expression is correlated with the Pax2 expression as we detected them only in P19, brain, kidney and eye. Currently we are performing RNA in situ hybridization experiments and we are planning to carry out microarray experiments to systematically analyze the putative expression pattern of all identified UCEs. These experiments will allow us to have a panoramic view of the expression of UCEs and will allow us to understand if tissue specific expression of these sequences exist.

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