The 5′ terminal region of mouse p53 mRNA: structure and function

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Abstract

Protein p53 is a transcription factor that regulates the pool of genes responsible e.g. for the response to cellular stress, regulation of cell cycle, apoptosis, ageing, metabolism, functioning of stem cells and metastasis. Investigation of the role of p53 protein in the cell and on the expression of *TP53* gene have mainly concerned their functioning in the human organism, although for better understanding of the processes involving them, a number of transgenic mice have been studied. However, even though mice have been often used as a model system, literature provides scarce information on the regulation of expression of *Trp53* gene in mice. In particular, as expected, in the p53 mRNA in mice an important element regulating the process of translation initiation is its 5' non-coding region.

The aim of this study was a comprehensive structural and functional analysis of the 5'- terminal region of p53 mRNA in mice.

The length of the 5' non-coding region of p53 mRNA was evaluated using the method of 5' RACE to establish that this length can vary and for further studies two transcripts were chosen: the most often occurred one and the longest identified one, containing the 5' non-coding region of the length of 122 (mRNA(-122)) and 247 nucleotides (mRNA(-247)), respectively.

On the basis of biochemical mapping, the secondary structures of the 5'-terminal regions of the studied mRNA and selected isolated elements of these secondary structures were analyzed. Moreover, the secondary structure of the 166-nucleotide-long 5' non-coding region (in mRNA(-166)) was studied. This transcript was not identified in the 5' RACE experiment, however it had been used in earlier published papers.

In the next stage of the investigation, the small angle X-ray scattering method was employed to generate the *ab initio* structures of the 5'-terminal region of mRNA(-122) and its selected fragments. The obtained *ab initio* models were compared with those generated by the RNA Composer program. The temperatures of phase transitions and basic thermodynamical parameters of the studied RNA molecules were determined by the circular dichroism spectroscopy.

As a result of the *in vitro* translations performed in the presence of increasing concentration of the m^7GpppG cap analog, it was found that the initiation of translation of mRNA(-122) and mRNA(-166) is a process depending on the presence of the cap at the 5' terminus of mRNA, while the initiation of translation of mRNA(-247) is independent of the cap presence.

A comparative analysis of the relative amounts of the protein and mRNA p53 in the cell line of mice fibroblasts proved that the endoplasmic reticulum stress leads to slight decrease in the p53 protein, while the genotoxic stress leads to its accumulation. In both cases studied, the level of mRNA did not change. The results suggest that p53 regulation in response to stress takes place at the level of translation.

The next objective of the study was to find out which one of two potential initiation codons, localized in mRNA p53 at a distance of only six nucleotides starts the synthesis of p53 protein. In order to establish this, the mRNA mutants were constructed with changes in the nucleotide triplets which initiate the synthesis of p53 protein and the effects of these mutations on the *in vitro* and *in cellulo* translation reactions were examined.

Finally, the conservation of structural elements localized in the 5'-terminal region of the studied mRNA(-122) and mRNA(-247) transcripts was analyzed. Five mRNA segments characterized by high conservation were distinguished. In order to identify the proteins that can potentially interact with the 5'-terminal region of mRNA(-247), the *in silico* analysis using the program RBPmap was performed.