Catalytic nucleic acids as a tool to regulate the expression of mitochondrial genes.

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Abstract

Genetic information in eukaryotic cells is divided between the nucleus and cytoplasmic organelles, i.e. mitochondria in non-plant organism, mitochondria and chloroplasts in plants. Mitochondria carry the respiratory chain and ensure fundamental function in energy production, redox status, metabolic pathways, programmed cell death, aging and in signaling cascades involving reactive oxygen species. The vast majority of mitochondrial proteins is encoded by nuclear genes and imported into the organelles upon cytosolic translation, but the organelles cannot function without the contribution of their own genetic system.

Mitochondria show a specific autonomy therefore there are no effective methods of intervention in their genetic program. So far, attempts to use protein transport pathways have proved to be ineffective and non-specific.

The development of methods for introducing new genetic material into individual cell compartments has become the basis for the design of mitochondrial transformation strategies using various selection markers. Despite many attempts, the mitochondrial DNA genetic transformation system was developed for only two unicellular organisms (yeast and Chlamydomonas reinharditi algae). The search for effective strategies to deliver DNA and RNA to the mitochondria in vitro or in the cellular system remains a great challenge. The transfection of mitochondria and the understanding of the genetics of these organelles remain in the mainstream of innovative basic research as well as applications in the context of the therapy of mitochondrial diseases.

Therefore, the search for and development of new strategies to deliver catalytic RNAs to mitochondria could be a breakthrough in the study of mitochondrial genomes. The introduction of in trans-acting ribozymes into the mitochondria will degrade the target RNA, resulting in functional changes in these organelles. This unprecedented and novel approach opens up a completely new path for the genetic study of organelle DNA, reading regulatory processes, and identifying unrecognized functions of certain regions of the mitochondrial genome.

In the first part of the work, the system of delivering molecular tools developed in our laboratory with the use of tRNA-like structures for the manipulation of plant cell

mitochondria was used. Genes whose function in plants was not fully known so far (matR and mttB) were selected as target sequences. Catalytic nucleic acids (hammerhead ribozymes) and antisense oligonucleotide sequences were used as the passenger sequence in the plant system. The results obtained in the course of the work confirmed the effectiveness of the method developed by us in reducing the expression of target mitochondrial genes. Moreover, it was possible to confirm the involvement of the matR gene in the splicing of mitochondrial genes belonging to group II introns.

In the second part of the work, an attempt was made to adapt the system for manipulating the human mitochondrial genome developed in a plant model. In this approach, two tRNA-like sequences were selected and their ability to penetrate the mitochondria of human cells in vitro and in vivo were tested. In this approach the catalytically active molecule was a hammerhead ribozyme. The obtained results confirmed the ability of tRNA-like molecules to penetrate into the mitochondria of human cells. It has also been shown that ribozymes carried by the transporter molecule are able to effectively reduce the level of expression of mitochondrial genes without showing non-specific and cytotoxic effects in the tested cell lines.

The obtained results confirmed that the system we use can be successfully used to study the functions of mitochondrial genes both in the plant system and in human cell lines. This innovative approach opens a new path for mitochondrial genetic research, reading regulatory processes and identifying as yet unrecognized functions of the mitochondrial genome.