Changed Profile of Expression of Splicing Regulator Genes in Response to Exercise

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 147, No. 6, pp. 674-678, June, 2009
Original article submitted April 7, 2009

Intensive exercise triggers the cascade processes of body adaptation, including modulation of splisosome functioning, and can lead to modification of its activity and choice of alternative exons. We studied the effect of exercise of the maximum aerobic power on activation of transcription of genes involved in the splicing process. Short-term exercise resulted in a significant increase of mRNA expression of genes encoding proteins involved in the formation of precatalytic splisosome: DDX17, DDX46, HNRNPR, PRPF4B, and SRPK2. The role of the detected regulators in initiation of splisosome assembly under conditions of maximally intensive exercise is discussed.

Key Words: *splicing; exercise; profile of expression*

Coding fragments of the gene in higher organisms are alternating with noncoding DNA, which is not used in protein expression, and hence, the majority of pre-mRNA are subjected to splicing during maturation. It is an obligatory stage of gene expression, during which the intron (noncoding) sequences are cut out, while the exons (encoding sequences) are ligated. The significance of pre-mRNA splicing was recently confirmed by the results of sequencing of genomes from various organisms. This study showed that the protein variety in the majority of higher eukaryotes is a result of alternative splicing sharply increasing the number of unique mRNA formed in the organism [3].

Splisosome is an intricate macromolecular complex consisting of several minor nuclear ribonucleoproteins (mnRNP) and a great number of proteins reacting with them at different stages. Each mnRNP consists of one mnRNP molecule and seven Sm or LSm proteins and of several proteins specific of each particle. The main splisosome mnRNP are U1, U2, U4, U5, and U6; they are responsible for splicing of the overwhelming majority of introns.

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Active catalytic center, in which splicing takes place, is assembled anew for each intron during a process including the formation of a sequence of intermediates: complexes A (presplisosome), B (precatalytic splisosome), B* (activated splisosome), and C (catalytic splisosome) (Fig. 1).

The assembly of a splisosome at the initial stage of the cycle is initiated by ATP-dependent interactions of U1 mnRNP with the pre-mRNA conservative 5' splice sites (5'SS) (Fig. 1). At the next stage U2 reacts with the ramification point, and A-complex forms. Then the triple U4/U6.U5 mnRNP complex reacts with A-complex, destabilizing U1 and forming B-complex, after which global restructuring of the splisosome takes place, including the final elimination of U1 and U4 mnRNP. All this leads to the formation of catalytically active splisosome: B*-complex. The first catalytic step is associated with further restructuring with the formation of splisosome C-complex. After the second step of splicing, the splisosome is dissociated, mature mRNA is released, and the splisosome components participate in subsequent splicing acts (Fig. 1) [11].

Hence, the splisosome at different stages consists of 5 mnRNP, about 70 proteins of mnRNP complexes,

0007-4888/09/1476-0733

and more than 100 other proteins, including RNA helicases, GTPases, peptidylprolyl isomerases, and other proteins involved in the RNA-protein and protein-protein interactions. Initiation (assembly of precatalytic splisosome and its activation) is the process limiting splicing velocity.

Here we studied the effects of exercise of maximum aerobic intensity on the expression of mRNA of genes involved in the formation of precatalytic splisosome.

MATERIALS AND METHODS

The expression of mRNA was studied in 5 trained skiers (aged 18.6±1.9 years, body weight 67.4±4.6 kg, height 176.4±6.2 cm). Maximum possible exercise with intensity increasing in a step-wise manner served as the model exercise. The test exercise was carried out on a treadmill (Venus, h/p/Cosmos). During exercise, the gas exchange values were measured (Oxycon Pro, Viasis), heart rate (S810, Polar) and lactate concentration were evaluated, and the maximum oxygen consumption was estimated (58.8±2.4 ml/min/kg).

Total mRNA was isolated using PAXgene Blood RNA Kit (PreAnalytiX GmbH). The volume of isolated RNA was evaluated on a 2100 Bioanalyzer (Agilent).

Analysis of genes expression was carried out using mRNA chips (Affymetrix) according to the instruction: 100 ng total RNA isolated from blood leukocytes served as the template for the synthesis of double-stranded cDNA. An aliquot of the resultant cDNA was used for the synthesis of biotin-labeled cRNA by the reverse transcription method.

Biotinilated cRNA was cut into 70-100-nucleotide fragments and hybridized on a HuGene 1.0 ST Array chip (Affymetrix) in a hybridization oven at 45°C and stirring for 16 h. After hybridization, the chips were washed from free cRNA and stained with streptavidin-phycoerythrin on a Fluidics Station 450 (Affymetrix). Stained chips were scanned on a GeneChip Scanner 3000 (Affymetrix).

The quality of hybridization, washing, and staining of the chips was evaluated using Gene Expression Console software (Affymetrix). All internal controls on the chips corresponded to the standards specified by the manufacturer.

The sample intensities obtained using Affymetrix Command Console were imported into medium R using xps library (*Stratowa C.* Vienna and Austria, xps: Methods for Processing and Analysis of Affymetrix Oligonucleotide Arrays including Exon Arrays and Gene Arrays. R package version 1.2.8).

The expression was calculated using FARMS algorithm realized in xps [4]. Based on the Gene Ontology database (records GO:0000398; 145 genes) and our unpublished data (271 genes), we formed a list of 303 splicing-associated genes present in the Affymetrix HuGene 1.0 ST chip. The list was reduced to 13 genes by I/NI filtration algorithm [10] at $p<0.005$. The "before" and "after" values for each gene were compared using Student's t test. For estimation of False Discovery Rate (FDR), the p values were corrected by Benjamini—Hochberg amendment. A total of 5 genes with statistically significant changes in expression were distinguished at FDR 0.05 (Table 1).

RESULTS

Exercise of the maximum aerobic power is a complex stress factor with such components as increased concentrations of free radicals, local hyperthermia, changes in electrolyte balance, acidosis caused by lactate accumulation in the blood, and high concentrations of free fatty acids. These factors lead to activation of cascade cell processes [1] and increase in the expression of mRNA [2], which results in triggering of protein neosynthesis. Due to this, the concomitant increase of splicing velocity can lead to reduction of the negative effects of physiological stress and sooner recovery after exercise.

Physiological stress characteristic of many kinds of sports was observed in examined athletes subjected to maximum exercise. The duration of exercise was

TABLE 1. Genes Involved in Splicing with mRNA Expression Increased as a Result of Exercise

Short name of the gene according to NCBI database	Protein coded for by the gene	Increase of expression, %±0.95 confidence interval
DDX17	ATP-dependent RNA helicase p72	16.0±11.9
DDX46	ATP-dependent RNA helicase Prp homolog	23.8±14.9
HNRNPR	Heterogeneous nuclear ribonucleoprotein R	29.2±19.2
PRPF4B	Pre-mRNA processing factor 4	32.9±17.6
SRPK2	Serine/arginine proteinkinase 2	23.4±10.9

Note. Increase in mRNA expression is presented as a change of expression in %±0.95 confidence interval for 5 genes.

15.0±0.5 min. This period is very short, but sufficient for triggering the processes of adaptation to exercise. The duration of work after attaining the lactate threshold was 4-6 min, which was comparable to the time needed for *in vitro* assembly of a precatalytic splisosome.

The expression of mRNA was analyzed by a modern method based on hybridization of labeled oligonucleotides on a chip and detection of the complementary samples fluorescence. Using this method, it is possible to evaluate the expression of mRNA of all human genes (28,000 samples) and carry out extensive screening of adaptation processes in the organism. The mRNA samples were isolated from blood leukocytes, because they are the most convenient for work and fully reflect the processes taling place in the organism.

Statistical processing and use of databases made it possible to single out of all genes, whose expression changed as a result of exercise, only those associated with splicing and increase of whose expression was statistically significant.

The mean values of the expression of these genes are presented in Table 1. The reaction to stress is an individual parameter, and the values vary greatly. However, all athletes retained the trend to an increase in the expression of these genes, the increase reaching 50%. This pronounced increase within a short time suggests that these genes play an important role in the development of early response to stress.

All proteins translated from the above genes possess enzymatic activity (helicase or kinase), due to which they are involved into the splicing cycle at different stages.

Despite the involvement of all 5 mnRNP in the formation of precatalytic splisosome, as well as of numerous protein factors acting separately and in cooperation, several major cooperating factors can be distinguished (Table 2) [8].

Interestingly, that the genes whose mRNA expression increased, encode proteins related to or reacting with proteins of virtually all main groups (Table 2). For example, HNRNPR is a member of the hnRNP protein family. It reacts with SF3A2 protein and hence, is involved in the formation of SF3a complex/U2 mnRNP [9]. In turn, DDX46 protein, belonging to group DExH/D helicases, forms a bridge between U1 and U2 mnRNP, thus forming a presplisosome [12].

In order to form a precatalytic splisosome, the presplisosome is to bind tri-mnRNP. The key step at this stage is phosphorylation of U5-100 (DDX23) protein, also a member of the helicase DExH/D group. Phosphorylation of this protein is an absolutely obligatory stage for integration of tri-mnRNP, it is realized by serine/arginine SRPK2 kinase [7]. Another enzyme involved in the formation of active tri-nmRNP complex is PRPF4B serine/threonine kinase. Being specifically bound to U5 mnRNP, it phosphorylates PRP6, which forms a bridge between U4/U6 and U5 mnRNP. It was shown on the yeast models that it reacted with DDX46 [5].

DDX23 plays an important role at the stage of splisosome activation. Due to its ATPase activity it can force out U1 mnRNP for the formation of the catalytic center. Presumably, DDX17 DExH/D helicase plays a role in this process. It is highly homologous (90%) to DDX5, with which it forms heterodimers in a cell. Though functions of this protein are little studied, it was shown that DDX17 is released together with U1 mnRNP. In turn, it was previously shown for DDX5 that it also reacts with U1 mnRNP and 5'SS duplex, destroying it [6]. Presumably, DDX5/DDX17

TABLE 2. Key Factors Involved in Assembly of Precatalytic Splisosome

Factors	Function
U1 mnRNP	Recognizes and binds 5'SS by means of U1C protein, which promotes the formation of complementary relationships between pre-mRNA and U1 RNA. Reacts with SR proteins and is thus involved in recognition of exons. Leaves the splisosome after formation of B-complex
U2 mnRNP	Forms a stable relationship with the ramification point during formation of A-complex. Includes SF3a and SF3b protein complexes, which form extra relationships in the ramification site region. Is involved in formation of the catalytic center together with U6
U4/U6.U5 tri-mnRNP	Regularly binds to splisosome, forming B-complex. U4 mnRNP shortly reacts with the splisosome, integrating catalytically inert U6 into it. U5 mnRNP reacts with pre-mRNA, coordinating the exons for chemical reactions. The U5 protein components are involved in formation of active center as cofactors. U6 mnRNP forms the catalytic center together with U2
hnRNP	Various RNA binding proteins, having no RS domain, react with the emerging transcripts and regulating (positively or negatively) the initiation of splisosome assembly
SR proteins	Participate in exon choice by binding to U1 mnRNP and U2AF35. RS domains form a series of successive contacts with ramification point and 5'SS during splisosome assembly

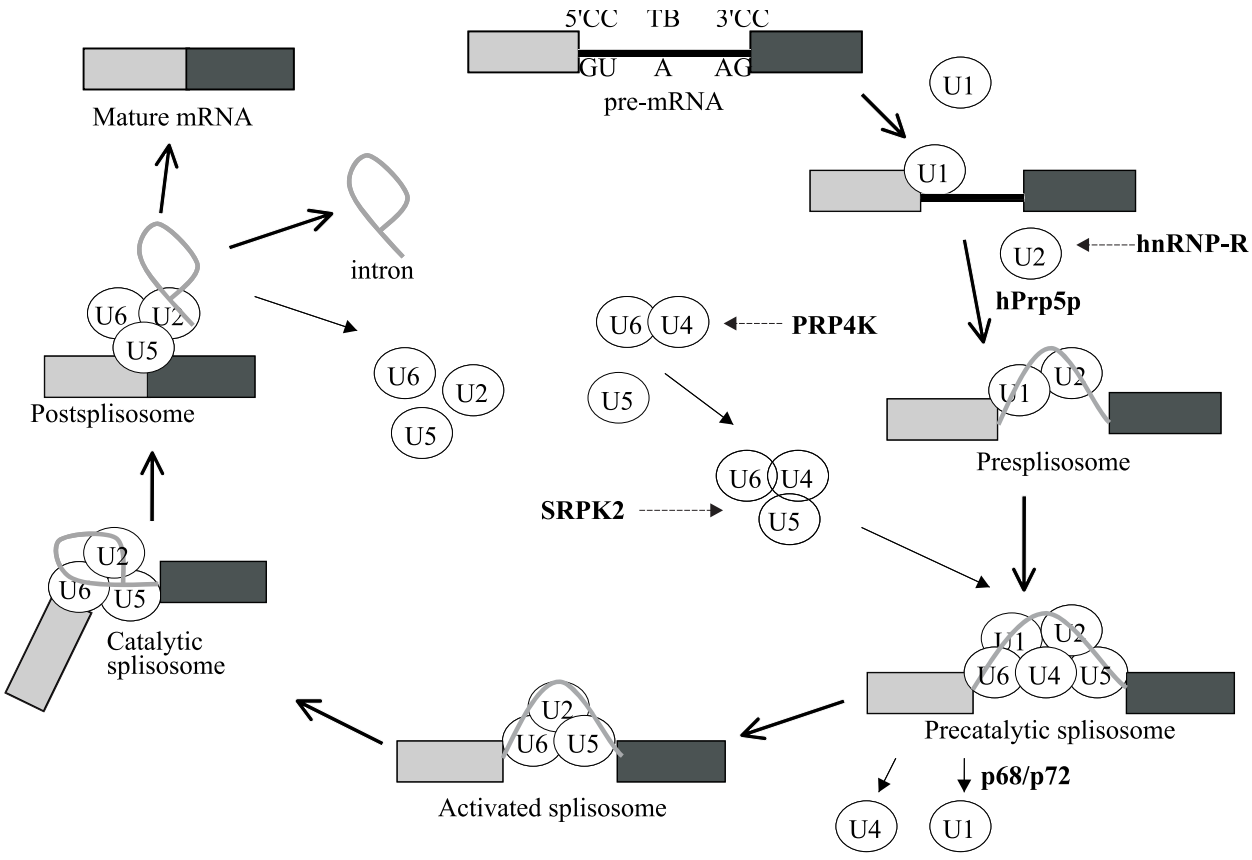

Fig. 1. Splicing scheme. Light gray rectangles: exons; U1-U6: mnRNP.

and DDX23 are involved in initiation of active splisosome formation.

The processes of urgent adaptation to work of maximum aerobic power were studied for the first time at the mRNA level and their detailed study is an interesting task. It was shown that response to physiological stress in athletes is individual and develops within a short time.

The study of genes expression for the whole human genome detected genes, whose expression has changed as a result of exercise. A group of splicing regulators, participating in the development of cell response to stress, was identified. This group includes DDX17, DDX46, HNRNPR, PRPF4B, and SRPK2. Due to their enzymatic activity, these regulators stimulate the formation of precatalytic splisosome and its transition into activated state and participate in many key stages of splisosome complex assembly.

Hence, the effects of short-term highly intensive exercise on the expression of mRNA of proteins involved in pre-mRNA splicing were studied in detail.

The authors are grateful to S. N. Aseev for his assistance in the experiments on whole genome studies of mRNA expression.

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