Comparative genomics analysis of thiamine-pyrophosphate riboswitches in fungal genomes

M.A.Moldovan

MSU, 119991, Russia, Moscow, Leninskie gory 1 , mika.moldovan@gmail.com

S.A.Petrova

MSU, 119991, Russia, Moscow, Leninskie gory 1 plazmoliz@yandex.ru

Riboswitches are conserved upstream elements on mRNAs that can bind small molecules. This binding induces conformational changes that modulate gene expression. Bacteria use various riboswitches extensively, whereas only thiamine-pyrophosphate (TPP) riboswitches were found in eukaryotes such as fungi, plants and green algae, where they regulate expression of genes, responsible for TPP-biosynthesis.

It was previously experimentally shown, that riboswitch-mediated regulation in fungi is carried out on splicing level, whereas in bacteria it is carried out mostly on transcriptional or translational level [1].

Also today a conciderable amount of known fungal genomes is availible. Thus, our aim was to find out which fungal genes are regulated via TPP-riboswitches, how conserved are principles of this regulation in different orthological groups and taxa and how conserved is the secondary structure of fungal TPP-riboswitch.

We performed a pattern-search using bacterial and some known eukaryotic riboswitch sequences and found 256 homologous RNA structures in 186 fungal genomes. These structures appeared to be associated with 5 orthological groups, three of which were previously shown to be regulated via TPP-riboswitches in Neurospora crassa [2]. Two of them: thiazole-synthase (*thi4*) and hydroxymethilpyrimidine-synthase (*nmt1*) correspond to TPP-biosynthesis enzymes, catalyzing key reactions of two TPP-metabolism branches. The other three seem to posess 11-13 transmembrane segments, thus being putative transporters. In our study we analyzed two enzymes and one putative transporter.

We examined regions, predicted to be TPP-riboswitches and found all structural features of TPP riboswitch in 40 fungal species for *nmt1* orthological group. We have also found in most cases other components of regulatory system existing in N.crassa such as alternative donor

splice sites and regions complement to P4-P5 riboswitch stem located upstream from the TPP-riboswitch sequence.

The same was shown for *thi4* in 50 species. The principle of TPP-riboswitch action in case of *thi4* wasn't characterized previously, but we suggest that it is similar to that of nmt1 concidering analoguos mutual arrangement of regulation components.

In the case of putative transporter, we managed to identify all TPP-riboswitch features and all regulation components for all predictions, thus showing that the second principle of fungal TPP-riboswitch action, which was previously experimentally shown for a homolog from N.crassa [3], is very conserved.

Then we analyzed presence of regulation via riboswitches in different taxons and in different orthological groups. In the case of phylum Ascomycota we observed that species are clearly separated in two groups: those with riboswitch-dependent regulation of both enzymes – *thi4* and *nmt1*, belonging to subphylum Pezizomycotina and those with no riboswitches in enzyme genes, belonging to subphylum Saccaromycotina. In cases of phylums Basidiomycotina and Zygomycotina we coudn't observe such a clear bias probably due to moderate sample volume. However, it can be said that in these phylums three situations are possible: i.Riboswitch-dependent regulation of both enzymes: *thi4* and *nmt1*. ii. Riboswitch-dependent regulation of only one enzyme. iii. Absence of riboswitch-dependent regulation for both enzymes. In the case of putative transporter, we found that every homolog from our sample is regulated via TPP-riboswitches and that transporter genes are present in genomes independently from enzyme genes.

Our data suggest that the principle of riboswitch action observed in putative transporter is presumably more conservative than the one observed in enzymes *thi4* and *nmt1*, besause in cases of enzymes we couldn't identify other features of regulation in some organisms apart from riboswitch itself.

We are planning to continue our work and examine two other orthological groups and genes, that were excluded from our sample due to lack of some riboswitch structural features or features of regulation.

This is a joint work with M.S.Gelfand

1. Serganov A, Nudler E. (2013) A decade of riboswitches, *Cell*, 1-2:17-2
2. Cheah MT, Wachter A, Sudarsan N, Breaker RR. (2007) Control of alternative RNA splicing and gene expression by eukaryotic riboswitches, *Nature* 447:497-500
3. Li S, Breaker RR. (2013) Eukaryotic TPP riboswitch regulation of alternative splicing involving long-distance base pairing, *Nucleic Acids Res.,* 5:3022-3031