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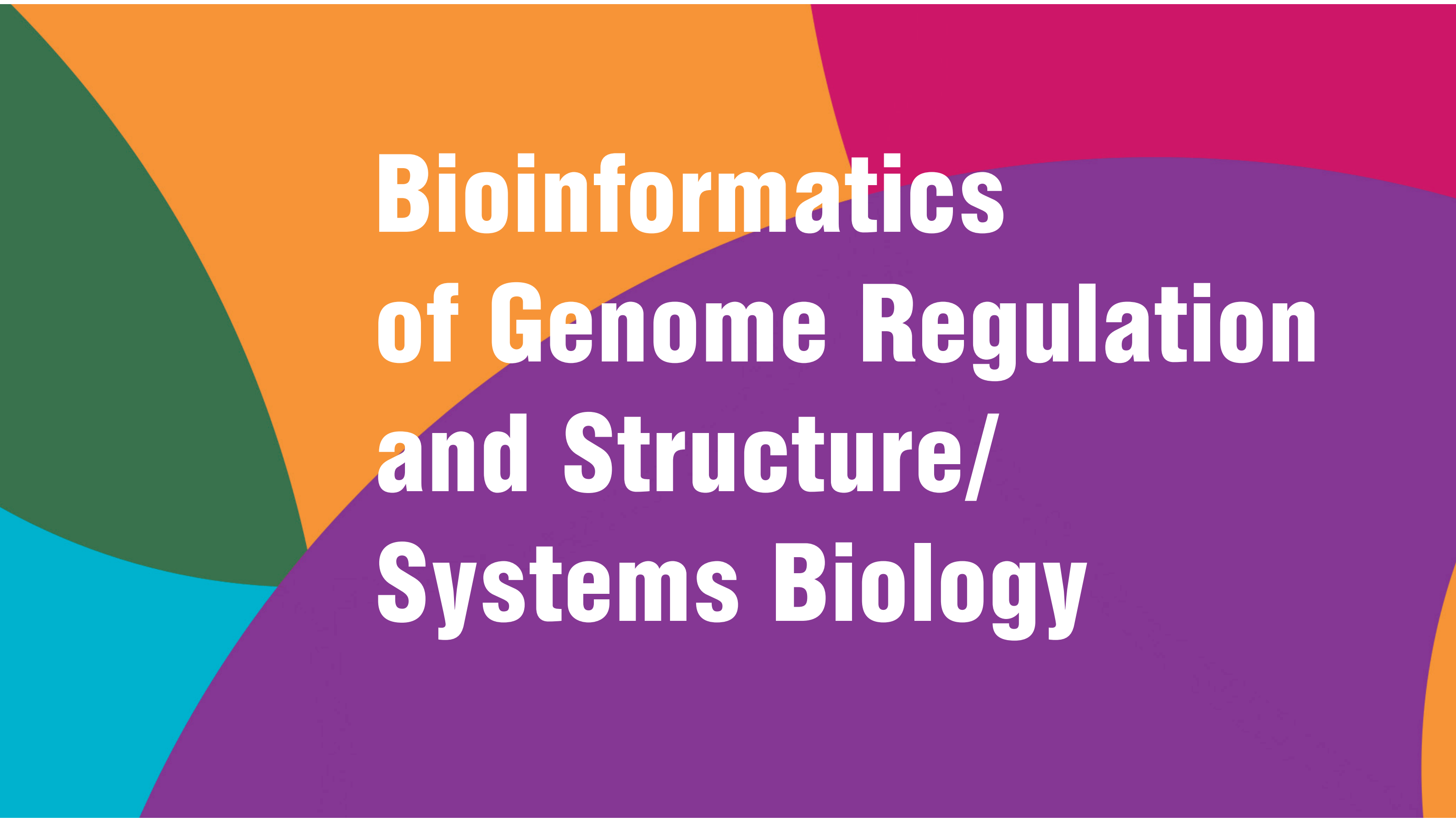

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Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences

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Contacts
All questions, please contact the official address
of the organizing committee of the conference: bgrs2020@bionet.nsc.ru

Organizing Committee Chairman: Zubova Svetlana
Address: Lavrentieva Avenue, 10, Novosibirsk, 630090
Phone: +7 (383) 363 4977, e-mail: svetazubova@gmail.com

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Characteristics of interaction of miRNA with mRNA of C2H2, ERF and GRAS transcription factors of arabidopsis, rice and maize

Aizhan Kazievna Rakhmetullina
SRI of biology and biotechnology problems
Al-Farabi Kazakh National University
Almaty, Kazakhstan
zhanullina1994@gmail.com

Svetlana Kazbekovna Turasheva
SRI of biology and biotechnology problems
Al-Farabi Kazakh National University
Almaty, Kazakhstan
svetlana.turasheva@kaznu.kz

Anna Yurevna Pyrkova
SRI of biology and biotechnology problems
Al-Farabi Kazakh National University
Almaty, Kazakhstan
anna.pyrkova@kaznu.kz

***Abstract* — The miRNA binding sites with mRNA of genes encoding C2H2, ERF, GRAS transcription factors (TFs) were identified for Arabidopsis thaliana, Oryza sativa and Zea mays. The free energy (ΔG) of interaction of miRNA with mRNA target genes, the maximum of free energy (ΔGm), the ratio ΔG/ΔGm, and location of the potential binding sites were calculated using MirTarget program. In mRNA of C2H2, ERF, GRAS genes of all studied plants, miRNA binding sites were located in the protein-coding part (CDS) and 5'-untranslated region (5'UTR). The ath-miR5658-5p, ath-miR5021-5p, osa-miR2102-5p, osa-miR5075-3p had binding sites in mRNA of three studied families, with the value of ΔG/ΔGm from 91% to 98%. The miR171a-3p had binding sites in mRNA GRAS transcription factors family of all studied plants, with the value of ΔG/ΔGm equal 100%. The nucleotide sequences of ath-miR171a-3p, osa-miR171a-3p, and zma-miR171a-3p were similar, and their quantitative characteristics of interaction with mRNA of LOC_Os02g44360.1, GRMZM2G037792_P01, and AT2G45160.1 genes, were also similar. The obtained results indicate the dependence of expression TF of C2H2, ERF, GRAS families on miRNA.**

Keywords — miRNA, mRNA, gene, binding site, plant, transcription factor

Introduction

Most plant target miRNAs are transcription factors (TFs) that are involved in the regulation of plant growth and development. Studies have shown that miRNAs are able to regulate a range of biological processes in plants, such as: regulation of flower shoots and seeds, formation of nodules, development and responses to environmental stresses [1-3]. miRNAs and TFs provide combinatorial gene regulation that includes a variety of functions that can later be used to improve crop yields. Therefore, it was important to identify *A. thaliana, O. sativa, Z. mays* miRNAs that bind to mRNA of C2H2, ERF, GRAS transcription factors families.

Materials and methods

The nucleotide sequences of *A. thaliana, O. sativa, Z. mays* miRNAs were downloaded from miRBase (http://mirbase.org, release 22.1). The nucleotide sequences of genes of the C2H2, ERF, GRAS family were obtained from Plant Transcription Factor Database v4.0 (http://planttfdb.cbi.pku.edu.cn) and NCBI GenBank (http://www.ncbi.nlm.nih.gov). A search for the miRNA target genes was performed using the MirTarget program. This program defines the following binding characteristics: the start of the miRNA binding site on the mRNA; the location of the miRNA binding site (5'UTR, CDS, 3'UTR); the interaction free energy (ΔG, kJ/mole), the ΔG/ΔGm value (%) [4]. The MirTarget program found hydrogen bonds between adenine (A) and uracil (U), guanine (G) and cytosine (C), G and U, A and C. The numbers of hydrogen bonds in the G-C, A-U, G-U and A-C interactions were found to be 3, 2, 1 and 1, respectively.

Result

The search of interaction of 428 A. thaliana, 738 O. Sativa, 325 Z. mays miRNAs with mRNA of C2H2, ERF, GRAS transcription factors genes was completed. Using the MirTarget program, we found miRNA binding sites in the protein-coding region (CDS) and 5'-untranslated region (5'UTR). Table presents the quantitative characteristics of plant miRNAs interaction with mRNA of the C2H2, ERF, GRAS TF genes. The ath-miR5658-5p, ath-miR5021-5p, osa-miR2102-5p, osa-miR5075-3p had binding sites in mRNA of three studied families. The targets for ath-miR5658-5p were mRNA of AT1G34370.1 (C2H2), AT1G44830.1 (ERF), and AT5G59450.1 (GRAS) genes, with the value of ΔG/ΔGm equal 96% and 94%. The mRNA of AT1G34370.1 and AT5G59450.1 genes contain binding sites for ath-miR5658-5p located in the 5'UTR with the free energy of interaction (ΔG) equal -98 kJ/mole. The mRNA of AT1G44830.1 gene bound to ath-miR5658-5p in the CDS. The ath-miR5021-5p had binding sites in the mRNA of AT1G02030.1 gene of C2H2 family, AT1G22810.1 gene of ERF family and AT4G37650.1 gene of GRAS family with ΔG/ΔGm ratio of 93% or more. The osa-miR2102-5p and osa-miR5075-3p can interact with the free energy (ΔG) more than -109 kJ/mole, which indicates a strong binding to mRNA of LOC_Os09g38610.1, LOC_Os06g10470.1 (C2H2), LOC_Os04g32790.1, LOC_Os04g32790.1 (ERF), LOC_Os12g04380.1, LOC_Os06g40780.1 (GRAS) genes. All detected osa-miR2102-5p, osa-miR5075-3p binding sites were located only in the CDS. ath-miR414-5p, ath-miR867-5p, ath-miR171a-3p had binding sites in mRNA of *AT5G14010.1*, *AT1G53170.1*, *AT2G45160.1* genes with free energy from -98 kJ/mole to -113 kJ/mole and ΔG/ΔGm value equal 92% and 100%. It should be noted that nucleotide sequences of ath-miR171a-3p, osa-miR171a-3p were identical, and their quantitative characteristics in mRNA of *AT2G45160.1* and *LOC_Os02g44360.1* genes were the same: the location of the miRNA binding site (CDS), the free energy of interaction (ΔG) -113 kJ/mole, the ΔG/ΔGm value of 100%. This indicates a strong miRNA interaction with the mRNA. Length of zma-miR171a-3p was less by one nucleotide, and was different by free energy binding in mRNA of *GRMZM2G037792_P01* gene, while the ΔG/ΔGm value remained the same (100%). These genes belong to the GRAS transcription factor family in *A. thaliana, O. sativa, Z. mays.* The zma-miR159e-5p bound in the CDS mRNA of *GRMZM2G060206_P01* ERF TF family gene, and *GRMZM2G146018_P01* GRAS TF family gene. The remaining zma-miR390a,b-5p, zma-miR169o-3p, zma-miR166n-5p, zma-miR160f-5p, zma-miR529-5p, zma-miR394a,b-5p had one target in the mRNA of *GRMZM2G114660_P01*, *GRMZM2G105224_P01*, *AC211702.2_FGP002* (C2H2), *GRMZM2G474326_P01*, *GRMZM2G060876_P01* (ERF), *GRMZM2G024973_P01* (GRAS) genes, respectively. Discovered binding sites were located in the CDS mRNA target genes, with the ΔG/ΔGm value equal or greater than 90%.

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TABLE - CHARACTERISTICS OF MIRNA BINDING SITES IN MRNA OF C2H2, ERF, GRAS TF GENES OF *A.THALIANA, O. SATIVA, Z. MAYS*

Gene	miRNA	Start of site, nt	Region	ΔG, kJ/mole	ΔG/ΔGm, %	Length, nt
C2H2 family						
AT1G34370.1	miR5658-5p	154	5'UTR	-98	96	21
AT1G02030.1	miR5021-5p	987	5'UTR	-91	93	20
AT5G14010.1	miR414-5p	449	CDS	-98	92	21
LOC_Os09g38610.1	miR2102-5p	229	CDS	-117	96	20
LOC_Os04g39520.1	miR5809-3p	66	CDS	-113	96	20
LOC_Os06g10470.1	miR5075-3p	158	CDS	-115	93	21
GRMZM2G114660_P01	miR390a,b-5p	484	CDS	-108	93	21
GRMZM2G105224_P01	miR169o-3p	1023	CDS	-102	92	20
AC211702.2_FGP002	miR166n-5p	9	CDS	-104	91	21
ERF family						
AT1G22810.1	miR5021-5p	22	5'UTR	-96	98	20
AT1G44830.1	miR5658-5p	133	CDS	-96	94	21
AT1G53170.1	miR867-5p	143	5'UTR	-93	92	21
LOC_Os02g54050.1	miR11343-3p	304	5'UTR	-100	96	21
LOC_Os04g32790.1	miR2102-5p	810	CDS	-113	93	20
LOC_Os04g32790.1	miR5075-3p	340	CDS	-113	91	21
GRMZM2G474326_P01	miR160f-5p	525	CDS	-108	91	21
GRMZM2G060876_P01	miR529-5p	760	CDS	-100	90	21
GRMZM2G060206_P01	miR159e-5p	583	CDS	-102	89	21
GRAS family						
AT2G45160.1	miR171a-3p	1304	CDS	-113	100	21
AT4G37650.1	miR5021-5p	374	CDS	-93	96	20
AT5G59450.1	miR5658-5p	119	5'UTR	-98	96	21
LOC_Os02g44360.1	miR171a-3p	1397	CDS	-113	100	21
LOC_Os12g04380.1	miR5075-3p	1311	CDS	-117	95	21
LOC_Os06g40780.1	miR2102-5p	662	CDS	-110	91	20
GRMZM2G037792_P01	miR171a-3p	1280	CDS	-106	100	20
GRMZM2G146018_P01	miR159e-5p	585	CDS	-106	93	21
GRMZM2G024973_P01	miR394a,b-5p	2039	CDS	-98	90	20

Conclusion

The presence of miRNA binding sites with strong interaction in mRNA of C2H2, ERF, GRAS TF genes of *A.thaliana, O. sativa, Z. mays* was shown. All binding sites of these miRNAs were located in the CDS and 5'UTR of mRNA target genes. The predicted miRNA binding sites with mRNA of target genes important for the regulatory mechanisms of miRNA in the process of plant growth and development.

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