GENE EXPRESSION

POLYMERASE II BINDING AND TRANSCRIPTION DYNAMICS OF DEVELOPMENTAL ENHANCERS

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Enhancers are central regulators of spatiotemporal patterns of gene expression during development. Accessing and predicting enhancer activity in the genome is therefore the primary objective towards understanding of the complex regulatory networks underlying cell differentiation and development.

In this study, we utilized several genomic approaches to access the potential rate of enhancer activation throughout the time course of Drosophila embryonic development and characterize the dynamic properties of enhancers such as Pol II binding, enhancer RNA (eRNA) transcription, and enhancer-specific epigenetic marks. In particular, we performed ChIP-seq on initiating and elongating forms of Pol II during mesoderm and nervous system development and complemented this approach with severaltypes of RNA-seq, such as strand-specific ribo-depleted RNA-seq and PRO-cap which enables highly sensitive identification of nascent capped transcripts. To access chromatin dynamics, we made use of the publicly available data such as ChIP-seq (H3K4me1 and H3K4Ac27) and DNase-seq.

We have found that genome-wide initiating Pol II occupancy is highly dynamic over open chromatin regions in mesoderm and neuronal development and is associated with active enhancers. We show that eRNA transcription is significantly correlated with the timing of *in vivo* characterized enhancer activity and transcription factor binding in fly embryo tissues, consistent with previous studies in mammalian cell culture systems. We characterize specific properties of Drosophila eRNA, such as abundance and directionality. Finally, we show that eRNA can be used for prediction of putative active enhancers and assign potential enhancer activity to a large set of thousands of previously uncharacterized regions within Drosophila genome.

INVESTIGATION OF PROMOTER REGION POLYMORPHISM OF TASAP-A1 GENE IN THE COLLECTION OF WINTER WHEAT (*TRITICUM AESTIVUM* L.) VARIETIES AND LINES

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TaSAP1 is a member of the stress association protein (SAP) gene family from wheat. It is involved in response to several abiotic stresses, including drought, salt and cold. TaSAP-A1 gene is located on chromosome 7A like the loci affecting yield-related traits, including thousand-kernel weight (TKW), spike length (SL) and the total number of spikelets per spike (TNSS). According to Chang et al., TaSAP-A1 gene is also associated with these traits. The highest polymorphism level was revealed in the promoter region of TaSAP-A1 gene. The objective of this study was to investigate the promoter region polymorphism of TaSAP-A1 gene and evaluate the association between its alleles and yield-related traits in the collection of winter wheat varieties and lines used in Belarusian breeding.

The promoter region polymorphism of TaSAP-A1 gene has been investigated in a collection of 72 varieties and lines of wheat used for the breading process in Scientific and Practical Center of NAS of Belarus on agriculture (Zhodino). TKW, SL and TNSS measurements were conducted in the laboratory of winter wheat of the above institution. DNA was isolated from grains by the method proposed by Plaschke et al. Isolation was carried out from two kernels for each cultivar or line. An analysis of promoter region polymorphism of TaSAP-A1 gene was conducted according to the method proposed by Chang et al., 2013, with modifications.

On the basis of conducted investigation of TaSAP-A1 promoter region in the presence of 5 bp indel at position -1.810 bp and 39bp indel at position -1.637 bp using Sap5 and Sap39 markers, respectively, and a SNP (A-C) at position -2.606 bp using Sap2606 marker all investigated varieties and lines were assigned to 4 haplotypes. Among them, 17 (22.7%) belonged to haplotype I, 34 (47.2%) – to haplotype II, 13 (18.1%) – to haplotype II and 8 (11.1%) – to haplotype IV. In investigated collection an averaged TKW was 51.5 g. Among the cultivars and lines with haplotype I it amounted 51.1g, with haplotype II – 51.4 g, with haplotype III – 51.9 g and with haplotype IV – 52.6 g, that confirm the literature data about haplotypes III and IV being a superior haplotypes for TKW. An averaged SL was 9.1 cm and among the cultivars and lines with haplotype I it amounted 9.4 cm, with haplotype II – 9.0 cm, with haplotype III – 9.0 cm and with haplotype IV – 8.9 cm. An averaged TNSS among investigated cultivars and lines was 17.8: among the cultivars and lines with haplotype I it amounted 18.1, with haplotype II – 17.9, with haplotype III – 17.4 and with haplotype IV – 17.4. It confirms the literature data about haplotype I and being a superior haplotype for TNSS compared with haplotypes II, III and IV.

Thus, the results of investigation of promoter region polymorphism of TaSAP-A1 gene in the winter wheat varieties and lines used in Belarusian breeding provides useful information for marker-assisted selection for yield-related traits.

BEST PRACTICES FOR GENE EXPRESSION MICROARRAY AND RNA-SEQ DATA ANALYSIS IN INTEGRATIVE STUDIES

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Modern gene expression study design usually implies operating with multiple sources of information. Such sources are not limited by simple control-experiment groups of samples produced in one go, but also comprised from datasets made within different timeframes and by different technologies. Moreover, last trends in massive gene expression data analysis shifted towards integrative approaches, where data from different experiments are combined directly or through meta-analysis to increase statistical significance of results. Here we summarized our experience in gene expression microarray and RNA-seq data analysis to provide optimal pipeline for joint data processing of both types.

Necessary steps for quality control analysis of raw microarray and rna-seq data were compiled. Time and technology dependent batch-effect and its negative influence on differential expression analysis were studied. We confirmed, that Illumina BeadChips, which contain several single arrays, each targeting one sample, are subject to unnecessary technical variations, especially if combined with RNA amplification and isolation batches. Based on quality control results global normalization methods for RNA-seq should be accompanied with specific methods such as RUVseq or svaseq to minimize the effect of different amount of RNA and library preparation. General-purpose Empirical Bayes methods such as ComBat can be applied to both microarray and RNA-seq data. Since microarrays contain ambiguous probesets to genes representations, we identified the best strategy for probesets selection to obtain more uniform result between different platforms and when comparing with RNA-seq data. Such strategy rely on actual probesets signal intensities contrast to mapping probe sequences to genome. Finally the methods based on estimating the expression from RNA-seq reads overlapping the microarray probe regions were compared with RNA-seq transcripts quantification on gene level with featureCounts and normalization to counts per million (CPM) in log scale with voom method.

We highlighted important steps and difficulties in gene expression microarray and RNA-seq data analysis and provided guidelines and scripts to further facilitate integrative studies.

CREATION OF GENE EXPRESSION DATABASE ON PREECLAMPSIA-AFFECTED HUMAN PLACENTA

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Publication of gene expression raw data in open access to online resources like NCBI or ArrayExpress made it possible to use these data for cross-experiment integrative analysis and make new insights into biological phenomena. However, most popular of the present online resources are meant to be archives rather than ready for immediate access and interpretation databases. Data uploaded by independent contributors is not standardized and sometimes incomplete and needs further processing before it is ready for the analysis. Hence, the need for a specialized database appears.

Given in this article is the description of the database that was created after processing a collection of 33 relevant datasets on pre-eclampsia-affected human placenta. Data processing includes the choice of relevant experiments from ArrayExpress database, the experiment sample attributes standardization according to MeSH term dictionary and Experimental Factor Ontology and the completion of missing data using information from the corresponding articles and authors.

A database of more than 1000 samples contains sufficient sample-wise metadata for them to be arranged into relevant case-control groups. Metadata includes information on biological specimen, donor's diagnosis, gestational age, mode of delivery etc. The average size of these groups will be higher than it is in separate experiments. This will reduce experiment bias and enhance statistical accuracy of the subsequent analysis such as search for differentially expressed genes or inferring gene networks. The article concludes with the guidelines for the microarray experiment metadata uploading for future contributors.

HYPOXIC REGULATION OF THE EXPRESSION OF A SUBSET OF PROLIFERATION RELATED GENES IN U87 GLIOMA CELLS: EFFECT OF IRE1 INHIBITION

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The endoplasmic reticulum stress is an important component of tumor growth, including glioblastoma. IRE1 (inositol requiring enzyme-1) signaling pathway of endoplasmic reticulum stress is a central mediator of the unfolded protein response and inhibition of this signaling pathway leads to a suppression of glioma growth through down-regulation of proliferation processes as a result of metabolic reprogramming of cancer cells. The endoplasmic reticulum stress controls the expression of numerous regulatory and proliferation related genes, which are responsible for glioma growth. We have studied the effect of inhibition of IRE1 signaling enzyme on the expression of a subset of genes encoding important regulatory proteins in U87 glioma cells.

We have used U87 glioma cells and their subline stably transfected with cDNA-construct expressing dnIRE1 (without kinase and endoribonuclease activities). The expression levels of BRCA1, DEK, BCL2L1, COL6A1, TPD52, GLO1, HOMER3, and STC2 mRNAs as well as ACTB mRNA were measured in these glioma cells by real-time quantitative polymerase chain reaction.

It was shown that the expression level of breast cancer 1 early onset (BRCA1), a nuclear phosphoprotein which coordinates a diverse range of cellular pathways and transcriptional regulation to maintain genomic stability, tumor protein D52 (TPD52), which inhibits growth and metastasis in renal cell carcinoma cells through the PI3K/Akt signaling pathway, and stanniocalcin 2 (STC2) mRNAs is significantly up-regulated (+83, +135, and +514%, correspondingly) in U87 glioma cells by inhibition of IRE1 signaling enzyme in comparison with the control cells. At the same time, the expression level of collagen, type VI, alpha 1 (COL6A1), which play an important role in tumorigenesis, DEK oncogene (DEK), glyoxalase I (GLO1), which promotes tumor growth, and homer homolog 3 (HOMER3), which is implicated in diverse biological functions, is strongly down-regulated (-72, -18, -91 and -36%, correspondingly) in glioma cells without IRE1 signaling enzyme function. It was also shown that hypoxia up-regulated the expression level of COL6A1, TPD52, and STC2 mRNAs (+60, +91, and +604%, correspondingly) and down-regulated – BRCA1, DEK, and GLO1 mRNAs (-51, -28, and -12%, correspondingly) in control glioma cells and that IRE1 inhibition modifies the effect of hypoxia on the expression of COL6A1, BCL2L1, HOMER3, and STC2 mRNAs.

Thus, the expression of most studied genes is responsible for IRE1-mediated endoplasmic reticulum stress signaling in gene specific manner. Therefore, the changes in expression level of genes encoding BRCA1, DEK, BCL2L1, COL6A1, TPD52, GLO1, HOMER3, and STC2 proteins possibly reflect metabolic reprogramming of glioma cells by IRE1-mediated endoplasmic reticulum stress signaling and correlate with suppression of glioma cell proliferation upon inhibition of the IRE1 signaling enzyme.

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IRE1 MEDIATED REGULATION OF *NAMPT*, *HSPB8*, *RAB5C*, *BIRC5*, *PSAT1*, *KRT18*, *CLU*, *GPI*, AND *TSPAN13* GENES EXPRESSION IN U87 GLIOMA CELLS

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The endoplasmic reticulum stress is an important component of tumor growth, including glioblastoma. IRE1 (inositol requiring enzyme-1) signaling pathway of endoplasmic reticulum stress is a central mediator of the unfolded protein response and inhibition of this signaling pathway leads to a suppression of glioma growth through down-regulation of proliferation processes as a result of metabolic reprogramming of cancer cells. The endoplasmic reticulum stress controls the expression of numerous regulatory and proliferation related genes including tumor suppressors, which are responsible for glioma growth. We have studied the effect of inhibition of IRE1 signaling enzyme on the expression of a subset of genes encoding important proliferation related proteins in U87 glioma cells.

We have used U87 glioma cells and their subline stably transfected with cDNA-construct expressing dnIRE1 constructs without kinase and endoribonuclease activities. The expression levels of *NAMPT*, *HSPB8*, *RAB5C*, *BIRC5*, *PSAT1*, *KRT18*, *CLU*, *GPI*, and *TSPAN13* genes as well as ACTB as reference gene were measured in these U87 glioma cells by real-time quantitative polymerase chain reaction and Western-blot analysis.

It was shown that the expression level of nicotinic acid phosphoribosyltransferase (NAMPT), a key enzyme in the biosynthesis of nicotinamide adenine dinucleotide, which is involved in many important biological processes, heat shock 22 kDa protein 8 (HSPB8), which is involved in carcinogenesis, tetraspanin 13 (TSPAN13), RAB5C, member RAS oncogene family (RAB5C) mRNAs is significantly down-regulated (-95, -86, -82, and -77%, correspondingly) in U87 glioma cells by inhibition of IRE1 signaling enzyme in comparison with the control cells. Inhibition of IRE1 enzyme had significantly lesser suppressive effect on the expression of baculoviral IAP repeat containing 5 (BIRC5), phosphoserine aminotransferase 1 (PSAT1), and clusterin (CLU) mRNAs (-40, -61, and -54%, correspondingly).

At the same time, the expression level of keratin 18 (KRT18), which plays an important role in cell proliferation, and glucose-6-phosphate isomerase (GPI), which is involved in glycolysis, is up-regulated (+170 and +30%, correspondingly) in glioma cells without IRE1 signaling enzyme function. It was also shown that hypoxia up-regulated the expression level of most studied genes and that IRE1 inhibition modifies the effect of hypoxia on their expression.

Thus, the expression of most studied genes is responsible to IRE1-mediated endoplasmic reticulum stress signaling in gene specific manner. Therefore, the changes in expression level of genes encoding BRCA1, DEK, BCL2L1, COL6A1, TPD52, GLO1, HOMER3, and STC2 proteins possibly reflect metabolic reprogramming of glioma cells by IRE1-mediated endoplasmic reticulum stress signaling and correlate with suppression of glioma cell proliferation upon inhibition of the IRE1 signaling enzyme.

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IRE1 MEDIATED REGULATION OF THE EXPRESSION OF A SUBSET OF NUCLEAR GENES ENCODING MITOCHONDRIAL PROTEINS IN U87 GLIOMA CELLS

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Mitochondria play an important role in the regulation of tumor growth and apoptosis through numerous metabolic pathways. The functional activity of mitochondria is controlled through numerous nuclear-encoded mitochondrial proteins and most of these factors and enzymes are responsible for metabolic reprogramming of mitochondria in cancer as well as in other diseases. The endoplasmic reticulum stress is an important component of tumor growth and inhibition of IRE1 (inositol requiring enzyme-1) signaling pathway, which is a central mediator of the unfolded protein response, leads to a suppression of tumor growth through down-regulation of the angiogenesis and proliferation processes. This stress contributes to the expression profile of many regulatory genes resulting in proliferation, apoptosis, angiogenesis, and mitochondrial functions. We have studied the effect of IRE1 inhibition on the expression of a subset of nuclear genes encoding mitochondrial proteins in U87 glioma cells.

We used U87 glioma cells and their subline stably transfected with cDNA-construct expressing dnIRE1 (no kinase, no endoribonuclease). The expression level of NNT, NR3C1, FAM162A, PRSS15 and ETHE1 mRNAs as well as ACTB mRNA were measured in U87 glioma cells by real-time quantitative polymerase chain reaction.

It was shown that the expression level of nicotinamide nucleotide transhydrogenase (NNT), glucocorticoid receptor (NR3C1), and pro-apoptotic protein FAM162A (family with sequence similarity 162 member A) mRNAs is significantly up-regulated (+64, 50, and 176%, correspondingly) in U87 glioma cells by inhibition of IRE1 signaling enzyme in comparison with the control cells. At the same time, the expression level of mitochondrial ethylmalonic encephalopathy 1 (ETHE1), which represents a sulfur dioxygenase that localizes within the mitochondrial matrix and suppresses p53-induced apoptosis, is strongly down-regulated (-76%) in glioma cells without IRE1 signaling enzyme function. It was also shown that IRE1 inhibition decreases the expression level of mitochondrial serine protease 15 (PRSS15), also known as hLON ATP-dependent protease, which mediates the selective degradation of misfolded, unassembled or damaged polypeptides in the mitochondrial matrix and participate in the regulation of mitochondrial gene expression.

The expression of all studied genes is responsible for IRE1-mediated endoplasmic reticulum stress signaling in gene specific manner, because IRE1 knockdown significantly affects their expression. Therefore, the changes in expression level of nuclear genes encoding NNT, NR3C1, FAM162A, PRSS15, and ETHE1 proteins possibly reflect metabolic reprogramming of mitochondria by IRE1-mediated endoplasmic reticulum stress signaling and correlate with suppression of glioma cell proliferation upon inhibition of the IRE1 enzyme function.

NGS-SEQUENCING AS A NEW METHOD OF FORENSIC IDENTIFICATION

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Advent of the next-generation sequencing (NGS) of DNA-analysis at the beginning of the 1980's brought about a revolution in biology and medicine.

Frederick Sanger was the first scientist to report the basic principles for determination of amino acid and nucleotide sequence of DNA and RNA in 1977.

But the determination of the whole human genome as the fundamental capability of sequencing was not suggested at the time. An international consortium of 16 laboratories accomplished this task in 2003.

To remain up-to-date with evolving technology in the field of DNA-analysis and human identification SSRFC of MIA of Ukraine introduced Ion Torrent PGM™ sequencer in 2016. It enables us to get a robust data about DNA sequence in record-breaking time. The instrument can simultaneously analyze more than a dozen samples in less than 24 hours.

Depending on its processor configuration it is capable of determining from 10 million to 1 billion base pairs with high precision.

Ion Torrent PGM™ enables us to sequence specific stretches of genome of various organisms, search for somatic and hereditary mutations, analyze expression of genes and noncoding RNA, conduct metagenomic analysis by the sequence of 16S pRNA as well as analysis of protein-DNA interactions (ChIP-Seq), Y-DNA and mtDNA.

In 2016-2017 we conducted a number of studies with Ion Torrent PGM™ that demonstrated the efficiency of mtDNA sequencing for establishing maternal relationship between parents and children as well as between siblings.

The results obtained are reproducible, repeatable and robust and have the following advantages over the results of Sanger mtDNA sequencing:

– mitotypes are more informative and discriminative since the whole mtDNA chain is sequenced (~16570 nucleotides) which is not the case with the Sanger method (~830 nucleotides);

– it takes 10 times less messenger DNA to get the result which allows us to analyze samples with extremely low DNA concentration.

Quantitative and differential analysis of gene expression plays a key role in performing various research tasks particularly in correlating gene expression with phenotype which can result in establishing such human characteristics as sex, age, color of hair, eyes, skin, etc.

DEPENDENCE OF EXPRESSION OF GENES INVOLVED IN AUXIN SIGNALING AND TRANSPORT ON GRAVISTIMULATION IN TOMATO LEAVES

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Gravitropism (geotropism) is an ability of roots and various above-ground plant organs to change their growth in response to gravity. Under alteration of gravity vector the asymmetric growth of upper and lower halves of an organ is stimulated and its gravitropic bending occurs. A key factor in mediation of the gravitropic bending is redistribution of phytohormone auxin between cells of gravistimulated organs. Such auxin redistribution in the above-ground organs induces extrusion of protons into the apoplast, acidification and weakening of the cell wall, "acid growth" of cells of the lower half of an organ and its subsequent bending. At the same time, the sensitivity of expression of genes associated with auxin metabolism and signaling to gravistimulation remains poorly understood.

The aim of this research was to evaluate influence of gravistimulus on expression of genes associated with auxin signaling and transport such as some isoforms of small auxin upregulated RNA (SAUR) gene family, auxin receptor TIR1, auxin efflux and influx transporters LAX1 and SiPIN1.

Influence of gravistimulus on gene expression was determined in the apical leaves of tomato plants. For gravistimulation the experimental group of tomatoes were turned by 90º so their stems were horizontal and exposed at different time intervals. Relative quantitative real-time RT-PCR was used to measure the change in mRNA expression levels of target genes.

It was found that expression of SAUR15, SAUR58, TIR1, LAX1, and SiPIN1 was increased in 15 min after the beginning of gravistimulation. The expression of SAUR15, SAUR58, SiPIN1, TIR1 remained high at the late stages of observations, meanwhile the expression of LAX1 was decreased.

The obtained results suggest the changes in expression of target genes at the transcriptional level can play an important role in development of gravitropic response in plant leaves.

STUDY ON DGAT1, ALPHA-LACTALBUMIN AND GHRELIN GENES POLYMORPHISM IN MAZANDARAN PROVINCE BUFFALOES AND THEIR ASSOCIATION WITH MILK QUANTITY AND QUALITY

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The aim of the present study was to investigate DGAT1, Alpha-lactalbumin and Ghrelin genes polymorphisms and their associations with milk production and compositions in buffaloes of Mazandaran Province using PCR-RFLP and PCR-SSCP methods. In this study the blood samples were collected from 100 buffaloes and DNA was extracted using modified salting out extraction protocol. A fragment of DGAT1 gene with the length of 411 bp including part of exon 3, and a fragment of Ghrelin gene with the length of 402 bp amplified by polymerase chain reaction (PCR). In order to PCR products were digested with CfrI and Hpa II restriction enzymes for DGAT1 and ghrelin genes, respectively. Polymorphism at Alpha-lactalbumin gene was detected by single strand conformation polymorphism (SSCP) method. In order to study association of studied genes with milk production and composition on buffalo, from GLM procedure of SAS software was used. The PCR or digested PCR products were electrophoresed on 1.5% agarose and 12% polyacrylamide gel. In the RFLP method for DGAT1 gene, only K allele and for ghrelin two allele's i.e. T and C were found. In this population TT, TC and CC genotypes have been identified with the 0.55, 0.3 and 0.15 frequencies, respectively. T and C allele's frequencies were 0.7 and 0.3. Using SSCP method, four banding patterns were found at Alpha-lactalbumin site with frequencies of 0.17, 0.66, 0.15 and 0.02 for AB, BC, BB and CC, respectively. A, B and C allele's frequencies were 0.085, 0.565 and 0.35, respectively. The results showed significant effect of Ghrelin gene on milk fat, protein and dry matter percentage. These traits were more favorable in the group of individuals with CC genotype, which may indicate an advantage of T allele over C. The Alpha-lactalbumin genotype was not significantly affected the studied. In general, results indicated possible potential for using DGAT1, Alpha-lactalbumin and ghrelin genes in marker-assisted selection programs.