activity at 0–35°C as well as relative thermostability. It was half-inactivated only after 45 min heating at 90°C.

To reveal molecular basis of EstPc unusual characteristics we have solved its 3D structure by X-ray crystallography. Structure was solved at 2.15 Å resolution and refined to R_f=18.7% and R_{free}=25.4%. Despite the protein has typical α/β hydrolase fold and its structure is similar to known esterases, comprehensive structure analysis revealed some special features possibly responsible for unusual enzyme characteristics.

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Keywords: cold-active enzymes, esterase, Protein structure.

TUE-508
Structure features of TBN1, a P1/S1-like nuclease

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Tomato bifunctional nuclease 1 is a multifunctional zinc-dependent, acidic, plant nuclease with P1/S1-like fold. Exact role of TBN1 is not known but it plays an important role in specific apoptotic functions, cell senescence vascular system development, stress response and tissue differentiation in plants. It shows anti-cancerogenic properties which were proven using mice bearing human tumours [1]. Variants of TBN1 used in our studies were produced recombinantly in *Nicotiana benthamiana* leafs. Presence of zinc in the protein was confirmed by X-ray fluorescence and absorption edge scan. The phase problem was solved using a combination of multi-wavelength anomalous dispersion and real space molecular replacement [2]. TBN1 has a P1/S1-like nuclease fold with a zinc cluster placed in the active site in the centre of the wide groove. Three oligosaccharides bonded on the surface serve primarily as a shielding of the hydrophobic regions and therefore contribute to solubility and stability of the enzyme. TBN1 acts as phosphodiesterase (and phosphomonoesterase) cleaving the bond between phosphorus and 3' hydroxyl group in both single stranded and double stranded forms of DNA and RNA and it also shows 3'-nucleotidase activity. Newly, a phospholipase C-like activity was also discovered. Comparison of TBN1 with single-strand specific P1/S1-like nucleases and other zinc dependent enzymes led to our better understanding of their substrate promiscuity and natural properties [3].

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Keywords: P1/S1-like nuclease, zinc dependence.

TUE-509
Study of anti-Warburg effect of AMPK activators and antimetabolite drugs on MCF7 cell model

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AMPK is an energy sensor that regulates cellular metabolism. When activated by a deficit in nutrient status, AMPK stimulates mitochondrial energy production, while turning off energy-consuming processes to restore energy balance. We analyzed AMPK activation in MCF7 ductal breast cancer cells.

AMPK can work as an anti-Warburg agent via enhancing mitochondrial biogenesis.

In MCF7 cells we studied the combined action of the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR), and the antimetabolite folate analog, Methotrexate. We observed increased mitochondrial activity, while glycolytic flux decreased. The combination of drugs also disrupted the phases of the cell cycle, and the cells were accumulated in the G1 and G2 phase. These data suggest that the combined application of AICAR and MTX brings about synthetic lethality through an anti-Warburg effect.

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Keywords: AMPK, anti-Warburg, mitochondria.

TUE-510
Study of enzyme activity in mutant lines of soft wheat obtained under the surfactants action

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Over the past decade, a large number of mutants were obtained experimentally for agricultural crops, which differ from the original in several features. One of the most reliable indicators of changes in the genetic apparatus is enzyme activity alteration. Those are the enzymes, influencing the intensity of the most important parts of metabolism, leading to an accurate estimation of the viability of obtained mutant genotypes.

It has been shown that exposure to aqueous solutions of various surfactants (1%) prior to sowing induced changes in traits, which are inherited through generations M_1-M_4.

The aim of this work is study on activity of key enzymes of nitrogen and energy metabolism in the obtained mutant lines (glutamate dehydrogenase – GDH, enzyme complex of malate dehydrogenase and glutamate aminotransferase – EC MDH-GOAT, malate dehydrogenase – MDH, alcohol dehydrogenase – ADH). Seeds of third generation mutant lines of common wheat and the original varieties Zhenis, Kazakhstanskaya 3, Shagala were used as research material. The reaction mixtures for estimation of enzyme activity contained: for GDG – NADH, 2-oksoglyutarat, ammonium sulfate for EC MDH-GOAT – NAD, malate, glutamate, for MDH – NAD and malate, for ADH – NAD and 96% ethanol. Estimation of enzyme activity (μM coenzyme/mg protein per min) was determined at the length of 340 nm, the total protein content (mg/ml) – at 330 nm.

It has been noted that mutant lines differ in content of soluble protein. For instance, in Shagala variety lines 3,4,5 its content is

increased up to 9% (0.198 ± 0.02 mg/ml, 0.205 ± 0.01 mg/ml, 0.200 ± 0.02 mg/ml, respectively, in control – 0.188 ± 0.01 mg/ml). Study of four enzyme systems activity shows the lines variation in their spectrum of activities. Lines selection on decreased GDH activity (10 lines in the range of 53.22 ± 0.01 to 64.51 ± 0.01 mM/mg; the initial variety Shagala-72.58 ± 0.01 mM/mg) and increased activity of EC MDH-GOAT (5 lines in the range of 403.17 ± 0.01 to 443.27 ± 0.01 mM/mg; the initial variety Shagala-344.44 ± 0.01 mM/mg) was conducted. For genetic and breeding work mutants with reduced GDH activity are interesting, since this enzyme releases toxic ammonia destroying biomembrane. MDH-GOAT plays an important role in the detoxification of products of protein degradation under abiotic and biotic stresses such as salinity, drought, etc. Plants with reduced GDH and high MDH-GOAT activities survive better in case of infection and maintain high productivity. Also the number of lines displayed an increase in ADH and MDH enzyme activity in comparison to the initial variety, the latter might indicate the activation of the respiratory processes. All of this suggests a large term use of surfactants for creation of valuable genotypes of major crops.

Keywords: crop breeding, enzyme activity.

TUE-511
Study of the expression pattern of L-Dopa decarboxylase (DDC) in human β-pancreatic cells: indications of involvement in the insulin-biosynthesis pathway

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The link between the dopaminergic system and glucose metabolism is largely supported by in-vitro and in-vivo studies in animals, and clinical data from psychiatric individuals. However, little is known about the exact molecular pathways underlying this relationship. Herein, we sought to investigate the expression pattern of 3,4-Dihydroxy-L-phenylalanine (*L*-Dopa) decarboxylase (DDC), the enzyme catalyzing the biosynthesis of the neurotransmitters dopamine and probably serotonin, under insulin-pathway stimulating conditions in human β-pancreatic cells.

Methods: The 1.2B4 immortalized human β-cell line was subjected to a titration of human insulin concentrations corresponding to normal insulin blood levels (0–100 μU/ml). In another set of experiments, insulin was added in higher concentration (14.5 mU/ml) with and without the presence of the LY294002 PI3K inhibitor (50 μM). The DDC mRNA and its predicted regulator miR-145 levels were estimated by qRT-PCR, while DDC protein levels by Western blot. The intra- and extra-cellular insulin contents were measured with a commercial ELISA assay. Each condition was tested in duplicates and in 3 independent experiments.

Results: Our data revealed negative correlation between the *DDC* mRNA levels and the amount of insulin added to the culture ($r = -0.88$, $p = 0.01$), as well as the intra- ($r = -0.78$, $p = 0.03$) and extra-cellular insulin content ($r = -0.99$, $p < 0.0001$). Yet, when insulin was added in higher concentration the DDC mRNA levels were increased (~1.5-fold) compared to untreated cells, and that was reversed by the addition of the LY294002 insulin pathway inhibitor. MiR-145 levels were by ~2-fold up-regulated by insulin addition, and by ~4-fold following LY294002 treatment. Likely changes in DDC protein levels were proved not possible to be detected by the method used.

Conclusion: Our preliminary data indicate significant association of the mRNA levels of *DDC*, the key-molecule in neurotransmitters' biosynthesis, with the insulin pathway in human β-cells supporting the widely accepted notion of communication between the dopaminergic system and glucose metabolism, that is, herein, for the first time studied in-vitro on cells of human origin. Further investigation is needed to elucidate the subtending molecular mechanisms that may serve as potential targets for the treatment of type 2 diabetes.

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Keywords: human β-pancreatic cells, insulin biosynthesis, L-Dopa decarboxylase.

TUE-512
Study on responses of ceramide metabolism pathway enzymes in mice liver tissue culture treated with withaferin A and withanolide A

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Ceramide play important roles in intracellular signaling involve in differentiation, proliferation and apoptosis. Withaferin A and Withanolide A are main bioactive compound that traditionally use to cure ulcer, rheumatism and leucoderama. In this investigation, we evaluated ceramide synthase, serine-palmitoyl transferase and dihydroceramide desaturase as anabolic pathway enzymes and ceramidase activity as anabolic enzyme in liver tissue culture treated with 0 to 120 μg/kg. Results showed markedly significant inhibition on ceramidase activity (61%) at 80 to 120 μg/kg of withaferin A and slightly increase (18%) on ceramide synthase and dihydroceramide desaturase (15%) activities with respect to control. There was no considerable effect on activity of serine-palmitoyl transferase in liver for both of these compounds. In addition, sphingosine level did not vary considerably in response to each compound. However, ceramide level increased in a dose dependent manner of withaferin A treatment and reached the highest level at 80 μg/kg exposure. On the other hand Withanolide A treatment did not elevated ceramide considerably as compared with control.

Our findings clarified the role of ceramide elevation in response to withaferin A in comparison to Withanolide A treatment that may involve in reported biological activities of this medicinal plant compound.

Keywords: Withaferin A, ceramide synthesis, sphingosine, liver tissue.

TUE-513
Substrate specific gene expression profiles on different kidney cell types associated with Fabry disease

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Fabry disease is an X-linked inborn error of glycosphingolipid metabolism associated with deficient alpha-galactosidase A activity. The major clinical feature of fabry disease, such as fibrosis in

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