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ments are needed. Resveratrol (3,4,5'-trihydroxy-trans-stilbene), a natural polyphenol, has been shown to exert anticancer effects in different systems based on its ability to inhibit diverse cellular events associated with tumor initiation, promotion, and progression. Resveratrol inhibits the growth of several cancer cell lines, which suggests that it also has an inhibitory effect on cancer progression.

In this study, we investigated the effect of resveratrol on neuroblastoma cells (Neuro-2a) treated with hydrogen peroxide or nitric oxide-releasing compound, NOC-18.

After 24 h incubation of Neuro-2a cells with H_2O_2 or NO respectively reduced the viability of the cells in dose-dependent manner. The concentration of the mentioned oxidants which caused 50% reduction in cell viability was 0.02 mM and 0.25 mM for H_2O_2 and NOC-18, respectively. Cells treated with resveratrol 3, 6 or 12 h prior to H_2O_2 or NOC-18 treatment exhibit significant reduction in survival compared to cells treated with hydrogen peroxide or nitric oxide alone. After resveratrol treatment, the apoptosis of the neuroblastoma cells exposed to hydrogen peroxide or NOC-18 increased significantly.

In conclusion, resveratrol in combination with hydrogen peroxide or nitric oxide, increases cellular cytotoxicity and inhibits the proliferation of neuroblastoma cells.

SW06.W30–46
Interaction of *Mycoplasma gallisepticum* with host-cell organelles

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The genus *Mycoplasma* relates to Gram-positive bacteria that lack a cell wall and are capable to cause chronic disease in humans and animals. The capability to affect the development of apoptosis in the host cells was shown for a number of mycoplasmas.

The object of the present study is a Mollicutes class bacteria *Mycoplasma gallisepticum* owning parasitic life style in poultry. A number of studies show that *M. gallisepticum* is capable to penetrate into eukaryotic cells. The ability of *M. gallisepticum* to *persist* in the host, as well as its participation in the development of apoptosis suggests that it could affect the host cell's and intracellular organelles' metabolism. In particular, possible cooperation between mycoplasma and mitochondria and especially their effect on the initiation of the mitochondrial apoptosis pathway is exiting.

We analyzed the interaction of *M. gallisepticum* with mitochondria isolated from *Gallus domesticus* liver tissue using confocal laser scanning microscopy (CLSM) with fluorescent staining. We demonstrate that *M. gallisepticum* is able to adhere to the mitochondrial surface. Trypsin treatment abolishes such interactions, which may indicate its protein-protein nature. However, we show that other species of the Mollicute class (*Acholeplasma laidlawii, Spiroplasma melliferum*) do not have such ability to interact with mitochondria. Interestingly, we did not observed interaction of *M. gallisepticum* with other intracellular organelles (for example, chloroplasts isolated from *Pisum sativum*).

We suggest that Mycoplasma – mitochondria interaction can affect the functional activity of mitochondria in the host cell and also play role in the triggering of the mitochondrial apoptosis pathway.

SW06.W30–47
Induction of cell responses to the endogenous expression of *Bacteroides fragilis* toxin in culture HEK-293

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The development of high-tech sequencing of nucleic acids resulted in the start of human metagenome projects. The microbial communities composition colonizing the large intestine, led to the creating a database of bacterial genomes. Despite the variety of learning mechanisms of bacterial and epithelial cell interaction, the role of some bacterial toxins is unknown. Remains uncertain role of toxins, that are freely enter to epithelium cells. The research purpose was in the creation and study of intracellular induction of cell responses to the endogenous expression of *Bacteroides fragilis* toxin (BFT) in the cell line HEK-293. To identify the BFT protein produced in cell culture as a result of gene expression Western blot has been used. In our experiments it was shown, that only immature inactive form of toxin is produced in epithelium cells line HEK-293. However, the intracellular effects of the toxin have identified a number of quantitative changes in the protein level and RNA transcription. We demonstrate the absence of the mature form of toxin that is expected as a result of the BFT protein processing. Although, we detected high levels of toxin mRNA in HEK-293. This suggests either, rapid degradation of processed BFT protein or lack of the normal processing. This effect may indicate that host cell is altered by the plasmid encoding BFT or by the transcribed BFT RNA and that such alteration promote the described changes of host cell translation and transcription.

SW06.W30–48
The role of nitric oxide in testicular sperm extraction (TESE)

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Many conditions or events associated with male infertility are inducers of oxidative stress. Such stress conditions can cause changes in the dynamics of testicular microvascular blood flow, endocrine signaling, and germ cell apoptosis. Testicular oxidative stress appears to be a common feature in much of what underlies male infertility. In recent years, scientists have studied the role of nitric oxide (NO) in the male infertility. There are different physiological roles for NO in male reproductive system. NO is a potent vasodilator and cell signaling molecule can play its own role in amplifying testicular injury. The aim of study is to evaluate NO and oxidative stress in testicular tissue in infertile men with azoospermia cases either do not have spermatozoa or have any spermatozoa. In this study, testicular biopsies were obtained from 20 men with azoospermia who were attended to infertility center for diagnosis or infertility treatment. TESE samples were divided to two groups as spermatozoa were detected and not detected for azoospermic men. Immunohistochemistry was used to localize the all three of nitric oxide synthase (NOS) isoforms in these tissues. Chemiluminescence

measurement of NO is based on the reaction of hydrogen peroxide and NO to peroxynitrite. Endothelial NOS (eNOS) reaction in spermatozoa detected group was considerably higher than spermatozoa absent group which shows that eNOS plays an important role in spermatogenesis detection. Inducible NOS (iNOS) reaction was also higher in comparison to eNOS reaction. It was observed that iNOS reaction was higher than spermatozoa absent groups. There was no significant difference in the neuronal NOS (nNOS) reaction between the spermatozoa detected and spermatozoa absent groups. Superoxide radical generation in spermatozoa detected group was significantly lower than spermatozoa absent group. Peroxynitrite ratio in spermatozoa absent group was significantly higher than spermatozoa detected group. These results were showed that three isoforms of eNOS and iNOS play an important role in spermatogenesis process in azoospermic men However; nNOS may act as a signal molecule for spermatogenesis process. In conclusion, testicular oxidative stress plays a role in male infertility.

SW06.W30–49
Out-of-peak ChIP-seq signal analysis and approach to ChIP-seq peaks and protein–protein interaction usage for protein complex reconstruction

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ChIP-seq is very important method for protein-DNA interaction examination. Usually it is used for examination of relatively constant protein-DNA binding that is represented by peak in signal but out-of-peak signal seems to be not used. In this work out-of-peak signal analysis and its applications are described. Integrative approach for ChIP-seq peaks and protein-protein interaction usage is described as well.

Out-of-peak ChIP-seq signals were derived from general ChIP-seq signal by annotated peaks exclusion. UCSC Browser was used as a source of ChIP-seq signals. ChIP-seq signals for different transcription factors and RNA polymerase II were used. Firstly it occurred that out-of-peak signal can be used for chromatin remodeling complex and general chromatin events reconstruction. One of applications was RNA polymerase II elongation complex. It occurred that out-of-peak signal is higher in genes and especially in exons than out of genes and exons respectively. Moreover out-of-peak signals of RNA polymerase II and its transcription factors were positively correlated but RNA polymerase II out-of-peak signal was not positively correlated with RNA polymerase III transcription factors. Original controls were used and strong underestimation of genes regions in genome was taken into account. According to results new model for elongation complex was suggested were RNA polymerase II was bound to transcription factors during elongation process. Model where it stays connected to promoter through transcription factors is also discussed.

ChIP-seq peaks were used for database approach where it was used along with protein-protein interactions. Many potential protein complexes were reconstructed. Those complexes included histone modifiers and chromatin remodeling complexes. This information can be used in docking for better understanding of orientation of proteins subunits in complexes.

Reference

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SW06.W30–50
Rule-based model of bacterial transcription initiation

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RNA-polymerase of *E. coli* is able to recognize more than 7000 promoter areas on the bacterial chromosome (Salgado, 2012). The key missing part in current understanding of the mechanism of transcription regulation is the mechanistic understanding of the process of promoter location by RNAP and its consequences for the promoter efficiency. It was estimated that the number of RNA-polymerase (RNAP) molecules vary between 1500 and 11 000 per bacterial cell (Klumpp, 2008). It was also shown that about 80% of RNAP are not engaged in transcription process as they are bound to the chromosome in non?specific manner. The subunit required by RNAP for promoter recognition and transcription initiation, σ-subunit, even more rare: there are 500 σ^{70}, 95 σ^{28} and 55 σ^{54} molecules per cell (Ishihama, 2000). Each promoter has slightly different sequence and as the consequence, RNAP interacts differently with each promoter.

We have developed *E. coli* transcription initiation model with the rule-based approach (Danos, 2009). The key feature of this model is the presence of non-specific DNA and rules that account for difference in interaction of RNAP with promoter and non-promoter DNA.

To make simulation setup similar to the *in vivo* environment we have developed new analysis technique called *concurrent sensitivity analysis*. Global sensitivity analysis probes behavior of the model by its simulation with a number of parameter sets evenly covering some hypercube in the parameter space. Usually such simulations are performed similar to *in vitro* experiment: model is simulated with each parameter set like each promoter tested in its own test tube. Parallel setup is. A rule-based simulation allows us to combine submodified models with all parameter sets into one model in a concurrent environment, which more reminding *in vivo* experiment.

The global sensitivity analysis of model simulations in concurrent shows that signs and values of sensitivity coefficients are similar in both *in vivo* and *in vitro* setups. What is interesting on that graph is that non-specific binding coefficients has sensitivity close to parameters of 'open' complex formation, which are known to be the key parameters in the transcription initiation model.

That results emphasize that in concurrent environment where some molecules are in deficit, weak non-specific interactions could be more important then strong interactions, which found significant during *in vitro* experiments.

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