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THE FUNCTIONAL ROLE OF PPN1 AND PPX1 POLYPHOSPHATASES UNDER STRESSES ACTION AND FOR ADAPTIVE RESPONSE DEVELOPMENT

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Enzymes of phosphorus metabolism play an important role in maintaining the life of cells of microorganisms under different environmental conditions. Two main polyphosphatases (poly(P)ases) PPN1 and PPX1 are known in the yeast cells. These enzymes participate in the polyphosphates metabolism and, thus, directly or indirectly involved in a number of intracellular processes. The ***purpose*** *of our study was to study the genetic relationships between these poly(P)ases under stresses and their involvement in the process of an adaptive response in yeast cells.* ***Methods.*** *Strains of yeast defective by PPN1 and PPX1 poly(P)ases genes were used in the study. Genetic interaction was assessed by phenotypic traits using the fitness-test method. To establish the mechanism of participation of poly(P)ases in the processes of cellular response to the action of stresses, the activity of enzymes of the dehydrogenase complex was evaluated. The viability degree was used as an assessment of the adaptive response (AR) of yeast cells to the action of various stresses (acid, peroxide, hypertension). The induction of the AR in yeast cells was performed by means of exposure with the electromagnetic field (EMF) of the ultra-high frequency range (40.68 MHz).* ***Results.*** *It was shown that enzymes are involved in the work of different metabolic pathways and therefore regulate different aspects of cell activity. Both enzymes (PPN1 and PPX1) showed to be involved in the formation of the stress response by the yeast cells, however, only PPN1 had a direct impact on the formation of the AR since cells with a lack of this enzyme were unable to start AR as well. The deficiency on PPX1 stimulated the formation of AR in yeast cells even without EMF pretreatment, but its mechanism was different from the mechanism of AR induction under the action of EMF. The dehydrogenases showed to be important for the formation of cells response to the action of stress factors but did not play a direct role in the AR formation.* ***Conclusions.*** *These results expand the understanding of the poly(P)ases PPN1 and PPX1 functional role under stresses and for the AR formation.*

Keywords: *polyphosphatases, yeast, stress, fitness-test.*

Living organisms are constantly influenced by factors of physical, chemical and biological nature. Many of these factors cause physiological, biochemical and molecular genetic perturbations in cells, resulting in the launch of a cascade of intracellular processes aimed at counteracting the impact of stress factors. Most of such processes are energy-consuming because they require *de novo* synthesis of proteins, enzymes, nucleic acids (mRNAs, tRNAs), lipids, sterols, hormones, polysaccharides and so on. Among the enzymes of energy metabolism, polyphosphatases (poly(P) ases) play an important role due to transforming the phosphorus compounds and thus directly or indirectly influence on various cellular processes. Several poly(P)ases are known in yeast: the PPN1 that possess both endo- and exopoly(P)ase activities and located in the vacuolar membranes of yeast cells, while in mammalian cells its analog is located in the nucleus and named endopoly(P) ase PPN1. Another one is an exopoly(P)ase PPX1 that is localized in the cytoplasm of yeast and mammalian cells. Another poly(P)ase could be present in the nuclei of yeast cells as well [1], probably it is DDP1 [2], while one more poly(P) ase PPN2 was described recently [3]. These poly(P)ases are encoded by the respective genes. The structural, physiological and biochemical role of poly(P)ases have been extensively studied, but differences in their localization in mammalian and yeast cells impose some specific features on the functioning of these enzymes. The main work

performing by these enzymes is polyphosphate (poly(P)) chains hydrolysis. The exopoly(P)ase hydrolyzes the terminal phosphate groups from the poly(P) chain, while the endopoly(P)ase catalyzes the cleavage of long poly(P) chains to the shorter ones [4]. Due to the fact that poly(P) is necessary for phosphorylation/dephosphorylation of proteins (which is one of the key reactions required for the implementation of receptor-dependent cell response to external influences), the poly(P)ases are thus indirectly involved in various cellular processes, including gene expression, maintenance of physiological functions, ensuring the mobility of microorganisms, the formation of virulence factors, resistance to stress, etc. [5]. The deletion of poly(P)ases leads to qualitative and quantitative changes in the content of poly(P) in various cellular compartments and in particular in the cytoplasm and cell wall of yeast [4]. The defect in both poly(P)ases is not lethal, and this suggests that the functional role of these enzymes (at least under optimal conditions for existence) may be partially offset by other enzymes. Yeasts defective by *ppn1* accumulate long-chains poly(P) and unable to grow on minimal media. In the case of deletion of both poly(P)ase genes (*ppn1* and *ppx1*), such a mutant rapidly loses viability in the stationary phase [4].

Both poly(P)ases (PPN1 and PPX1) are involved in the regulation of the JAK/STAT (Janus kinase/signal transducers and activators of transcription) signaling pathway, which transmits information from outside of a cell to DNA. PPN1 have a major function in this process because it inhibits SOCS1 (cytokine signaling-1 suppressor) [5]. In mammalian cells, PPN1 carries out the process of dephosphorylation of proteins in the nucleus, without which such important factors as, for example, a protein transducer and an activator of STAT1 transcription cannot leave the nucleus and fulfill its regulatory functions in the cytoplasm [5]. A defect in one or both poly(P)ases entails a malfunction of the signaling pathway and the inability of the cell to respond to an external stimulus.

Thus, poly(P)ases do not perform vital functions under optimal conditions of existence and cells may well exist in their absence. However, little is known about the functioning of these enzymes under stress. Obviously, at least PPN1 is needed to ensure the resistance of yeast cells to stresses. However, issues regarding the functioning of these enzymes, as well as their interaction under different types of stress remain unresolved. The **aim** of this work was to study the participation of these enzymes in the formation of the yeast cells resistance to the chemical and physical stress factors and to determine whether poly(P)ase are involved in the adaptive response formation, in particular.

Materials and methods

Yeast strains and cultivation.

Saccharomyces cerevisiae yeast defective by *ppn1* and *ppx1* were used in the study (Table 1). Individual haploid strains with deleted *ppn1* and *ppx1* genes were obtained previously as yeast knock-out collection (YKO) [6]. The deletion strains contain a genetic marker of resistance to geneticin conferred by the KanMX4 cassette inserted into a deleted ORF and uniquely tagged with one or two 20mer sequences (bar code). The homologous transformation was used to replace the former cassette with NatMX4, resulting in resistance to nourseothricin. These markers are neutral when compared with unmarked wild-type strains [7, 8]. After that the genomic DNA from one deletant (PPX1:KanMX4 alpha) was isolated. The whole deleted antibiotic cassettes were cloned with flanking sites for the specific gene (*ppx1*) and homologous transformation of the strains with other deletion (PPN1:NatMX4) was performed. In this way the transformed (double mutant) cells that possessed resistance both to geneticin and nourseothricin were obtained.

Yeasts were grown on agar nutrient medium YEPD at 28° C for 24 ± 1 hours. Cells were washed from nutrient residues with PBS (pH 7.2) by centrifugation at ×300g for 5 min, three times, and resuspended in sterile distilled water to a

Table 1

Features of the *Saccharomyces cerevisiae* yeast strains genotype used in the study

Strain	Genotype	Description
024-60	*MATα his3 leu2 lys2 ura3 +*	*Parental strain for all other strains was got from the strain BY4743, the analog of BY4742.*
025-72	*MATα his3 leu2 lys2 ura3 + ppn1Δ:: NatMX4*	*Defective by ppn1*
025-74	*MATα ppx1Δ::KanMX4*	*Defective by ppx1*
025-76	*MATα ppn1Δ::NatMX4 ppx1Δ::KanMX4*	*Defective by ppn1 and ppx1*

concentration of 2×10^8 cells/ml according to optical density.

RF-EMF experiments

Solenoid connected to the generator of electromagnetic field (EMF) with 40.68 MHz frequency (27.5 V/m, 22 A/m, the capacity of radiation 30 W, polarized in a horizontal plane) was used as a source of EMF. The EMF parameters were measured with Magnetometer (TESTLA DKP-B-2827, Czech Republic). Cells exposure was performed under strict thermostatic conditions (28° C) for 60 min.

Hypertonicity, oxidative and acidic stresses

To create the conditions of oxidative, acid and hypertonic stresses, yeasts were placed in 0.3 mM and 10 mM solutions of hydrogen peroxide, 100 mM and 150 mM solutions of acetic acid and 1M solution of sorbitol, respectivelly. Stock solutions were prepared for this purpose that were mixed with yeasts cells suspensions in a ratio of 1:1. Cells were treated at 28° C for 60 min and after that, all samples were washed three times in PBS by centrifugation at ×300g for 5 min.

Dehydrogenases activity

The activity of dehydrogenase complex enzymes was determined by the intensity of 2,3,5-triphenyltetrazolium chloride (TTC) conversion to formazan [9]. Yeasts at a concentration of 10^8 cells/ml were added to the reaction mixture (1/15 M phosphate buffer (pH 7.0), 0.1M magnesium sulfate, 0.1M glucose, 0.5% TTC). The reaction was carried out at 37° C for 60 min. To convert insoluble formazan into its dissolved form, dimethyl sulfoxide was added to the mixture. The activity was determined by the amount of formazan formed according to the optical density at 538 nm.

Quantitative characterization of genetic interaction between gene pairs

The interaction between *ppn1* and *ppx1* genes was evaluated by phenotypic features as described in [10]. The colonies area was determined after 24h of growth on the YEPD medium (with 2.5% agar) using ImageJ 1.64 software [http://rsb.info.nih.gov/ij/] for processing of the digital images of the yeast colonies that were obtained with the Nikon SMZ-1000 stereomicroscope (Nikon, Japan) and the Canon PowerShot G12 camera. The average values were calculated from at least 300 colonies in each sample. The size of the genetic interaction (ε) between pairs of genes was determined by the formula $\varepsilon = f_{ab} - f_a \times f_b$, where f_a, f_b, and f_{ab} are quantitative indicators of phenotypic parameters of two single and one double mutant strains respectively.

Statistical analysis

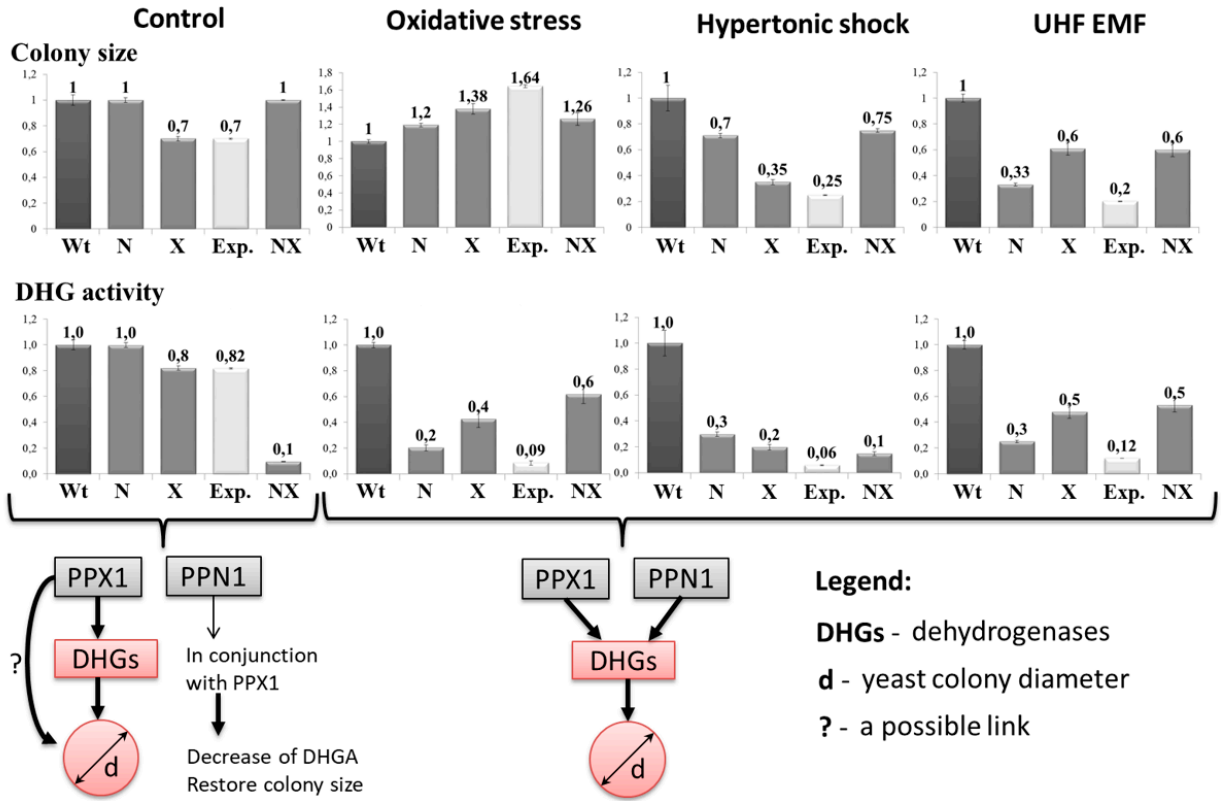
The experiments were conducted in triplicate and each assay was conducted in triplicate. Statistical data processing was carried out with STATISTICA (data analysis software system), version 10 (StatSoft, Inc. 2001, www.statsoft.com). The significance of differences between the mean values was evaluated by the Fisher-test at $p \leq 0.05$.

Results and Discussion

1. Growth kinetics of yeast cells defective by *ppx1* and *ppn1* genes

The physiological and biochemical changes, that take place in a cell in case of gene deletion, indicate the involvement of proteins encoded by these genes in certain biochemical processes. At the same time, the corresponding phenotypic changes in the double mutant serve to quantify the genetic interaction (ε) between the pair of genes [11]. This method is used to map interactions between genes and suggests the use of cells of parental and mutant strains (single and double mutants) grown on the nutrient medium to estimate their colonies' sizes. The deletion of vital genes resulted in a so-called "lethal phenotype" at which cells are unable to form colonies, whereas the deletion of most other genes may affect the colony size in different ways. The size of the colonies of the defective strains allows inferring the link between the pair of genes and to locate the products of these genes on the metabolic map.

The average area of colonies formed by yeast cells on the surface of the agar nutrient medium after 24 h was 1.51-1.57 mm^2. The *ppn1* deletion did not affect colony size and the phenotype index (*f*) value remained equal to 1, while the *ppx1* gene deletion resulted in a 30% decrease of the colonies area and the phenotype index was 0.7 (Fig. 1). Thus, the expected (theoretical) value of the phenotype index of the double mutant ($f_{ppn1::ppx1} = f_{ppn1} \times f_{ppx1}$) was 0.7. However, the actual area of colonies formed by cells with double mutation did not differ from the size of the colonies of the parent strain (1.60 ± 0.19 mm^2). Therefore, the magnitude of the genetic interaction (**ε**) for double mutant cells was positive (**ε** = 0.3) and asymmetric. Such a result indicates that the effect of deletion of one of the genes is suppressed by the deletion of another gene, which is common for the genes whose functions occur on different metabolic pathways or whose products (proteins/enzymes) are part of a larger protein complex [12].



F i g. 1. Phenotypic indexes (according to colony size and dehydrogenases activity) of yeast cells defective by poly(P)ases PPN1 (N) and PPX1 (X), and double mutant cells (NX) under the action of stress factors. The diagrams graphically represent the obtained result.

Under optimal conditions, the size of the colonies was significantly reduced by an average of 30% only in PPX1 defective cells and, along with that, the same cells showed a 20% lower DHG-activity compared with cells of the parental strain. The double mutant cells formed colonies that did not differ in size from the wild-type cells, while the DHG-activity in these cells was the lowest. This result may indicate that there is a direct relationship between PPX1 functions and the activity of DHGs and the growth processes: a decrease in growth activity may be associated with a decrease in the activity of the DHGs enzymes. However, in the case of double mutation, there is obviously a change in metabolism from energetic to constructive, which is reflected in the low activity of DHGs enzymes and growth rates close to normal. At this stage, it is not clear whether PPN1 has a direct effect on the activity of DHGs, or whether its absence is offset by the activity of PPX1 (Fig. 1). Therefore, we draw attention to the response of yeast cells to the action of stress factors that trigger in the yeast cells a cascade of stress-induced processes [13].

Deficiency in poly(P)ases negatively affected the radial growth of the colonies of cells pretreated with hypertonic stress and EMF. Changes of DHG-activities matched with the changes in the colonies' sizes under these conditions. In the case of oxidative stress, all mutations were beneficial, since the size of the colonies increased. However, DHG-activity under oxidative stress was reduced.

Cells with a double mutation showed a tendency to restore the colony size under the action of all stresses. However, DHG-activity was higher than in cells with single mutants only under oxidative stress. Whereas under the action of EMF, it was equal to the value of DHG-activity of cells defective by PPX1, and under the actions of hypertonicity it was almost equal to the expected value.

The changes of the colonies' size of double mutant cells indicate a positive (on the principle of suppression) mechanism of interaction between PPX1 and PPN1 genes. This indicates that the processes initiated in yeast cells defective by both poly(P)ases, although do not able to restore the phenotype completely still quite versatile and allow the functioning of yeast cells under different conditions of the environment.

The decrease of DHG-activity in cells with single deletions under the action of stresses indicates that there is a close relationship between DHG-activity and the activity of poly(P)ases. While

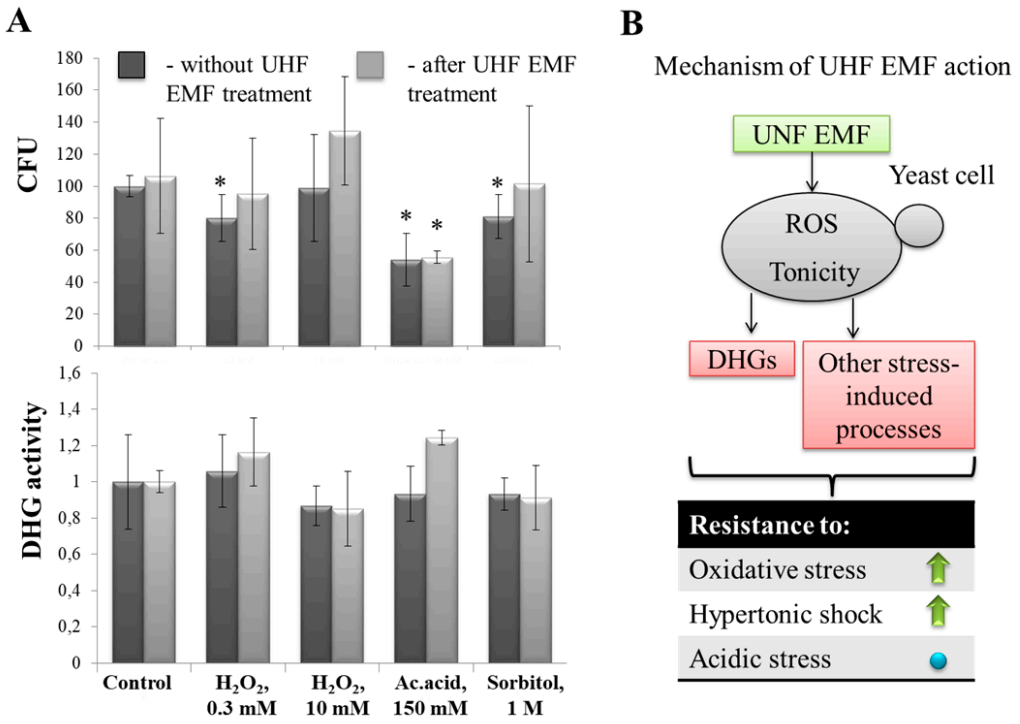
the difference between the DHG-activity profiles marked in case of the stresses action and under the normal conditions indicates that poly(P)ases are more important for the stress response formation than for existence under optimal conditions. And thus, both these poly(P)ases can be considered within the group of stress response enzymes.

2. Adaptive response in the poly(P)ases defective cells

The decrease in the DHG-activity under the action of stress in all defective strains may indicate the cytotoxic properties of the studied stresses (including EMF) and, correspondingly, a decrease in the proliferative activity of yeast cells defective by poly(P)ases. Cytotoxicity may cause the decrease of the colony sizes of cells defective by poly(P)ases marked after treatment with stresses (except for the acid stress). The type of EMF used in the current study possesses a binary action (stimulation and inhibition of growth processes) on yeast cells [14]. Given that the changes in DHG-activity cannot be related to the viability of the yeast cells (because, regardless of the mutations, the viability of yeast cells treated with EMF remained unchanged), and thus, we can assume, that the cytotoxic effect of this type of EMF is realized at the level of regulation of the proliferative activity that is slows down, but not stop the cells division.

The pretreatment of yeast cells with this type of EMF can increase the resistance of yeast cells to some stresses [15]. This effect can be explained by the induction of an "adaptive response" (the ability of a factor (usually weak stress) to trigger defense mechanisms in cells and thereby increase their resistance to stronger stress) in yeast cells caused by EMF action.

We checked whether this type of EMF would have a similar effect in the absence of poly(P)ases. As was expected, stresses decreased the number of viable cells of the wild type strain, while the activity of DHGs did not change (Fig. 2A). Pretreatment of these cells with EMF increased their viability (except for the acetic acid stress) and increased DHG-activity after the action of acetic acid. It should be noted that we point only to the overall trends observed in the studies and not to the absolute values of the DHG-activity, as it varied in experiments, while the overall trend remained unchanged. However, as previously stated, we noted that the pretreatment of cells with this type of EMF increased their resistance to stress factors.



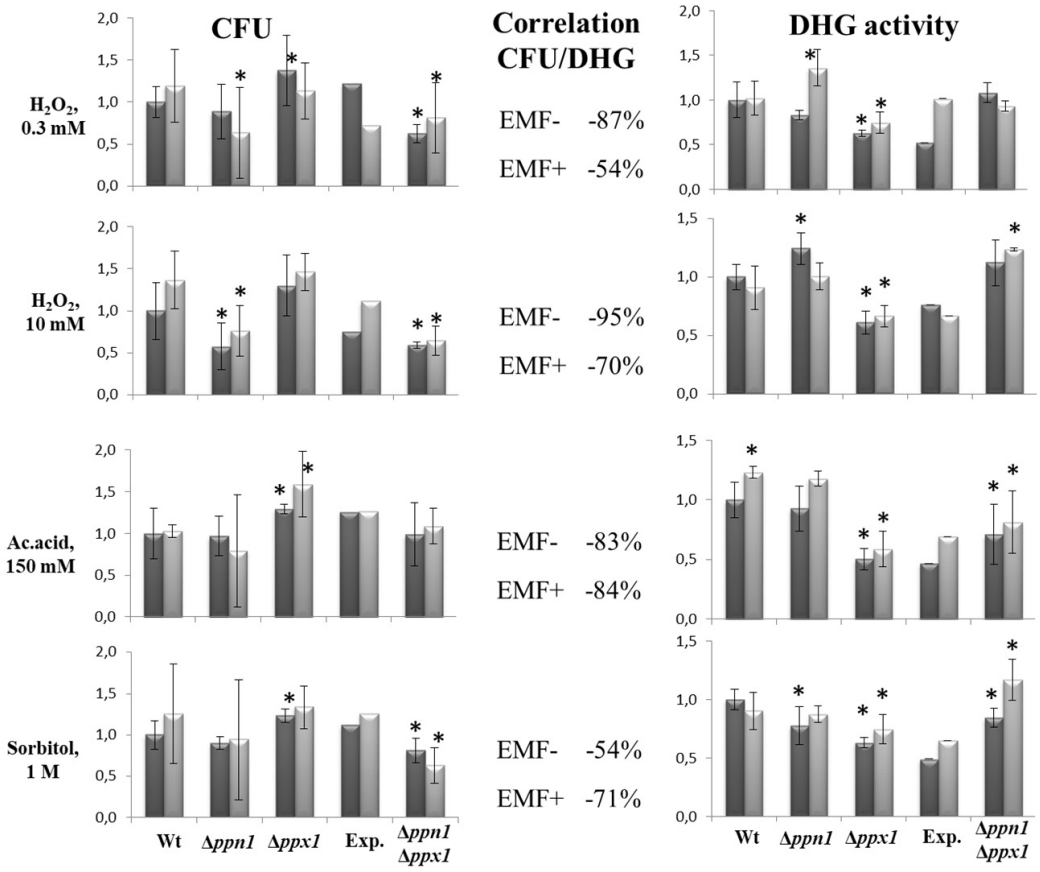
F i g. 2. Effect of the ultrahigh-frequency EMF on the yeast of wild type cell viability (by the colony-forming unite) and dehydrogenases activity under stresses action (A). A possible mechanism of resistance stimulation in yeast cells to the action of stresses after treatment with ultrahigh-frequency EMF (B). Asterisk (*) marks statistically significant differences compared with the corresponding values in the control samples.

The ability of EMFs to increase cell viability and DHG-activity under some stresses may only indirectly indicate the mechanism by which this EMF influences on the cells: formation of reactive oxygen species and change in tonicity are possible consequences of the EMF influence on the yeast cells (Fig. 2B). We marked in our previous studies that this type of EMF affects cell wall structure, which in turn may affect intracellular tonicity [16]. According to the literature data, this and other types of radiofrequency EMFs cause the formation of ROS [17] and, possibly, may change the intercellular pH. Therefore, obtained data confirm the literature data and indicate a possible mechanism of action of the ultrahigh-frequency EMF on yeast cells.

Decrease of viability was observed in the cells defective by PPN1 under the action of hydrogen peroxide (10 mM), as well as in cells defective by both poly(P)ases under the action of hydrogen peroxide and sorbitol (Fig. 3). The action of UHF EMF showed no protective effect and, instead, increased the sensitivity of exposed cells to the action of hydrogen peroxide (0.3 mM), and, possibly, to the action of acetic acid and sorbitol. Defectiveness on PPX1, on the contrary, itself had a beneficial effect and increased resistance of cells to all stresses. It seemed like a PPX1 deficiency was an indirect inducer of the adaptive response, and that was why the additional treatment of cells with the UHF EMF was ineffective.

That is, the defect in PPN1 had a negative effect on resistance to stress factors, whereas the defect in PPX1 had a positive effect. The deletion of both genes, in general, had a negative impact on the viability and the pretreatment with EMF was ineffective to increase it as well.

In contrast to the viability index, the PPX1 deletion had a negative effect on the DHG-activity under the effects of all stress factors. In general, the correlation between CFU and DHG-activity was negative. The defect in PPN1 resulted in an increase of the DHG-activity caused by the treatment of cells with hydrogen peroxide (10 mM), and a decrease of the DHG-activity was observed after the hypertension shock. In all cases (except



F i g. 3. Effect of ultrahigh-frequency EMF on the viability (by the colony forming unite) and dehydrogenases activity of the yeast cells defective by poly(P)ases PPN1 and PPX1 under the action of the stress factors. Asterisk (*) marks statistically significant differences compared with corresponding values in the control samples.

for the action of low concentrations of hydrogen peroxide), the level of DHG-activity of the cells with double mutation was between the values of DHG-activity of cells with single mutations, indicating a positive suppressive interaction of these genes. The pretreatment of cells with the UHF EMF in most (but not all) cases led to an increase of the DHG-activity. Most important in this case was that the activity of DHGs did not correlate with the adaptive response, because the values of DHG-activity could both increase and decrease depending on the situation and regardless of the level of resistance of cells to stress factors. Although we marked no direct link between the DHGs and the adaptive response nevertheless it is definitely possible that certain DHGs still can be involved in the adaptive response.

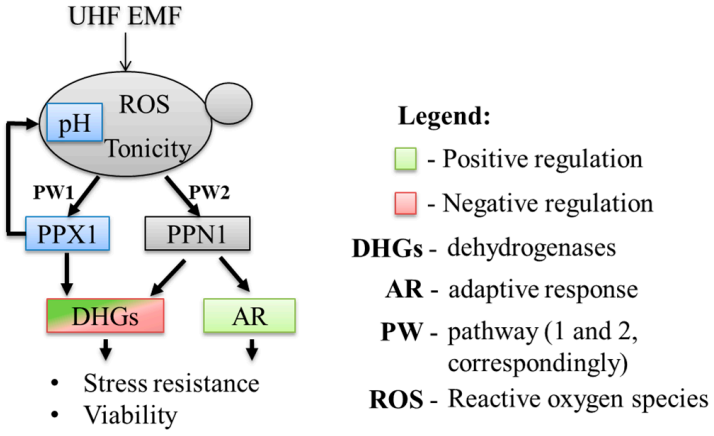
Thus, it is clear that PPN1 is directly involved in the formation of the adaptive response of yeast cells to stresses because in its absence the cells lost such ability. While PPX1 showed no connection with it. In the cells with double deletion, the mean survival rate for stress factors (except for hypertensive shock) was higher after UHF EMF however this difference was not significant. In addition, the level of cells viability with double deletion, in general, achieved the viability level of PPN1 defective cells. Therefore the adaptive response in these cells was suppressed due to PPN1 deletion.

Conclusions

The data obtained indicate that both poly(P)ases play an important role in ensuring the resistance of yeast cells to stress factors. The mechanism of interaction of the genes encoding these enzymes is positive and suppressive, which indicates that PPN1 and PPX1 perform their tasks on the functionally different metabolic pathways [12]. Location of different metabolic pathways may explain the marked differences in the effects caused by the deletion of PPN1 (decreased resistance to stress factors and affected one group of DHGs) and PPX1 (increased resistance to stress factors and affected another group of DHGs). The PPN1 showed to be exclusively involved in the formation of the adaptive response by yeast cells since its functions couldn't be restored by any other enzyme.

Obtained data let to propose the next mechanism of the adaptive response formation in yeast cells with the participation of DHGs, poly(P)ases PPN1 and PPX1, and a number of processes involved in the formation of the adaptive response (Fig. 4). The weak stress factor (which does not change the viability of cells) such as ultrahigh frequency EMF, initiates the ROSs formation in cells and changes of tonicity. The poly(P)ases PPN1 and PPX1 perform their functions at the different metabolic pathways and regulate the activity of DHGs, which involved in the formation of cellular response to the action of various stresses. The absence of a relationship between the level of DHG-activity and the adaptive response indicated that the latter has no direct dependence on the activity of DHGs, and therefore in the above scheme, these two blocks are separated from each other. At the same time, PPN1 participates in the initiation of the adaptive response processes, while PPX1 has no direct action on this process, but in case of its deletion, intracellular properties (including pH) change, and processes of adaptive response initiate, thus, increasing cell resistance and viability of the PPX1 deficient cells to various stresses.

It should be emphasized here that the triggers that start the adaptive response process in yeast cells under the action of UHF EMF are different



F i g. 4. Scheme of induction of the adaptive response in yeast cells under the action of ultrahigh-frequency electromagnetic field (UHF EMF)

from those triggers that initiate the defectiveness by PPX1. This follows from the observation that the action of UHF EMF did not cause an increase in the resistance of yeast cells to acetic acid, whereas the deletion of PPX1 did.

Thus, we showed that PPN1 and PPX1 can be associated with the stress response enzymes because they involved in the formation of cellular response on stresses. These enzymes, being at different metabolic pathways, regulate different aspects of the cell life, but only PPN1 participate in the adaptive response formation by the yeast cells.

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ФУНКЦІОНАЛЬНА РОЛЬ ПОЛІФАЗ PPN1 І PPX1 В УМОВАХ СТРЕСОВОГО НАВАНТАЖЕННЯ І ПРИ ФОРМУВАННІ АДАПТИВНОЇ ВІДПОВІДІ

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Резюме

Ферменти фосфорного метаболізму відіграють важливу роль у підтримці життєдіяльності клітин мікроорганізмів за різних умов існування. Для клітин дріжджів відомі дві основні поліфосфатази (полі(Ф)ази) PPN1 і PPX1, які залучені до метаболізму поліфосфатів і прямо або ж опосередковано залучені до роботи низки внутрішньоклітинних процесів. **Метою** нашого дослідження було вивчення генетичних взаємозв'язків між цими полі(Ф)азами за умов стресового навантаження та їх участь у формуванні клітинами дріжджів адаптивної відповіді. **Методи**. В роботі використано дріжджі, дефектні за генами полі(Ф)аз PPN1 і PPX1. Генетичну взаємодію оцінювали за фенотиповими ознаками методом фітнес-тесту. Для встановлення механізму участі полі(Ф)аз в процесах клітинної відповіді на дію стресових факторів оцінювали активність ферментів дегідрогеназного комплексу. Ступенем оцінки прояву адаптивної відповіді слугував показник життєздатності клітин дріжджів за дії різних стресових чинників (кислотний стрес, перекисний стрес, гіпертонічність). Індукцію адаптивної відповіді в клітинах дріжджів проводили за допомогою електромагнітного опромінювання ультрависокочастотного (ЕМВ УВЧ) діапазону. **Результати**. В результаті проведених досліджень було показано, що обидві полі(Ф)ази PPN1 і PPX1 залучені до формування клітинами дріжджів відповіді на дію досліджених факторів стресу. Ці ферменти перебувають на різних метаболічних шляхах і тому регулюють різні аспекти клітинної діяльності, проте лише полі(Ф)аза PPN1 грає безпосередньо ключову роль у формуванні клітинами дріжджів явища адаптивної відповіді. За відсутності цього ферменту цей ефект відсутній. Дефектність за PPX1 викликає в клітинах індукцію адаптивної відповіді, проте механізм, за яким це відбувається, відрізняється від механізму індукції адаптивної відповіді під дією ЕМВ УВЧ-діапазону. Ферменти дегідрогеназного комплексу відіграють важливу роль у формуванні клітинами дріжджів відповіді на дію факторів стресу, проте не приймають безпосередньої участі у формуванні явища адаптивної відповіді. **Висновки.** Представлена робота розширює уявлення про функціональну роль полі(Ф)аз PPN1 і PPX1 в умовах стресового навантаження і при формуванні явища адаптивної відповіді.

Ключові слова: поліфосфатази, дріжджі, стрес, фітнес-тест.

ФУНКЦИОНАЛЬНАЯ РОЛЬ ПОЛИФАЗ PPN1 И PPX1 В УСЛОВИЯХ СТРЕССОВОЙ НАГРУЗКИ И ПРИ ФОРМИРОВАНИИ АДАПТИВНОГО ОТВЕТА

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Резюме

Ферменты фосфорного метаболизма играют важную роль в поддержании жизнедеятельности клеток микроорганизмов в различных условиях существования. Для клеток дрожжей известны две основные полифосфатазы (поли(Ф)азы) PPN1 и PPX1, вовлеченные в метаболизм полифосфатов и прямо или косвенно вовлечены в работу ряда внутриклеточных процессов. **Целью** нашего исследования было изучение генетической взаимосвязи между этими поли(Ф)азами в условиях стрессовой нагрузки и их участие в формировании клетками дрожжей адаптивного ответа. **Методы.** В работе использованы дрожжи, дефектные по генам поли(Ф)аз PPN1 и PPX1. Генетическое взаимодействие оценивали по фенотипическим признакам методом фитнес-теста. Для установления механизма участия поли(Ф)аз в процессах клеточного ответа на действие стрессовых факторов оценивали активность ферментов дегидрогеназного комплекса. Степенью оценки проявления адаптивного ответа служил показатель жизнеспособности клеток дрожжей под действием различных стрессовых факторов (кислотный и перекисный стрессы, гипертоничность). Адаптивный ответ в клетках дрожжей индуцировали с помощью электромагнитного облучения ультравысокочастотного (ЭМИ УВЧ) диапазона. **Результаты.** В результате проведенных исследований было показано, что обе поли(Ф)азы PPN1 и PPX1 вовлечены в формирование клетками дрожжей ответа на действие исследованных факторов стресса. Эти ферменты находятся на разных метаболических путях и поэтому регулируют различные аспекты клеточной деятельности, но только поли(Ф)аза PPN1 играет непосредственную ключевую роль в формировании клетками дрожжей явления адаптивного ответа и при отсутствии этого фермента этот эффект отсутствует. Дефектность по PPX1 также индуцирует адаптивный ответ, однако механизм, по которому это происходит, отличается от механизма индукции адаптивного ответа под действием ЭМИ УВЧ-диапазона. Ферменты дегидрогеназного комплекса играют важную роль в формировании клетками дрожжей ответа на действие факторов стресса, однако не принимают непосредственного участия в формировании явления адаптивного ответа. **Выводы**. Данная работа расширяет представления о функциональной роли поли(Ф)аз PPN1 и PPX1 в условиях стрессовой нагрузки и при формировании явления адаптивного ответа.

Ключевые слова: полифосфатазы, дрожжи, стресс, фитнес-тест.

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