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V.S. Pidgorskyi, S.I. Voychuk, E.N. Gromozova

Zabolotny Institute of Microbiology and Virology,
National Academy of Sciencies of Ukraine,
154 Zabolotny St., Kyiv, 03143, Ukraine

THE ROLE OF POLYPHOSPHATASES IN THE CELL WALL AND CYTOPLASMIC MEMBRANE RESPONSE TO THE ACTION OF STRESS

The impact of an altered phosphorus metabolism on the content of the cell wall and cytoplasmic membrane components under the influence of physical and chemical stress factors was investigated. In this study, Saccharomyces cerevisiae strains with deleted PPN1 and PPX1 exopolyphosphatases were used. Osmotic shock, peroxide and acidic shocks, and radiofrequency electromagnetic radiation were applied to induce stress response reactions. The strains with deleted PPN1 and PPX1 had an increased content of the weakly linked and alkali–sensitive components of the cell wall proteins and a two–fold decreased content of glucosamines. The cellular fatty acid composition did not change, while the content of sterols varied depending on the strain peculiarities. The efficiency and direction of stress factors action showed an individual factor–specificity. The minor components were found to be the most labile and actively responding units to the effects of stress. That demonstrates a potential for their application as biomarkers and in industrial biotechnology. Thus, PPN1 and PPX1 polyphosphatases are involved in regulation of the biosynthesis of the cell wall and membrane components; they support their structure and are responsible for the changes in response to the influence of stress factors.

K e y w o r d s: yeast, polyphosphatases, stress, cell wall, membrane.

The main focus of modern biology is the study of functional patterns in biological systems at molecular and genetic levels. Together with physiological, biochemical and morphological studies, it enables to determine the underlying mechanisms of interaction between cells and environmental factors or other biological objects. Environmental stress factors, including electromagnetic radiation, can significantly affect the functioning of microorganisms in their natural habitats and the efficiency of biotechnological processes. All molecular, genetic, physiological and biochemical mechanisms activated by intracellular regulatory systems as a response to external signals are associated with the biosynthesis of the cytoplasmic membrane and cell wall components. The latter connect the adaptation of eukaryotic organisms to the action of physical and/or chemical stresses and are considered to be the primary outpost, which perceives the non–ionizing electromagnetic radiation exposure. However, the real biological molecules, providing the perception, transformation, and transduction of non–ionizing EMFs, are still unestablished. In the last decades, great attention was devoted to polyphosphates, which are thought now to be the fundamental biological units, which originated from the inorganic world and evolved to be a part of every living matter. They are short and long linear molecules that significantly vary in length and are found in all cellular compartments in association with other molecules or as separate structures

(volutin grains, for example). It is known that inorganic polyphosphates take part in the stress response of cells. However, their role in the synthesis of the cell wall and cytoplasmic membrane components induced by adverse factors is poorly understood [5]. There is only some evidence that polyP is able to activate cell wall enzymes such as glucan transferase Bg12p and may be other glycolytic enzymes too, inducing cell wall autolysis [4]. Radiobiological reactions of cells largely depend on the induced changes in the structure and functions of their membranes. The effects of ionizing radiation are studied in detail within radiation membranology, and the mechanisms of their biological action are thought to be established. However, the mechanisms of radiofrequency EMFs influence on the structure, chemical composition and characteristics of membrane functions (transport functions, signal perception, bioenergetic processes, etc.) are still under study.

We obtained data suggesting that the biosynthesis of the yeast cell wall and cytoplasmic membrane is sensitive to the exposure to radiofrequency EMF and that the outcome depends on the activity of two polyphosphatases (polyPases) PPN1 and PPX1, which are the best–known polyPases of yeast.

PolyPases are the enzymes that participate in the cellular energy metabolism, by means of elimination (dephosphorylation) of phosphoric acid residues from polyphosphate molecules. Inorganic polyphosphates are multifunctional molecules directly or indirectly involved in numerous processes, including gene expression, energy metabolism, motility, virulence, etc. One of the main causes of violations in the plurality of biological processes after a deletion of one of the polyPases may be explained by the accumulation of polyP in the cells that leads to an inhibition of cell wall components synthesis [10, 12].

Both polyPases (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 external chemical signals to the genes promoters on the DNA, which trigger the process of transcription activation. PPN1 performs the basic function in this process, as it provides inhibition of SOCS1 (suppressor of cytokine signaling–1), an important component of the JAK/STAT signaling pathway [15]. The functional role of PPN1 is extremely important, as it provides for dephosphorylation processes in the nucleus. Without its function, such important factors, as the transducer and activator protein of STAT1 transcription, cannot leave the nucleus to the cytoplasm and perform their regulatory functions [15]. Defects in one or both polyphosphatases resulted in a malfunction of signaling pathways and an inability to generate a response to an external stimulus.

Using the yeast *Saccharomyces cerevisiae* strains with deletions in the genes of the polyPases PPN1 and PPX1 (Table) we determined that the strains lacking polyPases possess an increased content of at least two types of cell wall proteins and changed quantities of various sugar residues. Cell wall components are the main factors of biological adhesion thus an increased quantity of proteins with weak non–covalent bonds or disulfide linkages along with a decreased content of glucosamine may be assumed as the main factors causing perturbations of yeast adhesion in the case of PPN1 and PPX1 deletion. Deficiency in the PPX1 resulted in an increased content of the *alpha*–form of D–mannose and D–glucose (to 17–25 %) and of Neu5Ac (to 31–33 %) and a corresponding decrease of GlcNAc (to 4–6 %) and GalNAc (to 11–19 %). A defect in PPN1

led to a decrease of GalNAc (to 19 %) and an increase of the Neu5Ac content (13 %). These changes are associated with an increased hardness of the cell walls of the mutant strain. However, this parameter decreased in the polyPases defective strains after the EMFs exposure and increased in the wild–type strain. The EMF treatment, in addition, caused a formation of the outer extracellular polymeric layer [13] and we assume that this may happen because of the breakage of weak protein bonds, induced by EMFs, and a consequent leakage of the cell wall components. However, the EMFs–induced layer was observed in the wild–type strains only. This may suggest that the specific polyP are necessary to preserve the cells as they form an obvious surrounding structure. At the same time, in the case of polyPases defectiveness, the components of this layer were eluted and wasted after the cell washing procedure.

Table

Genome peculiarities of *Sacchromyces cerevisiae* strains used in the study*

Strain	Genotype	Reference
CRY	*MAT*a *ade2 his3 leu2 trp1 ura3*	[14]
CRN	*MAT*a *ade2 his3 ura3 ppn1Δ*::*CgTRP1*	[10]
CRX	*MAT*a *ade2 his3 trp1 ura3 ppx1Δ*::*LEU2*	[14]
CNX	*MAT*a *ade2 his3 ura3 ppn1Δ*::*CgTRP1 ppx1Δ*::*LEU2*	[10]

* Yeast strains were obtained by A. Kornberg and co–authors and were kindly provided by the laboratory of I.S. Kulaev of the Skryabin Institute of Biochemistry and Physiology of Microorganisms, RAS (Pushchino, Russia).

These results clearly indicate that both polyPases (PPN1 and PPX1) and their products (polyP) are the essential units supporting cell wall integrity and ensuring protein binding to other cell wall components, mainly polysaccharides.

The action of chemical stresses, induced by oxygen peroxide, acetic acid, and sorbitol, causes major changes (index of variability achieved 40 %) in the content of minor components of the cell wall polysaccharides (CW PS) indicating that just these components are the main stress–response units. The osmotic shock and acetic acid affected mainly the quantities of D–galactose and *alpha* forms of D–mannose and D–glucose, while hydrogen peroxide caused a slight effect on the content of *beta*–forms of D–mannose and D–glucose, GlcNAc and GalNAc. The osmotic shock showed linear dose–effect dependence on the total PS content, while acetic acid had a strong nonlinear effect. At the same time, the RF EMF changed the character of dose–response curves for the content of GlcNAc and GalNAc under the influence of stress factors. In most cases, the curves became the opposite of the results obtained for the untreated control samples.

These results indicate that RF EMFs, along with the individual impact on the cell wall structure, may affect the influence of other stresses.

It was shown that this type of RF EMF (40.68 MHz) may increase the cell membrane permeability [13]. Thus, the character of changes of CW PS content after irradiation may be a result of the necessity of cellular adaptation to such

EMF's impact. We evaluated the fatty acids and sterols content in cells treated with the RF EMF and detected that the total content of fatty acids was fairly stable for the yeast strains without any obvious dependence on the polyPases defectiveness or EMF treatment.

However, the analysis of sterols showed that a defectiveness in one or both polyPases leads to an altered sterol composition that easily lets to recognize the wild–type strains from the mutants. A direct correlation between the yeast cell viability and squalene concentration in the PPX1 lacking strains (as well as in the double mutant) was observed. No similar dependence was observed for the wild type and PPN1 defective strains and thus this result may suggest the participation of PPX1 enzyme in the regulation of synthesis or transformation of squalene, at least.

The action of stress factors such as peroxide and acetic acid caused a linear dose–dependent reduction of various sterol quantities. The wild type appeared to be sensitive to the action of acetic acid, while the PPX1 deficient strain depended mainly on the action of peroxide. The double mutant showed (with a few exceptions) a negative linear dependence on the action of acetic acid and a nonlinear (quadratic) dependence on the action of peroxide. The PPN1 mutant had a very low content of sterols in membranes and, in this case, their quantity did not decrease additionally after the EMF treatment, thus, indicating some stability of the content, but in fact being a result of the presumably critical biological minimum of sterols necessary for maintaining cell viability and functioning.

Thus, all the studied features of the yeast cell walls and membranes were found to be dependent on the action of the polyPases PPN1 and PPX1, which have a direct impact on both the initial state of the tested structures and their changes in response to stresses. Our findings indicate that polyPases are the necessary components for the correct fulfillment of the polysaccharide chains biosynthesis in cell walls, as well as for the formation of strong non–covalent protein bonds. Polysaccharides are important structural elements of all living organisms. A lot of them have a commercial potential in various biotechnological processes and provide for a set of functions including the resistance to chemical and physical stresses. They are very labile cellular structures, which change in response to environmental perturbations and, thus, can be good indicators of biological effects of non–ionizing electromagnetic fields. There is a direct correlation between the quantity of alkali–sensitive polyphosphates (fraction polyP4) and mannoproteins in yeast cell walls [5]. In the case of PPN1 mutation the level of polyP4 decreased almost two–fold, which did not happen after the PPX1 deletion [6]. Nevertheless, there is no data on any connection between PPN1 and PPX1 and the synthesis of mannoproteins. However, in our study, we did not observe any significant changes in mannose amounts but determined a two–fold decrease in glucosamine content. This may be due to the accumulation of polyP fractions within cell walls, as it was suggested by [12].

Additionally, it is obvious that the biosynthesis of sterols is also associated with the functional properties of these two polyPases, and their absence will affect all levels of intracellular processes associated with the functional role of sterols. The genes of sterols biosynthesis in yeast and humans are orthologues and can be successfully replaced. This indicates common metabolic pathways

of synthesis of these polymers [3]. Thus, it can be assumed that the involvement of polyPases in this process, determined for the yeast cells, can be adopted for human biology as well. The possible cause of disorders in the biosynthesis of sterols in the absence of polyPases may be explained by the impossibility of implementation of the key proteins dephosphorylation, that is assumed for PPN1, which affects the process of dephosphorylation of the STAT1 protein (the transducer and activator of transcription) [15]. STAT1 induces the synthesis of cholesterol 25–hydroxylase, an enzyme necessary for the production of 25–hydroxycholesterol – the main cholesterol derivative formed by macrophages [7].

An overexpression of PPX1, which leads to a shortage in the accumulation of polyphosphates in cells, influences the expression of stress–inducible genes *rpoS* (which encode the σ^{38}–subunit of RNA polymerase responsible for the expression of approximately 50 genes) and *recA* at the level of their transcription. Thus, it additionally affects the expression of the related genes that are induced by stressful conditions (genes of SOS–regulon, actually *recA* and *umuDC*), or DNA damage with mitomycin and ultraviolet, or the osmotic (*osmB*) and heat shocks (*otsBA*), or an oxidative stress (*katE*), while also being activated during the cell's stationary phase [11]. Therefore, it is obvious that both, the accumulation and the absence of polyphosphates in cells will have a negative impact on the function of intracellular processes and can even lead to the cell death.

In summary, the results of our study demonstrate that polyPases PPN1 and PPX1 are important units in the integrity support of the yeast cell wall and membrane. They are needed to provide strong linkages in proteins–protein and proteins–polysaccharide complexes and seem to participate in sterol biosynthesis. In the absence of any of these two polyPases the cell reaction to the influence of various stresses changes, however, the cell response is still specific to each of the factors.

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В.С. Підгорський, С.І. Войчук, О.М. Громозова

Інститут мікробіології і вірусології ім. Д.К. Заболотного НАН України, вул. Заболотного, 154, Київ , 03143, Україна

РОЛЬ ПОЛІФОСФАТАЗ У РЕАКЦІЯХ КЛІТИННОЇ СТІНКИ І ЦИТОПЛАЗМАТИЧНОЇ МЕМБРАНИ НА СТРЕС

Резюме

Досліджено вплив змін у фосфорному метаболізмі клітин на вміст компонентів клітинної стінки і цитоплазматичної мембрани під дією фізичних і хімічних факторів. У дослідженні було використано штами дріжджів *Saccharomyces cerevisiae,* дефектні за екзополіфосфатазами PPN1 і PPX1. Стрес індукували за допомогою осмотичного шоку, перекісного і кислотного шоку, а також за допомогою радіочастотного електромагнітного випромінювання. Штами з видаленими PPN1 і PPX1 мали

підвищений вміст слабозв'язаних і лужно-чутливих білків клітинної стінки та вдвічі знижений вміст глюкозаміну. Склад жирних кислот не змінювався, у той час як вміст стеролів варіював у залежності від штамових особливостей. Ефективність і направленість дії стресових факторів була індивідуальною, фактор-специфічною. Мінорні компоненти виявилися найбільш лабільними і активно реагуючими на дію стресів. Це вказує на їх потенціал для застосування як біомаркерів, а також у біотехнологічних процесах. Таким чином, поліфосфатази PPN1 і PPX1 беруть участь у регуляції біосинтезу компонентів клітинної стінки і мембрани; вони підтримують їх структуру і несуть відповідальність за зміни у відповідь на вплив стресових факторів.

Ключові слова: дріжджі, поліфосфатази, стрес, клітинна стінка, мембрани.

В.С. Подгорский, С.И. Войчук, Е.Н. Громозова

Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины, ул. Заболотного, 154, Киев, 03143, Украина

РОЛЬ ПОЛИФОСФАТАЗ В РЕАКЦИЯХ КЛЕТОЧНОЙ СТЕНКИ И ЦИТОПЛАЗМАТИЧЕСКОЙ МЕМБРАНЫ НА СТРЕСС

Резюме

Исследовано влияние изменений в фосфорном метаболизме клеток на содержание компонентов клеточной стенки и цитоплазматической мембраны под действием физических и химических факторов. В исследовании были использованы штаммы дрожжей *Saccharomyces cerevisiae,* дефектные по экзополифосфатазам PPN1 и PPX1. Стресс индуцировали с помощью осмотического шока, перекисного и кислотного шока, а также с помощью радиочастотного электромагнитного излучения. Штаммы с удаленными PPN1 и PPX1 имели повышенное содержание слабосвязанных и щелоче–чувствительных белков клеточной стенки и вдвое сниженное содержание глюкозамина. Состав жирных кислот не изменялся, в то время как содержание стеролов варьировало в зависимости от штаммовых особенностей. Эффективность и направленность действия стрессовых факторов была индивидуальной, фактор-специфической. Минорные компоненты оказались наиболее лабильными и активно реагирующими на действие стрессов. Это указывает на их потенциал для применения в качестве биомаркеров, а также в биотехнологических процессах. Таким образом, полифосфатазы PPN1 и PPX1 участвуют в регуляции биосинтеза компонентов клеточной стенки и мембраны; они поддерживают их структуру и несут ответственность за изменения в ответ на воздействие стрессовых факторов.

Ключевые слова: дрожжи, полифосфатазы, стресс, клеточная стенка, мембраны.

1. *Campbell J.W., Morgan–Kiss R.M., Cronan J.E., Jr.* A new *Escherichia coli* metabolic competency: growth on fatty acids by a novel anaerobic betaoxidation pathway. Molecular Microbiology. 2003; 47(3): 793–805.
2. *François J.M.* A simple method for quantitative determination of polysaccharides in fungal cell walls. Nature Protocols. 2007; 1: 2995–3000.
3. *Kachroo A.H., Laurent J.M., Yellman C.M., Meyer A.G., Wilke C.O., Marcotte E.M.* Systematic humanization of yeast genes reveals conserved functions and genetic modularity. Science. 2015; 348(6237): 921–25.

4. *Kalebina T.S., Egorov S.N., Arbatskii N.P., Bezsonov E.E., Gorkovskii A.A., Kulaev I.S.* The role of high–molecular–weight polyphosphates in activation of glucan transferase Bgl2p from *Saccharomyces cerevisiae* cell wall. Doklady Biochemistry & Biophysics. 2008; 420(1): 142–45.
5. *Kulaev I.S., Vagabov V.M., Kulakovskaya T.V.* The Biochemistry of Inorganic Polyphosphates. Wiley & Sons, Ltd. 2005; 294.
6. *Kulakovskaya T.V., Trilisenko L.V., Lichko L.P., Vagabov V.M., Kulaev I.S.* The effect of inactivation of the exo–and endopolyphosphatase genes *PPX1* and*PPN1* on the level of different polyphosphates in the yeast*Saccharomyces cerevisiae.* Microbiology. 2006; 75(1): 25–8.
7. *Matsumiya T., Imaizumi T.* How are STAT1 and cholesterol metabolism associated in antiviral responses? JAKSTAT. 2013; 2(3): e24189.
8. *Pitarch A., Nombela C., Gil C.* Cell wall fractionation for yeast and fungal proteomics. Methods Mol Biol. 2008; 425: 217–39.
9. *Quail M.A., Kelly S.L.* The extraction and analysis of sterols from yeast. In Methods in Molecular Biology, Ed. Evans IV. School of Chemical and Life Sciencies, Greenvich Univ., London, UK. 1996; 53: 123–31.
10. *Sethuraman A., Rao N.N., Kornberg A.* The endopolyphosphatase gene: Essential in *Saccharomyces cerevisiae.* Proc Natl Acad Sci USA. 2001; 98(15): 8542–47.
11. *Shiba T, Tsutsumi K, Ishige K, Noguchi T.* Inorganic polyphosphate and polyphosphate kinase: heir novel biological functions and applications. Biochem. (Russia). 2000; 65(3): 375–84.
12. *Thayil S.M., Morrison N., Schechter N., Rubin H., Karakousis P.C.* The role of the novel exopolyphosphatase MT0516 in *Mycobacterium tuberculosis* drug tolerance and persistence. PLoS ONE. 2011; 6:e28076.
13. *Voychuk S.I., Gromozova E.N., Lytvyn P.M., Pidgorskyi V.S.* Changes of surface properties of yeast cell wall under exposure of electromagnetic field (40.68 MHz) and action of nystatin. The Environmentalist. 2005; 25: 139–44.
14. *Wurst H., Shiba T., Kornberg A.* The gene for a major exopolyphosphatase of Saccharomyces cerevisiae J Bacteriol. 1995; 177: 898–906.
15. *Yamada S., Shiono S., Joo A., Yoshimura A.* Control mechanism of JAK/STAT signal transduction pathway. FEBS Letters. 2003; 534: 190–6.

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