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Peculiarities of the formation of indole-3-acetic acid–protein complexes in yeast *Saccharomyces cerevisiae* plasmalemma

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The aim of our work was to elucidate whether the yeast *Saccharomyces cerevisiae* can receive the phytohormone IAA signal in the plasmalemma and to transduce it to the nucleus, eliciting changes in RNA synthesis peculiar also to the cells of the higher plants (wheat coleoptiles). IAA has been found able to form complexes with proteins in yeast strain α'1-1JA-R^+N cell plasmalemma, which enhanced RNA-polymerase II activity in the system of isolated nuclei of the same yeast strain cells. However, in the plasmalemma of this yeast strain cells one type of IAA–protein complexes of a relatively low affinity has been found, and according to fixed characteristics of IAA–protein complex formation in yeast cell plasmalemma in the Scatchard co-ordinates (K_a » 1361.034; $K_d \approx 7.35 \cdot 10^{-4}$ M; $n \approx 2.07 \cdot 10^{-8}$ mol/μg protein) these complexes cannot be considered receptory.

Key words: IAA–protein complexes, yeast, plasmalemma

INTRODUCTION

The background of the growth-regulating indole-3-acetic acid (IAA, auxin) system is IAA–protein receptory complexes. So far it is not clear how the related processes of plant growth and differentiation are regulated or what is the physiological role of receptory IAA–protein complex compartmentation in plant cells. There were reports on auxin-binding proteins localized in the external surface of higher plant cell plasmalemma (ABP-1) [1, 2], which were considered as possible IAA-receptory proteins. At the Laboratory of Plant Physiology, characterized were IAA–protein complexes whose receptory protein is located in wheat coleoptile plasmalemma [3, 4], and their response is manifested in the cell nucleus activity under intensified transcription processes.

Over the recent years attention has been given to the general physiological regularities of receiving and transducing external signals, including those of IAA. The signal received in the cell, the phytohormone having bound to specific receptors, is transduced to various mediators [5], and response specificity is achieved through changes in protein phosphorylation, with the participation of protein kinases and phosphatases [6]. Receptory proteins located in yeast and animal plasmalemma receive the signal and transduce it to secondary messengers – Ca^{2+}, cAMP, inositetriphosphate and protein kinase cascades [5]. Due to these changes the signal reaches the nucleus and induces changes in gene expression there [7].

The aim of the present work was to elucidate whether the yeast S*accharomyces cerevisiae* can receive the phytohormone IAA signal in the plasmalemma and to transduce it to the nucleus, evoking analogous changes in RNA synthesis peculiar to flowering plant (wheat coleoptiles) cells.

MATERIALS AND METHODS

The object of the work was baker's yeast *Saccharomyces cerevisiae* (strain α'1-1JA-R^+N) [8].

The plasmalemma and nucleus fractions from yeast cells were isolated according to [9, 10] with modifications [11]. The plasmalemma from wheat (*Triticum aestivum* L., 'Selpek') coleoptile cells was isolated by differential centrifugation and purification in sucrose gradient [12, 13].

To evoke the activity of RNA-polymerase II, a system of isolated yeast nuclei was elaborated according to [14], and IAA or plasmalemma preparations obtained upon incubation with IAA as well as dialysis and containing the resulting IAA–protein complexes were added. The RNA-polymerase II activity was evaluated with the aid a scintillation counter (Beckman, LS 1801; USA) according to the involved volume of ^{14}C-ATP (0.1 mM, rel. act. 3.1 MBq · g^{-1}; Amersham).

IAA specific binding with plasmalemma proteins was evaluated as a difference between the total binding of ^{3}H-IAA label (4.6 TBq · g^{-1}; Amersham) and the residual amount of the label upon exposing the study fractions to the same concentrations of marked IAA ($1 \cdot 10^{-9}$–$5 \cdot 10^{-7}$ M) and very high concentrations of the cold hormone. The free IAA excess was eliminated by centrifugation or equilibrium dialysis.

The protein amount was determined according to [15]. The tests, 3–4 replications each, were repeated 4 to 8 times, and the average error of the mean value was calculated to evaluate the results.

RESULTS AND DISCUSSION

IAA exerts a positive effect on the growth of the yeast *S. cerevisiae* strain α'1-1JA-R$^+$N cells containing less cAMP. The stimulating effect of IAA became manifested at the exponential phase of yeast growth (12–16 h), when into the growth medium the hormone was added at a concentration of $1 \cdot 10^{-7}$ – $1 \cdot 10^{-8}$ M. IAA had no enhancing effect on the cell growth of other strains (15 B-P4 and SP 1) characterized by a higher cAMP content [11]. Based on the ability of yeast strain α'1-1JA-R$^+$N cells to respond to the effect of the phytohormone IAA, attempts were made to elucidate the formation and action peculiarities of IAA–protein complexes in yeast cell plasmalemma by performing tests with the cells of this strain.

In wheat cell plasmalemma (pH 7.2) IAA–protein complexes in the system of isolated nuclei of the yeast strain α'1-1JA-R$^+$N enhanced RNA-polymerase II activity by 167% as compared to the control system to which the plasmalemma not exposed to IAA was added. Thus, IAA–protein complexes formed by wheat coleoptile cells caused changes in the system of yeast cell nucleus RNA synthesis. The question arose whether in the cell plasmalemma of yeast itself IAA–protein complexes can be formed and whether they would be active in the system of isolated nuclei of the yeast cells themselves. Addition of yeast strain α'1-1JA-R$^+$N cell plasmalemma vesicles exposed to IAA into a system of isolated yeast nuclei increased RNA-polymerase II activity by about 40% as compared to the control system of nuclei into which the plasmalemmal fraction not exposed to IAA (free IAA was eliminated during dialysis) was added (Table 1).

Different results were obtained in experiments with plasmalemma and nucleus fractions isolated from the yeast strain 15 B-P4, – addition of both free IAA and the cell plasmalemma from the same yeast exposed to IAA induced no changes in RNA-polymerase II activity (Table 2).

In the plasmalemma of yeast strain α'1-1JA-R$^+$N cells, one type of IAA–protein complexes which exhibited a low affinity was observed. The pH optimum (7.2) of the formation of these complexes corresponded with the pH optimum of analogous complexes in wheat coleoptile cell plasmalemma [3], however, the characteristics of these complexes calculated in the Scatchard co-ordinates [16] differed: $K_a \approx 1361.034$; $K_d \approx 7.35 \cdot 10^{-4}$ M; $n \approx 2.07 \cdot 10^{-8}$ mol/μg protein. Judging by the number of binding sites, such complexes could be receptory, however, K_a and K_d showed a low level of affinity not characteristic of the hormone–receptor complexes.

Thus, on the grounds of experimental data on the ability of IAA–protein complexes formed in wheat coleoptile cell plasmalemma to induce in a system of nuclei isolated from yeast cells a response similar to that in an analogous wheat coleoptile cell RNA synthesis system and considering the fact that the described changes in RNA synthesis are characteristic only of the yeast strain whose cell growth under the effect of IAA is intensified, it is possible to state that the yeast cell can receive and transduce IAA signal in a way similar to that in the higher plant cell. However, the characteristics of IAA–protein complexes formed in yeast cell plasmalemma

Table 1. **The effect of IAA–protein complexes formed in yeast *Saccharomyces cerevisiae* strain α'1-1JA-R$^+$N cell plasmalemma on the activity of RNA-polymerase II in the system of nuclei isolated from the same yeast strain cells**

Variants	RNA-polymerase II activity	
	cpm/100 μg protein	%
System of nuclei + + plasmalemma (control)	1472.0 ± 140.0	100
System of nuclei + + plasmalemma exposed to IAA ($1 \cdot 10^{-8}$M)	1982.0 ± 231.0	135
System of nuclei + + plasmalemma exposed to IAA ($1 \cdot 10^{-6}$M)	2124.0 ± 200.0	144

Table 2. **The effect of IAA–protein complexes formed in yeast *Saccharomyces cerevisiae* strain 15 B-P4 cell plasmalemma on the activity of RNA-polymerase II in the system of nuclei isolated from the same yeast strain cells**

Variants	RNA-polymerase II activity	
	cpm/100 μg protein	%
System of nuclei (control)	859.5 ± 183.2	100
System of nuclei + +IAA ($1 \cdot 10^{-8}$M)	885.5 ± 265.0	103
System of nuclei + +plasmalemma exposed to IAA ($1 \cdot 10^{-8}$M)	741.0 ± 150.0	86

show that these complexes cannot be considered receptory.

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INDOLIL-3-ACTO RÛGÐTIES IR BALTYMØ KOMPLEKSØ SUSIDARYMO MIELIØ *SACCHAROMYCES CEREVISIAE* PLAZMOLEMOJE YPATYBËS

S a n t r a u k a

Mûsø darbo tikslas buvo nustatyti, ar gali mielës *Saccharomyces cerevisiae* priimti fitohormono IAR signalà plazmolemoje ir já perduoti á branduolá, sukeldamos RNR sintezës pokyèius, bûdingus ir aukðtesniøjø augalø (kvieèiø koleoptiliø) làstelëms. Buvo nustatyta, kad mieliø kamieno α'1-1JA-R^+N làsteliø plazmolemoje IAR gali sudaryti kompleksus su baltymais, kurie suaktyvina RNR polimerazës II aktyvumà izoliuotø ið to paties mieliø kamieno làsteliø branduoliø sistemoje. Taèiau ðio kamieno làsteliø plazmolemoje buvo aptiktas tik vienas palyginti maþo gimiringumo IAR baltymø kompleksø tipas. Pagal nustatytas IAR baltymø kompleksø susidarymo mieliø làsteliø plazmolemoje charakteristikas Sketèardo koordinatëse – $K_a \approx 1361{,}034$; $K_d \approx 7{,}35 \cdot 10^{-4}$ M; $n \approx 2{,}07 \cdot 10^{-8}$ molio/μg baltymo – tø kompleksø negalima vertinti kaip receptoriniø.

Raktaþodþiai: IAR baltymø kompleksai, mielës, plazmolema