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Alterations of ciliate phosducin phosphorylation in *Blepharisma japonicum* cells

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Abstract

We have previously reported that motile photophobic response in ciliate *Blepharisma japonicum* correlates with dephosphorylation of a cytosolic 28 kDa phosphoprotein (PP28) exhibiting properties similar to those of phosducin. Here we demonstrate in in vivo phosphorylation assay that the light-elicited dephosphorylation of the PP28 is significantly modified by cell incubation with substances known to modulate protein phosphatase and kinase activities. Immunoblot analyses showed that incubation of ciliates with okadaic acid and calyculin A, potent inhibitors of type 1 or 2A protein phosphatases, distinctly increased phosphorylation of PP28 in dark-adapted cells and markedly weakened dephosphorylation of the ciliate phosducin following cell illumination. An enhancement of PP28 phosphorylation was also observed in dark-adapted ciliates exposed to 8-Br-cAMP and 8-Br-cGMP, slowly hydrolysable cyclic nucleotide analogs and 3-isobutyryl-1-methylxanthine (IBMX), a non-specific cyclic nucleotide phosphodiesterase (PDEs) inhibitor. Only slight changes in light-evoked dephosphorylation levels of PP28 were observed in cells treated with the cyclic nucleotide analogs and IBMX. Incubation of ciliates with H 89 or KT 5823, highly selective inhibitor of cAMP-dependent protein kinase (PKA) and cGMP-dependent protein kinase (PKG), respectively, decreased PP28 phosphorylation levels in dark-adapted cells, whereas the extent of light-evoked dephosphorylation of the phosphoprotein was only slightly influenced. Cell treatment with higher Ca^{2+} concentration together with ionophore A23187 in culture medium resulted in marked increase in PP28 phosphorylation levels, while quite an opposite effect was observed in cells exposed to Ca^{2+} chelators, EGTA or BAPTA/AM as well as calmodulin antagonists, such as trifluoperazine (TFP), W-7 or calmidazolium. Light-dependent dephosphorylation was not considerably affected by these treatments. The experimental findings presented here suggest that an endogenous light-dependent protein kinase-phosphatase system may be engaged in the alteration of phosducin phosphorylation in ciliate *B. japonicum* thereby to modulate the cell motile photophobic behavior.

Keywords: *Blepharisma japonicum*; Phosducin; Protein kinases/phosphatases; Photophobic response; Photosensory transduction

1. Introduction

Reversible protein phosphorylation is widely accepted as an essential mediating mechanism by which many intracellular processes are influenced by external stimuli [1–6]. Such a mechanism is also proposed for regulation of sensory transduction pathway in visual systems of higher organisms. In vertebrate retinas, it has been postulated that the process of phosphorylation and dephosphorylation of phosducin, a major cytosolic phosphoprotein, contributes to downregulation of the phototransduction cascade [7–9]. Under dark conditions, phosducin exists in the photoreceptor cells in highly phosphorylated state catalyzed by protein kinase A, Ca^{2+}-calmodulin kinase II or

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G-protein-coupled receptor kinase 2 [10–15]. Dephosphorylation of the phosphoprotein may occur by activation of type 1 or 2A protein phosphatases upon cell exposure to light [8,16]. Under illumination, phosducin in its dephosphorylated state forms a complex with Gβγ and prevents reassociation of Gα with Gβγ, resulting in lower signal amplification at the G-protein level [9,11,12,17–20].

Phosducin protein was first discovered in vertebrate retinas and developmentally related pineal glands [21,22]. Nowadays, there are increasing evidence that phosducin and also many phosducin-related proteins are widely expressed in other mammalian cells [9,18,23–26]. The existence of proteins of the phosducin family has also been reported in some lower eukaryotic organisms. In the yeast *Saccharomyces cerevisiae*, two phosducin-like proteins, PLP1 and PLP2 were detected, that in vivo can bind and regulate Gβγ activity [27]. In the case of fungus *Cryphonecteria parasitica*, one gene, *bdm-1* was identified, which was shown to encode phosducin-like protein involved in regulation of Gβγ function as well as in Gα accumulation [28]. Moreover, three genes, designated as *phlp-1*, *phlp-2* and *phlp-3*, encoding distinct phosducin-like proteins phosducin-1, phosducin-2 and phosducin-3, were discovered in *Dictyostelium discoideum* and it was firmly established that at least one of them, phosducin-1 also plays a role in G-protein-coupled signaling control [29].

Recently, a cytosolic phosphoprotein of 28 kDa (PP28), which displays properties of phosducin protein, has been identified in lower eukaryote, photosensitive protist *Blepharisma japonicum* [30,31]. In the ciliates adapted to darkness PP28 is highly phosphorylated and is reversibly dephosphorylated by illumination. The dephosphorylation of the phosphoprotein coincides with cell photomotility modification, known as the photophobic response. Such motile event results from an activation of a specific cellular photoreceptor system consisting of numerous photoreceptive units (pigment granules). Each of these granules contains hipericin-like pigment blepharismin, a pink-colored quinone [32–36]. The photoreceptor system in the ciliate is coupled to its locomotor system via G-protein-mediated signaling [37].

The studies reported here, were undertaken to further characterize ciliate PP28 to gain insight into the detailed mechanism of light transduction resulting in the photophobic behaviour of *B. japonicum*. To attain this we tested whether light-induced alterations in phosphorylation levels are governed by endogenous protein kinase-phosphatase system of the cell. Therefore, we examined in vivo the effect of selected substances, known to modulate protein kinase or phosphatase activities, on the ciliate phosducin phosphorylation state in ciliates under different light conditions.

2. Materials and methods

2.1. Cells

Stock cultures of ciliate *B. japonicum* were grown in glass dishes in Pringsheim solution (pH 7.2) at room temperature under semidark conditions [38]. Ciliates were fed twice a week with *Tetrahymena pyriformis* ciliates, grown axenically in a medium consisting of 1.0% proteose peptone (Difco, USA) with 0.1% yeast extract (Difco, USA). Before feeding *B. japonicum* the ciliates of *Tetrahymena pyriformis* were centrifuged and washed in Pringsheim solution. For each experiment, the *B. japonicum* cells were collected by a low speed centrifugation and subsequently washed in an excess of fresh culture medium lacking nutritional components. Finally, the selected cell samples incubated in dark or light in fresh culture medium (referred to as a control medium) as well as in test solutions of designed compositions were used for biochemical assays.

2.2. Cell photostimulation

For each test, chosen samples of ciliates adapted to darkness and control medium (control cells) or test solutions were first left at rest for about 15 min to avoid any mechanical disturbances and then exposed to illumination. Cell stimulation by light was provided by a light system including a 150 W fiber-optic light source (MLW, Germany) and an electromagnetic programmable shutter (mod. 1 22-841, Ealing Electro-Optics Co., England).

2.3. Electrophoresis and Western immunoblotting

Biochemical quantification of protein phosphorylation levels were carried out according to a method reported by Laemmli [39]. Briefly, ciliate samples were mixed with 4-fold concentrated electrophoresis buffer, supplemented with protease and phosphatase inhibitors (50 mM NaF, 2 mM PMSF, 5 μM okadaic acid, 10 μg/ml aprotinin, 10 μM leupeptin) to terminate biochemical reactions currently taking place within cells and then boiled for 5 min. Samples were then solubilized and 30 μg amounts of protein were separated by 10% SDS–PAGE using Hoefer System (Amersham, USA). Subsequently resultant gels were transferred as described by Towbin et al. [40] to nitrocellulose membranes (Bio-Rad, USA) during 60 min at 100 V in a transfer buffer consisting of 192 mM glycine, 20% methanol and 25 mM Tris (pH 8.3). The membranes were then blocked for 2 h at room temperature by incubation in TBS buffer composed of 150 mM NaCl and 10 mM Tris (pH 7.5) and supplemented with 2% BSA and 0.2% Tween-20 (TBS–BSA–Tween blocking solution). For detection of phosphoserine-containing proteins, blots were incubated

overnight at 4 °C with a monoclonal antibody raised against phosphoserine, clone PSER 4A9 (Alexis, Switzerland), at concentration of 0.1 μg/ml in TBS–BSA–Tween solution. The blots thus obtained were next washed several times in TBS buffer with addition of 0.1% Tween-20 and then incubated for 60 min at room temperature with a secondary antibody, anti-mouse IgG-horseradish peroxidase conjugate (Calbiochem, Germany) at a 1:10,000 dilution in TBS–BSA–Tween blocking solution. In the end, the membranes were washed several times in TBS–Tween buffer and developed with a detection system (ECL, Amersham Biosci., Austria). The intensities of immunoreactive protein bands were quantified using a laser densitometer equipped with ImageQuant software (Bio-Rad, USA). Protein molecular weights were estimated based on their relative electrophoretic mobilities with specified markers of molecular weights (Bio-Rad, USA). In the control set of experiments, incubations with primary antibody were omitted. The protein concentration in each tested cell sample was estimated with a method described by Bradford [41] using BSA as a standard. Tests for equal loadings and protein quantities were carried out throughout experiments with a ciliate *B. japonicum* photoreceptor protein of about 46 kDa showing distinct pink fluorescence upon ultraviolet irradiation.

2.4. Chemicals

Materials for electrophoresis were purchased from Bio-Rad Laboratories (USA). BAPTA/AM, membrane-permeable Ca^{2+} chelator was obtained from Molecular Probes Inc. (USA). Protein kinase and phosphatase inhibitors were obtained from Alexis Co. (Switzerland). The protein kinase and phosphatase inhibitors were stored at −20 °C as concentrated stock solutions. The substances diluted in DMSO were prepared as 10 mM stock in 100% DMSO and diluted with 10% DMSO to give appropriate final concentration of less than 0.1% of DMSO. Unless otherwise specified, all the other chemical substances were purchased from Sigma–Aldrich Co. (USA).

3. Results

3.1. Effect of protein phosphatase inhibitors

To check whether dephosphorylation of the cytosolic 28 kDa phosphoprotein (PP28) in *B. japonicum*, could be attributed to the activity of cellular protein phosphatases, we analyzed this aspect in vivo using two selected phosphatase inhibitors, okadaic acid and calyculin A. Each of these agents appeared to be a potent inhibitor of protein serine-threonine phosphatases (type 1 or type 2A) in all mammalian cells so far examined and highly selective inhibitors of type 1 phosphatase in *Paramecium tetraurelia* cells [42–46]. The results of a quantitative densitometric analysis of protein phosphorylation pattern in lysates from ciliates under different experimental conditions are presented in Fig. 1. These experiments indicated that 10 min treatment of ciliates with okadaic acid or calyculin A, consistently lowered protein phosphatase activity and overall levels of PP28 phosphorylation were enhanced (Fig. 1(a); lanes 3, 5 and 7) as compared with control (untreated) cells (Fig. 1(a); lane 1). The influence of these inhibitors was dose-dependent, as the phosphorylation levels of PP28 in cells treated

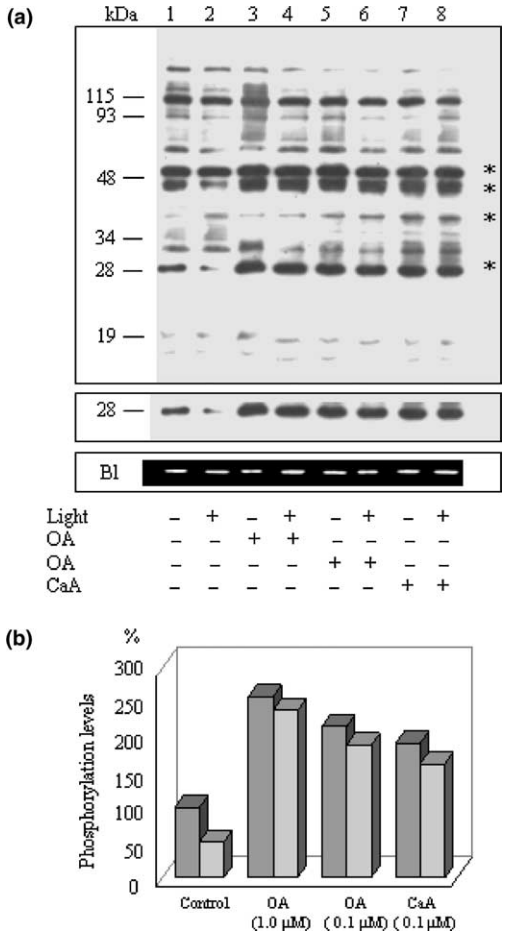

Fig. 1. The effects of okadaic acid (OA) and calyculin A (CaA) on in vivo phosphorylation of phosducin in the whole-cell homogenates from dark-adapted (filled bars) and light-stimulated *Blepharisma*s (open bars). (a) Immunoblotting analysis of phosducin phosphorylation in absence (−) or in presence (+) of light and/or indicated drugs; Bl – ciliate photoreceptor protein used as test for equal protein loadings. Lanes 1 and 2 – cells incubated in control solution without additions; cells incubated in control solution supplemented with 1 μM OA (lanes 3 and 4) or 0.1 μM OA (lanes 5 and 6) and with 0.1 μM CaA (lanes 7 and 8). Phosphoproteins present in the cell extracts that showed no significant changes in phosphorylation levels, under experimental condition tested are marked with asterisk. (b) Quantification diagrams of phosducin phosphorylation levels in cells under different experimental conditions. Data are shown as average values obtained for at least five separate experiments.

with 0.1 μM okadaic acid was doubled (Fig. 1(b); lane 5), while cell incubation with 1.0 μM for the same incubation period increased protein phosphorylation by about 150% (Fig. 1(b); lane 3) over that in control cells (Fig. 1(b); lane 1). Incubation of ciliates with 0.1 μM calyculin A for the same time also elicited enhancement of PP28 phosphorylation by almost 100% (Fig. 1(b); lanes 1 and 7). The other phosphoproteins present in tested cell lysate showed only small changes in their phosphorylation levels (marked with asterisks in Fig. 1(a)). In dark-adapted ciliates following application of okadaic acid or calyculin A, light stimulation elicited much weaker dephosphorylation of PP28 in comparison with that in non-treated ciliates (Fig. 1(a); lanes 2, 4, 6 and 8). Under these conditions, at 1 μM okadaic acid phosphorylation level of PP28 was less than 10% lower than in non-illuminated cells (Fig. 1(b); lanes 3 and 4). Slightly lower inhibitory effect on light-elicited PP28 dephosphorylation was observed in cells after treatment with calyculin A (Fig. 1(b); lane 8). Phosphorylation levels of PP28 were higher by about 50% in lysates from dark-adapted cells than those from cells exposed to light (Fig. 1(b); lanes 1 and 2).

3.2. Cyclic nucleotides-dependent phosphorylation

To evaluate the engagement of cyclic nucleotide-dependent protein kinases in phosphorylation of PP28 in tested ciliates we assayed in vivo phosphorylation levels in lysates from cells treated with 8-Br-substituted cyclic nucleotide analogs, 8-Br-cGMP and 8-Br-cAMP, and a non-specific phosphodiesterase (PDE) activity inhibitor, IBMX. The lipophilic analogs of cAMP and cGMP have considerable high cell membrane permeability and are relatively poorly hydrolyzed by cellular PDEs [47–49]. The IBMX is widely known to effectively raise intracellular cyclic nucleotide levels as a result of inhibition of cyclic nucleotide degradation by PDEs [50–52]. This line of experiments showed that PP28 phosphorylation levels were substantially influenced by these substances in tested lysates obtained from cells adapted to darkness (Fig. 2). This is clearly evidenced by an increase in PP28 phosphorylation level by about 75% at micromolar concentration of IBMX (Fig. 2(b); lane 3) and more than 2-fold elevation of phosphorylation levels at millimolar concentrations of both cyclic nucleotide analogs (Fig. 2(b); lanes 5 and 7) over that in control cells. However cell treatment with either cyclic nucleotide analogs or IBMX exerted little effect on PP28 light-dependent dephosphorylation event (Fig. 2(b); lanes 2, 4, 6 and 8). Under light conditions, phosphorylation levels of PP28 in control cells and those treated with cyclic nucleotide analogs or IBMX were between 50% and 60% of that measured in dark-adapted (control) cells (Fig. 2(b); lanes 2, 4, 6 and 8). These observations suggest that PP28, which is intensely

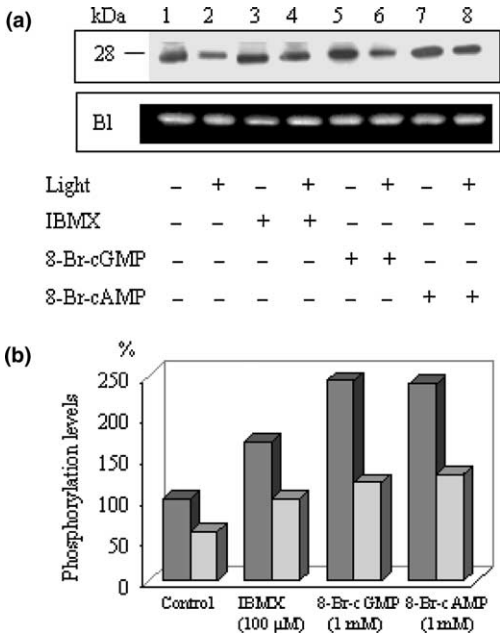

Fig. 2. The cyclic nucleotide-dependent phosphorylation in vivo of phosducin in cell homogenates. (a) Immunoblotting assay and (b) quantification diagrams of phosducin phosphorylation. Lanes 1 and 2 – cells incubated in control solution; cells incubated in control solution with additions of 100 μM IBMX (lanes 3 and 4), 1 mM 8-Br-cGMP (lanes 5 and 6), 1 mM 8-Br-cAMP (lanes 7 and 8). Other details are as in Fig. 1.

phosphorylated upon cell treatment with IBMX or both cyclic nucleotide analogs, represents a substrate of the cAMP-dependent protein kinase (PKA) or cGMP-dependent protein kinase (PKG) but the dephosphorylation process seems to be independent of cyclic nucleotides.

To test whether these kinases are indeed involved in the phosphorylation process of PP28 in tested ciliates, we used two highly specific inhibitors of PKG and PKA, KT 5823 and H 89, respectively. As shown in Fig. 3, incubation of dark-adapted cells for 10 min with 1 μM KT 5823 resulted in a decrease in phosphorylation level of PP28 by 25% compared to the control (Fig. 3; lanes 1 and 3). A marked dose-dependent reduction in the phosphorylation state of PP28 was also observed following treatment of dark-adapted cells by H 89. In cells exposed to this inhibitor at concentrations of 1 or 10 μM the levels of PP28 phosphorylation were suppressed by 32% and 55%, respectively (Fig. 3; lanes 5 and 7). Illumination of cells adapted to the applied protein kinase inhibitors induced stronger PP28 dephosphorylation by 27–44% of that in cells under control conditions (Fig. 3; lanes 4, 6 and 8).

3.3. Calcium- and calmodulin-dependent phosphorylation

To assess the role of Ca^{2+} on phosphorylation of PP28, we analyzed in vivo the phosphorylation pattern of the phosphoprotein in lysates from control ciliates

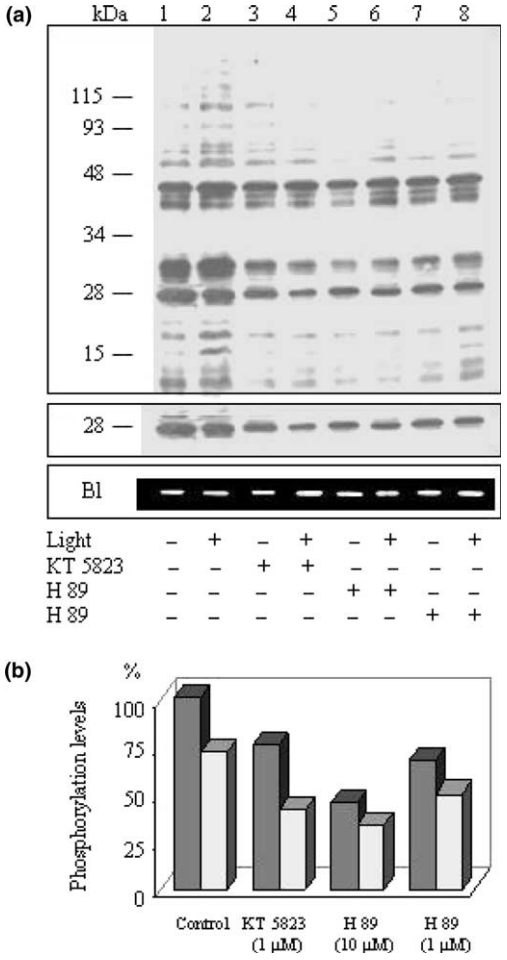

Fig. 3. The effect of specific inhibitors of cGMP-dependent protein kinase (PKG), KT 5823 and cAMP-dependent protein kinase (PKA), H 89 on in vivo phosphorylation of phosducin. Lanes 1 and 2 – ciliates adapted to control solution; cells incubated in control solution supplemented with 1 μM KT 5823 (lanes 3 and 4) and 10 μM (lanes 5 and 6) or 1 μM H89 (lanes 7 and 8). For other details see Fig. 1.

and cells adapted to control solutions supplemented with higher concentration of Ca^{2+} alone or with addition of lipophilic ionophore A23187. It translocates Ca^{2+} across biological membranes and tends to equilibrate the free concentrations of this cation on both sides of the cell membrane, i.e., in the cytoplasmic and extracellular compartments. These experiments showed that dark-adapted cells kept at 4 mM Ca^{2+} for 10 min revealed an increase in phosphorylation state of PP28 by about 20%. Application of ionophore A23187 at concentration of 2 μM in the presence of 4 mM Ca^{2+} intensified the effect of Ca^{2+} causing almost 2-fold increase in phosphorylation level of PP28 (Fig. 4; lanes 5 and 7). As expected, an opposite effect was observed in cells exposed to Ca^{2+} chelators, such as EGTA or BAPTA (Fig. 5). Incubation of cells with 1 μM or 10 μM BAPTA/AM and 2 mM EGTA for 10 min reduced the initial levels of phosphoprotein by 38% or 45%, respectively

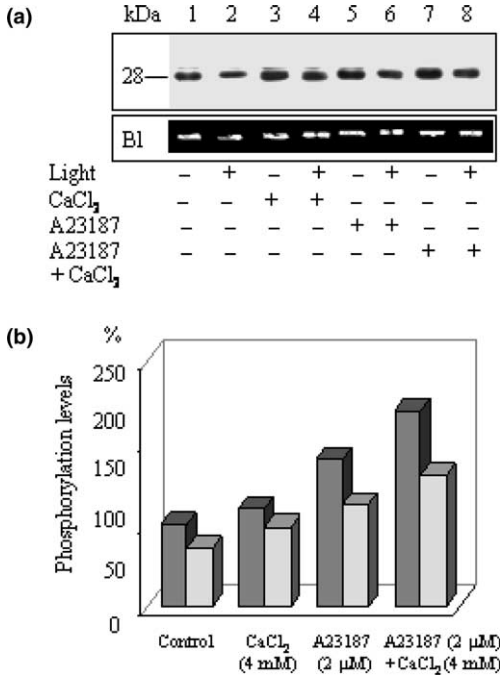

Fig. 4. The Ca^{2+}-dependent phosphorylation in vivo of ciliate phosducin. Lanes 1 and 2 – ciliates adapted to control solution; ciliates adapted to control solution with additions of 4 mM $CaCl_2$ (lanes 3 and 4), 2 μM A23187 (lanes 5 and 6) and mixture of 2 μM A23187 with 4 mM $CaCl_2$ (lanes 7 and 8). For other details see Fig. 1.

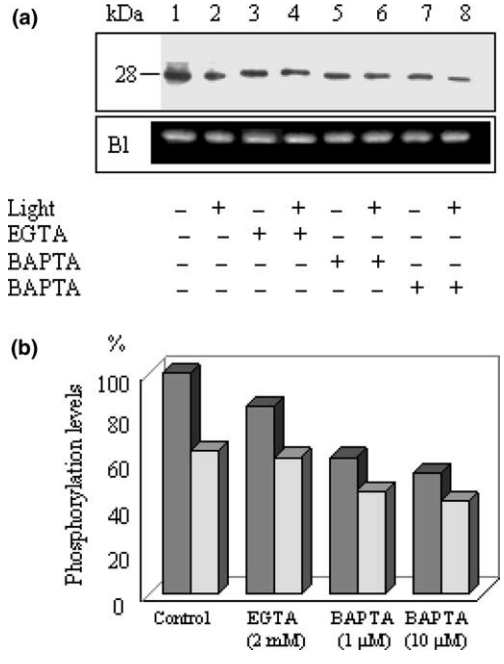

Fig. 5. Modulation of phosphorylation of phosducin by EGTA and BAPTA/AM. Lanes 1 and 2 – ciliates adapted to control solution; ciliates adapted to control solution with additions of 2 mM EGTA (lanes 3 and 4) and 1 μM BAPTA/AM and 2 mM EGTA (lanes 5 and 6) or 10 μM BAPTA/AM and 2 mM EGTA (lanes 7 and 8). For other details see Fig. 1.

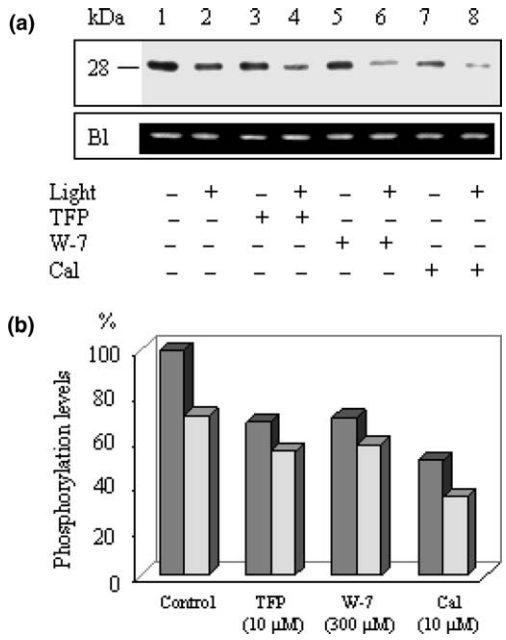

Fig. 6. Modulation of phosphorylation of ciliate phosducin by selected calmodulin antagonists. Lanes 1 and 2 – ciliates incubated in control solution; ciliates incubated in control solution supplemented with 10 μM TFP (lanes 3 and 4), 300 μM W-7 (lanes 5 and 6) or 10 μM calmidazolium (lanes 7 and 8). For other details see Fig. 1.

(Fig. 5; lanes 1, 3 and 5). Slightly weaker, 15% decrease in PP28 phosphorylation was observed in ciliates treated for the same incubation time with 2 mM of EGTA (Fig. 5; lane 3). In light-adapted cells, phosphorylation of PP28 was about half of that measured in cells under dark conditions (Fig. 5; lane 2).

To evaluate the influence of calmodulin on PP28 phosphorylation calmodulin antagonists, TFP, W-7 and calmidazolium were applied to ciliates via bath additions. As seen in Fig. 6, calmodulin antagonists decreased PP28 phosphorylation in dark-adapted ciliates by about 30–50% in relation to control (Fig. 6; lanes 1, 3, 5 and 7). Pretreatment of cells with calmodulin antagonists influenced dephosphorylation of PP28 only slightly indicating low effect of used substances on phosphatase activity (Fig. 6; lanes 4, 6 and 8). The differences in phosphorylation level of PP28 in dark-adapted and light-stimulated ciliates in the presence of calmodulin antagonist at the same incubation times and micromolar concentrations are between 20% and 30%.

4. Discussion

We have recently reported that ciliate protist *B. japonicum* possesses phosphoprotein (PP28), phosducin localized within the cell cytoplasm [31]. This ciliate phosphoprotein exists, as in the case of other photoreceptor cells [9], in highly phosphorylated form in dark-adapted cells and cell illumination eliciting motile photophobic response results in distinct dephosphorylation of the PP28. Our results of an in vivo phosphorylation assay presented in this study showed that the phosphorylation events are significantly influenced by protein phosphatase and kinase modulators. Both okadaic acid and calyculin A, well known as the inhibitors of type 1 and type 2A protein phosphatases [44,53,54] applied in micromolar concentrations increase in a great measure the PP28 phosphorylation levels in dark-adapted as well as light-exposed organisms compared with protein phosphorylation in control (untreated) cells (Fig. 1). The results of these experiments indicated that the cell endogenous kinases responsible for PP28 phosphorylation, seem to be active under both dark and light conditions and that protein dephosphorylation may occur by the endogenous protein phosphatase function, as it was reported for phosducins of vertebrate photoreceptor cells [16,55].

In addition, as shown in Figs. 4 and 5, the degree of phosducin phosphorylation in ciliates is controlled to some extent by cellular Ca^{2+} concentrations. Incubation of dark-adapted ciliates with higher Ca^{2+} concentration in the presence of A23187 resulted in marked increase in phosphorylation level of the ciliate phosducin (Fig. 4). As expected, an opposite effect was observed in cells treated with Ca^{2+} chelators, EGTA and BAPTA/AM (Fig. 5(b); lanes 3–8). The dephosphorylation level of PP28 by light was not essentially affected by incubation of cells with higher concentration of external Ca^{2+}. These data evidence that enhanced phosphorylation of PP28 occurring in dark-adapted cells may be mediated by one of the Ca^{2+}–calmodulin-dependent kinases. This interpretation is supported by the results of experiments with use of specific calmodulin antagonists, such as TFP, W-7 and calmidazolium [56]. All these pharmacological agents decreased the protein phosphorylation in dark-adapted cells, however, failed to inhibit the protein dephosphorylation in light-stimulated cells (Figs. 5 and 6). Since dephosphorylation of the ciliate phosducin by light was not influenced by Ca^{2+} chelation with EGTA and BAPTA/AM as well by specific calmodulin antagonists, the involvement of the Ca^{2+}–calmodulin-dependent phosphatase in PP2B phosphorylation changes seems to be ruled out [44].

Several previous studies suggested that phosphorylation of phosducins in photoreceptor cells of different organisms may occur by an activation of protein kinase A as a result of increase in cellular cAMP [8,10,12,55]. The data reported here show that phosducin from the tested ciliate protist could also be phosphorylated in a cyclic nucleotide-dependent fashion. The cyclic nucleotide analogs, 8-Br-cAMP and 8-Br-cGMP as well as IBMX, the compounds that are expected to elevate the cellular levels of cyclic nucleotides, significantly increased phosphorylation of PP28 in ciliates kept in

darkness, while low dephosphorylation effect was observed in cells following illumination (Fig. 2(b); lanes 4, 6 and 8). These findings may indicate that the ciliate phosducin represents substrate for protein kinase A (PKA) or/and G protein kinase (PKG). This supposition is evident from the experiments with application of H 89 and KT 5823, highly specific inhibitors of PKA and PKG, respectively. These data conclusively indicate that both protein kinases may operate in tested ciliate, since a dose-dependent reduction in the levels of PP28 phosphorylation was observed after treatment of dark- and light-adapted cells with these agents (Fig. 3; lanes 4, 6 and 8). Some difference in inhibitory effect of KT 5823 and H 89 on the levels of PP28 phosphorylations in cells exposed to darkness or light suggest that PKG may phosphorylate ciliate phosphoprotein under light conditions more effectively. Although phosducin derived from bovine rod outer segments is not noted to be phosphorylated by PKG [57,58], however phosducin obtained from nuclei of bovine retinal cells exhibits phosducin phosphorylation by this kinase type [59]. It has been deduced from purified and sequenced bovine retinal phosducin that potential phosphorylation sites on this phosphoprotein are available for protein kinases A and G, as well for protein kinase C and casein kinase II [21].

In conclusion, the results reported here together with other recent reports [30,31] showed that phosducin in *B. japonicum* exists in highly phosphorylated state under darkness. This phosphoprotein is possibly phosphorylated by the endogenous cyclic nucleotide-dependent protein kinases similar to those phosphorylating vertebrate phosducin [8,10,59,60]. Phosphorylation of PP28 revealed also distinct dependence on calcium and calmodulin activity. Ciliate phosducin undergoes rapid dephosphorylation when cells are exposed to light intensity eliciting motile photophobic response. The observed light-induced dephosphorylation of PP28 during photophobic response may occur by an activation of cellular protein phosphatases as shown for vertebrate phosducins [16,55]. On the basis of presented data, we propose for the first time for ciliate protists that endogenous light-dependent protein kinase-phosphatase system might be involved in the control of light signal transduction in *B. japonicum*. A complete clarification of a role of this system in the cell motile photophobic events awaits continuation of studies in this lower eukaryote.

5. Abbreviations

8-Br-cAMP	cyclic AMP analog
8-Br-cGMP	cyclic GMP analog
BAPTA/AM	1,2-bis(o-amino-5-bromophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid
BSA	bovine serum albumin
EGTA	ethylenebis(oxyethylenenitrilo)tetraacetic acid
H 89	PKA activity inhibitor
IBMX	3-isobutyryl-1-methylxanthine
KT 5823	PKG activity inhibitor
PKA	cAMP-dependent protein kinase
PKG	cGMP-dependent protein kinase
PDE	cyclic nucleotide phosphodiesterase
PMSF	phenylmethylsulfonylfluoride
TFP	trifluoperazine

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