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REVIEW

Mechanisms of the 14-3-3 Protein Function: Regulation of Protein Function Through Conformational Modulation

V. OBSILOVA[1], M. KOPECKA[1], D. KOSEK[1,2], M. KACIROVA[1,2], S. KYLAROVA[1,2], L. REZABKOVA[1,2], T. OBSIL[1,2]

[1]Department of Protein Structure, Institute of Physiology Academy of Sciences of the Czech Republic, Prague, Czech Republic, [2]Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Prague, Czech Republic

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Summary

Many aspects of protein function regulation require specific protein-protein interactions to carry out the exact biochemical and cellular functions. The highly conserved members of the 14-3-3 protein family mediate such interactions and through binding to hundreds of other proteins provide multitude of regulatory functions, thus playing key roles in many cellular processes. The 14-3-3 protein binding can affect the function of the target protein in many ways including the modulation of its enzyme activity, its subcellular localization, its structure and stability, or its molecular interactions. In this minireview, we focus on mechanisms of the 14-3-3 protein-dependent regulation of three important 14-3-3 binding partners: yeast neutral trehalase Nth1, regulator of G-protein signaling 3 (RGS3), and phosducin.

Key words

14-3-3 protein • Regulator of G-protein signalling (RGS3) • Phosducin • Neutral trehalase • Bmh1 • Conformation • Signaling

Corresponding authors

T. Obsil, Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Hlavova 2030, 12843 Prague, Czech Republic. E-mail: obsil@natur.cuni.cz and
V. Obsilova, Institute of Physiology, Academy of Sciences of the Czech Republic, v.v.i., Videnska 1083, 14220 Prague, Czech Republic. E-mail: obsilova@biomed.cas.cz

Introduction

The 14-3-3 proteins are a family of highly conserved acidic 30 kDa molecules that form stable homo- and heterodimers (Liu *et al.* 1995, Xiao *et al.* 1995). Each monomer consists of a bundle of nine anti-parallel α-helices and the 14-3-3 protein dimer possesses a characteristic cup-like shape with a large central channel containing two amphipathic binding grooves (Fig. 1). The inner walls of the central channel and the dimer interface are form by conserved residues whereas the less conserved residues are located on the outer convex surface. The extensive interactions between α-helices make the structure of the 14-3-3 protein dimer highly rigid and only small differences between apo- and ligand-bound forms were observed (reviewed by Obsil and Obsilova 2011). This structural rigidity suggests that the 14-3-3 protein can behave as a rigid platform on which the bound target protein can be reshaped (Yaffe 2002). The most flexible region of the 14-3-3 protein molecule is the C-terminal segment which also exhibits highest sequence variability among the 14-3-3 isoforms. This region, due to its flexibility and the presence of negatively charged residues, has been shown to play an important role in the regulation of binding properties of the 14-3-3 protein isoforms (Truong *et al.* 2002, Obsilova *et al.* 2004, Silhan *et al.* 2004, Veisova *et al.* 2010).

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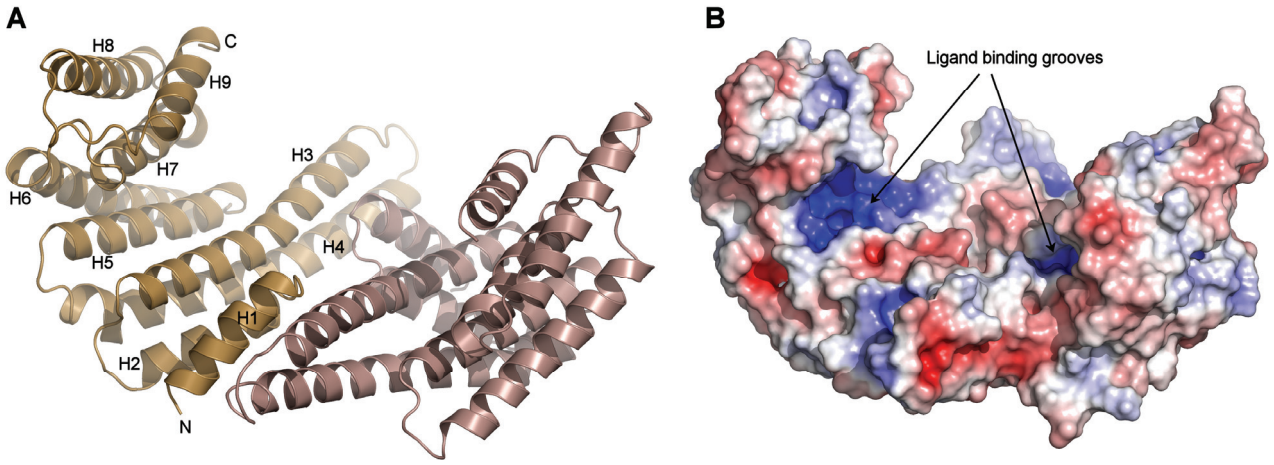

Fig. 1. Structure of the 14-3-3 protein. **(A)** The ribbon representation of the crystal structure of the 14-3-3ζ protein dimer (Obsil *et al.* 2001). Each monomer is colored in different color. Helices of one monomer are labeled H1-H9. **(B)** Molecular surface of the 14-3-3ζ protein dimer. The surface is colored according to the electrostatic potential, ranging from –6 kT/e (red) to +6 kT/e (blue). The electrostatic potential was calculated using APBS package (Baker *et al.* 2001) and the figure was created using PyMOL (www.pymol.org). Arrows indicate the position of the ligand binding grooves.

The 14-3-3 proteins specifically recognize phosphoserine/phosphothreonine-containing motifs (Muslin *et al.* 1996). Three types of consensus 14-3-3 binding motifs were identified: R[S/Φ][+]pSXP (mode I), RX[Φ/S][+]pSXP (mode II), and pS-X_{1-2}-COOH (motif III) where pS is phosphoserine (the phosphorylated residue may also be a threonine), Φ is an aromatic residue, + is a basic residue, and X is any type of residue (Yaffe *et al.* 1997, Rittinger *et al.* 1999, Ganguly *et al.* 2005). These motifs are not absolute as a number of the 14-3-3 protein binding partners identified to date contain either phosphorylated or even not-phosphorylated sequences that significantly differ from these optimal motifs (Masters *et al.* 1999, Wang *et al.* 1999, Johnson *et al.* 2010).

The mechanism of the 14-3-3 protein function can be generally classified into three modes of action: (1) direct conformational change of the target protein; (2) physical occlusion of sequence-specific or structural features; and (3) scaffolding that anchors proteins within close proximity of one another (reviewed by Fu *et al.* 2000, Tzivion and Avruch 2002, Bridges and Moorhead 2004, Obsil and Obsilova 2011). The exact mechanisms behind these modes are still mostly elusive, mainly due to the absence of high-resolution structures of the corresponding 14-3-3 protein complexes. This minireview focuses on mechanisms of the 14-3-3 protein-dependent regulation of three important 14-3-3 binding partners studied in our laboratory: (i) the yeast neutral trehalase Nth1; (ii) the regulator of G-protein signaling 3 (RGS3); and (iii) phosducin (Pdc). While Nth1 is an enzyme that catalyzes the hydrolysis of trehalose and its 14-3-3-dependent regulation seems to be an example of mode 1 mechanism (direct structural change), RGS3 and Pdc are proteins involved in the regulation of G-protein signaling and both of them seem to be regulated by a mechanism that includes both modes 1 and 2 (structural change accompanied by physical occlusion of the binding surface).

The 14-3-3 protein-dependent activation of yeast neutral trehalase Nth1

The neutral trehalase Nth1 belongs to the Glycoside hydrolase family 37 (EC 3.2.1.28) of O-Glycosyl hydrolases (EC 3.2.1.) which includes enzymes with the mutual trehalase activity (App and Holzer 1989, Kopp *et al.* 1993). Trehalase hydrolyzes disaccharide trehalose [α-D-glucopyranosyl-(1-1)-α-D-glucopyranoside] into two glucose subunits monitoring the stable trehalase concentration by that way. Trehalose, as a non-reducing disaccharide of glucose functions as a storage carbohydrate and stress metabolite because its concentration increases during various adverse environmental conditions like dehydration, heat, cold, oxidation and desiccation. In yeast and plants it also may serve as a regulatory or signaling molecule, to control certain metabolic pathways or even to affect growth (Elbein *et al.* 2003). It has been shown that in the yeast *Saccharomyces cerevisiae*, Nth1 can be fully activated by the cAMP-dependent protein kinase (PKA) phosphorylation, Ca^{2+} ions and yeast 14-3-3 protein binding (Panni *et al.* 2008, Veisova *et al.* 2012).

Bmh1 and Bmh2 (yeast 14-3-3 protein isoforms), share a great degree of homology and participate in the regulation of crucial physiological processes (van Heusden and Yde Steensma 2006, Veisova *et al.* 2010). Many interaction partners of the 14-3-3 proteins are phosphorylated on multiple amino acid residues and very often two phosphorylated motifs can be simultaneously involved in the interaction (Yaffe 2002). Nth1 is phosphorylated by PKA on multiple sites and phosphorylated Nth1 (pNth1) then interacts with Bmh proteins leading to the enhancement of pNth1 activity through an unknown mechanism (Panni *et al.* 2008). The identity and the role of individual PKA phosphorylation sites remain unclear. Thevelein group has recently shown using phosphospecific antibodies that glucose and nitrogen activation of trehalase *in vivo* is associated with phosphorylation of Ser21 and Ser83 (Schepers *et al.* 2012). It must be noted, however, that in this study phosphospecific antibodies just for pSer21 and pSer83 were used because the anti-pSer20 and anti-pSer60 couldn´t properly recognize between phosphorylated and not-phosphorylated forms of Nth1. Additional regulatory mechanism (beyond Bmh stimulation) for activation of trehalase *in vivo* have also been identified (De Mesquita *et al.* 2003). Dcs1, an mRNA decapping enzyme, acts as the negative regulator of trehalase activity and thus prevents Nth1 from the 14-3-3 protein binding. The deletion of the inhibitor Dcs1 causes the activation and phosphorylation of Nth1 but the deletion of both Bmh1 and Bmh2 abolishes the Nth1 phosphorylation and activation (Schepers *et al.* 2012).

Our group has recently shown by MALDI-TOF MS analysis that four serine residues from the disordered N-terminal part of yeast Nth1 (Ser20, Ser21, Ser60 and Ser83) are phosphorylated by PKA *in vitro* (Veisova *et al.* 2012). Consequent biochemical characterization of the 14-3-3 protein-dependent activation of pNth1 showed that both yeast 14-3-3 isoforms form stable complexes with pNth1 (with the molar stoichiometry 2:1) and substantially increase its enzymatic activity. Site-directed mutagenesis revealed that phosphorylation sites Ser60 and Ser83 are essential for pNth1 activation both *in vitro* and *in vivo* and very likely function as the 14-3-3 binding motifs (Fig. 2A). In addition, it has previously been suggested that Ca^{2+} ions form the indivisible component of the active trehalase (Franco *et al.* 2003). However, we observed much weaker activation of pNth1 in the presence of Ca^{2+}-only compared with the 14-3-3-dependent activation (Veisova *et al.* 2012).

In order to obtain structural insight into the mechanism of the 14-3-3-dependent activation of pNth1, we performed a biophysical study of the Bmh1-pNth1 complex using hydrogen/deuterium exchange coupled to mass spectrometry (HDX-MS) and circular dichroism spectroscopy (CD) (Macakova *et al.* 2013). The HDX-MS was used to characterize the binding interface between these two proteins and we showed that the Bmh1 protein binding to pNth1 affects the structural properties of several Nth1 regions: the N-terminal segment containing phosphorylation sites essential for pNth1 binding to Bmh, the region 102-185 containing the Ca^{2+}-binding domain (including peptide 102-110 whose change in deuteration kinetics upon the Bmh1 binding is shown in Figure 2B), and several regions from the catalytic trehalase domain. For structural interpretation of HDX-MS data we mapped the Bmh1-induced changes in the deuteration kinetics of pNth1 on the structural model of its catalytic domain (sequence 295-721). Peptides for which we observed significant changes in their deuteration kinetics upon the Bmh1 binding are shown in Figure 2C in orange and, as can be noticed, these peptides surround the active site of the catalytic trehalase domain. In addition, CD spectroscopy revealed that the complex formation is accompanied by change in the protein's tertiary structure. On the basis of these data, we proposed a model depicting the possible mechanism of the 14-3-3 protein-dependent activation of pNth1 (Fig. 2A). The active site of Nth1 in the absence of Bmh is buried within the structure of the trehalase domain and the enzyme is catalytically inactive. Interaction between pNth1 and Bmh induces a conformational change within both the Ca^{2+}-binding and the catalytic trehalase domains in the close proximity of the active site. We can speculate that this structural change increases the accessibility of the active site and leads to the pNth1 activation (Macakova *et al.* 2013). Our data also show that the interaction surface of the Bmh1 molecule in the Bmh1-pNth1 complex includes not only the surface of the ligand binding groove, where the phosphorylated N-terminal segment of pNth1 binds, but also the surfaces outside the central cavity of the Bmh1 dimer.

Thus, our proposed model for the 14-3-3 protein-dependent activation of pNth1 seems to be an example of mode 1 mechanism and resembles the 14-3-3 protein-dependent activation of serotonin N-acetyltransferase (Obsil *et al.* 2001). In that case the 14-3-3 protein also enhances the enzymatic activity by binding to the phosphorylated form of the enzyme and inducing a structural change in close proximity of its active site but remote from the phosphorylated 14-3-3 binding motif.

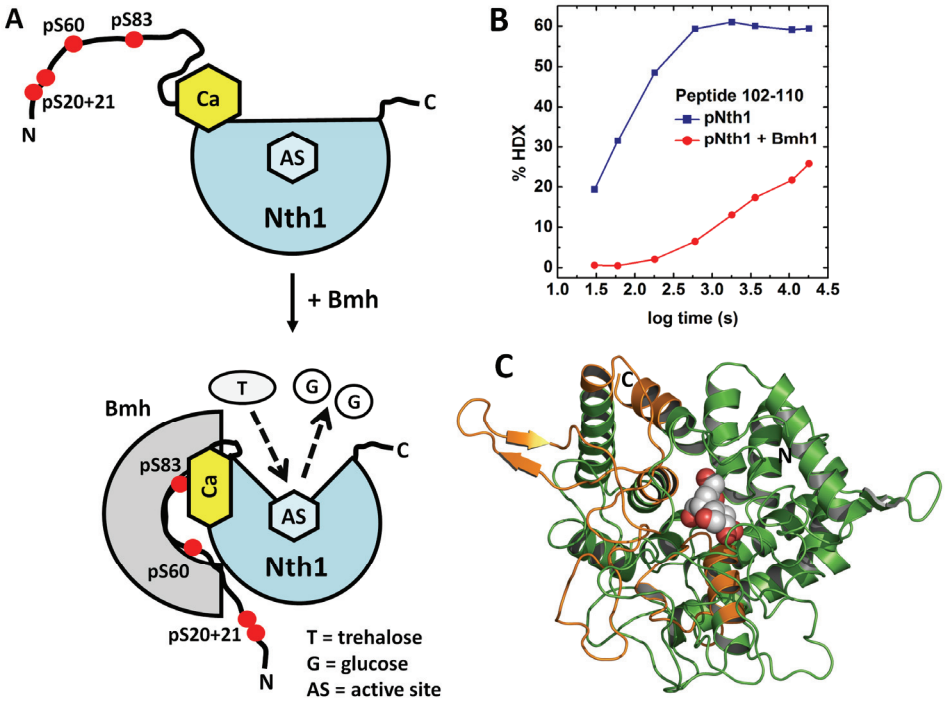

Fig. 2. Proposed mechanism for the Bmh-dependent activation of Nth1. **(A)** The active site of Nth1 in the absence of Bmh is buried within the structure of the trehalase domain and the enzyme is catalytically inactive (Macakova *et al.* 2013). Phosphorylated Nth1 is recognized by Bmh protein (yeast 14-3-3 isoform) and its binding to the N-terminal pSer60 and pSer83 induces conformational change within both the Ca^{2+}-binding domain and the catalytic domain in close proximity to the active site. This structural change enables substrate and product entry and departure, respectively, hence the enzyme activation. AS denotes the active site, T and G denote trehalose and glucose, respectively, serines that are phosphorylated by PKA are shown as red circles. The Ca^{2+}-binding domain, the catalytic trehalase domain and the Bmh protein are shown in yellow, cyan and grey, respectively. **(B)** The Bmh1-dependent decrease in the deuterium exchange kinetics for the peptide 102-110 that surrounds the calcium binding domain. The deuteration kinetics is expressed as percentages relative to the maximum theoretical deuteration level for pNth1 alone (blue circles) and pNth1 in the presence of Bmh1 (red circles) after 10 minutes of deuteration. Time units are in seconds. **(C)** Homology model of the catalytic trehalase domain of Nth1 (sequence 295-721). Regions that show slower deuterium exchange kinetics upon the Bmh1 binding are shown in orange. The active site contains trehalase inhibitor validoxylamine (shown as spheres) based on the crystal structure of trehalase Tre37A (PDB code 2JF4) used as a template to build this homology model (Gibson *et al.* 2007).

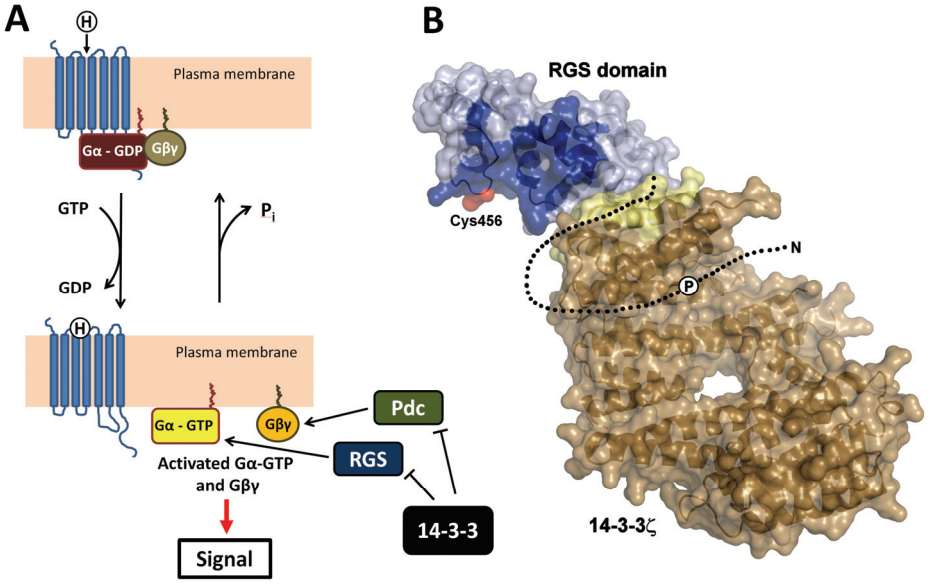

Fig. 3. Role of RGS3 and Pdc in the regulation of G-protein signaling. **(A)** Ligand binding, H, to the G-protein coupled receptor (GPCR, shown in blue) induces a conformational change in the receptor, which is transduced to the inactive heterotrimeric G-protein (Gαβγ). This enables the exchange of GDP for GTP on the Gα subunit and the dissociation of now active Gα and Gβγ subunits (shown in yellow and orange, respectively) that can mediate downstream signals. The amplitude and duration of the signal is dependent on the rate of activation as well as the rate of inactivation through GTP hydrolysis. This process is accelerated by RGS proteins that increase the rate of GTP hydrolysis, thus returning the G-protein to its heterotrimeric GDP-bound inactive state (Jean-Baptiste *et al.* 2006). Pdc, on the other hand, regulates visual signal transduction by modulating the amount of transducin $G_t\alpha\beta\gamma$ heterotrimer through competition with the $G_t\alpha$ subunit for binding to the $G_t\beta\gamma$ complex (Bauer *et al.* 1992, Lee *et al.* 1987). Both RGS3 and Pdc are inhibited by means of the 14-3-3 protein binding. **(B)** The low-resolution solution structure of the 14-3-3ζ-RGS3 complex (Rezabkova *et al.* 2011). The 14-3-3ζ protein and the RGS domain of RGS3 are shown in brown and light blue, respectively. The Gα interaction surface of the RGS domain is shown in dark blue. The less conserved regions on the outer surface of the 14-3-3ζ dimer outside its central channel involving helices H6 and H8 that participate in interaction with RGS3 are shown in light yellow. Residue Cys456 is shown in red.

The 14-3-3 protein-dependent inhibition of the regulator of G-protein signaling 3 (RGS3)

The amplitude and duration of G-protein signaling are regulated at the G-protein level by two different mechanisms. The first one involves the enhancement of the low intrinsic GTPase activity of the Gα subunit (Berman *et al.* 1996, Watson *et al.* 1996), whereas the second one is based on the inhibition of Gβγ interactions with Gα-GDP and downstream effectors (Lee *et al.* 1988, Willardson *et al.* 1996) (Fig. 3A). The first mechanism is carried out by a highly conserved regulator of G-protein signaling (RGS) domain of RGS proteins that specifically recognizes the GTP-bound form of Gα and function as GTPase-activating proteins (GAP) for Gα by stabilizing the transition state (Hepler *et al.* 1997, Tesmer *et al.* 1997). The RGS domain consists of ~125 amino acid residues and more than 25 proteins containing RGS or RGS homology domains have been identified to date. Some RGS proteins consist of little more that the RGS domain (e.g. RGS1, RGS2, RGS4) while others possess long N-terminal or C-terminal extensions (e.g. RGS3, RGS7, RGS9) that usually contain additional protein-protein interaction motifs and domains (Ishii and Kurachi 2003). Through these non-RGS regions RGS proteins can also serve many non-canonical functions distinct from inactivation of Gα (reviewed by Abramow-Newerly *et al.* 2006b, Sethakorn *et al.* 2010).

Various mechanisms are involved in the regulation of RGS functions including the post-translational modifications and the interaction with other signaling proteins (Kim *et al.* 1999, Schiff *et al.* 2000, Roy *et al.* 2003). Certain RGS proteins, including RGS3-5, RGS7, RGS8, and RGS16, have been found to interact with various 14-3-3 isoforms (Benzing *et al.* 2000, 2002, Schreiber *et al.* 2001, Niu *et al.* 2002, Abramow-Newerly *et al.* 2006a). Some of these studies showed that the 14-3-3 protein binding inhibits GAP function of, at least, RGS4, RGS7 and RGS16 presumably by blocking RGS-Gα interaction through unknown mechanism (Benzing *et al.* 2000, Abramow-Newerly *et al.* 2006a). Two different 14-3-3 binding sites have been identified on RGS3 and RGS7. Benzing *et al.* (2000, 2002) suggested that the 14-3-3 binding site is located within the RGS domain at a conserved Ser-Tyr-Pro motif. However, other studies showed that Ser^{264} of RGS3 located outside of its RGS domain serves as the primary 14-3-3 binding site (Niu *et al.* 2002, Ward and Milligan 2005). This residue is presumably phosphorylated by PKA, and its sequence (R^{259}RRTH<u>S</u>EG^{266}) is consistent with the type II binding motif for 14-3-3 (Yaffe *et al.* 1997). In addition, while phosphorylation of RGS3 and RGS7 has been shown to be required for their binding to 14-3-3 (Benzing *et al.* 2000, 2002), it seems that RGS4, RGS5 and RGS16 can interact with 14-3-3 in a phosphorylation-independent manner (Abramow-Newerly *et al.* 2006a).

RGS3 belongs to a B/R4 subfamily of RGS proteins and it has been shown that their interacting partners within GPCR pathways are $G\alpha_i$, $G\alpha_q$ and $G\beta_1\gamma_2$ (Shi *et al.* 2001, 2002). While most members of this subfamily are relatively small and consist of only the RGS domain flanked by short N- and C-termini, two isoforms (splice variants) of RGS3, called RGS3L and PDZ-RGS3, contain large N-terminal extension (Kehrl *et al.* 2002). Niu *et al.* (2002) showed that the 14-3-3 protein binding to RGS3 phosphorylated at Ser^{264} within this N-terminal extension blocks the interaction between RGS3 and G-proteins. To investigate the mechanism by which 14-3-3 interferes with the RGS3-G-protein interaction, we used several biophysical methods to obtain structural insight into this interaction. The time-resolved tryptophan fluorescence of two RGS3 mutants containing single-Trp residue either at position 295 (within the N-terminal extension close to the phosphorylation site Ser^{264}) or at position 424 (within the RGS domain) revealed that the 14-3-3ζ binding induces significant structural changes in the vicinity of both these residues (Rezabkova *et al.* 2010). This suggested that not only the region containing the phosphorylated 14-3-3 binding motif but also the remote C-terminally located RGS domain physically interact with the 14-3-3 dimer and/or undergoe a structural change upon the 14-3-3ζ-RGS3 complex formation. Since Trp^{424} is located in the center of the RGS domain close to its Gα interaction surface, it is reasonable to speculate this 14-3-3 protein binding-dependent structural change within the RGS domain could be responsible for the inhibition of RGS3-Gα interaction.

To obtain a more detailed structural insight into the interaction between 14-3-3ζ and RGS3, we next used small angle x-ray scattering (SAXS), hydrogen/deuterium exchange kinetics (HDX-MS), and Förster resonance energy transfer measurements to determine the low-resolution solution structure (hybrid structure) of the 14-3-3ζ–RGS3 complex (Rezabkova *et al.* 2011). This

structural model revealed that the RGS domain of RGS3 interacts with less conserved regions on the outer surface of the 14-3-3ζ dimer outside its central channel involving helices H6 and H8, thus providing a possible mechanistic explanation for the 14-3-3-dependent inhibition of RGS3 GAP function (Fig. 3B). The model suggests that the 14-3-3ζ protein binding sterically occludes the RGS3-Gα complex formation due to its binding in close proximity to the Gα binding interface of the RGS domain. In addition, the time-resolved AEDANS fluorescence measurements confirmed the results of tryptophan fluorescence experiments and showed that the 14-3-3ζ protein binding changes the structure of the RGS domain within its Gα-interacting portion (Rezabkova *et al.* 2011). The most significant 14-3-3ζ binding-induced change in fluorescence was observed for AEDANS-labeled Cys^{456} located directly within the Gα interaction surface of the RGS domain (Fig. 3B). Thus, our results suggest that the 14-3-3ζ protein binding affects the structure of the Gα interaction portion of RGS3 as well as sterically blocks the interaction between the RGS domain and the Gα subunit of heterotrimeric G-proteins.

The 14-3-3 protein-dependent regulation of phosducin

Phosducin (Pdc) is a highly conserved acidic phosphoprotein involved in the regulation of visual signal transduction, the transcriptional control, the regulation of transmission at the photoreceptor-to-ON-bipolar cell synapse, and the modulation of sympathetic activity and blood pressure (Lee *et al.* 1987, Bauer *et al.* 1992, Zhu and Craft 2000, Beetz *et al.* 2009, Herrmann *et al.* 2010). The best understood role of Pdc is the regulation of visual signal transduction by modulating of the amount of transducin $G_t\alpha\beta\gamma$ heterotrimer through competition with the $G_t\alpha$ subunit for binding to the $G_t\beta\gamma$ complex (Fig. 3A) (Lee *et al.* 1987, Bauer *et al.* 1992). The crystal structure of the Pdc-$G_t\beta\gamma$ complex showed that Pdc consists of two separate domains that wrap around $G_t\beta\gamma$ to form an extensive binding interface (Gaudet *et al.* 1996). The N-terminal domain seems to be highly flexible and partly disordered while the C-terminal domain possesses the thioredoxin-like fold. The stability of the Pdc-$G_t\beta\gamma$ complex is strongly dependent on the phosphorylation state of Pdc molecule as the phosphorylation of several serine residues within its N-terminal domain reduces its binding affinity through a mechanism that is still unclear (Lee *et al.* 2004). The *in vitro* studies revealed that residue Ser73 is phosphorylated by PKA while residues Ser36, Ser54, Ser73, and Ser106 are targets of the Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) (Lee *et al.* 1990, Thulin *et al.* 2001). However, only the phosphorylation of Ser54 and Ser73, that are both located within the N-terminal domain, seems to be involved in the regulation of Pdc function *in vivo* and it has been shown that Pdc phosphorylated at these two sites forms a stable complex with the 14-3-3 protein (Nakano *et al.* 2001, Thulin *et al.* 2001, Lee *et al.* 2004). The role of the 14-3-3 protein in the regulation of Pdc is still unclear. The 14-3-3 protein may completely block the interaction between Pdc and $G_t\beta\gamma$ by interacting and/or affecting the structure of the $G_t\beta\gamma$ binding surface within the N-terminal domain of Pdc. Another possibility is that the 14-3-3 binding decreases the rate of Pdc dephosphorylation and/or degradation. The 14-3-3 protein binding could also modulate interactions between Pdc and its other binding partners the transcription factor CRX and a subunit of the 26S proteasome complex SUG1 which were both reported to interact with the C-terminal domain of Pdc (Zhu and Craft 1998, 2000).

To better understand the interaction between Pdc and the 14-3-3 protein, we recently performed a biophysical analysis of the interaction between Pdc doubly phosphorylated at Ser54 and Ser73 and the 14-3-3ζ isoform (Rezabkova *et al.* 2012). Analytical ultracentrifugation showed that the simultaneous phosphorylation of both Ser54 and Ser73 is important for the interaction between Pdc and 14-3-3ζ as well as that these two proteins form a complex with the 1:2 stoichiometry (one molecule of Pdc interacts with the 14-3-3 dimer). The combination of data from analytical ultracentrifugation and dynamic light scattering also suggested that the Pdc molecule binds to the central channel of the cup-shaped 14-3-3ζ dimer. The time-resolved AEDANS fluorescence spectroscopy experiments revealed that the 14-3-3ζ-Pdc complex formation affects the structure and reduces the flexibility of both the N- and C-terminal domains of Pdc, although both 14-3-3 binding motifs are located within the N-terminal domain. These structural changes involve the $G_t\beta\zeta$ binding region within the N-terminal domain of Pdc, and thus could explain the inhibitory effect of 14-3-3 on Pdc-$G_t\beta\zeta$ interaction. In addition, the 14-3-3 binding-induced structural change within the C-terminal domain of Pdc suggests that 14-3-3 might be involved in the modulation of its interactions with CRX and SUG1.

Conclusions

Mechanisms of the 14-3-3 protein-dependent regulation of all three proteins mentioned in this minireview, Nth1, RGS3 and Pdc, show one common aspect – a conformational change induced by binding to the 14-3-3 protein molecule involving regions that are remote from the segment containing phosphorylated 14-3-3 binding motif(s). This confirms that the interactions between 14-3-3 and its ligand extend beyond those involving the ligand binding groove. HDX-MS experiments revealed that the 14-3-3 protein directly interacts with its binding partner using regions other than that of the central channel including the less conserved regions of helices H6 and H8 outside the central channel of the 14-3-3 dimer. The involvement of such less conserved regions may explain the observed isoform-specific interactions between 14-3-3 and their ligands. In addition, in the case of RGS3 as well as Pdc the 14-3-3 protein sterically blocks the binding surface of these proteins, thus inhibiting their interactions with other binding partners. In both cases these steric occlusions affect the same regions where we observed conformational changes (the N-terminal domain of Pdc and the RGS domain of RGS3).

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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