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MINIREVIEW

14-3-3 Proteins: A Family of Versatile Molecular Regulators

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Summary

The 14-3-3 proteins are a family of acidic regulatory molecules found in all eukaryotes. 14-3-3 proteins function as molecular scaffolds by modulating the conformation of their binding partners. Through the functional modulation of a wide range of binding partners, 14-3-3 proteins are involved in many processes, including cell cycle regulation, metabolism control, apoptosis, and control of gene transcription. This minireview includes a short overview of 14-3-3 proteins and then focuses on their role in the regulation of two important binding partners: FOXO forkhead transcription factors and an enzyme tyrosine hydroxylase.

Key words

14-3-3 protein • Forkhead transcription factor FOXO4 • Tyrosine hydroxylase • Conformation

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Structure and function of 14-3-3 proteins

The 14-3-3 proteins are a family of acidic regulatory proteins found in all eukaryotes (Fu *et al.* 2000). 14-3-3 proteins function as molecular scaffolds by modulating the conformation of their binding partners (Fu *et al.* 2000, Aitken 2006, Hermeking and Benzinger 2006). Through the functional modulation of a wide range of binding partners, 14-3-3 proteins are involved in many biologically important processes, including cell cycle regulation, metabolism control, apoptosis, and control of gene transcription (Fu *et al.* 2000, Aitken 2006, Hermeking and Benzinger 2006). The unusual name of these proteins, "14-3-3", originates from their elution and migration pattern on two-dimensional DEAE-cellulose chromatography and starch gel electrophoresis (Moore and Perez 1967). Since the initial discovery of 14-3-3 proteins, members of this protein family have also been given other names, e.g. BAP-1, Bilardo, Exo1, Leonardo, Stratifin etc., when they have been rediscovered due to their involvement in many regulatory processes (Aitken 2006).

The 14-3-3 proteins are highly conserved over a wide range of eukaryotic species and many organisms express multiple isoforms (Fig. 1). While lower eukaryotes, e.g. yeast, contain only two 14-3-3 genes, higher eukaryotes possess up to 15 14-3-3 genes. For example, in mammals seven isoforms (β, ε, η, γ, τ, ζ and σ) have been identified to date. With exception of mammalian sigma isoform, all 14-3-3 proteins can form both homo- and heterodimers (sigma isoform preferentially forms homodimers) (Wilker *et al.* 2005). Initial structural studies confirmed a dimeric nature of 14-3-3 proteins and revealed that each monomer consists of nine antiparallel α-helices (Liu *et al.* 1995, Xiao *et al.* 1995) (Figs 2A and 2B). A large 40 Å wide deep channel located in the center of a cup-shaped 14-3-3 protein dimer contains two amphipathic grooves. Detailed analysis of interactions between 14-3-3 proteins and their targets and the use of an oriented peptide library screening approaches resulted in identification of two optimal 14-3-3 binding motifs: RSXpSXP and RX(Y/F)XpSXP (X denotes any amino acid residues except cysteine) (Muslin *et al.* 1996, Yaffe *et al.* 1997, Rittinger *et al.*

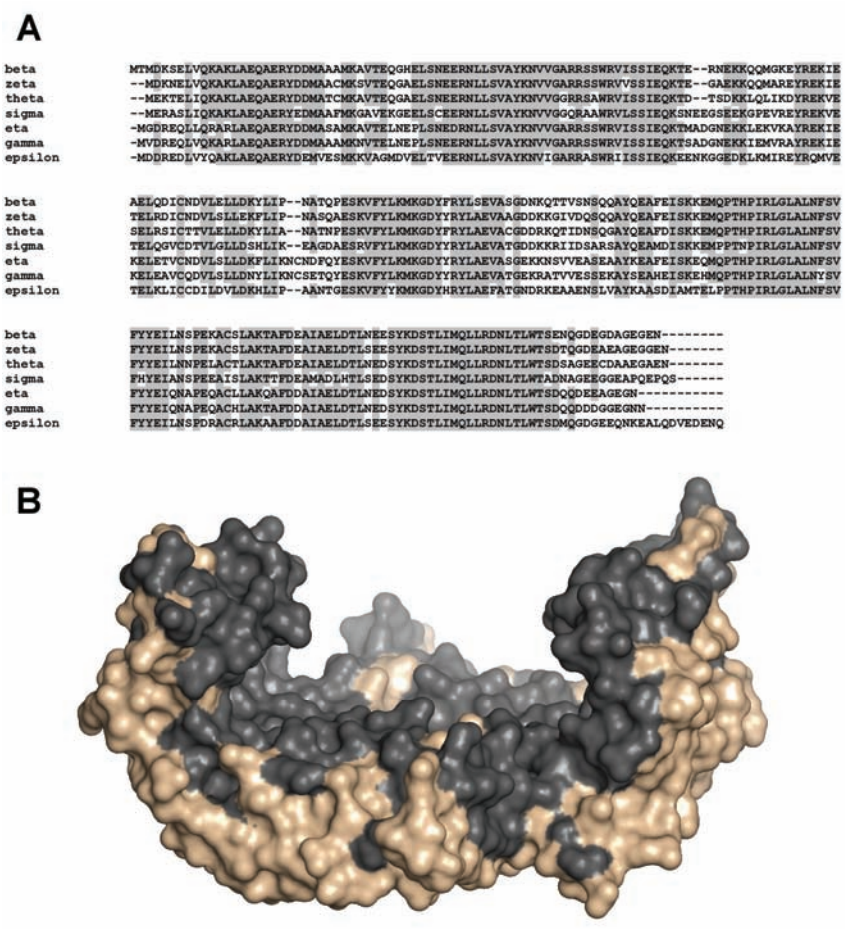

Fig. 1. Sequence conservation of 14-3-3 isoforms. (**A**) Sequence alignment of human 14-3-3 protein isoforms. Residues that are conserved at least in five sequences are shaded in gray. (**B**) Structure of human 14-3-3ζ isoform (surface representation) shaded according the sequence conservation (Rittinger *et al.* 1999). Residues that are totally conserved among all human isoforms are shaded in dark gray.

1999). Crystal structures of phosphopeptide-bound 14-3-3 complexes demonstrated that pS (or pT)-containing segments (pS and pT denotes phosphoserine and phosphothreonine, respectively) are bound within amphipathic grooves at either edge of the central channel of the 14-3-3 dimer and adopt an extended main chain conformation (Fig. 2) (Yaffe *et al.* 1997, Petosa *et al.* 1998, Rittinger *et al.* 1999). The phosphate group of phosphoserine (or phosphothreonine) is coordinated by salt bridges to the side chains of R56, R127, and K49, and a hydrogen bond to the hydroxyl group of Y128 (Figs 2C and 2D). The proline residue in the phosphopeptide adopts a *cis*-conformation, producing a sharp change in chain direction and allowing the remaining portions of the peptide to exit the binding cleft (Yaffe *et al.* 1997, Rittinger *et al.* 1999).

The whole molecule of the 14-3-3 dimer is very rigid likely as a result of extensive interactions between helices. The most flexible region is the C-terminal segment which is also the most variable region among 14-3-3 isoforms. The highly conserved regions map to the interior surface of the central channel (Fig. 1B). The dimeric nature of 14-3-3 proteins seems to be very important for their functions. Many binding partners of 14-3-3 proteins possess more than one 14-3-3 binding motif and the presence of two binding grooves within the 14-3-3 dimer might enable simultaneous binding of two phosphorylated motifs and thus more efficient ligand binding (Tzivion *et al.* 1998, Tzivion *et al.* 2000, Yaffe 2002, Shen *et al.* 2003). A synthetic peptide containing two 14-3-3 binding motifs binds with 30 times higher affinity compared to a peptide containing only one phosphorylated motif (Yaffe *et al.* 1997). Based on these observations a model for 14-3-3/ligand interaction has been proposed. This model suggests that 14-3-3 binding relies initially upon the interaction of a gatekeeper motif (a dominant binding motif) with one monomeric 14-3-3 subunit. This is followed by the binding of one or more weaker secondary sites to a second 14-3-3 subunit thus facilitating ligand conformation that is not favorable in the unbound state (e.g. exposing one or more regions of the protein that are inaccessible in the free or monomer-bound form) (Yaffe 2002).

The function of 14-3-3 proteins can be generally classified on the basis of three modes of action (Bridges and Moorhead 2004):

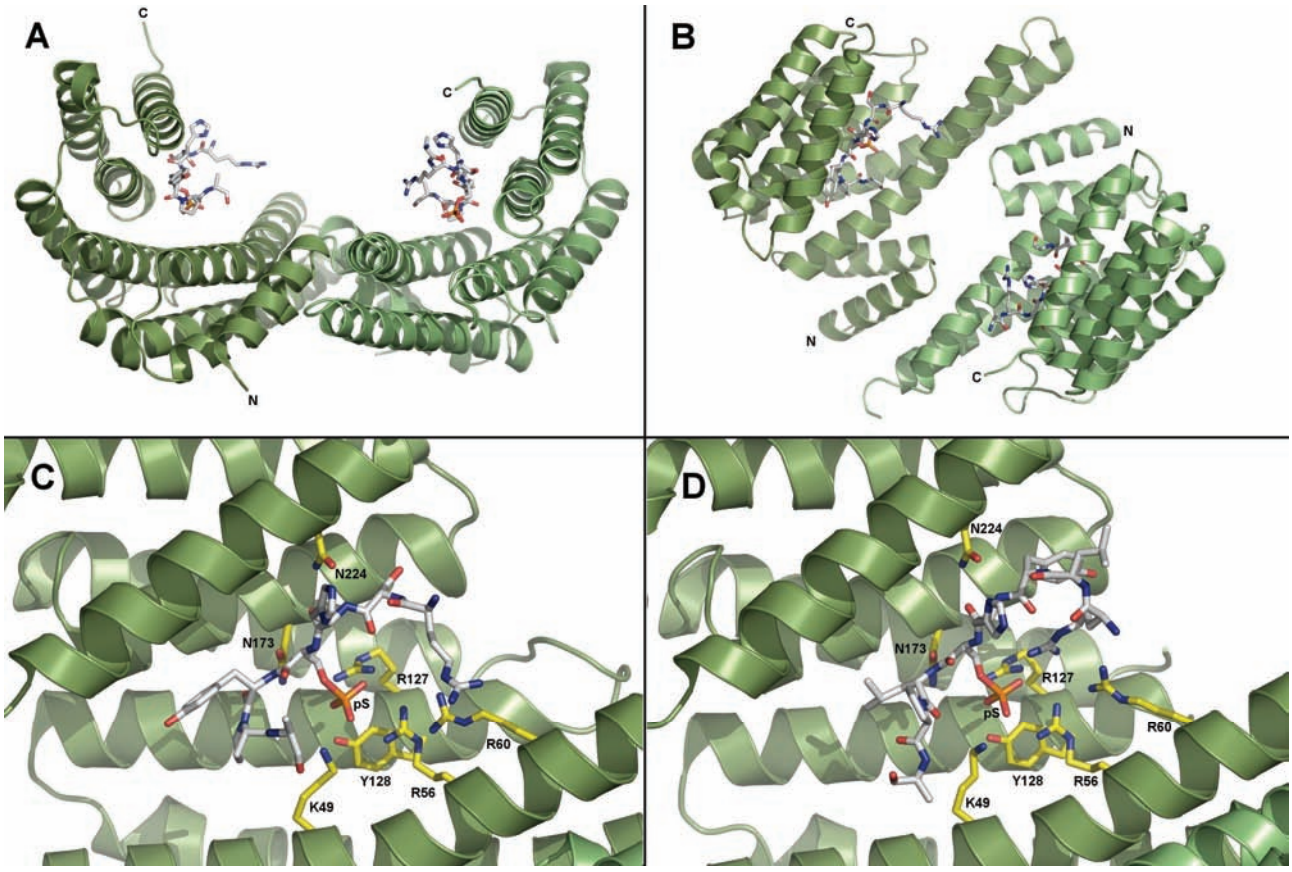

Fig. 2. Crystal structure of human 14-3-3ζ isoform with bound phosphopeptides containing optimal 14-3-3 binding motif (Rittinger *et al.* 1999). (**A** and **B**) Two orthogonal views of 14-3-3ζ dimer (ribbon representation) with bound phosphopeptide (stick representation). (**C**) A detailed view of the phosphopeptide ARSHpSYPA bound to 14-3-3ζ (Rittinger *et al.* 1999). (**D**) A detailed view of the phosphopeptide RLYHpSLPA bound to 14-3-3ζ (Rittinger *et al.* 1999).

(1) *14-3-3 protein changes conformation of its binding partner.* The 14-3-3 dimer is a very rigid molecule thus it could serve as a base on which the target protein can be reshaped. If the binding partner is an enzyme, this can affect the enzymatic properties of the binding partner. An example of this mode of regulation is the 14-3-3 protein-dependent activation of serotonin N-acetyltransferase (AANAT) (Ganguly *et al.* 2001, Obsil *et al.* 2001). This enzyme is in its free form catalytically inefficient mainly due to the low affinity for its substrates. Serotonin K_M of free AANAT is ~170 μM while the cytoplasmic concentration of serotonin in the nighttime ovine pineal gland is ~1 μM (Ganguly *et al.* 2001). However, upon the phosphorylation the 14-3-3 protein binds to and stabilizes a region of AANAT molecule involved in substrate binding (Obsil *et al.* 2001). This structural stabilization increases the substrate affinity 10-fold and activates the enzyme.

(2) *14-3-3 protein physically occludes sequence-specific or structural features of its binding partner.* The 14-3-3 binding can also mask important features of the surface of the target protein. For example, several well characterized binding partners (e.g. FOXO transcription factors, protein phosphatase Cdc25C, histone deacetylases) contain a nuclear localization sequence (NLS) in close proximity of a 14-3-3 binding motif (Muslin and Xing 2000, Graves *et al.* 2001, Kao *et al.* 2001, Giles *et al.* 2003, Van Der Heide *et al.* 2004). It has therefore been suggested that the 14-3-3 protein binding inhibits the interaction between NLS and nuclear import machinery thus blocking nuclear localization of these proteins.

(3) *14-3-3 protein functions as a scaffold molecule to anchor proteins within close proximity of one another.* Third possible function of 14-3-3 proteins is to act as scaffold molecule that stimulates protein-protein interactions. One monomer of the 14-3-3 dimer binds to a first protein and the other subunit binds to the second protein. Examples of this mode of action are 14-3-3-mediated interactions between Tau and glycogen synthase kinase 3β (Agarwal-Mawal *et al.* 2003), or the Ron receptor tyrosine kinase and 6β4/3β1 integrin (Santoro *et al.* 2003).

Regulation of 14-3-3 proteins

Most of the 14-3-3 protein isoforms, with the exception of mammalian σ isoform, are ubiquitously expressed in all tissues and they bind their targets with similar affinity. Therefore the binding properties of 14-3-3 proteins seem to be regulated through post-translational modifications and/or the binding of cofactors (Athwal *et al.* 1998, Wurtele *et al.* 2003, Bridges and Moorhead 2004, Aitken 2006). From various post-translational modifications the phosphorylation of 14-3-3 isoforms on specific residues is now well established mechanism of 14-3-3 regulation (Mackintosh 2004, Aitken 2006). Phosphorylation sites are not conserved among 14-3-3 isoforms thus this post-translational modification can enable selective isoform regulation. 14-3-3 protein isoforms are phosphoryted at four sites by a number of Ser/Thr kinases that are known to be involved in cell signaling and regulation (Mackintosh 2004, Wilker and Yaffe 2004, Aitken 2006). Two sites are located at the dimer interface (Ser58 and Ser63 in human 14-3-3ζ numbering) and it has been shown that phosphorylation of Ser58 promotes the formation of 14-3-3 monomers (Powell *et al.* 2003, Woodcock *et al.* 2003). Monomerization of otherwise dimeric 14-3-3 proteins might have profound effect on their function. The other two sites (Ser184 and Ser/Thr232) are located in the vicinity of the ligand binding groove and phosphorylation of both these sites have been shown to reduce ligand binding (Dubois *et al.* 1997, Aitken *et al.* 2002, Dumaz and Marais 2003, Obsilova *et al.* 2004, Tsuruta *et al.* 2004).

Phosphorylation site Ser/Thr232 is located within the C-terminal segment, a region that is believed to be flexible. The structure of this part of 14-3-3 molecule is unknown because it cannot be seen in any of the available 14-3-3 crystal structures presumably due to disorder (Liu *et al.* 1995, Xiao *et al.* 1995, Yaffe *et al.* 1997, Rittinger *et al.* 1999, Obsil *et al.* 2001). Several reports indicated that the C-terminal segment is involved in the regulation of ligand binding (Liu *et al.* 1995, Liu *et al.* 1996, Truong *et al.* 2002). Phosphorylation site Ser/Thr232 is present in the C-terminal segment of vertebrate ζ and τ isoforms only and can be phosphorylated both *in vitro* and *in vivo* by casein kinase Iα (Dubois *et al.* 1997). It has also been shown that in human embryonic kidney 293 cells 14-3-3ζ is phosphorylated exclusively at Thr232 (Dubois *et al.* 1997). Since in these cells only non-phosphorylated 14-3-3ζ is bound to Raf-1 kinase (Rommel *et al.* 1996), it has been concluded that *in vivo* phosphorylation at Thr232 inhibits interaction between 14-3-3ζ and Raf-1 kinase. Our group studied the conformational changes of 14-3-3ζ C-terminal stretch induced by phosphopeptide binding and phosphorylation at Thr232 (Obsilova *et al.* 2004, Silhan *et al.* 2004). Time-resolved fluorescence measurements revealed that the phosphopeptide binding changes the conformation and increases the flexibility of 14-3-3ζ C-terminal stretch, demonstrating that this region is directly involved in the ligand binding. The Förster resonance energy transfer (FRET) measurements between Trp residue inserted into the C-terminal segment and a dansyl group (attached at two different cysteine residues) indicated that, in the absence of the ligand, the C-terminal segment occupies the ligand binding groove of the 14-3-3 protein. Upon the phosphopeptide binding the C-terminal segment is displaced from the ligand binding groove and its flexibility increases. Phosphorylation of 14-3-3ζ at Thr232 changed the structure of the C-terminal segment and resulted in inhibition of phosphopeptide binding (Obsilova *et al.* 2004, Silhan *et al.* 2004). The precise mechanism of this inhibition is still unknown, but it is possible to speculate that phosphorylation-induced conformational change could affect interactions between the C-terminal segment and the ligand binding groove thus making its displacement from the groove more difficult.

The interaction between the 14-3-3 protein and its target can also be regulated by the presence of small molecule ligands. One of the best studied examples is fusicoccin, a terpenoid secreted by a fungal plant pathogen *Fusicoccum amygdali*. Fusicoccin specifically enhances the 14-3-3 protein binding to the plant plasma membrane H^+-ATPase by stabilizing the interaction between the C-terminus of the H^+-ATPase and 14-3-3 ligand binding groove (Wurtele *et al.* 2003). The crystal structure of the ternary complex between a plant 14-3-3 protein, fusicoccin and a phosphopeptide derived from the C-terminus of the H^+-ATPase revealed that the compound fills a cavity in the protein-phosphopeptide interaction surface. Calorimetric measurements showed that the toxin alone binds only weakly to 14-3-3 and that peptide and toxin mutually increase each others' binding afinity ~90-fold (Wurtele *et al.* 2003).

Role of 14-3-3 proteins in the regulation of FOXO forkhead transcription factors

The forkhead box (FOX) transcription factors contain an approximately 110 amino acid long winged

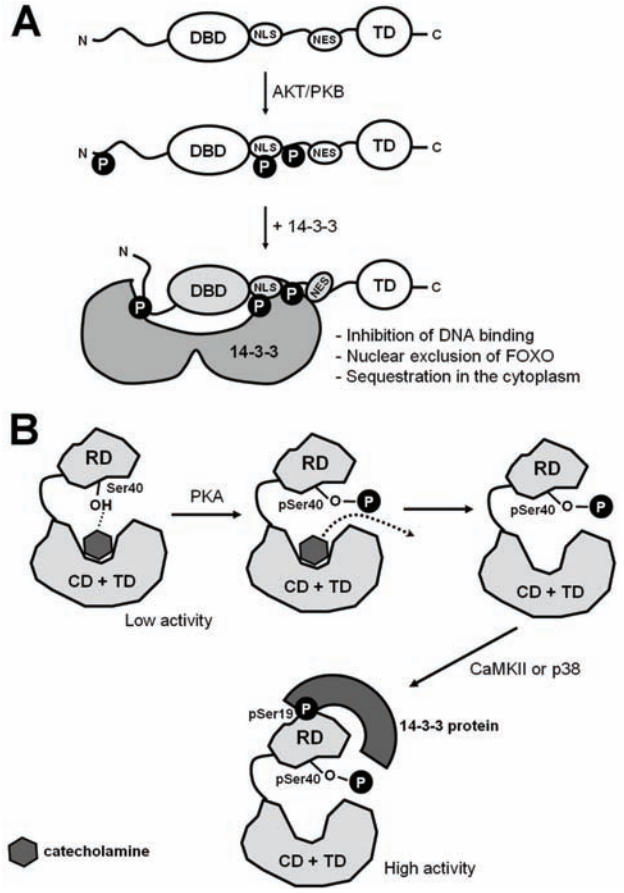

Fig. 3. (**A**) Schematic representation of primary structure of FOXO proteins. All FOXO proteins have the same domain organization and contain forkhead DNA binding domain (DBD), nuclear localization signal (NLS), nuclear export sequence (NES) and transactivation domain (TD). The AKT/PKB phosphorylation sites are labeled by black circles. (**B**) Diagram of tyrosine hydroxylase domain structure. Tyrosine hydroxylase contains three domains: regulatory domain (RD), catalytic domain (CD) and tetramerization domain (TD). It seems that the hydroxyl group of Ser40 stabilizes the catecholamine-bound inhibited form of TH (Ramsey and Fitzpatrick 1998). Phosphorylation of Ser40 induces a conformational change of the regulatory domain thus permitting easier dissociation of catecholamine and activation of TH (Wu *et al.* 1992, Bevilaqua *et al.* 2001, McCulloch *et al.* 2001). Phosphorylation of Ser19 induces TH binding to the 14-3-3 protein. That leads to the enhancement of the TH activity probably through the conformational change of regulatory domain (Obsilova *et al.* 2008).

helix DNA binding domain (DBD) known as the forkhead domain (Weigel and Jackle 1990, Kaestner *et al.* 2000). The FOX proteins display large functional diversity and play a wide range of roles in development, proliferation, differentiation, stress resistance, apoptosis and control of metabolism (Greer and Brunet 2005, van der Horst and Burgering 2007). Among the forkhead box family of transcription factors the "O" subgroup consists of four members (FOXO1, FOXO3, FOXO4, and FOXO6). They are the vertebrate orthologs of the *Caenorhabditis elegans* DAF-16 transcription factor and constitute key components of a highly conserved signaling pathway that connects growth and stress signals to transcriptional control (Lin *et al.* 1997, Ogg *et al.* 1997).

Transcriptional activity of FOXO proteins is regulated through insulin-phosphatidylinositol 3-kinase – protein kinase B (PI3K-AKT/PKB) signaling pathway. In response to growth signals, the PI3K activates AKT/PKB and related SGK (serum and glucocorticoid inducible kinase) that then phosphorylate FOXO proteins at three sites (Biggs *et al.* 1999, Kops *et al.* 1999, Brownawell *et al.* 2001) (Fig. 3A). Phosphorylation by AKT/PKB induces phosphorylation of additional sites by casein kinase 1 (CK1) and dual-specificity tyrosine-regulated kinase 1A (DIRK1). The AKT/PKB phosphorylates substrates that carry an RXRXX(S/T) motif, which is close to the consensus 14-3-3 binding motifs (Alessi *et al.* 1996, Yaffe *et al.* 1997, Rittinger *et al.* 1999). The AKT/PKB-dependent phosphorylation of sites at the N-terminus and in the forkhead domain of FOXO proteins creates two 14-3-3 binding motifs and induces FOXO binding to nuclear 14-3-3 proteins (Brunet *et al.* 1999, Brunet *et al.* 2002). It has been shown that simultaneous use of both AKT/14-3-3 motifs is necessary for optimal FOXO binding to 14-3-3 proteins (Brunet *et al.* 1999, Brunet *et al.* 2002, Obsil *et al.* 2003, Zhao *et al.* 2004). These two motifs border the DNA binding domain, raising the possibility that 14-3-3 proteins could participate in disruption of FOXO binding to the DNA. Such 14-3-3 protein-dependent inhibition of DNA binding has been observed for DAF-16 and FOXO4 (Cahill *et al.* 2001, Obsil *et al.* 2003, Boura *et al.* 2007). However, the exact mechanism of this 14-3-3-dependent inhibition of DNA binding is still unclear. Since the second AKT/PKB motif is embedded in the C-terminal part of forkhead domain, the 14-3-3 protein could affect the binding of this region to the DNA, mask other parts of FOXO DNA binding interface, or change the conformation of forkhead domain.

The association of FOXO factors with 14-3-3 proteins has another important consequence. Upon the 14-3-3 protein binding the resulting FOXO–14-3-3 complexes are rapidly transported out of the nucleus and retained within the cytoplasm (Biggs *et al.* 1999, Brunet *et al.* 1999, Nakae *et al.* 1999, Brunet *et al.* 2002). The exact mechanism of this process is unknown but 14-3-3 proteins have been shown to bind to phosphorylated FOXO in the nucleus where, along with the inhibition of DNA binding, could induce a conformational change in FOXO molecules

and expose their NES for interaction with Exportin/Crm1 (Brunet *et al.* 2002). In addition, all FOXO proteins contain a sequence that represents a nonclassical bipartite NLS. In general, nuclear localization signals do not conform to a specific consensus sequence but form two distinct classes termed monopartite NLS, consisting of a single cluster of basic amino acid residues, and bipartite NLS, consisting of two basic clusters separated by a variable spacer. These two basic clusters are independent and usually are both required for nuclear targeting (Dingwall and Laskey 1991). NLS found in FOXO proteins consists of two clusters containing in total 12 arginine and lysine residues positioned on both sides of the second AKT/14-3-3 binding motif in the C-terminus of forkhead domain. Therefore, it has been suggested that 14-3-3 proteins may prevent nuclear reimport of FOXO proteins by masking their NLS (Brunet *et al.* 1999, Brownawell *et al.* 2001, Rena *et al.* 2001) (Fig. 3A). To provide experimental evidence showing a direct interaction between the 14-3-3 protein and NLS of FOXO our group investigated whether the 14-3-3 protein binding affects the structure of FOXO4 NLS by means of fluorescence spectroscopy. We showed that the 14-3-3 protein binding significantly changes the environment around AEDANS-labeled NLS and reduces its flexibility. On the other hand, the phosphorylation itself and the binding of double-stranded DNA have a small effect on the structure of this region. Crystal structures of various 14-3-3 protein complexes showed that the 14-3-3 ligand-binding groove can accommodate a polypeptide chain about 9 amino acid residues long with the pS or pT in the middle (Yaffe *et al.* 1997, Rittinger *et al.* 1999, Obsil *et al.* 2001). Therefore, the first part of FOXO4 bipartite NLS (three arginine residues located upstream to the second AKT/PKB site) would likely be directly buried within the ligand-binding groove upon the 14-3-3 protein binding. However, the second part of FOXO4 NLS consisting of 7 basic residues located between Lys199 and Lys211 is far enough from the second AKT/PKB motif to be buried within the 14-3-3 ligand-binding groove. Significant reduction of fast segmental movements of AEDANS-labeled NLS together with changes in the polarity of the microenvironment around this group indicated that the second part of FOXO4 NLS either directly interacts with the 14-3-3 protein or dramatically changes its conformation as a result of complex formation. Therefore we concluded that the binding of the 14-3-3 protein can affect the whole region of FOXO4 NLS (Obsilova *et al.* 2005).

In addition to AKT/PKB-mediated phosphorylation, the function of FOXO proteins is also controlled by other types of post-translational modifications including non-AKT/PKB-mediated phosphorylation, acetylation, and ubiquitination (van der Horst and Burgering 2007). The mechanisms by which post-translational modifications regulate FOXO functions are mostly elusive, but in many cases they seem to affect DNA binding potential of FOXO proteins, function of their nuclear localization signal (NLS) and nuclear export sequence (NES), or interactions of FOXO with other proteins.

Role of 14-3-3 proteins in the regulation of tyrosine hydroxylase

Tyrosine hydroxylase (TH, EC 1.14.16.2) catalyzes the first step in the biosynthesis of catecholamines and its activity is controlled by multiple mechanisms including feedback inhibition by catecholamines, allosteric activation by heparin, phospholipids, and RNA, and activation by protein phosphorylation (Nagatsu *et al.* 1964, Fitzpatrick 1999). N-terminal regulatory domain of TH consists of 160-190 amino acid residues (in human isoforms) and can be removed without a significant decrease in catalytic activity (Daubner *et al.* 1993). Regulatory domain can be phosphorylated at four serine residues Ser8, Ser19, Ser31, and Ser40 *in vitro*, *in situ* and *in vivo* (Haycock 1990, Haycock and Haycock 1991, Fitzpatrick 1999). Among these, the effects of Ser19 and Ser40 phosphorylation are the best characterized. Residue Ser40 can be phosphorylated by a number of protein kinases and its phosphorylation by cyclic AMP-dependent protein kinase (PKA) induces the most potent activation of TH (Daubner *et al.* 1992, Ramsey and Fitzpatrick 1998). It has been proposed that phosphorylation of Ser40 alters conformation of the regulatory domain and its interaction with the catalytic domain. Phosphorylation of Ser40 is known to increase the sensitivity to proteolysis in its surrounding, while dopamine binding has the opposite effect, suggesting that there is a link between the conformation of the regulatory domain and the enzyme activity (McCulloch and Fitzpatrick 1999). It has been proposed that the hydroxyl group of Ser40 contributes to the stabilization of the catecholamine-bound inhibited form of TH (Ramsey and Fitzpatrick 1998). Phosphorylation of Ser40 probably induces a conformational change of the regulatory domain thus permitting easier dissociation of catecholamine and

activation of TH (Wu *et al.* 1992, Bevilaqua *et al.* 2001, McCulloch *et al.* 2001). In contrast to phosphorylation at Ser40, the activation of TH through the phosphorylation of Ser19 requires the presence of regulatory 14-3-3 protein (Yamauchi and Fujisawa 1981, Ichimura *et al.* 1987, Itagaki *et al.* 1999) (Fig. 3B).

Regulatory domain of TH contains two 14-3-3 protein binding motifs around residues Ser19 and Ser40. Detailed analysis of interactions between TH and different 14-3-3 protein isoforms using surface plasmon resonance confirmed that phosphorylation of Ser19 is required for stable association of human TH isoforms with bovine 14-3-3ζ isoform (Kleppe *et al.* 2001). On the other hand, Kleppe *et al.* (2001) also showed that yeast 14-3-3 protein isoform (BMH1) can bind with high affinity to all four human TH isoforms phosphorylated only at Ser40. The reason why yeast but not mammalian 14-3-3 protein isoforms bind to TH phosphorylated only at Ser40 is unknown.

The role of 14-3-3 protein in the regulation of TH activity is still unclear. While one study found that 14-3-3 protein binding to TH phosphorylated at Ser19 increased V_{max} threefold (Toska *et al.* 2002), other studies observed no 14-3-3-dependent enhancement of TH activity (Haycock and Wakade 1992, Sutherland *et al.* 1993). It has also been suggested that 14-3-3 protein might protect proteolytically very sensitive phosphorylated regulatory domain of TH, and/or slow-down dephosphorylation of phosphorylated Ser19 and Ser40 (Kleppe *et al.* 2001, Toska *et al.* 2002). Phosphorylation status of regulatory domain seems to be important for overall stability of TH because it has been shown that multiphosphorylated form of TH is more stable than the single and unphosphorylated forms, regardless of phosphorylation sites (Bevilaqua *et al.* 2001, Toska *et al.* 2002, Royo *et al.* 2005). Since 14-3-3 proteins are known to modulate the structure of their binding partners (Obsil *et al.* 2001, Yaffe 2002, Obsilova *et al.* 2005), it is reasonable to speculate that 14-3-3 protein changes the structure of phosphorylated regulatory domain of TH, thus making it less sensitive to proteolysis and/or dephosphorylation (Fig. 3B). To test this hypothesis we recently investigated whether the 14-3-3ζ protein binding affects conformation of the regulatory domain of human TH isoform 1 (denoted as TH1R) phosphorylated at both Ser19 and Ser40 (Obsilova *et al.* 2008). Site-directed mutagenesis was used to generate five single tryptophan mutants with tryptophan residue located at different positions within the regulatory domain (positions 14, 34, 73, 103, and 131). Time-resolved tryptophan fluorescence intensity decays revealed that phosphorylation of Ser19 and Ser40 by itself does not induce significant conformational changes in the regions surrounding inserted tryptophans. However, the binding of the 14-3-3ζ protein induces distinct structural changes in the N-terminal part of doubly phosphorylated regulatory domain. In addition, tryptophan fluorescence quenching experiments with acrylamide revealed that 14-3-3ζ protein binding decreases the exposure of tryptophan residues at positions 14 and 34. The 14-3-3ζ protein binding also reduces the sensitivity of regulatory domain to proteolysis by protecting the cleavage site between residues 33 and 34. Circular dichroism measurements showed that regulatory domain of TH isoform 1 is an unstructured protein with low content of secondary structure and that neither phosphorylation nor the 14-3-3ζ protein binding change its secondary structure. These results are consistent with the hypothesis that the 14-3-3 protein affects the structure of phosphorylated regulatory domain of TH, thus increasing its stability and helping to keep TH in the activated state .

Conclusions

The 14-3-3 isoforms play various roles in the regulation of many cellular proteins. While they activate or stabilize some proteins, and inactivate others, for many proteins 14-3-3 isoforms play an organizational role as a "scaffold" molecules. Through the functional modulation of a wide range of targets, 14-3-3 isoforms are involved in many processes, including cell cycle regulation, metabolism control, apoptosis, and control of gene transcription. Due to the multitude of 14-3-3 binding partners and physiological activities, a specific definition of their cellular role or function is difficult. Recent studies have made great progress towards elucidating the functions of 14-3-3 proteins and proteomic approaches has allowed us to better understand the interactions of 14-3-3 with other proteins. However, much yet remains to be discovered. The mechanisms of 14-3-3 protein action are now known to be complex and their better understanding will require the crystal structure of additional 14-3-3-targets, both free and in complex with 14-3-3 proteins.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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