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Review

The 14-3-3 proteins in regulation of cellular metabolism

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ABSTRACT

Thirty years ago, it was discovered that 14-3-3 proteins could activate enzymes involved in amino acid metabolism. In the following decades, 14-3-3s have been shown to be involved in many different signaling pathways that modulate cellular and whole body energy and nutrient homeostasis. Large scale screening for cellular binding partners of 14-3-3 has identified numerous proteins that participate in regulation of metabolic pathways, although only a minority of these targets have yet been subject to detailed studies. Because of the wide distribution of potential 14-3-3 targets and the resurging interest in metabolic pathway control in diseases like cancer, diabetes, obesity and cardiovascular disease, we review the role of 14-3-3 proteins in the regulation of core and specialized cellular metabolic functions. We cite illustrative examples of 14-3-3 action through their direct modulation of individual enzymes and through regulation of master switches in cellular pathways, such as insulin signaling, mTOR- and AMP dependent kinase signaling pathways, as well as regulation of autophagy. We further illustrate the quantitative impact of 14-3-3 association on signal response at the target protein level and we discuss implications of recent findings showing 14-3-3 protein membrane binding of target proteins.

Contents

Abbreviations: 4E-BP1, eIF4E-binding protein 1; AANAT, arylalkylamine *N*-acetyltransferase; ACC, acetyl-CoA carboxylase; AKT, protein kinase B; AMPK, AMP dependent protein kinase; AS, ATP synthase; AS160, AKT substrate 160; ATG, autophagy related; BAD, Bcl-2 antagonist of death; BAX, Bcl-2 associated X protein; BIM, Bcl-2-interacting mediator of death; CRTC, cAMP-regulated transcriptional coactivator; FAS, fatty acid synthase; GAPDH, glyceraldehyde-3-phosphase dehydrogenase; GLUT, glucose transporter; GSK3, glycogen synthase kinase 3; HIOMIT, hydroxyindole-*O*-methyltransferase; LKB1, liver kinase B1/STK11; MARK1–4, MAP/microtubule-regulating kinase 1–4; mTOR, mammalian target of rapamycin; PFK-2, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; PK, pyruvate kinase M; PKA, cAMP dependent protein kinase; PRAS40, proline rich AKT substrate 40; RAPTOR, regulatory-associated protein of mTOR; RICTOR, rapamycin-insensitive companion of TOR; SK61, p70 ribosomal S6 kinase 1; SIK1–3, salt-inducible kinase 1–3; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase; TSC1/2, Tuberous sclerosis protein 1/2; ULK1, unc-51-like kinase 1.

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1. Introduction

In unicellular as well as multicellular organisms, metabolism needs to be tightly regulated according to demand of cellular processes such as cell growth, proliferation, heat production and mechanical transformations, e.g. muscle cell contraction and migration. At the organism level, different tissues are formed to cope with specialized functions ensuring optimal energy conservation and nutrient availability for life and reproduction. Key signaling pathways elicit the co-regulation of metabolism at the cellular and tissue level and during the past decades, evidence has accumulated about the involvement of 14-3-3 proteins in this regulation. The 14-3-3 proteins are being implicated in a growing number of cell biology processes, attesting to the multifunctionality of this ubiquitous eukaryotic adaptor protein family. Here we review examples of 14-3-3 involvement in some metabolic processes, emphasizing their regulatory roles in energy metabolism and biosynthetic reaction pathways.

Affinity purification of cellular 14-3-3 binding proteins in proteomic studies provide evidence for several hundred different binding partners (possibly >500) associated with most cellular processes [1–8]. Although the associations with many of these binding partners have not been completely verified yet, these proteomic and interactomic studies clearly illustrate the diverse biological functions associated with this protein family. The extensive interactome of the 14-3-3 proteins and its regulation by protein phosphorylation events suggest a fundamental function of these proteins in signaling related to cellular metabolic states. The archetypical peptide sequence requirements for binding to 14-3-3 have been known for a long time [9] and have recently been reviewed [10], though structural features outside the binding motif can also contribute [11].

The 14-3-3 proteins seem to be expressed in all eukaryotic cell types and are highly abundant in the mammalian nervous system [12]. Among the seven mammalian 14-3-3 isoforms (α/β, ε, η, γ, τ/θ, δ/ζ, σ) there are reported differences in the isoform expression pattern between cell-types, tissues and various laboratory cell lines [13,14]. The underlying regulatory mechanisms responsible for controlling the cellular levels of different 14-3-3 isoforms are still poorly understood. Still, evidence points to a rich and dynamic regulation of 14-3-3 expression, exemplified by epigenetic regulation of 14-3-3σ in cancer [15], transcriptional regulation [16–18], and regulation by micro-RNA [19,20].

The two 14-3-3 isoforms in yeast seem to provide overlapping functionality as suggested by viable single versus lethal double knockout. Similar gross redundancy is likely to exist for the mammalian 14-3-3 proteins, but with more genetically modified models becoming available and the use of refined whole animal analysis, one will expect more isoform-specific functions to emerge. Thus, disruption of the 14-3-3ε isoform (*YWHAE* gene product) has led to its functional association with brain development and neuronal migration and it is found deleted in individuals with Miller–Dieker syndrome [21]. It has been reported that a YWHAE polymorphism is associated with schizophrenia [22], however, in other samples this association has not been replicated [23]. Furthermore, 14-3-3η (*YWHAH* gene product) has been associated with psychotic bipolar disorder [24,25]. Less functional specialization seems to be associated with 14-3-3γ based on its disruption in mouse [26], yet a link to brain and heart development has been found by similar studies in zebrafish [27]. The 14-3-3σ isoform has been mainly associated with regulation of cell proliferation and appears to be the most specialized of the mammalian isoforms, which may explain why it preferentially forms homodimers. This brings up a second unresolved issue of 14-3-3 function – homodimers versus heterodimers, and the severe effects of 14-3-3ε disruption might be related to its ability to form heterodimers with most of the other isoforms [28–30]. Interestingly, some cellular functions have been associated with specific 14-3-3 heterodimers such as aldosterone stimulated sodium channel translocation (14-3-3β/ε) and keratinocyte migration by the more unusual 14-3-3ζ/τ [28,31].

The main focus of 14-3-3 biology in mammalian systems has been on their modulation of cellular signaling pathways, cell death, cell-cycle and cytoskeletal dynamics. However, the first functional targets for 14-3-3 proteins were enzymes of specialized amino acid metabolism, i.e. tyrosine- and tryptophan hydroxylase [32] (TH and TPH, respectively), giving rise to their name tyrosine- and tryptophan hydroxylase activators (YWHAs) and putting them in a central position as modulators of catecholamine and serotonin biosynthesis. In plants, 14-3-3 function has been mainly associated with metabolic regulation (reviewed in [33]), though several examples of targets in signal transduction have been reported and cell signaling is an increasingly established function of 14-3-3s in plants [33–35].

2. Direct modulation of metabolic enzyme function by 14-3-3 proteins

Historically, the first reported targets of protein Ser/Thr kinases were enzymes involved in central metabolic pathways [36], discoveries which contributed to the understanding of hormonal control of cellular metabolism. In fact, metabolic regulation by Ser/Thr phosphorylation is crucial to cellular function. However, the impact of 14-3-3 proteins on regulation of metabolic enzyme activities is still poorly understood in mammals. In a large scale affinity purification study of 14-3-3 binding partners from HeLa cell lysates, using yeast 14-3-3 (BMH1) as bait and elution with the 14-3-3 binding peptide ARAApSAPA, MacKintosh and colleagues identified several key enzymes in central metabolism as potential binding partners of 14-3-3 [2]. These included enzymes involved in glycolysis, pentose phosphate pathway, fatty acid synthesis, nucleotide synthesis, methionine metabolism, and reductive metabolism. Interestingly, in a similar study using mammalian 14-3-3ζ as bait, several of these metabolic enzymes were confirmed, including pyruvate kinase M (PK), ATP-synthase (AS), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), fatty acid synthase (FAS) and the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2) [1] (Fig. 1). Many of these enzymes and additional proteins have been found to interact with 14-3-3 in subsequent investigations [7,37]. Interestingly, the heart isoform of PFK-2 was identified as a 14-3-3 target upon phosphorylation by AKT but not 5′AMP-activated protein kinase (AMPK), locking the enzyme in its glycolysis stimulating state in response to insulin [38] (Fig. 1).

Together, these findings indicate that 14-3-3 proteins may directly interact with and modify the functions of enzymes that are of central importance in metabolic regulation. However, for most of the protein targets that were identified in the above screening studies, the binding kinetics and biological implications of the interactions are not yet fully elucidated. Instead, most of our knowledge of 14-3-3 action on metabolic regulation comes from detailed studies on a limited number of regulatory enzymes involved in specialized biological pathways. The regulation of monoamine biosynthesis by the aromatic amino acid hydroxylases is of particular importance both for historical reasons and because the details of interactions have been studied in quantitative terms by many different groups.

The biosynthesis of catecholamine and serotonin neurotransmitters and hormones is tightly regulated at the level of the rate-limiting enzymes in these pathways (tyrosine hydroxylase

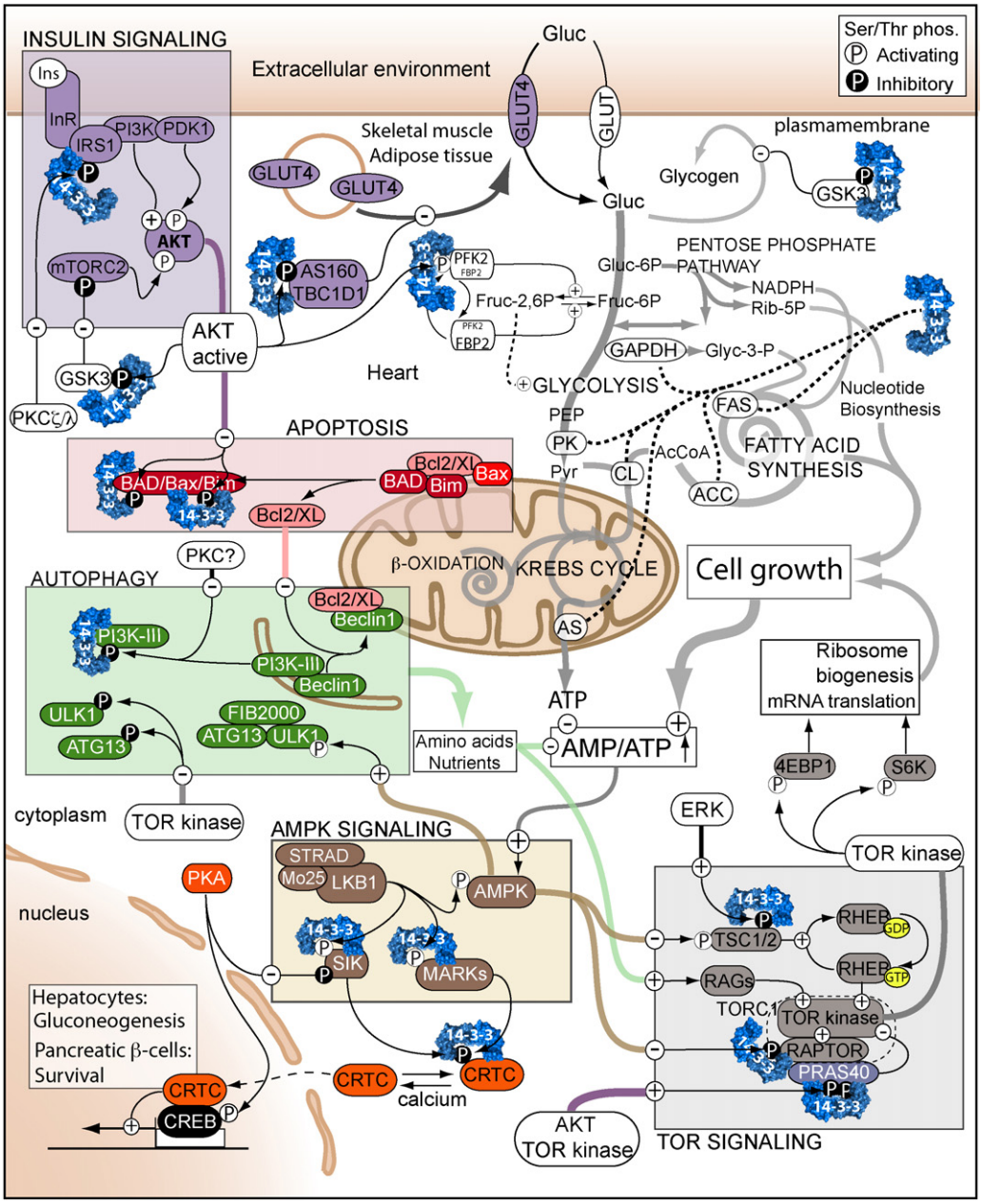

Fig. 1. Involvement of 14-3-3 proteins in regulation of cellular energy metabolism. The figure summarizes binding of 14-3-3 (blue) to important targets and their placement in core metabolic pathways and regulatory signaling pathways. The metabolic pathways are illustrated with broad arrows or spirals (fatty acid synthesis or oxidation) and only selected intermediates and enzymes, which are reported as 14-3-3 binding partners, are shown. Interactions with metabolic enzymes that are reported in interactomics studies are shown with dotted lines. Phosphorylation events are shown as (P) with arrows from the kinase involved, where stimulating phosphorylation (and 14-3-3 binding) are shown on white background and inhibitory phosphorylation on black. Metabolic intermediates are abbreviated Gluc (glucose), Gluc-6P (glucose-6-phosphate), Fruc-6P (fructose-6-phosphate), PEP (phosphoenolpyruvate), Pyr (pyruvate), AcCoA (acetyl coenzyme A), Glyc-3-P (glycerol-3-phosphate), Rib-5P (ribose-5-phosphate) and the enzymes CL (citrate lyase), ACC (acetyl-CoA carboxylase) and PKC (protein kinase C). For other abbreviations we refer to the text and abbreviations section.

(TH) and tryptophan hydroxylase (TPH), respectively). Before any other functional roles of 14-3-3 proteins had been discovered, in a series of pioneering studies it was shown that activation of TH and TPH by phosphorylation was dependent on an additional protein factor that could be separated from the hydroxylases [32]. Upon subsequent identification of the activator protein as a mixture of 14-3-3 proteins [39], access to purified enzymes and specific antibodies, it has been shown that all seven human 14-3-3 isoforms, as well as the proteins BMH1 and BMH2 bind to phosphorylated TH and TPH, albeit with different affinities and binding specificities [40–43].

In rats and humans TH is encoded by a single gene that is subject to alternative splicing, resulting in multiple isoforms with slightly different N-terminal sequences, also affecting their phosphorylation and 14-3-3 binding sites [41,44]. The 14-3-3-dependent TH activation/stabilization requires the phosphorylation at Ser19 [41,44]. Phosphorylation at this position also increases the rate of Ser40 phosphorylation by PKA, a modification that releases the feedback inhibition by catecholamines [44–47]. Binding to 14-3-3 preserves the stability of the highly active and polyphosphorylated TH, and it also appears to be important for the subcellular enzyme localization and coordinated dopa/dopamine synthesis

and release (see below). TPH is encoded by two separate genes, resulting in the homologous enzymes TPH1 and TPH2, with different tissue distributions, regulatory properties and physiological functions [48]. As for TH, 14-3-3 binding to TPH1 and TPH2 consolidates the enzyme activation that is induced by phosphorylation, protects the phosphoproteins against dephosphorylation, and increases their thermal stability [40,43]. Another example of fine regulation of a metabolic pathway by regulation of enzyme activity and stability through complex formation with 14-3-3 is found for melatonin synthesis in the pineal gland. Melatonin synthesis constitutes the final stage in this biosynthetic pathway from tryptophan (serotonin → N-acetylserotonin → melatonin), where arylalkylamine *N*-acetyltransferase (AANAT) is the penultimate enzyme. Both the intrinsic circadian clock and light exposure regulate AANAT at the transcriptional and translational level in concert with TPH and hydroxyindole-*O*-methyltransferase (HIOMT) that operate in the preceeding and succeeding steps in the pathway. But fine regulation of AANAT activity occurs through light-dependent phosphorylation at two sites in the enzyme, i.e. Thr31 and Ser205, that differently affect the affinity of the enzyme for 14-3-3ζ [49].

3. Regulation of metabolic processes by signaling pathways – impact of 14-3-3

Cellular functions, growth and proliferation are controlled by signaling pathways that sense the energy and nutrient status of the cell. The AMPKs (AMPK1–3) are important cellular sensors of the AMP:ATP ratio and are key regulators of energy consuming and energy producing pathways. These heterotrimeric (α, β, γ) enzymes are activated by AMP binding to their γ-subunit, making the activation loop of the catalytic α-subunit available for activation by Thr172 phosphorylation by an upstream AMPK Kinase [50]. In most cells the tumor suppressor liver kinase B1 (LKB1/STK11) acts as the master regulator, not only of AMPK, but also of other kinases in the AMPK family, including salt-inducible kinase 1–3 (SIK1–3) and the MAP/microtubule-regulating kinase 1–4 (MARK1–4) [51] (Fig. 1).

Control of localization and activity of AMPKs by 14-3-3 proteins was first described for the MARKs (also called Partitioning defective (Par) 1a–d), which are involved in regulating cellular polarity [52] (Par-5 is also a 14-3-3 protein in Caenorhabditis *elegans*), but is also reported for the SIKs [53]. In particular SIK2, but also MARK2 and AMPK play an important role in metabolic regulation through the repression of the CREB coactivator, cAMP-regulated transcriptional coactivator (CRTC) and thereby suppress hepatic gluconeogenesis [54]. Phosphorylation-dependent binding of 14-3-3 to CRTC with resulting localization to the cytosol seems to be an important regulatory mechanism [55], suggesting that 14-3-3 proteins act to consolidate suppression of gluconeogenesis through SIK2 and CRTC as well as insulin signaling (below) (Fig. 1).

There is considerable cross-talk between the AMPK pathway and other key energy regulatory pathways such as the Target of rapamycin (TOR) signaling complex 1 (TORC1) and insulin signaling. The TORC1 plays a key role in adapting cellular growth to nutrient availability [56]. The regulatory-associated protein of TOR (RAPTOR) containing complex stimulates ribosome biogenesis, protein translation through phosphorylation of S6 kinase 1 (S6K1) and eIF4E-binding protein 1 (4E-BP1) and inhibits autophagy by phosphorylation of unc-51-like kinase 1 (ULK1) and autophagy related 13 (ATG13) [56,57] (Fig. 1). The mammalian TORC1 (mTORC1) also depends on stimulatory input from the GTPase RHEB (Ras homologue enriched in brain), which receives inhibitory input from the GAP containing protein complex of Tuberous sclerosis protein 1 and 2 (TSC1/Hamartin and TSC2/Tuberin) [56]. Much of the modulation of mTORC1 is mediated through TSC1/2, such as growth factor signaling, AMPK and hypoxia. Inhibitory phosphorylation of TSC1/2 by AKT and MAPKAPK2 facilitates binding of 14-3-3 [58,59], and a prevailing hypothesis of hypoxia-induced inhibition of mTORC1 is by Rebb1 induction and sequestration of 14-3-3 away from TSC2 [60] (Fig. 1).

AMPK is reported to inhibit mTORC1 by activation of TSC1/2 and by inhibition of RAPTOR by phosphorylation-induced binding of 14-3-3 [61], both of which will stimulate autophagy. Recently, a direct stimulatory path from AMPK to autophagy was described through phosphorylation of ULK1 [62], and complex formation between ULK1, mTORC1 and AMPK has been found to coincide with RAPTOR phosphorylation and binding of 14-3-3 [63] (Fig. 1). Judging from the 14-3-3:RAPTOR interaction, 14-3-3 seems to be on both sides of the equation for autophagy. Interestingly, 14-3-3ζ has recently been reported to inhibit autophagy through inhibition of the class III phosphatidylinositol-3-kinase (PI3K-III) [64], which is necessary for autophagosome vesicle nucleation. 14-3-3ζ is also an interaction partner of BH3-only family members of the Bcl-2 protein family, such as Bcl-2 antagonist of death (BAD) and BCL-2 interacting mediator of cell death (BIM), as well as the pore forming Bcl-2 associated X protein (BAX) [65–67]. Hence, increased 14-3-3ζ should shift the balance of BCL-2 proteins towards survival, but also release more BCL-2/BCL-XL for binding to BECLIN-1 (ATG6) and thereby suppress autophagy of mitochondria and ER [68] (Fig. 1).

Downstream of insulin and growth factor stimulation, AKT is activated by PDK1 and the RICTOR (rapamycin-insensitive companion of TOR) containing TOR complex 2 (TORC2), leading to AKT and ERK mediated inhibition of TSC1/TSC2 and stimulation of the mTORC1 pathway. A second path of insulin to mTORC1 activation is mediated by AKT phosphorylation and inhibition of the TORC1 inhibitory protein Proline-rich AKT substrate 40 (PRAS40), which associates with RAPTOR [69]. Binding of 14-3-3 to phosphorylated PRAS40 is important to release its suppression of TORC1 [70], but the exact mechanism is still not settled. The presence of PRAS40 seems to be important for TORC1 activity, whereas TORC1 mediated phosphorylation of PRAS40 is implicated in the release of PRAS40 suppression [71] (Fig. 1).

A major effect of insulin in skeletal muscle and adipose tissue is increased uptake of glucose from the blood. This is mediated by increased translocation of glucose transporter 4 (GLUT4) to the plasma membrane by Rab-mediated vesicular transport. In resting cells this transport is inhibited by Rab-GAPs such as Akt Substrate 160 (AS160/TBC1D4) and TBC1D1, which are both phosphorylated by AKT in response to insulin [72,73]. AKT phosphorylation facilitates binding of 14-3-3 and cytosolic translocation of AS160 and TBC1D1 with subsequent release of its suppressive activity [74] (Fig. 1). Interestingly, a knock-in mouse of AS160 with a disrupted phosphorylation- and binding site of 14-3-3 showed decreased insulin-stimulated GLUT4-translocation in muscle and insulin insensitivity with respect to glucose tolerance [75]. Parallel to increased glucose uptake, insulin stimulates glucose storage as glycogen in liver and muscle by AKT-mediated inactivation of glycogen synthase kinase 3 (GSK3), which suppresses glycogen synthesis. The β-isoform of GSK3 is a known binding partner for 14-3-3 upon Ser9 phosphorylation, but this interaction has received more attention for its role in GSK3β mediated phosphorylation of Tau [76] (Fig. 1). The relative roles of GSK3α and β in regulation of glycogen synthesis in different tissues are yet not resolved. However, a recently discovered link between GSK3β and inactivation of RICTOR of TORC2 [77], important for insulin induced uptake of glucose in muscle [78], suggests a possible role of 14-3-3 in GSK3β-mediated insulin resistance.

The apparent involvement of 14-3-3 proteins in the regulation of energy metabolism, mTOR signaling and autophagy in addition to their well-known roles in apoptotic and cell-cycle signaling,

also makes them interesting for understanding carcinogenesis (see review by Yaffe et al. in this issue) [93].

4. Effector mechanisms of 14-3-3 proteins

4.1. Localization to membranes

The regulatory functions of 14-3-3 proteins extend beyond activation, inhibition, stabilization or orientation of its partners, as 14-3-3 also modulate the specific subcellular localization of their cargos [79]. It is well established that 14-3-3 sequesters proteins in the cytoplasm, inhibiting nuclear or mitochondrial localization of specific partners, though less is known about isoform-specific differences [80,81]. However, there is also increasing evidence on the role of 14-3-3 in the transfer of binding partners to membranes [82,83].

In addition to the unique role of biological membranes in cell compartmentalization, membranes allow the formation and function of specialized, multi-enzyme units. Several of the protein components of these units interact with membranes peripherally and reversibly and this interaction can be mediated in several ways, including phosphorylation and modulations of charge–charge relationships [84]. The lowered dielectric constant and lower pH in the vicinity of a biological negatively charged membrane are often the driving forces for peripheral interactions. We have recently shown that 14-3-3γ interacts reversibly with negatively charged membranes [84,85]. The membrane affinity of 14-3-3γ is high for phospholipid compositions that mimic that of synaptic vesicles, with a high content of 1-stearoyl-2-docosahexaenoyl-phosphatidylserine (SDPS) and increases when a Ser19-phosphorylated TH-derived peptide is bound to 14-3-3γ [84], suggesting a role of this 14-3-3 isoform in membrane localization of TH. Though mainly cytoplasmatic, a fraction of TH is also found as membrane-bound both in brain, notably at nerve endings and synaptic vesicles, and in catecholamine secretory granules in adrenal medulla [86]. The significance of the binding of TH to membranes is not clear but it is assumed to have a role in the coordination of dopamine synthesis and release since dopa decarboxylase also forms functional complexes with TH at membranes [87].

4.2. Effects on target protein phosphorylation

By specifically associating with the phosphorylated state of the target proteins, the 14-3-3 proteins will shield against dephosphorylation. The efficiency of the protection will depend on the dissociation constant (K_d) of the complex and the effective concentrations of the binding partners. For 14-3-3 target protein (TP) association, K_d-values in the sub-nanomolar to micromolar range are generally reported [41,80,88]. The influence of 14-3-3 binding on the steady state level of target phophorylation can therefore be predicted, as exemplified by the simple case of monovalent phosphorylation and TP binding to 14-3-3 (Fig. 2A and B). In this model, even for moderate binding affinities (K_d = 0.5 μM), a ~6-fold amplification of the signal (phospho-TP) response is observed, and for higher affinities significant sensitization is predicted in addition to prominent signal amplification.

Binding of dimeric 14-3-3 covers a considerable surface area of the TP and this may affect its localization, oligomerization/complex formation, or further protein modifications. 14-3-3 Binding can be considered efficient and dynamic means of inducing secondary effects of phosphorylation on protein function, as compared to other proteins that obtain similar responses through conformational changes. The 14-3-3 proteins are able to simultaneously bind two phospho-target sites, either to obtain high affinity interaction (coincidence detector) or to elicit structural effects upon binding

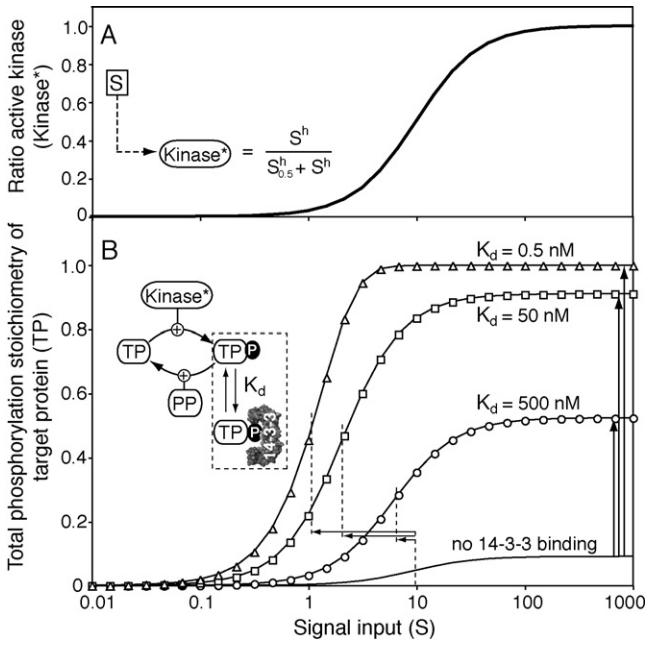

Fig. 2. The consolidating action of 14-3-3 on target phosphorylation. Panel (A) shows part of a signaling pathway where a protein kinase is activated (modeled by fractional activation) by a signal input (e.g. external signal or internal second messenger) according to a sigmoid activation function, where S is the signal input, $S_{0.5}$ is the signal input that gives half fractional activation of the kinase (here 10 nM) and h is the Hill coefficient of the kinase signal response (here 1.5). Panel (B) shows a downstream target protein (TP) ([TP] = 0.5 μM) of the activated kinase (Kinase*), which is dephosphorylated by a protein phosphatase (PP) and which may interact with 14-3-3 proteins (set to 5 μM) in phosphorylation dependent manner. The steady state amount of total TP phosphorylation in response to the signal input is calculated for TP with different affinities to 14-3-3 (K_d = 0.5, 50, 500 and ∞ nM). Thus, from a maximal TP phosphorylation response of 9% in the absence of 14-3-3 binding, decreasing the K_d (500 nM, ○; 50 nM, □; 0.5 nM, △) both amplifies (5.8-, 10- and 11-fold, respectively) and sensitize (about 2-, 5- and 10-fold, respectively) the TP phosphorylation response to S. The modeling was performed in Copasi (v4.5) [90] using rate of phosphorylation $V_{K^*} = k_{phos}(S^h/(S^h_{0.5} + S^h)) \cdot [\text{Kinase}_{tot}] \cdot [\text{TP}]$, where $[\text{Kinase}_{tot}] = 1$, k_{phos} = 0.5 μM^{-1} s^{-1} and rate of dephosphorylation V_{PP} = 5 μM^{-1} s^{-1} $\cdot [\text{PP}_{tot}] \cdot [\text{pTP}]$ ($[\text{PP}_{tot}] = 1$), whereas the association with 14-3-3, k_a was set to 0.5 μM^{-1} s^{-1} and K_d to fulfill the K_d [39].

(gatekeeper phosphorylation, molecular anvil [89]). These issues are discussed in [10], and the coincidence detector property of 14-3-3 can easily be visualized from Fig. 2B, where two versus one phosphorylation events dramatically increase the binding affinity, resulting in both signal amplification and sensitization.

5. Concluding remarks

The regulation of cellular metabolism by 14-3-3 proteins is an area ripe for further investigations. The 14-3-3 interactomic studies suggest that key metabolic enzymes are well represented among 14-3-3's cellular binding partners. The current understanding of how 14-3-3 proteins regulate their targets is likely to be valid for other metabolic enzymes as well. This will also provide additional mechanistic insight into how such enzymes are regulated by phosphorylation. Potential avenues for future research include how 14-3-3 proteins control enzyme activities and turnover, modulate single- or multiple phosphorylation events, and regulate metabolic activities at different cellular locations.

The presence of many alternative binding partners for 14-3-3s in the cell poses several important and unresolved issues related to our understanding of their ability to modulate cellular processes. How do different 14-3-3 ligands compete for complex formation? The total cellular level of 14-3-3 proteins is quite high, but the many interaction partners means that only a proportion of the 14-3-3

proteins will be free to engage partners in the cell. Unfortunately, the concentration of free 14-3-3 for the various 14-3-3 isoforms remains unknown.

The 14-3-3 proteins are certainly involved in many cellular processes that already are in focus for pharmacological intervention, but their broad involvement makes one wonder whether 14-3-3 proteins are realistic as targets for drugs either inhibiting or stabilizing their interactions in general. A feasible approach may be to target the unique protein–protein interface present for each 14-3-3:target protein complex. It remains to be seen if this specificity can be achieved at the organism level (see reviews by Fu et al. for discussion) [12,91]. Recently, the fungal phytotoxin fusicoccin, which stabilizes the interaction between plant 14-3-3 and H^+-ATPase, was shown to promote platelet aggregation, presumably via stabilization of the 14-3-3-glycoprotein Ibα complex [92]. Clearly, more basic knowledge and improved models and drug candidates are required to exploit the potential of the 14-3-3-proteins as drug targets.

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