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Targeting of PKA, PKC and protein phosphatases to cellular microdomains

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Summary The intracellular responses to many distinct extracellular signals involve the direction of broad-based protein kinases and protein phosphatases to catalyse quite specific protein phosphorylation/dephosphorylation events. It is now clear that such specificity is often achieved through subcellular targeting of distinct pools of kinase or phosphatase towards particular substrates at specific subcellular locations. Given the dynamic nature of protein phosphorylation reactions, coordinated control of both kinase and phosphatases is often required and complexes formed by common scaffold or targeting proteins exist to direct both kinase and phosphatase to the same subcellular location. In many cases more than one kinase or phosphatase is required and binding proteins which target more than one kinase or phosphatase have now been identified. This review summarizes recent findings relating to the concept of targeting PKA, PKC and the major serine/threonine phosphatases, PP1, PP2A and PP2B, through the formation of multi-enzyme signalling complexes. © Harcourt Publishers Ltd

INTRODUCTION

The classical picture of extracellular signals leading to a rise in the intracellular concentration of second messengers like cAMP and calcium, subsequent activation of protein kinases, like PKA and/or PKC, and phosphorylation of substrates illustrates the fundamental basis of any signal transduction system. However within this context, it is clear that more complex and intricate mechanisms are evoked to produce functional specificity in response to different extracellular signals that use the same basic second messenger systems. Furthermore, whilst it is also clear that regulation of PKA and PKC is essential for control of cellular signalling, it is important to give consideration to the dynamic nature of protein phosphorylation reactions. Thus, protein dephosphorylation must also be carefully controlled in order to sustain a given level of protein phosphorylation in response to a particular signal. Whilst PKA, PKC and protein phosphatases appear to have a wide intracellular distribution, gradients of second messengers are formed within the proximity of associated receptors which are believed to regulate distinct subcellular pools of co-localized kinases and perhaps phosphatases which then modulate similarly localized substrates [1]. A wide range of targeting proteins (collectively termed scaffold proteins, anchoring proteins or adaptor proteins) which play the role of recruiting the kinases and the phosphatases into signalling complexes has now been discovered [2]. These complexes may include some substrates and/or bring the enzymes close to other substrates. In some cases, even kinases and phosphatases are held within the same complex by the same anchoring or scaffold protein. In this review we discuss the current concepts and roles of the binding proteins involved in PKA, PKC and protein phosphatase targeting to cellular microdomains. Specific attention will be given to those proteins that possess multiple targeting roles by bringing together kinases and phosphatases in the same complex.

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TARGETING OF PKA

PKA is a multimeric protein composed of two catalytic subunits (C) and two regulatory subunits (R). In this conformation the C subunits are maintained in an inactive state; activation requires the dissociation of the C subunits from the complex. Dissociation of the complex occurs in response to binding of cAMP to the R subunits, thereby allowing for control over PKA activity in response to extracellular signals [3]. Although cAMP is absolutely required for activation of PKA, other regulatory mechanisms exist to dictate precisely where and when pools of the kinase are activated in response to specific stimuli. Although many extracellular signals use the same second messenger system, the opposing actions of adenylate cyclase and phosphodiesterases generate localized gradients of cAMP which will have greatest influence where concentrated pools of PKA are co-localized in an inactive conformation. For the most part, this targeting of inactive PKA is achieved by a family of proteins, AKAPs (A-Kinase Anchoring Proteins), which bind PKA through the regulatory subunits (RII) [4]. AKAPs are a family of more than 30 proteins that often share little or no sequence homology, but which all functionally bind PKA in the same way [5]. Most importantly, individual AKAPs possess distinct subcellular targeting sequences or mechanisms that allow for the exposure of distinct pools of PKA to localized changes in concentration of cAMP. Up to 75% of the total cellular PKA is believed to be associated with some member of the AKAP family [6]. Functional consequences of disrupting AKAP–PKA interactions have been demonstrated, illustrating the importance of this regulatory mechanism in vivo. Thus far, disruption of AKAP–PKA interaction has been shown to modify insulin secretion, L-type calcium channel activity, glutamate receptor function and calcium-activated K-channel activity [5]. In this way, AKAPs are seen to provide a means of coordinating different extracellular signals to regulate a given cell function.

AKAP–PKA interaction requires dimerisation of RII and involves isoleucines 3 and 5 side chains on each RII subunit [7]. Thus, AKAPs bind only the PKA holoenzyme and, therefore, target the inactive enzyme. AKAPs bind the RII subunits at a site distinct from the cAMP-binding site such that association with the AKAPs does not impede activation of PKA. The AKAP binding site for RII is a sequence of approximately 20 amino acids that may or may not share sequence homology between AKAPs. However, in each case the binding sequence forms an amphipathic helix such that, topologically, AKAP–PKA binding requires protein–protein interactions between three polypeptide chains [8].

The binding of AKAPs to RII represents a particularly strong interaction and peptides that span the AKAP helix region bind RII with high affinity (K_d=4nM). Such peptides have proved useful tools to disrupt AKAP–PKA interactions inside cells and demonstrate the functional roles of AKAPs in vivo [9–11].

Whilst anchoring of PKA to AKAPs via the RII subunit represents an extremely strong association, some AKAPs have now been shown to anchor PKA through association with RI. Although the affinity of binding is a fraction (approximately 1%) of that of RII, this is still within the physiological concentrations of RI and AKAPs such that anchoring of type I PKA may also occur in regions of the cell where RII concentrations are limited [12].

In addition to an RII binding domain, AKAPs also possess unique domains responsible for tethering the complex to different subcellular locations. Subcellular fractionation and immunochemical localization studies have demonstrated AKAPs at virtually every distinct subcellular locus. Thus, AKAPs exist which target PKA to centrosomes, endoplasmic reticuli, Golgi apparatus, mitochondria, microtubules, cell membranes, nuclear matrices, secretor granules and postsynaptic densities and dendrites [5]. Details of the specific targeting determinants are only beginning to emerge. Microtubule targeting of the AKAP, MAP2, is mediated by an octadecapeptide-repeat residing at the C terminus [13], whilst a unique peroxisomal targeting sequence (PTS-1) at the C terminus of AKAP220 appears to target it to peroxisomes [14]. Furthermore, different mechanisms exist for targeting different AKAPs to the same loci. For example, three linear sequences in AKAP79 are responsible for targeting to cell membranes [15]. Each sequence is enriched in basic amino acids and whilst constructs containing only one linear sequence do not target AKAP79 to the periphery, any combination of two basic regions will result in targeting. Membrane targeting is mediated by binding PtdIns (4,5) P2 and other acidic phospholipids to these regions. Binding appears to be a relatively non-specific electrostatic interaction, although some higher-affinity preference has been shown for certain phospholipids [15]. Furthermore, membrane binding is disrupted by PKC-mediated phosphorylation of a site within the membrane-binding region. Calcium-calmodulin is also able to disrupt membrane binding. In contrast, AKAP18, which also targets PKA to cell membranes, does so through palmitoylation and myristoylation of two out of possible three N-terminal residues [16]. A single lipid moiety was unable to sustain membrane targeting, which is consistent with the evidence that a single acyl group is insufficient to mediate stable protein–lipid interactions of a wide range of proteins.

Thus, the prevailing mechanism of PKA regulation appears to involve tethering pools of the inactive kinase to distinct subcellular locations to facilitate coordinated activity in response to localized increases in cAMP

concentration. The predominant means of PKA targeting is through association with AKAPs that have distinct subcellular locations and a variety of mechanisms have evolved for ensuring the correct targeting of the correct AKAP along with its associates. It is, therefore, unlikely that substantial amounts of 'untargeted' PKA exist inside the cell. As the mapping of AKAP targeting domains proceeds, we will undoubtedly learn more of the exquisite mechanisms responsible for determining the specificity of PKA activation.

TARGETING OF PKC

Protein kinase C is a family of isozymes that are known to undergo translocation from one subcellular compartment or location to another in response to extracellular stimulation [17]. Although most members of the PKC family rely upon diacylglycerol (DAG), phospholipid (phosphatidylserine, PS) and calcium, there are at least three sub-families whose response to these activators is distinct. The classical PKC subfamily (cPKC), which includes PKCα, PKCβI, PKCβII, require calcium, PS and DAG for activity. This family is also activated by phorbol ester [18]. The second PKC family (nPKC) does not require calcium for activation [19], largely as a consequence of an altered C2 domain found in the cPKC family. An aPKC family (atypical) lacks the C2 domain, is not activated by calcium and is also insensitive to DAG and phorbol ester. The insensitivity to DAG and phorbol ester has been mapped to the absence of part of domain C1 that is present in both cPKC and nPKC families [20]. A fourth distant relative of the PKC family, termed μPKC or PKD, has also been described [21–23]. PKD is closest to nPKC in functionality, being insensitive to calcium but requiring PS or phorbol ester for activation. PKD also contains a number of unique domains including a PH domain and a distinctive catalytic domain. Although detailed characterization of this form is not yet available, PKD is rapidly and potently activated by a variety of stimuli [24,25]. Interestingly, activation of PKD appears to occur via activation of other PKC family members such as PKCε and PKCφ, but direct phosphorylation of PKD by the other PKCs has not yet been demonstrated [26].

Despite the large number of isozymes, the role of individual PKCs in cellular function is still not clear. Whilst individual PKC isozymes possess a degree of substrate specificity and sensitivity to activators, most cells synthesise a range of PKC isozymes such that cell-specific expression is likely to represent only part of the mechanism for generating specificity of PKC-mediated signalling. Within a particular cell type, isozymes are distributed to different subcellular locations suggesting that targeting occurs as a means of regulating this kinase as well as PKA. PKCs have been found in a range of subcellular compartments including cell membranes, cytoskeleton, nucleus and cytosol in both active and inactive configurations.

Extracellular stimuli or cell permeable agonists lead to, in many cases dramatic, re-distribution of several PKC isoforms [20]. PKCs possess two targeting domains, C1 which contains DAG and phorbol ester binding sites and C2 which contains acidic lipid and/or calcium binding sites, indicating that protein–lipid interactions are likely to be involved in PKC targeting [21]. However, this is unlikely to be the full picture since these domains are absent in some PKCs that also undergo translocation. The role of protein–protein interactions in PKC redistribution is now beginning to emerge and at least four classes of PKC binding protein have now been identified.

The members of the first group of PKC binding proteins identified were all shown to be substrates [27]. Using an overlay technique, these PKC substrates all bound PKC in a PS-dependent manner. Importantly, while PS was required for binding, additional factors (DAG, calcium) were required for substrate phosphorylation suggesting that PS is predominantly involved in targeting.

A number of other PKC binding proteins are not substrates and are, therefore, candidates for a targeting role. Receptors for activated C-kinase (RACKs) comprise a group of proteins that bind PKC at a site distinct from the substrate binding domain [28]. Binding is insensitive to PS, suggesting that a direct protein–protein interaction is involved. As the name suggests, these proteins only bind activated PKC and appear to enhance activity. The first RACK to be identified (RACK1) is a protein which enhances the activity of PKC several fold, thereby promoting specific substrate phosphorylation by both targeting and activation of the kinase [28]. Another RACK, the coatomer protein β'COP, appears to be selective for epsilon PKC providing a mechanism for isoform-specific PKC regulation [29]. Since RACKs bind only the activated forms of PKC, it has been suggested that another group of proteins bind PKC in its inactive state [19]. Thus, it has been proposed that RICKs (receptor for inactive C kinase) target PKC to different subcellular locations from RACKs and specific PKC mediated phosphorylation is regulated by the movement of PKC from RICK to RACK [19].

The concept of RICKs resembles that of AKAPs, which also target inactive enzyme, but it still requires experimental substantiation. The protein InaD in *Drosophila* is a strong candidate for a RICK, being specific for inactive PKC in the eye. InaD maintains a signalling network comprising not only PKC but also a calcium channel (TRP), calmodulin and PLCβ, and is functionally essential for normal light sensitivity [30].

TARGETING OF PP1

Protein phosphatase 1 (PP1) is a member of the PPP gene family of phosphatase, which includes PP2A and PP2B [31]. Together, the members of this family account for the majority of serine/threonine dephosphorylation in vivo [32]. The catalytic subunit of PP1 (PP1c) is highly conserved and is involved in the dephosphorylation of multiple substrates and the control of a wide range of cellular functions [33]. Whilst PP1c is subject to inhibition by cdk-2 phosphorylation [34], the major mechanism of regulation is through binding a range of PP1-regulatory (or targeting) subunits [35]. These regulatory subunits direct PP1 specificity by their distinct subcellular locations (targeting) or by modulating activity towards different substrates. For example, the regulatory subunit, G_L, targets PP1 to glycogen particles in liver and inhibits PP1 activity towards glycogen phosphorylase, thereby enhancing glycogenolysis [35]. A different regulatory subunit G_M targets PP1c to glycogen in muscle[36]. The targeting role of G_M is modulated by PKA-mediated phosphorylation which results in dissociation of PP1c from the complex. G_M is dephosphorylated by PP2A and PP2B, the latter providing a potential means of regulating PP1 subcellular location by calcium [37]. Similarly, the binding of inhibitor-1, a cytosolic PP1c inhibitor is regulated by PKA phosphorylation. Inhibitor-1 binds and inactivates PP1c in a phosphorylation-dependent manner. Dephosphorylation of inhibitor-1 by PP2A and/or PP2B releases active PP1c [33]. The dephosphorylation of Inhibitor-1 by the calcium-dependent phosphatase, PP2B, illustrates an elegant mechanism for signal mediated regulation of PP1 activity and for the convergence of signals operating through distinct second messengers. Thus, elevation of cAMP by adrenalin or glucagon action leads to phosphorylation of inhibitor-1 and subsequent inactivation of PP1c, whilst elevation of intracellular calcium promotes dephosphorylation of inhibitor-1 and subsequent activation of PP1 [37]. Similarly, DARPP-32, an I-1 homolog that is enriched in brain, is rapidly dephosphorylated in response to the influx of extracellular calcium that follows glutamate receptor activation, which, in turn, leads to activation of PP1c [38]. Inhibitor-1 and DARPP-32 are essentially cytosolic proteins, such that signal-mediated dissociation (and activation) of PP1c renders it available for binding to other targeting proteins as well as the dephosphorylation of substrates. It is unlikely that substantial free PP1c exists inside cells.

Approximately 20 distinct PP1 regulatory subunits have been characterized to date and many more have been shown to exist through the use of microcystin-affinity chromatography and interactive cloning techniques [39]. Known PP1 regulatory subunits have distinct subcellular locations and target the otherwise broadly distributed PP1c towards co-localized substrates. Interestingly, little or no sequence homology exists between the known PP1 regulatory subunits. However, recent mutation and crystallography studies have identified a small PP1 binding motif that is conserved in all these regulatory subunits [40]. Thus, any protein containing an R/KVXF sequence can be predicted to bind PP1. However, the presence of this short motif alone is unlikely to determine functional binding since the binding efficiencies of each regulatory subunit vary considerably. Additional binding sites may be required or conformational alterations, promoted, for example, by phosphorylation, may be necessary to present the binding surface to PP1c. Furthermore, whilst inhibitor-1 also contains this motif, additional binding sequences, incorporating the PKA phosphorylation site, are required for inhibition [31].

TARGETING OF PP2A

Protein phosphatase 2A, like PP1 has a broad substrate specificity and subcellular distribution, in many cases overlapping with PP1 [41]. Consequently, PP2A has been implicated in the control of many cell functions, including metabolism, cell cycle progression, gene transcription, cell transformation, and exocytosis from neuronal and non-neuronal cells [41,42]. Although the broad functional involvement of PP2A implies strict control of its activity, the mechanisms whereby specificity of PP2A action is regulated, particularly by second messengers, are not yet fully understood.

PP2Ac is subject to C-terminal methylation [43] and tyrosine phosphorylation [44]. Whilst, in vitro, these modifications do alter activity (activation and inhibition respectively), the precise role in vivo is not yet known. Interestingly, both these modifications occur within the same three amino acid region at the C-terminus but have opposing functional consequences. The potential for dynamic alteration of PP2A activity through methylation and phosphorylation is an intriguing concept. Inside cells, PP2A exists predominantly as a heterotrimer [41] and it is possible that free catalytic subunit rarely, if ever, exists. It is possible that these post-translational modifications are associated with modulation of heterotrimer composition but supporting data is still required. The catalytic subunit (PP2Ac) is highly conserved and shares approximately 50% primary amino acid sequence homology with PP1c [31]. PP2Ac forms a constitutive dimer with a 65kDa scaffold protein (A subunit) which is also highly conserved. Although the AC dimer has reduced activity when compared to the free PP2Ac, the chief role of the A subunit is believed to involve recruitment of additional regulatory subunits (B subunit) to complete the trimeric complex.

Currently three different gene families, encoding proteins of 55kDa (B), 61kDa (B′, also known as B56) and 72kDa (B″), contribute to the B subunit component [41,42,45]. B subunit binding in general, reduces PP2A activity and the extent of inhibition depends on which B subunit is involved [41]. Each product of the B subunit gene family comprises several isoforms such that, in theory, approximately 50 different configurations of the PP2A heterotrimer can exist [45] and might, therefore, explain at least some of the PP2A specificity. However, some B subunits also have multiple subcellular locations (for example Bα is present in cytosol, cell membrane and cytoskeleton) such that additional regulatory factors must exist.

Additional proteins that bind PP2A are beginning to emerge and in several cases an intact heterotrimer is involved. Whilst the SV40 small t antigen inhibits PP2Ac by displacing some forms of B subunit [46], the adenovirus protein E4orf4 binds a PP2A trimer comprising the Bα subunit [47]. Furthermore, two HIV-encoded proteins, NCp7 and Vpr bind and, in this case, activate the B′-containing PP2A trimer [48]. Other proteins known to bind different PP2A trimers include cyclin G, which binds B′ subunit in response to p53 induction [49], Tau, a cytoskeletal substrate of the B subunit-PP2A trimer [50] and p107, which binds the B″ subunit-containing trimer [51]. A number of protein kinases including CaM Kinase IV [52], PKC (see later) and p70 S6 kinase [53] also form complexes with PP2A heterotrimers. Additional complexity in PP2A molecular complex regulation has arisen from the discovery of two heat-stable inhibitor proteins, originally identified as I_1^{PP2A} and I_2^{PP2A}, and now known to be Phap-I [54] and the myeloid leukemia associated protein SET1 [55] respectively. Both these inhibitors are distinct from the heat-stable inhibitors of PP1 and bind the catalytic subunit of PP2A independently of A or B subunit binding. Other proteins, such as HRX, a fusion protein associated with acute leukemia, bind PP2A through I_2^{PP2A} such that a phosphatase complex comprising five different proteins is possible [56]. The wnt signaling pathway involved in embryonic development illustrates even more complexity of PP2A–protein interactions. In this case, the adenomatous polyposis coli (APC) protein binds the B′ subunit whilst axin binds PP2Ac [57,58]. Both APC and axin, together with GSK3β, form a complex that is involved in phosphorylation-dependent destablization of β-catenin. Thus, PP2A appears to be involved in regulation of wnt gene transcription through the formation of a complex containing at least six proteins where the A-subunit represents the cornerstone and the C subunit may be involved in regulation of GSK3β activity. Despite the existence of these, often elaborate complexes, as yet, no means of regulation of PP2A in response to extracellular signals has been defined. Understanding the roles of these additional proteins in regulating PP2A function presents a major challenge and the discovery of more PP2A binding proteins in the future is likely to add to the complexity of understanding PP2A regulation.

MULTI-ENZYME TARGETING

The coordination of cellular signalling in most cases involves a series of phosphorylation and dephosphorylation events requiring the control of multiple protein kinases and phosphatases. It is beginning to emerge that in many cases coordination of these multiple signals involves bringing together the relevant kinases, phosphatases and indeed substrates at the same subcellular location by common scaffold or anchoring proteins [2,59]. In the following sections the current evidence for such complexes involving PKA and/or PKC is summarized.

PKA AND PKC

Whilst the targeting of kinases by AKAPs was originally believed to involve only PKA, it is now clear that several members of this anchoring protein family also bind PKC. PKC (α, βI and βII isoforms) binds AKAP79 in the presence of Ca^{2+} and phosphatidyl serine, at a site distinct from the PKA binding site [59]. AKAP79–PKC interaction inhibits PKC and confocal microscopy of primary hippocampal neurons showed co-localization of AKAP79 and PKC. Although PKC can phosphorylate AKAP79, this has little influence over binding efficiency. AKAP79 is concentrated at post-synaptic densities [15] such that it appears to target two different protein kinases which are activated by distinct second messengers towards important functional compartments in neurons and maintains them in an inactive configuration until signals are generated. AKAP250 (otherwise known as Gravin) also binds both PKA and PKC. Gravin is a cytosolic protein recognized by sera from Myasthenia Gravis patients and in this case targets both PKA and PKC to the membrane cytoskeleton and filopodia [60]. In an additional level of complexity, AKAP79 also binds the calcium-dependent phosphatase, PP2B and co-localization of all three enzymes with AKAP79 inside living cells has been demonstrated [61]. The coordination of multienzyme signalling complexes by AKAPs may, therefore, be a common mechanism for the integration of distinct signals to control specific phosphorylation/dephosphorylation events.

PKA AND PP1

The co-localization of PKA with PP2B raises the possibility that other phosphatases are targeting in this way.

Recent evidence indicates that at least two AKAPs bind and functionally target PP1. PKA and PP1 are both broad specificity signalling enzymes with opposing actions but which often share the same substrates. In order to regulate the level of phosphorylation of these substrates, coordination of kinase and phosphatase activity is essential. AKAP220 was originally identified by interaction cloning as a novel AKAP with peroxisomal location [62]. Sequence analysis revealed that AKAP220 posessed the amino acid sequence KVQF that was consistent with the PP1 binding motif. Surface plasmon resonance and immunoprecipitation studies showed that fragments of AKAP220 containing this motif bound PP1c, and AKAP220, and PP1 were able to be co-purified from rat brain extracts by microcystin affinity chromatography. Evidence for complex formation in vivo was obtained by co-localization studies using confocal microscopy of rat hippocampal neurons [63]. Interestingly, comparable binding fragments from rat and human AKAP220 bound PP1c with 20-fold different binding affinity. Sequence analysis revealed that the substitution of a glycine for a valine in the KV/GQF motif significantly reduces binding, indicating the importance of this motif for PP1 binding. AKAP220 at μmolar concentrations also inhibits PP1c and this involves additional, as yet unidentified, residues [63]. This finding raises the possibility that localized concentrations of AKAP220 could displace other PP1 regulatory subunits to not only alter the subcellular location but also the activity of distinct pools of PP1.

Another AKAP, called Yotaio, also binds PP1c [64]. Yotaio also contains a putative PP1 binding motif, but in this case maintains PP1c in an active conformation. Interestingly, Yotaio also binds the NMDA receptor (a PKA substrate). Functional studies showed that Yotaio anchoring of PKA (inactive), PP1 (active) and the NMDA receptor limits ion flow through the channel by maintaining the dephosphorylation state of the channel. PKA activation by cAMP alters the balance between kinase and phosphatase activity to rapidly enhance the receptor-mediated current [64].

The binding of PKA and PP1 by both AKAP220 and Yotaio is likely to account for only a small proportion of the cellular PKA and PP1. Given the broad overlapping substrate specificity of these two enzymes, it is likely that additional molecules responsible for coordinating specific PKA and PP1 signalling events will be identified.

PKC AND PP2A

Although little is known about the mechanism of regulation of PP2A-mediated phosphorylation by extra-cellular signals, a large number of protein kinases have been shown to be substrates of PP2A or to form stable complexes with it. Thus, signalling which requires control of PP2A may be facilitated in a similar manner to that described for PP1. Binding proteins may exist to achieve a localized level of constitutively active (or inactive) PP2A to ensure the appropriate levels of phosphorylation of substrates, mediated by activation of the kinase present in the same complex. In the context of PKC signalling, PP2A may be required for dephosphorylation of PKC substrates or, indeed, PKC itself. Down regulation of PKC can be a PP2A mediated event [65]. Furthermore, phosphorylation of PKC is a requirement for DAG activation [66] and PP2A dephosphorylation of Thr^{500} and Ser^{660} in PKCβII is necessary to produce a kinase capable of autophosphorylation [67]. Thus, recruitment of PP2A to PKC locations is likely to be necessary for normal signalling through PKC. In-vitro studies have shown that PKC can be dephosphorylated by a PP2A trimer containing the B subunit and a membrane-bound PP2A heterotrimer has been shown to inactivate PKC in COS cells [68]. Although the mechanism or molecule responsible for recruitment of PP2A to the membrane has not been identified, inhibition of PP2A with okadaic acid prevented PKC down regulation [68]. PP2A is traditionally considered to be a cytosolic enzyme. However, in neurons, high levels of irreversibly inactivated PP2A are found associated with the cell membrane [69] and the cytoskeleton [70] such that it is becoming clear that pools of PP2A are also compartmentalized inside the cell. Heterotrimers present in the cell membrane comprise both the B and B′ subunits, which are also present in other locations of the cell. Thus, other molecules are likely to be responsible for membrane targeting of PP2A. In a recent study (Ludowyke et al. unpublished), antigen-mediated secretion of histamine from mast cells was shown to coincide with a transient translocation of PP2A to the cell membrane. Antigen-mediated secretion also requires the translocation of PKC to the membrane and receptor-mediated secretion can be by-passed using PMA and calcium ionophore. Interestingly, PMA and ionophore also cause a transient translocation of PP2A suggesting a close link between not only PP2A translocation and secretion, but also PKC and PP2A translocation. It is possible that, at least in these cells, PKC and PP2A are held together in the same complex by a common anchor, since a direct effect of PMA on PP2A has not been demonstrated.

SUMMARY AND PERSPECTIVES

The discovery of binding proteins of individual kinases and phosphatases has undoubtedly shed important light on the regulation of these individual enzymes. It is clear that a wide range of proteins exist that bind and target subcellular pools of PKA, PKC, PP1 and PP2A in all cells. A great proportion of those that have thus far been

characterized have functional roles associated with binding, demonstrating their importance to cell signalling. The relative concentrations of these enzymes in cells and the multitude of functions with which they are associated indicate that many more targeting proteins await discovery. The characterization of these multi-enzyme signalling complexes, and those yet to be discovered, affords even greater opportunity for understanding cellular signalling events that more often than not require the coordination of different signals using different second messengers. The co-localization of PKA and PKC at different cellular microdomains allows for the control of specific events by different second messengers and may indeed require dual phosphorylation of the same substrate. Identifying molecules responsible for targeting both these kinases and understanding the mechanisms of targeting and binding in relation to cellular functions will undoubtedly lead to a new comprehension of the complexity of signal transduction regulation. Tight control of protein kinase location and activity is meaningless if similarly widespread and broad substrate specificity phosphatases are not also controlled. The evidence that PKA and PKC can be contained within the same complexes as PP1, PP2B or PP2A is a rational outcome but one that also adds to the exquisite complexity that is inherent in cell signalling. The challenge now being faced, not only involves identifying and characterizing further binding proteins for each individual kinase or phosphatase, but also determining how each fits together in bringing together multi-enzyme complexes responsible for distinct signalling events.

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