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ROLE OF PP2A IN INTRACELLULAR SIGNAL TRANSDUCTION PATHWAYS

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1. ABSTRACT

Intracellular signal transduction pathways play a crucial role in a variety of cellular processes, such as differentiation, proliferation, or apoptosis, and the reversible phosphorylation of their components is a major regulatory mechanism to control their activities. While much has been learned about the contribution of kinases, the involvement of phosphatases in these events is less clear and has only recently received more investigative attention. The availability of various natural product inhibitors of phosphatases has helped enormously to gain insight into the role that these enzymes exert in various signal transduction processes. This review will focus on serine/threonine protein phosphatase type 2A (PP2A) and will present findings pertaining to its involvement in cellular signal transduction pathways. Since the majority of these studies were done with the use of phosphatase inhibitory compounds, some pros and cons of their application will be presented.

2. INTRODUCTION

Control of cellular growth and differentiation is a prerequisite for the proper development of higher eukaryotic organisms. Extracellular molecules, such as hormones or growth factors, are important agents in determining this control. The genetic response of cells to these molecules often requires signal receptors, signal transduction (second and third messengers), and usually alterations in the activity of transcription factors which activate or repress target genes. The importance of adequate regulation of these signal transduction pathways has been emphasized by the finding that many protein products of protooncogenes are components of this network. If mutated or inappropriately expressed, they become oncoproteins that are able to cause unrestricted cellular growth and carcinogenesis (1).

A hallmark of these growth-regulatory signal transduction pathways is the reversible phosphorylation of proteins. Many growth factor receptors are kinases; upon binding of their respective ligand, their enzymatic activity becomes activated and initiates kinase cascades that transmit the signal to the nucleus. There, the gene-proximal targets, transcription factors, are being phosphorylated and cause changes in the activity of gene expression. As a consequence, the altered pattern of gene expression will generate the respective phenotypic response, such as, for example, cell proliferation, differentiation, or apoptosis (2, 3).

Many of the (proto)oncogene products are kinases, and it has been demonstrated that this enzymatic activity is an absolute requirement for their normal function, as well as for the process of tumorigenic transformation by these proteins. Similarly, several tumor suppressor gene products are strictly regulated by reversible phosphorylation reactions as well. For example, the retinoblastoma (RB) protein is active (growth suppressive) when it is unphosphorylated, but becomes inactivated by hyperphosphorylation (4, 5).

While the role of protein kinases in the above mentioned processes has been well established, the contribution of protein phosphatases is less clear and has only recently received more investigative attention. However, the finding that some phosphatases are crucial components of pathways that regulate cellular growth and therefore may play a role in the process of tumorigenic transformation, has brought them to the forefront of cancer research (6). So far, only very few phosphatases have been directly implicated in the etiology of tumors: the dual-specificity protein

Table 1. Inhibition of Phosphatases by Okadaic Acid

Phosphatase[3]	IC50 (nM)	References
PP1	20-100	(11, 13-15)
PP2A	0.1-1.0	(11, 13, 14)
PP2B	>5000	(16, 17)
PP2C	no[1]	(16, 17)
PP3	3.0-5.0	(18)
PP4	0.2	(19)
PP5	<1.0	(20)
PP6	n.d.[2]	(21)
PP7	no[1]	(22)

[1] no inhibition, [2] not determined, [3] two novel type 2C protein phosphatases, wip1 and FIN13, are insensitive to okadaic acid (23, 24)

Table 2. Inhibition of PP2A by Natural Product Inhibitors

Inhibitor	IC50 (nM)	References
Okadaic Acid	0.02-2.0	(11, 14-16, 30)
Calyculin A	0.25-7.3	(14-16, 30)
Nodularin[1]	0.03-1.0	(14, 31, 32)
Microcystin-LR[1]	0.04-2.0	(14, 33-35)
Tautomycin	10-23.1	(14, 33, 36, 37)
Fumonisin B1	$3x10^5$	(38)
Cantharidin	160	(39, 40)
Thyrsiferyl[2]	$4\text{-}16x10^3$	(41)
Motuporin	0.1	(42)
Fostriecin	3.0-40	(43, 44)

[1] not cell permeable; liver cells appear to have an uptake system capable, of transporting this compound, [2] Thyrsiferyl-23-acetate

phosphatases CDC25A and CDC25B, which are able to transform cells in culture (7), and thus can be classified as oncogenes; the dual-specificity protein phosphatase PTEN (MMAC1) which is frequently found mutated or deleted in advanced cancers and behaves like a tumor suppressor gene (8). A further important regulator of signal transduction and cell growth is the serine/threonine protein phosphatase type 2A (PP2A) which will be discussed in detail below.

3. NATURAL PRODUCT INHIBITORS OF PHOSPHATASES

One compound that has proven to be extremely useful for the study of gene regulation by phosphatases is okadaic acid, a complex polyether derivative of a 38-carbon fatty acid. It is synthesized by marine dinoflagellates and accumulates in filter feeding organisms such as shellfish or the black sponge Halichondria okadaii from which it was first isolated (9, 10). Okadaic acid is a recognized threat to human health through its ability to cause diarrhetic shellfish poisoning. Its only cellular targets that could be identified so far are certain members of the serine/threonine protein phosphatase family, including PP2A (see table 1). Okadaic acid binds to the catalytic subunit and inhibits its enzymatic activity (11). Because of this specific repression of phosphatase activity, okadaic acid quickly became a ubiquitous tool to investigate the cellular functions of the respective okadaic acid-sensitive phosphatases (12).

Okadaic acid inhibits different phosphatases differentially, i.e. the concentration of the drug that inhibits phosphatase activity by 50% (IC50) varies greatly among the different members of this enzyme family (see table 1). Cellular effects that are observed in response to low concentrations of okadaic acid are often contributed to the inhibition of PP2A, as this particular phosphatase is inhibited at subnanomolar concentrations of the drug. However, this conclusion could be misleading, because there are other, less abundant phosphatases that are also affected by low okadaic acid concentrations, such as PP4 and PP5 (see table 1). Furthermore, PCR analysis indicated there are more phosphatases of this type yet to be discovered (25). Therefore, the cellular or molecular consequences of okadaic acid treatment, even at low concentrations, cannot be ascribed unequivocally to the inhibition of one particular phosphatase (12, 26).

In addition to okadaic acid, several other naturally occurring compounds have been found that are also able to inhibit phosphatase activity (see table 2). Although these inhibitors constitute a structurally diverse group of toxins that are produced by different organisms, computational analysis revealed that many of them possess similar three-dimensional motifs that are involved in binding to the phosphatase catalytic subunit (14, 27, 28). As is the case with okadaic acid, the inhibitory potency of the different compounds varies greatly among the different types of phosphatases. For example, the IC50 of tautomycin is approximately 10-fold higher for PP2A than for PP1, whereas the reverse is true for microcystin; fostriecin inhibits PP2A >10,000-fold more potently than PP1 (see refs. in table 2). Because of these differential effects, the combinatorial use of various phosphatase inhibitors may prove helpful to further narrow the list of candidate phosphatases that may be involved in the cellular processes under investigation (29).

It has to be kept in mind, however, that there is significant variation among the published IC50 values, which are dependent on the concentration of phosphatase as well as on the type of substrate used (17). Furthermore, these values are derived from measurements performed in vitro, i.e. by the use of cellular lysates or purified enzymes. To study the role of phosphatases in signal transduction pathways and gene regulation, these inhibitors need to be added to cells in culture. In this case, however, the efficient concentrations are significantly higher, and therefore the IC50 values do not reliably apply to these cell culture conditions. For instance, the published IC50 for okadaic acid is around 1 nM with respect to PP2A activity in vitro (when added to diluted cellular lysate) (11, 15, 30), whereas in cell culture (when added to growing cells) the IC50 was found to be 30 nM and nearly 1 microM for NIH3T3 fibroblasts and MCF-7 breast cancer cells, respectively (45, 46).

Another caveat has to be considered when interpreting results obtained with the use of phosphatase inhibitors. It cannot be completely excluded that these

compounds exhibit certain effects on cellular processes due to their potential interaction with yet unknown, non-phosphatase targets. Okadaic acid has been extensively studied, and no other cellular targets have been identified so far. However, some of the other phosphatase inhibitors do affect the activity of non-phosphatase proteins; most notably fostriecin, which inhibits partially purified type II topoisomerase, although at much higher concentrations (47).

Potential unknown targets of phosphatase inhibitors might contribute to the paradox that some of these compounds have tumor promoting activity, whereas others exhibit antitumor activity. Alternatively, or in addition, it may be of importance which combination of the various phosphatases is targeted by a certain inhibitor. Okadaic acid, calyculin A, microcystin-LR, and nodularin are potent tumor promoters or liver carcinogens (48-52), whereas tautomycin has not been found to promote tumors on mouse skin or rat glandular stomach (37). Moreover, fostriecin, cantharidin, and cantharidin derivatives have demonstrated antitumor properties (40, 53-55). Fostriecin in particular exhibits antitumor activity against a wide spectrum of tumor cells in vitro, and is under evaluation as an antitumor drug in clinical trials (56-58).

4. SIGNAL TRANSDUCTION PATHWAYS AFFECTED BY OKADAIC ACID

Originally, it was suspected that the tumor promoting activity of okadaic acid was due to its regulation of the same pathway that is affected by the well-established phorbol ester tumor promoters, namely the protein kinase C (PKC) pathway (50, 59, 60). The idea was that activation of PKC by phorbol esters initiated a kinase cascade that lead to the increased phosphorylation/activation of various downstream components of this signal transduction pathway. Similarly, okadaic acid, through the inhibition of phosphatases that dephosphorylate/inactivate the same components, would generate the same net effect, namely the increased activity of this pathway. Further support for this hypothesis was provided by the finding that several growth-regulatory genes that are activated by the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) are also activated by okadaic acid (see detailed references in (12)). Most prominent among these genes are the proto-oncogenes c-fos and c-jun (61-64), whose protein products are able to interact and form the heterodimeric transcription factor AP-1 (65). And indeed, either agent, TPA or okadaic acid, causes increased AP-1 expression and activity (66-70). (For a detailed list of growth-regulatory genes that are regulated by okadaic acid, see ref. (71)). Activation of the c-fos gene is regulated through the reversible phosphorylation of ternary complex factor (TCF) by kinases of the mitogen-activated protein kinase (MAPK) family, and both agents, TPA and okadaic acid, stimulate TCF via the activation of the MAPK pathway (72). There are further examples of transcription factors that are activated by TPA and by okadaic acid. For example, the activity of nuclear factor kappa B (NF-kappaB) is stimulated by either agent (73, 74). Activation of NF-kappaB requires the degradation of protein inhibitors, IkappaB-alpha and IkappaB-beta, which is induced by the hyperphosphorylation of these proteins. It has been demonstrated that treatment of cells with okadaic acid or calyculin A results in the increased phosphorylation and subsequent degradation of these NF-kappaB inhibitors (75, 76). In the case of IkappaB-alpha, increased phosphorylation appears to be mediated through the (indirect) activation of ERK1, a member of the MAPK family, in response to okadaic acid treatment (76). Activation of the MAPK pathway by okadaic acid has been described by several groups (77-79).

However, while several cellular responses to drug treatment have been documented that are similar between okadaic acid and phorbol esters, there are also numerous differences. For example, using high definition two-dimensional gel electrophoresis, Guy et al. (80) identified 74 proteins that exhibited altered levels of phosphorylation in response to okadaic acid treatment of human fibroblasts. However, when the same cells were treated with TPA, a rather different pattern of protein phosphorylation was found. Moreover, in other studies physiological differences between okadaic acid-generated and TPA-generated transformed cells have been reported (61, 81).

In addition, depending on the experimental approach, TPA and okadaic acid are able to antagonize each other's effects. For instance, it has been well documented that okadaic acid is able to induce apoptosis in various primary as well as transformed cells (82-85). In Balb/c 3T3 fibroblasts, this effect was found to be dependent on the presence of the p53 tumor suppressor protein (86), whereas in human breast carcinoma cells p53 function was not found to be required for this process (87). Further, in H-ras oncogene transformed cells okadaic acid-induced apoptosis appeared to involve the modulation of raf-1, PKC, and MAPK activities (88). Interestingly, the simultaneous treatment of certain breast cancer cell lines with okadaic acid and TPA has been shown to greatly diminish the induction of apoptosis by okadaic acid (89). Similarly, in THP-1 meyloid leukemia cells, cell death induced by okadaic acid is strongly reduced in the presence of TPA (90).

The various cellular responses to treatment with phosphatase inhibitors can differ enormously, and sometimes even yield contradicting results, which likely is due to variations in the length of treatment, the applied concentration, or the cell type used. In this regard, it was observed that okadaic acid, calyculin A, and cantharidin were able to prevent apoptosis in short term, but not in long term experiments (91). Similarly, the level of phosphorylation of the retinoblastoma (Rb) tumor suppressor protein, an important regulator of cell cycle progression, is affected differentially by okadaic acid, depending on the time of incubation and concentration of the drug that is used (92-95). Moreover, several groups have reported okadaic acid as an inhibitor of transformation in different in vitro transformation assays (96, 97), which is in contrast to those reports discussed further above that established this compound as a potent tumor promoter. Whereas the details of these discrepancies remain to be investigated, the above data argue against the view that TPA

and okadaic acid exert their effects through two sides of the same coin, i.e. the phosphorylation versus the inhibition of dephosphorylation of the same substrates of signal transduction pathways.

5. PP2A AS A TUMOR SUPPRESSOR

The finding that okadaic acid is a tumor promoter, combined with the observation that this compound efficiently inhibits PP2A, has led to the suggestion that PP2A, and potentially some other phosphatases, may function as tumor suppressors (60). It is thought that the tumor suppressing function could be accomplished by the enhanced dephosphorylation of activated kinase cascades, which would revert oncoprotein-activated signaling pathways back to their inactive state. As many oncogene products cause the sustained activation of growth-regulatory kinase cascades, it is a distinct possibility that increased serine/threonine protein phosphatase activity might counteract elevated levels of protein phosphorylation and block cellular transformation (48).

Further support for such a negative role of phosphatases in growth-regulatory signal transduction pathways came from observations that treatment of cells with okadaic acid led to the increased expression of several proto-oncogenes (see detailed references in (12)). Elevated expression of such genes had been shown before to contribute to cellular transformation (1). Thus, these results suggested that the respective okadaic acid sensitive phosphatases contribute to the repression of these proto-oncogenes in normal cells.

Moreover, when added to cells synchronized in the S phase of the cell cycle, okadaic acid caused an increase in the enzymatic activity of cyclin-dependent kinase (histone H1 kinase), an enzyme that is necessary for cell cycle progression. The drug also stimulated premature mitosis and increased the phosphorylation of mitosis-specific proteins (see detailed references in (12). When added at low concentrations, okadaic acid caused quiescent fibroblasts to progress to S phase of the cell cycle (98); and in thyroid cells, the drug increased the fraction of thyrotropin-stimulated quiescent cells entering S phase (99). Further, a link between protein phosphatases and cellular transformation has been established by the finding that small DNA tumor viruses, such as Simian virus 40 (SV40) and Polyoma virus, synthesize proteins (small and medium T antigens) that bind to and inhibit PP2A (100). The currently available evidence indicates that alteration of phosphatase activity and subsequent changes in phosphorylation levels is a crucial step in transformation by these viruses (55, 100). Another viral protein, E4orf4 of Adenovirus, has been found to associate with PP2A as well (101).

Further evidence for the involvement of phosphatases in the neoplastic process has been provided by the finding that certain cis-platin resistant human cancer cells are resistant to growth inhibition by okadaic acid, and exhibit increased phosphorylation of certain nuclear proteins (102, 103). Resistance to okadaic acid has also been observed in cells that exhibit the multidrug resistance (mdr) phenotype (104-108). Mdr cells were established by chronic exposure of cells to increasing concentrations of okadaic acid. In this case, two different mechanisms were found to generate the mdr phenotype.

First, mdr cells had amplified the gene encoding the 170-kDa P-glycoprotein, which is a drug efflux pump with broad specificity, i.e. it is capable of extruding intracellular anticancer agents of diverse structures and mechanisms of action (105, 106). Consequently, these cells were not only resistant to okadaic acid and related phosphatase-inhibitory compounds, but also to other structurally unrelated anticancer drugs, such as vinblastine, taxol, or cisplatin. In addition, the activity of P-glycoprotein appears to be regulated via its phosphorylation status, which is increased in response to treatment of cells with okadaic (109). It should be noted, however, that the increased activity of the P-glycoprotein pump could not be demonstrated in all okadaic acid resistant cell types; in some cells no differences could be found in the accumulation or efflux of okadaic acid between drug resistant and normal cells (104, 107, 110).

A second mechanism that was found to generate okadaic acid resistant cell lines is the mutational alteration of the PP2A catalytic subunit. Upon sequencing of the PP2A gene from okadaic acid resistant hamster cells, a point mutation was found that resulted in the exchange of cysteine 269 for glycine (111). This mutation resulted in a PP2A protein that was much more resistant to inhibition by okadaic acid than the wild type protein. Further mutational analysis of this region established that the amino acids 265 to 269 are critical for inhibition of PP2A by okadaic acid (112). Mdr cell lines that harbored such a mutation of PP2A were found to express the mdr phenotype in a stable fashion. In contrast, cell lines without PP2A mutations but with amplified P-glycoprotein, tended to loose the mdr phenotype after cessation of long-term drug exposure (110).

In addition to gene amplification, the treatment of cells with okadaic acid has also generated other genetic changes in cultured cells, such as mutations endowing diphtheria-toxin resistance, sister chromatid exchange in the presence of bromodeoxyuridine, loss of exogenous transforming oncogenes, and minisatellite mutations (26, 113-116). Although the molecular mechanisms underlying this genotoxic activity of okadaic acid have not been elucidated, it is suspected that the alteration of the phosphorylation status of cellular proteins, and the resulting changes in the gene expression pattern, might be a crucial epigenetic event contributing to these processes. In this regard, it is important to note that okadaic acid induces elevated and sustained expression of the c-fos proto-oncogene (63, 105). Since c-fos has been shown to increase the spontaneous level of chromosomal aberrations (117-119), it is conceivable that okadaic acid may stimulate these processes through its continuous activation of c-fos expression. Furthermore, since okadaic acid exerts its effect on c-fos expression through inhibition of PP2A (120, 121), one could envision that PP2A, through its negative effects on the c-fos gene, may contribute to the maintenance of genomic integrity. Thus, these observations give further

credence to the idea that PP2A indeed may act as a tumor suppressor.

6. EXPERIMENTALLY INCREASED PP2A ACTIVITY

The down-regulation of phosphatase activity by the use of various natural product inhibitors has yielded lots of preliminary insight into phosphatase function, although this approach has been rather restricted due to the simultaneous effects on several different enzymes (see further above). A different avenue has been pursued with the use of the small tumor (T) antigens of polyomavirus or SV40. These proteins have been found to form stable complexes with PP2A and reduce its enzymatic activity (55, 100, 122), and therefore have served as rather specific tools to analyze in more detail the involvement of PP2A in signal transduction pathways. For example, introducing SV40 small T antigen into cells has helped establish a negative role for PP2A in the regulation of the mitogen activated protein (MAP) kinase pathway (78, 123). (The details of these interactions will be discussed elsewhere in this volume and thus will not be pursued here.)

The opposite experimental approach, the stably increased expression of selected protein phosphatases, would also be helpful to further study their role in signal transduction pathways. However, these types of studies have proven difficult to accomplish. Although it has been shown in mammalian cells that PP2A can be efficiently expressed after transfection of an expression vector containing the cDNA of the catalytic subunit of PP2A (124), there appears to be a potent autoregulatory mechanisms that keeps the overall amount of PP2A protein (catalytic subunit), as well as its enzymatic activity, at constant levels (125). As a consequence, it seems very difficult to establish cell lines with significantly increased overall activity of PP2A.

The observed autoregulatory control of PP2A expression may also provide an explanation for earlier seemingly contradictory findings by others who investigated PP2A expression. For example, in various mammalian cells and in fission yeast it has been shown that the level of PP2A protein remains constant throughout the cell cycle (126-128). In contrast, analyzing the amount of mRNA, others have demonstrated increased PP2A mRNA levels during the early stages of G1 in mammalian cells (129, 130). Moreover, Kakinoki et al. (131), by performing partial hepatectomy, presented evidence of almost constant levels of PP2A protein in regenerating liver, despite a 30-fold increase in PP2A mRNA. These observed discrepancies between elevated mRNA levels and rather constant protein levels can now be explained by the finding of a potent autoregulatory mechanism of PP2A synthesis that appears to work at the level of translation (125). In addition, in human keratinocytes a post-translational level of PP2A regulation was suggested (132).

In order to neutralize and overcome the autoregulatory feedback loop of PP2A expression, different experimental approaches may be useful. For example, microinjection studies have been used to introduce into cells various components of the phosphatase holoenzyme (121, 133). It is likely that in these experiments the large amount of microinjected protein is able to overwhelm any autoregulatory mechanism, at least for a short time, and induce the respective cellular responses. Experiments of this type, although limited by the small number of cells that can be used per experiment, have indeed provided valuable insights into phosphatase function (121, 134-136).

A further strategy to manipulate PP2A activity in cells was recently presented by Ruediger et al. (137). These authors generated an N-terminal mutant of the regulatory A subunit that was able to bind to the catalytic C subunit, but not to the regulatory B subunit. Expression of this A subunit mutant in cells resulted in an increase in the amount of PP2A core protein (A-C heterodimers) and a decrease in the amount of PP2A holoenzyme (B-A-C heterotrimers). Concomitantly, the relative activity of PP2A towards two different substrates, phosphorylase-a and a retinoblastoma (Rb) peptide, was altered. In the case of phosphorylase-a, PP2A activity was slightly stimulated, whereas with Rb peptide a significant inhibition of phosphatase activity was obtained (137). This differential effect of PP2A enzymatic activity generated consequences at the molecular and cellular level as well: transcription from the human immunodeficiency virus (HIV-1) long terminal repeat (LTR), as well as virus production, was inhibited in these cells (137). Thus, these results demonstrated that the manipulation of PP2A subunits, other than the catalytic C subunit, may be a useful experimental approach to manipulate PP2A expression and activity in living cells.

7. INTERACTION OF PP2A WITH OTHER REGULATORY PROTEINS

The involvement of PP2A in intracellular signal transduction pathways can be inferred most directly from experiments that establish the interaction of this enzyme with other cellular regulatory components. For example, most recently it was found that the trimeric PP2A holoenzyme can form a stable complex with Ca^{2+}- calmodulin-dependent kinase IV (CaMKIV) (138). In this interaction, PP2A serves to ensure the transient nature of CaMKIV activation: after the kinase has phosphorylated its substrates, CaMKIV activity is down-regulated by PP2A. Thus, this association of a phosphatase with a kinase, where PP2A functions as the negative regulator of CaMKIV, allows tight control of the corresponding signal transduction pathway.

In other studies, PP2A has been shown to interact with casein kinase 2alpha (CK2alpha) in mitogen starved cells (139). CK2alpha was found to negatively regulate the activity of the mitogen-activated protein kinase (MAPK) pathway. This inhibition appears to be mediated through the phosphorylation/activation of PP2A by CK2alpha, and the subsequent dephosphorylation/deactivation of MAPK kinase (MEK) by PP2A (78, 139). Because the MAPK pathway is one of the major growth regulatory pathways, these findings provide yet another example of the apparently crucial role of PP2A in cellular growth control.

Several other cellular signaling proteins have been identified that interact with PP2A (140-142). Notably HOX11, a homeo box gene product which is able to transform cells, associates with the catalytic subunit of PP2A (143). This interaction is thought to affect alterations of cell cycle progression induced by HOX11. In other studies, the translation termination factor eRF1 (eukaryotic release factor 1) has been found complexed with PP2A, which may serve to bring the phosphatase into contact with putative targets among the components of the translational apparatus (144).

In addition, the recruitment of PP2A to signal transduction pathways and other regulatory events may be regulated through its B-type subunits, which may add yet another layer of control and specificity. In this regard, it has been found that the B'alpha and the B'beta subunits of PP2A form complexes with cyclin G, a protein whose expression is regulated by the tumor suppressor p53 (145). Because induction of p53 protein appears to be required for this complex formation, the existence of specific cross-talk between PP2A and p53-mediated pathways has been suggested (145).

8. CONCLUSIONS

Great strides have been made towards a more complete understanding of phosphatase function in cellular signal transduction. However, because in most studies okadaic acid or other phosphatase inhibitors were used, it was often not possible to unequivocally establish which of the various drug-sensitive phosphatases was responsible for the observed effects. Rather, a combination of various approaches is necessary in order to characterize the precise roles of phosphatases in signal transduction pathways. For example, biochemical and immunological analysis together with cellular studies may be most useful. Moreover, the recent discoveries of complexes consisting of a kinase and a phosphatase suggest a tight coupling of activators with their respective inactivator; thus, the analysis of this type of interaction may lead the way to further unravel the contribution of various phosphatases to the regulation of cellular signal transduction pathways.

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