Second messenger systems and adaptation

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Adaptive changes represent shifts in the equilibrium state of biologic systems in response to long standing alterations in their surrounding environment. Adaptation encompasses a variety of cellular changes ranging from very specific responses, such as variation in the production of a single protein, to broader responses including alterations in the rate of cell growth. It is certain that the effectors for adaptive responses involve a complex and multilevel cellular network. As indicated by Figure 1, it can be anticipated that these effectors will include various ligands and their receptors, signal transduction pathways, and transcriptional and translational mechanisms. Our current understanding of each of these components is growing, and warrants a consideration of potential mechanisms whereby the signals for adaptive responses may be transmitted to their intracellular targets.

Adaptation appears to be governed by two general types of transduction mechanisms. The first acts through the modulation of traditional second messenger pathways known to be directed by calcium, lipids, and cyclic nucleotides. A second type of

transduction system is typified by highly specific ligand receptor interactions which appear to activate unique transduction pathways. It is anticipated that changes in a protein kinase or phosphatase activity will often represent the final common pathway for both of these systems. A comprehensive review of both potential transduction systems or detailed discussions of the various targets of the transduction systems is beyond the scope of this paper. Therefore the following will focus on the factors which influence the traditional transduction pathways.

There are two important emerging features of the traditional transduction pathways which relate to the modulation of their activity. First, each pathway shows various patterns of autoregulation. Second, the various transduction pathways directly interact at a number of important modulatory steps. Before considering these types of regulatory activities in detail, the salient features of the traditional transduction pathways will be reviewed.

Traditional transduction pathways

Three types of second messenger systems appear to be ubiquitous and include those activated by calcium, lipids, and cyclic nucleotides (Fig. 2). The cellular pathways responsible for the generation of each of these second messengers and their mechanisms of coupling to receptors are currently being elucidated. As discussed below, the generation of the primary calcium signal and the release of neutral lipid and arachidonate are closely linked to the breakdown of phosphatidyl inositol (PI), while the increase in cyclic nucleotide levels involves the stimulation of adenylate cyclase. Some of the most important investigative advances regarding these two pathways have been made over the past several years.

Pathways involved in phosphatidyl inositol breakdown

Recent studies by a number of workers, most notably Berridge and his collaborators,¹ have helped to decipher the biologic importance of the PI pathway as first observed by Hokin and Hokin.² The breakdown of PI, mediated through the action of a series of

Fig. 1 Schematic representation of the sequence of events which result in an adaptive response. In some systems, the transduction elements may directly act on translational events to effect an adaptive response.

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Fig. 2 Summary of the basic elements of the second messenger pathways. Receptor ligand interaction can alter the activity of the pathways which control the levels of adenylate cyclase or PI turnover. The regulation of adenylate cyclase is mediated through a group of associated proteins termed G proteins (AC refers to the adenylate cyclase and G proteins). The G proteins can have either a stimulatory or inhibitory effect on the adenylate cyclase. The products of PI degradation can influence the messenger levels for three pathways. These messengers include inositol 1,4,5-trisphosphate (IP₃), neutral lipids (DAG), and icosanoids. Although a GTP binding protein(s) can stimulate the breakdown of PI, its relationship to the adenylate cyclase system is unclear as implied by the dashed line. Abbreviations: AC-adenylate cyclase system including G proteins; PI-phosphatidylinositol; DAG-diacylglycerol; IP₃-inositol 1,4,5-trisphosphate; AA-arachidonate.

phospholipase C's and kinases, results in the generation of three important messengers. These include neutral lipid, inositol-trisphosphate (IP₃), and arachidonic acid.³⁴

As indicated in Figure 3, neutral lipid activates protein kinase C (C-kinase) by increasing its affinity for calcium.⁵ Although phorbol esters can substitute for neutral lipids in C-kinase activation,⁶ 'caution has to be exercised in making conclusions about the physiologic role of C-kinase based on this effect for the following reasons. First, phorbol esters have much longer half-lives than the neutral lipids generated by PI turnover. Second, cells exposed to phorbols may develop an unusual subcellular distribution of C-kinase and down regulate the enzyme.⁷ The physiologic role of C kinase is not completely understood in any system. Nonetheless, studies involving the action of some transforming oncogenes on PI turn-

Fig. 3 Summary of the primary effectors of the second messenger pathways. While neutral lipid appears to stimulate only C-kinase, two types of cAMP dependent protein kinase are recognized. In contrast, the Ca²⁺/calmodulin system can stimulate a variety of protein kinase activities (see text).

over,⁸ the influence of phorbol esters on cell transformation,⁹ and effect of C-kinase on the EGF receptor¹⁰ and transferrin receptor,¹¹ offer central role for C-kinase activation in some adaptive responses.

The second important messenger generated from the PI pathway is IP₃. This soluble product is generated immediately after ligand-receptor interaction, and appears to be responsible in large part for the generation of the cytosolic calcium transient seen in many systems.³⁴ IP₃ appears to stimulate the release of calcium from a vesicular, non-mitochondrial pool¹² through the activation of calcium conductive pathway and calcium-potassium exchange.¹³

The calcium transient stimulated by IP₃ is likely to be responsible for the activation of a number of enzymatic activities through the modulatory effects of the calcium binding protein, calmodulin. Among the most important groups of enzymes regulated by calcium/calmodulin are protein kinases. Two general classes of calcium/calmodulin regulated protein kinases are recognised. Included are a group showing a broad substrate specificity – for example, Type II calmodulin kinase and a group with narrow substrate specificity – for example, myosin light chain kinase, phosphorylase kinase. An important property of several calmodulin regulated enzymes relative to their sustained response to a calcium transient is that of hysteresis or up regulation.¹⁵ Up regulation, manifested by a decreased calcium requirement for enzyme activation, appears to occur through at least two different mechanisms. First, calmodulin may increase its affinity for calcium after filling its first two calcium binding sites and binding to its target molecule.¹⁶

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Second, the catalytic activity of calcium/calmodulin regulated enzymes may become calcium independent following activation as described below.

The third messenger system influenced by PI breakdown involves the generation of icosanoid mediators which are dependent on the availability of arachidonate. Because most tissue systems appear to have the ability to generate arachidonate through the action of phospholipase A2 on phosphatidylcholine or phosphatidylethanolamine,⁹ the relative contribution of PI breakdown is unclear. The mechanism of action of many of the prostaglandins, and arachidonate itself, is currently being elucidated. While some members of this group clearly alter cyclic nucleotide metabolism, a recent investigation suggests that arachidonate has the ability to stimulate a transient increase in cytosolic calcium by a mechanism that differs from that described for IP3.¹⁷ Thus, the various members of the icosanoid family may influence several second messenger pathways.

A growing body of information suggests that GTP binding proteins may influence PI turnover (Fig. 3). In cell-free systems, a non-hydrolysable GTP analogue stimulates the breakdown of PI through the activation of a phospholipase C.¹⁸ Moreover, micro-injection of this GTP analogue into intact sea urchin eggs results in the stimulation of calcium mediated exocytosis.¹⁹ Although these findings support close relationship between GTP proteins and PI hydrolysis through phospholipase C activation, in most instances the identity of the GTP protein is unknown. Therefore, a direct link from adenylate cyclase to PI hydrolysis is regarded as tentative (Fig 3).

Pathways involved in the generation of cyclic nucleotides

The adenylate cyclase system consists of a group of proteins which are responsible for the generation of cyclic nucleotides. This complex is directly coupled to receptors which stimulate or can inhibit cyclase activity. The G proteins of the cyclase system appear to influence the affinity of receptors for ligand and may be directly involved in the process of receptor desensitisation.²⁰ Adenylate cyclase has three primary components: a stimulatory subunit, termed G_s , an inhibitory subunit known as G_i , and a catalytic subunit.²¹ G_i and G_s contain three similar peptide subunits.²¹ Although their precise mechanisms of action remain controversial (and may differ in various tissue systems), several pharmacologic probes are used to dissect the roles of the various components of the cyclase system. Pertussis toxin catalyses the ADP-ribosylation of G_i which in turn appears to decrease receptor affinity for inhibitory ligands. The net result of these actions is an increase in the sensitivity of the

cyclase system to stimulatory agonists.²² Cholera toxin stimulates the ADP-ribosylation of G_s , thereby decreasing the affinity of G_s for G_i . This reaction appears to remove inhibitory constraints on G_s by G_i , making the cyclase system more sensitive to stimulation ligands.²³ Finally, the plant derived peptide, forskolin, directly stimulates the catalytic portion of the adenylate cyclase system, bypassing the regulatory effects of the G proteins.²¹

Autoregulation of individual transduction pathways

The transduction pathways acting through calcium, neutral lipids, and cyclic nucleotides have all shown the potential to be autoregulatory. Three patterns of autoregulation are evident. These include autonomy, modulation of the second messenger signal, or modulation of the transduction effector (in this case protein kinase levels). It must be emphasised that these autoregulatory properties can only be viewed as potential physiologic mechanisms in that most have only been produced *in vitro* or demonstrated through pharmacologic perturbations.

Type II calmodulin kinase shows a regulatory pattern consistent with autonomous autoregulation. Soon after its discovery, this enzyme was found to undergo calcium/calmodulin stimulated autophosphorylation. This phosphorylation event is associated with several changes in the properties of the enzyme including a change in the affinity of this enzyme for cytoskeletal elements²⁴ or calmodulin,^{25,26} and the regulation of the catalytic activity of this kinase.^{27,29} The effects of autophosphorylation on the catalytic activity of this enzyme are summarized in Figure 4. Brief autophosphorylation of type II calmodulin kinase results in an enzyme that retains its catalytic activity in the absence of calcium. In contrast, prolonged autophosphorylation of the kinase is associated with a marked decrease in its catalytic activity and insensitivity to calcium/calmodulin. Although the mechanism of the down regulation of the enzyme associated with prolonged autophosphorylation is uncertain, Kennedy and her coworkers observed that this form of the enzyme can be returned toward its original activity by treatment with phosphatase.²⁷ These data suggest that autophosphorylation of type II calmodulin kinase may have a central role in the activation and inactivation of this enzyme. Because the autophosphorylation of type II calmodulin kinase is an intramolecular event and independent of the concentration of kinase, it has the potential to occur in systems which contain only small amounts of the enzyme.

Although protein kinase regulation by autophosphorylation has been shown,^{30,31} the widespread tissue distribution and substrate recognition of the type II

Fig. 4 Effect of autophosphorylation on the activity of calmodulin kinase II. In the native state, the kinase can be stimulated at least 10 fold by the addition of Ca^{2+} and calmodulin. Brief autophosphorylation, followed by calcium removal, dramatically augments basal activity. In contrast, prolonged autophosphorylation is associated with diminished basal activity and insensitivity to Ca^{2+} and calmodulin (down-regulation). Treatment of the down-regulated enzyme with phosphatase returns it toward its highly active, Ca^{2+} -independent state. Abbreviations: Pk – protein kinase; CAM – calmodulin. Redrawn from reference with permission.

Fig. 5 Summary of the three potential patterns of calmodulin kinase II activation in response to a Ca^{2+} transient. The stimulated activity may remain at a continuous high level (a), or gradually or rapidly decay (b and c, respectively). Based on the activation/inactivation requirements shown in Figure 4, the pattern of kinase activity followed in a particular system will represent a balance between the duration of autophosphorylation and the action of phosphatases on the autophosphorylated enzyme.

calmodulin kinase makes the property of autoactivation one of particular interest and importance. This pattern of selfactivation suggests that the type II calmodulin kinase may only require a transient increase in calcium to be brought to its full catalytic potential. Furthermore, the duration of cellular activation of type II calmodulin kinase will likely be a balance between further autophosphorylation of the enzyme and the action of phosphatases on the kinase

(Fig. 5). A theoretical model of how a similar system might function to convert a transient signal into a long-lasting response has been proposed.³²

Unfortunately, there is little evidence that type II calmodulin kinase undergoes autophosphorylation *in situ*. In order to address this question, our laboratory has examined exocrine pancreas for evidence of type II calmodulin kinase autophosphorylation. In unpublished investigations we find the phosphorylation of a M_r 51 000 calmodulin binding protein (corresponding to the subunit of type II calmodulin kinase) is stimulated by the addition of neurohumoral agents. Currently our efforts are directed at determining whether this represents autophosphorylation of the type II calmodulin kinase and correlating these findings with the activity of the enzyme.

A second pattern of messenger modulation is present in the cAMP pathway. The cellular levels of cAMP represent a balance between its generation by adenylate cyclase and its degradation by phosphodiesterase. Wang and his coworkers find the M_r 60 000 subunit of brain phosphodiesterase is a substrate for cAMP dependent protein kinase.³³ This phosphorylation event results in a prominent decrease in the activity of the phosphodiesterase. The net result of this event is amplification of the cAMP signal.

A third type of modulatory event is observed with C-kinase in several systems. Phorbol esters activate C-kinase *in vitro*.⁶ The effects of phorbol esters in intact systems is likely much more complex. Not only do phorbols influence phosphorylation events unrelated to C-kinase,³⁴ they exert a biphasic effect on C-kinase activity. Short term exposure to these agents is associated with activation of C-kinase. In contrast, Kyo and his coworkers find that long term exposure to phorbol esters may markedly diminish C-kinase activity.⁷ The latter effect is associated with a dramatic decrease in the cellular levels of C-kinase. Thus, the duration of exposure to phorbol esters may influence an increase or a decrease in the cell content and activity of C-kinase.

Interactions of transduction pathways

In addition to the abilities of transduction pathways to modulate their own transduction systems, they may influence the levels of second messengers in other pathways. These modulatory effects may alter the levels of second messenger or change the temporal relationship between stimulation and the cellular response. A survey of the Table suggests that the pathways may interact at steps which generate the messengers – for example calmodulin stimulates adenylate cyclase to increase cAMP levels, or at steps involved in the termination of the signal – for example, calmodulin stimulates phosphodiesterase to

Table Interactions between second messenger pathways

Target	Modulator	Result
Cyclic nucleotide PDE	CaM	increased activity ³³
Cyclic nucleotide PDE	cAMP-PK	decreased activity ³³
Cyclic nucleotide PDE	CaM-PK	sl decreased activity ³⁵
Cyclic nucleotide PDE	CaM-phosphatase	increased activity ³³
Adenylate cyclase	CaM	activation ³⁶
IP3 phosphatase	C-kinase	activation ³⁷
Calcium channel	cAMP-PK	opens channel ³⁸
Phospholipase C	G-protein	PI hydrolysis ¹⁸
Arachidonic acid	PI hydrolysis	calcium transient ¹⁸

decrease cAMP levels). As can be seen from the previous example, a second messenger can conceivably exert opposing modulatory effects on another messenger pathway. The outcome of these potential modulatory effects will depend on several factors. First, the absolute amount and relative abundance of the regulatory mediators may vary between tissues. For example, it is unclear whether all tissues have calmodulin sensitive phosphodiesterase and calmodulin stimulated adenylate cyclase activities. Second, the isozymic forms of these enzymes and their sensitivity to cofactors (in the previous example, calmodulin) may differ among tissues. Therefore, while the Table outlines a series of intriguing potential interactions between the transduction pathways, the importance of these interactions relative to the control of second messenger levels is still poorly understood.

This work was supported by a donation in the memory of Cecil Hinkel and NIH grant AM31506.

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