

Protein kinase C: Ports of anchor in the cell

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The cytoplasm is afloat with highly specialized proteins that have defined chores in signal transduction.

Distinguishing among the myriad signals transduced by members of the protein kinase C family may be helped by specific anchoring of these enzymes to scaffold proteins that localize particular signalling cascades.

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Nature has chosen a remarkable array of 'motifs' to ensure that the right enzymes are in the right place at the right time. Precision of placement is particularly critical in signal transduction, where background noise must be kept to a minimum as second messengers navigate through the forest of kinases and phosphatases whose uncontrolled activation would wreak havoc on the cell. Invaluable insight into how specific motifs mediate precise targeting has come from the identification, biochemical characterization, and structural elucidation of modules such as SH2 (Src homology 2), SH3, C2, LIM and PH (pleckstrin homology) domains [1–3]. An alternative mechanism for targeting involves anchoring specific components of a signalling cascade to scaffold proteins, producing a large protein complex. A paradigm for how such 'signal transductionsomes' function is the sequestration of components of the mating pheromone pathway in yeast, elegant studies of which have identified Ste5 as a protein that localizes the MAP (mitogen-activated protein) kinase cascade specific to that pathway [4]. A recent report from John Scott's lab [5] provides a tantalizing insight into how mammalian cells might use a multi-functional scaffold protein to poise a key signal transducer, protein kinase C, at its site of action.

The protein kinase C isozymes were among the first signalling proteins to be shown to change their subcellular location upon activation. Early reports revealed that signals promoting lipid hydrolysis resulted in the 'translocation' of protein kinase C, typically to the plasma membrane [6]. The cloning of protein kinase C isozymes, extensive membrane enzymology, mutagenesis and recent structural studies have revealed that the archetypal protein kinase C contains two membrane-targeting motifs: the C1 domain, whose membrane affinity is increased dramatically upon binding its ligand, diacylglycerol (or analogues, such as phorbol esters); and the C2 domain, whose affinity for negatively charged phospholipids, notably phosphatidylserine,

increases upon binding Ca^{2+} (in the case of conventional protein kinase Cs) [7]. The high-affinity binding of lipid ligands activates the enzyme by inducing a conformational change that removes an autoinhibitory (pseudosubstrate) domain from the active site. Tethering of the enzyme to the membrane by these two domains effectively ensures that the extraordinarily high affinity enzyme–membrane interaction, and the resultant activation of the enzyme, occur only when the correct second messengers are present [2].

The molecular mechanism by which the binding of ligands to the C1 and C2 domains activates protein kinase C has been fairly well characterized *in vitro*, but it is also clear that the regulation of the enzyme *in vivo* must depend on other macromolecular interactions. In particular, different isozymes are found in different subcellular locations, where they would be expected to interact with different partners. There has been considerable experimental support in the past few years for the possibility that specific protein–protein interactions concentrate the enzyme at discreet locations within the cell, so favouring the phosphorylation of substrate proteins that are found at these sites [8].

Proteins that bind protein kinase C

A rapidly growing number of proteins that bind to protein kinase C are being identified by overlay assays (a modification of 'western' immunoblotting that uses protein kinase C, rather than an antibody, to probe protein bands); by interaction cloning (in which a cDNA expression library is probed with protein kinase C); and using the yeast two-hybrid genetic screen for protein–protein interactions. Binding proteins identified by such methods include several that also serve as direct substrates for protein kinase C, such as adducin and a perinuclear protein (PICK1), as well as proteins containing interaction motifs such as PH domains, and a class of proteins referred to as RACKs (receptors for activated C kinase) [9–12]. The substrate/binding proteins are thought to position inactive protein kinase C at specific cellular locations, with activation of the kinase releasing it from its substrate; other binding proteins — notably the RACKs — are thought to localize activated protein kinase C. Sites for interaction with other proteins have been identified in the regulatory and catalytic regions of protein kinase C, in conserved regions (such as the C2 and pseudosubstrate domains) and in sequences unique to particular isozymes. The idea that these specific interaction sites contribute to targeting of protein kinase C has been supported by the use of peptides, derived from defined regions of the

enzyme, which inhibit stimulus-dependent translocation *in vivo* [13].

The studies described above involve the localization of 'mature' protein kinase C, which is phosphorylated post-translationally at three positions [14]. 'Precursor' or immature protein kinase C is also localized: the dephosphorylated and partially phosphorylated forms interact in a phosphorylation-dependent manner with cytoskeletal components [14,15]. Thus, a plethora of proteins interact with protein kinase C by a variety of mechanisms: some proteins bind precursor protein kinase C, others bind mature but inactive protein kinase C, and yet others bind mature, active protein kinase C.

The model that follows from localization and mechanistic studies is that particular binding proteins differentially target protein kinase C isozymes to defined cellular locations, with activation ensuing as a result of a common mechanism mediated by second-messenger binding. This model, which ensures fidelity and specificity in isozyme activation, is now gaining widespread experimental support [16]. Now, the first report of protein kinase C binding to a multi-enzyme scaffold reveals that this kinase participates in signal transductionsomes within mammalian cells [5], where interplay with phosphatases and other kinases may modulate protein kinase C pathways.

A multi-enzyme scaffold

The binding of multiple proteins in the yeast MAP kinase signalling cascade to Ste5 epitomizes the use of a scaffold protein to localize the components of a particular pathway [4]. In mammalian cells, a similar function seems to be served by the AKAP family of proteins, so-named as a result of their original discovery as cAMP-dependent (A kinase) anchoring proteins [17,18]. The AKAPs contain at least two protein-protein interaction domains: one surface that tethers the AKAP to subcellular structures, and another that binds an amphipathic helix in the regulatory subunit of protein kinase A. The binding of cAMP to tethered dimers of the protein kinase A regulatory subunit (RII) releases the catalytic domain, and protein kinase A phosphorylates proteins close to the AKAP as a result. The finding that one family member, AKAP 79, is localized to postsynaptic densities led to the hypothesis that the anchoring protein positions protein kinase A for ready access to one substrate it regulates in the densities, namely the ionotropic glutamate receptor. In support of this idea, perfusion of hippocampal neurons with peptides that disrupt the interaction of RII with AKAPs reduces the protein kinase A-mediated desensitization of specific glutamate receptor ion channels [19].

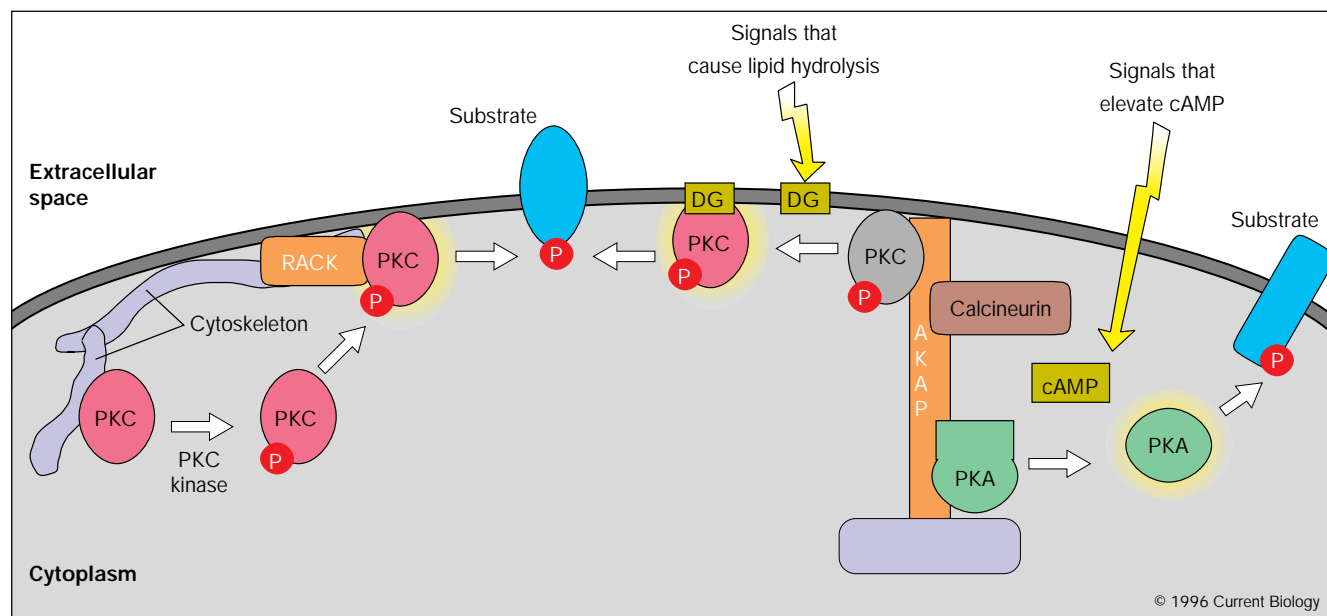
The report last year [20] that AKAP 79 binds the Ca^{2+} -calmodulin-dependent phosphatase, calcineurin, provided the first captivating evidence that AKAP 79 is, in

fact, a protein that anchors multiple enzymes. The colocalization of a kinase and phosphatase would allow fine-tuning of the sensitive balance between phosphorylation and dephosphorylation that regulates protein function. Kinetic studies revealed that calcineurin is inhibited by AKAP 79, suggesting that the anchoring protein tethers inactive phosphatase in a similar manner to its tethering of inactive protein kinase A. The events that would release active calcineurin from its binding site remain to be elucidated, but, curiously, do not appear to involve binding of Ca^{2+} to the regulatory domain of calmodulin. The coordinated binding of one kinase and one phosphatase to AKAP 79 suggest that it, like Ste5 in yeast, is a scaffold protein. But the story has only just begun to unfold.

Theresa Klauck and Maree Faux working in John Scott's lab have now shown that conventional (Ca^{2+} -regulated) protein kinase Cs bind to AKAP 79 [5]. As is the case for the other two enzymes associated with this AKAP, the bound protein kinase C is likely to be in its inactive conformation, because AKAP 79 inhibits its activity. The analysis of a series of AKAP 79 mutants, truncated at various positions, revealed that a basic stretch of peptide near the amino terminus of AKAP 79 inhibits protein kinase C activity in phosphorylation assays *in vitro*, and that this fragment also binds protein kinase C in overlay assays. The binding is stimulated by phosphatidylserine, suggesting that binding of lipid to the regulatory domain of protein kinase C helps the interaction, perhaps by exposing determinants on the enzyme that recognize the anchoring protein, or by positioning the enzyme in an orientation more amenable to the interaction.

How protein kinase C is released from this complex is not yet known. The kinase does phosphorylate AKAP 79, and although the functional consequences of this phosphorylation are unknown, the phosphorylation does not appear to affect the kinase's affinity for the AKAP. Perhaps the conformational change that occurs upon binding of diacylglycerol to the C1 domain of the kinase triggers release. Alternatively, the release signal for this conventional protein kinase C might be provided by Ca^{2+} . In this regard, it is interesting that Ca^{2+} -calmodulin prevents the inhibition of protein kinase C by an AKAP 79 peptide that encompasses the protein kinase C-binding domain, apparently by competing for binding to the peptide (Maree Faux and John Scott, personal communication).

Why nature chose to colocalize protein kinase C, protein kinase A and calcineurin to the same scaffold remains to be unveiled, but possibilities abound. In addition to the obvious interplay between phosphorylation and dephosphorylation of substrates, and possible synergism between phosphorylations catalyzed by protein kinase C and protein kinase A, mature protein kinase C is itself regulated by dephosphorylation. Indeed, calcineurin dephosphorylates

Figure 1

Ports of anchor for protein kinase C in the cell. Newly synthesized protein kinase C associates with a cytoskeletal component (far left, mauve); phosphorylation releases the mature enzyme into the cytosol (three phosphorylation sites simplified by single red circled P), where specific isoforms can interact with defined targeting or scaffold proteins (orange) to ensure specificity in the signals transduced. These targeting proteins are typically associated with the cytoskeleton. Two possibilities are shown: activated protein kinase C binds to a RACK and phosphorylates surrounding substrates (blue); on the right side of

the figure, inactive protein kinase C (gray) binds the scaffold protein, AKAP 79, and is colocalized with protein kinase A and calcineurin at postsynaptic densities (mauve oblong). Generation of diacylglycerol and/or Ca^{2+} releases active protein kinase C (pink) to phosphorylate adjacent substrates. On the far right, generation of cAMP is shown to release the catalytic subunit of protein kinase A to phosphorylate substrates such as ionotropic glutamate receptors. Active enzymes are shown with a yellow halo.

protein kinase C at one of the carboxy-terminal sites that regulate its function (E.M. Dutil and A.C.N., unpublished observations), suggesting that calcineurin might provide a rapid turn-off switch for the kinase. It is also interesting that both protein kinase A and protein kinase C phosphorylate AKAP 79, perhaps regulating the interaction of the scaffold protein with other components.

Figure 1 summarizes some of the interactions that control the subcellular location of protein kinase C. Newly synthesized protein kinase C associates with a cytoskeletal component in a lipid-independent manner, with release regulated by phosphorylation. Biochemical data suggest that protein kinase C is phosphorylated first by another kinase, with no effect on the enzyme's subcellular location; this transphosphorylation is followed by two carboxy-terminal autophosphorylations, the second of which appears to release the enzyme into the cytosol [16]. Mature protein kinase C then interacts with defined targeting or anchoring proteins, whose role may be to poise specific protein kinase C isoforms at their required sites of action. Some of these proteins (RACKs) bind protein kinase C in an active conformation, whereas others (not shown in the Figure) bind protein kinase C in an inactive conformation and may serve

as substrates themselves once the kinase is activated by the generation of diacylglycerol in the membrane.

The newest development in the targeting story is that protein kinase C also binds AKAP 79: this time the kinase, like protein kinase A and calcineurin, binds in an inactive conformation. The generation of Ca^{2+} , and/or perhaps diacylglycerol, would release protein kinase C, activated by the binding of its ligands in the C1 and C2 domains and primed to phosphorylate substrates in its immediate vicinity. Presumably, the metabolism of diacylglycerol would release protein kinase C from the membrane and allow it to re-bind AKAP 79. Whether AKAP 79 serves as a scaffold for a specific isoform of protein kinase C, and whether other scaffold proteins localize other isoforms of protein kinase C, remains to be determined. In the meantime, the glimpse we have obtained into one mechanism used to regulate how protein kinase C is in the right place at the right time shows how cleverly nature manages to maintain order within the complex network of signalling pathways.

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