Activating calcium release through inositol 1,4,5-trisphosphate receptors without inositol 1,4,5-trisphosphate

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Cytosolic calcium (Ca^{2+}) is a focal point of many signal transduction pathways and modulates a diverse array of cellular activities ranging from fertilization to cell death (1). Cells generate Ca^{2+} signals through both internal and external Ca^{2+} sources. In most cell types, the major internal Ca^{2+} stores are the endoplasmic reticulum/sarcoplasmic reticulum (ER/SR). One mechanism for mobilizing such stores involves the classical phosphoinositide pathway. Essentially, the binding of many hormones to specific receptors on the plasma membrane leads to the activation of an enzyme (phosphoinositidase C) that catalyses the hydrolysis of phospholipids to produce the intracellular messenger inositol 1,4,5-trisphosphate (InsP_3). Although derived from a lipid, InsP_3 is water soluble and diffuses into the cell interior where it encounters InsP_3 receptors (InsP_3Rs) on the ER/SR. The binding of InsP_3 changes the conformation of InsP_3Rs such that an integral channel is opened, thus allowing the Ca^{2+} stored at high concentrations in the ER/SR to enter the cytoplasm. A critical feature of InsP_3Rs is that their opening is regulated by the cytosolic Ca^{2+} concentration. This sensitivity to cytosolic Ca^{2+} allows them to act as Ca^{2+} -induced Ca^{2+} release (CICR) channels that promote the rapid amplification of smaller trigger events (1).

It was thought that the binding of InsP_3 was obligatory for channel opening. However, in an elegant study in this issue of PNAS, Foskett and colleagues (2) demonstrate that a protein could supplant the need for InsP_3 . Using a yeast two-hybrid screen, those authors demonstrate a high-affinity interaction between the NH_2 -terminal 600 aa of an InsP_3R and a member of a previously cloned group of proteins called Ca^{2+} binding proteins (CaBPs; ref. 3). These proteins belong to a superfamily of proteins that includes the well-known protein calmodulin, which bind Ca^{2+} by EF-hand motifs. CaBPs belong to a subfamily of EF-hand-

containing proteins known as neuronal calcium sensors (NCS) (4). Although members of this group of proteins show great structural similarities, they have very diverse functional properties. The NCS subfamily consists of small proteins (molecular mass ≈ 20 kDa) that are exclusively expressed in neurons or retinal photoreceptors. They have a high degree of sequence similarity to calmodulin, but differ with respect to the number of functional Ca^{2+} -binding EF hands. The proposed physiological roles of NCS proteins include modulation of neurotransmitter release and regulation of gene transcription (4).

The study by Foskett and colleagues (2) proposes a new function for the CaBP members of the NCS family—regulation of InsP_3Rs and Ca^{2+} release from intracellular stores. CaBPs interacted with InsP_3Rs only in the amino-terminal region that was used for bait, which also contains the domain where InsP_3 itself binds to the channel. Patch-clamp recording of single InsP_3Rs in the outer nuclear membrane of *Xenopus* oocytes indicated that CaBPs could activate the channels in the presence or absence of InsP_3 . In fact, CaBP alone gave rise to substantial opening of the InsP_3Rs ($P_o \approx 0.8$ in optimal conditions), with significant gating observed at 10 nM CaBP. The interaction between CaBPs and InsP_3Rs was greatly potentiated by increasing cytosolic Ca^{2+} over its physiological range (0.1–1 μM). This process critically depended on the EF hands, because mutations that inhibited Ca^{2+} binding to these domains abrogated the ability of CaBP to bind and activate InsP_3Rs .

The picture that emerges is that CaBPs can act as endogenous high-affinity ligands of InsP_3Rs , which can activate Ca^{2+} release without the need for InsP_3 production via the phosphoinositide pathway (see Fig. 1). Because the action of CaBPs depends on Ca^{2+} binding,

InsP_3R signaling would still operate in a CICR mode; CaBPs can act on InsP_3Rs to potentiate trigger Ca^{2+} signals arising from other sources, but presumably cannot initiate Ca^{2+} transients. At least five different CaBP isoforms with multiple splice variants exist, and they appear to have a clear tissue-specific expression profile (3). Some of the isoforms are abundantly expressed in the cerebral cortex and hippocampus, suggesting that CaBPs may be key mediators of Ca^{2+} signaling in the brain.

Although we can presently only speculate on the functions of CaBPs in neuronal Ca^{2+} signaling, their subcellular localization suggests some intriguing possibilities. Cellular fractionation and immunohistochemistry of CaBP-expressing neurons suggests that these proteins are present in synaptic and den-

dritic compartments, where InsP_3Rs are also located. These are strategic locations where information processing occurs. If CaBPs can participate in Ca^{2+} signals occurring during synaptic signaling they may have an important role in memory formation. Ca^{2+} signaling within spines underlie changes in synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD), which are models for learning and memory. Ca^{2+} signals underlie synaptic plasticity, and it is well established that InsP_3Rs are involved in some of these paradigms (5, 6). It has been proposed that InsP_3Rs act as “coincidence detectors” to interpret simultaneous signals arising from metabotropic receptors (producing InsP_3) and the Ca^{2+} entry through voltage-operated Ca^{2+} channel and receptor-operated Ca^{2+} channels

CaBPs can act as endogenous high-affinity ligands of InsP_3Rs .

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Fig. 1. Dual pathways for activation of InsP₃Rs. Shown are the alternative pathways leading to InsP₃R activation in neurons expressing CaBPs. Metabotropic receptors lead to InsP₃ production via phospholipase C, whereas ionotropic stimulation produces an influx of Ca²⁺ that engages CaBP-bound InsP₃Rs or recruits CaBP to the InsP₃R. Both metabotropic and ionotropic pathways lead to the activation of InsP₃Rs. InsP₃ requires the concurrent binding of Ca²⁺ to an activatory site on the InsP₃R. It is presently unclear whether the Ca²⁺-bound form CaBP is sufficient for InsP₃R opening, or if Ca²⁺ is also required to bind to InsP₃Rs independently.

(such as *N*-methyl-D-aspartate receptors) (7). CaBPs could obviate the need for metabotropic stimulation during LTD/LTP, thus enabling InsP₃Rs to participate in information processing in the absence of metabotropic receptor activation. The versatility of InsP₃Rs would be enhanced through this responsiveness to InsP₃ or CaBP, thus enabling them to operate after activation of metabotropic or ionotropic mechanisms.

Unless signals become amplified by CICR, buffering and sequestration usually determines that Ca²⁺ has a very limited diffusion within the cytosol of most cells. InsP₃ is more freely diffusible, although it is typically metabolized to the non-Ca²⁺-releasing messengers inositol 1,4-bisphosphate (InsP₂) and inositol 1,3,4,5-tetrakisphosphate (InsP₄) within seconds. The enzyme that catalyzes the latter reaction to produce InsP₄, InsP₃-kinase, has been proposed to act as a “firewall” within spines to stop the spread of InsP₃ into dendritic shafts and neighboring spines (8). If Ca²⁺-bound CaBP can act as a mobile signal, it may smuggle an activatory signal beyond the barrier that prevents InsP₃ diffusion, to allow InsP₃R-dependent Ca²⁺ signaling over significant distances. An analogous situation occurs with calmodulin, which can diffuse over tens of micrometers within cells to allow local Ca²⁺ signals to activate gene transcription (9, 10).

Just how mobile CaBPs are is not fully resolved. One feature of the NCS family is that its members contain consensus sequences for myristoylation (4). This posttranslational modification results in Ca²⁺-dependent translocation of these proteins, although along distinct routes

(11). Expression of isoform variants of a CaBP in Chinese hamster ovary cells revealed that it could have distinct cellular localizations—a short form was found at or near the plasma membrane, whereas a longer homologue was associated with the cytoskeleton (3). The ability of CaBPs to bind and activate InsP₃Rs would clearly be altered if they were tethered by lipid groups or structural proteins distant from InsP₃Rs.

Although the study from Foskett and colleagues (2) clearly demonstrates that CaBPs can regulate the activation of InsP₃Rs, there are clearly many outstanding questions concerning their mode of action. InsP₃ can have complicated effects on InsP₃Rs; it has been demonstrated to cause intrinsic inactivation of InsP₃Rs and at high concentrations to protect them from Ca²⁺-dependent inhibition (12). It will be interesting to see whether CaBPs can replicate all of the effects of InsP₃ on channel gating. Furthermore, it is possible that activation of InsP₃Rs is not the sole function of CaBPs. In common with other members of the NCS subfamily, they appear to interact with several targets (4). CaBPs have been demonstrated to activate calmodulin-dependent kinase II and G protein-coupled receptor kinases *in vitro* (3). Whether such interactions occur *in vivo* is not yet known, however, it points to a potential pleiotropic action of the CaBPs after a Ca²⁺ signal. In addition, the CaBPs have consensus sequences for phosphorylation and have been shown to be phosphorylated in hippocampal slices *in situ* (13). Their functions could therefore be modulated by upstream kinases. Another possibility is that the expression

of CaBPs could be modulated in a developmental- or activity-dependent manner, in which case their effects on the InsP₃Rs could vary substantially.

For the activation of calmodulin-dependent kinase II and G protein-coupled receptor kinases, CaBPs essentially mimicked the role of calmodulin (3). Interestingly, InsP₃Rs also have calmodulin binding regions, which can modulate the activity of the channels (14–16). One of the calmodulin binding sites has been mapped to the NH₂-terminal 159 aa of the InsP₃R (17), which is within the “bait” region used by Yang *et al.* (2) in their yeast two-hybrid screen. However, in their study, calmodulin had little effect on the interaction of CaBPs with InsP₃Rs, suggesting that they have distinct binding sites. In addition, calmodulin did not activate InsP₃R channel activity in membrane patches. Therefore, the effects of CaBP and calmodulin on InsP₃Rs appear to occur through distinct binding sites. It is plausible that these two proteins may have opposite effects on the opening of InsP₃Rs, with CaBPs causing Ca²⁺-dependent activation and calmodulin evoking Ca²⁺-dependent inhibition.

CaBPs are not the only members of the NCS family that regulate intracellular Ca²⁺ signals. Neuronal Ca²⁺ sensor 1/frequenin has been shown to activate phosphatidylinositol 4-kinase (18) and facilitate the activation of P/Q-type voltage-operated Ca²⁺ channels (19). Therefore, several of the NCS proteins have the capacity to both interpret and modulate neuronal Ca²⁺ signals.

The demonstration that CaBPs can activate InsP₃Rs in the absence of InsP₃ raises many important questions for future work. CaBPs essentially endow InsP₃Rs with the ability to display CICR without InsP₃ binding. This would make them functionally equivalent to ryanodine receptors (RyRs), which are intracellular Ca²⁺ release channels directly gated by Ca²⁺. Neurons expressing CaBPs, InsP₃Rs, and RyRs would therefore possess two channels capable of autonomous CICR and potentially causing steeply regenerative Ca²⁺ signals. It will be important to find out what decelerates CaBP-induced Ca²⁺ release to prevent it from simply running away with itself. Additional questions include the localization and translocation of CaBPs. Does this limit or enhance their effects on InsP₃Rs? The observation that CaBPs can bind to InsP₃Rs at low Ca²⁺ concentration (2) suggests that the Ca²⁺-free forms of these proteins can be prebound to InsP₃Rs. This may be a way of speeding up the responsiveness of InsP₃Rs, in a similar way that complexing apo-calmodulin does for P/Q-type voltage-

operated Ca^{2+} channels (20). Finally, are InsP_3 Rs engaged with CaBP still responsive to InsP_3 , or do CaBPs completely

decouple the phosphoinositide signaling pathway from Ca^{2+} release? Clearly, the novel function for CaBPs proposed by

Foskett and colleagues (2) opens up a new concept in the regulation of Ca^{2+} release.

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