Decoding Ca^{2+} signals: a question of timing

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Receptor-stimulated Ca^{2+} signals come in several flavors. The Ca^{2+} signals can be decoded linearly or by integration of the response. How the duration of the signal conveyed by cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) changes is regulated is not well understood. Liu et al. (Liu, Q., S.A. Walker, D. Gao, J.A. Taylor, Y.-F. Dai, R.S. Arkell, M.D. Bootman, H.L. Roderick, P.J. Cullen, and P.J. Lockyer. 2005. *J. Cell Biol.* 170:183–190) now report an example of decoding based on the differential regulation of Ras function by two Ca^{2+}-sensitive Ras inhibitors: Ca^{2+}-promoted Ras activator (CAPRI), which extends the duration of the effect of Ca^{2+} on Ras activity, and Ras GTPase activating-like protein (RASAL), which functions as a linear decoder of the Ca^{2+} signal.

The Ca^{2+} signal generated by physiological agonist concentrations is most often in the form of Ca^{2+} oscillations that initiate at discrete cellular sites and can either remain confined to a particular cellular microdomain (such as the apical pole of secretory cells) or propagate to all cellular domains in the form of repetitive Ca^{2+} waves (Petersen, 2004). The amplitude and frequency of the Ca^{2+} oscillations and speed of the Ca^{2+} waves are regulated by the intensity of the stimulus (Berridge et al., 2000). Furthermore, the pattern of the Ca^{2+} oscillations and waves are receptor specific in the same cell (Kiselyov et al., 2003). These intricate Ca^{2+} signals regulate cellular activities as diverse as vision and neurotransmitter release on a micro- or millisecond time scale and gene regulation on the scale of hours or days (Berridge et al., 2003).

How is the same Ca^{2+} signal decoded to regulate multiple functions with different spatial and temporal characteristics? A simple form of decoding is an exact translation of the Ca^{2+} signal to a physiological response. An example is direct binding of Ca^{2+} to effector proteins, such as the Ca^{2+}-activated Cl^- and K^+ channels in epithelia (Melvin et al., 2005). Another example is the uptake by the mitochondria of Ca^{2+} released from the ER to regulate enzymes involved in mitochondrial energy metabolism (Hajnoczky et al., 1995). A more complex form of decoding is achieved by differential Ca^{2+} sensitivity of the sensors. An example is the family of synaptotagmins, which display a range of apparent affinities for Ca^{2+} to confer specific Ca^{2+} dependence to exocytotic events (Sudhof, 2004). The most intricate form of decoding requires translating the information in the frequency and amplitude of Ca^{2+} oscillations into a physiological response, such as the activation of the NFAT, OAP, and NF-κB transcription factors (Dolmetsch et al., 1998; Li et al., 1998; Tomida et al., 2003). The molecular mechanism responsible for this form of decoding is not known.

Although we intuitively assume that decoding of complex Ca^{2+} signals involves activation of multiple Ca^{2+} sensors with different spatial and temporal characteristics that converge on the same pathway, to date there has been no clear example of this. The study by Liu et al. (2005) shows the selective response of two Ras GTPase-activating proteins (GAPs), Ca^{2+}-promoted Ras activator (CAPRI), and Ras GTPase activating-like protein (RASAL) to complex Ca^{2+} signals.

Ras proteins are binary switches that cycle between the GTP-active and GDP-inactive forms and regulate many signaling pathways, including the MAPK cascade, to regulate cell proliferation and differentiation (Downward, 2003). The intensity and duration of Ras activation is determined by the relative activity of the guanine nucleotide exchange factors (GEFs) that catalyze the exchange of GDP for GTP to activate Ras and the GAPs that catalyze the intrinsic Ras GTPase and inactivate Ras. Ras is activated by tyrosine kinase-linked receptors through recruitment and activation of Ca^{2+}-independent GEFs and GAPs (Takai et al., 2001), and by Ca^{2+}-mobilizing receptors, including G protein–coupled receptors, that recruit and activate Ca^{2+}-dependent GEFs and GAPs (Cullen and Lockyer, 2002). Previous work identified CAPRI and RASAL as Ca^{2+}-dependent Ras GAPs (Lockyer et al., 2001). CAPRI and RASAL are members of the GAP1 family that also includes $GAP1^{IP4BP}$ and $GAP1^m$ (Cullen, 1998). As illustrated in Fig. 1 A for RASAL and CAPRI, the family is typified by four conserved structural domains; tandem C2 domains; a GAP-related domain (GRD); a pleckstrin homology (PH) domain, and a Bruton's tyrosine kinase motif (Btk). The C2 domains of $GAP1^{IP4BP}$ and $GAP1^m$ do not mediate Ca^{2+}-dependent plasma membrane (PM) translocation (Lockyer et al., 1997); however, the C2 domains are essential for the Ca^{2+}-dependent translocation of CAPRI and RASAL.

In an earlier work, Walker et al. (2004) showed that RASAL faithfully translates the changes in $[Ca^{2+}]_i$ to PM translocation events and inactivation of Ras. Intense receptor

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Abbreviations used in this paper: CAPRI, Ca^{2+}-promoted Ras activator; GAP, GTPase-activating protein; GEF, guanine nucleotide exchange factor; PH, pleckstrin homology; PM, plasma membrane; RASAL, Ras GTPase activating-like protein.

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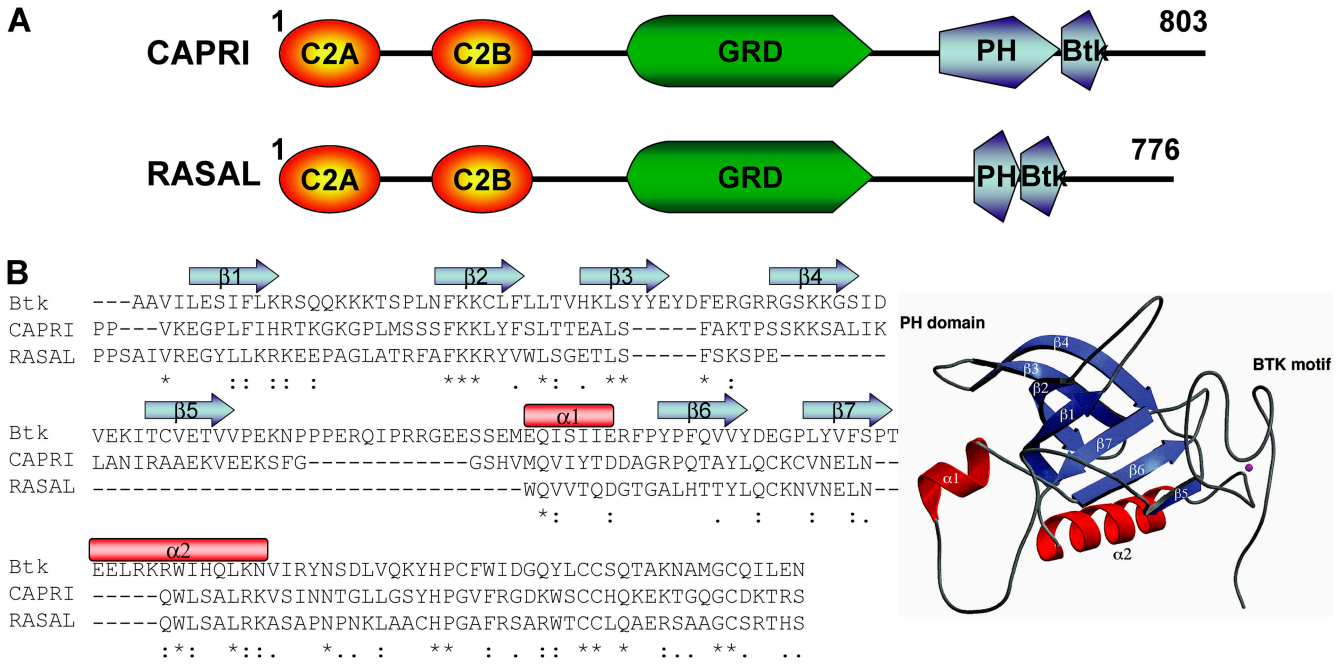

Figure 1. **Structural motif prediction and alignment of PH domains.** Structural motif prediction and alignment of PH domains was done using the 3D-PSSM Web Server V 2.6.0 at http://www.sbg.bio.ic.ac.uk/~3dpssm. The structure of BTK PH domain was taken from Hyvonen and Saraste (1997).

stimulation resulted in a single $[Ca^{2+}]_i$ transient and a matching transient translocation of RASAL to the PM, whereas weak receptor stimulation generated Ca^{2+} oscillations and a synchronized oscillatory translocation of RASAL. Translocation required RASAL's tandem C2 domains—a finding confirmed in Liu et al. (2005). Hence, the response of RASAL to $[Ca^{2+}]_i$ indicates that RASAL is a linear decoder of $[Ca^{2+}]_i$ signals.

Although the translocation of CAPRI to the PM is also dependent on its tandem C2 domains, Liu et al. (2005) report that CAPRI does not behave as a linear decoder of $[Ca^{2+}]_i$ changes. The initial $[Ca^{2+}]_i$ increase triggered by intense or weak stimulation caused C2 domain–dependent translocation of CAPRI to the PM. CAPRI remained at the PM despite the reduction of $[Ca^{2+}]_i$ to a plateau or maintained Ca^{2+} oscillations. Switching between RASAL and CAPRI C2 domains converted CAPRI to a linear decoder of Ca^{2+} oscillations and conferred persistent retention of RASAL at the PM. Importantly, Liu et al. (2005) found that the removal of external Ca^{2+} to prevent Ca^{2+} influx by store-operated channels converted CAPRI translocation from persistent to transient, and removal of the stimulus rapidly retrieved CAPRI from the PM. The dependence of CAPRI's retention at the PM on Ca^{2+} influx indicates that CAPRI is a sensor of Ca^{2+} influx. Ca^{2+} influx may maintain high Ca^{2+} levels next to the PM. The requirement for persistent agonist stimulation may reflect dependence on receptor-mediated lipid metabolism and binding of the PH domain of CAPRI to phosphatidylinositides because mutation of the conserved tryptophan of the PH domain of CAPRI (W664A) resulted in a transient, rather than persistent, translocation of CAPRI to the PM. Sequence analysis suggests that the PH domains of CAPRI and RASAL are most similar to that of Bruton's tyrosine kinase (Hyvonen and Saraste, 1997). The predicted CAPRI PH domain is more intact than the RASAL PH domain (Fig. 1 B) and it is functional, as evident from the effect of the W664A mutation. The RASAL PH domain lacks important structural motifs, which may render it nonfunctional. Indeed, Liu et al. (2005) show that PM retention segregates with CAPRI's PH domain.

The cooperation between the tandem C2 domains and the PH domain makes CAPRI an integrator of the Ca^{2+} signal. As a consequence, the duration of CAPRI's retention at the PM, and therefore its action as a GAP, is markedly extended. Hence, the study of Liu et al. (2005) provides an example for a mechanism by which the timing and duration of the Ca^{2+} signal can be differentially decoded. CAPRI and RASAL are both deactivators of Ras, and their combined action can be used to decode the complex forms of the receptor-evoked Ca^{2+} oscillations and waves.

The findings of Liu et al. (2005) raise several questions. For example, do the cells use the properties of the two GAPs to regulate physiological functions of different duration? Recent work showed that activation of Ras and the ERK/MAPK cascade is sensitive to the frequency of Ca^{2+} oscillations (Kupzig et al., 2005). What is the role of CAPRI and/or RASAL in this form of regulation? How do the tandem C2 domains of CAPRI mediate $[Ca^{2+}]_i$-dependent translocation while the PH domain mediates Ca^{2+} influx-dependent retention? Decoding of Ca^{2+} signals by CAPRI and RASAL is only one side of the equation. Two families of Ca^{2+}-dependent GEFs have been identified: Ras-GRF1 and Ras-GRF2, and the Ras-GRP/CalDAG GEFs (Cullen and Lockyer, 2002). At present, however, it is not known how these GEFs decode Ca^{2+} signals. Further insight into this process is needed before we can solve the puzzle of how a complex pathway like the Ras/MAPK pathway decodes agonist-evoked Ca^{2+} signaling.

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Note added in proof. An additional mechanism to regulate the duration of Ras signaling is through transcriptional regulation of RASAL and CAPRI expression levels. A recent study, searching for tumor suppressors that affect the activity of wild-type Ras, reported that the transcription factor PITX1 increased the expression of RASAL (Kolfschoten et al., 2005). The down-regulation of PITX1, resulting in a reduced level of RASAL and an increased level of active Ras, was found in several prostate, bladder, and colon cancers. Restoring PITX1 expression to colon cancer cell lines inhibited tumorigenesis in a Ras-dependent manner (Kolfschoten et al., 2005). These studies establish RASAL as a tumor suppressor whose long-term activity can be controlled by expression, and whose acute activity can be controlled by $[Ca^{2+}]_i$.

References

Berridge, M.J., P. Lipp, and M.D. Bootman. 2000. The versatility and universality of calcium signalling. *Nat. Rev. Mol. Cell Biol.* 1:11–21.

Berridge, M.J., M.D. Bootman, and H.L. Roderick. 2003. Calcium signalling: dynamics, homeostasis and remodelling. *Nat. Rev. Mol. Cell Biol.* 4:517–529.

Cullen, P.J. 1998. Bridging the GAP in inositol 1,3,4,5-tetrakisphosphate signalling. *Biochim. Biophys. Acta.* 1436:35–47.

Cullen, P.J., and P.J. Lockyer. 2002. Integration of calcium and Ras signalling. *Nat. Rev. Mol. Cell Biol.* 3:339–348.

Dolmetsch, R.E., K. Xu, and R.S. Lewis. 1998. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature.* 392:933–936.

Downward, J. 2003. Targeting RAS signalling pathways in cancer therapy. *Nat. Rev. Cancer.* 3:11–22.

Hajnoczky, G., L.D. Robb-Gaspers, M.B. Seitz, and A.P. Thomas. 1995. Decoding of cytosolic calcium oscillations in the mitochondria. *Cell.* 82:415–424.

Hyvonen, M., and M. Saraste. 1997. Structure of the PH domain and Btk motif from Bruton's tyrosine kinase: molecular explanations for X-linked agammaglobulinaemia. *EMBO J.* 16:3396–3404.

Kiselyov, K., D.M. Shin, and S. Muallem. 2003. Signalling specificity in GPCR-dependent Ca^{2+} signalling. *Cell. Signal.* 15:243–253.

Kolfschoten, I.G., B. van Leeuwen, K. Berns, J. Mullenders, R.L. Beijersbergen, R. Bernards, P.M. Voorhoeve, and R. Agami. 2005. A genetic screen identifies PITX1 as a suppressor of RAS activity and tumorigenicity. *Cell.* 121:849–858.

Kupzig, S., S.A. Walker, and P.J. Cullen. 2005. The frequencies of calcium oscillations are optimized for efficient calcium-mediated activation of Ras and the ERK/MAPK cascade. *Proc. Natl. Acad. Sci. USA.* 102:7577–7582.

Li, W., J. Llopis, M. Whitney, G. Zlokarnik, and R.Y. Tsien. 1998. Cell-permeant caged InsP3 ester shows that Ca^{2+} spike frequency can optimize gene expression. *Nature.* 392:936–941.

Liu, Q., S.A. Walker, D. Gao, J.A. Taylor, Y.-F. Dai, R.S. Arkell, M.D. Bootman, H.L. Roderick, P.J. Cullen, and P.J. Lockyer. 2005. CAPRI and RASAL impose different modes of information processing on Ras due to contrasting temporal filtering of Ca^{2+}. *J. Cell Biol.* 170:183–190.

Lockyer, P.J., J.R. Bottomley, J.S. Reynolds, T.J. McNulty, K. Venkateswarlu, B.V. Potter, C.E. Dempsey, and P.J. Cullen. 1997. Distinct subcellular localisations of the putative inositol 1,3,4,5-tetrakisphosphate receptors GAP1IP4BP and GAP1m result from the GAP1IP4BP PH domain directing plasma membrane targeting. *Curr. Biol.* 7:1007–1010.

Lockyer, P.J., S. Kupzig, and P.J. Cullen. 2001. CAPRI regulates Ca^{2+}-dependent inactivation of the Ras-MAPK pathway. *Curr. Biol.* 11:981–986.

Melvin, J.E., D. Yule, T. Shuttleworth, and T. Begenisich. 2005. Regulation of fluid and electrolyte secretion in salivary gland acinar cells. *Annu. Rev. Physiol.* 67:445–469.

Petersen, O.H. 2004. Local and global Ca^{2+} signals: physiology and pathophysiology. *Biol. Res.* 37:661–664.

Sudhof, T.C. 2004. The synaptic vesicle cycle. *Annu. Rev. Neurosci.* 27:509–547.

Takai, Y., T. Sasaki, and T. Matozaki. 2001. Small GTP-binding proteins. *Physiol. Rev.* 81:153–208.

Tomida, T., K. Hirose, A. Takizawa, F. Shibasaki, and M. Iino. 2003. NFAT functions as a working memory of Ca^{2+} signals in decoding Ca^{2+} oscillation. *EMBO J.* 22:3825–3832.

Walker, S.A., S. Kupzig, D. Bouyoucef, L.C. Davies, T. Tsuboi, T.G. Bivona, G.E. Cozier, P.J. Lockyer, A. Buckler, G.A. Rutter, et al. 2004. Identification of a Ras GTPase-activating protein regulated by receptor-mediated Ca^{2+} oscillations. *EMBO J.* 23:1749–1760.