J Korean Med Sci 2000; 15(Suppl): S46-8
ISSN 1011-8934

Receptor-Specific Ca^{2+} Signaling in Polarized Cells

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Signaling specificity is a central question in cell signaling. Important aspects of the problem are how receptors that use the same biochemical pathway can generate agonist-specific signals and how divergent signaling pathways to form signaling networks (1, 2). Ca^{2+} signaling in epithelial cells is particularly suitable to address these questions. The polarized function of epithelial cell requires specialized and polarized organization of signaling complexes (3).

Many dedicated functions of polarized cells, such as exocytosis and electrolyte and fluid transport, are regulated by the free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) on a milliseconds time scale. Cells regulate $[Ca^{2+}]_i$ by a coordinated action of active Ca^{2+} pumps that generate steep Ca^{2+} gradients, and passive Ca^{2+} channels that can rapidly dissipate these gradients (4). A Ca^{2+} signal can be initiated by G protein-coupled receptors through activation of PLC. The sequence of events initiated by receptor activation to generate a variety of precisely orchestrated Ca^{2+} signals, such as repetitive Ca^{2+} oscillations and propagated Ca^{2+} waves, have been described in several cellular systems including epithelial cells (3).

The G proteins-coupled Ca^{2+} signaling system in polarized cells can generate receptor-specific signals. An example is the receptor-specific Ca^{2+} waves generated by stimulation of the muscarinic, Bombesin (BS) and CCK receptors of the same pancreatic acinar cell (5). Hence, all three receptors use the same biochemical pathway but generate receptor-specific signals. It appears that cells use both targeting of signaling proteins to microdomains and regulatory mechanisms to achieve such receptor specific signaling responses.

How signaling specificity is gained by targeting of signaling proteins to signaling microdomains was reviewed recently (1) and is the subject of a special issue of Cell Calcium (November 1999). In the case of polarized cells, all isoforms of IP_3Rs are expressed at high levels in the luminal pole (6-9). Furthermore, these cells express SERCA and PMCA pumps in a polarized manner, which appears to play a role in propagation of Ca^{2+} waves (10). Finally, it seems that epithelial cells express various receptors in a non-uniform fashion, with high levels at the lateral borders, next to the tight junctions (11). At present, most of the evidence for a role for polarized expression of signaling proteins in signaling specificity is corollary in nature. However, it is now becoming increasingly possible to manipulate the expression of proteins in native cells so that the role of polarized expression of signaling proteins in signaling specificity can be studied more directly.

Another means in achieving signaling specificity is through biochemical manipulation of signaling pathways. Differential coupling or activation of Gq-coupled receptors by members of the Gq class of G proteins can be demonstrated (5). This differential coupling was not due to coupling to different members of the Gq class subunits or different combinations of G (5, 12). Furthermore, deletion of a single or several combinations of two subunits in mice revealed complete promiscuity of coupling of various receptors to Gq class subunits in pancreatic and submandibular gland cells (13). Rather, RGS proteins seem to determine coupling specificity.

In the turn over cycle of G proteins the intrinsic GTPase activity of the subunits is much slower than the rate of the receptor-stimulated GDP/GTP exchange reaction (14). This paradox was solved with the discovery of the Regulation of G protein Signaling (RGS) proteins (15, 16). Shortly after their discovery, it was found that RGS proteins catalyze the GTPase activity of the subunits of many members of the G proteins family (17). All RGS proteins have three domains, a variable N terminal domain, a homologous RGS box encompassing about 125 amino acids and a variable C terminal domain (16, 18). The GTPase accelerating (GAP) function of RGS proteins is confined to the RGS box (19). The mechanism responsible for the differential sensitivity of Gq-coupled receptor to guanine nucleotides was clarified once their interaction with RGS proteins was examined (20, 21). The muscarinic, BS and CCK receptors showed

Key Words: *Specificity; G Proteins; RGS Proteins*

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between 0-1,000 differential sensitivity to the same or different RGS proteins (20). Deletion mutations showed that the N terminal domain of RGS protein is essential for their activity in vivo and it mediates receptor recognition in vitro and in vivo (21). Hence, RGS proteins confer signaling specificity to G protein coupled receptors.

Considering the pivotal role of RGS proteins in regulating G protein-dependent signaling, it is important to understand how the activity of RGS proteins is regulated in vivo. Several clues suggest that the activity of RGS proteins is regulated during cell stimulation. RGS proteins seems to have a tonic inhibitory activity since they inhibit G proteins-dependent signaling when added as recombinant proteins to the cytosol (3). In unpublished work, we found that several mutants of RGS4 in the box domain act as dominant negative RGS proteins and were able to stimulate PLC activity and initialize Ca^{2+} oscillations and/or a large Ca^{2+} release from internal stores. In addition, antibodies that inhibit the action of RGS proteins also initiated Ca^{2+} oscillation followed by large Ca^{2+} release from internal stores.

The simplest interpretation of the action of RGS protein in vivo is that RGS proteins are active in resting cells and act as GAPs to inhibit signaling. Their activity can be antagonized by the dominant negative RGS proteins or the scavenging antibodies to initiate signaling. Agonist-bound receptors not only accelerate the GDP/GTP exchange on the subunits, but also inhibit the action of the native RGS proteins to amplify the signal and allow Ca^{2+} oscillations. Hence, RGS proteins may provide a biochemical control of Ca^{2+} oscillations. Since the discovery of the biphasic regulation of the IP_3R channel activity by Ca^{2+} (22) and the finding that non-metabolizable IP_3 analogues can trigger Ca^{2+} oscillations (23), it was generally assumed that Ca^{2+} oscillations is a biophysical phenomenon. However, the first measurement of IP_3 in single cells reported recently showed that Ca^{2+} oscillations follow oscillatory changes in IP_3 concentration (24). That is, Ca^{2+} oscillations may be a biochemical rather than a biophysical phenomenon. RGS proteins can provide the biochemical pathway to allow Ca^{2+} oscillation and control their frequency.

Another aspect of Ca^{2+} signaling complexes is the communication between the ER and the plasma membrane (PM). It is now clear that a small portion of the ER pool is responsible for gating the Icrac channel in the PM (25, 26). Recent work indicates that this allow the IP_3Rs to gate the store operated channels by conformational coupling (27, 28). This coupling is mediated by the N terminal domain of the IP_3R (28) and appears to be confined, at least in part, to defined sequences in the IP_3R (29). Conformational coupling can be shown with recombinant or native store-operated channels (30, 31). This mode of gating allows intimate communication between the ER and PM or between the cell interior and exterior.

Better understanding of targeting of signaling proteins to cellular microdomains and their interaction will help revealing how signaling specificity among different receptor complexes is achieved and how the activity of signaling networks is coordinated.

References

1. Hunter T. *Signaling-2000 and beyond. Cell 2000; 100: 113-27.*
2. Weng G, Bhalla US, Iyengar R. *Complexity in biological signaling systems. Science 1999; 284: 92-6.*
3. Muallem S, Wilkie TM. *G protein-dependent Ca^{2+} signaling complexes in polarized cells. Cell Calcium 1999; 26: 173-80.*
4. Berridge MJ. *Inositol trisphosphate and calcium signalling. Nature 1993; 365: 315-25.*
5. Xu X, Diaz J, Zeng W, Muallem S. *Spatial compartmentalization of Ca^{2+} signaling complexes in pancreatic acini. J Biol Chem 1996; 271: 24684-90.*
6. Lee MG, Xu X, Zeng W, Diaz J, Wojcikiewicz RJ, Kuo TH, Wuytack F, Racymaekers L, Muallem S. *Polarized expression of Ca^{2+} channels in pancreatic and salivary gland cells. Correlation with initiation and propagation of $[Ca^{2+}]_i$ waves. J Biol Chem 1997; 272: 15765-70.*
7. Sasaki T, Shimura S, Wakui M, Ohkawara Y, Takishima T, Mikoshiba K. *Apically localized IP3 receptors control chloride current in airway gland acinar cells. Am J Physiol 1994; 267: L152-8.*
8. Yamamoto-Hino M, Miyawaki A, Segawa A, Adachi E, Yamashina S, Fujimoto T, Sugiyama T, Furuichi T, Hasegawa M, Mikoshiba K. *Apical vesicles bearing inositol 1,4,5-trisphosphate receptors in the Ca^{2+} initiation site of ductal epithelium of submandibular gland. J Cell Biol 1998; 141: 135-42.*
9. Yule DI, Ernst SA, Ohnishi H, Wojcikiewicz RJ. *Evidence that zymogen granules are not a physiologically relevant calcium pool. Defining the distribution of inositol 1,4,5-trisphosphate receptors in pancreatic acinar cells. J Biol Chem 1997; 272: 9093-8.*
10. Lee MG, Xu X, Zeng W, Diaz J, Kuo TH, Wuytack F, Racymaekers L, Muallem S. *Polarized expression of Ca^{2+} pumps in pancreatic and salivary gland cells: role in initiation and propagation of $[Ca^{2+}]_i$ waves. J Biol Chem 1997; 272: 15771-6.*
11. Rios JD, Zoukhri D, Rawe IM, Hodges RR, Zieske JD, Dartt DA. *Immunolocalization of muscarinic and VIP receptor subtypes and their role in stimulating goblet cell secretion. Invest Ophthalmol Vis Sci 1999; 40: 1102-11.*
12. Zeng W, Xu X, Muallem S. *G Transduces $[Ca^{2+}]_i$ oscillations and Gq a sustained response during stimulation of pancreatic acinar cells with $[Ca^{2+}]_I$ mobilizing agonists. J Biol Chem*

1996; 271: 18520-6.

13. Xu X, Croy JT, Zeng W, Zhao L, Davignon I, Popov S, Yu K, Jiang H, Offermanns S, Muallem S, Wilkie TM. *Promiscuous coupling of receptors to Gq class subunits and effector proteins in pancreatic and submandibular gland cells. J Biol Chem 1998; 273: 27275-9.*
14. Gilman AG. *Nobel Lecture. G proteins and regulation of adenylyl cyclase. Biosci Rep 1995; 15: 65-97.*
15. Berman DM, Gilman AG. *Mammalian RGS proteins: barbarians at the gate. J Biol Chem 1998; 273: 1269-72.*
16. Siderovski DP, Strockbine B, Behe CI. *Whither goest the RGS proteins? Crit Rev Biochem Mol Biol 1999; 34: 215-51.*
17. Berman DM, Wilkie TM, Gilman AG. *GAIP and RGS4 are GTPase-activating proteins for the Gi subfamily of G protein alpha subunits. Cell 1996; 86: 445-52.*
18. Tesmer JJ, Berman DM, Gilman AG, Sprang SR. *Structure of RGS4 bound to AlF4--activated Gi 1: stabilization of the transition state for GTP hydrolysis. Cell 1997; 89: 251-61.*
19. Popov S, Yu K, Kozasa T, Wilkie TM. *The regulators of G protein signaling (RGS) domains of RGS4, RGS10, and GAIP retain GTPase activating protein activity in vitro. Proc Natl Acad Sci USA 1997; 94: 7216-20.*
20. Xu X, Zeng W, Popov S, Berman DM, Davignon I, Yu K, Yowe D, Offermanns S, Muallem S, Wilkie TM. *RGS proteins determine signaling specificity of Gq-coupled receptors. J Biol Chem 1999; 274: 3549-56.*
21. Zeng W, Xu X, Popov S, Mukhopadhyay S, Chidiac P, Swistok J, Danho W, Yagaloff KA, Fisher SL, Ross EM, Muallem S, Wilkie TM. *The N-terminal domain of RGS4 confers receptor-selective inhibition of G protein signaling. J Biol Chem 1998; 273: 34687-90.*
22. Bezprozvanny I, Watras J, Ehrlich BE. *Bell-shaped calcium-response curves of Ins(1,4,5)P3- and calcium-gated channels from endoplasmic reticulum of cerebellum. Nature 1991; 351: 751-4.*
23. Wakui M, Potter BV, Petersen OH. *Pulsatile intracellular calcium release does not depend on fluctuations in inositol trisphosphate concentration. Nature 1989; 339: 317-20.*
24. Hirose K, Kadowaki S, Tanabe M, Takeshima H, Iino M. *Spatiotemporal dynamics of inositol 1,4,5-trisphosphate that underlies complex Ca^{2+} mobilization patterns. Science 1999; 284: 1527-30.*
25. Broad LM, Armstrong DL, Putney JW Jr. *Role of the inositol 1,4,5-trisphosphate receptor in Ca^{2+} feedback inhibition of calcium release-activated calcium current Icrac. J Biol Chem 1999; 274: 32881-8.*
26. Krause E, Schmid A, Gonzalez A, Schulz I. *Low cytoplasmic $[Ca^{2+}]$ activates Icrac independently of global Ca^{2+} store depletion in RBL-1 cells. J Biol Chem 1999; 274: 36957-62.*
27. Kiselyov K, Xu X, Mozhayeva G, Kuo T, Pessah I, Mignery G, Zhu X, Birnbaumer L, Muallem S. *Functional interaction between InsP3 receptors and store-operated Htrp3 channels. Nature 1998; 396: 478-82.*
28. Kiselyov K, Mignery GA, Zhu MX, Muallem S. *The N-terminal domain of the IP3 receptor gates store-operated hTrp3 channels. Mol Cell 1999; 4: 423-9.*
29. Boulay G, Brown DM, Qin N, Jiang M, Dietrich A, Zhu MX, Chen Z, Birnbaumer M, Mikoshiba K, Birnbaumer L. *Modulation of $Ca^{(2+)}$ entry by polypeptides of the inositol 1,4,5-trisphosphate receptor (IP3R) that bind transient receptor potential (TRP): evidence for roles of TRP and IP3R in store depletion-activated $Ca^{(2+)}$ entry. Proc Natl Acad Sci USA 1999; 96: 14955-60.*
30. Zubov AI, Kaznacheeva EV, Nikolaev AV, Alexeenko VA, Kiselyov K, Muallem S, Mozhayeva GN. *Regulation of the miniature plasma membrane Ca^{2+} channel Imin by inositol 1,4,5-trisphosphate receptors. J Biol Chem 1999; 274: 25983-5.*
31. Ma HT, Patterson RL, van Rossum DB, Birnbaumer L, Mikoshiba K, Gill DL. *Requirement of the inositol trisphosphate receptor for activation of store-operated $Ca^{(2+)}$ channels. Science 2000; 287: 1647-51.*