Bridge between the channel and FRET of PtdIns(4,5)P_2 sensor

Masayuki X Mori*

Department of Synthetic Chemistry and Biological Chemistry; Graduate School of Engineering; Kyoto University; Kyoto, Japan

Itsuki K, Imai Y, Hase H, Okamura Y, Inoue R, Mori MX. PLC-mediated PI(4,5)P2 hydrolysis regulates activation and inactivation of TRPC6/7 channels. J Gen Physiol 2014; 143:183-201; PMID:24470487; http://dx.doi.org/10.1085/jgp.201311033

In 1953, Hokin MR and Hokin LE reported that stimulation of the pancreas by acetylcholine leads to a marked increase of phosophate incorporation to phospholipid, particularly in inositol-containing lipids.[1] This was the first observation that phosphoinositides are a link in the process of receptor-mediated cellular reactions. Later, Michell RH reported that the agonist-stimulated acceleration of Phosphatidylinositol (4,5)P_2 phosphate (PtdIns(4,5)P_2, PI(4,5)P_2 or PIP$_2$) catabolism was associated with a rise in cytoplasmic calcium levels.[2] Subsequent studies have shown that the hydrolysis response of PI(4,5)P$_2$ by phospholipase C (PLC) by a variety of stimuli is of fundamental biological significance in processes such as secretion, smooth muscle contraction, gene expression, and differentiation of cells. Consequently, PI(4,5)P$_2$ is now established as the source of the big-three secondary messengers- Diaclyglycerol (DAG), Inositol 1,4,5-triphosphate (Ins(1,4,5)P_3) and Phosphatidylinositol (3,4,5)P_3 phosphate.

A substrate of these secondary messengers, PI(4,5)P$_2$ is also well-known to regulate ion conducting proteins. The first evidence of this was reported by Hilgemans's group in the middle 90s in K$_{ATP}$ channel and Na/Ca exchanger.[3] The reason PI(4,5)P$_2$ is the regulator for numerous ion channels and transporters may seem rather obvious, given its relative abundance and preferential localization in the plasma membrane, as described in review by B. Hille's group.[4] Detection of PI(4,5)P$_2$ dynamics in living cells would strengthen our understanding of the role of PI(4,5)P$_2$ in channel regulation. To monitor PI(4,5)P$_2$, several types of PI(4,5)P$_2$ or PI(4,5)P$_2$ metabolites biosensors are available. Förster resonance energy transfer (FRET) based PI(4,5)P$_2$ sensors developed by Jalink's group utilize a PH-domain taken from PLCδ1 to detect PI(4,5)P$_2$ and has high sensitivity and high temporal resolution for detecting PI(4,5)P$_2$ dynamics.[5] This sensor was applied to the studies of KCNQ2/3 channels to demonstrate the positive role of PI(4,5)P$_2$, as excellently demonstrated by Hille's group.[6] This method was also applied to TRPM8 channel research in which the temporal correlation between PI(4,5)P$_2$ by hydrolysis through calcium-dependent PLC activity and channel desensitization was clearly demonstrated.[7]

However, PI(4,5)P$_2$ has a complicated role in TRPC3/6/7 channels, because these channels are activated by 'DAG', one of the products of PLC hydrolysis of PI(4,5)P$_2$. In order to study the link between the effect of PI(4,5)P$_2$ or DAG and TRPC3/6/7 currents during the PLC-coupled receptor stimulation, the simultaneous measurement of PI(4,5)P$_2$ or DAG and the TRPC6/7 currents is quite useful, but is not enough to gain a deep mechanistic insight. To fill a gap between the lipids dynamics and the channel activity, a model simulation was employed in our recent research.[8] Our model consisted of three parts as follows: 1) consecutive reaction scheme of PI(4,5)P$_2$ hydrolysis by PLC, 2) calculation of open probability which is controlled by PI(4,5)P$_2$ and DAG, where the channel gating was opened by DAG, and inhibited by reduction of PI(4,5)P$_2$, 3) calculation of FRET strength based on the concentrations of PI(4,5)P$_2$ and Ins(1,4,5)P_3. However, the building of the model simulation also led
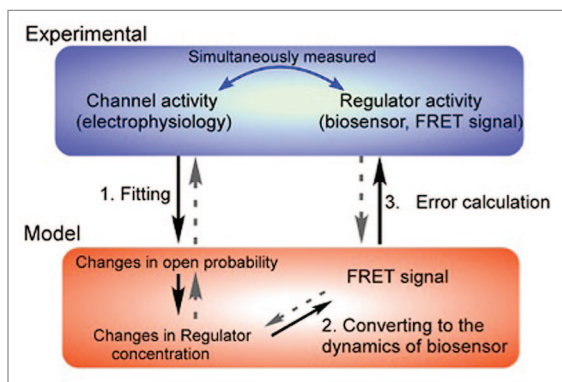
Figure 1. Diagram illustrating for the model evaluation.

to questions about how to assess the fidelity of this model?

We formulated a strategy for model evaluation as follows (**Fig. 1**). First; the receptor (muscarinic or vasopressin)-operated TRPC6/7 currents recording from the whole-cell patch clamp mode was fitted to the model with over 10 free parameters. Second; the fitted current in the model simulation turned out to give time-dependent changes in the free parameters including of $PI(4,5)P_2$ and $Ins(1,4,5)P_3$ concentrations. The concentration of $PI(4,5)P_2$ and $Ins(1,4,5)P_3$ were then converted to the strength of FRET. Third, the error between the calculated and the experimental FRET, the latter

of which was simultaneously obtained with the electrophysiological data, was evaluated by use of standard deviation. By using this validated model, the complex interplay between $PI(4,5)P_2$ or DAG and ionic currents could be simulated. From this simulation, the following conclusions were reached: (a) the effect of DAG dynamics predominated when the receptor stimulation was weak, (b) the inhibitory role of $PI(4,5)P_2$ reduction progressively emerged to inhibit TRPC6/7 currents, as receptor stimulation increased.

The regulation of ion channels is a complex phenomenon. To understand this complexity, simultaneous measurement of both the regulatory factors, which dynamics are measured by FRET biosensors, in conjunction with ion channel activity is becoming a generalized approach. In addition to experimental measurement, validated model simulation of the simultaneously measured data proves to be an insightful approach, especially when the channel activity is linked to cellular signaling.

References

1. Hokin MR, Hokin LE. Enzyme secretion and the incorporation of P32 into phospholipides of pancreas slices. J Biol Chem 1953; 203:967-77; PMID:13084667

2. Michell RH. Inositol phospholipids and cell surface receptor function. Biochim Biophys Acta 1975; 415:81-47; PMID:164246; http://dx.doi.org/10.1016/0304-4157(75)90017-9

3. Hilgemann DW, Ball R. Regulation of cardiac Na+,Ca2+ exchange and KATP potassium channels by PIP2. Science 1996; 273:956-9; PMID:8688080; http://dx.doi.org/10.1126/science.273.5277.956

4. Falkenburger BH, Jensen JB, Dickson EJ, Suh BC, Hille B. Phosphoinositides: lipid regulators of membrane proteins. J Physiol 2010; 588:3179-85; PMID:20519312; http://dx.doi.org/10.1113/jphysiol.2010.192153

5. van der Wal J, Habets R, Várnai P, Balla T, Jalink K. Monitoring agonist-induced phospholipase C activation in live cells by fluorescence resonance energy transfer. J Biol Chem 2001; 276:15337-44; PMID:11152673; http://dx.doi.org/10.1074/jbc.M007194200

6. Falkenburger BH, Jensen JB, Hille B. Kinetics of PIP2 metabolism and KCNQ2/3 channel regulation studied with a voltage-sensitive phosphatase in living cells. J Gen Physiol 2010; 135:99-114; PMID:20100891; http://dx.doi.org/10.1085/jgp.200910345

7. Yudin Y, Lukacs V, Cao C, Rohacs T. Decrease in phosphatidylinositol 4,5-bisphosphate levels mediates desensitization of the cold sensor TRPM8 channels. J Physiol 2011; 589:6007-27; PMID:22005680; http://dx.doi.org/10.1113/jphysiol.2011.220228

8. Itsuki K, Imai Y, Hase H, Okamura Y, Inoue R, Mori MX. PLC-mediated PI(4,5)P2 hydrolysis regulates activation and inactivation of TRPC6/7 channels. J Gen Physiol 2014; 143:183-201; PMID:24470487; http://dx.doi.org/10.1085/jgp.201311033