Multiple Enzyme Isoforms Provide the Cell With Tools to Precisely Control Second Messenger Levels

Second messengers are defined as small molecules that are synthesized in the cell in response to extracellular first messengers,1 and diffuse through the cytoplasm to mediate their effects. This definition is sometimes broadened to include hydrophobic molecules, such as diacylglycerol (DAG).2 Synthesis and degradation of second messengers are regulated by a number of enzymes expressed in mammalian cells. Such enzymes include, but are not limited to, adenylyl cyclase (AC) for synthesis of cyclic AMP (cAMP), guanylyl cyclase for synthesis of cyclic GMP (cGMP), cyclic nucleotide phosphodiesterase (PDE) for hydrolysis of cAMP and cGMP, and phospholipase C (PLC) for hydrolysis of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate (IP3) and DAG. Each of these enzyme groups consists of a plethora of isoforms. Thus, there are 10 different gene products in the mammalian AC gene family (AC1–9 and an atypical soluble AC isoform).3–5 Furthermore, different splice variants of AC4, AC5, and AC8 have been cloned.6–8 A large number of PDE and PLC isoforms have also been cloned. To date, 21 different PDE genes divided among 11 gene families have been identified in mammals.9 Most PDE families consist of more than 1 gene products, and most genes code for more than 1 messenger RNA. Although some PDE gene families hydrolyze cAMP exclusively (PDE4, PDE7, and PDE8), many of these enzymes hydrolyze both cAMP and cGMP (PDE1, PDE2, PDE3, PDE10, and PDE11), and some hydrolyze cGMP exclusively (PDE5, PDE6, and PDE9).9 There are at least 13 different PLC isoforms generating IP3 and DAG, which also exist in several different splice variants.10–11 Thus, the number of enzyme isoforms regulating synthesis and degradation of any given second messenger, even within 1 cell, is almost mind-boggling, and raises the question: Why are there so many different isoforms of enzymes catalyzing the synthesis or degradation of the same second messenger?

It is generally believed that the large number of second messenger-synthesizing and -degrading enzyme isoforms provide the cell with tools to precisely regulate second messenger levels in different subcellular compartments and following exposure to different stimuli.

Different Subcellular Pools of Second Messengers Result in Different Biological Responses

The cell uses a number of mechanisms to limit second messenger levels to different subcellular compartments. Three of these mechanisms, which are by no means mutually exclusive, are shown schematically in Figure 1, A–C. Not surprisingly, these subcellular second messenger pools have been linked to different downstream effects and biological responses, although the lack of sensitive tools to measure subcellular changes in second messengers and intracellular signaling events has hampered progress in this area. First, the localization of an isoform causing synthesis or degradation of a second messenger to different membrane structures, organelles, or cytosol, may determine the cellular response (Figure 1A). One example is the existence of the soluble AC (sAC) isoform in somatic cells, which was previously thought to be largely restricted in its expression pattern to sperm. AC1–9 are transmembrane proteins with a number of membrane spanning α-helices, whereas sAC lacks transmembrane spanning domains but nevertheless associates with a number of intracellular compartments.12 Recently, sAC was shown to play an important role in axon development.5 Likely, the action of sAC involves cAMP synthesis in a cellular compartment different from that affected by AC1–9.12 In some instances, different enzyme isoforms or splice variants contain different membrane-targeting sequences. For example, the N-terminal regions of cAMP-specific PDE4 isoforms are believed to play a role in targeting of the different PDE4 isoforms to distinct subcellular compartments.13 The PDE4A splice variant 1 (PDE4A1) contains an N-terminal sequence that localizes its binding to Golgi and Golgi vesicles, whereas other PDE4 isoforms contain other unique N-terminal regions.13 Subcellular localization of enzymes is regulated by a growing number of membrane binding domains, such as pleckstrin homology (PH) domains, Bin/Amphipathysin/Rvs (BAR) domains, and phox (PX) domains.14–15 The N-terminal PH domain of PLC-81 binds phosphoinositides with high affinity, although PH domains in other proteins show more nonspecific lipid-binding properties.11,14 Other domains confer binding to membranes in specific subcellular compartments. PX domains, for example, are specialized for endosomal targeting, and BAR domain dimers bind curved vesicular membranes, such as budding vesicles.14 Second, the biological response to a second messenger can depend on the scaffolding complex that binds enzymes that...
resulted in cytoskeletal reorganization and increased phosphorylation of the PKA substrate vasodilator-stimulated phosphoprotein (VASP).

 Consequently, PKA is activated and phosphorylates other AKAP targets in the correct cellular vicinity. Some AKAPs have also been found to associate with cAMP-hydrolyzing PDE isoforms, thereby providing a mechanism for fine-tuning local cAMP levels and downstream effects of PKA. The PDE4D splice variant PDE4D3 binds to muscle-selective AKAP (mAKAP), and this association results in low cAMP levels and prevents PKA activation under basal conditions. Following stimulation of cAMP synthesis, however, PKA is activated and phosphorylates mAKAP. These phosphorylation events result in a more efficient PDE4D3 action, and provides a negative feedback loop for termination of the cAMP signal.

Third, the biological response to a second messenger can be regulated by cross-talk between multiple intracellular signaling pathways, that are sometimes also being activated in the same subcellular compartment or scaffolding complex (Figure 1C). An interesting example of this mode of operation is the ability of PDE4D3 to recruit ERK5 to the mAKAP complex. Activation of ERK results in phosphorylation of PDE4D3 and subsequent inhibition of PDE activity, resulting in increased levels of cAMP.

In a study published in this issue of Circulation Research, Gros, Ding, Feldman, and colleagues address the question of whether different AC isoforms activate different biological responses in rat aortic smooth muscle cells (SMCs) and human HEK 293 cells. Overexpression of AC1 and AC3 (representative of the AC1, AC3, and AC8 subfamily), AC2 (representative of the AC2, AC4, and AC7 subfamily), and AC5 and AC6 (the 2 AC isoforms in this subfamily), at levels that resulted in similar forskolin-induced cAMP accumulation in the presence of a PDE inhibitor, was achieved by using adenoviral vectors. This approach generated several interesting findings, because it has been generally believed that the biological effects of specific transmembrane AC isoforms are primarily dependent on the upstream G-protein-coupled receptors and differential regulation of activity of the different AC isoforms. The present study demonstrates that overexpression of AC1 and AC3 (which, in contrast to AC1, is endogenously expressed in rat SMCs), resulted in growth inhibition, whereas overexpression of AC2 and AC6 had no such effect. A role for AC3 in regulation of SMC proliferation is consistent with previous studies on aortic SMC isolated from AC3-deficient mice. SMCs from these mice were insensitive to the growth-inhibiting effects of prostaglandin E2. Conversely, overexpression of AC6, but not the other AC isoforms, caused cytoskeletal rearrangements.

Another interesting observation is that overexpression of AC6, but not AC1 or AC2, resulted in PKA phosphorylation of the enzyme (E) to different scaffolding proteins (shaded gray and white ovals, respectively) may alter the biological response of the second messenger by bringing it in close proximity to different effectors. C, Cross-talk between signaling events induced by the enzyme (E) and other signaling molecules activated in the cell (black oval) can result in different biological effects. See text for specific examples of the mechanisms shown in A–C, D. In the study by Gros, Ding et al., adenylyl cyclase (AC1) and AC6 are shown to mediate distinct biological effects. It is likely that these responses are due to different scaffolding complexes for the different AC isoforms. Thus, overexpressed AC1 (and AC3) was found to bind to ERK1/2 and result in growth inhibition, whereas AC6 overexpression resulted in increased levels of cAMP.

How can these results be explained? As discussed above, at least 3 mechanisms can potentially result in differential biological effects in cells (see Figure 1A–C). The study by Gros, Ding et al. indicates that at least 1 of these mechanisms might be involved in the responses induced by the different AC isoforms, namely binding of distinct AC isoforms to different scaffolding proteins (Figure 1D). Thus, AC1 and AC3 were found to associate with ERK1/2, whereas AC2 and AC6 did not. Further studies are needed to evaluate the mechanisms of AC1/3-induced growth inhibition and the role of ERK1/2 association. It is also possible that the different AC isoforms show differences in subcellular localization (although overexpression might result in altered subcellular localization compared with that of the endogenous isoform), or that cross-talk with other signaling molecules modulates the biological response to overexpression of AC1 and AC3 versus AC6. There are previous examples of...
different subcellular localization of AC isoforms. In neurons, AC8 is targeted to excitatory synapses, whereas AC1 is fond in close proximity to this compartment.22

Together, these findings begin to question the validity of measuring levels of second messengers in total cell lysates, and add further evidence to the hypothesis that subcellular pools of second messengers are crucial in regulating different cellular responses. More specific studies of subcellular regulation of some second messengers can now be performed by using fluorescent indicators.23–24 With new tools in hand, our understanding of “subcellular” signal transduction will undoubtedly increase in the near future.

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References

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A Single Second Messenger: Several Possible Cellular Responses Depending on Distinct Subcellular Pools
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