Perspectives on: Cyclic nucleotide microdomains and signaling specificity

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The purpose of the Perspectives in General Physiology is to provide a forum where scientific uncertainties or controversies, or important problems, are discussed in an authoritative, yet open manner. The Perspectives are solicited by the editors—often based on recommendations by members of the editorial advisory board. To frame the issue, two or more experts are invited to present brief points of view on the problem; these are published consecutively in the Journal. One or more experts and the organizer review the contributions, but the comments and opinions expressed in the Perspectives are those of the authors and not necessarily those of the editors or the editorial advisory board. The Perspectives are accompanied by a few editorial paragraphs that introduce the problem and invite the submission of comments, in the form of letters to the editor, which usually are published four months after publication of the Perspectives. After the letters to the editor have been published, further responses are limited to full manuscripts.

The prototypical second messenger, cAMP, was discovered in 1957 (Sutherland, 1962), and many landmark discoveries since then have given us a basic biochemical description of cellular signaling events that are more widespread and more amplified in terms of their effects than membrane-delimited events such as synaptic transmission (Beavo and Brunton, 2002; Conti and Beavo, 2007; Willoughby and Cooper, 2007). We have a wealth of information about the identities, structures, and functions of the different proteins involved in this signaling pathway. Yet, if one delves deeper than what the average undergraduate biology major takes for granted about this classic signaling pathway, we still lack answers to two related questions that an engineer would consider to be fundamental to the description of a communication device: (1) How is signaling specificity achieved? For cyclic nucleotides and other second messengers, there is much to be learned about how information is relayed from a very large number of extracellular receptors for hormones, neurotransmitters, odorants, cytokines, etc., through a very small number of intracellular signaling molecules; and (2) How is the signaling compartmentalized? The morphological and biochemical basis for cellular microdomains that spatially segregate cyclic nucleotide signals remains largely a mystery.

The concept of compartmentation emerged more than 30 years ago in studies of cardiac myocytes to help explain how a variety of extracellular stimuli that primarily act through cAMP can have very different downstream effects on the cell (Corbin et al., 1977; Brunton et al., 1981; Steinberg and Brunton, 2001). For example, isoproterenol, a β-adrenergic agonist, triggers cAMP-dependent activation of PKA and subsequent phosphorylation of proteins associated with excitability and the strength and timing of heart muscle contraction. Prostaglandins cause similar changes in total cellular cAMP and PKA activity but no changes in contractility. Glucagon-like peptide, also working through PKA, regulates metabolism in heart muscle cells (Bers and Ziolo, 2001; Vila Petroff et al., 2001). Simple diffusion theory predicts that in the absence of any interference, cAMP will traverse the cytoplasm of a 20-μM cell in ∼0.2 s, and no appreciable accumulation of cAMP builds up around a single adenylyl cyclase molecule because it is a very slow enzyme (Rich et al., 2000). This is one way in which cAMP is very different from Ca^{2+}, which can accumulate to high concentrations at the mouth of a Ca^{2+} channel because of the high throughput rate. In essence, each cAMP diffuses away faster than the next one is produced. Thus, to explain the above observations, either there must be subcellular physical barriers that restrict the diffusion of cAMP, or the local variations in cAMP concentrations result from very high synthesis and degradation rates.

In the past 15 years, progress in four areas has provided more direct evidence for cAMP compartmentation and information on some of the key molecular processes that underlie this phenomenon: (1) the understanding of A kinase–anchoring proteins as molecular scaffolds that colocalize and organize signaling proteins has increased markedly (Jarnaess and Taskén, 2007; Dodge-Kafka et al., 2008; Welch et al., 2010); (2) a series of intracellular sensors for cAMP and cGMP were developed that have allowed cyclic nucleotide signals to be

measured with better spatial and temporal precision (Rich et al., 2001; Nikolaev et al., 2004; Ponsioen et al., 2004; Nausch et al., 2008; Willoughby and Cooper, 2008); (3) the extensive study of the biochemical properties and potential modes of regulation of phosphodiesterases (PDEs) has led to an increased appreciation of the critical role PDEs play in shaping and controlling cAMP signals (Conti and Beavo, 2007; Baillie, 2009; Blackman et al., 2011); and (4) computational modeling of cellular compartments has ruled out several hypotheses and led to a greater understanding of possible mechanisms (Rich et al., 2000, 2001; Saucerman et al., 2006; Iancu et al., 2007; Oliveira et al., 2010; Feinstein et al., 2012; Sample et al., 2012). Despite these advances, however, our overall understanding of cAMP compartments, or microdomains, remains fairly crude. It has seemed to some that we should be further along given the promise of the sensors and the wealth of molecular information that has been accumulated.

The four Perspectives in this issue summarize recent developments and provide guidance for future research in each of the four areas described above. Countering the sentiment expressed above, these articles provide reasons to be optimistic that current technical barriers can be overcome and that long-standing controversies can be resolved. Kapiloff et al. take the available molecular information about A kinase–anchoring proteins and provide a conceptual framework for how these scaffold proteins likely participate in feedback inhibition of cAMP levels and the shaping of cAMP signals in microdomains. Rich et al. critically evaluate the advantages and disadvantages of the available single-cell sensors for cAMP and cGMP, and they point out the pitfalls that can confound the interpretation of imaging data, such as overexpression and sensitivity to environmental variables like pH. They go on to describe recent improvements in fluorescent sensors in terms of dynamic range and the highly promising developments in hyperspectral imaging and automated analysis. These latter approaches should allow for improved signal-to-noise ratio of fluorescence and FRET measurements, the simultaneous use of multiple probes, measurements in tissue preparations, and unbiased data analysis. All of the techniques are evaluated in terms of their ability to detect a range of predicted cyclic nucleotide signals, which should make this a particularly useful guide for both future probe development and experiments. Conti et al. focus on the question of whether PDE activity is sufficient to generate cAMP microdomains in the absence of physical diffusion barriers. They present several lines of evidence against this simple notion, including the highly isoform-specific regulation of PDEs by receptor stimulation and other intracellular signaling events; the absence of spreading of certain signals when PDEs are inhibited (PDE inhibition usually, but not always, allows for the eventual spread of cAMP signals to other parts of the cell); the resistance of near-membrane cAMP signals to cellular washout in whole cell patch-clamp experiments; and new data showing that total stimulated PDE activity in neonatal cardiac myocytes is 100-fold lower than the levels required to generate cAMP gradients in the absence of physical barriers. Saucerman et al. summarize the progress that has been made with computational models of cAMP signaling over the last 13 years and reinforce the view that a combination of physical barriers and regulated PDE activity within the regions of restricted diffusion create cAMP microdomains and shape cAMP signals. Several different mechanisms can cause diffusion restrictions, including buffering, organelles and intracellular membranes, cytoskeleton, local properties of cytosol, and cell shape. A major challenge for the future will be to assess in a quantitative way how these different mechanisms contribute to cyclic nucleotide microdomains.

Letters to the editor related to these Perspectives should be received no later than Monday, March 3, 2014. The letters may be no longer than two printed pages (approximately six double-spaced pages) and will be subject to editorial review. They may contain no more than one figure, no more than 15 references, and no significant references to unpublished work. Letters should be prepared according to The Journal's Instructions and can be submitted electronically at http://www.jgp.org.

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REFERENCES

Baillie, G.S. 2009. Compartmentalized signalling: spatial regulation of cAMP by the action of compartmentalized phosphodiesterases. *FEBS J.* 276:1790–1799. http://dx.doi.org/10.1111/j.1742-4658.2009.06926.x

Beavo, J.A., and L.L. Brunton. 2002. Cyclic nucleotide research—still expanding after half a century. *Nat. Rev. Mol. Cell Biol.* 3:710–718. http://dx.doi.org/10.1038/nrm911

Bers, D.M., and M.T. Ziolo. 2001. When is cAMP not cAMP? Effects of compartmentalization. *Circ. Res.* 89:373–375.

Blackman, B.E., K. Horner, J. Heidmann, D. Wang, W. Richter, T.C. Rich, and M. Conti. 2011. PDE4D and PDE4B function in distinct subcellular compartments in mouse embryonic fibroblasts. *J. Biol. Chem.* 286:12590–12601. http://dx.doi.org/10.1074/jbc.M110.203604

Brunton, L.L., J.S. Hayes, and S.E. Mayer. 1981. Functional compartmentation of cyclic AMP and protein kinase in heart. *Adv. Cyclic Nucleotide Res.* 14:391–397.

Conti, M., and J. Beavo. 2007. Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling. *Annu. Rev. Biochem.* 76:481–511. http://dx.doi.org/10.1146/annurev.biochem.76.060305.150444

Corbin, J.D., P.H. Sugden, T.M. Lincoln, and S.L. Keely. 1977. Compartmentalization of adenosine 3′:5′-monophosphate and adenosine 3′:5′-monophosphate-dependent protein kinase in heart tissue. *J. Biol. Chem.* 252:3854–3861.

Dodge-Kafka, K.L., A. Bauman, and M.S. Kapiloff. 2008. A-kinase anchoring proteins as the basis for cAMP signaling. *Handbook Exp. Pharmacol.* 186:3–14. http://dx.doi.org/10.1007/978-3-540-72843-6_1

Feinstein, W.P., B. Zhu, S.J. Leavesley, S.L. Sayner, and T.C. Rich. 2012. Assessment of cellular mechanisms contributing to cAMP

compartmentalization in pulmonary microvascular endothelial cells. *Am. J. Physiol. Cell Physiol.* 302:C839–C852. http://dx.doi.org/10.1152/ajpcell.00361.2011

Iancu, R.V., S.W. Jones, and R.D. Harvey. 2007. Compartmentation of cAMP signaling in cardiac myocytes: a computational study. *Biophys. J.* 92:3317–3331. http://dx.doi.org/10.1529/biophysj.106.095356

Jarnaess, E., and K. Taskén. 2007. Spatiotemporal control of cAMP signalling processes by anchored signalling complexes. *Biochem. Soc. Trans.* 35:931–937. http://dx.doi.org/10.1042/BST0350931

Nausch, L.W., J. Ledoux, A.D. Bonev, M.T. Nelson, and W.R. Dostmann. 2008. Differential patterning of cGMP in vascular smooth muscle cells revealed by single GFP-linked biosensors. *Proc. Natl. Acad. Sci. USA.* 105:365–370. http://dx.doi.org/10.1073/pnas.0710387105

Nikolaev, V.O., M. Bünemann, L. Hein, A. Hannawacker, and M.J. Lohse. 2004. Novel single chain cAMP sensors for receptor-induced signal propagation. *J. Biol. Chem.* 279:37215–37218. http://dx.doi.org/10.1074/jbc.C400302200

Oliveira, R.F., A. Terrin, G. Di Benedetto, R.C. Cannon, W. Koh, M. Kim, M. Zaccolo, and K.T. Blackwell. 2010. The role of type 4 phosphodiesterases in generating microdomains of cAMP: large scale stochastic simulations. *PLoS ONE.* 5:e11725. http://dx.doi.org/10.1371/journal.pone.0011725

Ponsioen, B., J. Zhao, J. Riedl, F. Zwartkruis, G. van der Krogt, M. Zaccolo, W.H. Moolenaar, J.L. Bos, and K. Jalink. 2004. Detecting cAMP-induced Epac activation by fluorescence resonance energy transfer: Epac as a novel cAMP indicator. *EMBO Rep.* 5:1176–1180. http://dx.doi.org/10.1038/sj.embor.7400290

Rich, T.C., K.A. Fagan, H. Nakata, J. Schaack, D.M.F. Cooper, and J.W. Karpen. 2000. Cyclic nucleotide–gated channels colocalize with adenylyl cyclase in regions of restricted cAMP diffusion. *J. Gen. Physiol.* 116:147–162. http://dx.doi.org/10.1085/jgp.116.2.147

Rich, T.C., K.A. Fagan, T.E. Tse, J. Schaack, D.M.F. Cooper, and J.W. Karpen. 2001. A uniform extracellular stimulus triggers distinct cAMP signals in different compartments of a simple cell. *Proc. Natl. Acad. Sci. USA.* 98:13049–13054. http://dx.doi.org/10.1073/pnas.221381398

Sample, V., L.M. DiPilato, J.H. Yang, Q. Ni, J.J. Saucerman, and J. Zhang. 2012. Regulation of nuclear PKA revealed by spatiotemporal manipulation of cyclic AMP. *Nat. Chem. Biol.* 8:375–382. http://dx.doi.org/10.1038/nchembio.799

Saucerman, J.J., J. Zhang, J.C. Martin, L.X. Peng, A.E. Stenbit, R.Y. Tsien, and A.D. McCulloch. 2006. Systems analysis of PKA-mediated phosphorylation gradients in live cardiac myocytes. *Proc. Natl. Acad. Sci. USA.* 103:12923–12928. http://dx.doi.org/10.1073/pnas.0600137103

Steinberg, S.F., and L.L. Brunton. 2001. Compartmentation of G protein-coupled signaling pathways in cardiac myocytes. *Annu. Rev. Pharmacol. Toxicol.* 41:751–773. http://dx.doi.org/10.1146/annurev.pharmtox.41.1.751

Sutherland, E.W. 1962. The biological role of adenosine-3′,5′-phosphate. *Harvey Lect.* 57:17–33.

Vila Petroff, M.G., J.M. Egan, X. Wang, and S.J. Sollott. 2001. Glucagon-like peptide-1 increases cAMP but fails to augment contraction in adult rat cardiac myocytes. *Circ. Res.* 89:445–452. http://dx.doi.org/10.1161/hh1701.095716

Welch, E.J., B.W. Jones, and J.D. Scott. 2010. Networking with AKAPs: context-dependent regulation of anchored enzymes. *Mol. Interv.* 10:86–97. http://dx.doi.org/10.1124/mi.10.2.6

Willoughby, D., and D.M. Cooper. 2007. Organization and Ca^{2+} regulation of adenylyl cyclases in cAMP microdomains. *Physiol. Rev.* 87:965–1010. http://dx.doi.org/10.1152/physrev.00049.2006

Willoughby, D., and D.M. Cooper. 2008. Live-cell imaging of cAMP dynamics. *Nat. Methods.* 5:29–36. http://dx.doi.org/10.1038/nmeth1135