tion in the embryo appears to be very different. Here, CPEB and Maskin are mostly confined to animal-pole blastomeres, the cells that give rise to ectodermal structures such as the skin and nervous system. Within these blastomeres, CPEB and Maskin and *cyclin B* mRNA are associated with mitotic spindles and centrosomes (*12*), the microtubule machinery that separates chromosome pairs during the final stages of cell division. The abrogation of CPEB or Maskin activity by microinjection of antibodies or dominant-negative mutant proteins inhibits cell division and induces multipolar spindle assembly and accumulation of excess centrosomes. Importantly, injection of a mutated form of CPEB that is unable to associate with microtubules has little effect on cyclin B synthesis but causes *cyclin B* mRNA to detach from the spindles. This leads to a dramatic decrease in cyclin B protein accumulation at the spindles and the blocking of cell division. Disruption of spindle-associated translation of *cyclin B* mRNA thus appears to block normal progression through the cell cycle. This unexpected observation suggests that controlling the location of cyclin B production, in addition to regulating the time and place of cyclin B destruction, may be essential for cells to progress through the cell cycle.

It is unclear whether precise spatial control of cyclin mRNA translation is specific to early embryonic development, or is common to all cells. In early embryos, the cells are generally large and the production of mitotic cyclins at their site of action (the centrosome and spindle apparatus) may be particularly important for cell cycle progression. In smaller somatic cells, by contrast, there would appear to be relatively little need for local translation of *cyclin B* mRNA. However, cyclin B Cdk1 is capable of modifying an enormous number of cellular proteins, at least in vitro. Local translation of this relatively nonspecific kinase could help to generate critical substrate specificity, even in smaller somatic cells. Thus, cell cycle progression and patterning during embryonic development may be regulated by the local control of mRNA translation as well as by the temporal and local control of protein degradation.

References

1. R. W. King *et al.*, *Science* **274**, 1652 (1996).
2. J. Sonoda, R. P. Wharton, *Genes Dev.* **13**, 2704 (1999).
3. M. Asaoka-Taguchi *et al.*, *Nature Cell Biol.* **1**, 431 (1999).
4. B. Dalby, D. M. Glover, *EMBO J.* **12**, 1219 (1993).
5. J. Sonoda, R. P. Wharton, *Genes Dev.* **15**, 762 (2001).
6. S. Nakahata *et al.*, *J. Biol. Chem.* **276**, 20945 (2001).
7. C. Wreden *et al.*, *Development* **124**, 3015 (1997).
8. J. D. Richter, in *Translational Control of Gene Expression*, N. Sonenberg, J. W. B Hershey, M. B. Mathews, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2000), chap. 27.
9. R. Mendez *et al.*, *Nature* **404**, 302 (2000).
10. R. Mendez *et al.*, *Mol. Cell* **6**, 1253 (2000).
11. B. Stebbins-Boaz *et al.*, *Mol. Cell* **4**, 1017 (1999).
12. I. Groisman *et al.*, *Cell* **103**, 435 (2000).

PERSPECTIVES: SIGNAL TRANSDUCTION

Bringing Channels Closer to the Action!

Stéphane A. Laporte, Robert H. Oakley, Marc G. Caron

Cells are arrayed with a large number of surface receptors that enable them to recognize and respond to neurotransmitters, hormones, odorants, and growth factors. When these extracellular ligands bind to their receptors, they activate a cascade of intracellular signals that alter effector molecules such as enzymes or ion channels, leading to the generation of physiological responses. Many plasma membrane receptors belong to the extensive G protein–coupled receptor (GPCR) family. When bound to their ligands, GPCRs become activated and interact with heterotrimeric guanine nucleotide binding proteins (G proteins), which dissociate into Gα and Gβγ subunits. These subunits then amplify and propagate signals within the cell—by regulating the production of second messenger molecules such as adenosine 3′,5′-monophosphate (cAMP)—resulting in altered activity of effector proteins such as enzymes or ion channels.

There are many GPCRs at the cell surface that activate different G proteins and modulate different downstream effector molecules. Moreover, different GPCRs expressed in the same cell can activate the same G protein and effector molecule, yet elicit completely different physiological responses. How, then, do cells manage to ensure that one signaling pathway is selectively and rapidly engaged without the activation of other pathways? The idea is emerging that cells might achieve the required specificity and rapidity by organizing macromolecular signaling complexes in the plasma membrane that contain the GPCR, its G protein, the enzyme generating the second messenger, and the effector protein (see the figure). An elegant example of how such a signaling complex might work is presented by Davare *et al.* on page 98 of this issue (*1*).

Stimulation of the β_2 adrenergic receptor (β_2AR), a GPCR, by its ligand results in activation of a signaling pathway that ultimately increases the activity of the L-type class C calcium channel, $Ca_v1.2$. An increase in $Ca_v1.2$ channel activity results in altered contraction of heart muscle and modulation of nerve impulses in brain neurons. By immunoprecipitating β_2AR from rat hippocampal neurons, Davare and colleagues (*1*) provide evidence that β_2AR is associated with the central pore-forming α_{1C} subunit of $Ca_v1.2$. This association appears to be specific for β_2AR because the α_{1C} subunit was not detected in immune complexes containing other neuronal GPCRs. The authors pinpoint the carboxyl terminus of β_2AR as the site where this receptor interacts with the $Ca_v1.2$ α_{1C} subunit. They also show that β_2AR colocalizes with the $Ca_v1.2$ channel at postsynaptic sites (including the dendritic spines) of excitatory neurons.

Although these results indicate an association between the β_2AR and the $Ca_v1.2$ calcium channel, a much more elaborate complex presumably exists. Stimulation of β_2AR results in activation of cAMP–dependent protein kinase A (PKA), phosphorylation of the α_{1C} subunit, and increased activity of the $Ca_v1.2$ channel (*1*, *2*). Attenuation of channel activity depends on specific phosphatases that dephosphorylate the α_{1C} subunit. Both PKA and the phosphatase PP2A directly associate with the α_{1C} subunit and modulate its activity (*2*). Davare *et al.* now demonstrate that the α and βγ G protein subunits as well as adenylyl cyclase (the enzyme that catalyzes cAMP production) also associate with the α_{1C} subunit. Thus, β_2AR and the $Ca_v1.2$ calcium channel presumably assemble into a macromolecular complex that includes the G protein subunits, adenylyl cyclase, PKA, and the counterbalancing phosphatase PP2A (see the figure). But how does this concoction of proteins propagate signals within the cell?

The investigators address this question by recording the activity of the $Ca_v1.2$ calcium channel in rat hippocampal neurons before and after activation of β_2AR with albuterol. (Albuterol is a selective β_2AR agonist that mimics epinephrine, the natural

The authors are at the Howard Hughes Medical Institute Laboratories, Departments of Cell Biology and Medicine, Duke University Medical Center, Durham, NC 27710, USA. E-mail: caron002@mc.duke.edu

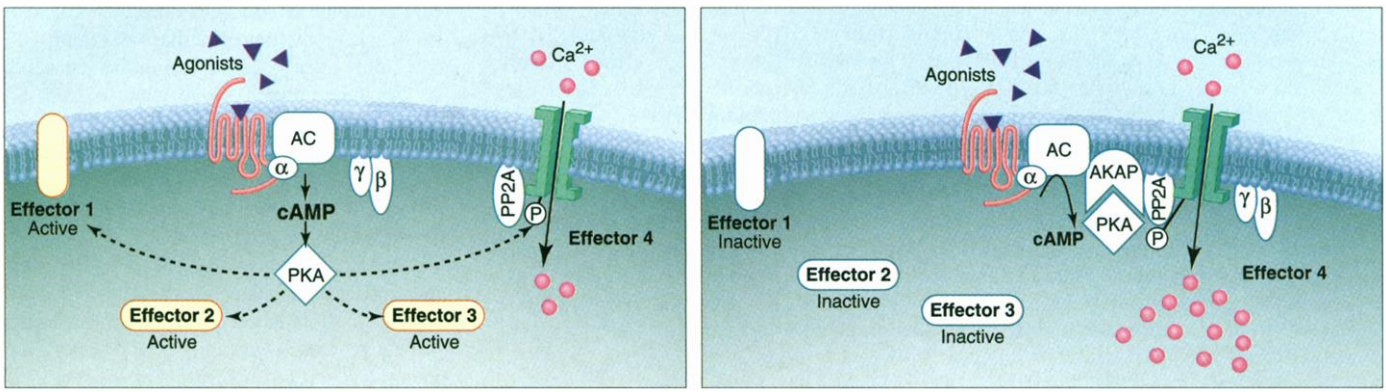
ligand of β_2AR.) When albuterol is applied to the whole cell, there is no change in $Ca_v1.2$ channel activity as measured by a patch electrode attached to the neuronal plasma membrane. In marked contrast, when albuterol is added to the recording electrode, thus specifically activating those receptors close to the channel, a robust increase in $Ca_v1.2$ channel activity is observed. These results suggest that assembly of the β_2AR and $Ca_v1.2$ calcium channel into a macromolecular signaling complex ensures that the receptor activates only this channel. When the full cellular complement of β_2ARs is stimulated, cAMP levels are presumably insufficient to elicit $Ca_v1.2$ channel activation. However, when receptors in the immediate vicinity of the channel are stimulated, the local concentration of cAMP is high enough to activate $Ca_v1.2$. This arrangement affords the cell an attractive solution to the problem of implementing a specific response to one of many GPCRs.

Besides providing insight into multiprotein signaling complexes, the Davare *et al.* work raises pharmacologically pertinent questions that may shed light on the molecular basis of partial agonism. Do partial agonists that would normally elicit only a partial response, such as albuterol, display enhanced efficacy when activating preassembled complexes (containing the receptor, G protein, and effector) compared with "unorganized" linear signaling pathways? The Davare *et al.* results suggest that this may be the case, because stimulation of the whole cell with albuterol did not elicit $Ca_v1.2$ channel activation, whereas addition of albuterol to the patch electrode resulted in robust channel opening. This response was even greater than that induced by addition of the full agonist isoproterenol, which stimulated the full cellular complement of β_2ARs. In this respect, it would be instructive to see whether isoproterenol added to the patch electrode would produce similar or greater channel activation than the apparently robust activation measured with albuterol in the same recording mode.

The notion that receptors and their downstream effector proteins assemble into signaling complexes raises several key questions. How general is this phenomenon? Can the β_2AR assemble and modulate the activity of other ion channels (such as ligand-gated channels) or transporter proteins in neurons? Is the β_2AR–calcium channel signaling complex restricted to neurons or does it assemble in, for example, heart muscle? A recent report suggests that β_2AR–calcium channel complexes do exist in the heart (*3*). Do all GPCRs that activate ion channels form such complexes? What are the determinants controlling complex assembly at the plasma membrane?

Recent evidence suggests that GPCRs form complexes with other signaling proteins in order to modulate their activity. For example, the dopamine D5 GPCR forms a complex with the ligand-gated $GABA_A$ (γ-aminobutyric acid type A) channel through a direct interaction of its carboxyl terminus with the γ subunit of the channel (*4*). This interaction is presumably crucial for the reciprocal modulation of dopamine and GABA signaling in hippocampal neurons. Furthermore, β_2AR interacts with the Na^+/H^+ exchanger regulatory factor (NERF), which modulates the activity of the Na^+/H^+ transporter protein in the kidney (*5*), raising the possibility that multiprotein signaling complexes might regulate other classes of membrane proteins. Finally, other proteins that desensitize GPCRs associate with agonist-stimulated receptors to recruit and assemble complexes containing signaling kinases (*6–8*).

The Davare *et al.* work goes beyond existing notions of how ion channels are regulated by GPCRs. Previous studies have shown that when ion channels are activated directly by G protein subunits, these events are confined to the plasma membrane (*9*). However, it is commonly assumed that activation of ion channels through second messenger–dependent kinases such as PKA can be sensed anywhere within the cell because of the rapid diffusion of small second messenger molecules. The Davare *et al.* findings certainly challenge the generality of this assumption. The assembly of complexes that contain the receptor and various components of the signal transduction machinery (as well as proteins that turn off the signal) may provide the cell with an exquisitely selective means to engage a specific signaling pathway in response to the binding of a ligand to its GPCR.



United we signal. **(Left)** Binding of a ligand (or agonist) to a G protein–coupled receptor (GPCR), such as the β_2 adrenergic receptor (β_2AR), leads to activation of heterotrimeric G proteins (Gα, G$\beta\gamma$) and the stimulation of adenylyl cyclase (AC), which increases production of the second messenger molecule cAMP. An increase in cAMP promotes activation of the cAMP-dependent protein kinase (PKA), which activates various downstream effector molecules (active effectors). One such effector is the L-type calcium channel (effector 4) whose activity is modulated by PKA-mediated phosphorylation of its α_{1C} subunit (*2*). This linear signaling pathway is nonselective because a single class of receptor can engage many pathways, and different classes of receptors can activate the same downstream effector. **(Right)** Davare *et al.* (*1*) propose a more integrated model of GPCR signaling in which, for example, β_2AR forms a multiprotein complex with specific signaling molecules such as the Gα and G$\beta\gamma$ subunits, AC, PKA and its anchoring protein AKAP, the phosphatase PP2A, and the final effector, the L-type calcium channel. Such multiprotein signaling complexes ensure rapid and specific activation of the correct signaling pathway.

References

1. M. A. Davare *et al.*, *Science* **293**, 98 (2001).
2. M. A. Davare, M. C. Horne, J. W. Hell, *J. Biol. Chem.* **275**, 39710 (2000).
3. Y. Chen-Izu *et al.*, *Biophys. J.* **79**, 2547 (2000).
4. F. Liu *et al.*, *Nature* **403**, 274 (2000).
5. R. A. Hall *et al.*, *Nature* **392**, 626 (1998).
6. L. M. Luttrell *et al.*, *Science* **283**, 655 (1999).
7. K. A. DeFea *et al.*, *J. Cell. Biol.* **148**, 1267 (2000).
8. P. H. McDonald *et al.*, *Science* **290**, 1574 (2000).
9. D. E. Clapham, E. J. Neer, *Annu. Rev. Pharmacol. Toxicol.* **37**, 167 (1997).