**Cyclic AMP Sensors in Living Cells**

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Dan Kelsay

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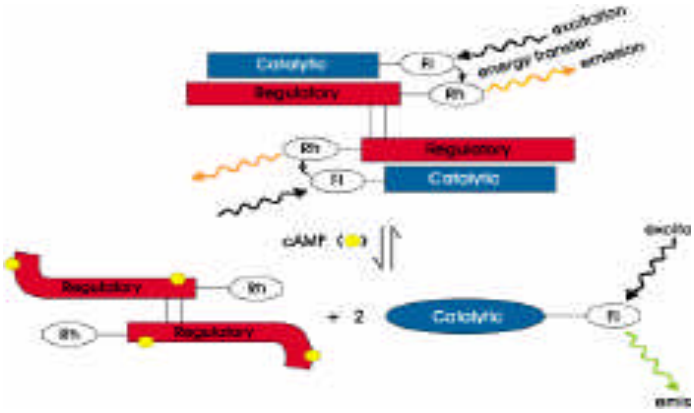
Cyclic Adenosine Monophosphate (cAMP) is an intracellular second messenger that transmits information to several proteins including nucleotide-gated ion channels and protein kinase A (PKA). These effectors then regulate a diverse range of cellular functions unique to each cell. Much is know about the enzymes that control cAMP levels but not much is know about the information that is assumed to be encoded in cAMP signals. This is because of the difficulties in measuring cAMP in living cells. It is believed that the frequency and location of the sub cellular compartments of cAMP is the source of this encoded information. These ideas are based on comparison to other second messenger systems in the cell especially cGMP located in photoreceptor cells. Until recently cAMP levels could not be measured with spatial or temporal resolution. Two methods in this paper are described.

The first technique is measuring fluorescence resonance energy transfer (FRET) from labeled PKA units. Each PKA type I were labeled with two different dyes, fluorescin and rhodamine. These were microinjected into the cells onto the catalytic and regulatory subunits respectively. In this complex FRET then occurs till cAMP binds to the regulatory subunits. Once cAMP binds to the regulatory subunit the catalytic disassociates. This reaction produces an increase in the fluorescent ratio that can then be measured. This method nicknamed for it elements FlCRhR or (‘flicker’) is promised to have real time results.

The second method uses cyclic nucleotide-gated channel as cAMP sensors. Ion channels that are directly activated by cyclic nucleotides (CNG Channels) respond to cGMP (guanosine monophosphate) signals in photoreceptor cells and olfactory receptor neurons. Olfactory neurons are equally sensitive to cAMP signals. Using electrophysiological or $Ca^{2+}$ imaging techniques on genetically modified sensitive olfactory neurons of rats a series of biological reactions are initiated to produce cAMP signals that can be measured




Changes in cAMP levels near the surface membrane are detected by measuring changes in the rate of $Ca^{2+}$ influx using fluorescent dye, fura-2.

Results from these two different methods greatly favor using nucleotide-gated channels for cAMP sensors. Reasons for this are that this method is simpler to implement, has a high signal to noise ratio, is sensitive to small changes in AC activity and that it can be adapted for use in cell populations or single cells. Also the promise of ‘flicker’ being able to operate in real time proved to be the opposite.

**Cyclic AMP Sensors in Living Cells: What Signals Can They Actually Measure?** Rich and Karpen, Department of Physiology and Biophysics, University of Colorado.