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Do you want a specific signal? Go local! Imaging our way to targeted therapy

Cyclic adenosine monophosphate (cAMP) is a ubiquitous intracellular second messenger, a small molecule that conveys the information carried by hormones, neurotransmitters and other extracellular stimuli to the intracellular environment. cAMP is generated at the plasma membrane when a hormone or neurotransmitter binds to their receptor. In the cell, cAMP diffuses to reach and activate the enzyme protein kinase A which, via phosphorylation of target proteins, triggers a functional response. This sequence of events is at the basis of the ancestral fight-or-flight response – a physiological response that occurs when the body is exposed to danger. On perception of danger, stress hormones such as adrenaline are released which trigger the release of cAMP and subsequent diversion of energy to the muscles so that one can take action. In addition, cAMP is responsible for the ability of the cell to react and adapt to the continuously changing extracellular environment, ensuring organ homeostasis. A key feature of this intracellular signaling system is the ability of cAMP to trigger the appropriate cellular response to each distinct extracellular stimulus. Disruption of cAMP signaling is implicated in various diseases. Thorough understanding of cAMP signaling pathway therefore may reveal novel therapeutic targets.

How cAMP signaling achieves diverse and distinct cellular responses that are hormone-specific has been investigated for decades as it was hard to reconcile the idea that once synthesized at the plasma membrane cAMP diffuses homogeneously throughout the cell and mediates the activation of various targets without resulting in unwanted responses. Researchers hypothesized therefore that distinct cAMP subcellular compartments must exist which contain confined cAMP pools and that these compartments are physically segregated to facilitate activation of separate arms of the cAMP pathway. It is well established that cAMP compartments are achieved in part by phosphodiesterases (PDEs) which are enzymes that degrade cAMP and control its diffusion from one compartment to another, and prevent unnecessary activation of PKA. A kinase anchoring proteins (AKAPs) – a group of structurally diverse proteins that localize in specific subcellular sites and anchor PKA in proximity to its phosphorylation targets, have also been shown to play a key role in signal compartmentalization. AKAPs also bind PDEs and phosphatases which dephosphorylate PKA targets, further contributing to achieve stimulus-specific local signals that are spatially and temporarily controlled.

Despite the hypothesis that distinct cAMP compartments must exist, it was not possible to directly demonstrate how the cell coordinates cAMP mediated responses with specificity until recently when a methodology based on Fluorescence Resonance Energy Transfer (FRET) reporters and imaging of cAMP with high spatial and temporal resolution was developed. FRET-based cAMP sensors contain a cAMP-binding site and two different fluorophores whose intensities' ratio is used as a read-out for cAMP levels. FRET-based sensors can be fused to targeting proteins of interest which direct the sensor to the subcellular compartment where the targeting protein is normally localized. This approach has been successfully exploited to target FRET-based cAMP sensors to distinct subcellular sites and helped reveal the fact that cAMP levels at the plasma membrane are higher than in the bulk cytosol. In addition, targeting of the sensor to the centrosome, nucleus, and sarcoplasmic reticulum (SR), showed that these sites deep in the cell also sense a higher cAMP than in the cytosol, thereby erasing the idea that cAMP propagates to generate a uniform gradient from its point of generation at the plasma membrane to the cytosol, and further consolidated the notion of cAMP compartmentalized signaling.

The exceptional advantages of FRET-based reporters are that they are genetically encoded and can be expressed in living cells, thereby rendering it possible to monitor cAMP dynamics in real time, as they occur in the complex intracellular environment, and within the intact microarchitecture of the cell. This was not possible before as conventional biochemical techniques to measure cAMP were not able to provide information on subcellular location of cAMP, and/ or measure free cAMP dynamics. Another important advantage of FRET-based reporters is that they can be expressed in living organisms as transgenes, thereby allowing for cAMP signaling read-out in free moving animals. FRET imaging has revolutionized our understanding of cAMP signalling as it clearly demonstrated that cAMP is compartmentalized i.e. does not diffuse homogenously within the cell and that this spatial regulation of cAMP, and of its effectors and regulators, is what guarantees the distinct specificity of the hormonal response. FRET-based technology enables researchers to identify the exact topography, and function of cAMP/PKA subcellular compartments, and identify individual PDE isoforms that play crucial roles in these compartments. Full understanding of this organization and function of individual cAMP domains by using FRET will greatly contribute to the development of drugs that target domain-specific cAMP pools rather than global intracellular cAMP in diseased human cells.

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