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The RAINmakers: how receptors orchestrate specific cell functions

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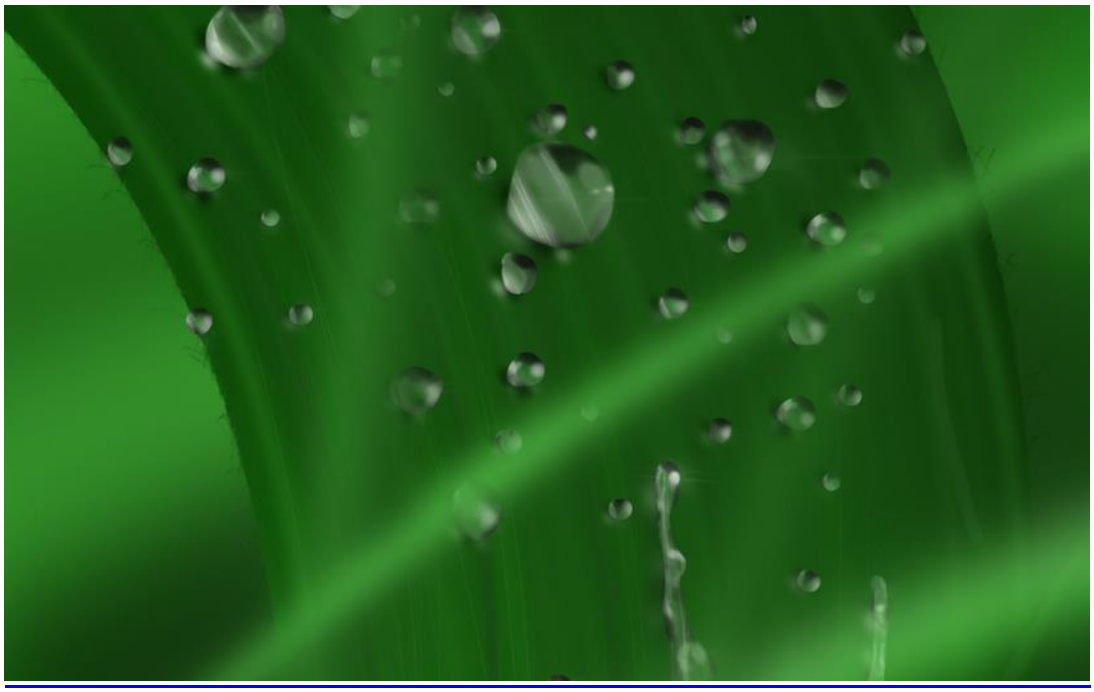
ABSTRACT

The exact interplay between receptors and their downstream signaling influences essentially all physiological functions. But how can a cell discriminate between hundreds of different receptors that share the same downstream signaling transducers? We find receptor-associated independent signaling nanodomains (RAINs) around single receptors which can specifically switch signaling cascades on or off.



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All physiological functions in humans are orchestrated by cell surface receptors and their intracellular signaling effects. Cell signaling typically consists of 5 components: *stimulus, receptor, transducer, messenger*, and *effector*. These components are modularly linked to each other to ensure that extracellular cues are relayed flawlessly into specific cellular responses. The basic mechanism appears rather simple: Cells sense extracellular stimuli such as hormones, neurotransmitters or therapeutic drugs that bind to cell membrane receptors. Consequently, the receptor proteins undergo conformational changes that lead to recruitment and activation of intracellular *transducer* proteins. These *transducers* can further regulate the intracellular concentration of so-called *second messengers* which then activate downstream *effector* proteins.

The topology of cell signaling modules follows a so-called *hourglass model*: in

particular, at the level of stimulus sensing, cells express several thousand receptors. The signaling pathways *converge* at the transducer level and even more so at the second messenger level. Then, receptor signaling *diverges* again to a myriad of effector proteins that orchestrate a highly receptor-specific cellular response. It is fascinating how cells can operate so precisely, i.e. how cells meticulously match different cell functions with activation of specific receptors while using only a handful of second messengers. As a matter of fact, it has remained one of the most fundamental questions in cell biology how cells achieve and maintain signaling specificity.

To address this question, we have studied G protein-coupled receptors (GPCRs) - the largest family of cell membrane receptors in humans and one of the most important drug targets in pharmacotherapy. The GPCR superfamily has more than 800 members that can be specifically activated by a multitude of extracellular stimuli. GPCR activation leads to coupling and activation of intracellular G proteins (the *transducers*) and subsequent modulation of the intracellular concentration of *second messengers* (i.e. cyclic adenosine monophosphate (cAMP) and others). Typically, cAMP activates downstream effectors that are responsible for eliciting the correct cell function. The GPCR/cAMP signaling module is a *bona fide* example of the hourglass architecture of cell signaling. For example, heart muscle cells can contract when they receive an adrenergic stimulus, however, they do not contract when stimulated by prostaglandin although under both conditions the increase in cAMP is identical. In order to explain these divergent effects, we and others have hypothesized that cAMP must be compartmentalized.

Compartmentation means that cAMP levels are inhomogeneously distributed inside a cell: there are areas with higher and lower cAMP concentrations which can influence distinct downstream processes. We have previously shown that cAMP compartmentation is rendered possible because cAMP is essentially immobile under non-stimulated conditions. This allows phosphodiesterases, the only enzymes that degrade cAMP, to form low-cAMP nanodomains. In these nanodomains cAMP effectors are protected from being activated at some locations in the cell but not at others. However, low-cAMP nanodomains do not explain how cells sense activation of an individual receptor and how they relay activation of this particular receptor into a highly specific cell function.

To address this question, we developed two types of fluorescent biosensors which measure changes in cAMP in intact cells. First, we directly fused a cAMP reporter to two different GPCRs: the glucagon-like peptide receptor 1, and the 2 adrenergic receptor. Second, we separated the cAMP reporter from the receptor in a stepwise manner using so-called nanorulers.
Our results are fascinating: stimulation of these receptors increases cAMP only in their direct vicinities. We have termed this local cAMP pools **RAINs**, **R**eceptor-**A**ssociated **I**ndependent cAMP **N**anodomain**s**. The cAMP inside RAINs does not equilibrate with cAMP in RAINs of other receptors nor with other cAMP pools in the cell. Using the second type of biosensors, the *GPCR nanorulers*, we

mapped the local cAMP pools and showed that they have a size of approximately 60 nm. Importantly, RAINs are self-sufficient signaling units, i.e. they contain all necessary proteins for signal amplification.

In summary, in our study we show evidence for highly local areas of increased cAMP that dictate GPCR signaling specificity in space and time. We believe that RAINs are a general phenomenon at all GPCRs or even at other receptor classes. This significantly changes the concept of cell signaling: our data would imply that a cell operates more like a microchip where each receptor builds an independent switch, which can turn single downstream signals on and off rather than turning the whole cell on and off. This might have important implications on future therapeutics: if we understand the molecular architecture and dynamics of RAINs, specific targeting of RAIN signaling might lead to more precise therapeutics with reduced side effects.