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The cyclic nucleotides – cyclic AMP (cAMP) and cyclic GMP (cGMP) – are the most important intracellular messengers. They link stimulation of receptors at the cell surface to cellular responses. We investigate how receptors at the cell surface become activated and how they trigger and regulate cAMP production. For these studies, we generated a number of fluorescent sensors for receptors and their downstream signaling proteins as well as fluorescent sensors for cAMP and cGMP. These sensors are used to generate images of receptor-generated signals in intact cells, resolved in space and in time. These new technologies give unprecedented views on receptor signaling. For example, we have observed that the exact localization of receptors within a cell determines how they signal and how a cell responds to stimuli. This has important (patho)physiological implications, which range from the control of thyroid hormone secretion to heart failure.

Fluorescent sensors for receptor signaling and second messengers

The strategy to create sensors for the various steps of receptor signaling – from receptor activation down to cyclic nucleotides – is based on a technique called fluorescence resonance energy transfer (FRET). FRET is the transfer of energy from one fluorescent moiety to another one in close vicinity. In our sensors, these moieties are generally cyan (CFP) and yellow (YFP) fluorescent proteins, but we are also experimenting with small dyes that can be attached to defined small epitopes in proteins.

FRET can cause an acceptor (for example YFP) to emit light when a nearby donor (for example CFP) is excited. FRET is very sensitive to changes in the distance between the two fluorescent moieties: even small increases in the distance lead to a large loss in FRET. Figure 1 shows an example, a sensor for cAMP. This sensor is built with three elements: two fluorescent moieties (CFP and YFP), which flank a binding domain for cAMP. When cAMP binds to this domain, it causes a movement of CFP away from YFP (dotted red arrow), and this results in a loss of FRET. Such changes in FRET can be monitored by measuring the signal intensities of the CFP- and YFP-emissions (and their ratio). They permit recording and imaging in real time, where and when signals change within an intact cell.

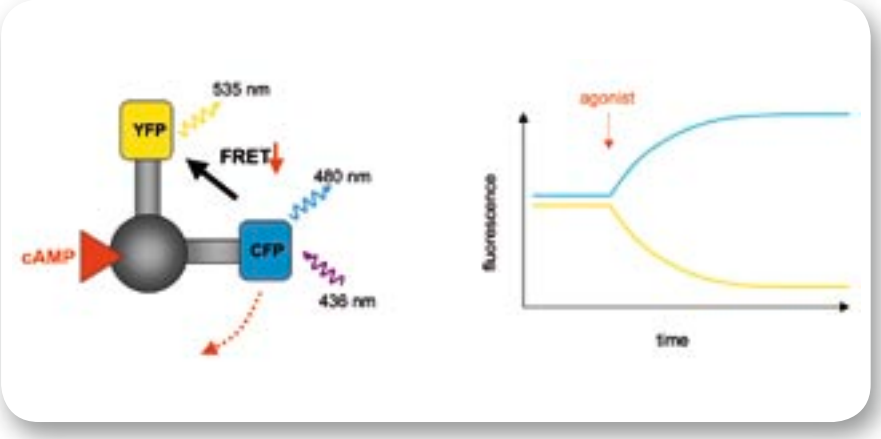

Fig. 1:
Principle of a FRET-sensor. This sensor for cAMP comprises a cAMP-binding domain (grey) fused to cyan (CFP) and yellow fluorescent proteins (YFP). Binding of cAMP moves CFP away from YFP and reduces FRET; therefore, cyan emission increases and yellow emission decreases.

Mechanisms of receptor activation and signaling

Activation of receptors is triggered by binding of agonists and a subsequent conformational change. The exact nature of this conformational change is still unclear. Our studies with fluorescently labeled receptors address the kinetics of these changes in intact cells and the responses to different types of ligands. We observed that they occur with high speed (usually 30-80 ms). Fast switching can also be exerted by allosteric ligands; these are ligands that bind to additional binding sites in receptors and appear to induce distinct specific conformations of receptors. This can decrease or increase the effects of regular, orthosteric ligands. The complex and rapid interactions that different types of ligands exert on receptors offer new strategies to alter receptor function, and may provide new venues drug treatment.

A different type of allosteric mechanisms has become a more recent research focus in our group: the fact that some receptors may form dimers, and that within such a dimer one receptor may alter the function of the other receptor. These dimers may result in complex interactions between drugs acting at different receptors and may have profound implications for drug therapy.

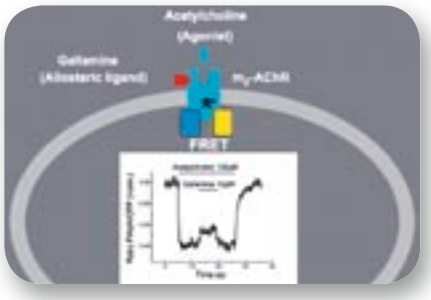

Fig. 2:
Orthosteric and allosteric effects on receptors. The m_2-muscarinic acetylcholine receptor (m_2AChR) can be activated via its "conventional" orthosteric site (e.g. by acetylcholine) and can be inhibited via a second, allosteric site (e.g. by gallamine). FRET-recording of a fluorescently labeled receptor show both the activation and the inhibition.

The importance of receptor localization for receptor signaling: a role in heart failure

It is generally assumed that receptors are evenly distributed over the entire surface of a cell, and that they exert their classical signaling function only from the cell surface. Recent studies in our group indicate that both assumptions may be wrong.
The first set of studies investigated beta-adrenergic receptors in cardiac myocytes, the muscle cells of the heart. These cells have two closely related receptors for the "stress hormones" adrenaline and noradrenaline, which are called beta$_1$- and beta$_2$-receptors. Both receptors cause an increase in cAMP production in the cells, but we observed earlier that the cAMP-signals produced by beta$_1$-receptors extend over the entire cell, while the cAMP-signals triggered by beta$_2$-receptors are locally confined. In a collaboration with the labs of J. Gorelik and Y. Korchev at Imperial College in London, we have been able to use very local stimulations, delivered through the tiny tip of the pipette of a scanning ion conductance microscope. This permits stimulation at two very distinct sites in the cells: directly at the cell surface, or in the t-tubules – long invaginations that emanate from the cell surface. We observed that beta$_2$-receptors cause cAMP-signals only upon stimulation at the t-tubules – suggesting that they are exclusively localized at these tubules. In contrasts, beta$_1$-responses are found all over the cell surface. The selective localization of the beta$_2$-receptors appears to be the reason for their distinct, local character of the cAMP-responses. Interestingly, both receptors increase cardiac contractions; however long-term stimulation of the beta$_1$-receptors causes growth and ultimately death of cardiac myocytes, whereas stimulation of beta$_2$-receptors does not. We postulate that this is due to the distinct localization of these receptors, and that this may provide a way of increasing cardiac contractility without damaging the heart.

A similar, but distinct role of receptor localization within a cell has become evident from studies of thyroid follicles. Here, receptors for the thyroid stimulating hormone (TSH) produce different signals, whether they are at the cell surface or when they move together with the TSH into the cell interior. In the latter case, they produce long-lasting cAMP signals that seem to regulate thyroid hormone secretion.

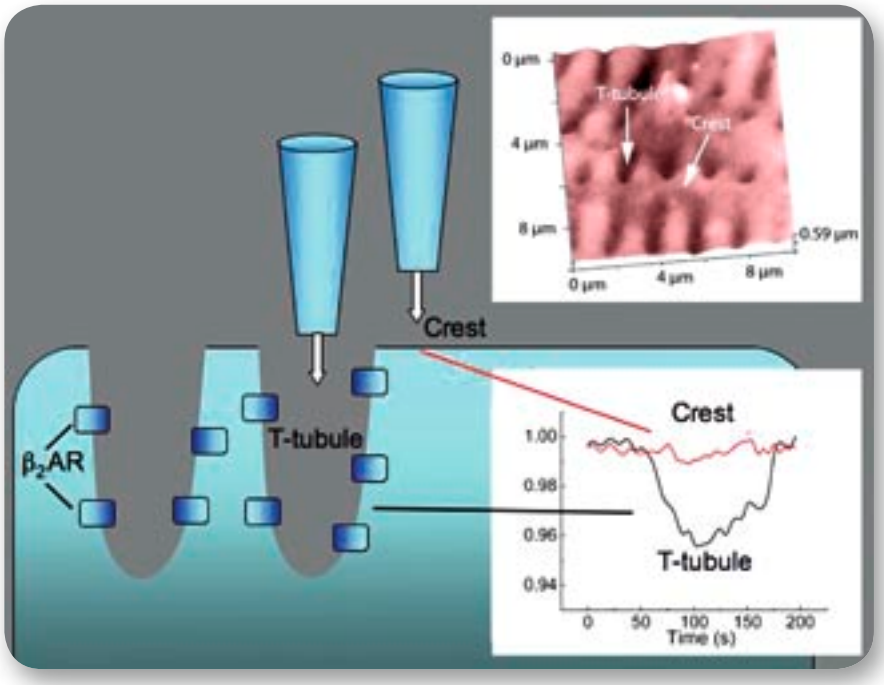

Fig. 3:
Specific localization of receptors. A cardiac myocyte (blue cell) has many invaginations called t-tubules (grey). Scanning ion conductance microscopy (SICM) reveals a pattern of t-tubular openings (top, right). Delivery of a highly localized β_2-adrenergic receptor stimulus through the SICM pipette causes a cAMP-signal (recorded by FRET) from the t-tubules but not from the surface crest. This indicates that these receptors are highly localized.

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Selected Publications

Calebiro, D., Nikolaev, V.O., Persani, L., and Lohse, M. J. (2010). Signaling by internalized G-protein-coupled receptors. **Trends Pharmacol Sci,** 31, 221-28.

Hoffmann, C., Gaietta, G., Zürn, A., Adams, S. R., Terillon, S., Ellisman, M. H., Tsien, R. Y., and Lohse, M. J. (2010). Fluorescent labelling of tetracysteine-tagged proteins in intact cells. **Nature Protoc,** 5, 1666-77.

Lohse, M. J. (2010). Dimerization in GPCR mobility and signaling. **Curr Opin Pharmacol,** 10, 53-58.

Maier-Peuschel, M., Frölich, N., Dees, C., Hommers, L. G., Hoffmann, C., Nikolaev, V. O., and Lohse, M. J. (2010). A FRET-based M_2 muscarinic receptor sensor reveals rapid kinetics of allosteric modulation. **J Biol Chem,** 285, 8793-8800.

Nikolaev, V. O., Moshkov, A., Lyon, A. R., Miragoli, M., Novak, P., Paur, H., Lohse, M. J., Korchev, Y. E., Harding, S. E., and Gorelik, J. (2010). Beta$_2$-Adrenergic receptor redistribution in heart failure changes cAMP compartmentation. **Science,** 327, 1653-57.