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Rapid monitoring of intracellular cGMP

Viacheslav O Nikolaev and Martin J Lohse*

Address: Institute of Pharmacology and Toxicology, University of Würzburg, Germany

Email: Martin J Lohse* - lohse@toxi.uni-wuerzburg.de

* Corresponding author

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In order to observe the spatial and temporal pattern of intracellular signalling in intact cells, we have developed a variety of sensors that are based on fluorescence resonance energy transfer (FRET) between CFP and YFP, or similar fluorophores, fused to various signaling proteins [1]. For the detection of the second messengers we have developed similar sensors based on cAMP or cGMP binding domains derived from various proteins that were likewise fused to CFP and YFP or analogous fluorescent proteins. These sensors respond to changes in intracellular second messenger concentrations with changes in FRET. For cGMP, various sensors were developed based on fragments of cGMP-dependent protein kinase or regulatory GAF-domains, which differed in amplitude, speed of reaction and in sensitivity to cGMP [2]. When expressed in primary cells or cell culture lines, these sensors allowed spatially and temporally resolved imaging of cGMP concentrations in intact cells. More rapid variants of these sensors also permit the monitoring of oscillating changes in cGMP levels, generated, for example, by pulsatile stimulation of cGMP synthesis by sodium nitroprusside.

In order to distinguish sub-membrane from global intracellular changes in cGMP, we have employed total internal reflection (TIRF) microscopy, using an instrument developed together with Leica that is capable of simultaneous recording of two different wavelengths (CFP and YFP) at the same depth. We then compared TIRF images and their changes over time with conventional wide-field microscopy of these sensors. TIRF-images were generated at various depths ranging up to 300 nm from the cell surface. These images showed uniform generation of cGMP in response to ANP, which were slightly faster when compared to global cellular cGMP responses, comparable with the signal diffusing rapidly from the cell surface through the cytosol.

Cyclic nucleotide-mediated signaling is, generally, terminated by the activity of phosphodiesterases (PDEs). In order to assess the effects of individual PDEs on such signals, we have recently generated fusions between sensors for cAMP or cGMP and various PDEs, attached to the C-terminal end of the sensors. These fusions allowed a specific analysis of the effects of individual PDEs (and their inhibition) on cyclic nucleotide levels, and also permitted the evaluation of a series of PDE inhibitors.

In summary, FRET sensors for cGMP allow the spatially and temporally resolved analysis of intracellular changes in this second messenger and also the assesssment of compounds that can affect changes in cGMP, either via a change in production or in generation.

References

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