Novel Techniques for Real-Time Monitoring of cGMP in Living Cells

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Contents

Abstract Recent developments of biophysical and electrophysiological techniques have enabled researchers to monitor levels of free intracellular cGMP in real-time and in intact living cells. These techniques are based on the use of cGMP sensors, which respond to cGMP with changes in transmembrane ion current or changes in fluorescence. Here, we describe the principles of these techniques, compare them in terms of sensitivity and discuss possible application for current cell biology and physiology.

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Abbreviations

CFP, enhanced cyan fluorescent protein; cGK, cGMP-dependent protein kinase; CNBD, cyclic nucleotide-binding domain; CNGC, cyclic nucleotide-gated channel; cpGFP, circularly permuted enhanced green fluorescent protein; FRET, fluorescence resonance energy transfer; GC, guanylyl cyclase; PDE, phosphodiesterase; RIA, radioimmuno assay; YFP, enhanced yellow fluorescent protein

1 Introduction

cGMP is a second messenger that regulates numerous cellular events and complex biological processes, including blood vessel tone (Hofmann et al. 2006; Juilfs et al. 1999; Rybalkin et al. 2003), neuronal excitability (Feil et al. 2005; Pfeifer et al. 1999), epithelial electrolyte transport (Hofmann et al. 2006; Jain et al. 1998; Lincoln et al. 1995), phototransduction in the retina (Arshavsky et al. 2002; Leskov et al. 2000; Pugh and Lamb 1993), cell adhesion (Hoffmeister et al. 2008; Massberg et al. 2004) and many others.

cGMP is produced in cells by two types of guanylyl cyclases (GC). Some natriuretic peptide receptors serve as membrane-bound or particulate GCs (Kuhn 2003; Potter and Hunter 2001). These receptors consist of three distinct domains: extracellular domain, transmembrane region and intracellular domain. The extracellular domain binds peptide ligands, members of the natriuretic peptide family (ANP, BNP and CNP), which induces a conformational change activating through the transmembrane helix, the intracellular domain of the receptor containing GC, which starts to synthesize cGMP (He et al. 2001). The second type of GCs is the soluble GC (Friebe et al. 2007; Pyriochou and Papapetropoulos 2005), which is located in the cytosol of many mammalian cells. This enzyme is a heterodimeric protein consisting of α- and β-subunits, and expression of both subunits is required for catalytic activity (Buechler et al. 1991; Harteneck et al. 1990; Kamisaki et al. 1986; Russwurm and Koesling 2004). Each subunit has an N-terminal regulatory domain and a C-terminal catalytic domain that shares sequence homology with the corresponding domains in membrane-bound GC and adenylyl cyclases (Chinkers et al. 1989; Krupinski et al. 1989). Soluble GC is activated by nitric oxide (NO), a paracrine vasorelaxant initially termed EDRF, for endothelium derived relaxing factor (Arnold et al. 1977a, b; Furchgott and Zawadzki 1980; Ignarro et al. 1987).

The effects of cGMP in cells are exerted by activation of three different types of effector molecules: cGMP-dependent protein kinase (cGK) (Hofmann et al. 2006; Schlossmann and Hofmann 2005), cGMP-regulated isoforms of phosphodiesterase (PDE) (Bender and Beavo 2006; Conti and Beavo 2007; Kass et al. 2007; Omori and Kotera 2007) and cyclic-nucleotide-gated ion channels (CNGC) (Craven and Zagotta 2006; Kaupp and Seifert 2002). Activation of all these proteins can be used to monitor changes in intracellular cGMP levels by real-time methods.

2 Classical Methods to Measure Intracellular cGMP

The classical biochemical approach to measure cGMP is based on radioimmuno assay (RIA). This is a destructive method, which requires a lysed batch of cells or tissue where GMP-concentrations can be determined using cGMP antibodies and radioactive cGMP. In addition to RIA, an ELISA method to measure cGMP has been introduced, which is based on the competition between cGMP and a cGMP tracer (e.g. cGMP linked to an acetylcholinesterase molecule). Although RIA and ELISA are sensitive methods, which allow to detect cGMP averaged over a batch of cells, these techniques require the destruction of the sample, they do not allow to monitor the physiologically relevant *free* cGMP levels, and they have very low temporal and no spatial resolution. The need for real-time cGMP measurements with high spatial resolution at the subcellular level is particularly dictated by the concept of cGMP compartmentation, which assumes different cGMP concentrations in various cellular compartments that are ultimately linked to the physiological functions (for review see (Fischmeister et al. 2006)).

3 Cyclic Nucleotide Gated Channels as the Sensors for cGMP

One type of intracellular effector proteins for cGMP is CNGCs, which are present in the retina and olfactory epithelium. These non-selective cation channels are comprised of four subunits, each with one intracellular cyclic nucleotide binding domain (CNBD) for cAMP and cGMP (Biel et al. 2002; Craven and Zagotta 2006; Kaupp and Seifert 2002). In the retina, CNG3 channels are kept open by high cGMP levels in the dark. The PDE6-mediated decrease of cGMP upon rhodopsin activation by light leads to a rapid closure of the channels and thus to hyperpolarization of photoreceptor cells (Arshavsky et al. 2002; Leskov et al. 2000; Pugh and Lamb 1993).

A real-time technique for cyclic nucleotide measurements in living cells has been developed based on olfactory CNG2 channels, which bind both cGMP and cAMP, with a slight selectivity for cGMP (Rich et al. 2000, 2001) (see Table 1). α-Subunits of these channels can be transfected into cells where they form functional homomultimeric complexes integrated into the plasma membrane. Increases in submembrane levels of cGMP open the channels, and this can be monitored by electrophysiological recording of the cation current or by calcium imaging (Fig. 1A).

3.1 Electrophysiological Recordings

CNGCs as membrane-localized sensors can effectively measure cGMP-concentrations at the plasma membrane. These sensors do not undergo desensitization, are not voltage-sensitive and respond rapidly to cGMP. However, cGMP-mediated activation of the channels leads to calcium influx, which might trigger some intracellular

Table 1 Sensitivity of different real-time sensors for cGMP. Reported EC_{50}-values for cAMP and cGMP and maximally measured changes in FRET are presented

Sensor	EC_{50} cGMP	EC_{50} cAMP	Selectivity cGMP/cAMP	ΔFRET max, %	References
CNGCs					
Wildtype CNG A_2	1.6 μM	36 μM	22.5	–	(Rich et al. 2000; 2001)
cGKIα-based FRET sensors					
CGY-Del1	0.02 μM	0.15 μM	7.5	24	(Nikolaev et al. 2006b; Sato et al. 2000)
Cygnet2.1	1.7 μM	>1,000 μM	>600	40	(Honda et al. 2001)
cGi-500	0.47 μM	>1,000 μM	>2,000	77[a]	(Russwurm et al. 2007)
cGi-3000	3.06 μM	>1,000 μM	>2,000	72[a]	
cGi-6000	5.64 μM	>1,000 μM	>2,000	57[a]	
Single-domain FRET sensors					
cGES-DE2	0.9 μM	115 μM	128	40	(Nikolaev et al. 2006b)
cGES-DE5	1.5 μM	630 μM	420	40	
Non-FRET FlincG sensors					
αFlincG	0.035 μM	40 μM	1,140	–	(Nausch et al. 2008)
βFlincG	1.1 μM	48 μM	30		
δFlinc	0.170 μM	48 μM	280		

[a]ΔFRET max measured for the cGi-500 sensor in living cells was ∼40%.

events. To overcome possible feedback effects caused by calcium, it is possible to measure the monovalent cation current (Rich and Karpen 2002; Rich et al. 2001).

CNGCs and their mutants with an increased sensitivity for cAMP were initially used to measure cAMP, and these studies supported the concept of compartmentation. In rat cardiac myocytes, β-adrenergic stimulation caused a compartmentalized cAMP signal distributed inside distinct subsarcolemmal domains (Rochais et al. 2004). Such membrane-confined cAMP signaling has been more recently directly visualized using FRET-sensors (Nikolaev et al. 2006a). Later, the same group went on to analyze cGMP dynamics in cardiac cells using the wild type CNG2 channels as sensors for cGMP. The authors tested whether the well known differences between the action of NO-donors and natriuretic peptides in the heart might be due to differential compartmentation of cGMP produced by the two types of GC. Indeed, the particulate pool of cGMP produced after natriuretic peptide stimulation was readily accessible at the plasma membrane and controlled exclusively by PDE2 activity, whereas cGMP produced by the soluble GC had no access to the membrane unless PDE5 was inhibited (Castro et al. 2006). Differential patterning of cGMP responses to NO-donors and natriuretic peptide was also a topic of another study (Piggott et al. 2006), which used CNGCs and measured intracellular calcium.

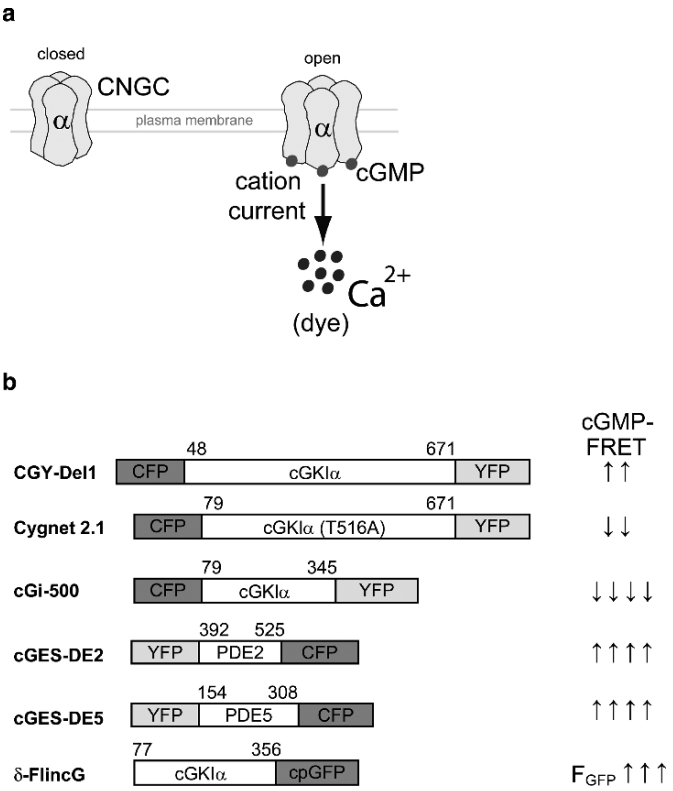

Fig. 1 Schematic structure and working principles of cGMP sensors based on CNGCs, FRET and cpGFP. **a**. Olfactory CNGCs are gated by subsarcolemmal pools of cGMP. Increases in cGMP result in a cation current, which can be measured electrophysiologically or by imaging intracellular calcium, e.g. using cell-permeable dyes. **b**. FRET-based cGMP sensors are constructed using parts of cGKIα or single GAF-domains of PDE2 and PDE5 fused between CFP and YFP. In these chimeric proteins, cGMP induces a conformational change which leads to an increase or a decrease in distance between the CFP and YFP monitored as a change in FRET. In case of FlincGs, the conformational change in the cGKI is transduced to the cpGFP leading to a change of its fluorescence intensity

3.2 Measurements Using Calcium-Sensitive Dyes

Another possibility to measure intracellular cGMP is to use a calcium-sensitive fluorescent dye, e.g. fura-2. A study by Rich and colleagues used HEK293 and vascular smooth muscle cells heterologously expressing CNG2 subunits and labelled them with fura-2 to measure cGMP-dependent calcium increases (Piggott et al. 2006). In these cells, ANP and NO-donors again showed different effects on intracellular cGMP. Particulate GC activated the second messenger production more readily than soluble GC, whose signal was highly confined to the cytosol even in the presence of the non-selective PDE inhibitor IBMX. In contrast, under PDE inhibition total

cGMP levels in both compartments were equally high, and so the authors concluded that natriuretic peptides and NO-donors stimulate cGMP synthesis in different compartments of the cell (Piggott et al. 2006).

Another calcium sensor used to image cGMP-dependent CNGC activity is the photoprotein aequorin (Wunder et al. 2005a, b, 2007). Calcium influx through cGMP-gated CNGCs leads to an increase in aequorin luminescence in the presense of appropriate co-factors (such as coelenterazine), which can be measured immediately after addition of compounds increasing cGMP levels. In this case, the reporter cell line is generated, which stably expresses the soluble GC, CNGCs and aequorin. The advantage of this system is a high signal-to-noise ratio and the possibility to use it in the pharmaceutical industry for high-throughput screening of such compounds as PDE inhibitors. In this case, the reporter cell line must be transfected with the PDE of interest (Wunder et al. 2005a, b). This method has been also used an as indirect assay to measure NO-production (Wunder et al. 2007).

4 FRET-Based Techniques

During the last decades, microscopic techniques have been developed for the real-time monitoring of biochemical events in living cells including the dynamics of the second messengers' calcium, cAMP and cGMP. Many of these techniques are based on fluorescence resonance energy transfer (FRET). FRET is a quantum mechanical phenomenon that occurs between a fluorescence donor and a fluorescence acceptor that are in molecular proximity of each other, provided that the emission spectrum of the donor overlaps with the excitation spectrum of the acceptor. FRET is very sensitive to the distance between the two fluorophores and thus allows the monitoring of changes in their distance that may occur either when they are attached to a pair of interacting proteins (*inter*molecular FRET) or when they are localized in a single protein that changes its conformation (*intra*molecular FRET). Examples of conformationally sensitive intramolecular FRET include sensors for the second messengers calcium and cyclic nucleotides (see below) as well as fluorescently labeled G-protein-coupled receptors that sense agonist activation (Vilardaga et al. 2003; Lohse et al. 2007). Examples of intermolecular FRET include cAMP-sensors based on the dissociation of the subunits of cAMP-dependent protein kinases (Zaccolo 2004; Mongillo et al. 2004) as well as those monitoring the activation state of G-proteins (Bünemann et al. 2003) or the interaction between receptors and G-proteins (Hein et al. 2005).

This methodology has been used to create a vast variety of fluorescent sensors (see the following review articles for details Giepmans et al. 2006; Miyawaki 2003; Lohse et al. 2008; Zaccolo 2004; Zhang et al. 2002). These approaches not only permit a detailed investigation of the kinetics of intracellular signaling, but they also permit the imaging of local changes in second messengers and, thus, the study of signaling compartments in intact cells (Fischmeister et al. 2006; Nikolaev et al. 2006a; Rich and Karpen 2002). Using appropriate sensors it is even possible to

image two second messengers simultaneously, such as calcium and cAMP (Landa et al. 2005). FRET has been applied in various approaches for cGMP measurements by developing and using FRET-based sensors, which report the activation of cGKs or cGMP-regulated PDEs.

4.1 CGY-Sensors Based on the Full-Length cGKIα

The first approach to measure real-time changes in intracellular cGMP has been developed using cGKIα as a backbone for a FRET monomolecular sensor (Sato et al. 2000). This sensor named CGY-Del1 is based on the well-established fact that the cGKI protein changes its overall conformation upon cGMP binding to the two sites in the regulatory domain, which results in an increase in FRET between CFP and YFP (Fig. 1B). In the CGY-Del1 sensor only 47 amino acids on the extreme N-terminus are truncated to prevent the dimerization of the cGKIα. However, the sensor includes the autoinhibitory region of the cGKIα present on the N-terminus, which might account for a very high sensitivity to cGMP of about 20 nM (see Table 1). Unexpectedly, such N-terminal deletion resulted in an almost complete loss of cGKIα selectivity towards cGMP, and cAMP activates this sensor half-maximally at 200 nM, making selective cGMP measurements difficult, because intracellular cAMP concentrations normally far exceed those of cGMP.

Recently, the same group has developed an approach for real-time NO-measurements using CGY-Del1 genetically coupled to α and β subunits of the soluble GC (Sato et al. 2005). This construct serves as an amplifier-coupled fluorescent indicator for NO and is capable of visualizing physiological nanomolar dynamics of NO in living cells with a detection limit of 0.1 nM. This approach demonstrated that 1 nM of NO, which is enough to relax blood vessels, is generated in vascular endothelial cells even in the absence of shear stress (Sato et al. 2005). More recently, the CGY-Del1 sensor has been used to create a reporter cell line, which can be used to monitor NO levels in the co-culture with the cells of interest. For this purpose, cells from a pig kidney-derived cell line PK15, which endogenously expressed soluble GC, are transfected with the CGY-Del1 sensor. The resultant reporter cell line called Piccell showed an outstanding sensitivity to NO and uncovered an oscillatory release of picomolar concentrations of NO from hippocampal neurons. Furthermore, the authors demonstrated that calcium oscillations in hippocampal neurons underlie the oscillatory NO release from the neurons during neurotransmission (Sato et al. 2006).

4.2 Cygnets Based on cGKIα

In parallel to the development of the CGY-Del1 sensor, another group headed by Wolfgang Dostmann generated cGKIα-based sensors for cGMP, which were slightly shorter on their N-terminus and did not include the autoinhibitory region

(Fig. 1B) (Honda et al. 2001). This might be the reason for the fact that these sensors called *cygnets* retained the exceptional selectivity of cGKIα for cGMP (Table 1). Cygnets reacted to natriuretic peptides and NO-donors in cell lines and Purkinje cells with cGMP-concentration-dependent decreases in FRET, allowing for real-time spatial and temporal analysis of this second messenger. In addition, the authors introduced a T516A point mutation to inactivate the catalytic domain of the kinase to prevent the down-stream intracellular events (Honda et al. 2001).

Cygnet 2.1 has been used in several studies to analyze real-time dynamics of cGMP in cultured vascular smooth muscle cells (Cawley et al. 2007; Honda et al. 2005a, b; Sawyer et al. 2003), rat neonatal cardiomyocytes (Mongillo et al. 2006; Takimoto et al. 2005), mouse teratocarcinoma cells (Ma and Wang 2007) and in thalamic neurons (Hepp et al. 2007).

In aortic smooth muscle cells transfected with cygnet 2.1, different NO-donors induced concentration dependent, transient cGMP responses irrespective of their rates of NO release. The kinetics of such cGMP peaks were governed by the concerted action of the soluble GC and PDE5, involving the cGKIα dependent phosphorylation of PDE5. Interestingly, cGMP transients could be elicited repeatedly without apparent desensitization of GC or by suppression of cGMP via long-term PDE5 activity, supporting the phasic nature of smooth muscle physiology (Cawley et al. 2007).

Measurements of cGMP in neonatal cardiac myocytes have further supported the idea of cGMP compartmentation. Inhibition of PDE5 with sildenafil enhanced the cGMP production and this effect was sensitive to the inhibition of NO synthase (NOS3), which might be coupled to the catabolic regulation of cGMP by PDE5 (Takimoto et al. 2005). Another study using neonatal cardiac myocytes and cygnet 2.1 demonstrated that activation of β_3-adrenergic receptor leads to the eNOS-dependent activation of cGMP synthesis which in turns activates PDE2 serving as a sink for cAMP produced after activation of β_1 and β_2 adrenergic receptor subtypes. Such a feedback regulatory loop is supposed to act in spatially defined signalling domains (Mongillo et al. 2006).

Very recent studies in living thalamic neurons used cygnet 2.1 expressed in brain slices by viral gene transfer (Hepp et al. 2007). In these cells, basal cGMP levels could be increased or decreased by NO-donors or PDE- and GC-inhibitors. Interestingly, PDE2 was shown to regulate the basal cGMP levels since an application of a selective PDE2- but not of PDE1- and PDE10-inhibitors increased the basal cGMP. In contrast, another cGMP-specific hydrolyzing enzyme, PDE9, was implicated in a decrease of cGMP levels after the stimulation with NO-donors, suggesting that different PDE isoforms may play distinct roles in the regulation of basal versus stimulated cGMP levels (Hepp et al. 2007).

4.3 cGi-500-Like Sensors Involving Regulatory Regions of cGKIα

To increase the sensitivity of the cygnet sensor for cGMP and to improve its speed and dynamic range, the group of Doris Koesling has introduced a systematic

approach to create FRET-based cGMP sensors based on cGKIα (Russwurm et al. 2007). The authors truncated a cygnet-like sensor C-terminally to obtain more sensitive constructs. Out of 24 cGMP-responsive constructs, three were selected to cover a range of cGMP affinities with an EC_{50} between 500 nM and 6 μM (Table 1). The most sensitive of these indicators called cGi-500 is a much shorter sensor than cygnet 2.1 because of the deleted catalytic domain (Fig. 1B). Although the larger C-terminal parts responsible for the interaction with the cGMP-binding regions and determining the ligand specificity (Landgraf et al. 1986) are deleted, cGi-sensors have been reported to possess excellent specificity for cGMP in vitro, fast binding kinetics and twice the dynamic range of existing cGMP sensors (Russwurm et al. 2007). These properties make the cGi-sensors the most sensitive FRET-based indicators to date. However, experiments in living cells showed a ∼two-fold lower dynamic range of the sensors with maximal amplitudes of only ∼40% which is comparable to that of cygnet 2.1 (Russwurm et al. 2007). Future studies should demonstrate whether these sensors retain their high selectivity for cGMP in vivo and can be used in physiologically relevant cells.

4.4 FRET Sensors Based on Single GAF-Domains from PDE2 and 5

The low selectivity of the CGY-Del1 sensor and the slow responsiveness of cygnet 2.1 in the physiologically relevant range of cyclic nucleotide concentrations have led our group to attempt the generation of sensitive and small FRET-sensors based on a single cGMP binding domain, as it had been achieved before for cAMP sensors (Nikolaev et al. 2004; Nikolaev and Lohse 2006). Such cAMP sensors have also been used to serve as indirect monitors of cGMP-concentrations. For example, in aldosterone producing cells, cAMP levels are subject to degradation by cGMP-activated PDE2 and are, therefore, regulated by natriuretic peptides (Nikolaev et al. 2005). For the development of cGMP-sensors, we focused on the cGMP binding domains of PDE2 and PDE5, which contains two in-tandem homologous sites of about 110 amino acids located at the N terminus and called regulatory GAF-domains (from c**G**MP-regulated phosphodiesterases, bacterial **A**denylyl cyclases, and transcription activator **F**lhA, where such domains were identified). The amino acid sequence of the GAF-domains is different from that of cGKI and CNGCs. The cyclic GMP binds to these allosteric sites and stimulates PDE activity via a conformational change, which is transduced from the GAF domains to the catalytic region (Martinez et al. 2002). Indeed, if a single cGMP binding GAF-domain was fused between CFP and YFP (Fig. 1A), an increase of FRET in such sensors was observed upon the stimulation of cGMP production in cells (Nikolaev et al. 2006b). This observation led to the development of cGES-DE sensors (see Table 1), which reacted rapidly to cGMP, showed good sensitivities (EC_{50} values in the range of 1 μM) and were well selective for cGMP, as demonstrated in living cells. Even in a cell line with low endogenous amounts of soluble GC, cGES-DE sensors showed robust

repetitive increases in FRET, which were followed by PDE5-dependent decreases of cGMP. In primary mesangial smooth muscle cells, such sensors reported the NO-dependent cGMP signals and could be used for real-time cGMP imaging (Nikolaev et al. 2006b). One important advantage of these sensors compared to the cGKI-based probes is their compactness, the presence of only one binding domain and absence of catalytic and other domains, which might be involved in protein–protein interactions. This might facilitate the use of these sensors for the generation of the transgenic in vivo models.

Very recently, another similar sensor based on the GAF-A domain of PDE5 has been developed to monitor cGMP levels based on bioluminescence resonance energy transfer (BRET) (Biswas et al. 2008). This method is similar to FRET with a difference that the energy is transferred from a donor enzyme, e.g. luciferase whose luminescence is monitored, to a fluorescent acceptor, typically a variant of GFP (Pfleger and Eidne 2006).

At present, a wide palette of FRET-based sensors for cGMP is available, which can be applied for real-time monitoring of this second messenger in living cells. These sensors differ substantially in their sensitivity, kinetic properties, signal-to-noise ratio and selectivity (see Table 1). The choice of the sensor depends on the type of application and the need for high sensitivity and cGMP/cAMP selectivity.

5 FlincGs: Novel Non-FRET Fluorescent Sensors

Recent developments of circularly permutated fluorescent proteins such as cpGFP offered a possibility to visualize the conformational changes in protein by just fusing them to one cpGFP molecule. The measurements are based on the monitoring of cpGFP intensity, which increases upon the change of conformation. This strategy was originally used to create fluorescent calcium sensors (Nagai et al. 2001; Nakai et al. 2001). Such calcium sensors proved useful for imaging in living cells and even in beating hearts in vivo (Tallini et al. 2006).

The group of Wolfgang Dostmann has recently created similar sensors for cGMP called FlincGs (fluorescent indicators of cGMP) (Nausch et al. 2008). Such sensors are comprised of the regulatory cGKIα regions similar in length to the cGi-500 sensor, which are N-terminally fused to the cpGFP. To prevent the dimerization of cGKIα, its extreme N-terminal domain was deleted to create the δFlincG sensor (Fig. 1B). This sensor showed a 1.7-fold increase in cpGFP fluorescence upon full stimulation with cGMP, rapid association and dissociation kinetics, high affinity and selectivity (280-fold cGMP/cAMP preference) for cGMP (see Table 1). Unexpectedly, δFlincG showed a second minor excitation peak at 410 nm, which allows ratiometric excitation measurements. This sensor was expressed in vascular smooth muscle cells and showed rapid kinetics of cGMP in response to various NO-donors. Finally, the authors investigated the previously discussed hypothesis that natriuretic peptides and NO donors stimulate cGMP synthesis in distinct subcellular compartments. For this purpose, they used confocal microscopy and aortic smooth muscle cells transfected with δFlincG using adenovirus. Sustained stimulation of soluble

GC with NO-donors induced transient global cGMP elevations with the decay phase dependent on the activity of PDE5. In contrast, stimulation of the particulate GC with natriuretic peptide elicited sustained submembrane elevations of cGMP which were converted to global cGMP elevations by PDE5 inhibition (Nausch et al. 2008). This highly interesting finding is the first direct optical visualization of cGMP produced in different compartments and supports previous observations in cardiac myocytes (Castro et al. 2006) and smooth muscle cells (Piggott et al. 2006) obtained using CNGCs as the sensor for cGMP.

6 Conclusions and Perspectives

The advent of FRET, fluorescent microscopy and electrophysiology has provided researchers with a variety of new tools to directly monitor rapid subcellular dynamics of cGMP with unprecedented spatial and temporal resolution, which are unobtainable by classical cGMP assays.

A large number of cGMP sensors mentioned in this review show different kinetic properties, sensitivity and selectivity for cGMP (Table 1). They are based on different techniques such as electrophysiology (CNGCs), FRET and simple fluorescence (FlincG). The choice of a sensor for each particular application will depend on the expected basal and stimulated cGMP concentrations in cells, cross-talk between cGMP and cAMP signalling pathways and the microscopic or electrophysiological method available for recording the sensor's signal.

Novel optical techniques, which allow direct visualization of cGMP, will ultimately facilitate our understanding of intracellular dynamics of this second messenger in different cell types, as well as in diverse in vivo models. They should give new insights into cGMP compartmentation as a mechanism of fine regulation of cellular functions.

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