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Optogenetic toolkit for precise control of calcium signaling

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ABSTRACT

Calcium acts as a second messenger to regulate a myriad of cell functions, ranging from short-term muscle contraction and cell motility to long-term changes in gene expression and metabolism. To study the impact of Ca^{2+}-modulated 'ON' and 'OFF' reactions in mammalian cells, pharmacological tools and 'caged' compounds are commonly used under various experimental conditions. The use of these reagents for precise control of Ca^{2+} signals, nonetheless, is impeded by lack of reversibility and specificity. The recently developed optogenetic tools, particularly those built upon engineered Ca^{2+} release-activated Ca^{2+} (CRAC) channels, provide exciting opportunities to remotely and non-invasively modulate Ca^{2+} signaling due to their superior spatiotemporal resolution and rapid reversibility. In this review, we briefly summarize the latest advances in the development of optogenetic tools (collectively termed as 'genetically encoded Ca^{2+} actuators', or GECAs) that are tailored for the interrogation of Ca^{2+} signaling, as well as their applications in remote neuromodulation and optogenetic immunomodulation. Our goal is to provide a general guide to choosing appropriate GECAs for optical control of Ca^{2+} signaling *in cellulo*, and in parallel, to stimulate further thoughts on evolving non-opsin-based optogenetics into a fully fledged technology for the study of Ca^{2+}-dependent activities *in vivo*.

Contents

1. Shedding light on Ca^{2+} signaling

Ca^{2+} acts as a versatile second messenger to regulate a myriad of cellular activities, ranging from short-term reactions occurring within seconds (*e.g.*, muscle contraction and neurotransmitter release) to long-term processes that last for hours or even days (*e.g.*, gene transcription) [1,2]. The location, amplitude and frequency of

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Ca^{2+} signals in mammalian cells undergo constant changes to maintain Ca^{2+} homeostasis while meeting the diverse requirements of different Ca^{2+}-modulated events. This challenging task is made possible through the coordinated actions of a repertoire of Ca^{2+} signaling components situated on the plasma membrane, across membranous organelles, or in the cytoplasm [2–4]. Over the past decade, the technical leap in Ca^{2+} imaging, propelled by the invention of a growing palette of genetically encoded Ca^{2+} indicators (GECIs), makes it a routine practice in numerous laboratories to monitor Ca^{2+} dynamics and report Ca^{2+}-triggered activity in both model cellular systems and living organisms [5–11]. In contrast to the rapid progress in developing optical reporters for Ca^{2+}, the quest for genetically encoded optical actuators to deliver Ca^{2+} signals with user-defined spatial and temporal properties remains relatively stagnant.

Earlier attempts to optically control Ca^{2+} signals in mammalian cells can be traced back to the invention of "caged" compounds in the 1980s. The caged substrates could be Ca^{2+} itself [12–17], or other signaling molecules, such as ATP [18], GTP [19], and IP_3 [20–23], that are intimately involved in the mobilization of intracellular Ca^{2+} in mammalian cells (Fig. 1A). When shielded from light, the encapsulated biomolecule is trapped in an inert or less active state via chelation or formation of covalent bonds with photolabile "cages" [24–27]. For instance, Ca^{2+} cages can be synthesized by introducing photolabile groups, such as *o*-nitrophenyl (Fig. 1A), into commonly used Ca^{2+} chelators (*e.g.*, BAPTA, EDTA or EGTA) [12–16,26]. In the dark, Ca^{2+} tightly binds to these modified metal chelators with a high affinity in the range of 5–150 nM [26]. Upon UV illumination, the photolytic products exhibit a dramatic decrease in affinity for Ca^{2+} by >10,000 fold, thereby unleashing the bound Ca^{2+} to produce Ca^{2+} spikes inside cells [12–16,26]. These photolabile Ca^{2+}-releasing compounds, commercially branded as DM-Nitrophen, NP-EGTA or nitr-5, are often used to trigger or drive Ca^{2+}-dependent 'ON' reactions in living cells [28,29]. Conversely, photoactivatable Ca^{2+} scavengers (*e.g.*, diazo-2) have been devised to chelate free intracellular Ca^{2+} to suppress or terminate Ca^{2+}-dependent activities [30,31].

Photo-inducible control of Ca^{2+} signaling offers two major advantages over conventional pharmacological approaches. First, the high temporal resolution enables the dissection of kinetic requirements of Ca^{2+} signals during mechanistic studies of Ca^{2+}-dependent 'ON' and 'OFF' reactions *in cellulo* [20,30,31]. For example, the fast release of Ca^{2+} makes it possible to photo-activate the contraction of skeletal muscle fibers within tens of milliseconds, a speed that is five times faster than the most rapid solution change method [32]. Another desirable feature of photo-induced Ca^{2+} and IP_3 uncaging is that the amplitude of chemical signals can be conveniently tuned by varying the intensities of incident light. Second, photorelease technology makes it feasible to conveniently program the spatial profiles of Ca^{2+} signals. Both global and local Ca^{2+} signals can be generated to modulate Ca^{2+}-dependent activities at subcellular precision by applying a focused beam of light on the whole cell or at user-defined areas [25,33–35]. However, the spatial resolution might be compromised owing to the rapid diffusion of caged compounds in the cytoplasm. Hurdles hampering the application of caged compounds *in vivo* include irreversibility, low delivery efficiency, limited depth of tissue penetration and strong phototoxicity associated with UV irradiation [24–26,36].

Optogenetics, which combines the use of light and genetics to control cellular activities at high spatiotemporal precision [37], offers an ideal solution to overcome the aforementioned hurdles whilst still preserving the advantages of photorelease technology. Originally designed and most widely adopted to manipulate neuronal activities, optogenetic tools are now gaining wide popularity in biomedical research beyond neuroscience [36,38–43]. At the heart of this revolutionary technology is the integration of genetically encoded photosensitive modules into cells of living tissues to achieve gain or loss of function of defined cellular events. Several photoactivatable domains and photosensory receptors derived from microbes or plants, including the most well-known channelrhodopsin 2 (ChR2) and its variants, light-oxygen-voltage-sensing domains (LOV), cryptochrome 2 (CRY2), phytochrome B (PhyB), UV-resistance locus 8 (UVR8) and Dronpa, have been successfully optimized and exploited to control a growing number of biological processes in mammals [36,38–42]. Very recently, photosensitivity has been engineered into the Ca^{2+} release-activated Ca^{2+} (CRAC) channel (*e.g.*, OptoSTIM1 and Opto-CRAC) [36,44–46], G-protein coupled receptors (*e.g.*, melanopsin and Opto-XRs) [47,48] and receptor tyrosine kinases (*e.g.*, Opto-RTKs) [49–51]. These exciting technical breakthroughs introduce a repertoire of highly Ca^{2+}-selective optogenetic tools to the Ca^{2+} signaling field (Fig. 1B). We name these tools collectively as 'genetically encoded Ca^{2+} actuators' or GECAs, which complement the existing toolbox of GECIs to allow simultaneous perturbation and recording of Ca^{2+} signals. In this review, we will present the current state of the art of the optogenetic toolkit tailored for Ca^{2+} signaling, outline engineering strategies and basic design principles for GECAs, and briefly discuss the strengths and weaknesses of the existing tools. Our goal is to provide a general guide to choosing appropriate GECAs based on the experimental requirements and the biological questions to be tackled.

2. Genetically-encoded photoactivatable Ca^{2+} releaser (PACR)

Inspired by photo-induced uncaging of Ca^{2+} with synthetic 'caged' compounds [13,15–17], Fukuda et al. devised a genetically encoded Ca^{2+}-releasing (PACR) molecular tool [52] by inserting a photosensitive domain LOV2 into a calmodulin (CaM)-M13 fusion protein (Fig. 1B), the latter of which contains four Ca^{2+}-binding sites with each adopting a pentagonal bipyramidal geometry to coordinate Ca^{2+} [3,4,53]. In the dark, owing to the formation of a complex composed of CaM and its target peptide M13, PACR binds Ca^{2+} with a dissociation constant (K_d) of ~16 nM [54], which falls into the physiological range of resting Ca^{2+} and renders PACR to act as a Ca^{2+} chelator in mammalian cells. When exposed to blue light, photoexcited LOV2 disrupted the CaM-M13 interaction, and therefore, restored CaM to its target-free state with subsequent reduction in the affinity for Ca^{2+} (K_d = 3.75 μM) by >200 fold. Consequently, the rate constant (k_{off}) of Ca^{2+} release increased from 0.77 s^{-1} in the dark to 181 s^{-1} following photostimulation [52]. Unfolded PACR recovered to its dark state in a reversible manner with a half time of 41.7 s to allow repeated Ca^{2+} release. The expression of PACR in HeLa cells was shown to moderately elevate cytosolic [Ca^{2+}] by 10–90 nM. The potential application of PACR *in vivo* was demonstrated in *C. elegans* by photo-triggering the firing of touch neurons to elicit a turning behavior [52].

The application of PACR in cellular context, however, will likely be impeded because of its limited Ca^{2+}-releasing capacity and perturbation to the host physiology. The majority of cellular responses require the fluctuation of cytosolic [Ca^{2+}] in the range of a few hundred nanomolar or micromolar, but PACR brings about no more than 90 nM increase in the cytosolic [Ca^{2+}]. Given that the amounts of sequestered Ca^{2+} is proportional to the intracellular concentrations of PACR, this concern might be partially alleviated through overexpression of PACR or PACR concatemers to push the Ca^{2+}-releasing capacity toward the upper limit. However, the presence of excessive amounts of PACR as a Ca^{2+} binding protein might run the risk of imposing buffering effects on intracellular Ca^{2+} and perturbing the host cell functions, particularly a multitude of bio-