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Commentary

Let there be light: a bright future for Ca^{2+} signaling

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Calcium ion (Ca^{2+}) is a ubiquitous second messenger in eukaryotic cells. In response to electrical, hormonal, or mechanical stimulation, cytosolic Ca^{2+} levels increase due to Ca^{2+} influxes through ion channels embedded in the plasma membrane or Ca^{2+} releases from intracellular Ca^{2+} stores, such as the endoplasmic reticulum (ER) [1]. The resulting Ca^{2+} signals control a vast array of cellular activities, ranging from short-term muscle contraction, neurotransmission and cell motility to long-term changes in gene expression and metabolism [2–5]. Accordingly, disruption of intracellular Ca^{2+} homeostasis can lead to many human diseases, including cancer, cardiovascular and neurodegenerative disorders. These highly versatile functions are often dictated by the spatial and temporal patterns of Ca^{2+} signals through the coordinated actions of a repertoire of Ca^{2+} signaling components [1,6]. Hence, the cure for diseases associated with deregulated Ca^{2+} signaling calls for maneuvers at high spatiotemporal resolution.

Optogenetics is a technology that combines optics with genetics to control cellular activities with high spatiotemporal precision. At the heart of this revolutionary technology involves the installation of photosensitive modules into target proteins in living cells or tissues to achieve the defined physiological actions. The prototypical optogenetic tool, channelrhodopsin-2 (ChR2), could mediate light-inducible Ca^{2+} entry under high extracellular Ca^{2+} concentrations, but it lacks the specificity for Ca^{2+}. By engineering of Ca^{2+} mobilizing machineries like G-protein coupled receptors (e.g., melanopsin and Opto-XRs), receptor tyrosine kinases (e.g., Opto-RTKs), optogenetics has enabled the usage of light to produce Ca^{2+} signals, but often involves the co-activation of other messengers or pathways and therefore lacks fidelity. To obtain more Ca^{2+} specific optogenetic tools, light operated Ca^{2+} binding proteins such as calmodulin (CaM, e.g., PACR) were made. Even though highly specific for Ca^{2+}, PACR has drawbacks, such as limited Ca^{2+} releasing capability and possible perturbation to resting cytosolic Ca^{2+} levels, thus limiting its cellular applications. To overcome these hurdles, OptoSTIM1 and Opto-CRAC were generated from STIM and ORAI that constitute the Ca^{2+} release-activated Ca^{2+} (CRAC) channel, which is among the most Ca^{2+} selective channels [7]. These genetically encoded calcium actuators (GECA) are generated by applying two general strategies: (I) utilizing light inducible dimerization (e.g. iLID/sspB) or oligomerization (e.g. CRY2) systems to crosslink and photo-activate STIM1 cytoplasmic domain, which remains largely inactive in the dark state; (2) using AsLOV2 to cage the action of SOAR, largely mimicking STIM1 autoinhibition mediated by CC1-SOAR interaction under physiological conditions [8]. The resulting OptoSTIM1 and Opto-CRAC could produce highly specific intracellular Ca^{2+} signals with high spatiotemporal resolution that are sufficient to drive the downstream hallmark responses, including the nuclear translocation of the Ca^{2+}-responsive transcription factor NFAT. Till now, these tools have been successfully applied to control the actions of immune cells and neurons [9] (see Review [10] for detailed descriptions). Given the abundant expression of STIM/ORAI in both excitable and non-excitable cells, these tools might find broad applications in a myriad of physiological responses, including photoswitchable gene expression [11], lymphocyte activation and neuromodulation [9,12]. It was shown that transgenetic mice with cardiac-specific CRAC defects have bradycardia syndrome [13]. Thus it is also possible to restore the heart rate back to normal by installing OptoSTIM1 or Opto-CRAC into animals with bradycardia (Fig. 1a).

While Opto-CRAC can photoactivate Ca^{2+} influx to trigger Ca^{2+}-dependent physiological actions, optogenetic tools that can do the opposite are also needed for both research and therapeutic purposes. To gain the ability to specifically switch off Ca^{2+} signaling with light, Zhou and Wang groups recently invented a new optogenetic platform (designated OptoRGK) that can inhibit voltage-gated calcium channels (VGCCs or Ca_V) channels [14]. Ca_V channels mediate Ca^{2+} influx in the excitable cells or tissues, such as the nervous and cardiovascular systems. The Ras-like GTPases, Rad/Rem/Gem/Kir (RGK) negatively regulate voltage-gated calcium channels (VGCCs or Ca_V) channels and are excellent candidates for optogenetic engineering. They installed an optical dimerizer into RGK core domain to spatially control the distribution of RGK in cells with light, thereby enabling tunable photo-inhibition of Ca_V channel activities. Unlike the other non-specific optogenetic tools that modulate cardiac function through altering the membrane potentials [5], OptoRGK could inhibit Ca_V channels, resulting in a substantial reduction or even termination of the rhythmic Ca^{2+} oscillations in tested cardiac cells. RGK proteins such as Gem were shown to suppress atrioventricular nodal conduction and to reduce heart rate in a model of porcine atrial fibrillation. Therefore,

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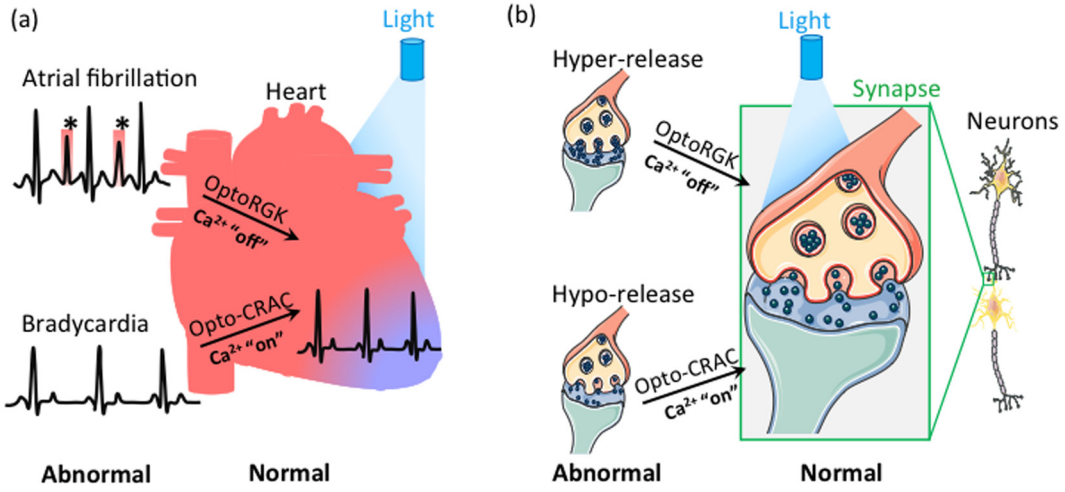

Fig. 1. (Color online) Cartoon illustration of potential applications of engineered Ca^{2+} channels for optogenetic intervention to treat human diseases. a) Restoring heart rate with light. b) Using light to restore Ca^{2+} dependent synaptic transmission back to normal.

OptoRGK may be exploited as a new interventional modality to treat cardiovascular disorders including high blood pressure, coronary artery disease and arrhythmia (Fig. 1a).

With these optogenetic tools, one can now use light to specifically turn on or off Ca^{2+} signaling and its downstream events with high spatiotemporal resolution, superb reversibility, and facile photo-tunability. In addition to applications in boosting immune cells to fight cancer by turning on Ca^{2+} influx with light, tools like OptoRGK might hold great promise to intervene cardiac or neuropsychiatric diseases associated with defective Ca_V channels (Fig. 1). It is anticipated that, OptoRGK, the newly developed photoswitchable inhibitor for VGCCs, will overcome the drawbacks associated with traditional VGCCs blockers to treat human diseases.

Current optogenetics still faces challenges during in vivo applications, mainly because of the limited tissue-penetrating ability of the blue light required to activate the widely used photosensory proteins (e.g. ChR2, CRY2, LOV2). Upconversion nanoparticles, which exhibit anti-Stoke's shift and are thus capable of converting near infrared-radiation (NIR) light into visible light might provide a tentative wireless solution to overcome this hurdle in the field. Zhou and Han groups have demonstrated the coupling of upconversion nanoparticles with the Opto-CRAC construct to devise a NIR optogenetic platform to mount photo-inducible immune responses for melanoma killing in rodent models [9]. Additionally, by combining the CRISPR/Cas9-based genome-engineering tool with Opto-CRAC, Zhou's group further developed a light-inducible transcription reprogramming tool (designated CaRROT for calcium-responsive transcriptional reprogramming tool) to precisely photo-induce the activation of targeted endogenous genes [11]. These new technologies and toolkits will certainly expedite the process of moving the field closer for the ultimate disease intervention in the clinical setting.

Going forward, several roadblocks are yet to be removed to enable broader applications in optical control of calcium signaling. First, OptoSTIM1 and Opto-CRAC systems are engineered from STIM1 and both rely on the presence of endogenous ORAI channels to make it work, and therefore may limit the applications in cells or tissues with low or no discernible ORAI expression. Second, the kinetics and dynamics of OptoRGK are yet to be improved to match the rhythm of heart beating or the speed of neuronal firing. Regardless of these caveats, it comes of age to remotely manipulate the Ca^{2+} signals within mammalian cells by hard-wiring photo-sensory modules into the calcium signaling network.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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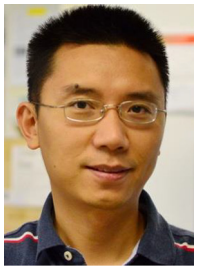

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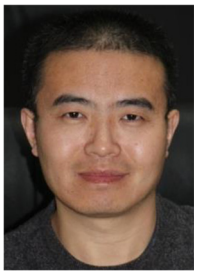

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