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Fast kinetics of calcium signaling and sensor design

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Fast calcium signaling is regulated by numerous calcium channels exhibiting high spatiotemporal profiles which are currently measured by fluorescent calcium sensors. There is still a strong need to improve the kinetics of genetically encoded calcium indicators (sensors) to capture calcium dynamics in the millisecond time frame. In this review, we summarize several major fast calcium signaling pathways and discuss the recent developments and application of genetically encoded calcium indicators to detect these pathways. A new class of genetically encoded calcium indicators designed with site-directed mutagenesis on the surface of beta-barrel fluorescent proteins to form a pentagonal bipyramidal-like calcium binding domain dramatically accelerates calcium binding kinetics. Furthermore, novel genetically encoded calcium indicators with significantly increased fluorescent lifetime change are advantageous in deep-field imaging with high light-scattering and notable morphology change.

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Introduction

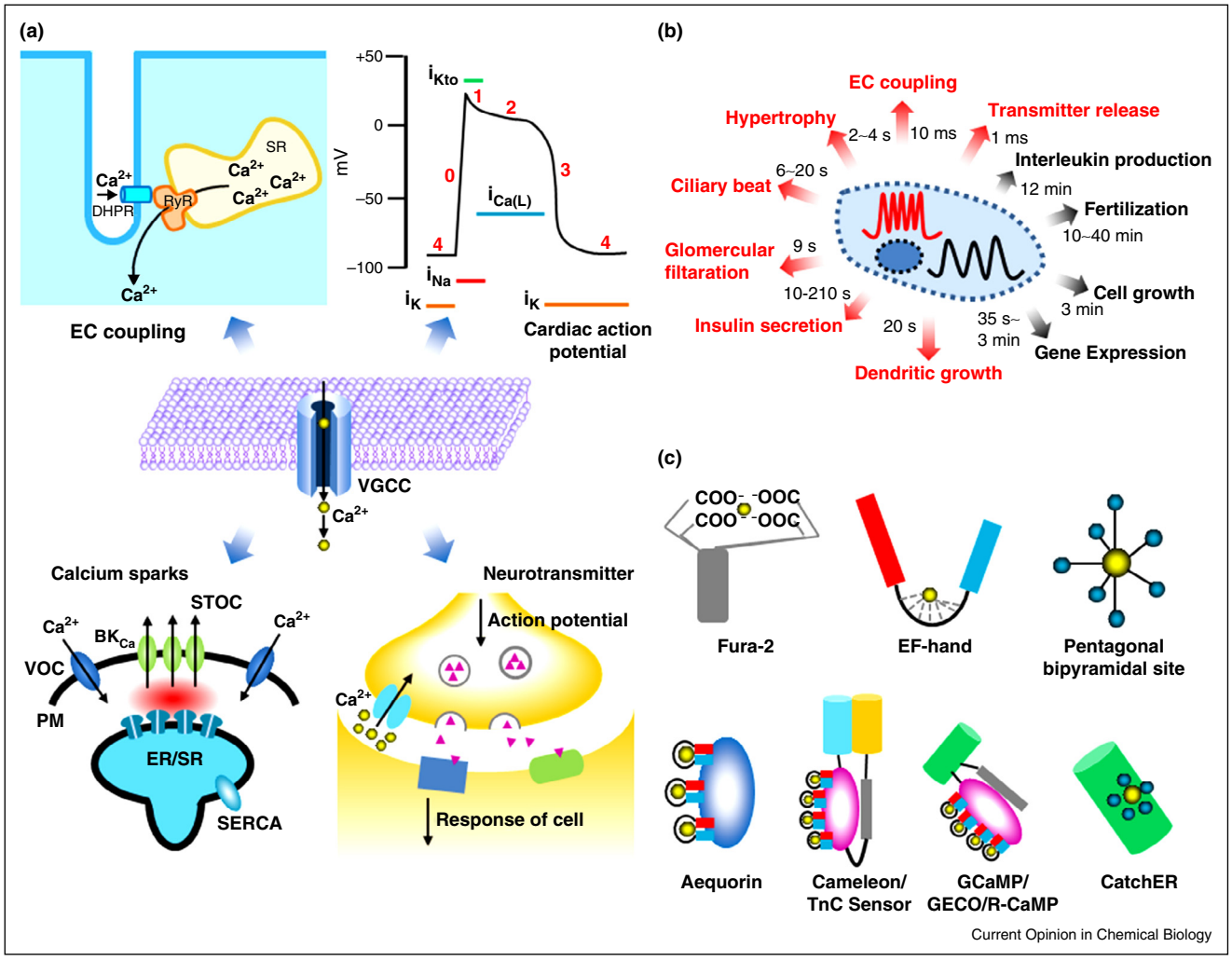
Calcium (Ca^{2+}), a second messenger and the most ubiquitous signaling molecule, plays an important role in regulating various biological functions in living organisms (Figure 1a). The time scale of calcium ion flow varies from milliseconds in muscle contractions to days for fertilization and development (Figure 1b) [1]. Rapid calcium signaling regulates calcium channels, excitation–contraction coupling, action potential, calcium sparks, and release of neurotransmitters (Figure 1a). Voltage gated calcium channels (VGCCs) exhibit a high open and close frequency and deliver fast calcium movement through a hydrophilic path in response to plasma membrane voltage changes, allowing precise calcium signaling within milliseconds [2,3]. During channel activation, calcium concentration is estimated to be hundreds of micromolar within several nanometers from the mouth of the channels, generating Ca^{2+} microdomains. A high Ca^{2+} gradient is generated between the microdomain and bulk cytosol [4,5].

In muscle cells, electrical stimuli applied to the plasma membrane can be converted to muscle contraction by a process known as excitation–contraction coupling (EC coupling). In skeletal muscle, an action potential activates the dihydropyridine receptor (DHPR) anchored in the T tubule of the sarcolemma. DHPR then physically interacts with ryanodine receptors (RyR) expressed in the sarcoplasmic reticulum (SR) membrane to induce SR calcium release; this interaction occurs within milliseconds. After stimulation, a transient asymmetric calcium spike lasting several to tens of milliseconds occurs in the cytosol, with a fast calcium recovery phase due to SERCA pump refilling of SR calcium and buffering effects of calcium binding proteins in the cytosol [6].

The VGCC is transiently activated after the initial Na^+ influx and K^+ efflux in cardiac muscles, forming a plateau and a sequential slow decayed phase of membrane potential lasting for about 200 ms, much longer than that of skeletal muscle or neurons lasting for only 2–4 ms. This limits the firing rate up to several Hz, preventing the tetanus contraction of cardiac muscles. The fast calcium influx through the calcium channel triggers SR calcium release through calcium-induced calcium release (CICR) to further elevate cytosolic calcium before decreasing. The Ca^{2+} influx is terminated by closing of the VGCC with cytosolic calcium pumped back into the SR by the SERCA pump or extruded to the extracellular space by the sodium–calcium exchanger (NCX) [7]. A normal contracting cardiac muscle cell exhibits a train of cytosolic calcium spikes with the time to peak around tens of milliseconds, and a decay phase within hundreds of milliseconds.

Calcium sparks, elementary events of the CICR through the RyR in cardiac EC coupling, were discovered by fast fluorescence imaging [8]. The opening of the RyRs in cardiac or skeletal muscle cells produces calcium transients with 10 ms to peak and 20 ms half-decay, restricted around 2 μm. Activation of numerous RyRs produces multiple simultaneous calcium sparks, ranging from 50 to 5000 in a cell [9], which is regulated by the SR calcium content. The summation of the sparks generates the cytosolic calcium change. The counterpart of the calcium sparks are Ca^{2+} blinks, the transient decrement of Ca^{2+} in SR exhibiting similar fast kinetics and a much smaller region.

Figure 1



Calcium signaling and fluorescent calcium sensors. **(a)** Fast calcium signaling regulated by the voltage-gated calcium channels (VGCC) includes EC coupling, cardiac action potential, calcium sparks and neurotransmitter release. **(b)** Different time scales of calcium signaling. **(c)** Representatives of calcium dye and genetically encoded calcium indicators. Fura-2 is synthesized calcium dye. Aequorin is used as genetically encoded calcium indicator. Cameleon and TnC sensor are composed by calmodulin and troponin C containing four EF-hands, respectively, flanked with FRET pair. GCaMP, GECO and R-CaMP are single fluorescent protein based sensor with calmodulin. CatchER is the single fluorescent protein with a designed calcium binding site on the surface of EGFP.

The neurotransmitter released from the presynaptic vesicles [10] triggered by presynaptic calcium channel activation will induce the postsynaptic receptors, for the synaptic transmission. Calcium microdomains and high calcium gradients are formed around the presynaptic calcium channels during the sub-millisecond calcium influx. In varying cell types, the local calcium concentration ranges from 10 μM to 200 μM for membrane fusion. Additionally, numerous calcium sensor proteins on the vesicle membrane surface are activated throughout the neurotransmitter release process [11]. Calcium dependent inactivation regulates the rapid termination of the calcium influx within 1–2 ms [12].

Need for calcium sensors with fast kinetics

The link between abnormal calcium channels and pathology requires precise tools to study them. Fluorescence microscopy with calcium sensors is indispensable in understanding live cell processes with high spatiotemporal resolution. Development of cell permeable calcium dyes has contributed greatly to our understanding of intracellular calcium signaling [1,13]. Dyes such as Fluo-5N with fast off rates are commonly used to monitor calcium dynamics (Table 1). However, calcium dyes without targeting are not suitable for microdomain imaging. The current genetically encoded calcium indicators (GECIs) possessing the EF-hand motif show slow