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Invited Review

Advances in malignant hyperthermia: novel insights into heat-induced Ca^{2+} release as a thermal signaling

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Abstract

Thermoregulation is essential for maintaining homeostasis in mammals under various environmental conditions. Impairment of this function can result in severe conditions, such as fever, heat stroke, and malignant hyperthermia (MH). In this review, we will focus on the role of the type 1 ryanodine receptor (RYR1), a Ca^{2+} release channel that is crucial for excitation-contraction coupling in skeletal muscles. Mutations in *RYR1* are associated with muscle disorders, including MH, which is characterized by dysregulated Ca^{2+}-induced Ca^{2+} release (CICR). Recent advances from genetically engineered mouse models of MH have provided new insights into the pathophysiological mechanisms underlying anesthetic- and heat-induced episodes, and revealed a heat-induced Ca^{2+} release (HICR) mechanism mediated by RYR1. Experimental evidences demonstrate that anesthetics induce simultaneous increases in cellular temperature and cytosolic Ca^{2+} concentration. Therefore, this review proposes that an increase in cellular temperature triggers further Ca^{2+} release via HICR, establishing a positive feedback loop that sustains excessive heat production during MH crises.

Key words: malignant hyperthermia, ryanodine receptor, thermal signaling, Ca^{2+} release, skeletal muscle

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Introduction

In mammals, body temperature is maintained constant through the regulation of heat production and dissipation even when the animal is exposed to fluctuating ambient temperature. Heat production is categorized into two types: shivering and non-shivering thermogenesis. Brown adipose tissue (BAT) is the primary site of the non-shivering thermogenesis, where heat is generated through uncoupling protein 1 (UCP1)-mediated mitochondrial respiration (1). Skeletal muscles contribute to thermogenesis mainly through shivering, however, recent studies have indicated their involvement also in non-shivering thermogenesis under certain conditions (2). These thermogenic and heat-dissipating processes are centrally coordinated by the hypothalamus, which integrates internal and external thermal signals and regulates effectors such as vasodilation, sweating, and muscle contraction (1). When this thermoregulatory function is impaired, severe conditions such as fever, heat stroke, and malignant hyperthermia (MH) are induced.

Recent studies have reported that Ca^{2+} leak from the sarcoplasmic reticulum contributes to heat production. This leak involves the ryanodine receptors (RYRs), a Ca^{2+} release channel (3).

RYRs are large homotetrameric Ca^{2+} release channels. There are three RYRs isoforms: RYR1 is predominantly expressed in skeletal muscle (4), RYR2 is primarily observed in cardiac muscle (5), and RYR3 is ubiquitously expressed in small amounts, including in smooth muscles (6). In skeletal muscle, RYR1 interacts with dihydropyridine receptors (DHPRs) on the T-tubule membrane and releases Ca^{2+} in response to membrane depolarization, a process referred to as depolarization-induced Ca^{2+} release (DICR) (7, 8). Additionally, RYRs can be directly activated by Ca^{2+}, a process known as Ca^{2+}-induced Ca^{2+} release (CICR) (9, 10). In skeletal muscle excitation-contraction coupling, DICR is the predominant Ca^{2+} release mechanism, whereas CICR is generally regarded as nonfunctional under physiological conditions. Excessive CICR activation contributes to the development of muscle diseases (11). These mechanisms are illustrated in Fig. 1. In contrast, in cardiac muscle excitation-contraction coupling, CICR is physiologically functional and serves as the primary mechanism of Ca^{2+} release via RYR2.

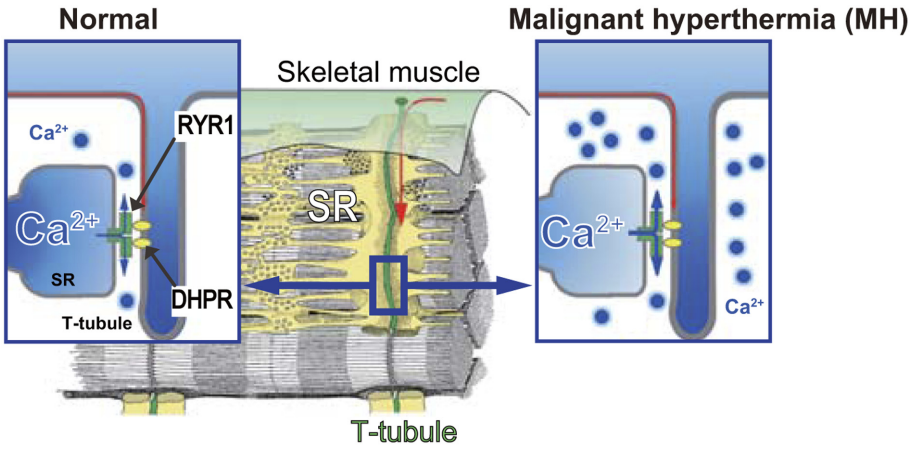

Fig. 1. Type 1 ryanodine receptor (RYR1) and malignant hyperthermia (MH). Schematic diagram illustrating the excitation-contraction coupling in skeletal muscles. Skeletal muscle contraction is initiated by depolarizing the plasma membrane of muscle fibers (red arrows) that activate dihydropyridine receptors (DHPR) located on the T-tubule membrane. This activation triggers the release of Ca^{2+} from the RYR1 on the sarcoplasmic reticulum (SR) membrane, resulting in the interaction of contractile proteins. During MH episodes, an abnormal increase in Ca^{2+}-induced Ca^{2+} release activity causes excessive Ca^{2+} release, resulting in sustained muscle contraction.

RYR1 mutations are associated with severe muscle diseases, such as MH and central core disease (CCD) (12). MH is a serious complication of general anesthesia involving inhalational anesthetics, characterized by a rapid increase in body temperature and the induction of a hypermetabolic state with skeletal muscle rigidity. CCD is a rare, non-progressive myopathy that presents in infancy and is characterized by hypotonia and proximal muscle weakness. Although MH is rare, occurring in only one in several tens of thousands of general anesthesia cases, it can be fatal without prompt treatment. Additionally, MH-associated *RYR1* mutations were implicated in certain cases of severe heatstroke (13–15). To date, over 300 *RYR1* mutations have been identified in patients with MH and related muscle diseases (11, 16, 17). However, not all MH cases are caused by RYR1 mutation. In rare instances, other genes may also play a role. For example, abnormal thermogenic responses were reported in transient receptor potential vanilloid 1 (TRPV1) mutant mice (18) and in mice lacking calsequestrin-1 (CASQ1), a Ca^{2+}-binding protein in the endoplasmic/sarcoplasmic reticulum (19). These findings support the notion that while RYR1 mutations are the major cause of MH, other molecular pathways may also contribute to MH-like syndromes. Dantrolene, an effective treatment for MH, was developed as a muscle relaxant in the 1960s and was later found to inhibit Ca^{2+} release from the SR through direct interaction with RYR1 (20).

Research on RYR1 structure began in 2010 with the determination of the X-ray crystal structure of its N-terminal domain (21). Subsequently, in 2015, three research groups reported its structure at a near-atomic level, facilitated by breakthroughs in cryo-electron microscopy technology (22–24). Additionally, the following year, the open-state structure of the channel was clarified, significantly advancing our understanding of the three-dimensional structure of RYR1 (25, 26). However, comprehensive structural and functional analyses of the disease variants are necessary to elucidate the mechanisms underlying MH pathogenesis. We performed functional analyses using HEK293 cells heterogeneously expressing a disease variant of *RYR1* (27). Our results revealed that the mutant exhibited various phenotypes, including enhanced CICR activity and reduced Ca^{2+} content in the lumen of the endoplasmic reticulum (ER) (27–29). Based on these findings, we generated a mouse model of MH to further elucidate the disease mechanisms.

This review highlights the significance and future prospects of this project, emphasizing the novel Ca^{2+} release mechanisms discovered through the analysis of MH model mice developed based on our previous results.

Animal Models of Malignant Hyperthermia

Various strains of mouse models carrying mutations in the *RYR1* gene have been developed to investigate the pathophysiology of MH. These include knock-in mice harboring mutations such as RYR1-p.R163C, p.Y523S (both located in the N-terminal domain of the RYR1 protein), p.G2435R (the helical domain 1), and p.T4826I (the C-terminal domain). These models reproduce key MH phenotypes, including isoflurane-induced hyperthermia and muscle rigidity, and have been widely used as valuable tools for elucidating disease mechanisms (30–33).

Among the MH-associated mutations, the RYR1-p.R2508C mutation has been reported to exhibit one of the highest levels of CICR activity when tested in heterologous expression systems using HEK293 cells (28). Based on these findings, RYR1-p.R2509C knock-in mice (R2509C mice), which carry the corresponding mutation in the mouse gene, were generated to further investigate MH pathogenesis *in vivo* (34). Homozygous R2509C mice were embryonically lethal, while heterozygous mice developed normally and exhibited no apparent abnormalities under standard conditions. However, exposure of the heterozygotes to isoflurane anesthesia

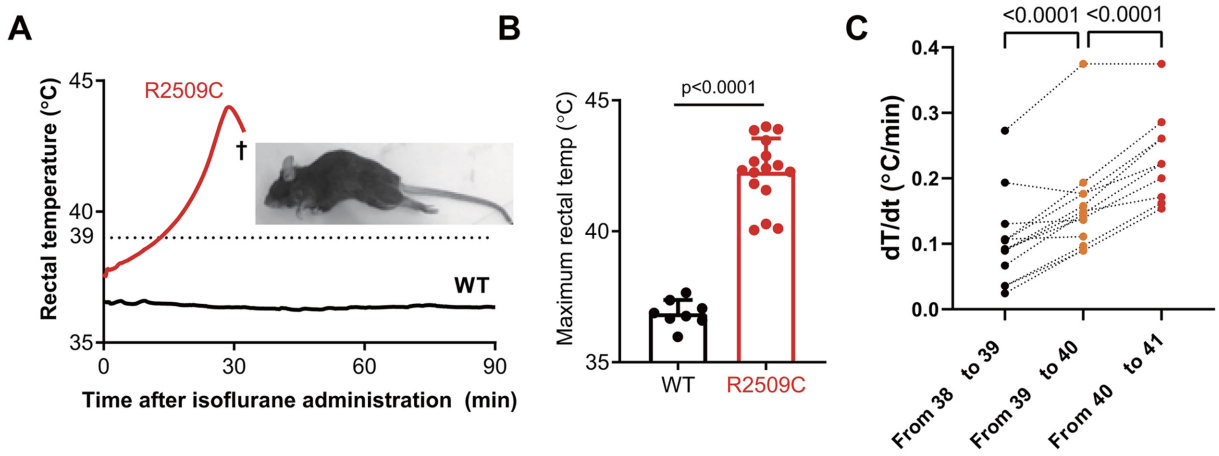

Fig. 2. Characterization of RYR1-p.R2509C (R2509C) mice. R2509C mice are generated using the CRISPR/Cas9 system. A. Alterations in rectal temperature in mice following isoflurane exposure. Heterozygous R2509C mice, but not wild-type (WT) mice, exhibited a significant increase in rectal temperature, thereby succumbing to fulminant malignant hyperthermia crisis (†). The inset indicates that R2509C mice exhibited full-body contractions, including arched backs and extended legs. B. Maximum rectal temperatures of WT (n=8) and R2509C (n=16) mice during isoflurane exposure. Data are presented as mean ± standard deviation. C. Rates of rectal temperature increase across three stages: early (38–39°C), middle (39–40°C), and late (40–41°C). Statistical analysis is performed using a paired *t* test. Significant differences are observed between the early and middle stages (n=15) and the middle and late stages (n=12). Figures are reproduced with modifications from Ref. (34).

induced rapid elevation of core body temperature above 40°C, accompanied by muscle rigidity and death within 90 min. In contrast, wild-type (WT) mice under the same conditions showed no significant changes in body temperature (Fig. 2A) (34). The maximum body temperature of R2509C mice was significantly higher than that of WT mice (Fig. 2B) (34).

Heat-Induced Ca^{2+} Release (HICR): a Novel Thermosensitive Mechanism

A notable characteristic observed in MH model animals is the accelerated rise in body temperature during exposure to volatile anesthetics such as isoflurane. In heterozygous R2509C mice, core body temperature gradually increased at first but then rose sharply after reaching a certain threshold, suggesting the existence of a positive feedback mechanism (Fig. 2C) (34). The cellular basis of this phenomenon was investigated in isolated skeletal muscle fibers from the flexor digitorum brevis (FDB) muscle, by subjecting to thermal stimulation using a focused near-infrared laser beam in a custom-designed live-cell imaging system (Fig. 3A, 3B).

Under these controlled heating conditions, FDB fibers from MH model mice carrying the RYR1 mutation exhibited abnormal Ca^{2+} release in response to increases in temperature. In contrast, WT muscle fibers showed no such response (Fig. 3C) (35). This thermally triggered Ca^{2+} release was termed heat-induced Ca^{2+} release (HICR), which represented a novel mode of RYR1 activation that was distinct from established mechanisms such as DICR, CICR, and nitric oxide (NO)-induced Ca^{2+} release (NICR) (36). NICR, which involves the *S*-nitrosylation of RYR1 by NO, has previously been reported in certain neuronal cells (36, 37).

The discovery of HICR introduced a fourth mode of RYR1 activation, in which small increases in temperature directly trigger Ca^{2+} release from the SR. In MH-susceptible muscle, this mechanism may amplify the pathological response to anesthetic or heat stress by establishing a self-perpetuating cycle: Ca^{2+} release leading to metabolic heat production, which further activates RYR1 through HICR, driving additional Ca^{2+} release.

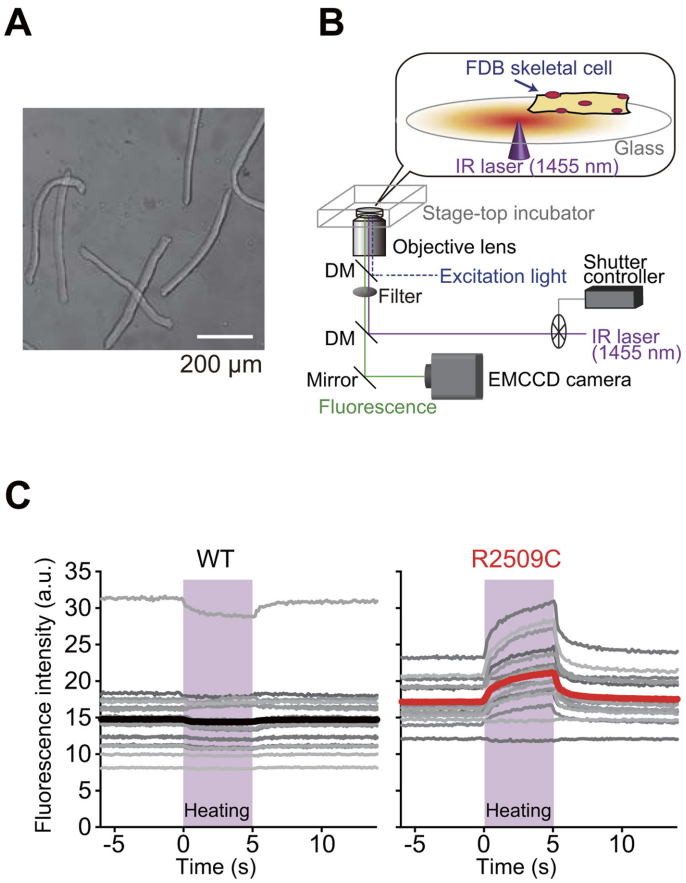

Fig. 3. Heat-induced Ca^{2+} release in skeletal muscles from wild-type (WT) and R2509C mice. A. A phase-contrast image of flexor digitorum brevis (FDB) muscles isolated from WT mice. B. Schematic illustration of the experimental setup. A 1,455-nm infrared laser (IR) beam is directed onto the sample stage using a dichroic mirror (DM) and an objective lens, focusing the beam on the medium. This setup allowed for localized (within the field of view of the optical microscope) and transient (within the order of second) temperature increase within the field of view. C. Time courses of fluorescence intensity in Cal-520-loaded muscles isolated from WT (left) or R2509C (right) mice. Each gray line represents the fluorescence intensity of an individual cell. The thick colored lines indicate average fluorescence intensities across cells. Pink vertical bars indicate the duration of heat pulses ($\Delta T = 3.5 \pm 0.5$°C, $T_0 = 23$°C). Figures are reproduced with modifications from Ref. (35).

This positive feedback loop provides a mechanistic explanation for the explosive rise in body temperature observed during MH episodes and suggests that HICR plays a central role in the thermopathology of the disorder.

Single-Cell Imaging Reveals Thermal Feedback Loop in MH Pathogenesis

Although previous studies have separately demonstrated increases in body temperature and cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) in MH model animals during anesthetic exposure, the direct temporal and spatial correlation between these parameters at the single-cell level had not been fully clarified. To address this gap, our laboratory developed a live-cell imaging system that enabled simultaneous measurement of intracellular temperature and $[Ca^{2+}]_{cyt}$ in isolated skeletal muscle cells. We employed ERthermAC (38), a small-molecule fluorescent thermometer that selectively targets the SR in flexor digitorum brevis (FDB) muscle cells

(Fig. 4A). In parallel, we used Cal-520, a fluorescent probe for detecting intracellular Ca^{2+} dynamics. Upon application of 1% isoflurane to FDB cells prepared from heterozygous R2509C mice, we observed a marked increase in Cal-520 fluorescence (indicative of Ca^{2+} release) and a concurrent decrease in ERthermAC signal (indicative of temperature rise) (Fig. 4B, right panels). These responses were not observed in WT cells under identical conditions (Fig. 4B, left panels) (39). Our findings provided direct evidence that volatile anesthetics induce simultaneous increases in $[Ca^{2+}]_{cyt}$ and temperature, specifically in MH-susceptible muscle cells.

The observed rise in SR temperature probably acts as a thermal stimulus that further activates mutant RYR1 channels through the HICR mechanism described earlier. This coupling between intracellular heat generation and Ca^{2+} release suggests the existence of a positive feedback loop, wherein Ca^{2+} release leads to metabolic heat production, which in turn exacerbates Ca^{2+} dysregulation through thermal activation of RYR1 (Fig. 5) (40).

Implications and Future Directions

The discovery of HICR as a novel activation mode of RYR1 adds a new dimension to our understanding of intracellular thermal signaling (40) and its role in muscle pathophysiology. Unlike classical DICR or CICR, HICR is uniquely triggered by temperature elevation and appears to play a central role in the pathogenesis of MH. Our findings suggest that mutant RYR1 channels can respond to even mild increases in intracellular temperature by releasing Ca^{2+}, thereby promoting further heat production through enhanced muscle metabolism. This creates a self-amplifying positive feedback loop that may underlie the explosive rise in body temperature seen in MH crises.

The ability to visualize this feedback loop at the single-cell level using simultaneous thermometry and Ca^{2+} imaging provides a powerful experimental platform to explore thermosensitive Ca^{2+} dynamics in real time. The ability to recapitulate MH-like responses at the single-cell level underscores the utility of this imaging approach for studying the molecular basis of MH. This approach could also be extended to assess the

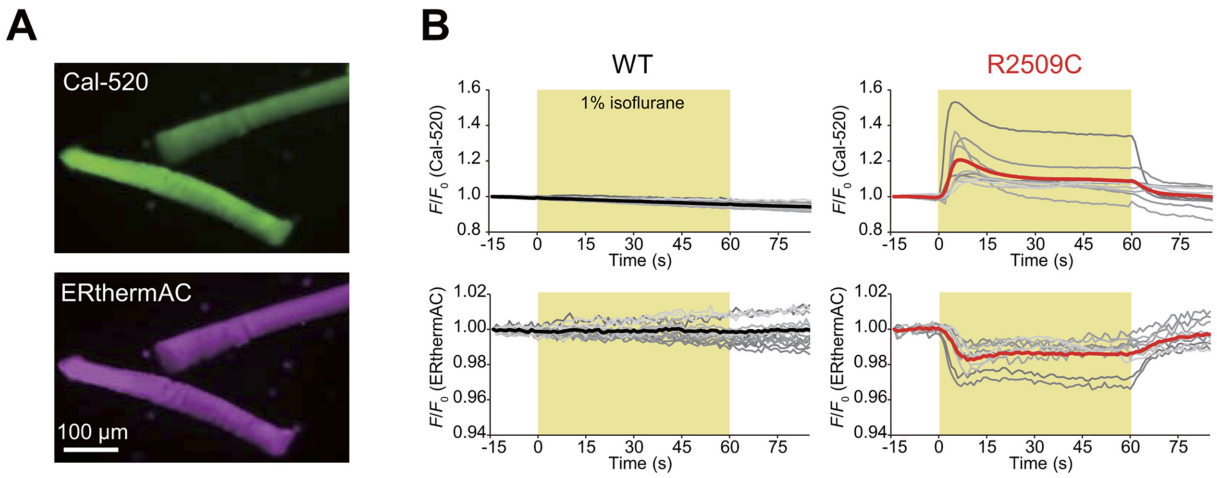

Fig. 4. Measurements of intracellular Ca^{2+} concentration and cellular temperature in flexor digitorum brevis fibers during isoflurane application. A. Fluorescence images of flexor digitorum brevis fibers isolated from mice, co-stained with Cal-520 and ERthermAC. B. Time courses of alterations in the relative fluorescence intensity of Cal-520 (top) and ERthermAC (bottom) in wild-type (WT, left) and R2509C (right) cells. Gray lines represent individual cellular responses, whereas thick colored lines indicate average fluorescence intensities. Isoflurane (1%) is applied at the time points marked by yellow horizontal bars. Figures are reproduced with modifications from Ref. (39).

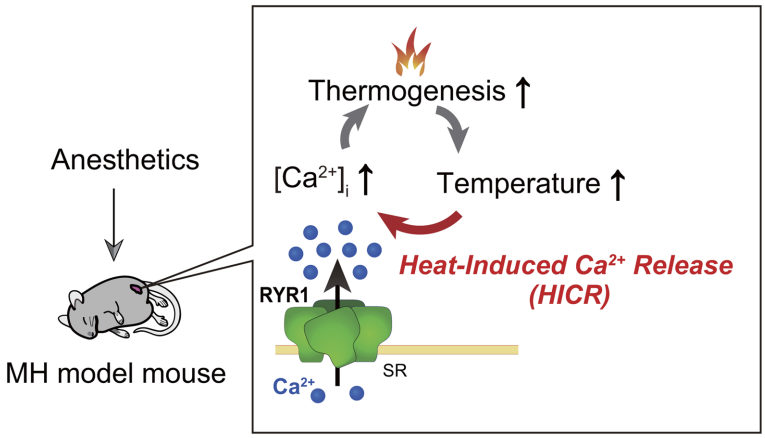

Fig. 5. Implications of heat-induced Ca^{2+} release in malignant hyperthermia (MH). Thermal stimulation of skeletal muscle cells triggers the release of Ca^{2+} from the sarcoplasmic reticulum (SR). This release occurs through type 1 ryanodine receptor (RYR1). The released Ca^{2+} stimulates heat production in skeletal muscle, resulting in an increase in body temperature. In the context of malignant hyperthermia (MH) models, the increase in temperature serves as a thermal stimulus or thermal signaling, further facilitating Ca^{2+} release. We named this positive feedback-regulating Ca^{2+} signal as heat-induced Ca^{2+} release (red arrow).

efficacy of therapeutic agents such as dantrolene which inhibits RYR1-mediated Ca^{2+} release, and also to study other heat-related disorders.

Moving forward, it will be pertinent to further dissect the molecular basis of HICR using advanced biophysical techniques and disease-model animals. Structural studies of RYR1 mutants, combined with functional assays, may reveal critical domains responsible for thermal sensitivity. Such insights could lead to the development of more targeted interventions for MH, as well as broaden our understanding of how intracellular temperature modulates signaling pathways in excitable cells.

Concluding Remarks

In this review, we summarized recent advances in our understanding of MH, with a particular focus on HICR as a novel RYR1-mediated mechanism. HICR provides a plausible explanation for the self-amplifying cycle of intracellular heat and Ca^{2+} dysregulation observed in MH, linking thermogenic stress to pathological Ca^{2+} signaling. The integration of genetic mouse models, real-time imaging techniques, optical microheating system, and molecular thermometry has enabled the visualization of this feedback mechanism at the single-cell level. These insights not only deepen our understanding of MH pathogenesis but also offer new perspectives for therapeutic development. Further studies on HICR and thermosensitive Ca^{2+} signaling pathways is essential for advancing both clinical care and basic research in muscle physiology and thermal biology. In particular, the future development of *in vivo* imaging and microheating technologies will likely allow us to capture dynamic heat-Ca^{2+} interactions in intact organisms, thereby providing a more comprehensive understanding of MH mechanisms under physiological conditions.

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Conflicts of Interest

Authors declare that they have no conflicts of interests.

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