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Calcium signaling in membrane repair

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Abstract

Resealing allows cells to mend damaged membranes rapidly when plasma membrane (PM) disruptions occur. Models of PM repair mechanisms include the “lipid-patch”, “endocytic removal”, and “macro-vesicle shedding” models, all of which postulate a dependence on local increases in intracellular Ca^{2+} at injury sites. Multiple calcium sensors, including synaptotagmin (Syt) VII, dysferlin, and apoptosis-linked gene-2 (ALG-2), are involved in PM resealing, suggesting that Ca^{2+} may regulate multiple steps of the repair process. Although earlier studies focused exclusively on external Ca^{2+}, recent studies suggest that Ca^{2+} release from intracellular stores may also be important for PM resealing. Hence, depending on injury size and the type of injury, multiple sources of Ca^{2+} may be recruited to trigger and orchestrate repair processes. In this review, we discuss the mechanisms by which the resealing process is promoted by vesicular Ca^{2+} channels and Ca^{2+} sensors that accumulate at damage sites.

Keywords

TRPML1; Ca^{2+}; lysosomal exocytosis; calcium sensor; membrane repair

Introduction

Plasma membrane (PM) disruptions occur in most cells, especially in those residing in mechanically-active environments, such as skeletal and cardiac muscle [1, 2]. Resealing is a repair process that allows cells to mend damaged membranes, preventing the loss of terminally-differentiated cells [1, 2]. Recent studies have suggested that damaged cells are able to restore the lipid bilayer barrier by adding membrane components from intracellular vesicles to the cell surface [3].

Three distinct mechanisms of PM lesion repair have been described (see Fig. 1). The first is the so-called “lipid-patch” model in which intracellular vesicles fuse with one another to form membrane patches, after which the patches fuse with the PM, thereby mending lesions [4, 5]. Among the intracellular vesicles, lysosomes are the primary candidate [4, 6]. The

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second mechanism is the so-called “endocytic removal” model, in which membrane lesions are removed through endocytosis [2, 7, 8]. Upon injury, acid sphingomyelinase (aSMase) is secreted to the extracellular space through lysosome exocytosis [2, 7, 9], and then aSMase-mediated hydrolysis of sphingomyelins (SMs) triggers ceramide-driven membrane invagination, mediating lesion removal [2, 7, 9]. The third mechanism is the recently-reported “macro-vesicle shedding” model, in which the damaged membranes undergo “outward” shedding upon injury [10–12]. This process involves the assembly of endosomal sorting complex required for transport (ESCRT) machinery [10, 13] to generate an outward curvature [14]. Depending on cell type, injury size, and type of injury, one or more of the aforementioned repair mechanisms may be recruited.

All three of the aforementioned repair process models entail a strict dependence on Ca^{2+} [1, 2, 7, 8, 10–12]. Membrane damage causes a significant increase in intracellular calcium concentration ($[Ca^{2+}]_{injury}$) at PM injury sites; [15–17]), and preventing the $[Ca^{2+}]_{injury}$ response with calcium chelators has been shown to block PM repair [15–17]. Furthermore, multiple calcium sensors, including synaptotagmin (Syt) VII and dysferlin, have been shown to promote lysosomal exocytosis in repair models 1 and 2 [4, 6]. In support of repair model 3, the Ca^{2+}-binding protein apoptosis-linked gene-2 (ALG-2) was shown to be essential for recruitment of ESCRT to damage sites [10, 13]. Cytosolic calcium ion concentration $[Ca^{2+}]$ is kept low at rest (~100 nM) in most cells. Conversely, the $[Ca^{2+}]$ in the extracellular space and in intracellular stores [e.g., in endoplasmic reticulum (ER) and endolysosomes] are maintained at 2 mM and 0.5–1 mM, respectively [18, 19]. Both the extracellular space and intracellular stores could contribute to $[Ca^{2+}]_{injury}$ fluxes. However, almost all previous studies have focused on extracellular Ca^{2+}. Very recently, Ca^{2+} release from intracellular stores was also shown to be important [20]. Hence, depending on injury size and the type of injury, one or both sources of Ca^{2+} may be used to trigger repair processes. In this review, we discuss the mechanisms by which PM resealing processes are promoted by intracellular Ca^{2+} channels and Ca^{2+} sensors.

Calcium acts locally

Virtually all aspects of cellular life are affected by Ca^{2+}, which is appreciated for being an evolutionarily conserved cellular signaling molecule with key functions in synaptic transmission, muscle contraction, granule secretion, gene expression, and membrane repair [21]. Ca^{2+} adds charge to Ca^{2+}-binding proteins, thereby initiating conformational changes and switching Ca^{2+} sensor protein functions “on” and “off” [19] [21]. There exist hundreds of Ca^{2+} sensor proteins with binding affinities in the nM to mM range that are known to trigger a wide variety of Ca^{2+}-sensitive cellular processes [19] [21]. There are 5,000- to 20,000-fold Ca^{2+} concentration gradients between the cytosol (~100 nM) and extracellular space (~2 mM) or intracellular compartments (0.5–1 mM) [18, 19]. These gradients are established by primary and secondary Ca^{2+} transporters localized at the cell surface or on the membranes of intracellular organelles [22]. Upon cellular stimulation, Ca^{2+} enters the cytosol through PM Ca^{2+} channels and organellar Ca^{2+} release channels [22].

To activate regulated signaling transduction involving cascades at specific cellular sites, intracellular Ca^{2+} signaling is shaped by diffusion and cytosolic buffering. Cytosolic buffers

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restrict the spread of a Ca^{2+} signal such that it remains close to source channels by reducing and localizing transient [Ca^{2+}] increases [19, 23–25]. Cytosolic buffering can produce ~10 μM to ~100 nM drops in intracellular [Ca^{2+}] over a distance of 30 nm within a few milliseconds [19, 23–25]. Hence, steep [Ca^{2+}] gradients around entry and release sites result in non-homogeneous activation of Ca^{2+} sensor proteins.

Sources of intracellular calcium flux

Extracellular space

The extracellular space provides a virtually unlimited supply of Ca^{2+} (~2 mM). Under physiological conditions, PM Ca^{2+} channels mediate Ca^{2+} influx upon stimulation. Under certain pathological conditions, PM disruptions may also cause Ca^{2+} influxes, resulting in $[Ca^{2+}]_{injury}$ [1, 2]. Such increases are also localized and transient due to cytosolic buffering and rapid resealing of membranes [1, 2]. Hence, the extracellular space is assumed to be the primary source of $[Ca^{2+}]_{injury}$. However, Ca^{2+} can also be released from specialized Ca^{2+} storage organelles [19], including the ER, endosomes, lysosomes, Golgi apparatus, and mitochondria (see Fig. 2).

ER

The ER, the largest intracellular Ca^{2+} store, is a heterogeneous organelle with a non-uniform distribution of Ca^{2+} [18, 19]. ER luminal [Ca^{2+}] (0.3–1 mM) is established and maintained by the sarcoendoplasmic reticulum Ca^{2+} (SERCA) pump [26]. ER Ca^{2+} channels include ryanodine receptors (RyRs) and inositol 1,4,5-triphosphate receptors (IP_3Rs) [27]. Activation of phopspholipase C-coupled, G-protein-coupled receptors induces IP_3-mediated Ca^{2+} release from the ER lumen. Additionally, both IP_3Rs and RyRs are activated by cytosolic Ca^{2+}, a mechanism known as Ca^{2+}-induced Ca^{2+} release [28]. Hence, ER Ca^{2+} may play an amplifying role by mediating a rapid augmentation of $[Ca^{2+}]_{injury}$. ER Ca^{2+} stores can be refilled through Ca^{2+} release-activated Ca^{2+} entry (see Fig. 2). Upon ER Ca^{2+} depletion, the so-called EF-hand domain in stromal-interacting molecule-1 proteins are oligomerized to activate Orai channels in the PM [29]. In the muscle cells, the sarcoendoplasmic reticulum is the primary source of Ca^{2+} [18, 19]. Given that the nuclear envelope is continuous with the ER membrane, the nucleus may also be viewed as a Ca^{2+} store [18].

Mitochondria

Mitochondria buffer Ca^{2+} released from the ER [29]. Recent studies have shown that mitochondria interact physically with the ER through mitochondria-associated membranes [30]. The mitochondria Ca^{2+} uniporter, which is localized to the inner mitochondrial membrane, conducts Ca^{2+} released from the ER Ca^{2+} into the mitochondrial matrix (see Fig. 2).

Lysosomes

Lysosomes are also Ca^{2+} stores with a luminal concentration of ~0.5 mM [31, 32]. Channels known to be involved in lysosomal Ca^{2+} release include the transient receptor protein mucolipin channel (TRPML; see below) and the as yet unconfirmed receptor for the second

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messenger nicotinic acid adenine dinucleotide phosphate (NAADP). Two-pore channel proteins are strong candidate NAADP receptors [33–38]. The ER and endolysosomes may cross-talk with each such that endolysosomal Ca^{2+} release may activate Ca^{2+}-induced Ca^{2+} release from the ER [39]; lysosomes may also sequester Ca^{2+} released from the ER [40].

Role of Ca^{2+} influx in membrane repair

Cells that reside in a mechanically-active environment, such as skeletal and cardiac muscle cells [1, 2], face a “reseal or die” challenge when they are injured. It has been long known that resealing of disrupted cell membranes of sea urchin embryo cells and 3T3 fibroblasts requires external Ca^{2+} [15–17]. It has been known for decades that resealing can be blocked by Ca^{2+} chelators, such as ethylene glycol tetraacetic acid (EGTA) and 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA) [41]. Follow-up studies performed in various *in vitro* damage/repair assays and cell types have shown that removal of extracellular Ca^{2+} impairs the resealing [15–17]. The $[Ca^{2+}]_{injury}$ influx is rendered transient by quick resealing, perhaps owing to extracellular Ca^{2+} triggers the rapid delivery, docking, and fusion of intracellular vesicles at the injury sites [41]. If resealing is blocked or delayed, uncontrolled rises in $[Ca^{2+}]_{injury}$ may trigger calpain-mediated cell death [42]. Hence, whether Ca^{2+} triggers resealing or cell death may be determined by the magnitude of $[Ca^{2+}]_{injury}$ flux.

The simple interpretation of PM resealing’s extracellular Ca^{2+}-dependence is that Ca^{2+} influx is required for $[Ca^{2+}]_{injury}$. However, it is questionable whether extracellular calcium is the sole source of calcium during membrane resealing. Passive depletion of ER Ca^{2+} stores triggers ER Ca^{2+} refilling through Ca^{2+} release-activated Ca^{2+} [29], extracellular Ca^{2+} is also required for the maintenance of intracellular Ca^{2+} stores (see Fig. 3). In a very recent study designed to examine the molecular mechanisms underlying lysosomal Ca^{2+} depletion and refilling (Garrity et al., in preparation), we found evidence indicating that extracellular Ca^{2+} plays an important role in the refilling of lysosome Ca^{2+} stores. We employed a lysosome-targeted genetically-encoded Ca^{2+} indicator, GCaMP3-ML1, to measure lysosomal Ca^{2+} release in intact cells, wherein transient Ca^{2+} increases, reflected by GCaMP3 fluorescence changes, are induced by an ML1-specific synthetic agonist in a Ca^{2+}-free external solution [43]. Remarkably, pre-incubation in Ca^{2+}-free solution for only 10 min resulted in a dramatic (~90%) reduction of lysosomal Ca^{2+} release, and lysosomal Ca^{2+} stores could be restored by exposure to a 2 mM Ca^{2+} external solution for a few minutes (Fig. 3). These results suggest that lysosomal Ca^{2+} may contribute, at least partially, to the Ca^{2+}-dependence of PM resealing. Hence, one or both sources of Ca^{2+} may be used to trigger membrane repair depends on the type and size of PM injury.

Role of Ca^{2+} release in membrane repair

Local and rapid $[Ca^{2+}]$ increases are critical for signal transduction and membrane trafficking [31, 44–46]. In cell-free vesicle fusion assays, late endosome-lysosome fusion is inhibited by the fast Ca^{2+} chelator BAPTA, but not by the slower chelator EGTA [45, 47]; the calculated association constant is 0.3 μs for BAPTA *versus* 1.2 ms for EGTA [48]. It has been estimated that a millisecond-elevation of Ca^{2+} within approximately 20 nm is required

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to trigger vesicle fusion [49]. These tight temporal and spatial requirements suggest that the vesicles themselves may provide the Ca^{2+} required for membrane fusion [46, 47]. Hence, PM injury may induce intracellular Ca^{2+} release to trigger membrane fusion and lysosomal exocytosis.

There are three ways to test the importance of intracellular Ca^{2+} release in membrane resealing: first, if intracellular Ca^{2+} release is critical for PM resealing, then depletion of specific intracellular stores would affect resealing; second, incubating cells with both membrane-permeable and non-permeable Ca^{2+} chelators would impair resealing; and third, and most telling, intracellular Ca^{2+} release channels would be required for membrane repair. These conditions were examined in a recent study in our laboratory [20]. First, we demonstrated that selective depletion of lysosome Ca^{2+} stores (with glycyl-L-phenylalanine-beta-naphthylamide) prevented PM resealing [20]. Second, we found that PM resealing was impaired in cells incubated with BAPTA-AM, a membrane permeable Ca^{2+} chelator [20]. And finally, we showed that PM resealing requires functional TRPML1, a lysosomal Ca^{2+} channel [20]. Although the dystrophin-glycoprotein complex and the known membrane repair proteins were expressed normally in TRPML1-null muscle fibers or when TRPML1-mediated lysosomal Ca^{2+} release was pharmacologically inhibited, membrane resealing was defective [20]. Hence, this work demonstrated that lysosomal Ca^{2+} channels and Ca^{2+} stores are both essential for membrane resealing. Furthermore, lysosomal Ca^{2+} imaging revealed that damage-induced $[Ca^{2+}]_{injury}$ flux from intracellular Ca^{2+} stores was reduced in TRPML1 knockout cells (Fig. 3). However, elevated $[Ca^{2+}]_{injury}$ lasted much longer in TRPML1 knockout cells when the cells were in 2 mM Ca^{2+} solution (Fig. 3), suggesting that PM resealing contributes to $[Ca^{2+}]_{injury}$ kinetics. Moreover, together these findings suggest that intracellular Ca^{2+} release may also play an important role in regulating the PM resealing process.

Given the observed influence of extracellular Ca^{2+} availability on lysosome Ca^{2+} stores, it is difficult to dissect the relative contributions of extracellular Ca^{2+} flux *versus* lysosomal Ca^{2+} release in membrane repair. Membrane repair is a complicated process involving a rapid (within seconds) initial resealing response followed by a prolonged (up to tens of minutes) remodeling phase [50]. Massive Ca^{2+} influx peaks within 10–20 s of a PM breach (Fig. 3). However, the fusion of lysosomes with other vesicles and the PM of the cell takes place tens of minutes after injury [51]. Live imaging in primary human myotubes showed that membrane repair and remodeling may last more than 10 min [52]. Hence, it is likely that both extracellular and intracellular store Ca^{2+} plays multiple roles in membrane repair. Consistently, even for sarcolemma repair, multiple Ca^{2+} sensors have been identified, including dysferlin, Syt-VII, myoferlin, and the annexins A1 and A2.

Intracellular Ca^{2+} release channels in membrane repair

TRPML1 was the first identified Ca^{2+} release channel in the lysosome to be implicated in resealing. It remains to be investigated whether other lysosome channels, such as TRPML3 and the two-pore channels, contribute to $[Ca^{2+}]_{injury}$ fluxes. Finally, ER Ca^{2+} channels could be involved in Ca^{2+}-induced Ca^{2+} release, further increasing $[Ca^{2+}]_{injury}$.

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TRPML1

The mucolipin subfamily of TRP channels comprises three members in mammals, namely TRPML1 (Mucolipin 1, MCOLN1), TRPML2 (MCOLN2), and TRPML3 (MCOLN3). Like other TRP channels, TRPML channels consist of six putative transmembrane-spanning domains (S1–S6) with the N- and C- termini facing the cytosol [53]. While TRPML1 is expressed ubiquitously in almost every tissue and cell type, TRPML2 and TRPML3 are expressed only in particular cell types [53, 54]. TRPML1–3 channels are localized predominately on the membranes of late endosomes and lysosomes [53, 55]. It is worth noting that TRPML1 is so important physiologically, presumably in large part because of its well-known role in lysosome-endosome trafficking of cellular constituents, that loss-of-function TRPML1 mutations result in Mucolipidosis type IV, a severe lysosomal storage disorder that manifests as a severe neurodegenerative disease characterized by psychomotor disabilities [56–58].

TRPML1 is a non-selective cation channel that conducts Ca^{2+} out of the endolysosomal lumen. Phosphatidylinositol 3,5-bisphosphate [$PI(3,5)P_2$], which is localized on endosomes and lysosomes, activates whole-lysosome TRPML1 currents [59]. In contrast, $PI(4,5)P_2$ and SMs in the PM inhibit TRPML1 [43, 60]. Besides TRPML1's endogenous activators, several synthetic small molecule activators of TRPML1 have been identified [43, 61], including ML-SA1 and ML-SA3, which activate TRPML1 robustly at low micromolar concentrations, producing a response comparable to that produced by $PI(3,5)P_2$ [43, 62].

Growing evidence support a direct role of TRPML1 in lysosomal exocytosis. The permeation and gating properties of TRPML1 suggest that the channel function of TRPML1 is to release Ca^{2+} from the lysosome lumen in response to various cellular cues [53, 63]. Consistent with a role of TRPML1 in lysosomal trafficking, several fusion defects were observed in TRPML1 knockout cells [64, 65]. Furthermore, several lines of evidence suggest that the role of TRPML1 in lysosomal exocytosis is likely to be direct. First, a gain-of-function mutation in TRPML1 results in enhanced lysosomal exocytosis in cells [66]. Second, acute ML-SA1 treatment has been shown to induce cell surface expression of lysosomal-associated membrane protein 1 (Lamp1) and lysosomal enzyme release in wild-type, but not TRPML1 knockout, macrophages [67]. And third, TRPML1 mediated lysosomal exocytosis is impaired in TRPML1 knockout or pharmacologically inhibited cells upon PM damage [20]. Importantly, TRPML1 knockout mice exhibit muscle repair defects and develop a muscular dystrophy phenotype [20].

The mechanism by which TRPML1 is activated by membrane damage is not known. Membrane damage causes influx of Ca^{2+} and oxidants [16]. It is conceivable that an event at injury sites may activate TRPML1; however, TRPML1 is unlikely to be directly activated by Ca^{2+} [59]. Oxidant levels are known to be increased at damage sites [16]. It remains to be determined whether intracellular reactive oxygen species may modulate TRPML1.

TRPML3

Like TRPML1, TRPML3 is also an endolysosomal Ca^{2+} release channel that is activated by $PI(3,5)P_2$ [59]. However, the restricted expression pattern of TRPML3 would seem to limit

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its role in resealing to specialized cell types. An interesting property of TRPML3 is that its channel activity is inhibited under low-pH conditions [68]. When biliary epithelial cells are infected by uropathogenic *E. coli* (UPEC), TRPML3 senses the UPEC-mediated lysosome neutralization and releases Ca^{2+}, triggering lysosome exocytosis to expel the exosome-encased UPEC [69]. Given the similarities in membrane repair and elimination of lysosome-localized pathogens, it is possible that TRPML3 may also play a role in membrane repair.

Calcium sensors in membrane repair

Consistent with a crucial role of Ca^{2+} in PM resealing, a number of Ca^{2+}-sensor proteins have been shown to be involved in resealing in various cell types, including Syts, ferlins, annexins, and ALG-2. Most Ca^{2+} sensor proteins contain one or more Ca^{2+}-binding domains, such as a C2 domain or an EF hand [70]. Ca^{2+} binding to a C2 domain may promote its interactions with phospholipids, altering the fusogenic potential of the lipid bilayer [70]. Likewise, EF hand-containing proteins may also be involved in membrane fusion [19].

Syts

The Syts are a group of transmembrane proteins with double C2 domains in their cytosolic regions [71, 72]. Syt-I, the most common isoform, functions as a Ca^{2+} sensor in synaptic vesicle exocytosis [73]. The plant homolog of Syt-I is required for the maintenance of membrane integrity in *Arabidopsis thaliana* [74, 75]. Several mammalian Syts, including Syt-VII, are expressed ubiquitously [76, 77]. The discovery that Syt-VII is a Ca^{2+} sensor that regulates lysosomal exocytosis is consistent with the possibility that it may play a role in membrane repair [17]. Indeed, Syt-VII is activated in Ca^{2+} triggered lysosomal exocytosis during membrane repair [7, 8, 78] via a mechanism that involves an interaction with SNARE proteins [73] (see Fig. 1).

Ferlins

Mammals have six ferlin proteins, including dysferlin, otoferlin, and myoferlin [79–82]. Ferlins, which share many structural and functional properties with Syts [83, 84], contain six C2 domains within their cytosolic regions [80] that confer Ca^{2+}-dependent phophospholipid-binding activity [83, 85].

Mutations in the dysferlin gene *DYSF* cause limb girdle muscular dystrophy type 2B and Miyoshi myopathy [86–88], and dysferlin knockout mice exhibit defects in muscle repair [89]. Dysferlin is proposed to mediate the fusion of intracellular vesicles with the PM [89], most likely via Ca^{2+}-dependent binding of the first C2A domain of dysferlin to phospholipids [90]. Full-length dysferlin is cleaved by calpains that are activated by injury specifically, resulting in mini-dysferlin$_{C72}$ (i.e. C2E~C2F) [91]. Mini-dysferlin$_{C72}$-postive vesicles are recruited rapidly to PM injury sites and fuse with PM compartments decorated with MG53, a potential oxidant sensor that accumulates at injury sites [15, 16]. Cleavage of dysferlin has been shown to be Ca^{2+}-dependent, suggesting that Ca^{2+} may play multiple roles in the PM resealing process [91, 92].

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Myoferlin is widely distributed in mammalian cells, with very high expression in myoblasts and particularly marked enrchment at sites where myoblasts are in contact with each other [93, 94]. Upon muscle injury, myoferlin levels become dramatically increased [79, 93, 95]. Like dysferlin, both myoferlin and otoferlin can be cleaved enzymatically to release their C-terminal fragments, which bear two C2 domains [92].

Annexins

Annexins (annexin A1–A13) bind to membranes exposing negatively-charged phospholipids in a Ca^{2+}-dependent manner [96, 97]. At resting cytosolic [Ca^{2+}], annexins are diffusely localized in the cytosol. Upon cytosolic [Ca^{2+}] increase, annexins translocate to the PM and organellar membranes [98]. Annexin 1, 2, and 5 have been associated with endosomal functions [99–103]. Annexins' fusogenic properties and their lipid preferences make them ideal mediators of resealing. In muscle cells, annexin A1 and A2 have been shown to promote the formation of lipid patches by way of their interactions with dysferlin. In zebrafish skeletal muscle, there is sequential accumulation of dysferlin, annexin A6, A2, and A1 at injury sites [104]. In human placenta, annexin A5 has been reported to play a role in the repair of human trophoblast membranes [105].

ALG-2

ALG-2 is a Ca^{2+}-binding protein that contains five serially-repetitive EF-hands, forming a penta-EF-hand moeity [106]. ALG-2 binds to annexin A7/A11, Sec31A, ESCRT proteins, and ALG-2-interacting protein X (ALIX) in a Ca^{2+}-dependent manner [106–111]. Luminal Ca^{2+} release from secretory organelles activates ALG-2, regulating vesicle transport in the secretory pathway [112].

ALG-2 is reported to be essential for resealing in the "macrovesicle shedding" model [10, 13] (see Fig. 1). Injury-triggered Ca^{2+} increases lead to ALG-2-regulated assembly of an ESCRT III-ALIX-Vps4 complex at PM injury sites, resulting in cleavage and shedding of damaged membranes [13]. Interestingly, ALG-2 is a Ca^{2+}-dependent interactor of TRPML1 [113], raising the possibility that TRPML1 may also be involved shedding-mediated membrane repair.

Calpains

Calpains are Ca^{2+}-dependent cysteine proteases that contain a "C2-like" domain near their N-terminal catalytic regions [114]. Mutations of muscle specific calpain-3 are associated with limb girdle muscular dystrophy type 2A [115]. Resealing of transected axons in cultured neurons has been shown to involve calpains [116, 117]. Furthermore, studies in various mammalian cell types have also revealed roles for the ubiquitously-expressed calpains-1 and -2 in PM resealing [118, 119]. Interestingly, a direct link between calpain and dysferlin was reported recently, suggesting that Ca^{2+} sensors may interact with one another in PM resealing [92].

Ca^{2+}-regulated ion channels

Anactamin (ANO) proteins are the best supported candidates for the once identity-elusive calcium-activated chloride channels [120–125]. Anoctamin 5 (ANO5) is highly expressed in

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skeletal and cardiac muscle cells and recessive ANO5 mutations in patients with muscular dystrophy are associated with sarcolemmal membrane lesions [124, 126]. It appears that ANO5 serves as a sensor for $[Ca^{2+}]_{injury}$ surges, to which it responds by releasing chloride [1, 84]. Notably, a chloride current has been observed after membrane wounding of sea urchin embryos [127]. Given that the ER and lysosomes interact closely and conduct crosstalk with each other [39, 40] and the observation that ANO5 is localized primarily in the ER [128], it is possible that ANO5 may modulate lysosomal exocytosis for PM resealing.

Perspective and future directions

PM resealing is essential for cell survival. Exocytic, endocytic, and exososme-mediated mechanisms have been proposed as mediators of membrane repair (see Fig. 1). It is likely that the type and size of wound dictates the repair mechanisms to be triggered. Both Ca^{2+} and oxidants are putative damage signals. Ca^{2+} may regulate multiple steps in the resealing process through the recruitment of multiple Ca^{2+} sensors. Significant $[Ca^{2+}]$ increases at injury sites last for only seconds, though it takes tens of minutes to complete PM repair. Consistent with the repair timeline, Ca^{2+} sensor proteins accumulate continuously at damage sites for tens of minutes. Given the regional specificity of $[Ca^{2+}]_{injury}$ fluxes, it is evident that the Ca^{2+} source of these fluxes is local, either from vesicle lumens or from the extracellular space. In the near future, we expect to see progress in the following areas:

- Development of vesicle-targeted genetically-encoded Ca^{2+} sensors that can be used to monitor cytosolic and luminal Ca^{2+} changes;
- Live cell imaging that enables Ca^{2+}-dependent vesicle movement to be recorded;
- Elucidation of the mechanisms by which Ca^{2+} release channels are activated upon PM damage;
- The existence of resealing of intracellular membranes.

A better understanding of the resealing process should help inform the design of approaches to treat diseases caused by defective membrane integrity, such as muscular dystrophy and cardiomyopathy.

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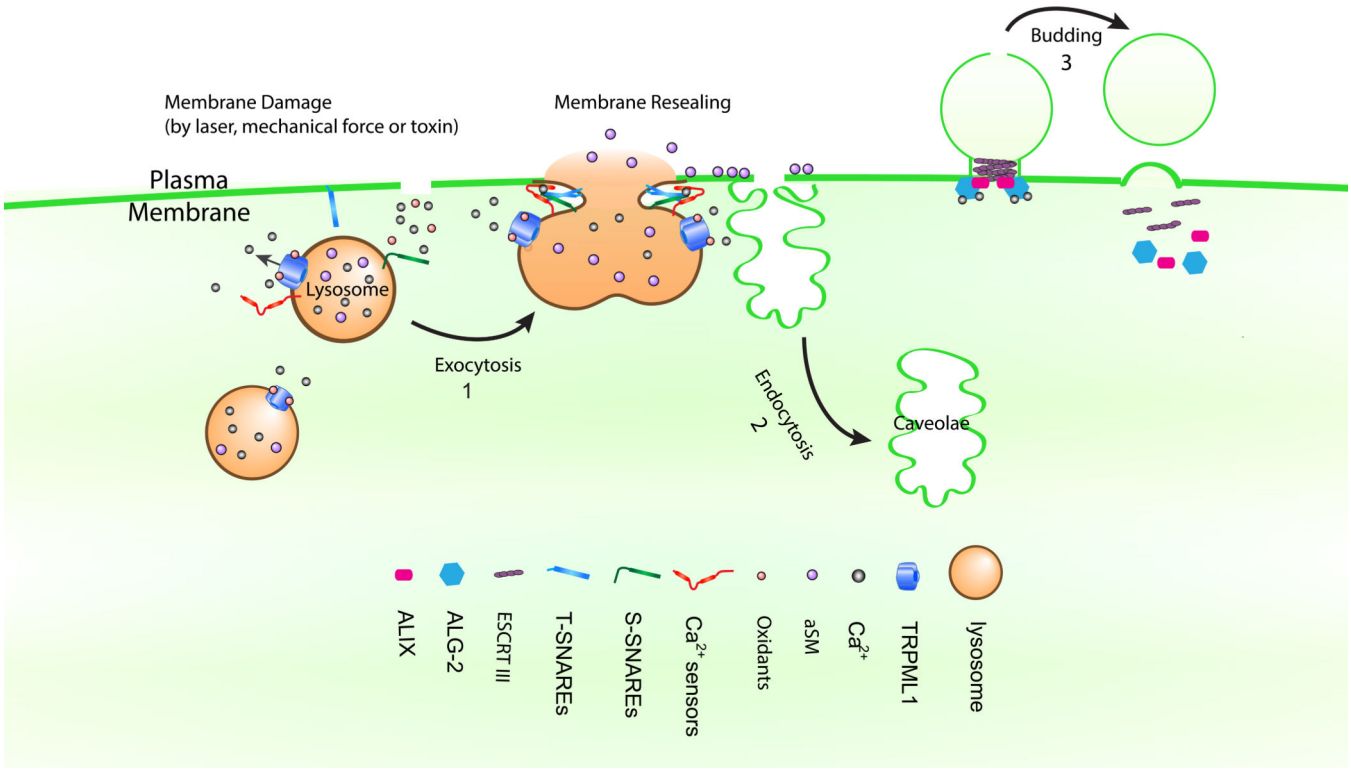

Fig. 1. Three working models for membrane repair

In the "lipid-patch" model (1), TRPML1, Syt-VII, dysferlin, and SNAREs participate in membrane repair. Upon the incursion of membrane damage, an influx of oxidants and Ca^{2+} promotes TRPML1 conducted lysosomal Ca^{2+} release, activating Syt-VII and other Ca^{2+} sensors. Subsequently, lysosomal exocytosis is triggered to reseal the disrupted membranes. In the "endocytic removal" model (2), lysosomal exocytosis mediates the release of aSMase to catalyze ceramide-dependent rapid lesion removal by caveolar endocytosis. In the "macro vesicle shedding" model (3), an injury-triggered Ca^{2+} surge recruits ALG-2 to the injury site. Accumulation of ALG-2 facilitates the assembly of ALIX and ESCRT III at the injury site, resulting in the cleavage and shedding of the damaged span of membrane.

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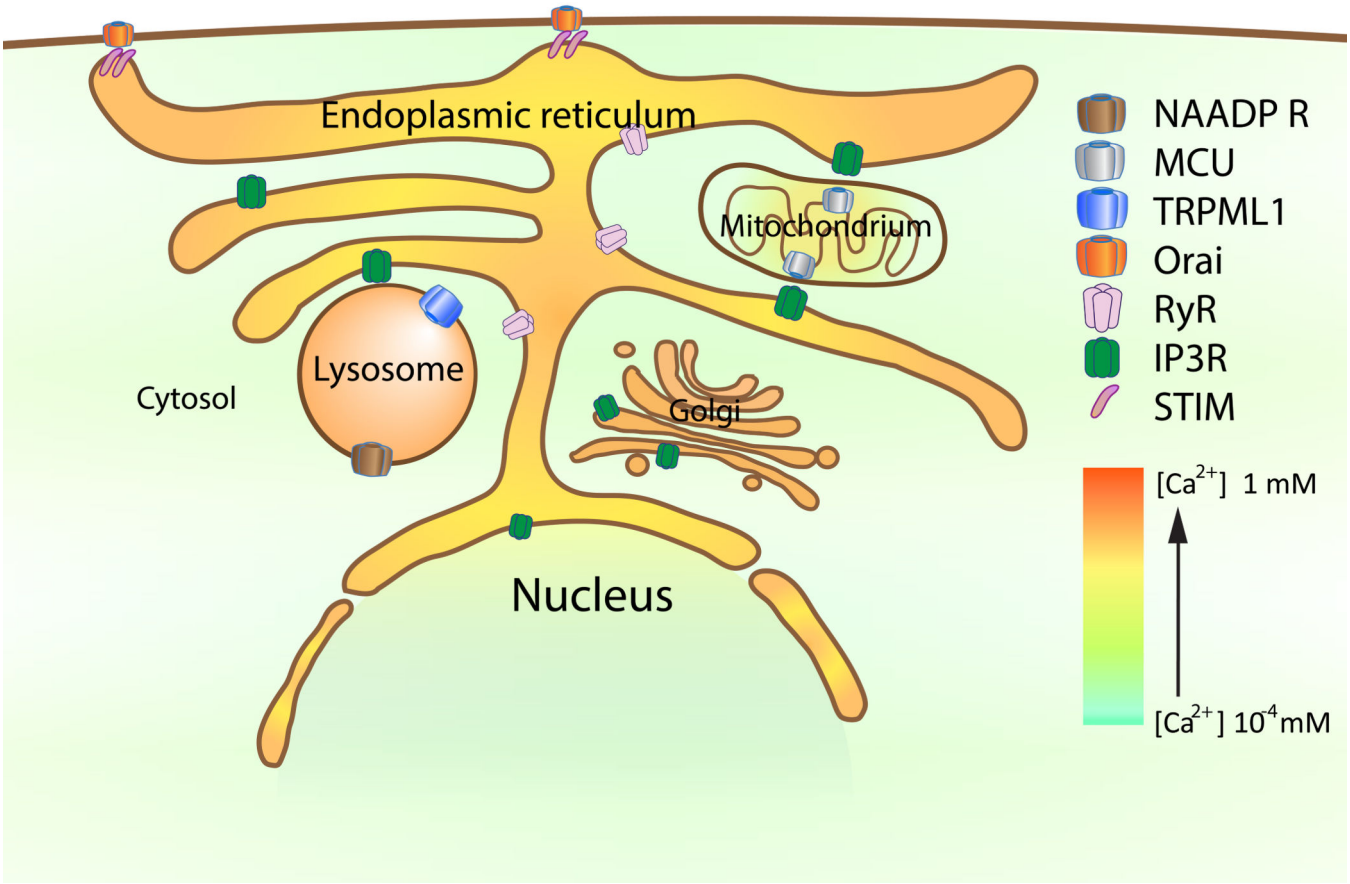

Fig. 2. Intracellular Ca^{2+} signaling pathways

ER Ca^{2+} depletion induces cytosolic entry of Ca^{2+} through the store-operated Ca^{2+} influx pathway, which is formed by the ER-localization stromal-interacting molecule 1 (STIM1) and the plasma membrane-localized Orai channels. Ca^{2+}-mediated exchange between ER and mitochondria is dependent on the IP_3R and the mitochondria Ca^{2+} uniporter (MCU). Ca^{2+} released through lysosomal TRPML1–3 channels and NAADP receptors may activate ER IP_3R and RyRs to release more Ca^{2+}.

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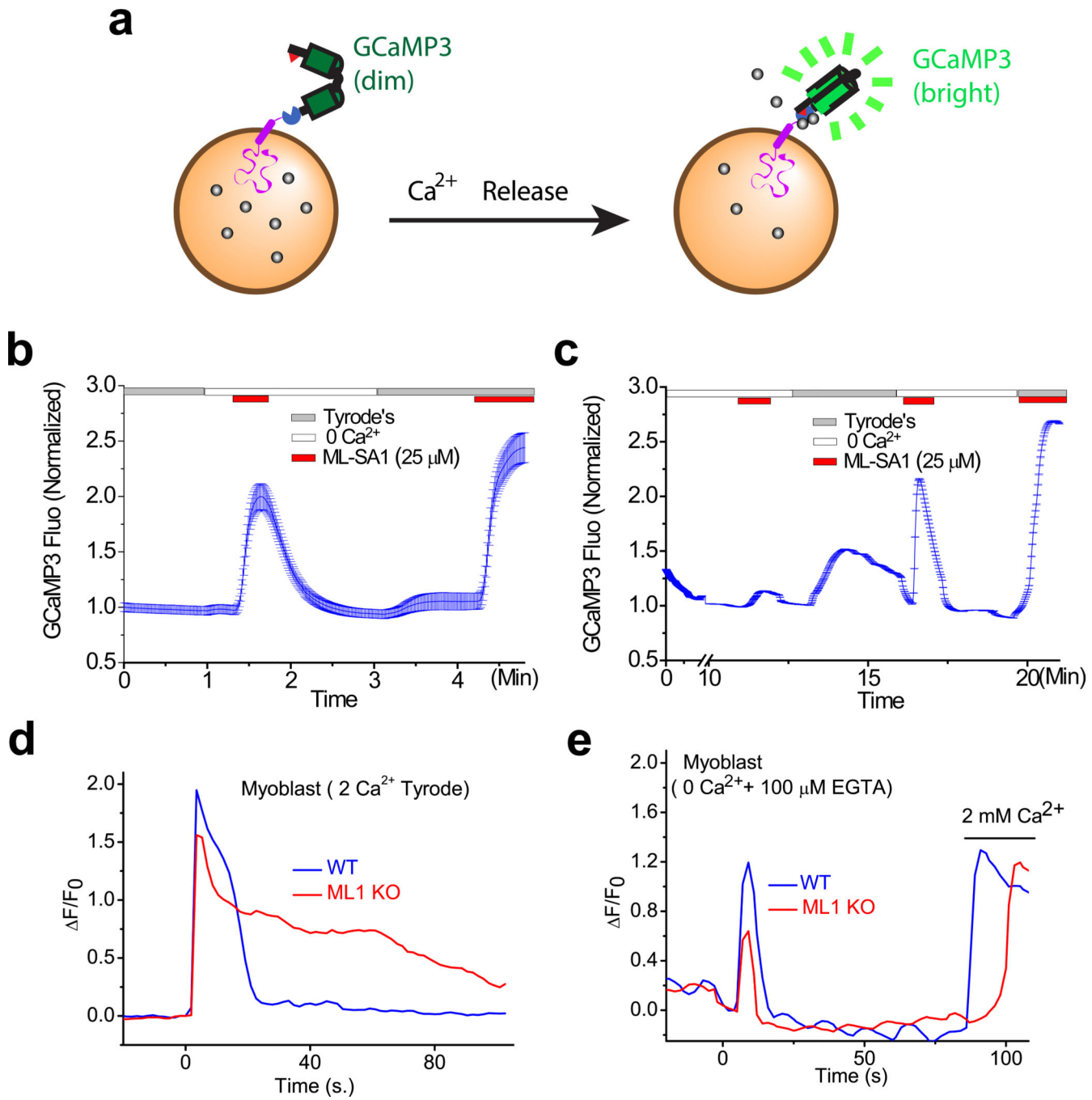

Fig. 3. Extracellular Ca^{2+}-dependence of lysosomal Ca^{2+} stores and membrane damage induced $[Ca^{2+}]$ change

(**a**) Regulation of lysosomal Ca^{2+} store depletion and refilling by extracellular Ca^{2+}. Lysosomal Ca^{2+} release is detected by a genetically-encoded Ca^{2+} indicator (GCaMP3) fused directly to the C-terminus of Lamp1 (Lamp1-GCaMP3) or the N-terminus of TRPML1 (GCaMP3-ML1). (**b**) TRPML1-mediated lysosomal Ca^{2+} release is triggered by the TRPML1-specfic synthetic agonist ML-SA1 in a zero Ca^{2+} external solution. (**c**) After a 10-min incubation period in the zero Ca^{2+} external solution, the ML-SA1-induced Ca^{2+}

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response was dramatically reduced. Re-incubation in Tyrode's solution (2 mM Ca^{2+}) for several minutes restored ML-SA1-induced responses. (**d, e**) Membrane damage induces intracellular [Ca^{2+}] changes in the presence and absence of extracellular Ca^{2+} in Lamp1-GCaMP3-transfected wild-type and TRPML1 knockout myoblasts. Panels d and e are re-plotted from Ref. [20] with permission.