# ROS and intracellular ion channels

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## Abstract

Oxidative stress is a well-known driver of numerous pathological processes involving protein and lipid peroxidation and DNA damage. The resulting increase of pro-apoptotic pressure drives tissue damage in a host of conditions, including ischemic stroke and reperfusion injury, diabetes, death in acute pancreatitis and neurodegenerative diseases. Somewhat less frequently discussed, but arguably as important, is the signaling function of oxidative stress stemming from the ability of oxidative stress to modulate ion channel activity. The evidence for the modulation of the intracellular ion channels and transporters by oxidative stress is constantly emerging and such evidence suggests new regulatory and pathological circuits that can be explored towards new treatments for diseases in which oxidative stress is an issue. In this review we summarize the current knowledge on the effects of oxidative stress on the intracellular ion channels and transporters and their role in cell function.

## Graphical Abstract
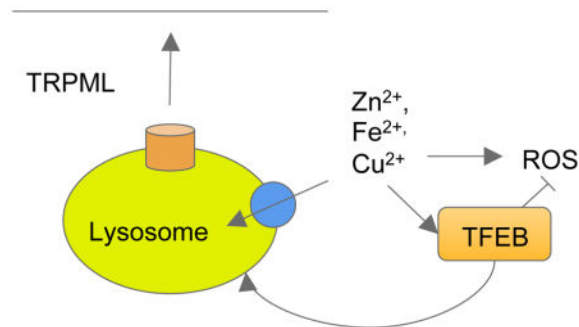


## Keywords

Reactive oxygen species; oxidative stress; calcium; transition metals; ion channels; endoplasmic reticulum; lysosomes

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## INTRODUCTION

Oxidative stress is an increase in cellular and tissue concentration of reactive oxygen species (ROS) leading to lipid, protein and DNA damage due to peroxidation and ultimately to cell death [1–10]. ROS include superoxide, hydrogen peroxide and hydroxyl radical. They are produced as part of the cell biological response by a number of cellular reactions, most prominently in the mitochondria [3]. Under normal physiological conditions, ROS and the peroxidized molecules are neutralized by a powerful antioxidant system involving superoxide dismutases, catalases, glutathione S-transferases and thioredoxins [11, 12]. However, when the ROS supply exceeds the antioxidant capacity, ROS buildup and damage the cells. This is the driving force behind a wide range of human maladies including ischemic stroke and reperfusion injury, diabetes, acute pancreatitis and Alzheimer's and other neurodegenerative diseases [1, 2, 13–21].

Mitochondrial ROS production is a normal consequence of electron leak in the electron transfer chain associated with oxidative phosphorylation. Such a leak results in superoxide formation, which is converted into hydrogen peroxide and hydroxyl radical. The leak is increased by mitochondrial damage and by hypoxia, converting some of the residual oxygen into superoxide, which further damages the mitochondria [3, 19, 22, 23]. Reperfusion provides additional oxygen fueling the reaction and accelerating the damage. Damaged mitochondria may continue leaking electrons, and thus the cycle continues until the damaged organelles are repaired or cleared [3, 18, 24–26].

Additional sources of ROS include the organellar and cytoplasmic transition metals, which catalyze a range of reactions that produce ROS [27]. Lysosomes are the main iron $Fe^{2+}$ entry pathway into the cells, which involves the endocytosis of $Fe^{3+}$ bound to transferrin or ferritin followed by their dissociation in the lysosomes, reduction to $Fe^{2+}$, and the efflux of $Fe^{2+}$ from the lysosomes into the cytoplasm [9]. $Fe^{2+}$ buildup in the lysosomes has been linked to oxidative stress in aging and in lysosomal storage diseases [9, 28]. In addition, lysosomes have emerged as an important component of zinc ($Zn^{2+}$) handling due to their role in endocytosis, autophagy of $Zn^{2+}$-rich organelles such as the mitochondria, and the presence of $Zn^{2+}$ uptake transporters in their membranes. Lysosomal $Zn^{2+}$ buildup followed by ROS release has been shown in brain cells and is thought to be a critical component of excitotoxicity due to massive amounts of $Zn^{2+}$ released by some neurons [29–31].

DNA damage, protein misfolding and aggregation, and lipid peroxidation are followed by the release of pro-apoptotic factors, such as cytochrome *c* and the lysosomal cathepsins, drive cell death caused by oxidative stress [4, 13, 31–34]. Indeed, these effects have been shown in a rage of pathological conditions linked to oxidative stress, including in the ischemic stress and in reperfusion injury models, Alzheimer's and Parkinson's disease models, as well as drug and metal toxicity models [1, 2, 13–21]. It should be noted that in addition to the "passive" damage to DNA, proteins and lipids, oxidative stress directly regulates molecules that are at the center of the cellular signaling circuits, such as ion channels and numerous protein kinases [35–44]. Whereas several reviews in this issue will discuss a role of ROS in regulation of plasma membrane ion channels, here we will focus on regulation of intracellular ion channels and transporters by ROS.

In addition to its pathologic aspects in the first place, ROS effect on ion channels and transporters is part of a regulatory circuit. Such a regulatory circuit may serve a compensatory role enabling the cells to respond to the cellular oxidative potential, similar to the transcription factor machinery that is activated by oxidative stress and resulting in expression of antioxidant components. In addition, activation by oxidative stress may function as a second messenger in a signaling cascade driven by changes in the ion channel activity in response to hormones and neurotransmitters. Indeed a low level of oxidative stress is important for proper cell function, including insulin secretion, a process firmly linked to intracellular $Ca^{2+}$ release. Both scenarios will be considered here.

The regulation of several intracellular channels by ROS, most notably IP$_3$ receptors and Ryanodine receptors (RyR), and their involvement in ROS generation has been discussed in numerous excellent reviews [45, 46] and in this Special Issue. In the current review, we will focus only on new or poorly understood aspects of ROS of these and of other intracellular ion channels and transporters.

## INTRACELLULAR CHANNELS AS TARGETS OF ROS

### ROS and IP$_3$R

The Inositol 1,4,5-trisphosphate (IP$_3$) receptors (IP$_3$Rs) is a family of cation channels comprising 3 subtypes with similar properties but distinct expression pattern [47–49]. They are present in the endoplasmic reticulum membrane (ER). Their main function is the release of $Ca^{2+}$ ions from the ER in response to activation of the plasma membrane receptors and phospholipase C. IP$_3$, the product of PI(4,5)P$_2$ cleavage by phospholipase C activity opens IP$_3$Rs, releasing the $Ca^{2+}$ stored in the ER into the cytoplasm. This cascade of reactions mediates the effect of many signaling circuits including glutamatergic, adrenergic and cholinergic signaling.

Modulation of the IP$_3$Rs-dependent $Ca^{2+}$ release by the cellular redox status has been demonstrated functionally following $Ca^{2+}$ release and biochemically using agonist binding in reconstituted systems [50–52]. A role for ROS in regulating IP$_3$Rs *activity* has been directly shown by several groups in a number of *in vitro* experimental models.

In human platelets, $H_2O_2$ induced $Ca^{2+}$ release, which was suppressed by the IP$_3$Rs antagonist Xestospongine C, by the ER SERCA $Ca^{2+}$ pump inhibitor thapsigargin and by the antioxidant DTT, but was unaffected by the phospholipase C inhibitor U73122 [53]. $H_2O_2$ triggered platelet aggregation, which was previously linked to IP$_3$Rs activation thus bridging the molecular and functional readouts of the ROS effects of $H_2O_2$ on IP$_3$Rs and $Ca^{2+}$ signaling. In human umbilical vein epithelial cells, $H_2O_2$ caused $Ca^{2+}$ mobilization, which was sensitive to IP$_3$Rs inhibitor heparin but not to U73122, and did not involve a spike in IP$_3$ production [54]. It was proposed that $H_2O_2$ directly targets IP$_3$Rs in this system. A spike in $Ca^{2+}$ release in response to $H_2O_2$ was shown in pulmonary arterial smooth muscle cells [55]. Such a spike was not abolished by extracellular $Ca^{2+}$ removal but it was eliminated by IP$_3$Rs antagonists. Furthermore, $H_2O_2$ triggered a wave of $Ca^{2+}$ influx due to the activation of store-dependent $Ca^{2+}$ influx channel brought about by ER $Ca^{2+}$ depletion.

These finding suggest that activation of IP$_3$Rs by ROS contributes to the H$_2$O$_2$-dependent vasoconstriction.

A recent study utilizing DT40 cells identified the IP$_3$Rs type mediating the superoxide-dependent Ca$^{2+}$ release [56]. Chicken DT40 cells express all three IP$_3$Rs isoforms. Triple IP$_3$Rs-deficient DT40 cells lost Ca$^{2+}$ release in response to superoxide, as did the cells expressing only the type 3 IP$_3$R3. These data suggest that IP$_3$R1 and IP$_3$R2 but not IP$_3$R3 respond to the ROS. These findings serve as the first step towards identification of the structural determinants of the ROS effect on IP$_3$Rs by analyzing the amino acid differences between the three IP$_3$Rs subtypes. Potential structural determinants accounting for regulation of IP$_3$Rs by ROS have been discussed before [45, 46] and will not be further discussed here.

Superoxide release by activated macrophages elicited a cytoplasmic Ca$^{2+}$ signal in endothelial cells with Ca$^{2+}$ release that was followed by Ca$^{2+}$ influx [57]. The influx was sensitive to ROS scavenge and to ER Ca$^{2+}$ depletion. As above, superoxide failed to elicit a Ca$^{2+}$ signal in triple IP$_3$Rs-deficient DT40 cells, clearly showing that IP$_3$Rs are involved in this process. Interestingly, in these studies U-73122 abolished the superoxide-induced Ca$^{2+}$ release, suggesting a completely different mechanism or release than direct activation of IP$_3$Rs described above and likely involving an increase in phospholipase C activity and in IP$_3$ production.

The activation of IP3R by ROS suggests signaling and pathological roles of this process. Accordingly, a role of ROS in neuronal plasticity has been discussed [58], and modulation of IP$_3$Rs activity is a good candidate for a molecular target for such a role. On the other hand, as discussed in the section on intracellular channels and ROS production, massive Ca$^{2+}$ release from the ER floods the mitochondria with Ca$^{2+}$, accelerates ROS production and activates apoptosis and then necrosis leading to cell death. It is likely that a balance between these processes defines the response of a given tissue to ROS.

While the experiments in macrophages illustrate one of the possible scenarios linking ROS and IP$_3$Rs function, in general, pathologically relevant *in vivo* evidence of ROS effect on IP$_3$Rs is very thin. It would be interesting to explore the role of IP$_3$Rs in the pathologies linked to mitochondrial dysfunction and ROS production, such as mitotoxic drugs and hypoxia/reperfusion injury. This is especially interesting in the context of the mitochondrial-ER interaction, of which IP$_3$Rs are an important component [59, 60]. Such an interaction places IP$_3$Rs in a close proximity to the potential source of ROS. How the mitochondrial-ER interaction affects the ability of ROS to affect Ca$^{2+}$ release via IP$_3$Rs is an interesting unanswered question.

## ROS and RyR

Ryanodine receptors (RyRs) is a family of three sarco/endoplasmic reticulum ion channels responsible for excitation-contraction coupling and for amplification of the Ca$^{2+}$ signals initiated by the IP$_3$Rs or by the plasma membrane Ca$^{2+}$ influx channels. Types 1, 2 and 3 RyRs mediate Ca$^{2+}$-induced Ca$^{2+}$ release that drives the amplification, while the type 1 RyR

is capable of directly sensing conformational changes in the L-type $Ca^{2+}$ channels to mediate the $Ca^{2+}$ release in response to membrane depolarization [61–63].

RyRs regulation by ROS is among the best understood in the ion channel field and has been shown directly using $Ca^{2+}$ imaging and application of ROS, or using various other experimental paradigms. The structural determinants of RyR regulation by ROS have been reviewed recently [45, 64, 65] and are discussed in other articles in this issue. RyRs activation by oxidative stress has been linked to muscle damage in aging, heart failure, cerebral ischemia and Duchenne muscular dystrophy [66–70]. Besides the well-illustrated role of ROS-dependent RyR-mediated $Ca^{2+}$ release in muscle and neurons, several recent findings illustrate possible new paradigms in membrane traffic.

RyRs activation by the ROS has been linked to glucose-dependent insulin secretion by the β-cells. Both glucose and $H_2O_2$ caused RyR glutathionylation and $Ca^{2+}$ release [71]. The ROS buildup due to glucose influx drives the $Ca^{2+}$ release *via* RyRs followed by insulin secretion. In another studies, ROS promoted insulin-dependent translocation of the GLUT4 transporter to the plasma membrane [72], a process that depends on the fusion of the GLUT4-containing vesicles with the plasma membrane. $H_2O_2$ increased GUT4 translocation, while antioxidants had an opposite effect. As in [71], insulin promoted RyRs glutathionylation and $Ca^{2+}$ release while stimulating GLUT4 translocation. These data show that RyRs activation by ROS may drive membrane traffic events including exocytosis.

The role of ROS-dependent $Ca^{2+}$ release via RyRs in membrane traffic events illuminates a possible new point of interaction between the ROS and $Ca^{2+}$ signaling and the cell clearance. Such an interaction may play an important role in facilitating the repair of damage caused by ROS. Organelles damaged by oxidative stress, especially lysosomes and mitochondria, tend to amplify the damage by releasing pro-apoptotic factors, such as cytochrome *c* and the lysosomal hydrolases. A timely utilization of such organelles should serve to minimize the ROS damage, and thus it is important to consider the possible regulatory circuits linking ROS and activation of cellular clearance pathways. Lysosomal exocytosis has been recently shown as a critical part of the cellular detoxification process by promoting the extrusion of protein aggregates and undigested autophagosome content, and to drive phagocytosis by supplying membrane material to the plasma membrane. Since RyRs present in the ER are able to actuate the exocytosis of insulin-containing vesicles, it is possible that they are able to drive lysosomal exocytosis as well. If RyRs-dependent $Ca^{2+}$ release promotes autosomal or lysosomal exocytosis and clearance, then a new regulatory circuit can be postulated that responds to oxidative stress by increasing cellular clearance via RyRs-dependent $Ca^{2+}$ release and lysosomal exocytosis.

## ROS and ion transporters

**SERCA**—The sarco/endoplasmic reticulum $Ca^{2+}$ ATPase pump (SERCA) mediates $Ca^{2+}$ pumping into the sarco/endoplasmic reticulum [73]. Although IP$_3$Rs and RyRs $Ca^{2+}$ release channels are activated by ROS, the supply side of the $Ca^{2+}$ signaling machinery is inactivated by ROS [45]. Such an inactivation has been demonstrated in numerous systems and its structural determinants have been identified and linked to pathological conditions [74–78]. It is possible that suppressed $Ca^{2+}$ uptake leads to a depletion of ER $Ca^{2+}$, which

could be of advantage under oxidative stress conditions by limiting the runaway damage caused by the feedback loop of ROS-driven $Ca^{2+}$ release and the resulting mitochondrial ROS production.

**ZnT, ATP7/ATP7B and DMT1**—ZnTs, ATP7 and DMT1 are intracellular divalent cation transporters responsible for handling the transition metals $Zn^{2+}$, $Cu^{2+}$ and $Fe^{2+}$, respectively [79, 80]. Given the critical role of transition metals in ROS production, as well as in antioxidant defense, it is surprising how little we known about their regulation by oxidative stress. Largely, all the information on this topic is limited to regulation of their expression by ROS, directly or indirectly. This information is summarized below.

The ZnT (SLC30) is a family of ion transporters comprising 10 members in mammals. The ZnT transporters are responsible for the removal of $Zn^{2+}$ from the cytoplasm into organelles or their efflux across the plasma membrane [79, 81, 82]. ZnTs are localized in different intracellular compartments and some are expressed in a tissue-restricted manner. For example, while the plasma membrane ZnT1 transporter is ubiquitously expressed, ZnT3 is limited to presynaptic vesicles into which it pumps $Zn^{2+}$ necessary for the release with glutamate [79, 81, 82]. Such a release modulates the neuronal excitability by acting on NMDA and AMPA receptors as well on the neuronal $Zn^{2+}$ receptor itself [83–88]. In the mammary gland intracellular ZnT2 and ZnT4 mediate $Zn^{2+}$ delivery during lactation[89, 90]. ZnT2 and ZnT4 dysregulation has been linked to $Zn^{2+}$ mishandling in mammary gland epithelium, impaired $Zn^{2+}$ delivery with milk and aberrant progression of mammary gland involution [91].

ZnT expression is regulated by the transcription factor MTF-1, which is activated by binding of $Zn^{2+}$ and other transition metals [92–94]. Free cytoplasmic $Zn^{2+}$ concentration is in the picomolar range due to organellar uptake and binding to cytoplasmic chelating metallothionein proteins [95]. ROS significantly decreases the metallothioneins affinity to transition metals, releasing them into the cytoplasm to increase cytoplasmic $Zn^{2+}$ [95, 96]. The free transition metals are recognized by MTF-1, leading to upregulation of ZnT expression. This powerful feedback system allows cells to adjust their $Zn^{2+}$ buffering capacity under oxidative stress and transition metal exposure. We are presently unaware of any evidence of a direct effect of ROS on ZnT activity or expression. Finding such an effect would suggest that cells are able to predict ROS buildup due to transition metal exposure and transition metals build up due to ROS exposure and prepare for it.

ATP7A and ATP7B export $Cu^{2+}$ from the cytoplasm. They are present in a vesicular pool recently linked to lysosomes [97, 98]. $Cu^{2+}$ exposure promotes translocation of ATP7 into the plasma membrane and $Cu^{2+}$ expulsion [99]. The translocation has been traditionally considered a preparative step for $Cu^{2+}$ expulsion through the plasma membrane. However, recent evidence indicates that a very large fraction of $Cu^{2+}$ export is in vesicles and occurs *en route* to the plasma membrane, so that $Cu^{2+}$ exocytosis rather than transport across the plasma membrane is the predominant $Cu^{2+}$ extrusion pathway [99]. ATP7 loss causes Wilson's and Menkes diseases, characterized by liver damage and developmental deficits [20]. ROS buildup has been shown in Wilson's disease, which is associated with $Cu^{2+}$ overload [20]. No direct evidence of ROS effect on ATP7 activity has been reported.

However, regulation of ATP7A expression by the hypoxia-inducible transcription factor Hif2α links ROS with the ATP7-dependent transport processes [100, 101].

Transition metals enter the cells via plasma membrane transporters and via the endocytic pathway. Indeed, receptor-mediated endocytosis of ferritin- or transferrin-bound $Fe^{2+}$ is the major $Fe^{2+}$ entry pathway in mammalian brain cells [80, 102]. DMT1 (SLC11A2) mediates the uptake of $Fe^{2+}$ that is then dissociated from ferritin or transferrin in the endolysosomes from which it is transported into the cytoplasm [80, 102]. Inefficient clearance of the lysosomal $Fe^{2+}$ has been linked to ROS production, lipofuscin buildup and aging [9, 103]. While the data on direct effect of ROS on DMT1 activity are lacking, ROS upregulates DMT1 expression via the transcription factor Hif-1 [104]. This is another example of a regulatory loop that increases the cellular transition metal clearance throughput to suppress oxidative damage.

## INTRACELLULAR TRANSPORTERS AND ROS PRODUCTION

While ion channels and transporters themselves do not contribute to ROS production or regulation, they frequently lead to dysregulation that promotes ROS production. Several such processes are reviewed in this section while focusing on poorly understood and novel aspects of this topic.

### ER $Ca^{2+}$ release and ROS production

Stimulation of the ROS production by the mitochondria, especially damaged mitochondria, has been shown in numerous systems, and is thought to drive or contribute to cell death in ischemic stroke and reperfusion injury, traumatic brain injury, myocardial infarction, acute pancreatitis and neurodegenerative diseases [1, 2, 13–21]. That the RyRs- and IP$_3$Rs-dependent $Ca^{2+}$ release contributes to these processes is well established. Indeed, ROS-dependent upregulation of IP$_3$Rs and RyRs activities has been linked to mitochondrial $Ca^{2+}$ overload and are associated with these maladies [105, 106]. Similarly, cell death in Alzheimer's disease models reported upregulation of the $Ca^{2+}$ release machinery and mitochondrial ROS production [107]. Upregulation of IP$_3$Rs activity by ROS and the subsequent $Ca^{2+}$ flooding of the mitochondria have been proposed to underlie endothelial apoptosis during ischemia/reperfusion episodes and to enhance the excitability of amygdala neurons and pain manifestation [57, 108]. It is therefore clear that the facilitation of $Ca^{2+}$ release by ROS leads to $Ca^{2+}$ flooding of the mitochondria and mitochondrial damage [105]. $Ca^{2+}$ flooding of the mitochondria, in turn, facilitates ROS production, creating a runaway damage feedback loop. Such a feedback loop is observed in cardiotoxic effects of cardiac glycosides, including digitoxin and digoxin [109]. It was proposed that cardiac glycosides activate the NADPH oxidase NOX2 to promote ROS production, which facilitates $Ca^{2+}$ release that floods the mitochondria with $Ca^{2+}$, which promotes further ROS production.

Why does such a loop exist? It is possible that the loop is an aspect of a cytoprotective mechanism that is revved up into a pathological zone under stress conditions. The loss of stimulated ATP production by moderate levels of $Ca^{2+}$ entering the mitochondria was shown to underlie several forms of cell death due to energy-dependent cytotoxicity. It is possible that moderate levels of ROS signal stress and stimulate cellular function by activating $Ca^{2+}$

release to stimulate ATP production. However, massive or sustained stress may overwhelm the mitochondrial $Ca^{2+}$ load capacity resulting in the damage loop. This is illustrated in Figure 1.

## Lysosomal dysfunction and ROS production

While commonly discussed as digestive organelles and energy sensors, lysosomes are gaining recognition as a cytoprotective organelles actively involved in limiting cellular damage. First, they are responsible for the autophagic degradation of damaged organelles such as mitochondria, which otherwise would continue to damage the cells. Second, lysosomes absorb transition metals from the cytoplasm, which suppresses oxidative stress [97, 110, 111]. Interestingly, not all endolysosomal ion channels limit oxidative stress: the endosomal $H^+/Cl^-$ exchanger ClC-3 (CLCN3) supports ROS production by leaking superoxide, or by sustaining NADPH oxidase through increased NOX activity in the endosome [112–114].

TRPML1 is an endolysosomal ion channel permeable to mono- and divalent cations including $Ca^{2+}$, $Fe^{2+}$ and $Zn^{2+}$ [115, 116]. TRPML1 has emerged as a major regulator of the endolysosomal function, and several aspects of its role in the endocytic pathway have been linked to oxidative stress. First, a buildup of $Fe^{2+}$ in the lysosomes of TRPML1-deficient fibroblasts has been shown [115]. Although oxidative stress was not observed initially, subsequent evidence linked TRPML1 loss to oxidative stress, which was aggravated by exposure to $Fe^{2+}$ [8]. Prominent oxidative stress in the brain of mouse model of mucolipidosis type IV, a lysosomal storage diseases caused by the TRPML1 loss, further supports a role for TRPML1 in oxidative stress [28].

Several mechanisms can explain the buildup of $Fe^{2+}$ in the lysosomes of TRPML1-deficient cells. First, TRPML1 was shown to function as $Fe^{2+}$-permeable channel [115]. While DMT1 is considered the endolysosomal $Fe^{2+}$ absorption transporter, the evidence for such a role is lacking in several cell types, most notably oligodendrocytes and brain capillary epithelial cells [117–119]. It is possible that TRPML1 substitutes DMT1 in these cells. Second, TRPML1 is required for lysosomal exocytosis [120–122]. Therefore, $Fe^{2+}$ retention could be due to delays in the traffic of transferrin/ferritin-bound $Fe^{2+}$ to the endolysosomes, or delayed exocytosis of $Fe^{2+}$-containing lysosomes. We have recently proposed a similar model for the extrusion of $Zn^{2+}$ as lysosomes accumulate $Zn^{2+}$ in the absence of TRPML1 and lysosomal exocytosis is critically important for $Zn^{2+}$ expulsion from cells [111, 123, 124]. Moreover, as illustrated in Figure 2, we showed that transition metals stimulate lysosomal biogenesis via activation of the lysosomal gene network, of which TRPML1 is a component. It is possible that this is an additional mechanism through which cells increase their transition metal clearance capacity in response to transition metal exposure and oxidative stress. Finally, it was proposed recently that TRPML1 may determine the correct localization of transition metal transporters and in the absence of which transition metals may accumulate to cause ROS build up [125].

At present, the data on TRPML regulation by ROS are lacking. Such a regulation may involve a direct effect of ROS on the channels or, since TRPML1 and possibly TRPML3 are regulated by phosphor- and sphingolipids [126, 127], lipid peroxidation. A finding that

TRPML are regulated by ROS would identify novel regulatory networks driven by oxidative stress and the lysosomal clearance function. If, for example, TRPML are inhibited by ROS then the membrane traffic processes controlled by these channels are likely to be inhibited by oxidative stress. Indeed, autophagy inhibition by oxidative stress has been shown [7, 34, 128]. This would suggest that beyond damaging the organelles, ROS delay the cellular repair by suppressing autophagy, lysosomal exocytosis (including pathogen expulsion) and other clearance processes that depend on the membrane traffic events driven by TRPML [121, 122, 129–133]. On the other hand, a finding that TRPML are activated by ROS would suggest a compensatory mechanism that increases membrane turnover in response to ROS. Such a feedback loop would enable cells to respond to oxidative stress by increasing the cellular repair. Clearly, further research into the regulation of TRPML channels by oxidative stress is likely to give new insights into the mechanisms of cellular damage by oxidative stress and the cytoprotective function of the lysosomes. Pharmacological approaches to TRPML regulation are beginning to emerge [121, 134–137], and with them – new possibilities of attacking the pathogenic processes driven by oxidative stress.

## SUMMARY

ROS regulate the function or expression of several intracellular transporters, which in turn regulate ROS levels. Such a regulation likely plays a cytoprotective role under physiological conditions, but may turn into a cycle of runaway cell and tissue damage when uncontrolled. Breaking such a cycle is likely to point to new treatments for diseases in which oxidative stress is an issue.

## References

1. Xia Z, Chen Y, Fan Q, Xue M. Oxidative stress-mediated reperfusion injury: mechanism and therapies. Oxidative medicine and cellular longevity. 2014; 2014:373081. [PubMed: 24803980]

2. Rodriguez-Rodriguez A, Egea-Guerrero JJ, Murillo-Cabezas F, Carrillo-Vico A. Oxidative stress in traumatic brain injury. Curr Med Chem. 2014; 21:1201–1211. [PubMed: 24350853]

3. Kalogeris T, Bao Y, Korthuis RJ. Mitochondrial reactive oxygen species: a double edged sword in ischemia/reperfusion vs preconditioning. Redox biology. 2014; 2:702–714. [PubMed: 24944913]

4. Repnik U, Stoka V, Turk V, Turk B. Lysosomes and lysosomal cathepsins in cell death. Biochim Biophys Acta. 2012; 1824:22–33. [PubMed: 21914490]

5. Ott M, Gogvadze V, Orrenius S, Zhivotovsky B. Mitochondria, oxidative stress and cell death. Apoptosis. 2007; 12:913–922. [PubMed: 17453160]

6. Baird SK, Kurz T, Brunk UT. Metallothionein protects against oxidative stress-induced lysosomal destabilization. Biochem J. 2006; 394:275–283. [PubMed: 16236025]

7. Van Beersel G, Tihon E, Demine S, Hamer I, Jadot M, Arnould T. Different molecular mechanisms involved in spontaneous and oxidative stress-induced mitochondrial fragmentation in tripeptidyl peptidase-1 (TPP-1)-deficient fibroblasts. Bioscience reports. 2013; 33:e00023. [PubMed: 23249249]

8. Coblentz J, St Croix C, Kiselyov K. Loss of TRPML1 promotes production of reactive oxygen species: is oxidative damage a factor in mucolipidosis type IV? Biochem J. 2014; 457:361–368. [PubMed: 24192042]

9. Kurz T, Terman A, Gustafsson B, Brunk UT. Lysosomes in iron metabolism, ageing and apoptosis. Histochem Cell Biol. 2008; 129:389–406. [PubMed: 18259769]

10. Allen CL, Bayraktutan U. Oxidative stress and its role in the pathogenesis of ischaemic stroke. International journal of stroke : official journal of the International Stroke Society. 2009; 4:461–470. [PubMed: 19930058]

11. Yoshioka M, Tanaka K, Miyazaki I, Fujita N, Higashi Y, Asanuma M, Ogawa N. The dopamine agonist cabergoline provides neuroprotection by activation of the glutathione system and scavenging free radicals. Neurosci Res. 2002; 43:259–267. [PubMed: 12103444]

12. Robinson NJ, Winge DR. Copper metallochaperones. Annu Rev Biochem. 2010; 79:537–562. [PubMed: 20205585]

13. Miller MW, Sadeh N. Traumatic stress, oxidative stress and post-traumatic stress disorder: neurodegeneration and the accelerated-aging hypothesis. Mol Psychiatry. 2014; 19:1156–1162. [PubMed: 25245500]

14. Hare D, Ayton S, Bush A, Lei P. A delicate balance: Iron metabolism and diseases of the brain. Frontiers in aging neuroscience. 2013; 5:34. [PubMed: 23874300]

15. Terman A, Dalen H, Eaton JW, Neuzil J, Brunk UT. Aging of cardiac myocytes in culture: oxidative stress, lipofuscin accumulation, and mitochondrial turnover. Ann N Y Acad Sci. 2004; 1019:70–77. [PubMed: 15246997]

16. Zheng Z, Lee JE, Yenari MA. Stroke: molecular mechanisms and potential targets for treatment. Curr Mol Med. 2003; 3:361–372. [PubMed: 12776991]

17. Cojocaru IM, Cojocaru M, Sapira V, Ionescu A. Evaluation of oxidative stress in patients with acute ischemic stroke. Romanian journal of internal medicine = Revue roumaine de medecine interne. 2013; 51:97–106. [PubMed: 24294813]

18. Neri M, Fineschi V, Di Paolo M, Pomara C, Riezzo I, Turillazzi E, Cerretani D. Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction. Current vascular pharmacology. 2015; 13:26–36. [PubMed: 23628007]

19. Cornelius C, Crupi R, Calabrese V, Graziano A, Milone P, Pennisi G, Radak Z, Calabrese EJ, Cuzzocrea S. Traumatic brain injury: oxidative stress and neuroprotection. Antioxid Redox Signal. 2013; 19:836–853. [PubMed: 23547621]

20. Scheiber IF, Mercer JF, Dringen R. Metabolism and functions of copper in brain. Prog Neurobiol. 2014; 116:33–57. [PubMed: 24440710]

21. Yu JH, Kim H. Oxidative stress and inflammatory signaling in cerulein pancreatitis. World J Gastroenterol. 2014; 20:17324–17329. [PubMed: 25516643]

22. Baffy G, Derdak Z, Robson SC. Mitochondrial recoupling: a novel therapeutic strategy for cancer? Br J Cancer. 2011; 105:469–474. [PubMed: 21712825]

23. Drose S, Brandt U. Molecular mechanisms of superoxide production by the mitochondrial respiratory chain. Adv Exp Med Biol. 2012; 748:145–169. [PubMed: 22729857]

24. Schafer MK, Pfeiffer A, Jaeckel M, Pouya A, Dolga AM, Methner A. Regulators of mitochondrial Ca(2+) homeostasis in cerebral ischemia. Cell Tissue Res. 2014; 357:395–405. [PubMed: 24715113]

25. Szydlowska K, Tymianski M. Calcium, ischemia and excitotoxicity. Cell Calcium. 2010; 47:122–129. [PubMed: 20167368]

26. Liou AK, Clark RS, Henshall DC, Yin XM, Chen J. To die or not to die for neurons in ischemia, traumatic brain injury and epilepsy: a review on the stress-activated signaling pathways and apoptotic pathways. Prog Neurobiol. 2003; 69:103–142. [PubMed: 12684068]

27. Brunk UT, Jones CB, Sohal RS. A novel hypothesis of lipofuscinogenesis and cellular aging based on interactions between oxidative stress and autophagocytosis. Mutat Res. 1992; 275:395–403. [PubMed: 1383780]

28. Grishchuk Y, Peña KA, Coblentz J, King VE, Humphrey DM, Wang SL, Kiselyov KI, Slaugenhaupt SA. Impaired myelination and reduced brain ferric iron in the mouse model of mucolipidosis IV. Disease models & mechanisms. 2015

29. Sensi SL, Paoletti P, Koh JY, Aizenman E, Bush AI, Hershfinkel M. The neurophysiology and pathology of brain zinc. J Neurosci. 2011; 31:16076–16085. [PubMed: 22072659]

30. Lee SJ, Park MH, Kim HJ, Koh JY. Metallothionein-3 regulates lysosomal function in cultured astrocytes under both normal and oxidative conditions. Glia. 2010; 58:1186–1196. [PubMed: 20544854]

31. Lee SJ, Koh JY. Roles of zinc and metallothionein-3 in oxidative stress-induced lysosomal dysfunction, cell death, and autophagy in neurons and astrocytes. Molecular brain. 2010; 3:30. [PubMed: 20974010]

32. Granzotto A, Sensi SL. Intracellular zinc is a critical intermediate in the excitotoxic cascade. Neurobiol Dis. 2015; 81:25–37. [PubMed: 25940914]

33. Brito R, Castillo G, Gonzalez J, Valls N, Rodrigo R. Oxidative Stress in Hypertension: Mechanisms and Therapeutic Opportunities, Experimental and clinical endocrinology & diabetes : official journal. German Society of Endocrinology [and] German Diabetes Association. 2015; 123:325–335.

34. Lee SJ, Cho KS, Koh JY. Oxidative injury triggers autophagy in astrocytes: the role of endogenous zinc. Glia. 2009; 57:1351–1361. [PubMed: 19229997]

35. Wang F, Reece EA, Yang P. Advances in revealing the molecular targets downstream of oxidative stress-induced proapoptotic kinase signaling in diabetic embryopathy. Am J Obstet Gynecol. 2015; 213:125–134. [PubMed: 25595581]

36. Luczak ED, Anderson ME. CaMKII oxidative activation and the pathogenesis of cardiac disease. J Mol Cell Cardiol. 2014; 73:112–116. [PubMed: 24530899]

37. Takahashi N, Kozai D, Kobayashi R, Ebert M, Mori Y. Roles of TRPM2 in oxidative stress. Cell Calcium. 2011; 50:279–287. [PubMed: 21616534]

38. Veit F, Pak O, Brandes RP, Weissmann N. Hypoxia-dependent reactive oxygen species signaling in the pulmonary circulation: focus on ion channels. Antioxid Redox Signal. 2015; 22:537–552. [PubMed: 25545236]

39. Syed Mortadza SA, Wang L, Li D, Jiang LH. TRPM2 Channel-Mediated ROS-Sensitive Ca(2+) Signaling Mechanisms in Immune Cells. Front Immunol. 2015; 6:407. [PubMed: 26300888]

40. Olschewski A, Weir EK. Redox regulation of ion channels in the pulmonary circulation. Antioxid Redox Signal. 2015; 22:465–485. [PubMed: 24702125]

41. Gamper N, Ooi L. Redox and nitric oxide-mediated regulation of sensory neuron ion channel function. Antioxid Redox Signal. 2015; 22:486–504. [PubMed: 24735331]

42. Sesti F, Wu X, Liu S. Oxidation of KCNB1 K(+) channels in central nervous system and beyond. World J Biol Chem. 2014; 5:85–92. [PubMed: 24921000]

43. Sahoo N, Hoshi T, Heinemann SH. Oxidative modulation of voltage-gated potassium channels. Antioxid Redox Signal. 2014; 21:933–952. [PubMed: 24040918]

44. Kozai D, Ogawa N, Mori Y. Redox regulation of transient receptor potential channels. Antioxid Redox Signal. 2014; 21:971–986. [PubMed: 24161127]

45. Hidalgo C, Donoso P. Crosstalk between calcium and redox signaling: from molecular mechanisms to health implications. Antioxid Redox Signal. 2008; 10:1275–1312. [PubMed: 18377233]

46. Donoso P, Sanchez G, Bull R, Hidalgo C. Modulation of cardiac ryanodine receptor activity by ROS and RNS. Front Biosci (Landmark Ed). 2011; 16:553–567. [PubMed: 21196188]

47. Vervloessem T, Yule DI, Bultynck G, Parys JB. The type 2 inositol 1,4,5-trisphosphate receptor, emerging functions for an intriguing Ca(2)(+)-release channel. Biochim Biophys Acta. 2015; 1853:1992–2005. [PubMed: 25499268]

48. Mikoshiba K. Role of IP3 receptor signaling in cell functions and diseases. Adv Biol Regul. 2015; 57:217–227. [PubMed: 25497594]

49. Mak DO, Foskett JK. Inositol 1,4,5-trisphosphate receptors in the endoplasmic reticulum: A single-channel point of view. Cell Calcium. 2015; 58:67–78. [PubMed: 25555684]

50. Bootman MD, Taylor CW, Berridge MJ. The thiol reagent, thimerosal, evokes Ca2+ spikes in HeLa cells by sensitizing the inositol 1,4,5-trisphosphate receptor. J Biol Chem. 1992; 267:25113–25119. [PubMed: 1334081]

51. Joseph SK, Ryan SV, Pierson S, Renard-Rooney D, Thomas AP. The effect of mersalyl on inositol trisphosphate receptor binding and ion channel function. J Biol Chem. 1995; 270:3588–3593. [PubMed: 7533153]

52. Renard-Rooney DC, Joseph SK, Seitz MB, Thomas AP. Effect of oxidized glutathione and temperature on inositol 1,4,5-trisphosphate binding in permeabilized hepatocytes. Biochem J. 1995; 310(Pt 1):185–192. [PubMed: 7646443]

53. Redondo PC, Salido GM, Rosado JA, Pariente JA. Effect of hydrogen peroxide on Ca2+ mobilisation in human platelets through sulphydryl oxidation dependent and independent mechanisms. Biochem Pharmacol. 2004; 67:491–502. [PubMed: 15037201]

54. Zheng Y, Shen X. H2O2 directly activates inositol 1,4,5-trisphosphate receptors in endothelial cells. Redox Rep. 2005; 10:29–36. [PubMed: 15829109]

55. Lin MJ, Yang XR, Cao YN, Sham JS. Hydrogen peroxide-induced Ca2+ mobilization in pulmonary arterial smooth muscle cells. Am J Physiol Lung Cell Mol Physiol. 2007; 292:L1598–1608. [PubMed: 17369291]

56. Bansaghi S, Golenar T, Madesh M, Csordas G, RamachandraRao S, Sharma K, Yule DI, Joseph SK, Hajnoczky G. Isoform- and species-specific control of inositol 1,4,5-trisphosphate (IP3) receptors by reactive oxygen species. J Biol Chem. 2014; 289:8170–8181. [PubMed: 24469450]

57. Madesh M, Hawkins BJ, Milovanova T, Bhanumathy CD, Joseph SK, Ramachandrarao SP, Sharma K, Kurosaki T, Fisher AB. Selective role for superoxide in InsP3 receptor-mediated mitochondrial dysfunction and endothelial apoptosis. J Cell Biol. 2005; 170:1079–1090. [PubMed: 16186254]

58. Massaad CA, Klann E. Reactive oxygen species in the regulation of synaptic plasticity and memory. Antioxid Redox Signal. 2011; 14:2013–2054. [PubMed: 20649473]

59. Annunziata I, d'Azzo A. Interorganellar membrane microdomains: dynamic platforms in the control of calcium signaling and apoptosis. Cells. 2013; 2:574–590. [PubMed: 24709798]

60. Grimm S. The ER-mitochondria interface: the social network of cell death. Biochim Biophys Acta. 2012; 1823:327–334. [PubMed: 22182703]

61. Van Petegem F. Ryanodine receptors: allosteric ion channel giants. J Mol Biol. 2015; 427:31–53. [PubMed: 25134758]

62. Eisner D. Calcium in the heart: from physiology to disease. Exp Physiol. 2014; 99:1273–1282. [PubMed: 25128325]

63. Amador FJ, Stathopulos PB, Enomoto M, Ikura M. Ryanodine receptor calcium release channels: lessons from structure-function studies. The FEBS journal. 2013; 280:5456–5470. [PubMed: 23413940]

64. Niggli E, Ullrich ND, Gutierrez D, Kyrychenko S, Polakova E, Shirokova N. Posttranslational modifications of cardiac ryanodine receptors: Ca(2+) signaling and EC-coupling. Biochim Biophys Acta. 2013; 1833:866–875. [PubMed: 22960642]

65. Sun QA, Wang B, Miyagi M, Hess DT, Stamler JS. Oxygen-coupled redox regulation of the skeletal muscle ryanodine receptor/Ca2+ release channel (RyR1): sites and nature of oxidative modification. J Biol Chem. 2013; 288:22961–22971. [PubMed: 23798702]

66. Andersson DC, Betzenhauser MJ, Reiken S, Meli AC, Umanskaya A, Xie W, Shiomi T, Zalk R, Lacampagne A, Marks AR. Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging. Cell Metab. 2011; 14:196–207. [PubMed: 21803290]

67. Santulli G, Xie W, Reiken SR, Marks AR. Mitochondrial calcium overload is a key determinant in heart failure. Proc Natl Acad Sci U S A. 2015; 112:11389–11394. [PubMed: 26217001]

68. Bull R, Finkelstein JP, Galvez J, Sanchez G, Donoso P, Behrens MI, Hidalgo C. Ischemia enhances activation by Ca2+ and redox modification of ryanodine receptor channels from rat brain cortex. J Neurosci. 2008; 28:9463–9472. [PubMed: 18799678]

69. Cooper LL, Li W, Lu Y, Centracchio J, Terentyeva R, Koren G, Terentyev D. Redox modification of ryanodine receptors by mitochondria-derived reactive oxygen species contributes to aberrant Ca2+ handling in ageing rabbit hearts. J Physiol. 2013; 591:5895–5911. [PubMed: 24042501]

70. Wang Q, Wang W, Wang G, Rodney GG, Wehrens XH. Crosstalk between RyR2 oxidation and phosphorylation contributes to cardiac dysfunction in mice with Duchenne muscular dystrophy. J Mol Cell Cardiol. 2015; 89:177–184. [PubMed: 26555638]

71. Llanos P, Contreras-Ferrat A, Barrientos G, Valencia M, Mears D, Hidalgo C. Glucose-Dependent Insulin Secretion in Pancreatic beta-Cell Islets from Male Rats Requires Ca2+ Release via ROS-Stimulated Ryanodine Receptors. PLoS One. 2015; 10:e0129238. [PubMed: 26046640]

72. Contreras-Ferrat A, Llanos P, Vasquez C, Espinosa A, Osorio-Fuentealba C, Arias-Calderon M, Lavandero S, Klip A, Hidalgo C, Jaimovich E. Insulin elicits a ROS-activated and an IP(3)-dependent Ca(2)(+) release, which both impinge on GLUT4 translocation. J Cell Sci. 2014; 127:1911–1923. [PubMed: 24569874]

73. Vandecaetsbeek I, Vangheluwe P, Raeymaekers L, Wuytack F, Vanoevelen J. The Ca2+ pumps of the endoplasmic reticulum and Golgi apparatus. Cold Spring Harbor perspectives in biology. 2011; 3

74. Lancel S, Qin F, Lennon SL, Zhang J, Tong X, Mazzini MJ, Kang YJ, Siwik DA, Cohen RA, Colucci WS. Oxidative posttranslational modifications mediate decreased SERCA activity and myocyte dysfunction in Galphaq-overexpressing mice. Circ Res. 2010; 107:228–232. [PubMed: 20508180]

75. Adachi T, Matsui R, Xu S, Kirber M, Lazar HL, Sharov VS, Schoneich C, Cohen RA. Antioxidant improves smooth muscle sarco/endoplasmic reticulum Ca(2+)-ATPase function and lowers tyrosine nitration in hypercholesterolemia and improves nitric oxide-induced relaxation. Circ Res. 2002; 90:1114–1121. [PubMed: 12039802]

76. Greensmith DJ, Eisner DA, Nirmalan M. The effects of hydrogen peroxide on intracellular calcium handling and contractility in the rat ventricular myocyte. Cell Calcium. 2010; 48:341–351. [PubMed: 21106236]

77. Pande J, Dimmers G, Akolkar G, Skelley L, Samson SE, Grover AK. Store operated Ca2+ entry dependent contraction of coronary artery smooth muscle: inhibition by peroxide pretreatment. Cell Calcium. 2012; 51:149–154. [PubMed: 22206647]

78. Grover AK, Samson SE. Peroxide resistance of ER Ca2+ pump in endothelium: implications to coronary artery function. Am J Physiol. 1997; 273:C1250–1258. [PubMed: 9357769]

79. Schweigel-Rontgen M. The families of zinc (SLC30 and SLC39) and copper (SLC31) transporters. Curr Top Membr. 2014; 73:321–355. [PubMed: 24745988]

80. Valerio LG. Mammalian iron metabolism. Toxicology mechanisms and methods. 2007; 17:497–517. [PubMed: 20020877]

81. Huang L, Tepaamorndech S. The SLC30 family of zinc transporters - a review of current understanding of their biological and pathophysiological roles. Mol Aspects Med. 2013; 34:548–560. [PubMed: 23506888]

82. Kambe T. Molecular architecture and function of ZnT transporters. Curr Top Membr. 2012; 69:199–220. [PubMed: 23046652]

83. Mlyniec K, Singewald N, Holst B, Nowak G. GPR39 Zn(2+)-sensing receptor: a new target in antidepressant development? J Affect Disord. 2015; 174:89–100. [PubMed: 25490458]

84. Mlyniec K. Zinc in the Glutamatergic Theory of Depression. Curr Neuropharmacol. 2015; 13:505–513. [PubMed: 26412070]

85. Koh JY, Choi DW. Zinc toxicity on cultured cortical neurons: involvement of N-methyl-D-aspartate receptors. Neuroscience. 1994; 60:1049–1057. [PubMed: 7936205]

86. Bouron A, Kiselyov K, Oberwinkler J. Permeation, regulation and control of expression of TRP channels by trace metal ions. Pflugers Arch. 2014

87. Manev H, Kharlamov E, Uz T, Mason RP, Cagnoli CM. Characterization of zinc-induced neuronal death in primary cultures of rat cerebellar granule cells. Exp Neurol. 1997; 146:171–178. [PubMed: 9225750]

88. Nolte C, Gore A, Sekler I, Kresse W, Hershfinkel M, Hoffmann A, Kettenmann H, Moran A. ZnT-1 expression in astroglial cells protects against zinc toxicity and slows the accumulation of intracellular zinc. Glia. 2004; 48:145–155. [PubMed: 15378655]

89. Hennigar SR, Seo YA, Sharma S, Soybel DI, Kelleher SL. ZnT2 is a critical mediator of lysosomal-mediated cell death during early mammary gland involution. Science Reports. 2015 In press.

90. Seo YA, Kelleher SL. Functional analysis of two single nucleotide polymorphisms in SLC30A2 (ZnT2): implications for mammary gland function and breast disease in women. Physiological genomics. 2010; 42A:219–227. [PubMed: 20858712]

91. McCormick NH, Hennigar SR, Kiselyov K, Kelleher SL. The biology of zinc transport in mammary epithelial cells: implications for mammary gland development, lactation, and involution. Journal of mammary gland biology and neoplasia. 2014; 19:59–71. [PubMed: 24338187]

92. Laity JH, Andrews GK. Understanding the mechanisms of zinc-sensing by metal-response element binding transcription factor-1 (MTF-1). Arch Biochem Biophys. 2007; 463:201–210. [PubMed: 17462582]

93. Saydam N, Adams TK, Steiner F, Schaffner W, Freedman JH. Regulation of metallothionein transcription by the metal-responsive transcription factor MTF-1: identification of signal

transduction cascades that control metal-inducible transcription. The Journal of biological chemistry. 2002; 277:20438–20445. [PubMed: 11923282]

94. Andrews GK. Cellular zinc sensors: MTF-1 regulation of gene expression. Biometals : an international journal on the role of metal ions in biology, biochemistry and medicine. 2001; 14:223–237.

95. Thirumoorthy N, Shyam Sunder A, Manisenthil Kumar K, Senthil Kumar M, Ganesh G, Chatterjee M. A review of metallothionein isoforms and their role in pathophysiology. World J Surg Oncol. 2011; 9:54. [PubMed: 21599891]

96. Maret W, Krezel A. Cellular zinc and redox buffering capacity of metallothionein/thionein in health and disease. Molecular medicine. 2007; 13:371–375. [PubMed: 17622324]

97. Polishchuk EV, Concilli M, Iacobacci S, Chesi G, Pastore N, Piccolo P, Paladino S, Baldantoni D, van ISC, Chan J, Chang CJ, Amoresano A, Pane F, Pucci P, Tarallo A, Parenti G, Brunetti-Pierri N, Settembre C, Ballabio A, Polishchuk RS. Wilson Disease Protein ATP7B Utilizes Lysosomal Exocytosis to Maintain Copper Homeostasis. Dev Cell. 2014

98. Harada M, Kawaguchi T, Kumemura H, Terada K, Ninomiya H, Taniguchi E, Hanada S, Baba S, Maeyama M, Koga H, Ueno T, Furuta K, Suganuma T, Sugiyama T, Sata M. The Wilson disease protein ATP7B resides in the late endosomes with Rab7 and the Niemann-Pick C1 protein. Am J Pathol. 2005; 166:499–510. [PubMed: 15681833]

99. Lutsenko S, Bhattacharjee A, Hubbard AL. Copper handling machinery of the brain. Metallomics. 2010; 2:596–608. [PubMed: 21072351]

100. Xie L, Collins JF. Transcription factors Sp1 and Hif2alpha mediate induction of the copper-transporting ATPase (Atp7a) gene in intestinal epithelial cells during hypoxia. J Biol Chem. 2013; 288:23943–23952. [PubMed: 23814049]

101. Xie L, Collins JF. Transcriptional regulation of the Menkes copper ATPase (Atp7a) gene by hypoxia-inducible factor (HIF2{alpha}) in intestinal epithelial cells. Am J Physiol Cell Physiol. 2011; 300:C1298–1305. [PubMed: 21346155]

102. Ji C, Kosman DJ. Molecular mechanisms of non-transferrin-bound and transferring-bound iron uptake in primary hippocampal neurons. J Neurochem. 2015; 133:668–683. [PubMed: 25649872]

103. Terman A, Brunk UT. Lipofuscin. Int J Biochem Cell Biol. 2004; 36:1400–1404. [PubMed: 15147719]

104. Qian ZM, Wu XM, Fan M, Yang L, Du F, Yung WH, Ke Y. Divalent metal transporter 1 is a hypoxia-inducible gene. J Cell Physiol. 2011; 226:1596–1603. [PubMed: 20945371]

105. Csordas G, Hajnoczky G. SR/ER-mitochondrial local communication: calcium and ROS. Biochim Biophys Acta. 2009; 1787:1352–1362. [PubMed: 19527680]

106. Waring P. Redox active calcium ion channels and cell death. Arch Biochem Biophys. 2005; 434:33–42. [PubMed: 15629106]

107. Muller M, Cheung KH, Foskett JK. Enhanced ROS generation mediated by Alzheimer's disease presenilin regulation of InsP3R Ca2+ signaling. Antioxid Redox Signal. 2011; 14:1225–1235. [PubMed: 20701429]

108. Li Z, Ji G, Neugebauer V. Mitochondrial reactive oxygen species are activated by mGluR5 through IP3 and activate ERK and PKA to increase excitability of amygdala neurons and pain behavior. J Neurosci. 2011; 31:1114–1127. [PubMed: 21248136]

109. Ho HT, Liu B, Snyder JS, Lou Q, Brundage EA, Velez-Cortes F, Wang H, Ziolo MT, Anderson ME, Sen CK, Wehrens XH, Fedorov VV, Biesiadecki BJ, Hund TJ, Gyorke S. Ryanodine receptor phosphorylation by oxidized CaMKII contributes to the cardiotoxic effects of cardiac glycosides. Cardiovasc Res. 2014; 101:165–174. [PubMed: 24104877]

110. Peña KA, Kiselyov K. Transition metals activate TFEB in overpexpressing cells. Biochem J. 2015; 470:65–76. [PubMed: 26251447]

111. Kukic I, Kelleher SL, Kiselyov K. Zn2+ efflux through lysosomal exocytosis prevents Zn2+-induced toxicity. J Cell Sci. 2014; 127:3094–3103. [PubMed: 24829149]

112. Hawkins BJ, Madesh M, Kirkpatrick CJ, Fisher AB. Superoxide flux in endothelial cells via the chloride channel-3 mediates intracellular signaling. Mol Biol Cell. 2007; 18:2002–2012. [PubMed: 17360969]

113. Liu J, Zhang FF, Li L, Yang J, Liu J, Guan YY, Du YH. ClC-3 deficiency prevents apoptosis induced by angiotensin II in endothelial progenitor cells via inhibition of NADPH oxidase. Apoptosis. 2013; 18:1262–1273. [PubMed: 23873092]

114. Fisher AB. Redox signaling across cell membranes. Antioxid Redox Signal. 2009; 11:1349–1356. [PubMed: 19061438]

115. Dong XP, Cheng X, Mills E, Delling M, Wang F, Kurz T, Xu H. The type IV mucolipidosis-associated protein TRPML1 is an endolysosomal iron release channel. Nature. 2008; 455:992–996. [PubMed: 18794901]

116. Wang W, Zhang X, Gao Q, Xu H. TRPML1: an ion channel in the lysosome. Handb Exp Pharmacol. 2014; 222:631–645. [PubMed: 24756723]

117. Moos T, Skjoerringe T, Gosk S, Morgan EH. Brain capillary endothelial cells mediate iron transport into the brain by segregating iron from transferrin without the involvement of divalent metal transporter 1. J Neurochem. 2006; 98:1946–1958. [PubMed: 16879716]

118. Moos T, Morgan EH. The significance of the mutated divalent metal transporter (DMT1) on iron transport into the Belgrade rat brain. J Neurochem. 2004; 88:233–245. [PubMed: 14675167]

119. Burdo JR, Menzies SL, Simpson IA, Garrick LM, Garrick MD, Dolan KG, Haile DJ, Beard JL, Connor JR. Distribution of divalent metal transporter 1 and metal transport protein 1 in the normal and Belgrade rat. J Neurosci Res. 2001; 66:1198–1207. [PubMed: 11746453]

120. Cheng X, Zhang X, Gao Q, Ali Samie M, Azar M, Tsang WL, Dong L, Sahoo N, Li X, Zhuo Y, Garrity AG, Wang X, Ferrer M, Dowling J, Xu L, Han R, Xu H. The intracellular Ca(2)(+) channel MCOLN1 is required for sarcolemma repair to prevent muscular dystrophy. Nat Med. 2014; 20:1187–1192. [PubMed: 25216637]

121. Samie M, Wang X, Zhang X, Goschka A, Li X, Cheng X, Gregg E, Azar M, Zhuo Y, Garrity AG, Gao Q, Slaugenhaupt S, Pickel J, Zolov SN, Weisman LS, Lenk GM, Titus S, Bryant-Genevier M, Southall N, Juan M, Ferrer M, Xu H. A TRP channel in the lysosome regulates large particle phagocytosis via focal exocytosis. Dev Cell. 2013; 26:511–524. [PubMed: 23993788]

122. Laplante JM, Sun M, Falardeau J, Dai D, Brown EM, Slaugenhaupt SA, Vassilev PM. Lysosomal exocytosis is impaired in mucolipidosis type IV. Mol Genet Metab. 2006; 89:339–348. [PubMed: 16914343]

123. Kukic I, Lee JK, Coblentz J, Kelleher SL, Kiselyov K. Zinc–dependent lysosomal enlargement in TRPML1-deficient cells involves MTF-1 transcription factor and ZnT4 (Slc30a4) transporter. Biochem J. 2013; 451:155–163. [PubMed: 23368743]

124. Eichelsdoerfer JL, Evans JA, Slaugenhaupt SA, Cuajungco MP. Zinc dyshomeostasis is linked with the loss of mucolipidosis IV-associated TRPML1 ion channel. J Biol Chem. 2010; 285:34304–34308. [PubMed: 20864526]

125. Cuajungco MP, Basilio LC, Silva J, Hart T, Tringali J, Chen CC, Biel M, Grimm C. Cellular zinc levels are modulated by TRPML1-TMEM163 interaction. Traffic. 2014; 15:1247–1265. [PubMed: 25130899]

126. Weiss N. Cross-talk between TRPML1 channel, lipids and lysosomal storage diseases. Communicative & integrative biology. 2012; 5:111–113. [PubMed: 22808310]

127. Zhang X, Li X, Xu H. Phosphoinositide isoforms determine compartment-specific ion channel activity. Proc Natl Acad Sci U S A. 2012

128. Vucicevic L, Misirkic-Marjanovic M, Paunovic V, Kravic-Stevovic T, Martinovic T, Ciric D, Maric N, Petricevic S, Harhaji-Trajkovic L, Bumbasirevic V, Trajkovic V. Autophagy inhibition uncovers the neurotoxic action of the antipsychotic drug olanzapine. Autophagy. 2014; 10:2362–2378. [PubMed: 25551567]

129. Miao Y, Li G, Zhang X, Xu H, Abraham SN. A TRP Channel Senses Lysosome Neutralization by Pathogens to Trigger Their Expulsion. Cell. 2015; 161:1306–1319. [PubMed: 26027738]

130. Venkatachalam K, Wong CO, Zhu MX. The role of TRPMLs in endolysosomal trafficking and function. Cell Calcium. 2014

131. Curcio-Morelli CFC, Micsenyi MC, Cao Y, Venugopal B, Browning MF, Dobrenis K, Cotman SL, Walkley SU, Slaugenhaupt SA. Macroautophagy is defective in mucolipin-1-deficient mouse neurons. Neurobiol Dis. 2010; 40:370–377. [PubMed: 20600908]

132. Martina JA, Lelouvier B, Puertollano R. The calcium channel mucolipin-3 is a novel regulator of trafficking along the endosomal pathway. Traffic. 2009; 10:1143–1156. [PubMed: 19497048]

133. Vergarajauregui S, Connelly PS, Daniels MP, Puertollano R. Autophagic dysfunction in mucolipidosis type IV patients. Hum Mol Genet. 2008; 17:2723–2737. [PubMed: 18550655]

134. Saldanha, SA.; Grimm, C.; Mercer, BA.; Choi, JY.; Allais, C.; Roush, WR.; Heller, S.; Hodder, P. Probe Reports from the NIH Molecular Libraries Program. Bethesda (MD): 2010. Campaign to Identify Agonists of Transient Receptor Potential Channels 3 and 2 (TRPML3 & TRPML2).

135. Chen CC, Keller M, Hess M, Schiffmann R, Urban N, Wolfgardt A, Schaefer M, Bracher F, Biel M, Wahl-Schott C, Grimm C. A small molecule restores function to TRPML1 mutant isoforms responsible for mucolipidosis type IV. Nat Commun. 2014; 5:4681. [PubMed: 25119295]

136. Saldanha, SA.; Grimm, C.; Allais, C.; Smith, E.; Ouizem, S.; Mercer, BA.; Roush, WR.; Heller, S.; Hodder, P. Probe Reports from the NIH Molecular Libraries Program. Bethesda (MD): 2010. Identification of Selective Agonists of the Transient Receptor Potential Channels 3 (TRPML3).

137. Grimm C, Jors S, Saldanha SA, Obukhov AG, Pan B, Oshima K, Cuajungco MP, Chase P, Hodder P, Heller S. Small molecule activators of TRPML3. Chem Biol. 2010; 17:135–148. [PubMed: 20189104]

## Highlgihts

- Reactive oxygen species suppress the lysosomal exocytosis.

- Stimulation of the endo-lysosomal ion channels TRPML1 and TRPML3 partially the suppression.

- Oxidative stress damages cells and inhibits the repair by suppressing the lysosomal exocytosis.
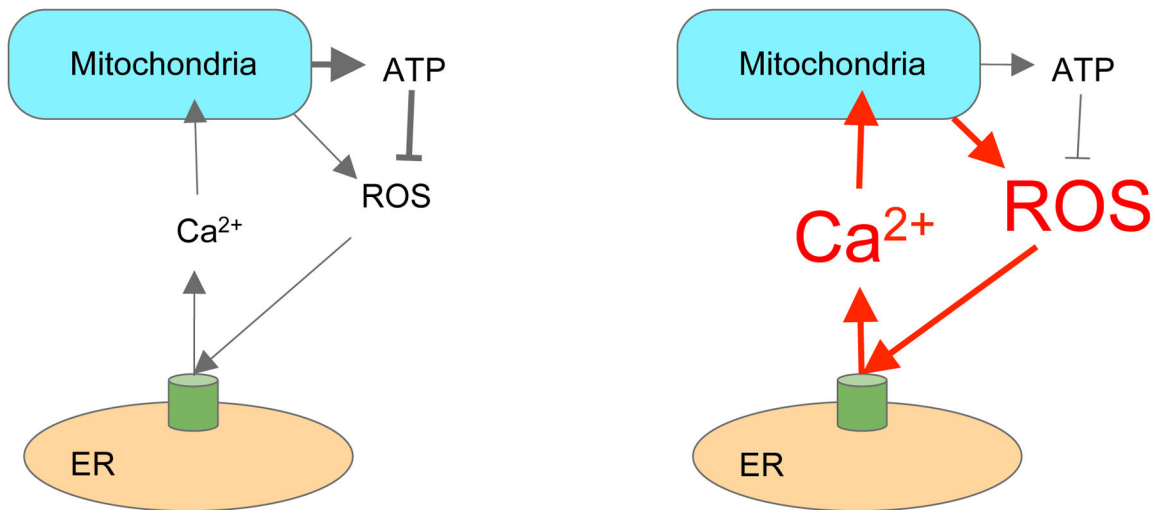
**Fig 1. The $Ca^{2+}$-release – mitochondria – ROS cycle**
Left: Within the physiological range, ROS signal to the ER facilitates $Ca^{2+}$ release, which stimulates ATP production in mitochondria. The ATP is used to handle pro-oxidants and counter the effects of ROS. Right: ROS overload stimulates $Ca^{2+}$ release, floods the mitochondria and promotes more ROS production, triggering a pathogenic cascade.
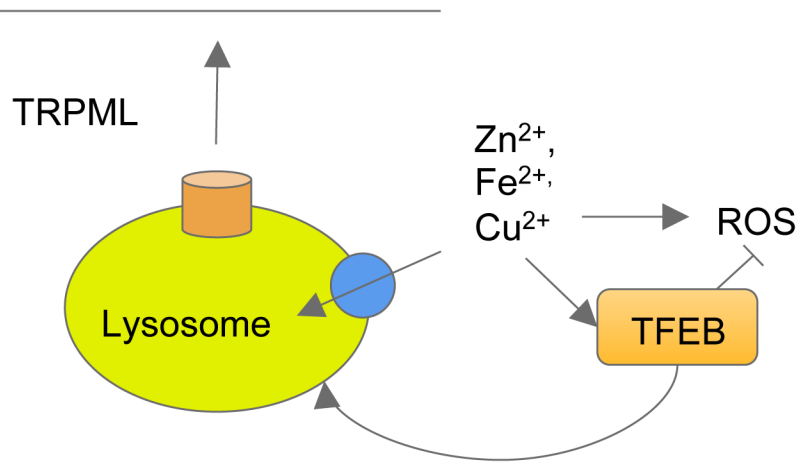
**Fig 2. The lysosomal metal sink**

The lysosomal metal transporters absorb $Zn^{2+}$, $Fe^{2+}$ and $Cu^{2+}$ from the cytoplasm into lysosomes, lowering their concentrations and the ROS production catalyzed by the metals. TFEB stimulation by transition metal enhances their clearance into the lysosomes. Furthermore, the enhanced lysosomes biogenesis counters the damage caused by oxidative stress.