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ITPRs/inositol 1,4,5-trisphosphate receptors in autophagy: From enemy to ally

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Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; ATP2A/SERCA, ATPase, Ca^{2+} transporting, cardiac muscle, fast twitch; BCL2, B-cell CLL/lymphoma 2; BECN1, Beclin 1, autophagy related; ER, endoplasmic reticulum; IP_3, inositol 1,4,5-trisphosphate; ITPR, inositol 1,4,5-trisphosphate receptor; LC3, microtubule-associated protein 1 light chain 3; TFEB, transcription factor EB; TGM2, transglutaminase 2; TMBIM6/BI-1, transmembrane BAX inhibitor motif containing 6; TPCN, 2 pore segment channel; WIPI1, WD-repeat domain, phosphoinositide interacting 1

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ITPRs (inositol 1,4,5-trisphosphate receptors), the main endoplasmic reticulum (ER) Ca^{2+}-release channels, were originally proposed as suppressors of autophagy. Yet, new evidence has accumulated over recent years supporting a crucial, stimulatory role for ITPRs in driving the autophagic flux. Here, we provide an integrated view on how ITPR-mediated Ca^{2+} signaling can have a dual impact on autophagy, depending on the characteristics of the spatio-temporal Ca^{2+} signals, including the existence of ER-mitochondrial and ER-lysosomal Ca^{2+} signaling microdomains.

During evolution, cells have gradually optimized their intracellular Ca^{2+}-signaling pathways into an intricate system of Ca^{2+} stores with intralumenal Ca^{2+}-buffering proteins, membrane-inserted Ca^{2+} pumps and membrane-release channels and cytosolic Ca^{2+}-dependent effectors, together constituting the Ca^{2+} signalosome.[1] The most important intracellular Ca^{2+} store in mammalian cells is the endoplasmic reticulum (ER), where the ubiquitously expressed ITPR (inositol 1,4,5-trisphosphate receptor) acts as the main intracellular Ca^{2+}-release channel.[2] Three isoforms (ITPR1, ITPR2 and ITPR3) contribute to the release of Ca^{2+} from the ER in response to inositol 1,4,5-trisphosphate (IP_3), which is produced at the plasma membrane upon exposure of cells to extracellular signals (e.g. ATP, hormones, antibodies, growth factors, neurotransmitters). In this manner, a variety of cellular processes, including cell death and survival, are regulated by ITPR-mediated Ca^{2+} signaling.[3] To control and regulate specific pathways or proteins, physiological Ca^{2+} signals are tightly but dynamically controlled in a spatiotemporal manner, often involving subcellular Ca^{2+} microdomains.[1,4]

ITPR-mediated Ca^{2+} signaling also influences autophagy. However, seemingly opposing concepts concerning the role of Ca^{2+} signaling and ITPRs in autophagy have been proposed, with evidence for intracellular Ca^{2+} signals activating as well as inhibiting the process.[5] ITPRs have been proposed as important negative regulators of autophagy since suppressing ITPR-mediated Ca^{2+} signaling by the depletion of IP_3, pharmacological inhibition using the selective ITPR inhibitor Xestospongin B, or the downregulation or knockout of ITPRs, results in an elevation of autophagy markers in vitro.[6–11] However, other findings indicate that ITPR-mediated Ca^{2+} signaling positively influences autophagic cell death in *Dictyostelium*,[12] whereas enhanced ITPR function is critical for driving canonical MTOR (mechanistic target of rapamycin [serine/threonine kinase])-dependent autophagy in mammalian cells exposed to nutrient starvation or rapamycin.[13,14]

Similar to ITPRs, intracellular Ca^{2+} signaling also appears to play a dual role in autophagy, leading to apparently contradictory results.[5] Increase of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) triggered by treatment of cells with extracellular agonist ATP, the ATP2A/SERCA inhibitor thapsigargin or Ca^{2+} ionophores such as ionomycin, induce an increase in LC3-II

levels and in the number of autophagosomes.[15] Such an increase in autophagic markers, however, does not necessarily imply the stimulation of autophagy, as it may represent the accumulation of autophagic vesicles due to an inhibition of the autophagic flux. Although Grotemeier et al. still observe a thapsigargin-mediated increase in LC3-II in Jurkat T cells, despite inhibition of the autophagic flux with lysosomal inhibitors,[16] other experiments using lysosomal inhibitors indicate that thapsigargin and Ca^{2+} ionophores rather inhibit the autophagic flux than stimulate autophagy,[17,18] thus thereby reducing the degradation of long-lived proteins.[19,20] This has both been linked to an effect of thapsigargin on autophagosome-lysosome fusion,[18] as well as to an impaired biogenesis of autophagosomes downstream of WIPI1-puncta formation.[20] Altogether, these results demonstrate that comparing autophagy in different conditions should be done with great care: treatment of the cells with either thapsigargin or ionophores leads to nonphysiological elevations in Ca^{2+} with amplitudes and spatio-temporal characteristics that are different from Ca^{2+} signals triggered by physiological agonists. Moreover, the nature and consequences of these Ca^{2+} signals are dependent on the applied concentrations of those Ca^{2+} mobilizers and the duration of the treatment. Finally, a similar Ca^{2+}-dependent inhibitory effect on autophagosome formation is proposed to occur downstream of the plasma membrane L-type Ca^{2+} channels.[17] Antagonists of the latter appear to induce autophagy by a mechanism involving cyclic adenosine monophosphate-dependent regulation of the IP_3 levels and calpain activation. Hence, inhibition of these Ca^{2+} signals by depleting cellular IP_3 levels with lithium chloride is proposed to activate autophagy and thereby to prevent protein aggregation in neurodegeneration.[11,17]

Different studies using pharmacological inhibitors or ITPR-knockdown approaches[6-10] also propose an inhibitory role for the ITPR and the IP_3-induced Ca^{2+} release with respect to autophagy, albeit via different mechanisms. Kroemer and coworkers propose a Ca^{2+}-independent scaffolding role for ITPRs by enhancing the formation of the anti-autophagic BCL2-BECN1/Beclin 1 complex.[7] Alternatively, Foskett and coworkers advocate the importance of ITPR-mediated Ca^{2+} oscillations that drive mitochondrial ATP production, thereby suppressing the activity of AMPK,[8] a positive regulator of autophagy.[21] As such, DT40 cells in which all 3 ITPR isoforms are genomically deleted display an increased AMPK activation and elevated basal autophagic flux.[8]

Although these studies indicate that ITPRs are able to inhibit basal autophagy levels, other studies reveal the requirement of ITPR-mediated Ca^{2+}-release during starvation-,[13] rapamycin-,[14] or natural killer cell[22]-induced autophagy in mammalian cells and during differentiation factor-induced autophagy in *Dictyostelium*.[12] The different outcomes and the proposed roles of the ITPR in autophagy are possibly due to a divergent role of the ITPRs with respect to basal vs. stress-induced autophagy. Indeed, our study shows that while ITPR inhibition by Xestospongin B stimulates the basal autophagic flux, it also abrogates the starvation-induced autophagic flux.[13] In line with the latter view, a recent report by Mikoshiba and coworkers, using the tandem red/green fluorescent protein reporter RFP-GFP-LC3 in HeLa cells reveals that knockdown of ITPR1 leads to an accumulation of autophagosomes.[23] Interestingly, the autophagosomes are not randomly located (as observed after treatment with bafilomycin A_1), but are restricted to the perinuclear space. Cells in which TGM2 (transglutaminase 2), an ITPR regulator, has been knocked down, show increased ITPR-mediated Ca^{2+} signaling and display mostly autolysosomes, similar to starvation-subjected cells, indicating enhanced autophagic clearance.[23] Although further independent confirmation will be needed, these first data support a concept in which ITPR-mediated Ca^{2+} release can enhance the trafficking of autophagosomes toward lysosomes, thereby promoting the autophagic flux. In any case, all these different reports strongly advocate the need for proper analysis of the autophagic flux when using Ca^{2+} mobilizers.

Another important aspect of the complex relation between Ca^{2+} signaling and autophagy, is the fact that the ER Ca^{2+} stores are remodeled during autophagy, and the functional properties of the ITPRs are modified by essential autophagy proteins.[13,14,24] These findings have implications for autophagy activity, because it has already been demonstrated that autophagy is dependent on the Ca^{2+} present in the intracellular Ca^{2+} stores rather than on the extracellular Ca^{2+}.[19] Nutrient starvation leads to an overall sensitization of Ca^{2+}-release events from the ER, by increasing the ER Ca^{2+}-store content and by promoting IP_3-induced Ca^{2+} release.[13] The former is linked to an increased ER Ca^{2+}-buffering capacity due to an upregulation of ER lumenal Ca^{2+}-binding proteins concomitant with a decreased passive Ca^{2+} leak from the ER, whereas the latter is linked to a direct interaction of BECN1 with the ITPR, thereby sensitizing the channel toward lower IP_3 concentration. Due to their mechanism of action, the use of compounds such as thapsigargin or Ca^{2+} ionophores will eliminate the functional consequences of these fine-tuned alterations in ER Ca^{2+} and ITPR function that are critical to drive the autophagic flux. It is interesting to note that autophagy-deficient T cells lacking ATG7 (autophagy related 7) expand their ER Ca^{2+} stores and increase ER Ca^{2+} levels by upregulating ATP2A, which may serve as a compensatory mechanism in an attempt to restore autophagic flux.[24]

Hence, there is clear evidence that Ca^{2+} and ITPRs are able to both stimulate and suppress autophagosome synthesis as well as to both enhance and inhibit the autophagic flux. ITPRs and Ca^{2+} can execute such opposing functions due to the different spatio-temporal characteristics of Ca^{2+} signals that can be generated, each having distinct impacts on different steps in the autophagy pathway (**Fig. 1**). Ca^{2+} signals can vary in the cellular space: large Ca^{2+} waves can spread out over the entire cell, while local Ca^{2+} signals, including basal Ca^{2+} oscillations, can act in a specific cellular microdomain. The probably best known example for this phenomenon is the Ca^{2+} transfer between ER and mitochondria with specific proteins regulating contact-site formation and efficient Ca^{2+} signaling between these 2 organelles.[25,26] This is in part achieved by the chaperone HSPA9/GRP75 (heat shock 70kDa

protein 9 [mortalin]), which physically links ITPRs to VDAC1 (voltage-dependent anion channel 1), the Ca^{2+}-entry channel located at the mitochondrial outer membranes.[27] These contact sites are most likely responsible for the ITPR-dependent Ca^{2+}-induced fueling of mitochondrial ATP production and the subsequent suppression of AMPK and autophagy,[8,26] as well as for triggering cell death by eliciting mitochondrial Ca^{2+} overload under specific conditions, and mitophagy by disturbing mitochondrial Ca^{2+} signaling.[26] Furthermore, it should be highlighted that changes in overall ER Ca^{2+} homeostasis can have very local effects. For instance, lowering the steady-state ER Ca^{2+} levels will limit the ITPR-driven Ca^{2+} oscillations and the local transfer of Ca^{2+} into the mitochondria, thereby compromising mitochondrial ATP production. This mechanism has been proposed to explain the role of TMBIM6/BI-1, an evolutionarily conserved cell-death suppressor[28] that acts as an ER Ca^{2+}-leak channel,[29,30] a sensitizer of ITPRs,[31] and as a positive regulator of autophagy.[32] Other possible space-restricted Ca^{2+} signals that regulate autophagy include local ITPR-mediated Ca^{2+} signals altering both phosphatidylinositol 3-phosphate-rich omegasome formation at the ER membranes *via* CAMK1 (calcium/calmodulin-dependent protein kinase 1)[33] and accumulation of the phosphatidylinositol 3-phosphate-binding protein WIPI1.[16] Downstream of WIPI1, the thapsigargin-induced impairment of autophagosome biogenesis is shown to be independent of bulk $[Ca^{2+}]_{cyt}$ changes, suggesting local Ca^{2+} variations account for this effect of thapsigargin.[20] Moreover, lysosomes have recently emerged as novel Ca^{2+} stores that generate Ca^{2+} signals and that functionally interact with the ER Ca^{2+}-handling mechanisms in a bidirectional way.[34–36] Close association of lysosomes with the ER enables rapid exchange of Ca^{2+} between these organelles, allows the ITPRs to influence the lysosomal Ca^{2+} concentration and subsequently Ca^{2+} release through lysosomal nicotinic acid adenine dinucleotide phosphate (NAADP)-dependent 2 pore segment channels (TPCNs), whereas NAADP-dependent Ca^{2+} release can stimulate ITPRs via Ca^{2+}-induced Ca^{2+} release. Interestingly, activation of TPCN-mediated Ca^{2+}-signaling inhibits autophagosome-lysosome fusion events by alkalinizing lysosomal pH through an unknown mechanism.[37] Underscoring the importance of lysosomal Ca^{2+} in autophagy, a very recent report demonstrates that nutrient starvation promotes Ca^{2+} release from the lysosomes through the Ca^{2+} channel MCOLN1/TRPML1 (mucolipin 1).[38] This Ca^{2+} results in the activation of the protein phosphatase PPP3/calcineurin (protein phosphatase 3) in a microdomain around the lysosomes, and the subsequent

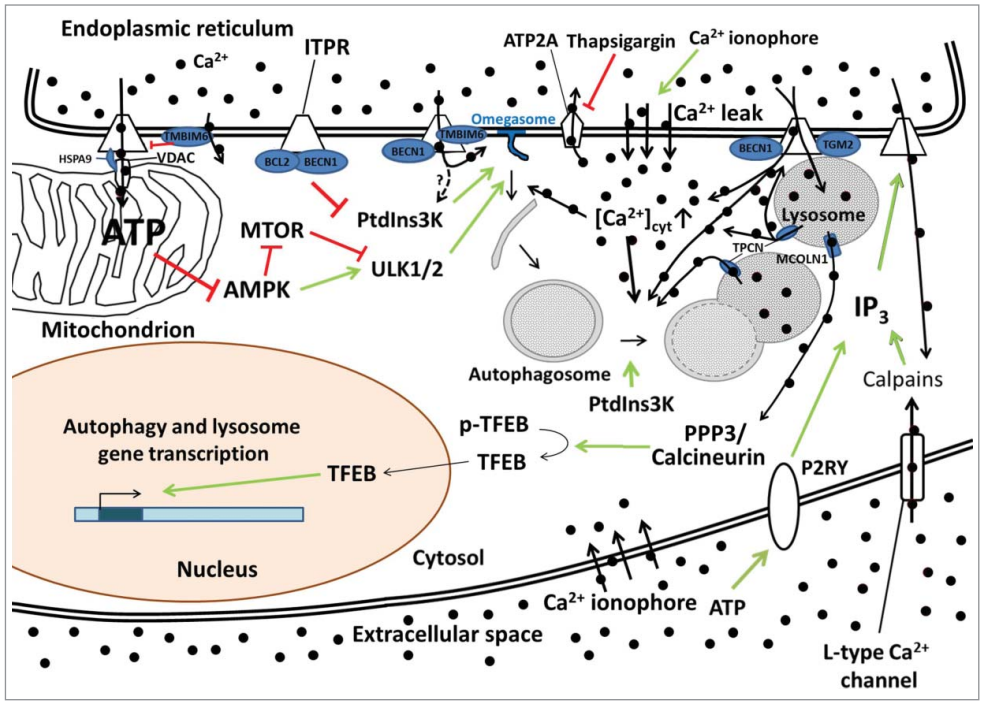

Figure 1. The various possible mechanisms of Ca^{2+}-ITPR-mediated control of autophagy. Constitutive ITPR-mediated Ca^{2+} release into mitochondria inhibits a proximal step in the autophagy pathway by fueling mitochondrial energetics and ATP production and limiting AMPK activity. The ER Ca^{2+}-leak channel TMBIM6 can impede ATP production by lowering the steady-state ER Ca^{2+} concentration and thus reduce the amount of Ca^{2+} available for transfer into the mitochondria. ITPRs can also function as scaffolding molecules, thereby suppressing autophagy independently of their Ca^{2+}-release activity by promoting the interaction of BCL2 with BECN1 and thus preventing the formation of the active class III phosphatidylinositol 3-kinase (PtdIns3K) complex. ITPR-mediated Ca^{2+} release can also be enhanced by BECN1 and TMBIM6 and dampened by TGM2, thereby influencing omegasome formation (possibly through PtdIns3K activation) and autophagosome maturation/trafficking. ITPR-mediated Ca^{2+} release can also influence the lysosomal Ca^{2+} concentration and lysosomal Ca^{2+} release through TPCNs, likely influencing lysosomal fusion events, or through MCOLN1, influencing autophagic and lysosomal gene transcription through a pathway involving PPP3/calcineurin and TFEB. TPCNs reciprocally also influence ITPRs via a Ca^{2+}-induced Ca^{2+}-release mechanism. Autophagosome synthesis, maturation and fusion are also affected by Ca^{2+}-mobilizing agents such as thapsigargin (that inhibits the ER Ca^{2+} pump ATP2A) and Ca^{2+} ionophores that increase the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$). IP_3 production and the subsequent IP_3-mediated Ca^{2+} release can also be regulated by a feedback loop involving calpain activation by L-type Ca^{2+} channel-mediated Ca^{2+} entry or the activation of P2RY (purinergic receptor, G-protein coupled). The black circles represent Ca^{2+} ions, with thick black arrows indicating the direction of the Ca^{2+} fluxes. Green arrows indicate stimulatory effects, red lines inhibitory ones.

dephosphorylation of TFEB, a major transcription factor coordinating lysosomal biogenesis. Dephosphorylated TFEB accumulates in the nucleus, promoting the transcription of genes involved in autophagy and the production of lysosomes.[38] Finally, Ca^{2+} signals from the ER or lysosomes could influence fusion events more directly, since autophagosome maturation is regulated by the Ca^{2+}-binding proteins ANXA1/annexin A1 and ANXA5.[39]

From all these studies, it is clear that there is an intimate interplay between autophagy and Ca^{2+} signaling from the ER, including via the ITPR channel, likely involving a tight control of the frequency and amplitude of Ca^{2+} signals in space and time. Furthermore, ITPRs and Ca^{2+} signaling not only affect autophagy, but reciprocally ITPRs and Ca^{2+} signaling are modulated by the autophagy process in general and by essential autophagy proteins in particular. Hence, considering the complex interrelation between ITPRs and Ca^{2+} signaling in autophagy, it can be questioned whether the direct pharmacological targeting of these Ca^{2+}-release channels holds potential as a future therapy in autophagy-dependent diseases. However, interesting possibilities lay within the fine-tuning of the Ca^{2+}-flux properties of the channels such as the ITPRs by affecting its dynamic regulation via associated proteins, as has been successfully done with respect to associated anti-apoptotic BCL2 proteins.[40,41] For example, BECN1 is recruited by ITPRs during starvation-induced autophagy and sensitizes the ITPRs to low levels of IP_3 (**Fig. 1**).[13] In contrast, TGM2, a protein that induces protein crosslinking, counteracts enhanced ITPR-mediated Ca^{2+} signaling during autophagy stimulation.[23] Modulating regulatory proteins acting on the ITPRs could thus fine-tune the ITPR-mediated Ca^{2+} signals in cells undergoing autophagy, thereby enhancing or reducing the autophagic flux as appropriate. For example, an increase in covalent posttranslational modifications of ITPR1 mediated by TGM2, resulting in a dampened ITPR1 activity, is already found in animal Huntington disease models and in primary B lymphocytes obtained from Huntington patients.[23] Limiting TGM2 activity could in those conditions enhance the ITPR-mediated Ca^{2+} release, stimulate the autophagy pathway and thus increase the autophagy-mediated degradation of mutant HTT (huntingtin). The advantage of such an approach will be that it may not lead to general elevations in cytosolic Ca^{2+} concentration, which has been linked to autophagy inhibition and an impaired clearance of aggregate-prone proteins in neurodegenerative diseases.[17]

In conclusion, identifying the molecular determinants underlying the formation of multiprotein complexes between the ITPRs and associated regulatory proteins may thus provide new therapeutic avenues to modulate autophagy in the context of human pathologies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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