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TORC2-SGK-1 signaling integrates external signals to regulate autophagic turnover of mitochondria via mtROS

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ABSTRACT

Macroautophagy/autophagy is an evolutionarily conserved cellular degradation and recycling process that is tightly regulated by external stimuli, diet, and stress. Our recent findings suggest that in *C. elegans*, a nutrient sensing pathway mediated by MTORC2 (mechanistic target of rapamycin kinase complex 2) and its downstream effector kinase SGK-1 (serum- and glucocorticoid-inducible kinase homolog 1) suppresses autophagy, involving mitophagy. Induced autophagy/mitophagy in MTORC2-deficient animals slows down development and impairs reproduction independently of the SGK-1 effectors DAF-16/FOXO and SKN-1/NFE2L2/NRF2. In this punctum, we discuss how TORC2-SGK-1 signaling might regulate autophagic turnover and its impact on mitochondrial homeostasis via linking mitochondria-derived reactive oxygen species (mtROS) production to mitophagic turnover.

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Changes in environmental conditions (stress) and aging may induce autophagy to maintain cellular homeostasis under unfavorable conditions. MTOR (mechanistic target of rapamycin kinase) and INS (insulin)-IGF (insulin like growth factor) are two of the prominent signaling pathways sensing shifts in nutrient availability, and function as critical autophagic regulators. Both pathways crosstalk with one another, however, many details of such crosstalks are still unknown. The transcription factor FOXO is the main effector of INS-IGF signaling, and its activity promotes autophagy induction. The kinase MTOR forms the enzymatic core of two distinct, but highly conserved protein complexes, MTORC1 and MTORC2. MTORC1 is a well-studied, potent repressor of predominantly autophagosome formation. The role of MTORC2 in autophagy is highly controversial, and may mediate either activating or repressing effects on autophagy, the mechanisms of which have remained elusive.

In *C. elegans*, we previously identified SGK-1 (serum- and glucocorticoid-inducible kinase homolog 1) as a prominent component of INS-IGF signaling that acts downstream of PDK-1 and in parallel to AKT-1 and AKT-2 (Figure 1). SGK-1 also integrates MTOR signaling downstream of RICT-1/RICTOR. SGK-1 regulates DAF-16/FOXO, lipid metabolism, and phase II ROS detoxification (by phosphorylating SKN-1/NFE2L2). SGK-1 activity is apparently modulated by external stimuli such as growth temperature and nutrients to either positively or negatively affect lifespan and other physiological responses.

Using reporters to monitor autophagy, we found that *rict-1* and *sgk-1* deficiency results in a strong increase of autophagy in different tissues, suggesting that MTORC2, like MTORC1, counteracts autophagy in *C. elegans* [1]. This observation was confirmed by knocking down additional components of MTORC2. Surprisingly, autophagy induction is independent of a prominent SGK-1 target, the FOXO transcription factor DAF-16. *daf-16* mutants, although fully suppressing the autophagy induction resulting from loss of *akt-1;akt-2* that act in parallel to *sgk-1* in the INS-IGF pathway, do not have an impact on *rict-1(-/-)*-induced autophagy.

Because our previous experiments had suggested that mutant *sgk-1* animals have moderately increased unfolded protein responses of the mitochondria (mtUPR) that might induce mitophagy, we next tested whether mitophagy was responsible for increasing autophagosomes in MTORC2-deficient animals. Fluorescent marker screens indeed suggested an increased sequestration of mitochondria that resulted in delivery to acidic autolysosomes.

Targeting defective mitochondria to the autophagosome requires the mitochondrial outer membrane interactors of LGG-1/LC3, DCT-1/BNIP3/NIX, PINK-1 and PDR-1/PRKN/Parkin. Mutants of each of these genes reduce puncta in *rict-1*- and *sgk*-1-deficient animals, implying that mutant MTORC2 indeed increases mitophagy. Both *sgk-1* and *rict-1* mutants display developmental retardation and low brood sizes. We found that these phenotypic aspects can partially be suppressed by inhibiting mitophagy.

Do *sgk-1* and *rict*-1 animals suffer from perturbed mitochondrial homeostasis that, in addition to activating the mtUPR, induces mitophagy? Apparently this is the case, because these mutants exhibit altered oxygen consumption rates and a strong reduction in TMRE staining, a dye that labels

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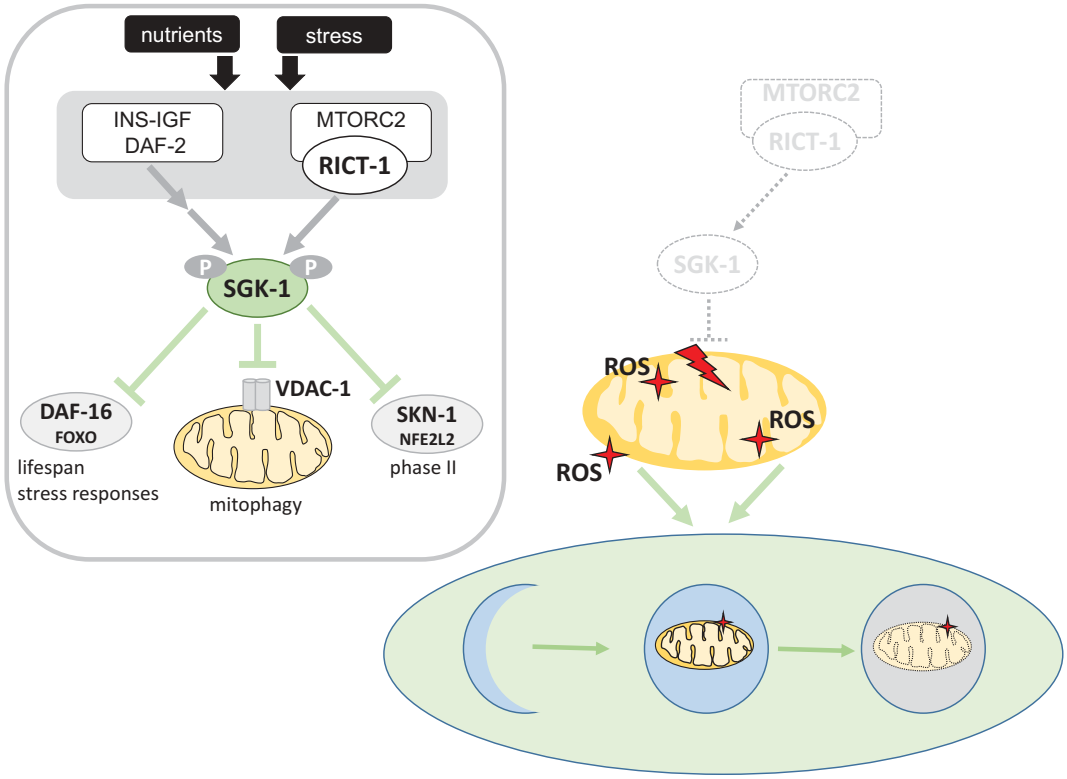

Figure 1. Loss of *sgk-1* or *rict-1* induces mitochondrial ROS, resulting in mitophagy. Insert: Simplified model of the regulatory network of SGK-1. SGK-1 phosphorylation and activity is modulated by nutrients and stress via INS-IGF signaling (via the receptor DAF-2) and the MTORC2/RICT-1 complex. SGK-1 has also been proposed to be phospho-regulated by temperature (not shown). SGK-1 negatively controls the transcription factors DAF-16 and SKN-1 involved in lifespan and stress responses. A number of SGK-1 substrates have been proposed, but only one candidate target, the mitochondrial outer membrane protein VDAC-1, is displayed.

active mitochondria. Therefore, increased mitochondrial depolarization, low membrane potential ($\Delta\Psi_m$) and an aberrantly increased mitophagy might contribute to the reproductive and developmental phenotype of MTORC2-deficient animals.

A low $\Delta\Psi_m$, conversely, should result in reduced ROS generation as a byproduct. However, MTORC2 mutants show greatly enhanced mitochondrial ROS levels that might diffuse to the cytoplasm to trigger autophagy. Indeed, *sgk-1* and *rict-1* deficiency induce cytosolic ROS. Scavanging by NAC reduces the number of autophagic puncta and also alleviates developmental defects in MTORC2 mutants. Thus, increased ROS levels induce mito/autophagy in MTORC2-deficient animals. Surprisingly, this induction does not depend on SKN-1/NFE2L2, the critical regulator of the phase II detoxification of cytosolic ROS that we and others have shown previously to be activated upon *sgk-1* downregulation. Instead, it requires HIF-1 (hypoxia inducible factor), CEP-1/TP53/p53, and AMPK, all of which have been previously implicated in autophagy regulation. In summary, these data suggest that MTORC2 might steer a novel transcriptional program that does not rely on DAF-16/FOXO or SKN-1/NFE2L2.

What are the candidate phosphorylation targets of altered RICT-1 and SGK-1 activity? In unpublished experiments, we had identified a short list of candidates, among them VDAC-1, a mitochondrial outer membrane protein proposed to function in the mPTP complex. VDAC-1 was recently confirmed by the Soukas lab as a substrate of SGK-1, suggesting that SGK-1 negatively regulates VDAC-1 by promoting its turnover. According to this model, loss of MTORC2 activity might therefore result in an uncontrolled opening of the mitochondrial permeability transition pore. How this could activate mitophagy is still not known, but we suggest that under low MTORC2 signaling, mtROS might couple mitochondrial perturbations to autophagy induction.

There are a number of unsolved questions in the mysteries around *sgk-1* and *rict-1*. Both our results and that of the Soukas lab suggest that MTORC2 deficiency decreases the mitochondrial membrane potential ($\Delta\Psi_m$), whereas other studies had suggested an increase in $\Delta\Psi_m$ in RICTOR mutants in human cancer cells and mouse keratinocytes. However, as indicated above, loss of *rict-1* and *sgk-1* may have adverse phenotypic consequences already in *C. elegans*, depending on alterations of external stimuli such as food source and temperature. Nevertheless, our experiments show that altered mitochondrial redox signaling plays a key role in autophagy regulation downstream of MTORC2, suggesting a mitohormetic function of ROS that differentially affects mitochondrial function and signaling during stress versus non-stress conditions.

Increasing autophagy by a number of measures are currently being proposed for treatment of many diseases, including cancer and neurodegeneration. MTORC2 inhibitors have already been discussed for the treatment of solid cancers, myelomas and lymphomas. In the light of malicious autophagy induction reported here, these should be considered with caution!

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Disclosure statement

No potential conflict of interests was reported by the authors.

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