Mitophagy, mitochondrial dynamics and the general stress response in yeast

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Abstract

Autophagy is a fundamental cellular process promoting survival under various environmental stress conditions. Selective types of autophagy have gained much interest recently as they are involved in specific quality control mechanisms removing, for example, aggregated proteins or dysfunctional mitochondria. This is considered to counteract the development of a number of neurodegenerative disorders and aging. Here we review the role of mitophagy and mitochondrial dynamics in ensuring quality control of mitochondria. In particular, we provide possible explanations why mitophagy in yeast, in contrast with the situation in mammals, was found to be independent of mitochondrial fission. We further discuss recent findings linking these processes to nutrient sensing pathways and the general stress response in yeast. In particular, we propose a model for how the stress response protein Whi2 and the Ras/PKA (protein kinase A) signalling pathway are possibly linked and thereby regulate mitophagy.

Quality control of mitochondria by selective autophagy

Autophagy is a highly conserved catabolic process in eukaryotic cells. It mediates the transport of proteins, lipids and even entire organelles into the vacuole/lysosome for hydrolytic degradation. Thus it allows the cell to adapt to changing nutrient conditions and to respond to different types of stress. Based on morphological characteristics two types of autophagy can be distinguished: macro- and micro-autophagy. During macroautophagy cytoplasmic components are sequestered via double-membrane compartments, termed autophagosomes, which then fuse with the vacuole/lysosome. Microautophagy involves the direct uptake of the components by the vacuolar/lysosomal boundary membrane [1,2]. Autophagy can be bulk, that is, non-specific, but also selective types of autophagy have been reported, among them the Cvt (cytoplasm-to-vacuole targeting) pathway, pexophagy or mitophagy [3–5]. Studies in yeast and other fungi have identified 35 autophagy-related (*ATG*) genes so far. Most of them are required for both selective and non-selective autophagy, but some, such as Atg11, Atg20 and Atg24, are specifically required for selective types of autophagy [6,7].

The selective degradation of mitochondria via autophagy is termed mitophagy. The mechanism and regulation of this process are only poorly understood. Two groups in parallel performed a genomic screening for yeast mutants defective in mitophagy using a library of non-essential deletion strains [8,9]. They identified approximately 40 genes involved in diverse pathways, such as membrane trafficking, protein modification/degradation, lipid metabolism or mitochondrial metabolism. Atg32 was found, in both screenings, to promote mitophagy. It is not required for bulk autophagy or other types of selective autophagy. Atg32 has been proposed to act as a mitochondrial receptor that during mitophagy interacts with Atg11, a known adaptor protein for selective types of autophagy, and thereby recruit the autophagy machinery to mitochondria [9,10]. In mammalian cells, PINK1 and parkin, both linked to Parkinsons's disease, are involved in targeting depolarized mitochondria to the autophagosome [11]. The mitochondrial kinase PINK1 recruits the E3 ubiquitin ligase parkin to the mitochondrial outer membrane, mediating ubiquitination of mitochondrial proteins such as VDAC (voltage-dependent anion channel) or mitofusin [12,13]. During terminal erythrocyte differentiation, NIX has been identified as a selective receptor for mitophagy interacting with LC3 proteins, essential components for autophagosome formation [14].

Mitochondria form a highly dynamic network constantly undergoing fusion and fission events [15,16]. Several neurodegenerative diseases such as Parkinson's disease or autosomal dominant optic atrophy type I are associated with alterations in mitochondrial dynamics [17]. It has been proposed that fusion and fission promote mitochondrial quality control [18]. On the one hand they allow mitochondrial content mixing and thereby contribute to the integrity and homogeneity of the mitochondrial network. In addition, mitochondrial dynamics could isolate dysfunctional mitochondria from the intact network, helping to target them for autophagic degradation. Indeed, in mammalian cells mitophagy is impaired when mitochondrial fission is blocked and dysfunctional mitochondria accumulate [19]. However, in yeast the role of mitochondrial dynamics in mitophagy and mitochondrial quality control has been debated over the last few years.

Key words: autophagy, general stress response, mitochondrial dynamics, mitophagy, Whi2, yeast.

Abbreviations used: Cvt, cytoplasm-to-vacuole targeting; PKA, protein kinase A; STRE, stress-responsive element; TOR, target of rapamycin.

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The function and morphology of mitochondria are linked

It has been proposed that mitochondrial dynamics and morphology are linked to the bioenergetic state of mitochondria; but how this is regulated on a molecular level remained unclear for quite some time. The dynamin-like GTPases Mgm1 (in yeast) and OPA1 (in mammals) were found to be key players in this process. Both are essential for mitochondrial fusion in the respective organisms [20,21]. Mgm1 exists in two isoforms, which are present in roughly equal amounts and which are localized in the inner membrane/intermembrane space [22,23]. The short isoform (s-Mgm1) is generated by limited intramembrane proteolysis by the rhomboid protease Pcp1, whereas the large isoform (l-Mgm1) is only processed by matrix processing peptidase, removing the N-terminal mitochondrial targeting sequence [23,24]. Interestingly, both isoforms have to be present to allow respiratory growth and to maintain mitochondrial DNA and mitochondrial morphology [23,25]. The formation of s-Mgm1 was shown to depend on a functional protein import machinery and on the ATP level in the matrix. Reduced ATP level led to a decreased level of s-Mgm1, increased levels of l-Mgm1 and to fragmentation of mitochondria *in vivo* [26]. A model for alternative topogenesis of Mgm1 was proposed linking the bioenergetic state of mitochondria to the ratio of the two Mgm1 isoforms and consequently to mitochondrial morphology.

Similarly, in mammalian cells different isoforms of OPA1 regulate mitochondrial dynamics in response to bioenergetics. The OPA1 protein exists in at least five mitochondrial isoforms. Mitochondrial dysfunction, for example, induced by dissipation of the membrane potential, leads to an increased proteolytic processing of the large OPA1 isoforms into the small OPA1 isoforms and subsequently to mitochondrial fragmentation [27,28]. Taken together, alternative topogenesis of Mgm1 in yeast and stress-induced proteolytic processing of OPA1 in mammals provide a molecular mechanism to specifically inhibit fusion competence when mitochondrial function is impaired. This would help us to distinguish functional from dysfunctional mitochondria based on their morphology. On the one hand this would lead to a spatial separation of damaged mitochondria from the intact network to minimize further damage. On the other hand it could enable the removal of these isolated mitochondria presumably by mitophagy. Indeed, mitophagy was shown to be increased in various systems of mitochondrial dysfunction in mammals and in yeast [19,29–31].

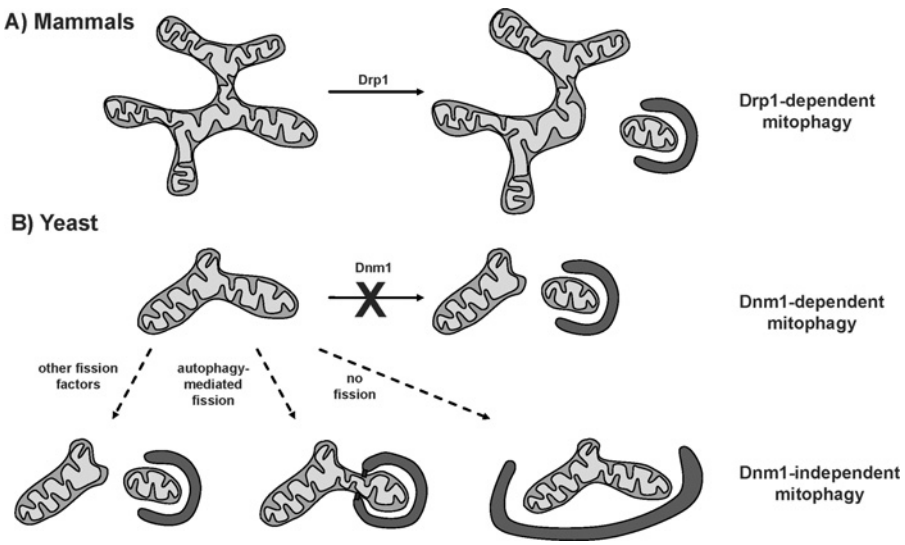
Mitophagy in yeast is independent of mitochondrial fission

In case segregation of dysfunctional mitochondria from the remaining intact network would target them for degradation, mitochondrial fission is predicted to be a prerequisite for mitophagy. Indeed, Twig et al. [19] could show that in mammalian cells Drp1-mediated fission is required for the degradation of mitochondria and that blocking of autophagy led to accumulation of oxidized mitochondrial proteins [19]. In yeast, conflicting results on the role of fission in mitophagy have been reported. In particular the involvement of the fission-promoting dynamin-like GTPase Dnm1, the orthologue of mammalian Drp1, remains under debate. In a genomic screening, Δ*dnm1* was found to show reduced levels of mitophagy, whereas the deletion of the other known fission factors Fis1, Mdv1 and Caf4 did not show such impairment [8]. Induction of mitophagy as observed on loss of Mdm38 was prevented by deletion of *DNM1*, further supporting the idea of the requirement for fission [30]. However, a second screening did not identify a role of Dnm1 or the other fission factors in mitophagy [9]. Recently, we investigated the effect of altered mitochondrial dynamics on mitophagy in detail, applying enzymatic, biochemical and fluorescence-based assays [32]. This study demonstrated that fragmentation of mitochondria alone was not sufficient to trigger mitophagy. Furthermore, drug-induced inhibition of oxidative phosphorylation also did not induce mitophagy. When mitochondrial fission was blocked by expressing a dominant-negative variant of Dnm1, the level of mitophagy induced by rapamycin was not altered compared with the wild-type control. In addition, strains lacking one of the fission factors Dnm1, Mdv1 or Caf4 did not show an impairment of mitophagy on rapamycin treatment, suggesting that fission is not essential for mitochondrial degradation. However, mitophagy and (to a lesser extent) also autophagy were reduced in the strain deleted for the fission factor Fis1. How can one explain this observation? Does Fis1 have a second, fission-independent role in mitophagy or does the Δ*fis1* strain contain a secondary suppressor mutation in a gene required for mitophagy? Indeed, a secondary point mutation in the *WHI2* locus introducing a premature stop codon could be identified in the Δ*fis1* strain used in this study, consistent with an earlier report [33]. Moreover, rapamycin-induced mitophagy was reduced in a Δ*whi2* strain resembling the effect in the Δ*fis1* strain containing the secondary *whi2* mutation. Expression of Whi2 but not Fis1 could complement the mitophagy- and autophagy-deficient phenotype of Δ*fis1*, showing that the reduced level of mitophagy in the Δ*fis1* strain was actually caused by the secondary loss-of-function mutation in *WHI2*. Taken together, several lines of evidence show that mitophagy is truly independent of mitochondrial fission in yeast. Furthermore, we identified Whi2 as a novel mitophagy-promoting factor [32].

These results differ from findings in mammalian cells and throw into question the hypothesis of mitochondrial dynamics as quality control checkpoints (Figure 1). However, it cannot be ruled out that basal levels of mitophagy depend on mitochondrial fission and might be too little to be detected by the assays. Additional triggers might be needed in addition to fragmentation for inducing fission-dependent mitophagy. Furthermore, an alternative, Dnm1-independent fission mechanism might exist that is able to mediate the spatial separation of individual mitochondria for degradation. Thus

Figure 1 | The interplay of mitochondrial dynamics and mitophagy in mammals and yeast

(**A**) In mammalian cells, Drp1-mediated fission is required for the selective removal of mitochondria. (**B**) In yeast, mitochondrial fission mediated by the Drp1 orthologue Dnm1 is not a prerequisite for mitophagy. Thus mitophagy appears to occur in a Dnm1-independent manner. Still, the following alternatives, possibly depending on the conditions applied, are not excluded yet: other unknown fission factors mediate the spatial separation of individual mitochondria. Mitochondrial fission is conducted during autophagosome formation by the autophagy machinery itself. Another possibility is that indeed no fission event is required because the mitochondrial networks present in yeast are small enough to be enclosed by autophagosomes.



it cannot be fully excluded that mitochondrial morphology is important for the selection of dysfunctional mitochondria or that it is involved in signalling of mitochondrial dysfunction to induce mitophagy in yeast. But how can the degradation of mitochondria occur without a preceding fission event? One possibility is that mitochondrial fission is mediated by an unknown mechanism or the autophagy machinery itself. It is also conceivable that the mitochondrial network in yeast creates tubules that are small enough to be entirely surrounded by the autophagosomes in contrast with the larger mammalian network. The different possibilities are summarized in Figure 1(B).

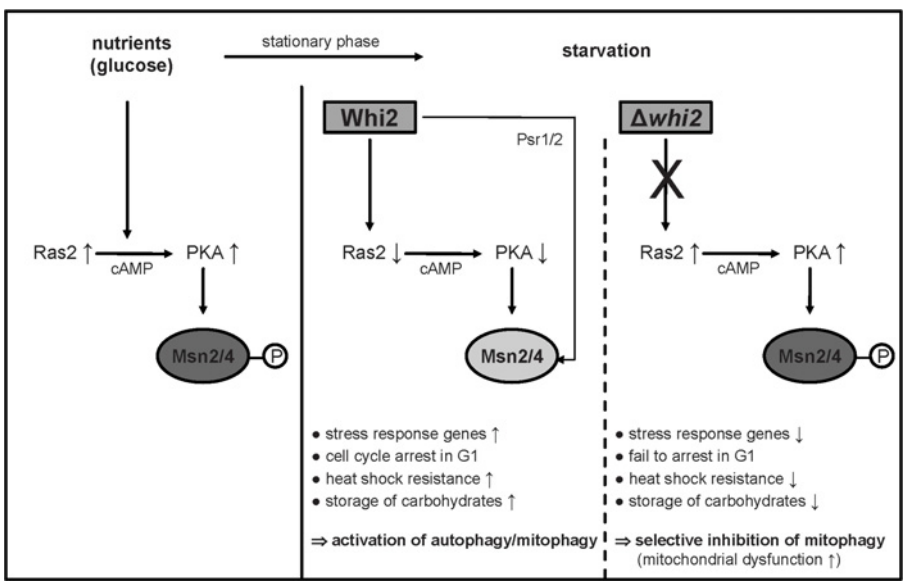
Whi2 links mitophagy to the general stress response

The level of mitophagy was shown to be reduced by ~40 % in cells deleted for *WHI2* compared with the wild-type control after treatment with rapamycin [32]. In contrast, autophagy was only slightly inhibited in Δ*whi2* cells under the same conditions. These observations point to a specific role for Whi2 in mitophagy. Furthermore, the Cvt pathway seems to be unaffected in strains lacking Whi2, suggesting no general function of this factor in other selective types of autophagy. Interestingly, overexpression of Whi2 seems to be able to positively modulate autophagic flux.

To date, not much is known about this novel mitophagy-promoting protein. Still, a few studies carried out over the last 30 years indicate that the fungi-specific protein Whi2 links proliferation and stress response to environmental sensing mechanisms. Sudbery et al. [34] isolated the *whi2* mutant and found that this gene is involved in cell cycle control. The Δ*whi2* cells continue proliferation during stationary phase and fail to arrest in the G_1-phase of the cell cycle. They retain the properties of exponentially growing cells and their arrest is randomly distributed in the cell cycle. Consequently, the cells become abnormally small and are predominantly budded compared with wild-type cells in stationary phase. Moreover, these cells exhibited a lower viability, failed to accumulate storage carbohydrates and became less resistant to environmental stresses such as heat shock [35,36]. *WHI2* transcriptional levels were shown to be increased in the fermentative phase of growth (glucose) and to be drastically reduced in non-fermentative, aerobic growth conditions (ethanol) similar to the transcription levels of genes required for glycolysis [37]. However, the expression of Whi2 is sensitive to the growth rate rather than to catabolite repression. Moreover, Whi2 seems to be a negative regulator of catabolite repressible functions. Thus cells deleted for Whi2 respired more actively in the presence of glucose and grew more rapidly on glycerol [38]. The mRNA levels of the G_1 cyclins *CLN1* and *CLN2* were found to be increased in the Δ*whi2* strain during exponential phase and to persist longer at high levels during stationary phase than in the wild-type cells. Otherwise the overexpression of *CLN1* in stationary phase wild-type cells resulted in a Δ*whi2*-like phenotype, confirming that Whi2 negatively regulates G_1 cyclin expression [39]. Interestingly, Radcliffe et al. [40] revealed that

Figure 2 | Whi2 links cell cycle regulation, stress response and signalling pathways to mitophagy in yeast

During exponential growth under nutrient-rich conditions the Ras/PKA pathway is activated and transcription of STRE-controlled genes is blocked via phosphorylation of Msn2/4. Whi2 functions as a regulator in adjusting the cellular response to the environmental situation. When nutrients become limited, cells enter stationary phase and Whi2 mediates the activation of the general stress response by ensuring dephosphorylation of Msn2/4. This can occur by activation of the phosphatases Psr1/2 and/or by inactivation of the PKA pathway. The cells arrest in G_1 phase, become heat shock resistant, accumulate carbohydrates and activate autophagy and mitophagy. Cells lacking Whi2 are not able to induce this general stress response under starvation. Interestingly, mitophagy seems to be preferentially inhibited in Δ*whi2* cells as opposed to autophagy and dysfunctional mitochondria accumulate.



overexpression of Whi2 leads to filamentous growth of yeast cells, suspecting also a function in budding and cytokinesis.

The identification of a regulatory function of Whi2 in the general stress response provided novel insights into the cellular role of Whi2 [41]. It was shown that Whi2 was necessary for full activation of gene expression controlled by STREs (stress-responsive elements). Furthermore, Whi2 can interact with the transcription factor Msn2 and the plasma-membrane-associated phosphatase Psr1, both necessary for the activation of stress response genes. Msn2 was found to be hyperphosphorylated in strains deleted for Whi2 or Psr1/2, suggesting a role for Whi2 in mediating the dephosphorylation of transcription factors by specific phosphatases.

Another study points to a regulatory role of Whi2 in the Ras/PKA (protein kinase A) pathway [42]. In Δ*whi2* cells, actin aggregation, mitochondrial fragmentation, dissipation of membrane potential, ROS accumulation and loss of viability were observed during diauxic shift. This was shown to result from the hyperactivation of the PKA pathway. Moreover, Ras2 was found to be abnormally activated and located to mitochondria, indicating the requirement for Whi2 for deactivating and degrading Ras2 during nutrient depletion.

Taken together, Whi2 seems to link cell cycle regulation, general stress response and the PKA pathway and thereby specifically modulates the regulation of mitophagy. Indeed, autophagy was shown to be regulated by highly conserved signalling pathways such as the TOR (target of rapamycin) pathway or the PKA pathway [6,43]. These two kinases negatively regulate autophagy either by directly phosphorylating components of the initial Atg1–kinase complex or by inactivation of transcription factors preventing the expression of autophagic genes. In addition, inhibition of TOR via rapamycin promotes the nuclear localization of Msn2 and Msn4, resulting in the activation of the general stress response [44]. It has also been shown that Msn2 subcellular localization was regulated by PKA activity and that TOR seems to signal through the PKA pathway to modulate stress response and induction of autophagy [45,46].

Altogether, Whi2 may be a key player in adapting the complex network of signalling pathways in response to the nutritional status of the cell (Figure 2). We propose a model according to which Whi2 is responsible for inactivation of the PKA pathway and activation of the general stress response during nutrient depletion, thereby inducing autophagy and mitophagy. Mutants lacking Whi2 are not able to inactivate PKA and/or to activate Psr1/2 when cells enter stationary phase. Thus Msn2 becomes hyperphosphorylated and is not capable of inducing the general stress response. Mitophagy seems to be selectively inhibited under these conditions, whereas the level of autophagy remains affected only modestly.

The finding that Whi2 selectively influences mitophagy has shed new light on the regulation of this process. The decreased

mitophagic response in cells lacking Whi2 after inhibition of the TOR kinase with rapamycin [32] resembles the previously observed failed response of these cells to certain stresses such as nutrient depletion in stationary phase [35,36]. In light of all these findings, mitochondrial dysfunction and fragmentation observed in Δ*whi2* during diauxic shift [42] could well be explained by reduced elimination of damaged mitochondria. Also, the observed activation of Ras2 might be a direct consequence of this, consistent with a recent report by Graef and Nunnari [47] showing that mitochondrial dysfunction negatively regulates autophagy via activation of PKA. Interestingly, earlier studies have shown that hyperactivated PKA inhibits early steps of autophagosome formation [48]. In mammalian cells, PKA was reported to phosphorylate and inactivate the mitochondrial fission factor DRP1 [49]. This resulted in hyperfused mitochondria that are less prone to be degraded by autophagy, demonstrating that PKA may inhibit mitophagy at various levels. Overall, several lines of evidence indicate an important role of Whi2 in regulating the PKA signalling and the general stress response pathway. It remains an open question how this is exerted mechanistically and why mitophagy is specifically promoted by Whi2.

Conclusions

The molecular mechanisms and the regulation of mitophagy are far from being understood yet. Furthermore, the molecular factors are only partly conserved between yeast and mammals. In the latter, mitochondrial dynamics seem to play an important role in mitophagy, ensuring quality control of mitochondria. However, in yeast, fission of mitochondria does not appear to be required for mitophagy. Possibly, here fission is only required under certain conditions yet to be identified, fission is exerted by unknown mechanisms, or fission is indeed generally dispensable for mitophagy. However, the fact that mitophagy is controlled by a complex network of signalling pathways is conserved between yeast and mammals. In particular, the role of the Ras/PKA signalling pathway has become more evident from recent studies. Future studies have to concentrate on deciphering the complex interplay between the pathways involved in regulating mitophagy, which will finally allow us to gain a better molecular understanding of mitochondrial quality control.

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References

1 Yang, Z. and Klionsky, D.J. (2010) Eaten alive: a history of macroautophagy. Nat. Cell Biol. **12**, 814–822
2 Nakatogawa, H., Suzuki, K., Kamada, Y. and Ohsumi, Y. (2009) Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat. Rev. Mol. Cell Biol. **10**, 458–467
3 Shintani, T., Huang, W.P., Stromhaug, P.E. and Klionsky, D.J. (2002) Mechanism of cargo selection in the cytoplasm to vacuole targeting pathway. Dev. Cell **3**, 825–837
4 Dunn, Jr, W.A., Cregg, J.M., Kiel, J.A., van der Klei, I.J., Oku, M., Sakai, Y., Sibirny, A.A., Stasyk, O.V. and Veenhuis, M. (2005) Pexophagy: the selective autophagy of peroxisomes. Autophagy **1**, 75–83
5 Lemasters, J.J. (2005) Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. Rejuvenation Res. **8**, 3–5
6 Inoue, Y. and Klionsky, D.J. (2010) Regulation of macroautophagy in *Saccharomyces cerevisiae*. Semin. Cell Dev. Biol. **21**, 664–670
7 Nazarko, V.Y., Nazarko, T.Y., Farre, J.C., Stasyk, O.V., Warnecke, D., Ulaszewski, S., Cregg, J.M., Sibirny, A.A. and Subramani, S. (2011) Atg35, a micropexophagy-specific protein that regulates micropexophagic apparatus formation in *Pichia pastoris*. Autophagy **7**, 375–385
8 Kanki, T., Wang, K., Baba, M., Bartholomew, C.R., Lynch-Day, M.A., Du, Z., Geng, J., Mao, K., Yang, Z., Yen, W.L. and Klionsky, D.J. (2009) A genomic screen for yeast mutants defective in selective mitochondria autophagy. Mol. Biol. Cell **20**, 4730–4738
9 Okamoto, K., Kondo-Okamoto, N. and Ohsumi, Y. (2009) Mitochondria-anchored receptor Atg32 mediates degradation of mitochondria via selective autophagy. Dev. Cell **17**, 87–97
10 Kanki, T., Wang, K., Cao, Y., Baba, M. and Klionsky, D.J. (2009) Atg32 is a mitochondrial protein that confers selectivity during mitophagy. Dev. Cell **17**, 98–109
11 Narendra, D.P., Jin, S.M., Tanaka, A., Suen, D.F., Gautier, C.A., Shen, J., Cookson, M.R. and Youle, R.J. (2010) PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. PLoS Biol. **8**, e1000298
12 Geisler, S., Holmstrom, K.M., Skujat, D., Fiesel, F.C., Rothfuss, O.C., Kahle, P.J. and Springer, W. (2010) PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. Nat. Cell Biol. **12**, 119–131
13 Ziviani, E., Tao, R.N. and Whitworth, A.J. (2010) Drosophila parkin requires PINK1 for mitochondrial translocation and ubiquitinates mitofusin. Proc. Natl. Acad. Sci. U.S.A. **107**, 5018–5023
14 Novak, I., Kirkin, V., McEwan, D.G., Zhang, J., Wild, P., Rozenknop, A., Rogov, V., Lohr, F., Popovic, D., Occhipinti, A. et al. (2010) Nix is a selective autophagy receptor for mitochondrial clearance. EMBO Rep. **11**, 45–51
15 Bereiter-Hahn, J. and Voth, M. (1994) Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. Microsc. Res. Tech. **27**, 198–219
16 Nunnari, J., Marshall, W.F., Straight, A., Murray, A., Sedat, J.W. and Walter, P. (1997) Mitochondrial transmission during mating in *Saccharomyces cerevisiae* is determined by mitochondrial fusion and fission and the intramitochondrial segregation of mitochondrial DNA. Mol. Biol. Cell **8**, 1233–1242
17 Schafer, A. and Reichert, A.S. (2009) Emerging roles of mitochondrial membrane dynamics in health and disease. Biol. Chem. **390**, 707–715
18 Tatsuta, T. and Langer, T. (2008) Quality control of mitochondria: protection against neurodegeneration and ageing. EMBO J. **27**, 306–314
19 Twig, G., Elorza, A., Molina, A.J., Mohamed, H., Wikstrom, J.D., Walzer, G., Stiles, L., Haigh, S.E., Katz, S., Las, G. et al. (2008) Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J. **27**, 433–446
20 Cipolat, S., Martins de Brito, O., Dal Zilio, B. and Scorrano, L. (2004) OPA1 requires mitofusin 1 to promote mitochondrial fusion. Proc. Natl. Acad. Sci. U.S.A. **101**, 15927–15932
21 Wong, E.D., Wagner, J.A., Scott, S.V., Okreglak, V., Holewinske, T.J., Cassidy-Stone, A. and Nunnari, J. (2003) The intramitochondrial dynamin-related GTPase, Mgm1p, is a component of a protein complex that mediates mitochondrial fusion. J. Cell Biol. **160**, 303–311

22 Wong, E.D., Wagner, J.A., Gorsich, S.W., McCaffery, J.M., Shaw, J.M. and Nunnari, J. (2000) The dynamin-related GTPase, Mgm1p, is an intermembrane space protein required for maintenance of fusion competent mitochondria. J. Cell Biol. **151**, 341–352
23 Herlan, M., Vogel, F., Bornhovd, C., Neupert, W. and Reichert, A.S. (2003) Processing of Mgm1 by the rhomboid-type protease Pcp1 is required for maintenance of mitochondrial morphology and of mitochondrial DNA. J. Biol. Chem. **278**, 27781–27788
24 McQuibban, G.A., Saurya, S. and Freeman, M. (2003) Mitochondrial membrane remodelling regulated by a conserved rhomboid protease. Nature **423**, 537–541
25 Zick, M., Duvezin-Caubet, S., Schafer, A., Vogel, F., Neupert, W. and Reichert, A.S. (2009) Distinct roles of the two isoforms of the dynamin-like GTPase Mgm1 in mitochondrial fusion. FEBS Lett. **583**, 2237–2243
26 Herlan, M., Bornhovd, C., Hell, K., Neupert, W. and Reichert, A.S. (2004) Alternative topogenesis of Mgm1 and mitochondrial morphology depend on ATP and a functional import motor. J. Cell Biol. **165**, 167–173
27 Duvezin-Caubet, S., Jagasia, R., Wagener, J., Hofmann, S., Trifunovic, A., Hansson, A., Chomyn, A., Bauer, M.F., Attardi, G., Larsson, N.G. et al. (2006) Proteolytic processing of OPA1 links mitochondrial dysfunction to alterations in mitochondrial morphology. J. Biol. Chem. **281**, 37972–37979
28 Ishihara, N., Fujita, Y., Oka, T. and Mihara, K. (2006) Regulation of mitochondrial morphology through proteolytic cleavage of OPA1. EMBO J. **25**, 2966–2977
29 Priault, M., Salin, B., Schaeffer, J., Vallette, F.M., di Rago, J.P. and Martinou, J.C. (2005) Impairing the bioenergetic status and the biogenesis of mitochondria triggers mitophagy in yeast. Cell Death Differ. **12**, 1613–1621
30 Nowikovsky, K., Reipert, S., Devenish, R.J. and Schweyen, R.J. (2007) Mdm38 protein depletion causes loss of mitochondrial K^+/H^+ exchange activity, osmotic swelling and mitophagy. Cell Death Differ. **14**, 1647–1656
31 Barsoum, M.J., Yuan, H., Gerencser, A.A., Liot, G., Kushnareva, Y., Graber, S., Kovacs, I., Lee, W.D., Waggoner, J., Cui, J. et al. (2006) Nitric oxide-induced mitochondrial fission is regulated by dynamin-related GTPases in neurons. EMBO J. **25**, 3900–3911
32 Mendl, N., Occhipinti, A., Muller, M., Wild, P., Dikic, I. and Reichert, A.S. (2011) Mitophagy in yeast is independent of mitochondrial fission and requires the stress response gene WHI2. J. Cell Sci. **124**, 1339–1350
33 Cheng, W.C., Teng, X., Park, H.K., Tucker, C.M., Dunham, M.J. and Hardwick, J.M. (2008) Fis1 deficiency selects for compensatory mutations responsible for cell death and growth control defects. Cell Death Differ. **15**, 1838–1846
34 Sudbery, P.E., Goodey, A.R. and Carter, B.L. (1980) Genes which control cell proliferation in the yeast *Saccharomyces cerevisiae*. Nature **288**, 401–404
35 Saul, D.J., Walton, E.F., Sudbery, P.E. and Carter, B.L.A. (1985) *Saccharomyces cerevisiae whi2* mutants in stationary phase retain the properties of exponentially growing cells. J. Gen. Microbiol. **131**, 2245–2251
36 Rahman, D.R., Sudbery, P.E., Kelly, S. and Marison, I.W. (1988) The effect of dissolved oxygen concentration on the growth physiology of *Saccharomyces cerevisiae* whi2 mutants. J. Gen. Microbiol. **134**, 2241–2248
37 Mountain, H.A. and Sudbery, P.E. (1990) Regulation of the *Saccharomyces cerevisiae WHI2* gene. J. Gen. Microbiol. **136**, 727–732
38 Mountain, H.A. and Sudbery, P.E. (1990) The relationship of growth rate and catabolite repression with WHI2 expression and cell size in *Saccharomyces cerevisiae*. J. Gen. Microbiol. **136**, 733–737
39 Radcliffe, P., Trevethick, J., Tyers, M. and Sudbery, P. (1997) Deregulation of CLN1 and CLN2 in the *Saccharomyces cerevisiae* whi2 mutant. Yeast **13**, 707–715
40 Radcliffe, P.A., Binley, K.M., Trevethick, J., Hall, M. and Sudbery, P.E. (1997) Filamentous growth of the budding yeast *Saccharomyces cerevisiae* induced by overexpression of the WHi2 gene. Microbiology **143**, 1867–1876
41 Kaida, D., Yashiroda, H., Toh-e, A. and Kikuchi, Y. (2002) Yeast Whi2 and Psr1-phosphatase form a complex and regulate STRE-mediated gene expression. Genes Cells **7**, 543–552
42 Leadsham, J.E., Miller, K., Ayscough, K.R., Colombo, S., Martegani, E., Sudbery, P. and Gourlay, C.W. (2009) Whi2p links nutritional sensing to actin-dependent Ras-cAMP-PKA regulation and apoptosis in yeast. J. Cell Sci. **122**, 706–715
43 Cebollero, E. and Reggiori, F. (2009) Regulation of autophagy in yeast *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **1793**, 1413–1421
44 Beck, T. and Hall, M.N. (1999) The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. Nature **402**, 689–692
45 Gorner, W., Durchschlag, E., Martinez-Pastor, M.T., Estruch, F., Ammerer, G., Hamilton, B., Ruis, H. and Schuller, C. (1998) Nuclear localization of the C2H2 zinc finger protein Msn2p is regulated by stress and protein kinase A activity. Genes Dev. **12**, 586–597
46 Schmelzle, T., Beck, T., Martin, D.E. and Hall, M.N. (2004) Activation of the RAS/cyclic AMP pathway suppresses a TOR deficiency in yeast. Mol. Cell. Biol. **24**, 338–351
47 Graef, M. and Nunnari, J. (2011) Mitochondria regulate autophagy by conserved signalling pathways. EMBO J. **30**, 2101–2114
48 Budovskaya, Y.V., Stephan, J.S., Reggiori, F., Klionsky, D.J. and Herman, P.K. (2004) The Ras/cAMP-dependent protein kinase signaling pathway regulates an early step of the autophagy process in *Saccharomyces cerevisiae*. J. Biol. Chem. **279**, 20663–20671
49 Gomes, L.C., Benedetto, G.D. and Scorrano, L. (2011) During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. Nat. Cell Biol. **13**, 589–598

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