Published in final edited form as:

Ageing Res Rev. 2015 September ; 23(0 0): 67–74. doi:10.1016/j.arr.2014.12.007.

Mitochondria to Nucleus Signaling and the Role of Ceramide in Its Integration into the Suite of Cell Quality Control Processes during Aging

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Abstract

Mitochondria to nucleus signaling has been the most extensively studied mode of inter-organelle communication. The first signaling pathway in this category of information transfer to be discovered was the retrograde response, with its own set of signal transduction proteins. The finding that this pathway compensates for mitochondrial dysfunction to extend the replicative lifespan of yeast cells has generated additional impetus for its study. This research has demonstrated crosstalk between the retrograde response and the target of rapamycin (TOR), small GTPase RAS, and high-osmolarity glycerol (HOG) pathways in yeast, all of which are key players in replicative lifespan. More recently, the retrograde response has been implicated in the diauxic shift and survival in stationary phase, extending its operation to the yeast chronological lifespan as well. In this capacity, the retrograde response may cooperate with other, related mitochondria to nucleus signaling pathways. Counterparts of the retrograde response are found in the roundworm, the fruit fly, the mouse, and even in human cells in tissue culture. The exciting realization that the retrograde response is embedded in the network of cellular quality control processes has emerged over the past few years. Most strikingly, it is closely integrated with autophagy and the selective brand of this quality control process, mitophagy. This coordination depends on TOR, and it engages ceramide/sphingolipid signaling. The yeast *LAG1* ceramide synthase gene was the first longevity gene cloned as such, and its orthologs *hyl-1* and *hyl-2* determine worm lifespan. Thus, the involvement of ceramide signaling in quality control gives these findings cellular context. The retrograde response and ceramide are essential components of a lifespan maintenance process that likely evolved as a cytoprotective mechanism to defend the organism from diverse stressors.

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Key terms

Saccharomyces cerevisiae; *Caenorhabditis elegans*; Lifespan; Sphingolipids; Retrograde response; Autophagy

1. Introduction

Cellular quality control has emerged as a central player in aging. This is not only the case in terms of resistance to age-related diseases, but also to longevity itself, at least in lower organisms. This issue of the journal reviews many aspects of these relationships, while considering various quality control mechanisms at the cell's disposal. It may not be as easy to discern, however, how all of these devices are integrated to provide a suite of mechanisms that tailor the response to the situation the cell encounters.

This article provides an overview of a compensatory response the cell deploys when it is faced with mitochondrial dysfunction. Rather than eliminating the source of the dysfunction, this response makes the best of a bad situation by adapting cell physiology to the worsening state of affairs. Thus, one could consider this to be a last ditch effort at survival.

Surprisingly, the cell (and organism) appears to thrive under these new circumstances, and it can live longer. This certainly surmounts any limitations of quality control mechanisms, and, indeed, it complements them nicely.

The view just presented would neglect an important feature of the compensatory response to mitochondrial dysfunction. This is its firm coordination with other cell quality control mechanisms. It is this aspect that draws our attention in this article. However, there are two additional issues that will be discussed. The first concerns the nature of the underlying coordination and the signals that are involved. The second, and perhaps related question, that will be touched upon briefly is the evolution of the compensatory response.

2. The yeast retrograde response

The compensatory response alluded to above is the retrograde response, first identified in yeast as retrograde signaling to denote a mechanism of communication from the mitochondrion to the nucleus (Butow and Avadhani, 2004). Subsequently, the moniker "retrograde response" was bestowed, as its broad impact on cell physiology became clear (Jazwinski, 1999). Activation of the retrograde response due to mitochondrial dysfunction, which can be induced in various ways, results in changes in the expression of some 400 nuclear genes (Epstein, et al., 2001, Traven, et al., 2001). *CIT2*, encoding peroxisomal citrate synthase, is one of these genes, and its induction is often used as a robust indicator of retrograde response activation (Liao, et al., 1991). The gene expression changes that encompass the retrograde response adjust cell metabolism to life without a fully functioning Krebs cycle. This is achieved through the activation of anaplerotic reactions and the conservation of carbon in the peroxisomal glyoxylate cycle, among others. Furthermore, pathways that enhance the ability to resist stress are induced, including xenobiotic stress.

Retrograde signaling consists of a bona fide, intracellular signaling pathway. A signal elicited by dysfunctional mitochondria is transduced by the Rtg2 protein, resulting in the translocation of the Rtg1-Rtg3 transcription factor from the cytoplasm to the nucleus. This deceptively uncomplicated set of events involves the interactions of several proteins, which will not be presented here. Similarly, the connection of retrograde signaling to epigenetic gene regulation and genomic stability will not be examined. These details have been thoroughly discussed recently (Jazwinski, 2012a, Jazwinski, 2012b, Jazwinski, 2014, Jazwinski and Kriete, 2012, Srinivasan, et al., 2010).

The nature of the mitochondrial signal that triggers the retrograde response deserves some comment, as it had been an enigma until recently. Fundamentally, there could be three signals emanating from dysfunctional mitochondria. One are reactive oxygen species (ROS); another is a drop in ATP levels; and, the third is a reduction in mitochondrial membrane potential. The third of these alternative signals has been shown to be the elusive, physiologic signal in dividing cells, while ROS were excluded (Miceli, et al., 2011). Recently, it has been shown that Rtg2 protein is released from the Mks1 protein, an inhibitor of retrograde signaling, in the presence of ATP (Zhang, et al., 2013). This reaction was demonstrated in vitro. Mks1 can then prevent the partial dephosphorylation of Rtg3 protein and the consequent translocation of Rtg1-Rtg3 to the nucleus. It can be argued that ATP levels fall in the cell when oxidative phosphorylation is blocked, and the cell generates ATP through fermentation. Interestingly, fermenting yeast cells grow faster than respiring ones. Furthermore, further taxing ATP pools in fermenting cells to reverse the decline in mitochondrial membrane potential actually suppresses retrograde signaling in living yeast cells (Miceli, et al., 2011). These facts would argue against the notion of a drop in ATP as the mitochondrial signal of the retrograde response. However, there is the possibility that a localized pool of ATP is involved in vivo, and that its reduction could act in tandem with the drop in mitochondrial membrane potential.

3. Crosstalk of retrograde signaling with other signaling pathways

There are three major signaling pathways with which retrograde signaling interacts. These are the target of rapamycin (TOR), the small GTPase RAS, and the high-osmolarity stressresponsive kinase HOG pathways (Fig. 1).

TOR complex 1 (TORC1) negatively regulates retrograde signaling, acting both upstream and downstream of Rtg2 (Breitkreutz, et al., 2010, Giannattasio, et al., 2005, Komeili, et al., 2000). TOR is a sensor of nutrient status in the cell. Glutamate availability activates TORC1 signaling, and its downstream outcomes such as ribosome biogenesis. The retrograde response is repressed by glutamate, and mutants in the *RTG* genes are glutamate auxotrophs (Jia, et al., 1997, Liao and Butow, 1993, Liu and Butow, 2006). Lst8, a component of TORC1, was shown to be a negative regulator of retrograde signaling in genetic studies, acting both upstream and downstream of Rtg2 (Roberg, et al., 1997). Those mutants of *LST8* which act upstream of Rtg2 resemble phenotypically mutations in a plasma membrane amino acid sensor.

TORC1 phosphorylates the AGC protein kinase Sch9, and this is markedly reduced in cells in which retrograde signaling activity occurs (Kawai, et al., 2011). This has important consequences, as phosphorylation of Sch9 inhibits the activation of stress responses dependent on the Msn2-Msn4 transcription factor, while activating ribosome biogenesis (Urban, et al., 2007). Thus, stress responses would be elevated with concomitant decline in ribosome production in the retrograde response. Phosphorylation of Sch9 also inhibits cAMP-dependent, protein kinase A activity, which facilitates the activation of Msn2-Msn4 mediated stress responses (Zhang, et al., 2011). Inhibition of protein kinase A would result in the inhibition of growth in the presence of nutrients. This does not happen, however, because protein kinase A feedback inhibits this effect of Sch9. The net effect of this feedback loop on inhibition of TORC1 in cells possessing dysfunctional mitochondria is to balance cell growth and metabolism with stress responses.

Ras2 is required for the retrograde response (Kirchman, et al., 1999). Mks1, the negative regulator of retrograde signaling, was first identified as a negative regulator of Ras2-cAMP signaling, which might implicate Ras2-cAMP in the crosstalk with retrograde signaling. However, a Ras2 pathway that is cAMP-independent has also been described (Mosch, et al., 1996, Sun, et al., 1994), and it could be this pathway that is relevant (see below). The downstream target of the Ras2-cAMP pathway is protein kinase A, which ties together retrograde signaling, TORC1, and Ras2 signaling.

The hyperosmolarity-activated, Hog1 protein kinase pathway regulates the Rtg1-Rtg3 transcription factor (Ruiz-Roig, et al., 2012). Translocation of Rtg1-Rtg3 into the nucleus requires Hog1, as does the binding of this transcription factor to chromatin and transactivation of gene expression. Interestingly, the osmotic stress that activates Hog1 also results in a transient reduction in Sch9 phosphorylation by TORC1 (Urban, et al., 2007). This would serve to upregulate, transiently, a plethora of cellular stress responses, while slowing ribosome production and growth. Clearly, the activities of the retrograde signaling pathway, TORC1, Ras2, and Hog1 communicate and are closely coordinated.

4. The retrograde response affects yeast lifespan

The significance of the retrograde response in aging first became apparent when it was demonstrated that the activation of the retrograde signaling pathway extended the yeast replicative lifespan (RLS) (Kirchman, et al., 1999). The RLS is defined by the number of times an individual yeast cell divides, or in other words the number of daughter cells produced. Subsequently, it was shown that activation of this pathway is necessary for the extension of chronological lifespan (CLS) (Barros, et al., 2004), which is the time yeast cells survive in a non-dividing, stationary phase that is usually measured in the absence of nutrients.

The retrograde response is not the only pathway connecting RLS and CLS. TORC1 signaling, as an expression of intracellular nutrient status, does so as well. This is amply demonstrated by the fact that deletion of *SCH9*, which encodes a protein kinase that is an effector of TORC1, extends both RLS and CLS (Fabrizio, et al., 2001, Kaeberlein, et al., 2005). This is curious because of the markedly different growth status of cells under the two

conditions. Yeast cells in stationary phase respire, albeit slowly. This is connected to ROS generation, and stationary phase cells launch antioxidant defenses to account for it (Piper, 2012). They must do this sufficiently. Dividing cells can “runaway” from damage, in essence leaving damage behind as clones expand exponentially. This is particularly feasible due to age asymmetry in the segregation of damaged and dysfunctional constituents (Jazwinski, 2012a). The resolution of the paradoxical effect of *SCH9* deletion on both RLS and CLS may reside in the balance struck by the feedback loop that connects TORC1 and Ras2 signaling through Sch9 and protein kinase A, discussed earlier.

Deletion of *RAS2* curtails RLS (Sun, et al., 1994), but it extends CLS (Fabrizio, et al., 2003). Significantly, it is the cAMP-independent pathway that is involved in the former and the cAMP-dependent protein kinase A pathway in the latter. These effects highlight the differences between RLS and CLS mentioned above, and they also point to the critical nature of the feedback loop just discussed. It appears that there is a trade-off that allows cells to proliferate when nutrients are available (RLS) and to survive in their absence (CLS) that represent opposite ends of the functional spectrum afforded by this feedback loop. Absence of Ras2 makes stationary phase survival efficient. However, it compromises the ability to rapidly proliferate. Overexpression of *RAS2* extends the RLS (Sun, et al., 1994), a situation that makes stationary phase arrest difficult. However, this RLS extension engages a Ras2 cAMP-independent pathway; activation of the cAMP-dependent pathway curtails RLS (Sun, et al., 1994).

In the discussion of the mitochondrial signal that triggers the retrograde response, one possible signal that was mentioned was ROS. The retrograde response in yeast cells dividing during the RLS does not involve ROS signaling (Miceli, et al., 2011). However, ROS signaling from the mitochondrion to the nucleus is involved in CLS extension in non-dividing cells, and this requires TORC1 (Pan, et al., 2011). It should be pointed out that such cells do not necessarily contain dysfunctional mitochondria, and they not only generate ROS but also upregulate ROS defenses, which may be among the many ROS-dependent, epigenetic changes in gene expression. Although mitochondrial dysfunction increases during the RLS (Lai, et al., 2002, Laun, et al., 2001), this is not necessarily equivalent to the drastic effects on mitochondrial function that are used to elicit the retrograde response experimentally (Borghouts, et al., 2004). Indeed, even modest decreases in mitochondrial membrane potential can activate the retrograde response and increase RLS (Jazwinski, 2000, Miceli, et al., 2011).

5. Retrograde response in other organisms

Certain metabolic changes that occur during aging in *Caenorhabditis elegans* resemble those that are found in yeast on retrograde response activation, which led to the proposal that there is an operative retrograde response in this round worm (Kirchman, et al., 1999). In fact, there appear to be three such pathways. One of them involves the hypoxia-inducible transcription factor HIF-1, which is activated by mitochondrial ROS (Lee, et al., 2010). Another engages the mitochondrial unfolded protein response and the transcription factors UBL-5 and DVE-1 (Durieux, et al., 2011), while the third is mediated by the predicted homeobox transcription factor CEH-23 (Walter, et al., 2011). The phenotypes associated

with the lifespan extension afforded by mitochondrial dysfunction in the worm can be varied (Yang and Hekimi, 2010), and they can incorporate hormonal effects by bile acid-related species (Liu, et al., 2012). Similar to the worm studies, knockdown of respiratory chain components in *Drosophila* also extends lifespan (Copeland, et al., 2009), as does defective coenzyme Q synthesis (Liu, et al., 2011), but the signaling pathways are not known.

Mouse lifespan has also been extended by disruption of mitochondrial function in several ways. Lowered coenzyme Q synthesis through reduced activity of the *MLCK1* gene is associated with increased longevity (Lapointe and Hekimi, 2008). This appears to be the result of a subtle effect on the balance of ROS production in the mitochondrion and in the cytoplasm. The model explaining these effects of lowered coenzyme Q synthesis proposes reduced cytoplasmic ROS production coupled to enhanced antioxidant defenses. The latter could be linked to mitochondria-generated ROS signaling gene expression changes in the nucleus. A *SURF1* conditional knockout, which interferes with cytochrome oxidase assembly also extends mouse lifespan (Dell'agnello, et al., 2007). Surprisingly, even a complete lack of the Surf1 cytochrome oxidase assembly factor was compatible with postnatal survival. Importantly, mild mitochondrial uncoupling produced by feeding dinitrophenol to mice not only extends lifespan, but it also improves a variety of metabolic parameters, including serum glucose, triglyceride and insulin levels (Caldeira da Silva, et al., 2008). In similarity, normal, diploid human fibroblasts show an increase in population doubling levels when supplied with dinitrophenol in the medium, while ROS are suppressed, telomere shortening is reduced, and DNA repair foci are sparse (Passos, et al., 2007).

6. Integration of the retrograde response into the cell quality control suite and ceramide

The retrograde response is firmly embedded in the cell quality control network. This is so much the case that it is virtually impossible to completely discuss this topic in a short review. There are three elements of quality control which will be touched upon here (Fig. 2). The first concerns the removal of damaged or excess proteins of the mitochondrial inner membrane with the assistance of the prohibitins, Phb1 and Phb2. The second involves removal of damaged or dysfunctional mitochondria through autophagy, which can be selective for this organelle (mitophagy). The third process depends on asymmetric segregation of damaged or dysfunctional mitochondria. These three processes will be discussed in order.

Expression of *PHB1* and *PHB2* is activated in the retrograde response (Traven, et al., 2001). The products of these genes perform a chaperone function in the assembly of mitochondrial inner-membrane protein complexes (Nijtmans, et al., 2000), and it has been suggested that prohibitins deliver excess mitochondrial membrane proteins to the inner membrane m-AAA protease for degradation (Kirchman, et al., 2003). Deletion of either or both of the prohibitin genes severely curtails RLS of rho⁰ cells (Kirchman, et al., 2003). Mitochondrial DNA (mtDNA) is missing in rho⁰ cells, which results in the lack of production of mtDNA-encoded components of the electron transport chain, and this defect activates the retrograde response which in some yeast strains is glucose-repressed (Kirchman, et al., 1999).

Importantly, deletion of *RAS2* completely suppresses the RLS deficit of ρ^0 cells in which prohibitin genes are deleted (Kirchman, et al., 2003).

Ras2 stimulates the synthesis of nuclear-encoded components of the mitochondrial respiratory chain (Dejean, et al., 2002, Kirchman, et al., 2003). Thus, deletion of *RAS2* would reduce the imbalance between the respiratory chain proteins synthesized in the cytoplasm and in the mitochondrion that occurs in ρ^0 cells lacking prohibitins. The phenotypic consequences of a prohibitin deficit in ρ^0 cells is also suppressed by deletion of *TOR1* or *SCH9* (Wang, et al., 2008). Elimination of the TORC1/Sch9 pathway depresses mitochondrial biogenesis, as mentioned earlier.

The second aspect of cell quality control relevant to our discussion is autophagy, and particularly selective autophagy in the form of mitophagy. Autophagy is a genetically-orchestrated process by which various cytoplasmic components are delivered to the lysosome, in yeast called the vacuole, for degradation. Whole organelles can be disposed of in this way, and in mitophagy these are the mitochondria. Autophagy can generate building blocks for biosynthesis, particularly in times of scarcity. However, it also removes damaged cell components, acting as a quality control mechanism. Autophagy and mitophagy would be deleterious to the cell if not balanced by relevant biosynthetic reactions and organelle biogenesis. It is not clear how this balance operates in detail in yeast. However, yeast cells attempt to compensate for accumulation of dysfunctional mitochondria during the replicative lifespan, though the mitochondrial biogenesis that occurs cannot keep up (Lai, et al., 2002). In mammalian skeletal muscle, there is a decline in mitochondrial biogenesis with age, coupled to a reduction in mitochondrial dynamics (fission/fusion) and mitophagy (Chistiakov, et al., 2014)

The retrograde response and autophagy have crosstalk with TORC1 in common. Inhibition of TORC1 triggers autophagy, sensed by the cell as a state of starvation. TORC1 blocks autophagy by phosphorylating Atg13, which is part of the Atg1 protein kinase complex that is essential for autophagy (Kamada, et al., 2010). Nitrogen starvation induces autophagy in yeast, and this is further stimulated by deletion of *IPT1* and *SKN1* (Thevissen, et al., 2010). The products of these two genes are involved in the synthesis of yeast sphingolipids, the mannosyldi-inositol phosphorylceramides. Thus, these molecules act as signals modulating the extent of autophagy. Autophagy is also inhibited by c-AMP-dependent, protein kinase A phosphorylation of both Atg1 and Atg13 (Stephan, et al., 2009). This is distinct from the regulation by TORC1, which targets different sites for phosphorylation on Atg13.

Like TORC1, TORC2 also has an AGC protein kinase as its target, Ypk2 (Aronova, et al., 2008). In this way, TORC2 regulates the activity of ceramide synthase, an enzyme essential for synthesis of the signaling molecule ceramide and all sphingolipids, which are derived from it. Ceramide synthase activity is thus stimulated in the presence of nutrients, which would attenuate autophagy. Cellular stress inhibits ceramide synthase activity, in contrast. This inhibition is mediated by the calcium/calmodulin-dependent protein phosphatase calcineurin. In this fashion, stress would enhance autophagy. In sum, both TORC1 and TORC2 regulate autophagy, and ceramide plays a role in this process.

In yeast, there are two homologous genes that encode ceramide synthase activity, *LAG1* and *LAC1* (Guillas, et al., 2001, Jiang, et al., 1998, Schorling, et al., 2001). *LAG1* was the first gene cloned as a longevity assurance gene (D'Mello N, et al., 1994, Jiang, et al., 1998). The human orthologs encode six ceramide synthases Lass1-6 (Guillas, et al., 2003, Teufel, et al., 2009, Venkataraman, et al., 2002). Interestingly, *LAC1* expression is regulated coordinately with the multi-drug resistance membrane transporter gene *PDR5*, which along with other members of this gene family is activated in the retrograde response (Hallstrom and Moye-Rowley, 2000, Kolaczowski, et al., 2004, Moye-Rowley, 2005). It would seem that ceramide synthase activity must be finely tuned. This certainly is the case when RLS extension by *LAG1* expression is concerned (Jiang, et al., 2004).

Deletion of *CRD1*, which encodes cardiolipin synthase, leads to delay of cell division in ρ^0 cells (Chen, et al., 2010, Vaena de Avalos, et al., 2005). The cause is the up-regulation of synthesis of the morphogenesis checkpoint protein Swe1, which requires activation of retrograde signaling. During diauxic shift, Isc1 translocates from the cytoplasm to the mitochondrial membrane where it exhibits cardiolipin-dependent inositol sphingolipid phospholipase C activity (Vaena de Avalos, et al., 2004). This results in production of ceramide, which activates a pathway parallel to retrograde signaling that is responsible for induction of a wide array of genes necessary for adaptation to stationary phase (Kitagaki, et al., 2009). The protein phosphatase 2A (PP2A), whose catalytic subunit is Sit4 protein is regulated by Isc1 (Barbosa, et al., 2011). Ceramide activates PP2A. This could well be the parallel pathway referred to here. PP2A, just like the retrograde response, is regulated by TORC1. Furthermore, inactivation of Sit4 stimulates autophagy, and this effect occurs downstream of TORC1 (Yorimitsu, et al., 2009). Ceramide arrests cells in the G₁ phase of the cell cycle by activating PP2A (Nickels and Broach, 1996). The *ISC1* and *SWE1* genes interact (Tripathi, et al., 2011). Thus, the retrograde response regulates expression of *SWE1*, while ceramide may regulate the activity of Swe1 protein. The deletion of *SIT4* in ρ^0 cells suppresses defects in mitochondrial protein import, cell proliferation, loss of mitochondrial membrane potential, autophagy deficit, and retrograde signaling (Garipler, et al., 2014). On the other hand, deletion of *SIT4* in *isc1* cells rescues their premature chronological aging and oxidative stress sensitivity (Barbosa, et al., 2011). Deletion of *TOR1* or *SCH9* in cells devoid of *ISC1* suppresses mitochondrial dysfunction, sensitivity to oxidative stress, and curtailed CLS (Teixeira, et al., 2014), further distinguishing dividing from non-dividing cells. Hog1 hyperphosphorylation is required for the effect of *SCH9* deletion but not for *TOR1* deletion. The mutual relationship of the TORC1-Sch9 signaling pathway and ceramide signaling is indeed complex. Sch9 regulates stress tolerance and longevity in yeast by acting as both an effector and a regulator of sphingolipid metabolism (Swinnen, et al., 2014).

Inhibition of the de novo sphingolipid synthesis pathway activates the HOG pathway at the level of the osmosensing machinery (Tanigawa, et al., 2012). Interestingly, Hog1 has recently been shown to be activated by hypoxia, in a manner and with consequences distinct from its activation by osmotic stress (Hickman, et al., 2011). Deletion of *ISC1* leads to activation of Hog1, and it also activates the cell wall integrity pathway in yeast (Barbosa, et al., 2012), which plays a role in RLS (Kaeberlein and Guarente, 2002) as well as CLS.

Atg32 tags mitochondria for selective removal in the process of mitophagy (Kanki and Klionsky, 2009, Kanki, et al., 2009a, Kanki, et al., 2009b, Okamoto, et al., 2009a, Okamoto, et al., 2009b). Mitophagy in stationary phase requires retrograde signaling (Journo, et al., 2009). The Aup1 protein phosphatase, which is located in the mitochondrial intermembrane space, is essential. Deletion of *AUP1* blocks retrograde signaling, and deletion of *RTG3* prevents mitophagy. Not surprisingly, the phosphorylation pattern on Rtg3 depends on Aup1. In addition to this connection to retrograde signaling, crosstalk with Hog1 is also involved. The Hog1 protein kinase functions specifically in mitophagy (Mao, et al., 2011).

The third issue related to quality control is the age asymmetry in segregation of mitochondria during cell division. During cell division, approximately equal amounts of mitochondrial material find their way into the mother and its daughter (Simon, et al., 1997). As the mother cell becomes older during the RLS, its mitochondria suffer damage and dysfunction, judging by many different parameters (Kirchman, et al., 2003, Klinger, et al., 2010, Lai, et al., 2002, Lam, et al., 2011, Laun, et al., 2001, McFaline-Figueroa, et al., 2011, Piper, et al., 2002, Seo, et al., 2007). These dysfunctional mitochondria are retained by mother cells (Lai, et al., 2002, McFaline-Figueroa, et al., 2011, Piper, et al., 2002). This age asymmetry is reflected in the fact that daughter cells have before them the prospects of a full RLS. However, this age symmetry breaks down as mother cells age (Egilmez and Jazwinski, 1989, Kennedy, et al., 1994). Structural and functional asymmetry is the basis for a theory of aging (Jazwinski, 1993). Mutants in age asymmetry have been isolated in which daughters are born old, revealing that at least one of its sources is the segregation of dysfunctional mitochondria to the mother (Lai, et al., 2002). It will be of interest to determine how this might tie in to the retrograde response which is activated by mitochondrial dysfunction.

It is important to note here that other sorts of damaging agents also display age asymmetry in their segregation. They include extrachromosomal rDNA circles (Sinclair and Guarente, 1997) and aggregated and oxidized proteins (Aguilaniu, et al., 2003, Liu, et al., 2010).

7. Retrograde signaling is a response to cytotoxic stress

Longevity regulation has recently been proposed to have evolved from cytoprotective pathways responsive to diverse triggers across species (Shore and Ruvkun, 2013). The *C. elegans* retrograde response was the primary consideration in this proposal. Among the stressors discussed were xenobiotics and pathogens that have plagued cells and organisms since early in evolution. The important point was that resistance to one specific stressor induced crossprotection to other stressors, which is typical of hormesis. This resistance includes detoxification of xenobiotics and induction of autophagy. On point, exposure to any stressor induces a common set of responses that protect against various stressors, repair the damage, or remove the defective cellular structures. As it happens, these are the same structures that are susceptible to damage during aging. According to the model, it is the damage itself rather than the damaging agents that are sensed, making this a relatively parsimonious response.

Exposure of yeast to the xenobiotic erythromycin, an agent that blocks mitochondrial protein translation was determined long ago to extend RLS (Holbrook and Menninger, 2002). This

is consistent with the cytoprotection model discussed above. Furthermore, activation of the retrograde response triggers the expression of *PDR5* and other genes of the cytoplasmic-membrane localized multi-drug resistance transporter family that pump xenobiotics out of cells, and this occurs coordinately with expression of the ceramide synthase gene *LAC1* (Hallstrom and Moye-Rowley, 2000, Kolaczowski, et al., 2004, Moye-Rowley, 2005). This links mitochondrial dysfunction, whether it occurs during aging or through the action of external agents, to cross cross-protection against a panoply of xenobiotics, and it is associated with RLS and CLS extension, as discussed earlier.

Certain genotoxic agents can deplete nucleotide pools in the cell, which can result in DNA replication stress, and induction of *RNR1*, encoding ribonucleotide reductase, can rescue this situation (Weinberger, et al., 2013). DNA replication stress is known to curtail the RLS (Weinberger, et al., 2013). The retrograde response genes *RTG1*, *RTG2*, and *RTG3* regulate a metabolic circuit that can buffer deficiencies in ribonucleotide reductase (Hartman, 2007). *ISC1* interacts with DNA integrity checkpoint genes in yeast (Tripathi, et al., 2011), implicating ceramide signaling in the response to DNA replication stress. Indeed, *Isc1* impacts both the DNA replication and DNA damage checkpoints in yeast. *ISC1* appears to be synthetic with the replication checkpoint mediators *MRC1*, *TOF1*, and *CSM3*, with *SWE1* downstream, and this is further connected to actin cytoskeleton dynamics. In parallel, *ISC1* cooperates with the damage checkpoint gene *RAD9*, whose readout is mediated by *RAD53* and its target *CDK1*. These two parallel checkpoint pathways may be interconnected. As mentioned earlier, the retrograde response regulates expression of *SWE1*, while ceramide may regulate the activity of *Swe1* protein. The findings summarized here provide additional support for the role of ceramide and sphingolipids in cytoprotection in yeast and connects them to the retrograde response.

One of the *C. elegans* homologs of *LAG1* is *hyl-2* (Jiang, et al., 1998). The ceramide species synthesized by *HYL-2* have a pro-survival effect. They are involved in the activation of *HIF-1* (Menuz, et al., 2009) whose activity extends worm longevity (Mehta, et al., 2009). It is noteworthy that ROS generated by mitochondrial dysfunction in the *C. elegans* retrograde response extends lifespan in a *hif-1*-dependent manner (Lee, et al., 2010). Another worm *LAG1* homolog is *hyl-1* (Jiang, et al., 1998), and the ceramide generated by the ceramide synthase it encodes has pro-apoptotic effects (Menuz, et al., 2009). Knockdown of *hyl-1* extends worm lifespan (Tedesco, et al., 2008). Recently, it has been shown that mitochondrial dysfunction in *C. elegans* extends lifespan by countering the classic apoptotic pathway (Yee, et al., 2014). This is the case even though the worm does not die due to wholesale apoptosis.

The critical role of ceramide and sphingolipids in longevity that was first found in the yeast *Saccharomyces cerevisiae* (D'Mello N, et al., 1994, Guillas, et al., 2001, Jiang, et al., 2004, Jiang, et al., 1998) has been highlighted recently in *C. elegans* (Liu, et al., 2014). A comparison of the genetic determinants of lifespan extension in the worm induced by various cytotoxic agents prominently featured ceramide and its metabolism. This is not surprising, as it is known that sphingolipids impact virtually every signaling pathway in the yeast cell that is relevant for longevity (Huang, et al., 2013, Liu, et al., 2013). This includes the various pathways and cellular processes with which the retrograde response cross-talks.

Thus, ceramide is central to the integration of the retrograde response into the suite of cellular quality control that is crucial in aging and lifespan regulation.

Acknowledgments

The research in the author's laboratory is supported by grant AG006168 from the National Institute on Aging of the National Institutes of Health (U.S.P.H.S.).

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Figure 1.

Crosstalk of retrograde signaling with other signaling pathways. Availability of nutrients activates TORC1, which inhibits retrograde signaling and activates Sch9. This activation of Sch9 is inhibited by the retrograde response. The protein kinase Sch9 activates ribosome biogenesis, while inhibiting protein kinase A and the Msn2-Msn4 transcription factor, which is responsible for activating stress response genes. Protein kinase A also inhibits this transcription factor while stimulating ribosome production. Protein kinase A feedback inhibits its own activation. One or more signals activate Ras2, which functions in a cAMP-dependent and a cAMP-independent pathway. The first of these is likely to block the retrograde response, as it is known to curtail RLS, which is maintained by progressive activation of the retrograde response during the RLS. The second pathway is known to extend the RLS, and Ras2 is required for the retrograde response that extends RLS. The Hog1 protein kinase is responsive to osmotic stress and to hypoxia. It activates the retrograde response directly and by inhibiting TORC1. Activation of the retrograde response extends lifespan.

Figure 2.

Integration of the retrograde response into the cell quality control network. The retrograde response activates prohibitin gene expression, promoting degradation of supernumerary and damaged mitochondrial inner membrane proteins. The retrograde response also activates expression of the ceramide synthase gene *LAC1*, whose product, ceramide, is a substrate for the synthesis of complex sphingolipids that inhibit general autophagy. This synthesis is mediated by Ipt1 and Skn1. Ceramide can block autophagy on its own, and it activates the Sit4-containing protein phosphatase 2A which has the same effect. This protein phosphatase is also activated by TORC1. Ceramide can also inhibit Hog1 protein kinase. Complex sphingolipids are also a source of ceramide when they are liberated by Isc1 after it translocates to the mitochondrion. Ceramide synthase is activated by TORC2 via the protein kinase Ypk2. Ceramide synthase is inhibited by the protein phosphatase calcineurin. TORC1 blocks the retrograde response. It also activates protein kinase Sch9, which is both an effector and a regulator of sphingolipid metabolism. TORC1 phosphorylates Atg13 in the Atg1-Atg13 complex that is essential for autophagy. Protein kinase A phosphorylates both of these proteins to block autophagy, and it also inhibits the retrograde response. The retrograde response stimulates stationary phase mitophagy with the participation of the Aup1 protein phosphatase. Hog1 plays an essential role in this process. The retrograde response also induces multi-drug resistance transporters, such as Pdr5, in the plasma membrane. I speculate that the retrograde response may also impinge upon age asymmetry in the segregation of damaged or dysfunctional mitochondria, either directly or through ceramide signaling.