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REVIEW

Comparing the yeast retrograde response and NF-κB stress responses: implications for aging

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Summary

The mitochondrial retrograde response has been extensively described in *Saccharomyces cerevisiae*, where it has been found to extend life span during times of mitochondrial dysfunction, damage or low nutrient levels. In yeast, the retrograde response genes (RTG) convey these stress responses to the nucleus to change the gene expression adaptively. Similarly, most classes of higher organisms have been shown to have some version of a central stress-mediating transcription factor, NF-κB. There have been several modifications along the phylogenetic tree as NF-κB has taken a larger role in managing cellular stresses. Here, we review similarities and differences in mechanisms and pathways between RTG genes in yeast and NF-κB as seen in more complex organisms. We perform a structural homology search and reveal similarities of Rtg proteins with eukaryotic transcription factors involved in development and metabolism. NF-κB shows more sophisticated functions when compared to RTG genes including participation in immune responses and induction of apoptosis under high levels of ROS-induced mitochondrial and nuclear DNA damage. Involvement of NF-κB in chromosomal stability, coregulation of mitochondrial respiration, and cross talk with the TOR (target of rapamycin) pathway points to a conserved mechanism also found in yeast.

Key words: Aging; retrograde response; stress; NFκB; RTG.

Introduction: evolutionary basis

Several stress-sensitive pathways, both oxidative stress related and others, have been conserved in humans. While humans and other mammals have developed specialized stress responses and methods to regulate them, such as the transcription factor nuclear factor – kappaB (NF-κB), the basis is still present in *Saccharomyces cerevisiae* and other simple eukaryotes such as *Caenorhabditis elegans* (Pujol *et al.*, 2001). One stress response mechanism in *S. cerevisiae* is the retrograde response, which is controlled by the stress-sensitive retrograde response (RTG) pathway that elicits transcriptional changes. The retrograde response links stress response to nutritional changes, mitochondrial dysfunction and aging, with adaptation of metabolism, chromatin-dependent gene regulation, and genome stability (Jazwinski, 2005). Hints that both pathways, NF-κB in humans and RTG in yeast, may be related, stems from the fact that NF-κB is activated in response to the introduction of mitochondrial dysfunction (Biswas *et al.*, 1999; Amuthan *et al.*, 2002). This warrants discussion of potential similarities between RTG genes and NF-κB.

Currently, there is a clear gap between the understanding of the NF-κB pathway that has been established in *C. elegans, Drosophila melanogaster,* and *Carcinoscorpius rotundicauda (horseshoe crab)* and higher organisms and the more primitive RTG pathway. Both pathways have in common that they are tuned to mitochondrial dysfunction and levels of oxidative stress (Butow & Avadhani, 2004). For instance, oxygen consumption and free radical production as the by-product of mitochondrial respiration, as initially outlined in the free radical theory of aging (Gerschman *et al.*, 1954; Harman, 1956), contribute to the accumulation of oxidized proteins and dysfunction that activates either pathway and extends life span. However, there are several clear differences between mammals and simple eukaryotes to consider that affect the conservation of these responses. The first is the development of organ systems and the fact that selection occurs at the organismal level – thus a response that is beneficial to a particular cell may not be so for the organism as a whole. The other is the development of an immune system and changes in stresses experienced, based on nutritional and environmental dependencies. The development of these other systems and stresses has led to the allocation of additional roles for these stress-mediating molecules. In this review, we take an evolutionary perspective to bridge the gap between these two well-characterized pathways: the yeast retrograde response and the mammalian/human NF-κB–mediated stress response.

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RTG pathway and aging

The retrograde response was discovered through the observation of a curious accumulation of nuclear transcripts in yeast cells lacking mitochondrial DNA (Parikh *et al.*, 1987). Subsequent studies identified some of these transcripts, and a genome-wide analysis demonstrated that the retrograde response activates genes involved in metabolism and stress responses that encode proteins destined for the mitochondrion, the cytoplasm, and peroxisomes (Epstein *et al.*, 2001). Concomitantly, several genes were implicated in the retrograde signaling pathway, including *RTG1*-3, discussed in detail here (Liao & Butow, 1993; Jia *et al.*, 1997a). Further efforts demonstrated the translocation of the Rtg1–Rtg3 transcription factor from the cytoplasm to the nucleus and the involvement of *RTG2* in this process (Sekito *et al.*, 2000). Rtg2 is a phosphatase with an ATP-binding domain similar to that of the Hsp70/actin/sugar kinase superfamily (Ferreira Junior *et al.*, 2005). When bound to Rtg2, the heterodimeric transcription factor complex Rtg1/Rtg3 can shuttle to the nucleus. Rtg3 contains several sites in its N-terminal region that are phosphorylated upon activation, and it translocates to the nucleus when dimerized with Rtg1. Otherwise, the Rtg3 nuclear localization sequence (NLS) is blocked, and the protein becomes dephosphorylated at these sites in the N-terminal region and returns to the cytoplasm (Ferreira Junior *et al.*, 2005).

A key element in the regulation of this pathway is the interaction of Rtg2 with Mks1 (Sekito *et al.*, 2002; Liu *et al.*, 2003; Ferreira Junior *et al.*, 2005). Mks1 is a negative regulator of the retrograde response; it was originally identified as a negative regulator of the Ras2-cAMP pathway (Matsuura & Anraku, 1993). In hyperphosphorylated form, Mks1 is bound to Bmh1 and Bmh2, yeast homologs of mammalian 14-3-3 proteins (Dilova *et al.*, 2004). This complex maintains Rtg3 in a hyperphosphorylated, cytoplasmic form. The efficient switch between the hypo- and hyperphosphorylated forms of Mks1 is facilitated by ubiquitination and degradation of partially phosphorylated Mks1. Strong activation of Mks1p therefore suppresses the retrograde response (Liu *et al.*, 2003, 2005).

The discovery of the involvement of Lst8, a component of the TOR (target of rapamycin) complex, in retrograde signaling was the first clue of the interaction between TOR signaling and the retrograde response (Liu *et al.*, 2001). It too is a negative regulator of the retrograde response like Mks1. However, TOR regulation of retrograde target gene expression is distinct from the activation of the retrograde response by mitochondrial dysfunction (Giannattasio *et al.*, 2005). In addition to its role in the translocation of Rtg1–Rtg3 to the nucleus, Rtg2 is a component of the transactivation complex SLIK (SAGA-like), and it is found at the promoters of retrograde target genes (Pray-Grant *et al.*, 2002). It is also essential for the suppression of trinucleotide repeat expansion (Bhattacharyya *et al.*, 2002). It has recently been shown that there is some overlap in the target genes of the retrograde response and caloric restriction in growing yeasts (Wang *et al.*, 2010). This overlap primarily involves the activation of the glyoxylate cycle, which facilitates the production of biosynthetic intermediates for cell growth.

Activation of the retrograde response increases yeast replicative life span (Kirchman *et al.*, 1999). This increase in yeast longevity requires *RTG2* and *RTG3* (Kirchman *et al.*, 1999; Borghouts *et al.*, 2004). It also requires *RAS2* (Kirchman *et al.*, 1999). Activation of the retrograde response in these studies was accomplished either by elimination of mitochondrial DNA to generate mitochondrial petite yeast or by the deletion of the *COX4* gene, both of which interrupt the electron transport chain to generate dysfunctional mitochondria. In some yeast strains, these manipulations do not activate the retrograde response and do not enhance replicative life span. However, switching to growth on raffinose rather than glucose, which relieves glucose repression allows activation and increased longevity (Kirchman *et al.*, 1999). Loss of mitochondrial DNA or lack of respiration ability is not essential for activation of the retrograde response. During normal yeast aging, there is a gradual loss of mitochondrial membrane potential, which is not the result of interruption of the electron transport chain, because the cells can still grow on nonfermentable carbon source (Muller, 1971; Lai *et al.*, 2002; Borghouts *et al.*, 2004). There is also a corresponding gradual activation of the retrograde response (Borghouts *et al.*, 2004). This activation compensates for the increasing mitochondrial dysfunction to enhance yeast longevity because it has been shown that the higher the induction of the retrograde response, the greater is the extension of yeast life span (Jazwinski, 2000). Consistent with the known negative effect of TOR signaling on the retrograde response, disruption of TOR signaling increases yeast replicative life span (Kaeberlein *et al.*, 2005). *RTG2* is also involved in chronological life span in yeast, as deletion of the gene curtails it (Barros *et al.*, 2004). Survival in stationary phase involves mitophagy in yeast, which is regulated by a conserved protein phosphatase, Aup1 (Journo *et al.*, 2009). The activation of retrograde signaling is defective in mutants missing this enzyme, and the deletion of *RTG3* results in defective autophagy in stationary phase (Journo *et al.*, 2009). This constitutes additional evidence that the retrograde response may be involved in chronological aging.

As mentioned earlier, *RTG2* is a component of the SLIK complex. SLIK is a transcriptional co-activator that shares many of its subunits with SAGA (Pray-Grant *et al.*, 2002). One of these is the histone acetyltransferase Gcn5. Deletion of *GCN5* attenuates the retrograde response in similar fashion to the deletion of *RTG2* (Kim *et al.*, 2004). SAGA overlaps with another transcriptional co-activator, TFIID, in its choice of promoters (Huisinga & Pugh, 2004). However, SAGA is specific for stress response genes, while TFIID activates housekeeping genes. These stress response genes are suppressed by corepressor complexes containing Rpd3 or Hda1 (Huisinga & Pugh, 2004). It has been suggested that the SLIK version of SAGA may extend the activity of this co-activator to the metabolic genes that are the targets of retrograde signaling (Jazwinski, 2005). *RTG2* links metabolism to stress responses, because it is proximal to the signal generated by dysfunctional mitochondria in the retrograde response,

and it is a component of SLIK which is involved in the activation of certain metabolic and stress response genes. Deletion of *RTG2* abrogates the retrograde response and prevents extension of replicative life span. Importantly, deletion of *RPD3* or *HDA1* has the expected opposite effect of enhancing yeast longevity (Kim *et al.*, 1999). The involvement of SLIK in the retrograde response links metabolism with chromatin-dependent gene regulation through *RTG2*.

The retrograde response also links metabolism with genome stability. *RTG2* is responsible for suppressing the generation of extrachromosomal ribosomal DNA circles or ERC (Borghouts *et al.*, 2004), whose accumulation can cause yeast death (Sinclair & Guarente, 1997). However, Rtg2 can only suppress ERC production when it is not engaged in retrograde signaling. Nevertheless, cells in which the retrograde response is induced are more resistant to the deleterious effects of ERC despite the unavailability of Rtg2 (Borghouts *et al.*, 2004). Disruption of the SLIK complex by deletion of *GCN5* prevents retrograde signaling, but it reduces ERC production in mitochondrial petites, presumably by making Rtg2 available to suppress their formation (Kim *et al.*, 2004).

NF-κB pathway and aging

NF-κB has accumulated various adaptive functions through its evolutionary history, and it has acquired 'multichannel processor' capabilities canalizing a spectrum of exogenous and endogenous stressors, immune responses, organelle dysfunction and aging (Salminen *et al.*, 2008a; Kriete & Mayo, 2009). The pathway participates in the signal transduction of the immune system, representing a bow-tie architecture with many upstream inputs, a number of central key proteins, and downstream fan outs to regulate a broad number of target genes (Kitano & Oda, 2006). The NF-κB family consists of several proteins that can be activated in part or jointly. For instance, TNF alpha induces five mammalian NF-kappaB/Rel proteins, c-Rel, NF-kappaB1 (p50/p105), NF-kappaB2 (p52/p100), RelA/p65, and RelB. These complexes associate with the cytosolic inhibitor IκB for retention in the cytoplasm. Phosphorylation and subsequent degradation of IκB by the IKK complex (IKKα, IKKβ, and IKKγ/-NEMO) unmasks a nuclear translocation signal, which shuttles NF-κB to the nucleus where it transcribes anti-apoptotic genes, cytokines, immunoreceptors, and adhesion molecules (Pahl, 1999; Hayden & Ghosh, 2004).

Activation of NF-κB by pro-inflammatory cytokines like TNF alpha is the canonical pathway, while activation in B-cells by a specific IKK signalosome complex is termed an alternative pathway. Other stress factors including oxidative stress are termed atypical mechanisms (Pahl, 1999). They include oxidative (Li & Karin, 1999), genotoxic (Janssens & Tschopp, 2006), organelle stress including the endoplasmic overdose response (Pahl & Baeuerle, 1995) and change in calcium homeostasis involving calcineurin and IκBβ (Biswas *et al.*, 2008). Dynamic regulation of NF-κB requires modulation of interorganelle trafficking by phosphorylation, masking and unmasking of the NLS. More recently, 14-3-3 phosphoserine and phosphothreonine-binding proteins, which play a role in many signaling pathways (Dougherty & Morrison, 2004), have been found to be involved in regulating location and binding of both IKBα and p65, both of which contain 14-3-3–binding domains (Aguilera *et al.*, 2006). Disrupting 14-3-3 activity by transfection with a dominant-negative 14-3-3 leads to the accumulation of nuclear p65–IκBα complexes and the constitutive association of p65 with the chromatin. It is unknown whether 14-3-3 proteins interact with other NF-κB heterodimers or whether 14-3-3 homologs, Bmh1p and Bmh2p, may play a similar role in RTG responses in yeast.

While most widely studied and characterized in mice and humans, the earliest traces of NF-κB can be found in the NF-κB p100 homolog in *C. rotundicauda* Relish (CrRelish), an early antibacterial defense mechanism in the horseshoe crab (Fan *et al.*, 2008). Orthologous proteins and domains have been found in even simpler organisms, such as the nematode *C. elegans*, though in much more primitive form and function (Fan *et al.*, 2008). The three main areas of NF-κB involvement are oxidative, immune-inflammatory, and protein stress responses. In addition to these cell-protective responses, NF-κB has been known to have pro-apoptotic functions at times, depending on the severity and genre of stress (Wang *et al.*, 2002).

Many transcription factors, particularly the ones that affect large regions of the genome, have been found to exert epigenetic effects – that is, alteration of the packaging of DNA that subsequently affects transcription of that area. NF-κB has been shown to act in this manner, perhaps contributing to the breadth of genes controlled by it (Vanden Berghe *et al.*, 2006). Epigenetic changes have been shown to contribute to the progression of aging as seen in the retrograde response as well (Pray-Grant *et al.*, 2002; Kim *et al.*, 2004), as discussed earlier. Both pathways are further implicated in epigenetic control, involvement in microRNA expression (Taganov *et al.*, 2006; Liang *et al.*, 2009), and in the control of mitochondrial processes through interactions with mtDNA (De Benedictis *et al.*, 2000). Previous work has found that although the epigenetic effects of NF-κB are variegated in their mechanisms, DNA methylation resulting in gene silencing is paramount (Vanden Berghe *et al.*, 2006).

Mitochondrial mutations and damage caused by ROS or otherwise have long been implicated in human aging (Harman, 1972; Bandy & Davison, 1990; Balaban *et al.*, 2005; Loeb *et al.*, 2005; Navarro & Boveris, 2007). It has been suggested that NF-κB, an important regulator of ROS scavengers (Xu *et al.*, 1999), may contribute to ubiquitously observed low ROS levels in aging cells and tissues, but damage such as oxidized proteins can accumulate and constitute another mechanism for NF-κB activation (Kriete *et al.*, 2010). Accordingly, NF-κB has been found constitutively upregulated in various aged tissues (Spencer *et al.*, 1997; Adler *et al.*, 2007) and in resting fibroblasts from older donors (Kriete *et al.*, 2008). Likewise, reduced ATP biosynthesis by damaged mitochondria may introduce an adaptive downregulation of biosynthesis through the TOR pathway, along with a compensatory activation of aerobic glycolysis

(Schieke *et al.*, 2006). Under these conditions, NF-κB and mTOR would work cooperatively and protectively but trap the cell in a lower-energy supplying state, beginning many of the trends we see in aging. However, without this particular stress response life span is reduced as shown in NF-κB knock-out mice (Lu *et al.*, 2006).

Protein homologies

Rtg1 and Rtg3 are basic helix-loop-helix/leucine zipper (bHLH/zip) transcription factors that heterodimerize to activate transcription from a novel R-box site (Jia *et al.* 1997b). bHLH/zip proteins comprise a large and important class of transcription factors (Moore *et al.*, 2000), including the Myc/Max/Mad network of proteins that play roles in cell proliferation, differentiation, and death (Grandori *et al.*, 2000). While the structures of the RTG proteins are not available yet, a structural modeling of Rtg1 and Rtg3 by I-TASSER server (Zhang, 2008) and the structural alignment using the *Vorometric* server (Sacan *et al.*, 2008) and protein visualization tools (Krieger *et al.*, 2002; Hanson, 2008) confirm the conservation of key bHLH/zip residues between Rtg1 and Myc and between Rtg3 and Max (Fig. 1). The residues around the DNA-binding sites of Rtg3 and Max are found to be particularly conserved (Fig. 2).

An amino acid sequence homology analysis shows the microphthalmia-associated transcription factor MITF to be the closest vertebrate homolog of both Rtg1 and Rtg3 (with 32% sequence identity between yeast Rtg1 and mouse MITF and 39% sequence identity between yeast Rtg3 and human MITF). MITF is a member of the MYC family of basic helix-loop-helix leucine zipper transcription factors (Hallsson *et al.*, 2007). It is conserved in both vertebrates and invertebrates and is important in the development of several different cell types, including melanocytes and retinal pigment epithelial cells (Steingrimsson *et al.*, 2004). The Rtg3 sequence is additionally found to be related to the transcription factor EB (38% identity with human TFEB), Max-like protein isoform X (35% identity with human MLX), transcription factor E3 (33% identity with human TFE3), and upstream stimulatory factor USF (29% identity with human USF2), all of which are bHLH/zip transcription factors. MITF, TFEB, and TFE3, along with TFEC, comprise a transcription factor family (MiT) that regulates key developmental pathways. They have been most widely studied in the context of renal translocation carcinomas (Argani & Ladanyi, 2005).

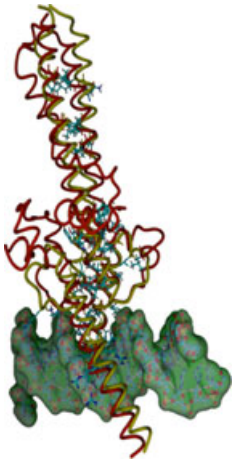

Fig. 1 Homology model of yeast proteins Rtg1 (front, red) and Rtg3 (back, red), structurally aligned with the human Myc protein (front, yellow) and Max protein (back, yellow) in complex with DNA (green molecular surface). The Myc/Max/DNA complex is taken from PDB id: 1nkp. The identical residues in the alignment are shown in stick configuration. Structures of Rtg1 and Rtg3 are modeled using I-TASSER server (Zhang, 2008), the structure alignments are obtained using Vorometric (Sacan *et al.*, 2008), and the figure is drawn using Yasara (Krieger *et al.*, 2002).

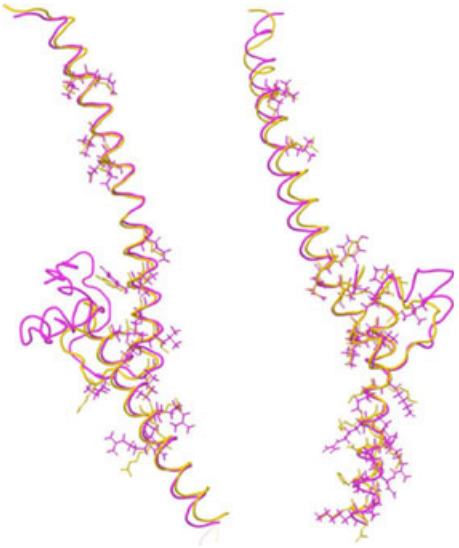

Fig. 2 A closer look into the structural alignments between Rtg1 (left, red) and Myc (left, yellow) and between Rtg3 (right, red) and Max (right, yellow). Identical residues in the alignments are shown in stick configuration. Myc/Max structures are taken from PDB id: 1nkp. I-TASSER (Zhang, 2008), Vorometric (Sacan *et al.*, 2008), and Jmol (Hanson, 2008) are used for modeling, structural alignment, and visualization, respectively.

The ubiquitously expressed USF factors are indicated to be key regulators of stress and immune responses, cell cycle and proliferation, and lipid and glucose metabolism. Interestingly, the overexpression of HINT1 tumor repressor gene inhibits both USF and NF-κB activity in human hepatoma cells (Wang *et al.*, 2009), which indicates the presence of a common regulation of USF and NF-κB. The structure of USF and its interaction with DNA are highly similar to those of Max (Ferre-D'Amare *et al.*, 1994).

The MLX protein mentioned previously encodes a MAX-like protein X and belongs to the class of bHLH/zip transcription factors. The MondoA-MLX heterodimers have been proposed as candidate sensors of glucose concentration (Sans *et al.*, 2006) and may be considered a phylogenetically related counterpart of Rtg1/3 in higher eukaryotes. Unlike other Myc proteins, MLX and its binding partner MondoA localize in the cytoplasm and are thought to be associated with the outer mitochondrial membrane (Sans *et al.*, 2006). MondoA:MLX complexes accumulate in the nucleus in response to glucose and activate a broad spectrum of metabolic genes (Stoltzman *et al.*, 2008). Note that MondoA:MLX has been proposed as a sensor for glucose levels,

and a direct correspondence between MondoA:MLX and RTG complex has not been previously suggested. It is not known whether MondoA:MLX can also respond to mitochondrial dysfunction.

It is worth noting that there are several difficulties in drawing parallels between related transcription factors across different organisms. Firstly, there is a lack of knowledge of their upstream regulators. Genetic screens have been particularly fruitful in determining some of the important players, such as Mks1p, a negative regulator of RTG-dependent gene expression (Liu *et al.*, 2003). However, the biochemical activity of these additional proteins is yet to be determined. Secondly, the genes identified in one organism may not have identifiable homologs in the other. For example, there is currently no known counterpart in higher eukaryotes for the Rtg2 protein as a sensor of mitochondrial dysfunction and a transducer of mitochondrial signals that activate Rtg1/3-like transcription factors. All that is known is that the sensors exist, as there are downstream transcription factors such as NF-κB that are associated with them. Thirdly, even for known homologs, not all of the protein domains are well characterized. For example, for Rtg1/3 proteins, the homology with Myc-related proteins is limited to the bHLH/zip domain, and we have little knowledge regarding the structure and function of the regions flanking that domain. Finally, the downstream targets of signaling modules diverge during evolution, while the modules themselves remain intact.

Conservation of the retrograde response

Is there a retrograde response that plays a role in determining life span in organisms other than yeast? It has been known for quite some time that with age there are changes in metabolism in *C. elegans* that resemble those found in the retrograde response, and similar changes are induced in *daf* mutants that display an increased life span (Vanfleteren & De Vreese, 1995). More recently, the use of RNAi to knock down gene expression has demonstrated the wide involvement of mitochondrial function in determining the life span of the worm (Dillin *et al.*, 2002; Lee *et al.*, 2003). The decline in mitochondrial function appears to trigger a retrograde response that increases life span (Cristina *et al.*, 2009). There may, however, be more than one pathway associated with this retrograde signaling in the worm (Yang & Hekimi, 2010).

In animal cells, the operation of a retrograde response has been discerned for some time (Butow & Avadhani, 2004) and is the topic of this article. The difficulty has been to find the common threads that link mitochondrial dysfunction with the induced patterns of gene expression in different cell types. A comparative study has found that there are some commonalities across cell types with many more differences noted (Miceli & Jazwinski, 2005a,b). The common changes in gene expression involve adaptations to the glycolytic production of energy necessary for cell survival, which bears significant similarities to the retrograde response in yeast. Furthermore, there is evidence that the induction of a retrograde response in human cells in culture results in a delay in replicative senescence and thus perhaps aging as well (Passos *et al.*, 2007).

As we develop evidence for relationships between human and yeast proteins participating in a retrograde response, we also find related pathways in yeast that are conserved in humans. One of the strongest relationships between the RTG genes and the oxidative stress-sensitive pathways lies in the TOR pathway; a protein linking the retrograde response and TOR, Lst8, provides this connection (Butow & Avadhani, 2004). Depending on the nature of the nutritional source, the degree of mitochondrial dysfunction, and the amount of Lst8p activity that acts as a switch-protein (Rosner *et al.*, 2009), different target genes downstream of *TOR1* and *RTG1/2/3* are activated (Giannattasio *et al.*, 2005). Both the kinase TOR and its mammalian counterpart mTOR have two functional complexes TORC1/2 and mTORC1/2 (Frias *et al.*, 2006). These complexes are dormant during stress and active when nutrients abound in the environment (Schmelzle & Hall, 2000; Wullschleger *et al.*, 2006). While TOR is usually bound to Lst8 or its mammalian ortholog, GβL (Diaz-Troya *et al.*, 2008), during stress it is separated, which inactivates the complex (TORC1/2) and leads to stress responses that contrast with the default TOR-mediated cellular functions. Notably, another mitochondrial back-signaling mechanism investigated in yeast involves the transcription factor and metabolic regulator SFP1 also interacting with the TORC1 kinase complex (Heeren *et al.*, 2009). In the NF-κB pathway, mTOR activates IKK resulting in activation of NF-κB. GβL is known to suppress TNFα-induced NF-κB signaling by interacting with IKKβ (Kim *et al.*, 2008). Conversely, AKT can stimulate IKK activity directed toward the phosphorylation of IκBα and RelA/p65 in cancer cells, and it in turn is controlled by mTOR (Dan *et al.*, 2008).

In addition to this connection to the TOR pathway, RTG pathways converge with the Ras pathways as well; these help to control stress resistance as well as life span in *S. cerevisiae* (Shama *et al.*, 1998). Whereas in humans *RAS* is best known as a proto-oncogene, it has critical functions in controlling respiration and redox balance in yeast (Heeren *et al.*, 2004). Both *RAS* genes are GTP-binding proteins and activate cAMP-dependent cascades and show strong homology; though evolution has led them to take different roles in each organism (Wigler *et al.*, 1988). As a result of exerting control of cell survival during periods of oxidative stress, as it does in humans, it can extend or limit life span, dependent on transcriptional levels. In fact, much of the pathway itself is similar in humans and yeast in terms of activation, function, and constituents. This is very similar to the effect of NF-κB, which diverts the cell to replicative or quiescent states depending on the amount, duration, and types of stresses faced by the cell (Schoemaker *et al.*, 2002; Wang *et al.*, 2002). Other related pathways have proven homologous or otherwise similar in simpler organisms including sirtuins, which have been shown to inhibit NF-κB and inflammation-associated cascades in humans. In *S. cerevisiae* and *C. elegans*, they have been shown to be involved in aging (Salminen *et al.*, 2008b). While NF-κB and sirtuins have a more complex role in humans, their primordial role in both

aging and inflammation has been established in *C. elegans*. Although no complete homology has been shown between the *RTG* genes in *S. cerevisiae* and NF-κB, strong homologies between inhibitors and pathways of both lead one to believe that the retrograde response is a potential predecessor of the now-central stress-regulator, NF-κB.

Intriguingly, the c-MYC protein, which shows homology with Rtg3, has two identified NF-κB–binding sites (Duyao *et al.*, 1990a,b) and is involved in the regulation of glycolysis, suggesting that these mechanisms are indeed related. Furthermore, upregulation of peroxisomal activities in mammalian cells contributes to ROS production, and peroxisome proliferator-activator receptors stimulate NADPH oxidase in macrophages (Teissier *et al.*, 2004), which in unison may represent an evolved defense mechanism under control of NF-κB with ancient roots in the yeast retrograde response, which is known to activate peroxisomal-related target genes.

Summary

As described here, the current research has closed in on a relationship between the RTG pathway in yeast and the more sophisticated NF-κB pathway. As an organism becomes more complex, especially in its immune system, it seems that NF-κB takes on a larger role, eventually becoming a central transcription factor in most major stress responses as in humans. Many studies have elaborated on the various patterns of gene expression resulting from different stressors in yeast, while others have looked at progressively less complex organisms and searching for NF-κB or homologous transcription factors. Not only has NF-κB been found in simpler organisms but its mechanisms of activation and relationship with various stressors have been found to be more primitive as well (Fan *et al.*, 2008). The variants or versions of NF-κB found in simpler organisms like *C. elegans* have little or no immune involvement; though, the human and fly variants are heavily connected with viral responses (Pujol *et al.*, 2001).

As summarized in Fig. 3, a differentiation has to be made under which conditions both stress responses occur. Basal levels of RTG and NF-κB contribute to chromosomal stability. If pharmacologically inhibited or in knock-outs, life span in both organisms is compromised. Under nutritional stress and intact mitochondria, RTG facilitates metabolic adaptations and survival. In mammalian cells, periods of inflammation are a comparable transient situation, and without NF-κB the cell would enter apoptosis. In some viral infections this may be accompanied by dysfunctional mitochondria. In aging, the respiratory capacity of mitochondria becomes compromised, and both pathways become chronically upregulated, which correlates with metabolic adaptations, which may be further modulated by involvement of the TOR pathway. While both pathways provide a pro-survival and adaptive characteristic, an important difference between RTG and NF-κB is their involvement in epigenetic regulation long term. High levels of NF-κB contribute to a tumor promoting chromosomal instability, particularly seen in many age-related diseases. In yeast, RTG2 supports chromosomal

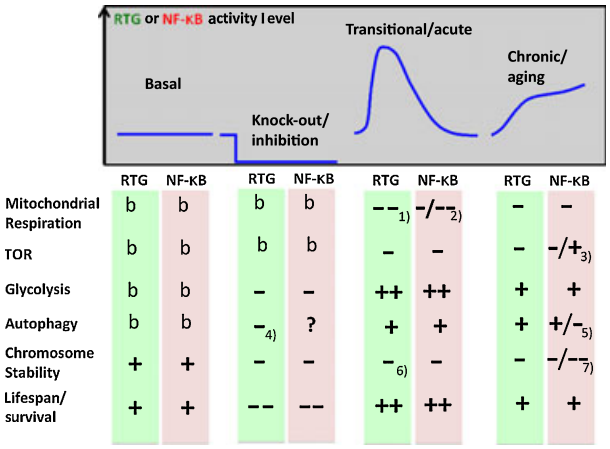

Fig. 3 Comparison of the RTG and NF-κB pathways. The four phases showing different levels of activity are a stationary phase, inhibition or knock-out, a transitional acute phase of nutritional stress in yeast and acute inflammation in mammalian cells, and chronic elevation in aging. Influential of both pathway are mitochondrial respiration and TOR activity, modulating glycolysis, autophagy, chromosome stability and lifespan. Activity is coded by 'b' = basal levels, '+' enhanced, '++' strongly enhanced, '−' suppressed and '−−' strongly suppressed. Explanations: 1) The retrograde response is compensatory for mitochondrial dysfunction; 2) In infection, mitochondria are working either at highest capacity, which activates the endoplasmic overdose response (EOR) and NF-κB, or the mitochondria are dysfunctional, but without either response cells go into apoptosis; 3) TOR can initially decrease, but later increase activity in senescence and age-related diseases; 4) Inhibition of Rtg3 reduces autophagy; 5) Autophagy is related to the behavior of TOR in 3); 6) Rtg2 contributes to epigenetic stability when it is not bound; 7) Chromosomal instability is high in aging and highest in cancer. See text for further details.

stability when it is not bound and not only when overexpressed (Borghouts *et al.* 2004)

Activation of the mitochondrial-to-nucleus pathway both in yeast and mammals and the bigenomic regulation of mitochondrial proteins opens the intriguing possibility that these mechanisms are connected, providing a closed feedback loop. Therefore, in times of stress involving the mitochondria, the retrograde response could, in principle, feedback to the mitochondria. In response to deficient mitochondria, some studies of cells harboring deleterious mtDNA mutations had indicated an increase in expression of genes encoding mitochondrial proteins to promote mitochondrial biogenesis (Heddi *et al.*, 1999), while in yeast ρ^0 petite cells no upregulation of these genes has been found (Epstein *et al.*, 2001). Similarly in mammalian cells, NF-κB has been shown to have a role in mitochondrial biogenesis involving the transcription factor YY1 (Sui, 2009), and at the same time, YY1 also activates c-MYC. In aging, however, reports on downregulation of mitochondrial genes encoded in the nucleus are ubiquitous throughout the literature, albeit counterintuitive. Lowering mitochondrial respiration under stress conditions reduces oxidative phosphorylation but promotes glycolysis for ATP production and prevents high ROS levels. Indeed, application of mild heat stress in yeast has shown a downregulation of mitochondrial genes encoded in the nucleus (Sakaki *et al.*, 2003) and inhibition of mitochondrial respiration prolongs longevity in nematodes (Cristina *et al.*, 2009). Because mitochondrial respiration is under bigenomic regulation, all COX

genes have been found to be under control of NRF as well as under regulation of the mitochondrial genome. Notably, IkBα/p50 dimers have been found to be directly located in the mitochondria. If activated by external stress factors, such as TNFα, p50 homodimers are formed interacting with the transcriptional machinery of the mitochondria and downregulating cytochromes and cox III encoded by the mitochondrial genome (Cogswell *et al.*, 2003). Furthermore, IKBα has been found to interact with ANT, the mitochondrial ATP/ADP translocator, upon induction of apoptosis (Bottero *et al.*, 2001).

Although no complete homology has been shown between the *RTG* genes in *S. cerevisiae* and NF-κB, strong homologies between inhibitors and pathways of both lead one to believe that the retrograde response is a potential predecessor of the now-central stress-regulator, NF-κB. We have pointed to the function of LST8p as a conserved connecting element of the TOR pathway and RTG/NF-κB complexes. Furthermore, we have mentioned 14-3-3 proteins, relevant to the nucleocytoplasmic transport of IKBα and p65, and the interaction of Mks1p with the yeast homolog of 14-3-3, Bmh1p. We also have discussed the c-MYC protein, which shows homology with Rtg3 and the role of the retrograde response in peroxisomal processes. Therefore, insight from investigations from either side may help to identify and decipher hitherto unknown mechanisms.

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