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Mitochondrial Signaling: The Retrograde Response

Review

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Mitochondrial retrograde signaling is a pathway of communication from mitochondria to the nucleus that influences many cellular and organismal activities under both normal and pathophysiological conditions. In yeast it is used as a sensor of mitochondrial dysfunction that initiates readjustments of carbohydrate and nitrogen metabolism. In both yeast and animal cells, retrograde signaling is linked to TOR signaling, but the precise connections are unclear. In mammalian cells, mitochondrial dysfunction sets off signaling cascades through altered Ca^{2+} dynamics, which activate factors such as NFκB, NFAT, and ATF. Retrograde signaling also induces invasive behavior in otherwise nontumorigenic cells implying a role in tumor progression.

Research on mitochondria has evolved from bioenergetics to biogenesis, the genetic functions of mitochondrial DNA (mtDNA), and diseases associated with mitochondrial dysfunction. Although these areas continue to be investigated vigorously, a new era in mitochondrial research has emerged that concerns the role of this organelle in intracellular signaling—a process that is likely to have far-reaching implications in development, aging, disease, and environmental adaptation. Retrograde regulation is the general term for mitochondrial signaling, and is broadly defined as cellular responses to changes in the functional state of mitochondria. Implicit in this definition is that mitochondrial signaling is opposite in direction to that of the more familiar anterograde regulation characterized by the transfer of information and material from the nucleus and cytoplasm to mitochondria. Retrograde responses are effected by metabolic cues or by more direct routes, such as mitochondria-related changes in intracellular Ca^{2+} dynamics, all of which culminate in wide-ranging changes in nuclear gene expression. The outcome is usually a recasting of metabolic, regulatory, or stress-related pathways. These retrograde responses are for the most part adaptive in that they represent cellular adjustments to altered mitochondrial states. They are distinct from mitochondria-initiated apoptosis—the most extreme example of mitochondrial signaling—which occurs upon release of cytochrome c and other proapoptotic factors from the organelle. With many excellent reviews on apoptosis (see, for example, Wang, 2001), we shall restrict our discussion here to an examination of adaptive retrograde signaling pathways, focusing largely on yeast and animal cells.

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The Big Picture

The most detailed information on the scope and regulation of the retrograde response has been obtained with the budding yeast, *Saccharomyces cerevisiae*. Analysis of the consequences of mitochondrial dysfunctions, for which *S. cerevisiae* is well suited, includes genome-wide transcriptional profiling of respiratory-competent (ρ^+) versus respiratory-deficient cells lacking mtDNA (ρ° petites). Two such studies (Epstein et al., 2001; Traven et al., 2001) have yielded somewhat different results. One study (Traven et al., 2001) concluded, for example, that ρ° cells upregulate the expression of many genes involved in the biogenesis and function of mitochondria, including those of the tricarboxylic acid (TCA) cycle, mitochondrial protein import pathways, and the oxidative phosphorylation apparatus. In the other study (Epstein et al., 2001), expression of many of those same genes was either unaffected or was downregulated in ρ° cells.

How might these differences be reconciled? One possibility is the choice of carbon source in the growth medium—glucose in one case (Traven et al., 2001) and raffinose, a nonrepressing but fermentable carbon source, in the other (Epstein et al., 2001). Many of the genes whose expression was elevated in ρ° cells in Traven et al. (2001) are glucose repressible, such as those encoding mitochondrial proteins, and are thus induced during the diauxic shift when glucose becomes exhausted from the medium (DeRisi et al., 1997). Superimposition of expression changes caused by derepression would not be a factor in cells grown on raffinose. Another possibility is strain difference: Traven et al. (2001) used a strain (W303) which has been reported to have a weak or nonexist retrograde response of *CIT2* (Kirchman et al., 1999), a prototypical retrograde-responsive gene (Liao et al., 1991), whereas Epstein et al. (2001) used a strain (PSY142) with a robust *CIT2* retrograde response. Although it is not clear what accounts for these strain differences, it is likely that parallel retrograde pathways with different targets and effectors operate in response to mitochondrial perturbations. This same generality also seems to apply to animal cells.

A key function of the retrograde response in respiratory-deficient yeast cells is the maintenance of glutamate supplies, which would otherwise be compromised because of the respiratory-deficient state (Figure 1). In hindsight, these metabolic readjustments make sense given that in respiratory-deficient cells, the TCA cycle fails to operate as a full cycle (succinate cannot be oxidized to fumarate). This limits the production of oxaloacetate (OAA), and in turn α-ketoglutarate, the direct precursor to glutamate. To compensate, respiratory-deficient cells induce the expression of many genes whose products function in (anaplerotic) pathways that would resupply mitochondria with OAA and acetyl-CoA. Thus, for example, there is a dramatic proliferation of

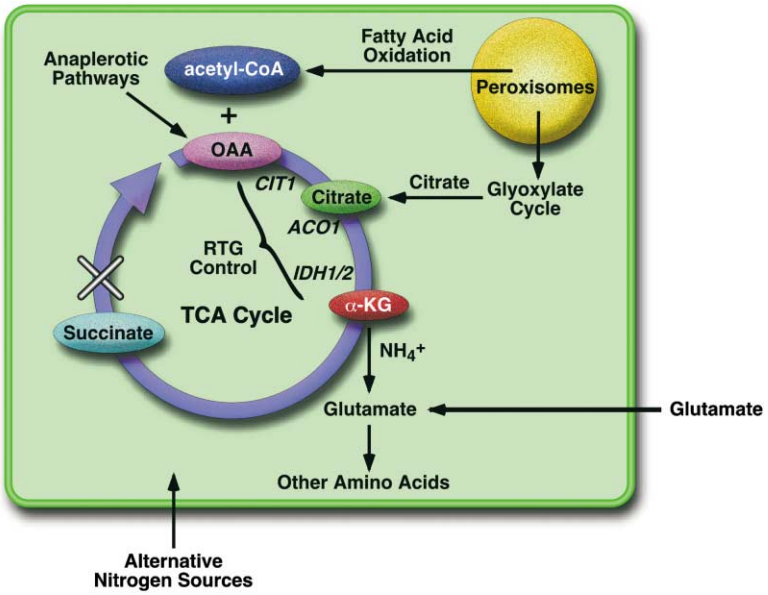

Figure 1. Retrograde Induction of Metabolic Pathways in Respiratory-Deficient Cells

The retrograde pathway enables respiratory-deficient cells to maintain glutamate supplies by providing substrates for α-ketoglutarate synthesis through anaplerotic pathways and increased transport into cells of glutamate and alternative nitrogen sources.

peroxisomes and an attendant increase in fatty acid oxidation (a peroxisomal activity in yeast) that results in an increase in acetyl-CoA production. In addition, glyoxylate cycle activity increases (the citrate synthase isoform encoded by *CIT2* is part of the glyoxylate cycle), and there is increased expression of genes encoding transporters that function to facilitate the transport of key metabolites such as acetyl-CoA into mitochondria and glutamate and other nitrogen sources into cells. Finally, expression of the genes encoding the first three steps of the TCA cycle which produce α-ketoglutarate comes under the control of retrograde-specific regulatory genes in cells that lack or have reduced mitochondrial respiratory activity (Liu and Butow, 1999). These findings reflect the drive of the retrograde pathway to synthesize α-ketoglutarate and hence to maintain glutamate supplies.

Directed defects in the TCA cycle in otherwise respiratory-competent cells also give rise to a robust retrograde response (Chelstowska and Butow, 1995). Genome-wide transcriptional analysis of the consequences of systematic inactivation of each of the 15 genes encoding a subunit polypeptide of the eight TCA cycle enzymes reveals a striking and complex regulatory network of genes whose expression in various combinations changes in response to specific TCA cycle defects (McCammon et al., 2003). For the most part, inactivation of genes encoding different subunits of the same TCA cycle enzyme complex elicit a similar response, which probably reflects a general change in gene expression away from respiratory metabolism.

A curious outcome of the systematic, one-by-one inactivation of TCA cycle genes is that the pattern of expression of responder genes (some of which also encode TCA cycle proteins) alternates between elevated and depressed expression as one moves pairwise around the TCA cycle; that is, responder gene expression is elevated in aconitase and isocitrate dehydrogenase mutants, depressed in α-ketoglutarate dehydrogenase and succinyl-CoA ligase mutants, elevated in succinate dehydrogenase and fumarase mutants, and so on. A plausible explanation for this unusual pattern of gene expression is that the levels of metabolites that are substrates or products of these pairwise, contiguous steps of the TCA cycle—oxaloacetate, isocitrate, succinyl-Co,A and fumarate—are themselves signaling molecules that effect these changes in gene expression. TCA cycle defects of this type have been shown to have significant consequences in humans. In particular, succinate dehydrogenase mutations give rise to brain tumors (Niemann and Muller, 2000), and fumarase defects were found to be associated with dominantly inherited uterine fibroids, skin leiomyomata, and renal cell cancers (Tomlinson et al., 2002). These findings suggest that these TCA cycle enzymes can function as tumor suppressors. Whether these effects relate to the similarity in patterns of gene expression in response to inactivation of succinate dehydrogenase and fumarase in yeast remains to be established.

The *RTG*-Dependent Pathway of Retrograde Signaling

The most detailed information on the mechanism of retrograde signaling comes from studies of the *CIT2* gene. Since its discovery as a retrograde-responsive gene (Liao et al., 1991), *CIT2* has served as a prototypical readout for the yeast retrograde response. Depending on the strain, *CIT2* expression may be upregulated as much as 50- to 60-fold in cells with dysfunctional mitochondria. Retrograde regulation of *CIT2* expression has turned out to be surprisingly (and richly) complex. Three positive regulatory factors, Rtg1p, Rtg2p, and Rtg3p (Liao and Butow, 1993; Jia et al., 1997), and four negative regulators, Mks1 (Dilova et al., 2002; Sekito et al., 2002; Tate et al., 2002), Lst8p (Liu et al., 2001), Bmh1p, and Bmh2p (Liu et al., 2003), have been described that control both basal and retrograde-induced *CIT2* expression (Figures 2 and 3). In addition, *CIT2* expression is positively regulated by Cyc8 (Conlan et al., 1999), a compo-

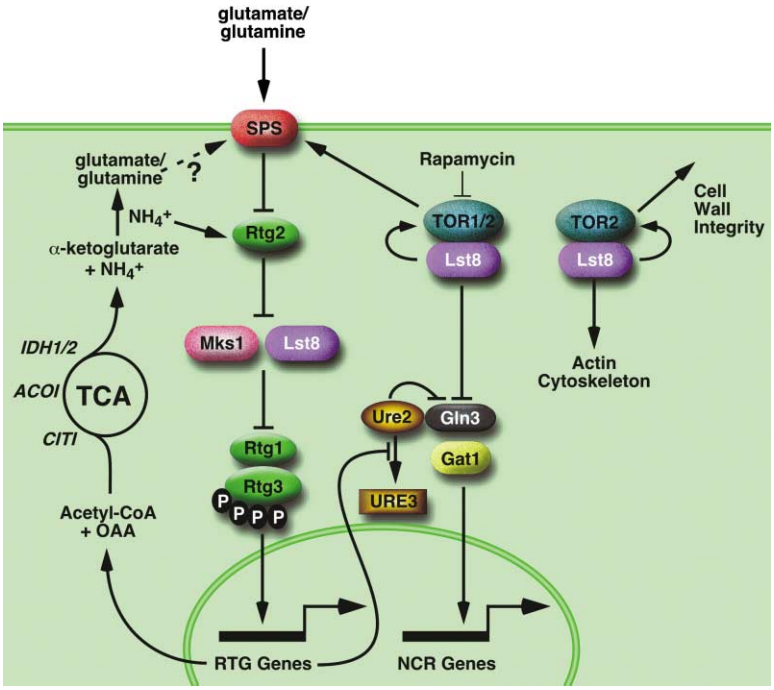

Figure 2. Regulation of *RTG*-Dependent Gene Expression

Positive and negative regulators of the RTG pathway and interactions with TOR signaling are shown. TOR is proposed to regulate the SPS amino acid sensor system that acts upstream of Rtg2. Expression of RTG genes also regulates the formation of [URE3] (Sekito et al., 2002), which is an inactive form of a negative regulator of the NCR pathway, Ure2 (Wickner, 1994).

nent of a complex (Ssn6) containing Tup1 that functions mostly as a general transcriptional corepressor.

Rtg1p and Rtg3p are basic helix-loop-helix-leucine zipper (bHLH-Zip) transcription factors that heterodimerize to activate transcription at a novel site, GTCAC, called an R box (Jia et al., 1997). Rtg3p contains both N- and C-terminal transactivation domains, whereas no transaction domain has been identified in Rtg1p. An AD1 activation motif important for interaction of certain transcription factors with the SAGA histone acetyltransferase/transcriptional adaptor complex has also been identified near the N terminus of Rtg3p (Massari et al., 1999). Rtg2p is novel regulator of the yeast retrograde response pathway. It has an N-terminal ATP binding motif, similar to that found in the Hsp70/actin/sugar kinase superfamily of ATP binding proteins (Koonin, 1994), whose integrity is essential for Rtg2p function (Liu et al., 2003). Rtg2p acts upstream of the Rtg1p/Rtg3p transcriptional complex (Sekito et al., 2000), where it is both a proximal sensor of mitochondrial dysfunction and a transducer of mitochondrial signals that enables the Rtg1/3p complex to activate transcription of target genes (Figure 2). Although there is much to be learned about how Rtg2p senses mitochondrial dysfunction, we are beginning to understand how it regulates the Rtg1/3p complex (Sekito et al., 2000). When the RTG pathway is off, Rtg1p and Rtg3p are sequestered together in the cytoplasm where Rtg3p is phosphorylated at multiple sites on the protein; when the retrograde pathway is activated, Rtg3p becomes partially dephosphorylated and enters the nucleus, as does Rtg1p, where these proteins assemble at R box sites to activate transcription. In cells lacking Rtg2p, Rtg3p becomes hyperphosphorylated and the Rtg1/3p complex remains cytoplasmic, no longer responsive to retrograde signals. Thus, the primary control point for the *RTG*-dependent retrograde response is the specification of the intracellular localization of the Rtg1/3p complex. That strategy of transcriptional control is used by many systems, but Rtg2p appears to play a unique role in dictating where the Rtg1/3p complex resides. The relevant kinase(s) and phosphatase(s) that modify Rtg3p are presently unknown.

How does Rtg2p control the location of the Rtg1/3p complex? Genetic screens designed to search for mutants that could bypass the requirement for Rtg2p have been particularly fruitful in addressing this question (Liu et al., 2001, 2003; Sekito et al., 2002). Among the factors revealed by this screen was Mks1p, which was independently identified by two other laboratories as a negative regulator of *RTG*-dependent gene expression (Dilova et al., 2002; Tate et al., 2002). As is the case for other negative regulators of the RTG pathway, *CIT2* expression in cells in which *MKS1* is inactivated is constitutively high, insensitive to glutamate repression and independent of Rtg2p. A key insight into the role of Rtg2p as a master regulator of the RTG pathway came from observations that Rtg2p interacts dynamically with Mks1p (Sekito et al., 2002; Liu et al., 2003). When the RTG pathway is on, Mks1p, a phosphoprotein, is present in a largely dephosphorylated form complexed with Rtg2p; when the pathway is off, Mks1p becomes more phosphorylated, no longer interacts with Rtg2p, and is in a complex with the 14-3-3 proteins Bmh1p and Bmh2p (Figure 3). Although the biochemical activity of Mks1p responsible for preventing nuclear accumulation of Rtg1/3p remains to be determined, it seems that Mks1p must be complexed with Bmh1p and Bmh2p for it to act as a negative regulator of the RTG pathway. In this respect, Bmh1p and Bmh2p, which have redundant functions (van Heusden et al., 1995), are also negative regulators of the pathway (Liu et al., 2003).

The interaction of Mks1p with Rtg2p is dependent on the integrity of the Rtg2p ATP binding domain (Liu et

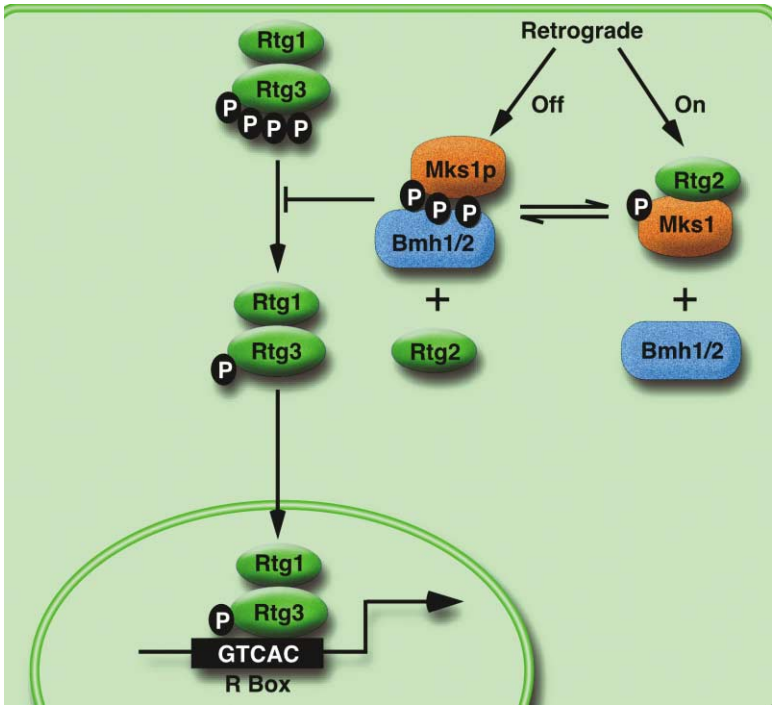

Figure 3. Regulation of *RTG*-Dependent Gene Expression by a Dynamic Interaction between Rtg2p and the Negative Regulator Mks1p

Mks1p, when bound to Rtg2p, is inactive; Mks1p bound to the 14-3-3 proteins Bmh1p and Bmh2p is the form that prevents Rtg1p and Rtg3p from translocating from the cytoplasm to the nucleus.

al., 2003). Rtg2p mutants with point mutations located in or close to conserved regions of that domain not only fail to interact with Mks1p, but they also fail to support *RTG* target gene expression. Most likely, ATP or ATP hydrolysis is required to cycle Mks1p binding to Rtg2p. Finally, despite a large body of evidence indicating that Rtg2p is cytoplasmic and regulates the RTG pathway there, evidence has been presented that Rtg2p might also have a function in the nucleus, acting directly at the *CIT2* promoter (Pray-Grant et al., 2002), and in triplet repeat instability of genomic DNA (Bhattacharyya et al., 2002). The latter effect, however, seems unrelated to retrograde signaling.

Because of the link between respiratory activity and glutamate biosynthesis, inhibition of the RTG pathway by inactivation of any one of the *RTG* genes in respiratory-deficient cells, or in ρ^+ cells with reduced mitochondrial respiration, results in glutamate auxotrophy (Liao and Butow, 1993; Liu and Butow, 1999). As noted previously, this is because the expression of TCA cycle genes responsible for the synthesis of α-ketoglutarate, *CIT1*, *ACO1*, *IDH1*, and *IDH2*, which are regulated by the Hap2/3/4 transcriptional complex in cells with robust respiratory activity (Rosenkrantz et al., 1994), come increasingly under the control of the *RTG* genes as respiratory activity declines (Liu and Butow, 1999). This transcriptional switch underscores the role of the RTG pathway in tailoring carbohydrate metabolism to the needs of respiratory-deficient cells. Even in respiratory-competent cells, glutamate starvation strongly activates the RTG pathway (Liu and Butow, 1999); in other words, glutamate is a potent repressor of *RTG*-dependent gene expression. Although the RTG pathway is clearly linked to glutamate homeostasis, there remain conflicting views as to what metabolite(s) actually signals *RTG*-dependent gene expression. Confounding this issue are profound strain-dependent differences in the responses of both the RTG and nitrogen catabolite repression (NCR) pathways to different nitrogen sources (Tate and Cooper, 2003). Glutamine rather than glutamate has been proposed to be the signaling molecule for the retrograde response (Crespo et al., 2002), and ammonium has been suggested to be a positive regulator of the pathway (Tate and Cooper, 2003). Because of facile metabolic interconversions, pinpointing regulatory metabolites is not straightforward, so that defining those molecules that directly regulate the RTG and NCR pathways may well require more precise biochemical reconstitution approaches.

Lst8p and the RTG-TOR Connection

Lst8p, an essential protein with seven WD40 repeats, is also a negative regulator of the RTG pathway (Liu et al., 2001). *LST8* was first identified from the properties of a mutant allele, *lst8-1*, as a synthetic lethal with *sec13* (Roberg et al., 1997). The *lst8-1* mutant, which contains a point mutation near the C terminus of the protein (Liu et al., 2001), was shown to cause a defect in the sorting of the general amino acid permease Gap1p to the plasma membrane (Roberg et al., 1997). Other mutant alleles of *LST8* were identified in an *rtg2*Δ bypass screen affecting *CIT2* expression, and the mutations clustered together in two adjacent WD repeats (four and five) located on the opposite side of the protein from the *lst8-1* mutation in repeat one (Liu et al., 2001). Subsequently, additional *lst8* mutant alleles were isolated (Chen and Kaiser, 2003), and together these mutants could be phenotypically grouped into two classes with respect to RTG retrograde signaling: class I mutants cannot bypass the requirement for Rtg2p, whereas class II mutants can. *CIT2* expression in both classes of mutants is constitutively high and relatively insensitive to glutamate

repression. Thus, Lst8p is not only a negative regulator of Rtg1/3p-dependent gene expression, but it appears to regulate the pathway at two sites, one upstream and the other downstream of Rtg2p (Figure 2). One attractive possibility is that these two sites of Lst8p regulation are the result of domain-specific interactions of the protein suggested by the different locations of the point mutations in the class I and class II mutants.

The inability of the class I mutants like *lst8-1* to sense external glutamate may be because of a defect in the SPS (*SSY1, PTR3, SSY5*) amino acid sensing system (Liu et al., 2001). The SPS sensor consists of Ssy1p, a plasma membrane amino acid sensor resembling amino acid permeases, and at least two peripheral membrane proteins, Ptr3p and Ssy5p, which probably associate with Ssy1p; together, these proteins function in a signal transduction cascade in response to external amino acids, such as glutamate (Forsberg et al., 2001). Inactivation of the SPS sensor system, or inactivation of *SHR3*, a gene that functions in the targeting of Ssy1p to the plasma membrane (Klasson et al., 1999), results in elevated *CIT2* expression and a loss of glutamate repression (Liu et al., 2001). Nevertheless, *CIT2* expression in SPS-inactivated cells still requires Rtg2p. In these respects, the effects of the *lst8-1* mutation and inactivation of the SPS sensor system on the RTG pathway are indistinguishable. By contrast, the class II *lst8* mutants, which are epistatic to *rtg2*Δ, must be downstream of the SPS-sensing pathway. A plausible interpretation of these findings is that the class I mutants affects the assembly or function of the SPS sensor system.

An intriguing connection between the RTG pathway and nutrient sensing was uncovered by the finding that Rtg1/3p-dependent gene expression is activated in cells in which TOR (target of rapamycin) signaling mediated by the PI kinase-related kinases, Tor1 and Tor2, is inhibited by the immunosuppressant rapamycin (Komeili et al., 2000). As is the case for retrograde signaling, activation of Rtg1/3p by rapamycin is completely dependent on Rtg2p. TOR signaling is conserved from yeast to man and is involved in diverse cellular processes, including growth control and nutrient sensing (Jacinto and Hall, 2003). Thus, for example, inhibiting TOR is a nitrogen starvation signal that results in the induction of genes normally repressed when good nitrogen sources (e.g., glutamine) are available. These genes are induced when cells are faced with nitrogen starvation or poor quality nitrogen sources (e.g., proline). The regulation of expression of genes in the NCR pathway is determined by the intracellular localization of the GATA transcription factors Gln3p and Gat1p/Nil1p (Beck and Hall, 1999; Cardenas et al., 1999), which move from the cytoplasm to the nucleus in rapamycin-treated cells, just as do Rtg1p and Rtg3p (Komeili et al., 2000) (Figure 2). Finally, a relation between TOR and mitochondrial activity has also been uncovered in animal cells in which compromised mitochondrial function or oxidative stress inhibits the kinase activity of the mammalian TOR cognate, mTOR (Kim et al., 2002). What the mitochondria-generated signals are that impinge on mTOR activity, however, is unknown.

The relation between TOR signaling and the RTG pathway is underscored by the finding that Lst8p together with other proteins is an integral component of the Tor1/2 and Tor2 complexes (Loewith et al., 2002; Chen and Kaiser, 2003; Wedaman et al., 2003), also referred to as TORC1 and TORC2 (Loewith et al., 2002). Moreover, the mammalian TOR cognate, mTOR, also contains a homolog of Lst8p (Loewith et al., 2002; Kim et al., 2003), suggesting an evolutionarily conserved function. What is the role of Lst8p in TOR signaling and does Lst8p have a function independent of TOR? Although it does not appear that the association of Lst8p with Tor1/2 is affected by rapamycin treatment (Wedaman et al., 2003) or by the *lst8* mutations (Chen and Kaiser, 2003), Lst8p depletion experiments showed that Rtg1/3p-dependent transcription was activated, similar to the effect of rapamycin treatment (Loewith et al., 2002); in addition, there was a defect in the polarization of the actin cytoskeleton, a function ascribed to TORC2.

Using a collection of *lst8* mutants, Chen and Kaiser (2003) came to similar conclusions regarding TORC1. Although they did not observe any effects of their *lst8* mutants on the actin cytoskeleton, they did note that cell wall integrity, a function of TORC2, was compromised in *lst8* mutant cells. Together with the Lst8p depletion studies (Loewith et al., 2002), these findings lead to the conclusion that Lst8p functions as a positive regulator of TOR. But as is often the case, the devil is in the details. Depletion of Lst8p, for example, activates Rtg1/3p-dependent gene expression but fails to activate Gln3p target genes (Loewith et al., 2002); rapamycin inhibition of TOR, however, activates both pathways. This differential effect of Lst8p depletion on the RTG and NCR pathways is also observed for some *lst8* mutant alleles (Chen and Kaiser, 2003); moreover, activation of the RTG pathway in those mutants is independent of Rtg2p. One possibility is that signaling by the Lst8p-TOR complexes can have different outcomes depending, say, on how specific targets might interact with Lst8p. Alternatively, not all of the cellular Lst8p may be functionally associated with TOR even though both classes of *lst8* mutants increase the intracellular glutamate and glutamine levels as a result of derepression of the RTG pathway (Chen and Kaiser, 2002). Indeed, biochemical studies suggest that not all of the Lst8p is associated with the TOR complexes (Loewith et al., 2002).

These complexities also raise the issue whether the effects of TOR on the RTG pathway are direct or indirect. Both RTG and TOR are involved in nutrient sensing, but do they operate in parallel, or are they part of a single nutrient sensing cascade? First, the *RTG*-dependent retrograde response is very robust in cells with defective mitochondria even when those cells are grown in rich medium—a condition in which the TOR kinases are active and the NCR genes are repressed. Second, rapamycin treatment of cells, which always leads to induction of NCR genes, sometimes fails to induce *CIT2* expression, an effect that is dependent on the nitrogen source in the medium (Tate and Cooper, 2003). These results are difficult to reconcile with the notion that TOR is a direct regulator of the RTG pathway. One explanation is that TOR affects the RTG pathway only indirectly, for example, by regulating the activity of SPS sensor system (Figure 2). That regulation could be a function of the TOR-Lst8p complex as suggested by the properties of the *lst8-1* mutant in which *CIT2* expression is

relatively insensitive to glutamate repression but is still dependent on Rtg2p. It would then follow that Lst8p regulation of Rtg1/3p target gene expression that is independent of Rtg2p might reflect the pool of Lst8p not associated with the TORC complexes. Clearly, more studies will be required to understand how Lst8p regulates both TOR and the RTG pathway.

Retrograde Signaling and Aging

The mechanisms that define and control an organism's life span are at once complex, controversial, and confounding. Mitochondria have figured prominently in aging, both causatively as a source of ROS and responsively through signaling pathways that may temper the aging process via metabolic readjustments. Here we restrict our discussion largely to the involvement of retrograde signaling in aging, but for further exploration of this vast topic see, for example, Guarente and Kenyon (2000).

A metric of aging in budding yeast is replicative life span, which is the number of buds that a mother cell produces before it dies (Mortimer and Johnston, 1959). Multiple factors, many of which may act independently, are contributing determinants of life span, such as Ras-cAMP signaling (Jazwinski, 1999), genomic silencing (Imai et al., 2000), nutrient availability (Jiang et al., 2000; Lin et al., 2000), and extrachromosomal levels of rDNA replicons (Sinclair and Guarente, 1997). That retrograde signaling influences life span in yeast was first noted by Kirchman et al. (1999), who observed that, for some strains, respiratory deficiency, induced either by loss of mtDNA or by nuclear mutation, resulted in a significant life span extension. Two lines of evidence suggested that retrograde signaling was an important determinant of the life span extension associated with respiratory deficiency. First, it was not seen in a strain with a weak retrograde response (as measured by the level of *CIT2* expression in respiratory-deficient cells), and second, the increased life span extension was reversed by inactivation of the *RTG* genes. One argument rationalizing a role for the retrograde response in aging is that mitochondrial function declines with age, and the activity of the retrograde pathway increases to compensate for this decline (Kirchman et al., 1999).

In *C. elegans,* life span can be extended in animals with mitochondrial dysfunction, for instance, in *clk-1* mutants, which are defective in the biosynthesis of coenzyme Q (coQ) (Felkai et al., 1999). *clk-1* mutants accumulate dimethoxyubiquinone (DMU), a coQ precursor that is a potent anitoxidant (Miyadera et al., 2002). Life span extension in those mutants may occur not only because of increased capacity of those animals to deal with ROS, but also because of reduced developmental rates, a property which can contribute significantly to worm longevity (Guarente and Kenyon, 2000). Two recent studies employing RNAi methodology have strongly implicated mitochondrial function and, indirectly, retrograde signaling as a critical determinant of *C. elegans* life span (Dillin et al., 2002; Lee et al., 2003). A comprehensive RNAi screen for long-lived mutants revealed that inactivation of a number of mitochondrial proteins which lowered oxidative phosphorylation capacity significantly extended life span (Lee et al., 2003). The increased longevity of animals with compromised mitochondrial function was suggested to be a combination of increased ability to handle oxidative stress and metabolic shifts toward anaplerotic pathways, similar to the retrograde responses in *S. cerevisiae*. Directed RNAi inactivation of specific components of the mitochondrial electron transport chain and ATP synthase complexes early in development also increased longevity in worms, and this was accompanied by reduced growth and behavioral vigor (Dillin et al., 2002). What was surprising was that RNAi inactivation of mitochondrial proteins of adult animals, which led to similar reductions in mitochondrial oxidative phosphorylation capacity, did not extend life span. Moreover, relieving the RNAi inhibition in adult animals that were treated with RNAi early in development did not restore the (reduced) ATP levels. Altogether, these studies suggest that interfering with mitochondrial function early in development initiates events imprinted later on that extends longevity.

As in all other organisms where it has been tested, caloric restriction (CR) in yeast, which is easily accomplished by limiting the amount of glucose in the growth medium, extends life span (Jiang et al., 2000; Lin et al., 2000). The mechanism(s) by which CR extends life span is a hotly debated issue. It has been suggested that life span extension by CR and by the retrograde pathway are mechanistically separate processes but that both impact an overlapping set of metabolic and stress-related genes (Jazwinski, 2002). Seemingly at odds with the findings that respiratory deficiency can extend life span are results showing that CR results in an *increase* in respiration in long-lived yeast cells and that the extended life span associated with the CR regime is reversed by blocking mitochondrial electron transport (Lin et al., 2002). The underlying argument to explain this effect centers on *SIR2*, which encodes an NAD^+-dependent histone deacetylase activity (Imai et al., 2000; Landry et al., 2000; Smith et al., 2000). Life span is shortened by inactivation of *SIR2* and is lengthened when it is overexpressed (Kaeberlein et al., 1999). The speculation is that the shift toward respiration during CR would increase the NAD^+/NADH ratio due to increased mitochondrial electron transport and hence activate Sir2p, increasing life span (Lin et al., 2002). But that view has recently been challenged based on the findings that CR reduces NAD^+ levels and altering the NAD^+/NADH ratios in vivo does not affect the activity of Sir2p (Anderson et al., 2003). In any case, that both respiratory deficiency and increased respiratory activity are associated with longevity argues for multiple pathways regulating life span.

Alternate Retrograde Pathways in Yeast

Transcriptional profiling of the consequences of mitochondrial dysfunction revealed that not all retrograde-responsive genes are regulated by the *RTG* genes (Epstein et al., 2001). Among that class is *ATO3* which encodes a putative outward ammonium transporter (Palkova et al., 2002) localized to the plasma membrane (Guaragnella and Butow, 2003). *ATO3* expression is elevated in ρ° cells to levels comparable to that of *CIT2* (Guaragnella and Butow, 2003), but unlike *CIT2*, its expression shows little dependence on the *RTG* genes;

instead, *ATO3* expression is tied more directly to amino acid regulation and amino acid sensing. In particular, *ATO3* expression is dependent on Gcn4p, a transcriptional regulator of the general amino acid control pathway, and on the integrity of the SPS amino acid sensing system. The latter is required for the induction of *ATO3* expression in respiratory-deficient cells. One rational for *ATO3* retrograde regulation is that an imbalance between ammonia and α-ketoglutarate levels could occur in respiratory-deficient cells, such that the excess ammonia would be toxic unless transported out of the cell. It is interesting that the physiological state of cells which secrete ammonia, namely, those in the late stages of growth, have much reduced mitochondrial respiration and have increased anaplerotic activity (Palkova et al., 2002), similar to that of respiratory-deficient cells.

Other examples of *RTG*-independent, retrograde-responsive genes are those that confer pleiotropic drug resistance (PDR) to cells (Hallstrom and Moye-Rowley, 2000; Traven et al., 2001; Zhang and Moye-Rowley, 2001). The expression of many of those genes, such as the ABC transporter, *PDR5*, is regulated by the closely related transcription factors, Pdr1p and Pdr3p (Hallstrom and Moye-Rowley, 2000); however, the induction of *PDR5* observed in ρ° petites is largely because of increased expression of Pdr3p. In addition to *PDR5* expression being for the most part independent of *RTG* regulation, the signals that result in the induction of *PDR5* in cells with mitochondrial dysfunction are probably different from those that induce the RTG pathway. For example, specific mutations in the inner mitochondrial membrane protein, Oxa1, which functions in the assembly of the cytochrome oxidase and ATP synthase complexes (Altamura et al., 1996), is sufficient to induce the expression of *PDR5* (Zhang and Moye-Rowley, 2001). How those lesions are transduced to increase Pdr3p expression is not yet clear.

Mitochondrial Signaling in Mammalian Cells

Assembly of the 13 polypeptides encoded by mtDNA in animal cells into functional inner membrane complexes requires interaction with proteins encoded by nuclear genes. The biogenesis of mitochondrial enzyme complexes and biosynthetic machinery requires two general types of regulatory controls, one involving the same or similar transcription factors acting directly or indirectly to regulate both nuclear and mitochondrial gene expression, and the other, metabolic signal(s) originating from mitochondria that modulate nuclear gene expression (Poyton and McEwen, 1996; Lenka et al., 1998). It appears unlikely that there is a unique mechanism for global regulation of all nuclear genes encoding mitochondrial proteins. Rather, the nuclear genes coding for various respiratory chain proteins, some genes involved in mitochondrial protein import, the mitochondrial transcription factors, mtTFA and mtTFB, and the voltage gated ion channel, VDAC, are regulated by NRF1, a serum factor with important roles in growth and development, and by NRF2/GABP, an Ets family heteromeric factor (Carter et al., 1992; Virbasius et al., 1993; Virbasius and Scarpulla, 1994; Lenka et al., 1998; Scarpulla, 2002). In addition, genes encoding cytochrome c, subunits of NADH dehydrogenase, and δ-aminolevulinate synthase are regulated by NRF1 (Virbasius and Scarpulla, 1994; Scarpulla, 2002), whereas genes involved in mitochondrial fatty acid metabolism and β-oxidation are regulated by peroxisome-proliferator activated receptor (PPAR) α and γ (Scarpulla, 2002). Thus, a limited but select number of transcription factors may be involved in the regulation of expression of different subsets of mitochondrial proteins.

Important insights into the coordinate regulation of nuclear and mitochondrial genes came from the finding that PGC-1 (PPARγ coactivator-1), a cold temperature and high carbohydrate diet-inducible coactivator of nuclear receptors, induces mitochondrial biogenesis and the rate of respiration in brown fat and skeletal muscle cells (Puigserver et al., 1998; St Pierre et al., 2003). PGC-1α-, PGC1β-, and PGC-1-related homologs of the PGC family of coactivators induce the transcriptional rates of many nuclear genes via physical and functional interactions with NRF1, NRF2/GABP, and the ligand-regulated transcription factors, PPARα and PPARγ (Wu et al., 1999; Vega et al., 2000; Puigserver and Spiegelman, 2003). In both adipose tissue and muscle, PGC-1 induces thermogenesis by inducing the expression of the mitochondrial uncoupler protein, UCP-1. An important observation was that PGC-1α also induced the expression of mtTFA (Wu et al., 1999), a mitochondrial-specific DNA binding protein that has a role in mitochondrial transcription (Larsson et al., 1998). This would provide a direct means of coordinating mitochondrial biogenesis through coordinated regulation of nuclear and mitochondrial transcription (Wu et al., 1999; Scarpulla, 2002). In transfected muscle cells, PGC-1β, a close homolog of PGC-1α, induces the expression of mitochondrial and nuclear genes for respiratory proteins and increases respiration and ATP generation, which together suggest a possible role of this coactivator in the induction of mitochondrial biogenesis during muscle cell differentiation and muscle contraction (Lehman et al., 2000; Moyes and Hood, 2003; St Pierre et al., 2003). The functional difference between PGC-1α and PGC-1β in inducing, respectively, thermogenesis and respiration-coupled ATP production, appears mainly because of the inability of PGC-1β to induce UCP-1 expression (St Pierre et al., 2003). PGC-1α and PGC-1β, in addition to NRF1 and NRF2/GABP, are induced in contracting muscle. Thus, it remains to be seen how the contrasting functional roles of the two PGC homologs are regulated to meet the increased energy demands of the tissue. In any case, the PGC family of coactivators presents an important means of coordinated regulation of spatially separated nuclear and mitochondrial genes.

Nitric oxide (NO), a physiologically important signaling molecule, also induces mitochondrial biogenesis, leading to increased respiration and energy metabolism in diverse cell types including brown adipose tissue and muscle through a cGMP signaling pathway (Clementi et al., 1999). Recent results show that modulation of mitochondrial biogenesis by NO occurs through increased expression of PGC1α and NRF1 (Nisoli et al., 2003).

Thyroid (T3) and glucocorticoid hormones also induce mitochondrial biogenesis and respiration. Recent studies show the localization of truncated forms of the T3 and glucocorticoid receptors in both the nucleus and

mitochondria (Wrutniak et al., 1995; Scheller et al., 2000). Although the ligand-dependent transcriptional activities of these factors in mitochondria are poorly understood, these receptors induce the transcriptional activity of mitochondrial promoter constructs under in vitro conditions (Scheller et al., 2000). Other nuclear transcription factors, such as CREB and p53, have also been found in mitochondria under certain physiological conditions (Cammarota et al., 1999; Mihara et al., 2003). Despite a lack of information on the role of these factors on transcription of mitochondrial genes, a regulated compartmentalization of the same transcription factor in both the nucleus and mitochondria may be a way of coordinating mitochondrial and nuclear gene expression.

Retrograde Pathways in Mammalian Cells

Altered nuclear gene expression in response to mitochondrial dysfunction in mammalian cells was suggested by studies showing increased mRNA levels coding for various mitochondrial proteins in different ρ° cell lines (Marusich et al., 1997; Wang and Morais, 1997). Mitochondrial retrograde signaling in mammalian cells (also referred to as mitochondrial stress signaling) was described initially in C2C12 skeletal myoblasts (rhabdomyoblasts) and later confirmed in human lung carcinoma A549 cells (Biswas et al., 1999; Amuthan et al., 2002). Mitochondrial stress was defined by altered mitochondrial membrane potential, $\Delta\psi_m$, induced either by treating cells with ethidium bromide to partially deplete their mtDNA content or with the mitochondria-specific ionophore, CCCP (carbonyl cyanide m-chlorophenyl hydrazone). These treatments resulted in elevated cytosolic free Ca^{2+} ($[Ca^{2+}]_c$) and activation of Ca^{2+}/calmodulin-responsive calcineurin and their related factors. In both cell types mitochondrial stress caused upregulation of a number of genes involved in Ca^{2+} transport and storage, including Ryanodine Receptor I or II (RyR1 or RyR2), calreticulin, and calsequestrin (Biswas et al., 1999; Amuthan et al., 2001). The biochemical and phenotypic changes, including expression of nuclear marker genes, increased cytosolic free $[Ca^{2+}]_c$, and altered cell morphology associated with partial depletion of mtDNA, were reversed to near control cell levels in reverted cells whose mtDNA content was increased following growth in medium devoid of ethidium bromide. These findings provide a direct link between mitochondrial stress induced by reduction in mtDNA content and disruption of $\Delta\psi_m$ and the observed alteration in cell physiology and nuclear gene expression.

A similar retrograde signaling involving increased cytosolic free $[Ca^{2+}]_c$ was observed in rat phecromocytoma cells treated with the uncoupler carbonyl cyanide p-(trifluoromethoxy)phenylhydrazone (FCCP) (Luo et al., 1997). Increased Ca^{2+} levels in FCCP-treated cells correlated with activation of ERK1 and REK2 kinases. Using ρ° human fibrosarcoma 143B cells and a MERRF (myoclonic epilepsy with ragged red fibers) cybrid cell line carrying the mutated mitochondrial $tRNA^{Lys}$ (A8344G), Arnould et al. (2002) showed that respiratory deficiency and the associated increase in cytosolic free Ca^{2+} induced the activation of CaMKIV, which in turn activated CREB by protein phosphorylation. In all of these cell systems, removal of free Ca^{2+} by specific chelators abrogated the activation of various (Ca^{2+}-responsive) factors and the induced transcription of nuclear target genes, confirming the role of Ca^{2+} in retrograde signaling.

Mitochondria also appear to have a stress response pathway superficially similar to the unfolded protein response in the endoplasmic reticulum (Mori et al., 1993; Travers et al., 2000). The accumulation of a mutant form of misfolded ornithine transcarbamylase (OTC) in the mitochondrial matrix induces a stress response that activates CEBP homology protein, CHOP, and induces expression of nuclear gene-encoded stress response proteins, Cpn60, Cpn10, mtDNAJ, and ClpP (Zhao et al., 2002). The transcriptional activity of CHOP is modulated by its association with CEBP/β and ATF2. Of interest is the finding that ATF2 and some members of the CEBP family of factors are also activated by retrograde signaling initiated by disruption of $\Delta\psi_m$ (Biswas et al., 1999; G. Amuthan and N.G.A., unpublished data). Moreover, because mtDNA depletion also causes increased expression of the same nuclear chaperone genes (Martinus et al., 1996), it is likely that the stress response mediated by misfolded OTC is associated with mitochondrial dysfunction and collapse of $\Delta\psi_m$. Figure 4 shows our current understanding of mitochondrial retrograde signaling in mammalian cells. It is likely that reduced entry of Ca^{2+} into mitochondria and reduced Ca^{2+} efflux from the cytosol due to a low cellular ATP pool might be responsible for the observed increase in the free Ca^{2+} (Biswas et al., 1999; Amuthan et al., 2002). It remains to be seen whether the retrograde signaling in mammalian cells is also induced by changing mitochondrial metabolite pools as in yeast and whether different signaling mechanisms affect different nuclear gene targets.

NO is also considered as an important intramitochondrial signaling molecule, which modulates mitochondrial respiration by direct binding to cytochrome c oxidase (Cleeter et al., 1994). This cGMP-independent action of NO is known to induce the production of superoxide anion, H_2O_2 (collectively referred to as ROS) and trigger redox signaling (Brookes et al., 2002, 2003). Although the pathological consequence of ROS production by NO is invariably linked to apoptosis (Dai et al., 2001), it is likely that the NO produced in mitochondria under physiological conditions may induce mitochondrial retrograde signaling through altered $\Delta\psi_m$.

Special Features of Retrograde Ca^{2+} Signaling in Mammalian Cells

Mitochondria in mammalian cells play an important role in Ca^{2+} storage and Ca^{2+} homeostasis. Ca^{2+} released either from intracellular stores or from outside the cell are taken up by mitochondria and stored transiently, and then released to the cytoplasmic compartment as part of the intracellular Ca^{2+} traffic and signaling (Rizzuto et al., 1993; Babcock et al., 1997; Ichas et al., 1997). Disruption of $\Delta\psi_m$ by genetic or metabolic stress causes an elevated cytoplasmic free Ca^{2+} partly because of the inability of mitochondria to participate in this important Ca^{2+} uptake (Luo et al., 1997; Biswas et al., 1999; Amuthan et al., 2002; Arnould et al., 2002). Ca^{2+} mediates a diverse array of cell functions through varying amplitude and frequencies of intracellular sparks and puffs that are determined by the nature and location of channels

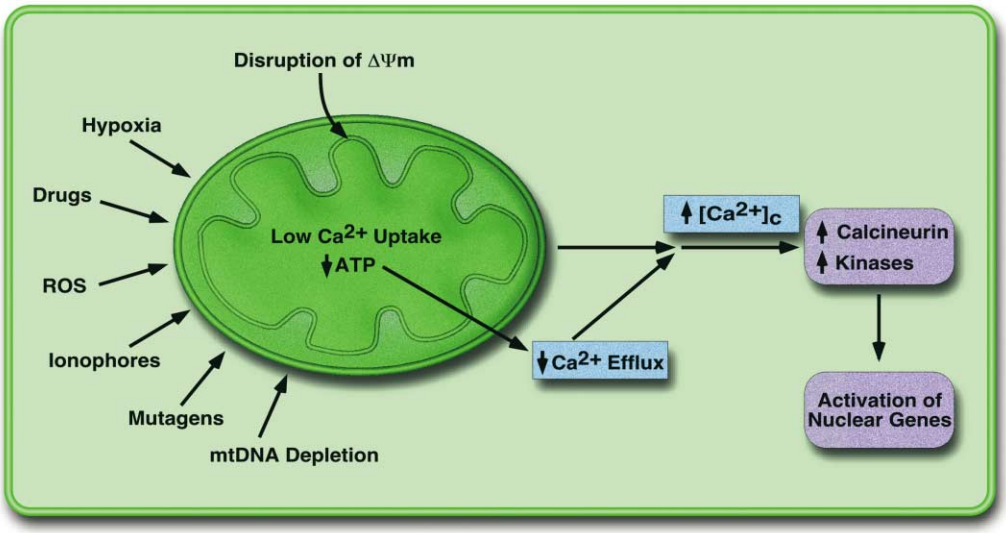

Figure 4. Retrograde Signaling in Mammalian Cells Occurs through Increased Cytosolic Ca^{2+}

Disruption of $\Delta\psi_m$ by various causes affects mitochondrial uptake of Ca^{2+} and reduced efflux into storage organelles or outside the cells due to reduced availability of ATP. Increased cytosolic Ca^{2+} in turn activates calcineurin and various Ca^{2+}-dependent kinases.

as well as the signaling event. Sustained oscillations lasting over 20 hr occur soon after fertilization (Berridge et al., 1998). In this respect, a sustained increase in free Ca^{2+} induced by mitochondrial stress might be reminiscent of that seen in fertilized eggs. A sustained 3- to 8-fold increase of steady-state Ca^{2+} as part of retrograde signaling leads to the activation of calcineurin, which in turn activates NFAT and NFκB. In addition, Ca^{2+}-dependent PKC, CamKIV, JNK, and MAPK are activated, which then activates ATF2, CEBP/δ, CREB, Egr-1, and CHOP in different cell types. The results indicate cell-specific differences in the activation of different Ca^{2+}-sensitive factors and pathways (Figure 5).

Activation of NFκB by retrograde signaling involves a novel mechanism, distinct from the known cytokines, chemokine, and other stress-mediated activation (Biswas et al., 2003). Under both genetic (mtDNA depletion) and chemical stress (CCCP treatment) conditions, cytosolic IkBβ levels are reduced by about 60%–70% of control values, and nuclear cRel and p50 levels increase 2- to 3-fold, suggesting activation of the NFκB pathway (Biswas et al., 1999). IkBα and IkBβ are the two major inhibitory factors which bind to and retain the NFκB homo- or heterodimers in an inactive form in the cytoplasm (Ghosh and Karin, 2002). It is believed that activation of NFκB in response to TNFα, IL-1, lipopolysaccharides, lymphotoxin, and other stress-mediated signaling occurs through inactivation of IkBα, whereas IkBβ is involved in constitutive or housekeeping functions of NFκB. It is interesting that the retrograde signaling-dependent activation occurs through inactivation (dephosphorylation) of IkBβ and the release of active NFκB/Rel dimers, which translocate to the nucleus (Biswas et al., 2003). Notably, inactivation of NFκB-bound IkBβ occurs through calcineurin-mediated dephosphorylation at S313 of IkBβ. Binding of phosphorylated IkBβ to calineurin and its subsequent inactivation was inhibited by an active site-specific peptide (RII peptide) that also inhibits the binding of other target proteins of calcineurin, including the NFAT family of transcription factors (Feske et al., 2003). Propagation of retrograde signaling, and hence induced expression of nuclear target genes, RYRI, cathepsin L, hexokinase, and PEPCK, requires intact IkBβ but proceeds normally in cell with mutated IkBα. Activation of Ca^{2+}-responsive factors in response to mitochondrial stress failed to occur in calcineurin $A\beta^{-/-}$ cells or cells treated with FK506, a calcineurin inhibitor, indicating the critical importance of calcineurin in retrograde signaling. IkBβ binding to calcineurin occurs through its C-terminal PEST domain; in particular, phosphorylation of Ser313 of human IkBβ is critical for both binding to calcineurin and propagation of retrograde signaling (Figure 6). Calcineurin-mediated activation of NFκB is thought to play a critical role in lymphocyte activation (Feske et al., 2003). However, it is not clear whether the retrograde signaling is involved in this activation.

Transcriptional activity of the putative target genes of retrograde signaling (RyR1, hexokinase, PEPCK, TGFβ, and cathepsin L), are activated by NFκB in conjunction with NFATc, ATF2, Egr1, and CEBP/δ, the last three of which are activated by various Ca^{2+}-dependent kinases (Figure 4). A mutant form of IkBβ (Ser313A) suppressed the transcription of the endogenous genes in transfected cells and also the transcriptional activity of promoter constructs in mtDNA-depleted cells (Biswas et al., 2003), suggesting a dominant-negative effect. The biochemical and transcriptional changes in response to retrograde signaling are completely reversed by withdrawal of stress modulators, thus suggesting a possible role for this signaling in physiological processes.

Role of Retrograde Signaling in Tumor Progression and Mitochondrial Dysfunctional Diseases

The role of respiration in cancer has been debated since Warburg's initial hypothesis in 1926, which proposed that increased glycolysis is an important causative or promoting factor. Despite contradictory views, a num-

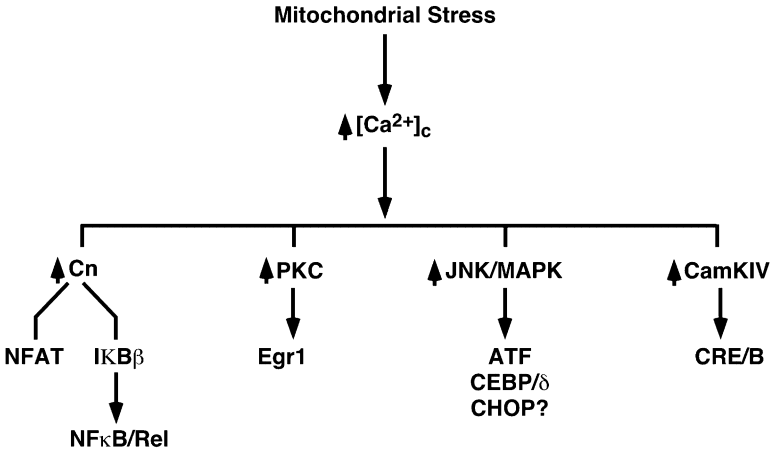

Figure 5. Activation of Cytoplasmic and Nuclear Factors in Different Cell Systems in Response to Mitochondrial Stress Induced by Different Causes

Increased cytoplasmic free Ca^{2+} in different cell systems activates protein phosphatase, calcineurin, and Ca^{2+}-sensitive kinases, PKC, JNK, MAPK, and CamKIV, which in turn activate different nuclear transcription factors.

ber of human and rodent tumors show reduced respiratory capacity and altered mitochondrial morphology. Furthermore, "cytoplast" from tumor cells can transfer tumorigenic property when fused with karyotypes from normal cells, suggesting that an unknown cytoplasmic factor may support the formation of malignant phenotypes (Howell and Sager, 1978). Recently, a large number of human tumors have been shown to contain point mutations or large-scale deletions of mtDNA, including homoplasmic mtDNA deletions in some of the aggressive tumors (Horton et al., 1996; Polyak et al., 1998; Yeh et al., 2000). However, experimental tumorigenesis studies carried out with ρ° tumor cells to determine the role of mitochondrial dysfunction in cancer have yielded mixed results, ranging from minimal effects to markedly increased tumor growth in animals (Giguere and Morais, 1981; Morais et al., 1994; Cavalli et al., 1997). Thus, it is not clear whether mtDNA mutations found in human cancers are contributing factors in tumorigenesis, or simply arise as part of secondary effects of increased cellular ROS production.

Studies using cells partially depleted of mtDNA provide some important clues to this long-standing question. Induction of mitochondrial retrograde signaling, either by mtDNA depletion or by treatment with CCCP, induces increased invasive behavior in otherwise nontumorgenic C2C12 rhabdomyoblasts and A549 human lung carcinoma cells (Biswas et al., 1999; Amuthan et al., 2001, 2002). Retrograde signaling induces the expression of a number of tumor-specific marker genes, such as cathepsin L, an extracellular matrix protease, TGFβ, epiregulin, and mouse melanoma antigen, as well as other genes involved in cellular metabolism discussed previously. The retrograde signaling also induces changes in cell shape and development of pseudopod-like structures often seen in invasive tumor cells. The increased invasive behavior of mtDNA-depleted C2C12 cells can be reversed by restoration of the mtDNA content. The mtDNA-depleted cells are poorly differentiated with no multinuclear structures, whereas control and reverted C2C12 cells showed the presence of multinucleated structures reminiscent of differentiated myocytes (Amuthan et al., 2001). These findings clearly establish a role for mitochondrial retrograde signaling in inducing phenotypic changes and tumor progression in C2C12 rhabdomyoblasts. Increased expression (excretion) of cathepsin L, a target gene of retrograde signaling, was an important factor in induced invasive behavior of tumor cells (Amuthan et al., 2002). PKC inhibitors, which adversely affect cathepsin L gene expression by inhibiting the activation of Egr-1, also markedly reduce the retrograde signaling-mediated invasive behavior (Amuthan et al., 2001, 2002). Finally, overexpression of a dominant-negative mutant of IkBβ (S313A), which abrogates retrograde signaling and thus reduces expression of nuclear gene targets, also causes reduced invasion (Amuthan et al., 2002; Biswas et al., 2003). These results confirm the synergistic roles of NFκB and Egr-1 on cathepsin L gene expression. Retrograde signaling therefore induces aggressively invasive phenotypes in C2C12 and A549 cells by way of induced expression of nuclear genes implicated in cancer progression.

An important question is whether retrograde signaling is activated in human tumor cells carrying heteroplasmic or homoplasmic mtDNA mutations, and if so, what nuclear gene targets are affected in these cells. Preliminary results with fibroblasts from human patients with MERRF and MELAS (mitochondrial encephalopathy, lactic acidosis, and stroke-like episode caused by an A3243G mutation in mitochondrial $tRNA^{Leu\ (UUR)}$) showed activation of CamKIV and its mediated activation of CREB, and activation of Egr1, which occurs through PKC-mediated phosphorylation (Arnould et al., 2002; Freyssenet et al., 2004). C2C12 and A549 cells partly depleted of mtDNA show consistently high invasive properties (Amuthan et al., 2001, 2002) by contrast to the mixed results reported with different ρ° tumor cells (Morais et al., 1994; Cavalli et al., 1997). The reasons for this difference may be the inherently slow growth of most ρ° cells, their special nutritional needs, and the relative sensitivity or resistance of different cell types to undergo apoptosis in response to mtDNA depletion or disruption of $\Delta\psi_m$. In situ analysis of tumor cells with mtDNA mutations or deletions will be necessary to confirm the role of retrograde signaling in tumor progression.

Initial studies from various laboratories using ρ° cells and mitochondrial inhibitors suggested a close association between the release of cytochrome c from mitochondria in response to apoptotic stimuli and collapse of $\Delta\psi_m$. More recent studies with established tumor cell lines show, however, that disruption of $\Delta\psi_m$ alone, either

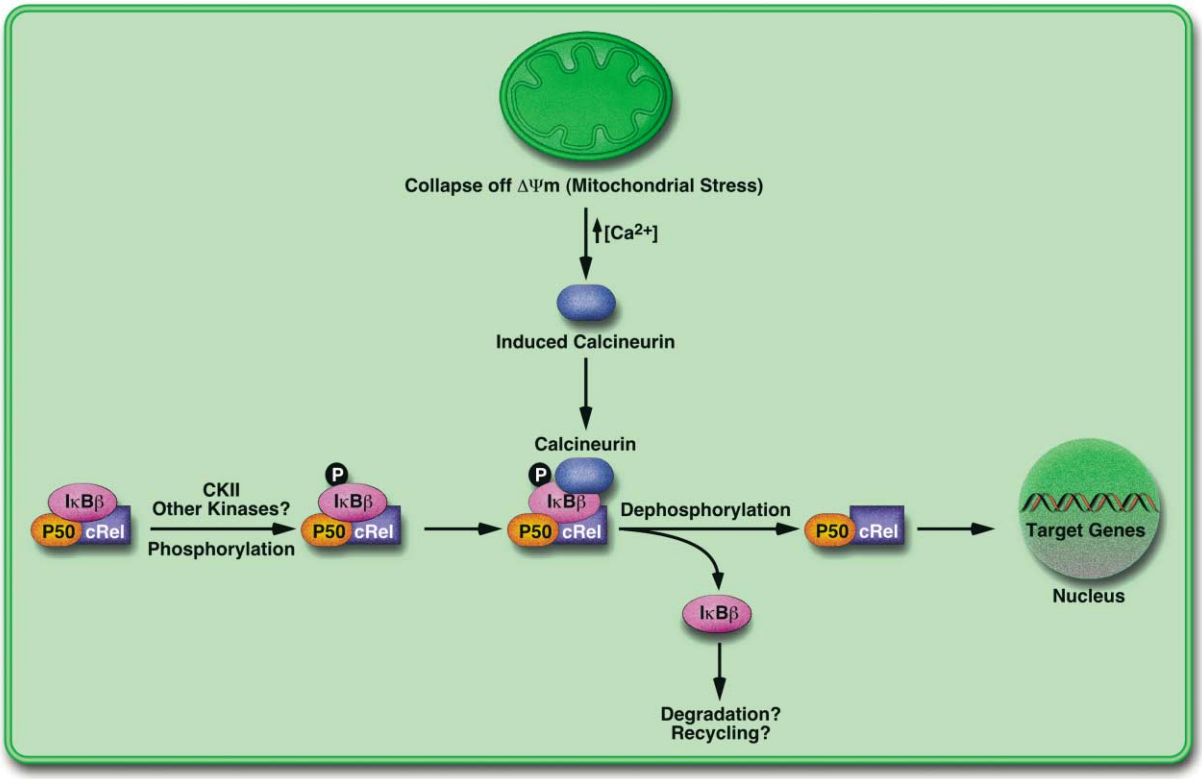

Figure 6. Mechanism of Mitochondrial Stress-Induced Activation of NFκB through Ca^{2+}

Activated calcineurin binds to IkBβ-heteromeric Rel protein complex through the C-terminal PEST domain of IkBβ. Phosphorylation of PEST domain Ser residues by CKII or other kinases is critical for calcineurin binding. Calcineurin-mediated dephosphorylation of IkBβ causes the release of active Rel dimers, which translocate to the nucleus. IkBβ released from the complex is either recycled or degraded probably by ubiquitination.

by mtDNA depletion or by metabolic inhibitors, is not sufficient for cytochrome c release and execution of apoptotic signals (Eskes et al., 1998). In fact, in some cases, partial or complete loss of mtDNA renders these cells more resistant to apoptosis (Dey and Moraes, 2000; Amuthan et al., 2002; G. Biswas and N.G.A., unpublished data). In these latter cases, mitochondrial retrograde signaling induced by mtDNA depletion, or by agents that cause disruption of $\Delta\psi_m$, induces the expression of antiapoptotic Bcl2 and also significantly reduces the processing of precursor Bid to active tBid. Resistance to apoptosis is a hallmark of cancer cells at advanced stages of tumor progression and cells with acquired resistance to cytotoxic drugs. Thus, induced expression of antiapoptotic BH3 domain proteins coupled to overexpression of genes involved in turmorigenesis might be important features of tumor progression mediated by mitochondrial retrograde signaling. It remains to be seen whether this mechanism is tumor cell or tissue specific and whether the retrograde signaling and its attendant changes in nuclear gene expression are factors in a heterogeneous group of neuromuscular retinal degenerative diseases, such as Parkinson's, cardiac dysfunction, diabetes, and inherited forms of deafness that are known to be associated with mtDNA mutations (Wallace, 1992).

Concluding Remarks

Retrograde signaling is an important mechanism of communication between mitochondria and nucleus in both unicellular eukaryotes and metazoan cells. Mitochondrial signaling pathways impact a wide spectrum of cellular activities under both normal and pathophysiological conditions. In yeast retrograde signaling is mediated by at least three positive and four negative regulatory factors and is used as a sensor of mitochondrial activities, allowing cells to adjust their metabolism appropriately. These adjustments involve the integration of carbohydrate and nitrogen metabolism. In both yeast and mammalian cells, retrograde signaling is also linked to TOR signaling, but the precise signals and connections that interlink these pathways are unclear. In mammalian cells, compromised mitochondrial function resulting from mtDNA lesions, membrane damage, insufficient O_2, or nutrient supply sets off signaling cascades through altered Ca^{2+} dynamics, which induces activation of factors such as NFκB, NFAT, ATF, and others. Retrograde signaling also induces invasive behavior in otherwise nontumorigenic cells implying its role in tumor progression. Abrogation of mitochondrial stress signaling under in vivo tumor settings through the use of specific inhibitors, such as the dominant-negative IkBβ, may offer new therapeutic intervention strategies in some cancers.

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