Review

Ischemic preconditioning: The role of mitochondria and aging

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ABSTRACT

Aging represents a triple threat for myocardial infarction (MI). Not only does the incidence of MI increase with age, but the heart becomes more susceptible to MI induced damage and protective interventions such as ischemic preconditioning (IPC) become less effective. Therefore, any rational therapeutic strategy must be built around the ability to combat the detrimental effects of ischemia in aged individuals. To accomplish this, we need to develop a better understanding of how ischemic damage, protection, and aging are linked. In this regard, mitochondria have emerged as a common theme. First, mitochondria contribute to cell damage during ischemia–reperfusion (IR) and are central to cell death. Second, the protective signaling pathways activated by IPC converge on mitochondria, and the opening of mitochondrial ion channels alone is sufficient to elicit protection. Finally, mitochondria clearly influence the aging process, and specific defects in mitochondrial activity are associated with age-related functional decline. This review will summarize the effects of aging on myocardial IR injury and discuss relevant and emerging strategies to protect against MI with an emphasis on mitochondrial function.

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1. Introduction

Stoppage of blood flow through the coronary arteries results in an acute lack of oxygen and deprives cardiomyocytes of substrates. When coupled with the restoration of blood flow, this results in ischemia–reperfusion (IR) injury. The subsequent development of myocardial infarction (MI) is a major cause of death, and the annual incidence of first-time MI in the US is 610,000. There are multiple risk factors for MI, but by far the greatest risk is age (Lloyd-Jones et al., 2010). Despite an increased focus on "heart health" in recent years, and a number of interventions that may reduce the risk of MI, there are few if any therapeutic avenues that reduce damage to the heart following an MI. Furthermore, co-morbidities such as obesity, type II diabetes, and aging ensure that MI is likely to remain a major clinical problem globally.

In 1986 it was discovered that "preconditioning" the heart with short periods of ischemia could alleviate damage incurred by a subsequent prolonged ischemic insult (Murry et al., 1986). It is also known that the prognosis following an MI is better for patients with unstable angina than those suffering an unpredicted MI (Ottani et al., 1995). These fundamental observations suggest that endogenous mechanisms exist in the heart to protect against future ischemic events. Many studies have attempted to define the signaling pathways induced by ischemic preconditioning (IPC) with the goal of developing pharmacologic mimics to provide protection (e.g. reduced infarct size, recovery of contractile function). The central problem has been that IPC itself is not effective in the aged heart (Fig. 1) (Boengler et al., 2009a; Jahangir et al., 2007; Bartling et al., 2003).

Aging is associated with an accumulation of pathologic changes leading to a progressive decline in cellular, organ and whole organism function. In addition, aging results in a lower resistance to stress (Shih and Yen, 2007). Mitochondria have been recognized to play a prominent role in aging, and a decline in mitochondrial function is thought to underlie some of the decline in tissue function with age. In addition, the mitochondrial signaling pathways leading to cell injury and death may be disrupted during aging.

Counteracting the role of mitochondria in pathology however, is the fact that they are known to be critically involved in the protection afforded by IPC. Thus developing a clear understanding of how aging influences the mitochondrial balance between ischemic damage and protection is essential to devise effective therapeutic strategies for cardioprotection in older adults.

2. Cardiac mitochondria: basic function, and role in IR injury

Cardiac contractile function relies on mitochondrial oxidative phosphorylation (Ox-Phos) for the bulk of its ATP. While mitochondria are the metabolic hub of all eukaryotic cells, mitochondria from different organs are adapted to specific functions (Johnson et al., 2007). Specifically, cardiac mitochondria are known to preferentially oxidize fatty acids (Stanley et al., 2005). Moreover, cardiac mitochondria exist in

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two subpopulations: subsarcolemmal (SSM) and interfibrillar mitochondria (IFM), which differ not only in their location but also function (Palmer et al., 1977). For example, IFM have an increased maximal respiration rate and ability to handle Ca^{2+} (Hofer et al., 2009; Lesnefsky et al., 2001b).

In addition to their central metabolic role, mitochondria are also key players in IR injury. IR injury is characterized by two events: ischemia and reperfusion. At the cellular level, ischemia is characterized by a lack of O_2 and substrates, and ischemic cells accumulate metabolites such as lactate. Via alterations in pH, Na^+, and ATP levels, ischemia and reperfusion then trigger a cascade of events including massive reactive oxygen species (ROS) generation, loss of nucleotide homeostasis, and disruption of Ca^{2+} homeostasis, culminating in the formation of the mitochondrial permeability transition (PT) pore and the activation of cell death signaling pathways (reviewed in detail (Lemasters et al., 2009; Murphy and Steenbergen, 2008; Brookes et al., 2004)). Thus, mitochondria have emerged as key arbiters of cellular survival in IR injury.

3. Cardiac mitochondria and IR injury: the effects of aging

As shown in Fig. 1, the aging heart is more sensitive to IR injury (Lesnefsky et al., 2006, 2001b). In addition, a significant decline in cardiac mitochondrial function is seen in aging, and this appears to differ between IFM and SSM (Judge et al., 2005; Fannin et al., 1999). This section will focus on two mitochondrial commonalties between aging and IR injury: (i) mitochondrial Ca^{2+} handling and (ii) mitochondrial ROS generation/oxidative damage.

The Ca^{2+} insult associated with pathology of cardiac IR injury results in mitochondrial Ca^{2+} overload and ultimately cell death (Lemasters et al., 2009; Murphy and Steenbergen, 2008; Brookes et al., 2004). In this regard, it is notable that mitochondria from the aged heart display a decreased capacity to accumulate and retain Ca^{2+}, resulting in a decreased tolerance to Ca^{2+} insult (Jahangir et al., 2001). This inability to handle a pathological Ca^{2+} insult may predispose the aged myocardium to IR injury.

In a similar manner, large scale mitochondrial ROS production is associated with IR injury (Fridovich, 1983), and increased mitochondrial ROS and oxidative damage, particularly in IFM, are also found in the aged heart (Judge et al., 2005; Lesnefsky et al., 2001b; Fannin et al., 1999).

The molecular mechanisms underlying defects in Ca^{2+} and ROS homeostasis find their roots at the level of the respiratory chain. Both aging and IR injury result in damage to the Ox-Phos machinery

(Kwong and Sohal, 2000; Fannin et al., 1999; Lenaz et al., 1997; Guerrieri et al., 1993), raising the possibility that an already age-diminished Ox-Phos may be susceptible to further decline with IR.

There are similarities between the molecular damage to Ox-Phos that occurs in aging and IR injury. For example, IR causes a loss of cardiolipin (Lesnefsky et al., 2001c). Similar effects on cardiolipin have been reported in the aging heart (Petrosillo et al., 2001; Pepe et al., 1999). In addition to its metabolic role, cardiolipin loss is also associated with increased ROS generation and cytochrome c release (Kagan et al., 2004; Pepe et al., 1999).

Alterations in complex III activity have been reported in both IR injury (Lesnefsky et al., 2001a) and aging (Lesnefsky et al., 2001b), although the molecular mechanisms may be distinct; complex III's cytochrome c binding site is the target of functional decline in aging, while ischemic damage is primarily at the iron–sulfur center within the complex (Lesnefsky et al., 2001a). Notably, both of these mechanisms manifest in increased ROS production. While this review focuses on changes in ROS production at the level of the Ox-Phos machinery, other mitochondrial ROS modulating components such as monoamine oxidase and p66[Shc] are also associated with aging. The inhibition of monoamine oxidase and p66[Shc] results in protection from IR injury (Carpi et al., 2009), their expression levels increase with age (Pandolfi et al., 2005; Saura et al., 1994), and genetic deletion of p66[Shc] increases life span (Migliaccio et al., 1999).

In addition to changes in ROS generation by the respiratory chain, a state of oxidative stress can also be precipitated by a decrease in antioxidant defenses. In this regard, IR injury is known to decrease the activity of enzymes involved in ROS removal, such as manganese superoxide dismutase and glutathione peroxidase (Shlafer et al., 1987). Antioxidant defenses are similarly compromised with aging (Ferrara et al., 2008; van der Loo et al., 2005; Moghaddas et al., 2003).

The increased mitochondrial ROS generation associated with aging and IR injury can have a profound impact on the formation of the mitochondrial permeability transition (PT) pore. The induction of the mitochondrial PT pore is associated with irreversible damage and the initiation of cell death (Lemasters et al., 2009). The increased ROS and altered calcium handling conditions set forth in the aged myocardium may be interrelated and ultimately sensitize the heart to the induction of mitochondrial PT pore opening.

It is not clear whether differences in mitochondrial ROS generation are an underlying factor in the differential responses to IR injury that occur between animals of varying aging rates. It is known that mitochondria from short-lived animals generate more ROS (Lambert et al., 2007). Interestingly, these animals also develop myocardial infarction more quickly (Downey and Cohen, 2009; Manintveld et al., 2007; Gersh et al., 2005; Barja and Herrero, 2000). While this connection between the rate of aging and infarct development is appealing, other hemodynamic parameters (e.g. collateral flow) should be taken into consideration when comparing different species. In addition, there have been a number of recent observations that ROS can be beneficial as well as detrimental and that ROS signaling may be important for adaptive responses to ischemia (see below). Hence, the role of ROS depends upon the context in which it is presented, including that of aging.

Collectively, a dysregulation of mitochondrial Ox-Phos, Ca^{2+} handling and ROS generation occurs in both IR injury and aging, and it is logical to suggest that the molecular mechanisms underlying these phenomena may be shared.

4. Ischemic preconditioning, aging and mitochondria

One strategy to protect the heart from IR injury is IPC (Murry et al., 1986), which can be used clinically in transplant and coronary surgeries (Ji et al., 2007). Similar to IPC, protection can also be achieved via ischemic postconditioning during reperfusion (Zhao et al., 2003). This

	Adult	Aged
IR		
IPC +IR		
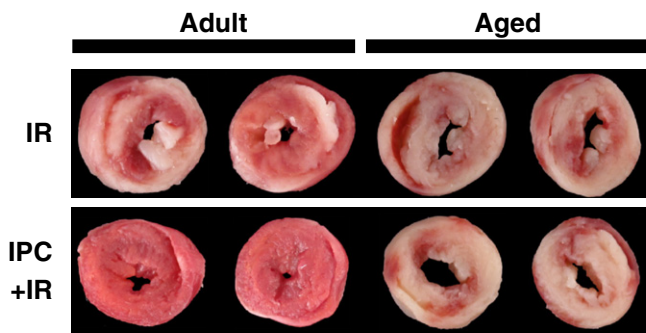
Fig. 1. Loss of ischemic preconditioning (IPC) protection in the aged heart. Adult (2 mo.) and Aged (18 mo.) C57BL/6 mouse perfused hearts were subjected to 25 min ischemia and 60 min reperfusion (IR) or preconditioned with three cycles of 5 min ischemia and 5 min reperfusion followed by index IR. Following protocols, hearts were sliced transversely, stained with triphenyltetrazolium chloride to delineate live (red) and infarcted (white) tissue. Typical slices demonstrate that while both adult and aged hearts were subject to damage from IR injury, IPC protected the adult but not the aged heart from damage.

is a process whereby short periods of ischemia are applied following the index ischemic insult. Like IPC, the effectiveness of postconditioning also decreases with age (Boengler et al., 2008), though whether these similarities persist at the mechanistic level is unclear.

IPC triggers a diverse array of signaling cascades (reviewed in (Heusch et al., 2008; Downey et al., 2007)) (Fig. 2), many of which converge at the mitochondrion (Garlid et al., 2009; Murphy and Steenbergen, 2007b). In addition to factors such as gender (Murphy and Steenbergen, 2007a), diabetes (Kersten et al., 2000), and many prescription drugs (Wojtovich et al., 2010; Shim and Kersten, 2008), a major confounder for the effectiveness of IPC is age (Boengler et al., 2009a; Jahangir et al., 2007; Bartling et al., 2003). Furthermore, even the effectiveness of drugs which activate similar protective signaling pathways as IPC (e.g. volatile anesthetics) is diminished with age (Schulman et al., 2001). As relates to IPC, the mechanism underlying its loss of effectiveness in aged individuals is thought to include: (i) disruption of the machinery that maintains cardiac redox status, (ii) inadequate energy supply due to impaired Ox-Phos function, (iii) defective IPC signaling, or (iv) a change in the trigger/threshold for IPC activation.

(i) A mild generation of ROS is required for IPC induced protection, and notably antioxidants render IPC ineffective (Dost et al., 2008). The aging heart, with a large increase in ROS production (Juhaszova et al., 2005; Judge et al., 2005; Lesnefsky et al., 2001b; Muscari et al., 1990) coupled with decrease in mitochondrial antioxidant defenses (e.g. manganese superoxide dismutase) (Ferrara et al., 2008) may render IPC ineffective by disrupting redox signaling. Several components of the IPC signal cascade (e.g. mitochondrial K_{ATP} channel) are redox sensitive (Queliconi et al., 2011), although the question remains whether antioxidants can restore redox balance and IPC to the aged heart.

(ii) The recovery from IR requires an adequate energy supply. The underlying loss of IPC efficacy in aging may be due to impaired Ox-Phos since, as previously discussed, components of the Ox-Phos machinery such as complex I activity are diminished with aging via irreversible oxidative modifications (Navarro and Boveris, 2007; Choksi et al., 2007; Fannin et al., 1999).

(iii) The protection resulting from IPC depends on a diverse array of signaling cascades (Garlid et al., 2009) and the loss of one or more of these with aging may compromise preconditioning. For example, the mitochondrial K_{ATP} channel (Wojtovich and Brookes, 2009; Facundo et al., 2006) and sirtuin activity (SIRT1) (Nadtochiy et al., 2011; Nadtochiy et al., 2010) are critical components of IPC, and both exhibit an age-dependent decline in activity (Braidy et al., 2011; Krylova et al., 2006).

In addition to upstream signaling cascades that converge on mitochondria, intrinsic features of mitochondria themselves may also modulate their ability to receive/respond to cardioprotective signaling cascades. For example, a prerequisite for IPC is the ability of the respiratory chain to undergo reversible modulation of its activity. Indeed, several reversible inhibitors of Ox-Phos are cardioprotective (Burwell et al., 2009). It has been shown that complex I mutant mice cannot be preconditioned, suggesting that pre-existing deficiencies in Ox-Phos may compromise the plasticity of electron transport chain activity during IPC. As such, age induced modifications to the Ox-Phos machinery (Choksi et al., 2007) may have a similar effect.

Similarly, the cellular processes that remove damaged mitochondria could also play a role in IPC. Autophagy acts as quality control by targeting and removing dysfunctional macromolecules and organelles (Gottlieb and Mentzer, 2010). This turnover is critical to maintaining a healthy pool of functional
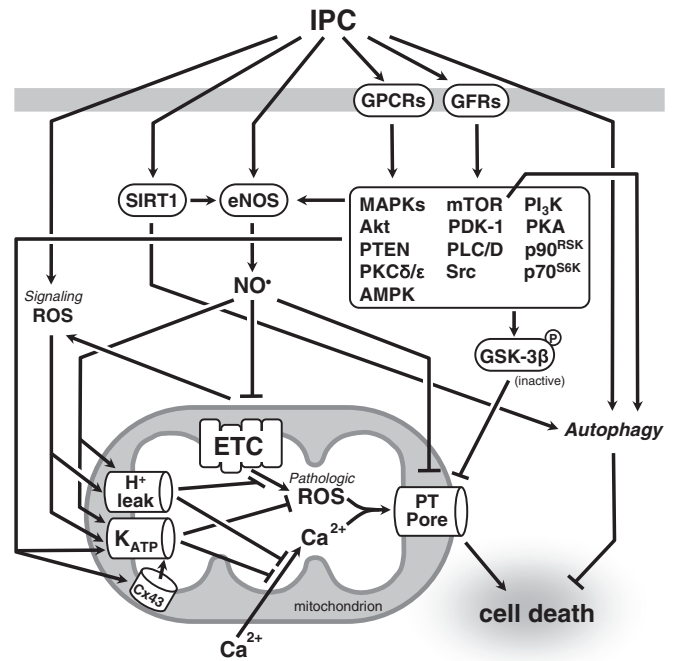
Fig. 2. Ischemic preconditioning (IPC): signaling pathways and targets. IPC elicits protection through a complex signaling cascade that converges on the mitochondrion. This schematic, while not comprehensive, focuses on functional and regulatory interactions discussed in this review. Aging results in the loss of IPC which may result from modifications to this signal transduction pathway, many components of which have been shown to be altered with age. For a comprehensive overview of IPC kinase signaling please refer to Downey et al. (2007). Abbreviations: IPC, ischemic preconditioning; GPCRs, G protein coupled receptors; GFRs, growth factor receptors; SIRT1, Sirtuin 1; eNOS, endothelial nitric oxide synthase; MAPKs, Mitogen-activated protein kinases; mTOR, mammalian target of rapamycin; PI$_3$K, phosphatidylinositol 3-kinase; Akt, Protein Kinase B; PDK-1, pyruvate dehydrogenase kinase; PKA, protein kinase A; PTEN, Phosphatase and tensin homolog; PLC/D, Phospholipase C/D; p90^{RSK}, p90 ribosomal S6 kinase; PKCδ/ε, protein kinase C δ/ε; p70S6K, p70S6 kinase; AMPK, AMP-activated protein kinase; ROS, reactive oxygen species; GSK-3β, glycogen synthase kinase-3β; ETC, electron transport chain; K_{ATP}, ATP-sensitive potassium channel; PT Pore, permeability transition pore.

mitochondria. It is well documented that autophagy is compromised with aging, and the resulting accumulation of damaged mitochondria may lead to impaired Ox-Phos function in older individuals (reviewed in (Green et al., 2011)). Moreover, autophagy is required for IPC (Huang et al., 2011) and the inhibition of autophagy results in exacerbated IR injury (Weber and Reichert, 2010). A simple hypothesis is that the accumulation of damaged mitochondria resulting from a reduction in autophagy during aging may impact the effectiveness of IPC. Coupled with an increased sensitivity to IR injury caused by damaged mitochondria, reductions in autophagy may represent a critical risk factor for MI. Since multiple IPC signaling pathways are also affected by age, however, it is not clear that stimulating autophagy alone would be sufficient to restore IPC in aged individuals. Nevertheless, it is likely that ridding the myocytes of age-associated dysfunctional mitochondria would increase ischemic tolerance and alter the threshold for inducing damage.

It is interesting that a particular sub-population of mitochondria has been implicated in IPC signaling. Specifically, it has been shown that the gap junctional protein connexin 43 (Cx43) is required for IPC, and cardiac Cx43 is localized exclusively in SSMs, not in IFMs (Rottlaender et al., 2010; Boengler et al., 2009b). This suggests that SSM rather than IFM are more influential in the IPC signal transduction cascade. While the overall expression of Cx43 is indeed decreased in aged myocardium (Boengler et al., 2007), this observation poses a dilemma, since aging appears

to primarily affect the metabolic function of IFM, not SSM (Lesnefsky et al., 2006; Lesnefsky et al., 2001b). Clearly, there may be subtle functions of SSM (e.g. their possession of Cx43), which render them more important in aging induced loss of IPC, versus the more generalized metabolic changes in aging which are assigned to IFM, and may not be important in loss of IPC. Alternatively, signaling between mitochondrial populations may play a role in IPC and this signaling could become defective with age.

(iv) It is known that the degree of protection afforded by a fixed IPC stimulus varies depending on the severity of index IR injury which follows (Hanley and Daut, 2005). Thus one reason underlying the loss of IPC efficacy in aging may be that a different amount of IPC stimulus is required to elicit protection in aging vs. young hearts. Interestingly, one study has found that a greater than normal IPC stimulus was able to elicit protection in elderly patients undergoing angioplasty (Lee et al., 2002).

In summary, there are several mechanisms which may underlie the loss of IPC in aging. Further elucidation of these mechanisms may aid in the development of therapeutics to restore IPC in the aged heart.

5. Current research

Despite numerous IPC mimicking drugs discovered in the laboratory, none has yet made the transition to clinical use, with the result that there are currently no FDA approved drugs for the indication of reducing myocardial infarct size. One reason for this may be that most MI patients are neither young nor healthy, whereas pharmacologic agents are often developed in the laboratory using young animals (Hausenloy et al., 2010; Downey and Cohen, 2009). Examples of normally-efficacious agents which are ineffective at providing cardioprotection in aged individuals include volatile anesthetics, mitochondrial K^+ channel openers and adenosine A1 agonists (Sniecinski and Liu, 2004; Schulman et al., 2001). Thus, an important avenue for current research is to develop broadly applicable therapies, particularly agents that work in the aging heart. In this regard, two areas of research that are currently promising are studies of caloric restriction and the use of model organisms with short life spans.

5.1. Caloric restriction (CR) and sirtuins

Recent research has shown that caloric restriction (CR) may activate signaling pathways which restore the ability to precondition aged hearts (Abete et al., 2010). A major advance in the CR field was the discovery of the silent information regulator (Sirtuin) family of proteins. There are 7 mammalian Sirtuins (SIRTs), some of which are up-regulated and activated by CR and may be responsible for the effects of CR (Morris et al., 2011).

SIRT3, which is localized in mitochondria, may decrease oxidative stress via deacetylation and activation of MnSOD (Qiu et al., 2010). In addition, SIRT3 dependent deacetylation of isocitrate dehydrogenase can elevate NADPH levels, which may keep antioxidant enzymes in a reduced state (Schlicker et al., 2008). SIRT3 can also deacetylate cyclophilin D and prevent its interaction with adenine nucleotide translocase (Hafner et al., 2010), which may inhibit mitochondrial PT pore opening.

SIRT1 is a cytosolic/nuclear protein, which has been implicated in cardiac stress responses in aging (Hsu et al., 2008). We found that pharmacologic inhibition or partial genetic ablation of SIRT1 blocked IPC in adult mouse hearts (Nadtochiy et al., 2011; Nadtochiy et al., 2010), suggesting an endogenous role for SIRT1 in protection. Thus, it is possible that age-dependent decline in SIRT1 activity (Braidy et al., 2011) may contribute to loss of IPC. Furthermore, overexpression of SIRT1 elicits resistance to oxidative stress and protects heart from IR injury (Nadtochiy et al., 2011; Hsu et al., 2008). This suggests that boosting SIRT1 activity in aging may be able to restore IPC.

The molecular mechanisms of SIRT1-mediated cardioprotection have not been fully elucidated, but SIRT1 is known to regulate a variety of potential protective events, such as eNOS activation and autophagy (Nadtochiy et al., 2011). In addition, SIRT1 is at the crossroad of the CR-mediated regulation of mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK) pathways (Ghosh et al., 2010; Shinmura et al., 2007), all of which are cardioprotective. The complexity and intertwinement of signaling cascades involved in reducing IR injury is illustrated by the finding that the cardioprotective effect of rapamycin was reversed by the mitochondrial K_{ATP} channel inhibitors (Khan et al., 2006), suggesting that both SIRT1 and CR may indirectly be involved in mitochondrial K_{ATP} activation. In addition, mTOR mediated protection may also include activation of autophagy (Gurusamy et al., 2010). Intriguingly, exercise has been shown to increase SIRT1 activity in aged rats (Ferrara et al., 2008) which raises the possibility that SIRT1 may contribute to exercise induced cardioprotection (Lawler et al., 2009).

5.2. Model systems of aging

As mentioned above, an important and emerging approach is to study IPC in aging animals. In this regard, invertebrate animal models are of increasing interest, owing to their short generation times and ease of use. Mechanistically, the process of triggering protection by IPC may exhibit certain species-dependent components, but the fundamental phenomenon of IPC is evolutionarily conserved (Dasgupta et al., 2007) and readily reproduced in organisms such as the nematode *C. elegans*.

C. elegans has been used extensively to study the relationship between aging, ROS and mitochondrial dysfunction, and more recently to identify genetic determinants of hypoxic sensitivity and hypoxic preconditioning (HPC). Early work showed that a mutation in mitochondrial complex II (*mev-1*) increased ROS production and decreased lifespan (Ishii et al., 1998). In addition, mutations that increased lifespan, such as in the canonical insulin signaling pathway, also increased resistance to ROS. These data were consistent with the free radical theory of aging, which states that increased mitochondrial metabolism and oxygen consumption will result in increased ROS production and a subsequently reduced lifespan.

However, this seemingly direct correlation between ROS and lifespan has recently been challenged, and there are multiple lines of evidence that in fact low levels of mitochondrial ROS formation can stimulate adaptive processes that increase both longevity and stress-resistance. The ability of sub-lethal mitochondrial stress to trigger protection has been termed mitohormesis (for comprehensive reviews, see Ristow and Schmeisser, 2011; Ristow and Zarse, 2010).

Relevant examples that dispute the free radical theory of aging include demonstrating that overexpression of the mitochondrial superoxide dismutase in *C. elegans* does not alter lifespan (Doonan et al., 2008) and that lifespan extension in *C. elegans* mitochondrial (mit) mutants with decreased Ox-Phos activity does not correlate with oxidative stress levels (Rea et al., 2007). In fact, mild mitochondrial dysfunction may signal a beneficial response (Ventura et al., 2009; Rea et al., 2007; Dillin et al., 2002). In this regard, localized ROS in mitochondria (Yang and Hekimi, 2010) may coordinate functional responses; it is possible that these responses are not limited to the cell in which the signal originates, either, as recent data suggests that mitochondria can contribute to protection through trans-cellular signaling (Durieux et al., 2011). This may be akin to the phenomenon of "remote preconditioning" observed in mammals.

The ability to elicit biphasic responses (i.e. adaptive or detrimental) by modulating Ox-Phos activity is consistent with the idea that mitochondria are central to both cell death and to protective signaling. These data also establish clear parallels between *C. elegans* and mammalian systems. Previous work indicating that ROS production is

increased and mitochondrial function is decreased in aged mammals, as detailed in Section 3, leads us to speculate that the balance between the adaptive and detrimental effects of ROS is altered with aging, though whether this is due to differing sites of ROS formation, the ability of signaling pathways to recognize ROS, or functional changes that occur at the level of the mitochondria themselves has yet to be determined.

A significant advantage to working with a genetic model organism is the ability to perform unbiased screens to answer the above type of question. However, C. elegans are extremely resistant to conventional hypoxia (~0.5% O_2), and instead relatively anoxic conditions (<0.1% O_2) have been used to identify genetic determinants of whole-organism ischemic sensitivity (Hayakawa et al., 2011; Mabon et al., 2009; Mendenhall et al., 2006; Scott et al., 2002) as well as IPC (LaRue and Padilla, 2011; Dasgupta et al., 2007). Novel targets identified thus far include tRNA synthetases (Anderson et al., 2009), Apoptosis Signal Regulating Kinase family proteins (Hayakawa et al., 2011), 5′ AMP-activated protein kinase (LaRue and Padilla, 2011), components of the unfolded protein response machinery (Mao and Crowder, 2010), and the Apaf1 ortholog ced-4, which has been shown to be required for IPC independent of its role in apoptosis (Dasgupta et al., 2007). Whether these results will translate to mammalian models is as-of-yet unclear given the fact that hypoxic and anoxic responses differ in worms.

It is not yet clear to what extent mitochondria are necessary to support the role of these proteins, but signaling pathways that coordinate adaptive responses in worms often target mitochondria. Our own work has shown that worm mitochondria, like those from mammals, have both K_{ATP} and big conductance potassium (BK) channels whose activities appear to be both necessary and sufficient for protection from ischemic damage (i.e. Wojtovich et al., 2008; Wojtovich et al., in press). Interestingly, metabolic profiling in worms with Ox-Phos mutations suggests a transition to alternate means of ATP production that do not require O_2, and these worms are both long-lived and resistant to ischemia (Butler et al., 2010; Rea et al., 2007). In this respect, it would be reasonable to ask whether Ox-Phos mutants are endogenously preconditioned.

In addition to the identification of novel targets that regulate anoxic/hypoxic sensitivity, novel mechanisms have been discovered for well-established targets. As in mammals, HIF-1 in worms mediates responses to non-lethal or moderate hypoxic exposure (Shen et al., 2005). HIF-1 was recently shown to regulate the expression of a tyrosinase in C. elegans ASJ sensory neurons. This enzyme is secreted and antagonizes p53-dependent germline apoptosis, thus linking hypoxia and programmed cell death through cell non-autonomous signaling (Sendoel et al., 2010). Whether increased tyrosinase expression is sufficient to confer protection is unknown, nor is it known whether this signaling pathway is similarly protective in other cell death paradigms. However, it is notable that multiple reports have shown that HIF-1 modulates lifespan in worms (Zhang et al., 2009; Chen et al., 2009; Mehta et al., 2009).

While validation in mammalian systems is undoubtedly necessary, these examples illustrate how the use of genetics has great potential to yield previously unheralded protective approaches and molecular targets. Although the understanding of invertebrate aging (Partridge, 2011) and mitohormesis (Ristow and Zarse, 2010) is advancing, significant gaps remain to be addressed. For example, does aging impact on HPC efficacy in worms? Does mitochondrial dysfunction preclude HPC? Can HPC be elicited cell non-autonomously? The use of genetic model organisms has and will continue to yield fundamental new insights into these questions.

6. Conclusions

Multiple signaling pathways interface at the mitochondrion to influence longevity and IPC. Elucidating the mechanisms of loss of IPC efficacy in aging remains a major problem in cardiovascular research, which demands the development of novel treatment strategies for myocardial infarction in aged individuals. Mitochondria both contribute to the pathology of IR injury and are critical mediators of cardioprotective signaling. Current research focusing on lifestyle interventions such as caloric restriction and exercise, pharmacologic approaches, and model organism research are promising avenues for learning how to restore IPC in aged patients.

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