Trends in Endocrinology and Metabolism

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--Manuscript Draft--

Manuscript Number:	TEM-D-24-00092R1
Article Type:	Forum
Keywords:	Mitochondria; mitochondrial diseases; OXPHOS; ISR; tissue-specificity
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ISR-pathway contribution to tissue specificity of mitochondrial diseases

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Abstract

Mitochondrial genetic defects caused by whole-body mutations ~~present in the whole body~~ typically affect different tissues differently. ~~To~~ elucidatinge the molecular determinants that cause certain cell-types to be primarily affected has become a critical research target within the field. We propose a differential activation of the Integrated Stress Response ~~(ISR)~~ as a potential contributor to this tissue-specificity.

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Diagnostic and phenotypic complexity of mitochondrial diseases

The mitochondrial proteome has a dual genetic origin. From approximately 1200 proteins functioning in mitochondria, only 13 are encoded in the mitochondrial DNA (mtDNA) and are synthesized inside the organelle, whereas the vast majority of them are encoded in the nuclear DNA, synthesized in cytoplasmic ribosomes, and translocated into mitochondria. Genetic defects in either mtDNA or nuclear genes encoding mitochondrial proteins have been linked to disease, mainly caused by defects in oxidative phosphorylation and decreased energy production. Diagnosis of mitochondrial disorders is a complex and challenging process: although there are some well-defined and easy-recognizable clinical syndromes, many patients present with one or a few of the clinical features. In addition, different patterns of inheritance (depending on which genome is affected) can be observed. Moreover, genotype-phenotype relationships are complex since mutations in different genes can cause the same phenotype or, conversely, the same pathogenic mutation can be linked to a range of different phenotypes. Indeed, mutations in the mtDNA can be found in **homoplasmy** (see Glossary) or **heteroplasmy**; ~~and~~ therefore, different mutational load across different tissues or individuals can modify the range of symptoms or the penetrance of the disease[1]. Altogether, it is remarkable that even though some mutations causing mitochondrial diseases are present in all body cells, only some tissues, or cell types, are affected by the energetic defect and contribute to the patients´ phenotype. The causes of this tissue-specificity are still under debate. Here, we explore the activation of cellular stress response mechanisms upon different mitochondrial insults in different scenarios and how the concomitant cellular consequences might differ among different cell types, therefore contributing to the tissue-specificity of these severe genetic disorders.

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Integrated stress response as a potential mediator of tissue specificity of mitochondrial diseases

Due to their importance for the proper function of the organelle, import, folding and quality control of the mitochondrial proteome is regulated by a transcription regulation program that responds to protein misfolding, known as mitochondrial unfolded protein response (UPR^{mt}). Increasing evidence has demonstrated that the UPR^{mt} protects cells from a broader range of different mitochondrial stresses such as OXPHOS dysfunction, protein import deficiency, ATP depletion or dissipation of mitochondrial membrane potential. Interestingly, studies on mammalian systems have highlighted the integrated stress response (ISR) as a central core of the UPR^{mt}[2]. The ISR promotes through activation of four different kinases the phosphorylation of **eIF2α**, therefore leading to reduced global translation [3]. Indeed, translation attenuation has been shown to increase mitochondrial activity and to protect cells from mitochondrial dysfunction. Interestingly, ISR concomitantly activates the expression of different transcription factors such as **CHOP, ATF4** or **ATF5** which promote different cellular pathways such as serine biosynthesis, one carbon metabolism, transulfuration and proline synthesis. In the same line, systemic metabolism rewiring is stimulated trough circulating hormones such as **FGF21** or **GDF15**.

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However, it seems that activation of mitochondrial ISR is context-dependent, not always exerting beneficial effects in cell lines with electron transfer chain (ETC) deficiencies [4]. For example, mouse models for aminoacyl-tRNA synthetase defects showed that chronic activation of ISR contributed to the axonal peripheral neuropathy observed and that accounts for different forms of Charcot-Marie-Tooth disease [5]. Another murine model for lung-specific complex I deficiency evidenced that inability to regenerate mitochondrial NAD^+ results in a hyperactivation of ISR, influencing cell fate determination and preventing the successful differentiation into alveolar epithelial type 1 (AT1) cells. The resulting defect in postnatal epithelial development leads to respiratory failure and death of animals [6]. An interesting finding of this work was that whereas complex I deficiency altered cell fate determination and could be rescued by re-introducing the yeast NADH dehydrogenase NDI1, complex II deficiency in the lung did not trigger hyperactivation of ISR, meaning that in lung cells, the molecular trigger that activated the pathological compensatory mechanism was the impairment of mitochondrial NAD^+ regeneration rather than a bioenergetic defect. In the same line, the molecular triggers for the activation of the ISR upon mitochondrial dysfunction vary among cell types with different metabolic states. In that regard, the analysis of global gene expression, bioenergetics and metabolism in muscle cells that were proliferating (myoblasts) or differentiated (myotubes) treated with a panel of small-molecule mitochondrial inhibitors revealed that, in proliferating myoblasts, the ETC inhibition is linked to an increased mitochondrial and cytosolic $NADH/NAD^+$ ratio that decreases aspartate synthesis, depletes asparagine and ultimately activates ISR. On the other hand, decreased ETC activity in differentiated myotubes showed an inhibition of ATP synthase, activating the ISR due to the hyperpolarization of the inner mitochondrial membrane [7].

Thus, if pharmacological ETC inhibition in different cell types result in distinct molecular and metabolic consequences that ultimately activates ISR, it is tempting to speculate that the same genetic defects in different cell types, may impact differently in their metabolic fitness and therefore influence the activation of compensatory mechanisms such as the ISR. Following this reasoning, it seems plausible that not all cell types might have the same intrinsic capacity to activate ISR or other compensatory mechanisms. Indeed, embryonic and adult cardiomyocytes showed differences in ISR activation upon complex III inhibition with antimycin A. Although cardiomyocyte maturation was followed by an increased expression of redox proteins to cope with high reactive oxygen species (ROS) levels, these cells were not able to activate ISR and were highly sensitive to antimycin A. On the contrary, embryonic cardiomyocytes induced ISR pathway and were therefore more resistant to complex III inhibition. Such plasticity of neonatal cardiomyocytes might be essential to outlast periods of unfavorable intrauterine conditions, guaranteeing proper heart growth and avoiding perinatal cardiac diseases [8]. In addition, other studies using murine models for mitochondrial cardiomyopathy elucidated the contribution of FGF21 to ISR activation in the heart. Surprisingly, this factor might be a modulator of stress signaling in mild-to-moderate mitochondrial dysfunction, but its effects are dispensable or overtaken by other compensatory mechanisms in severe mitochondrial dysfunction [9]. Interestingly, studies using cellular models for mitochondrial diseases carrying mutations in different aminoacyl-tRNA synthetases highlighted a tissue-specific activation of ISR. This compensatory

response might explain the much less effect on the OXPHOS system of patient-derived proliferating neuronal progenitor cells (iNPCs) compared to mature neurons. However, the compensatory mechanisms observed were unique to the different aminoacyl-tRNA synthetase mutants and further investigations are required to explore the determinants of these activation patterns[10].

To conclude, all the evidence presented suggests that ISR activation might be triggered differently in different cells depending on the cell-type, the metabolic state, or the developmental state. Therefore, we could envision three different scenarios after the appearance of a mitochondrial insult. First, cells that are able to activate ISR and compensate for the mitochondrial defect, showing mild or no cellular phenotype. Secondly, cells that do not undergo ISR-mediated cellular adaptation and fail to cope with mitochondrial damage would therefore suffer cellular consequences and alter the proper function of the tissue. Finally, cells where the hyperactivation of these compensatory mechanisms might negatively impact cell fate and contribute to the pathogenesis of the disease. Therefore, differential ISR activation potentially contributes not only to the tissue-specificity of mitochondrial disorders. but also to the heterogeneity of symptoms **(Fig. 1)**.

Concluding remarks and future perspectives

The understanding of compensatory mechanisms such as UPR^{mt} or ISR has been the focus of studies during the last decades to find new therapeutical strategies for mitochondrial diseases. Indeed, new molecular defects beyond OXPHOS dysfunction (such as mitochondrial DNA double strand breaks) have been recently described to activate ISR [11]. In addition, a specific branch of ISR has been recently defined to activate mitophagy and contribute to cellular content renewal, therefore increasing the known cellular consequences of ISR activation [12]. Since triggering of ISR may not always exert beneficial effects and chronic ISR activation has been shown to be detrimental and associated to certain pathological conditions [5,6], it seems plausible that differences in the molecular triggers and the different degree of ISR activation (ranging from absence or mild response to hyperactivation) in different cell types might contribute to the specificity of mitochondrial disorders. We need to elucidate the determinants of ISR activation and downstream targets in different cell types harboring the same genetic defects to understand the contribution of ISR or other compensatory mechanisms to the tissue-specificity of mitochondrial disorders.

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Acknowledgements

This work was supported by Agencia Estatal de Investigación (Grant PID2020-116970GA-I00 and Grant RYC2020-029544-I) funded by MCIN/AEI/10.13039/501100011033 and by “ESF Investing in your future”, by Instituto de Salud Carlos III (ISCIII-FIS-PI21/00229) and European Regional Development Fund (FEDER); Gobierno de Aragón (Grupos Consolidados B33_23R); Fundación Mutua Madrileña. The CIBERER is an initiative of the ISCIII. AVS was supported by a fellowship “contratación de personal investigador predoctoral en formación 2002-26” from Gobierno de Aragón.

Declaration of Interests

No interests are declared.

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Glossary

Homoplasmy- all mtDNA copies present in a cell harbor the same genotype (wildtype or mutant).

Heteroplasmy- mtDNA copies with wildtype or mutant genotype coexist within a cell. Denoted as % of mutant genotype.

eIF2α- eucaryotic translation initiation factor 2 subunit alpha. Participates in the early steps of protein synthesis by forming a ternary complex with GTP an initiator tRNA. ISR activation mediates phosphorylation of this factor and translation attenuation.

CHOP10- also known as DNA damage-inducible transcript 3 protein (DDIT3). Multifunctional transcription factor inducing cell cycle arrest and apoptosis in response to endoplasmic reticulum stress.

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ATF4- transcription factor that binds the cyclic AMP response element (CRE) and mediates metabolic and redox processes in addition to be the master regulator of ISR.

ATF5- transcription factor binding to the cyclic AMP response element (CRE) and also the amino acid response element (AARE). Participates in survival, proliferation and differentiation processes in the cell.

FGF21- fibroblast growth factor 21. Involved in glucose uptake, systemic glucose homeostasis and insulin sensitivity.

GDF15- growth/differentiation factor 15**.** Involved in metabolic responses related to food intake, energy expenditure and body weight activated by different stresses.

Figure Legends

Figure 1. ISR differential activation might contribute to the tissue-specificity of mitochondrial diseases. Different molecular triggers associated with mitochondrial dysfunction activate the ISR, phosphorylating eIF2α and attenuating translation. Concomitantly, increased expression of different translation factors (ATF4, ATF5, CHOP10) or circulating hormones (FGF21, GDF15) results in different cellular and metabolic responses. Depending on the degree of ISR activation, different cells would either not be able to compensate for the mitochondrial damage and present with clinical manifestations (yellow), successfully cope with the mitochondrial insult and remain healthy (green) or be negatively affected by ISR hyperactivation and favor the progression to the diseased phenotype (red). This response might be different between patients harboring similar or different mitochondrial dysfunction, modifying the tissues affected and therefore the clinical symptoms. Tissues differently colored in this figure represent an hypothetical scenario and not a particular described case. Figure created using BioRender

~~**Figure 1. ISR differential activation might contribute to the tissue-specificity of mitochondrial diseases.** Different molecular triggers associated with mitochondrial dysfunction activate the ISR, phosphorylating eIF2α and attenuating translation. Concomitantly, increased expression of different translation factors (ATF4, ATF5, CHOP10) or circulating hormones (FGF21, GDF15) results in different cellular and metabolic responses. Depending on the degree of ISR activation, different cells would either not be able to compensate for the mitochondrial damage and present with clinical manifestations (yellow), successfully cope with the mitochondrial insult and remain healthy (green) or be negatively affected by ISR hyperactivation and favor the progression to the diseased phenotype (red).~~

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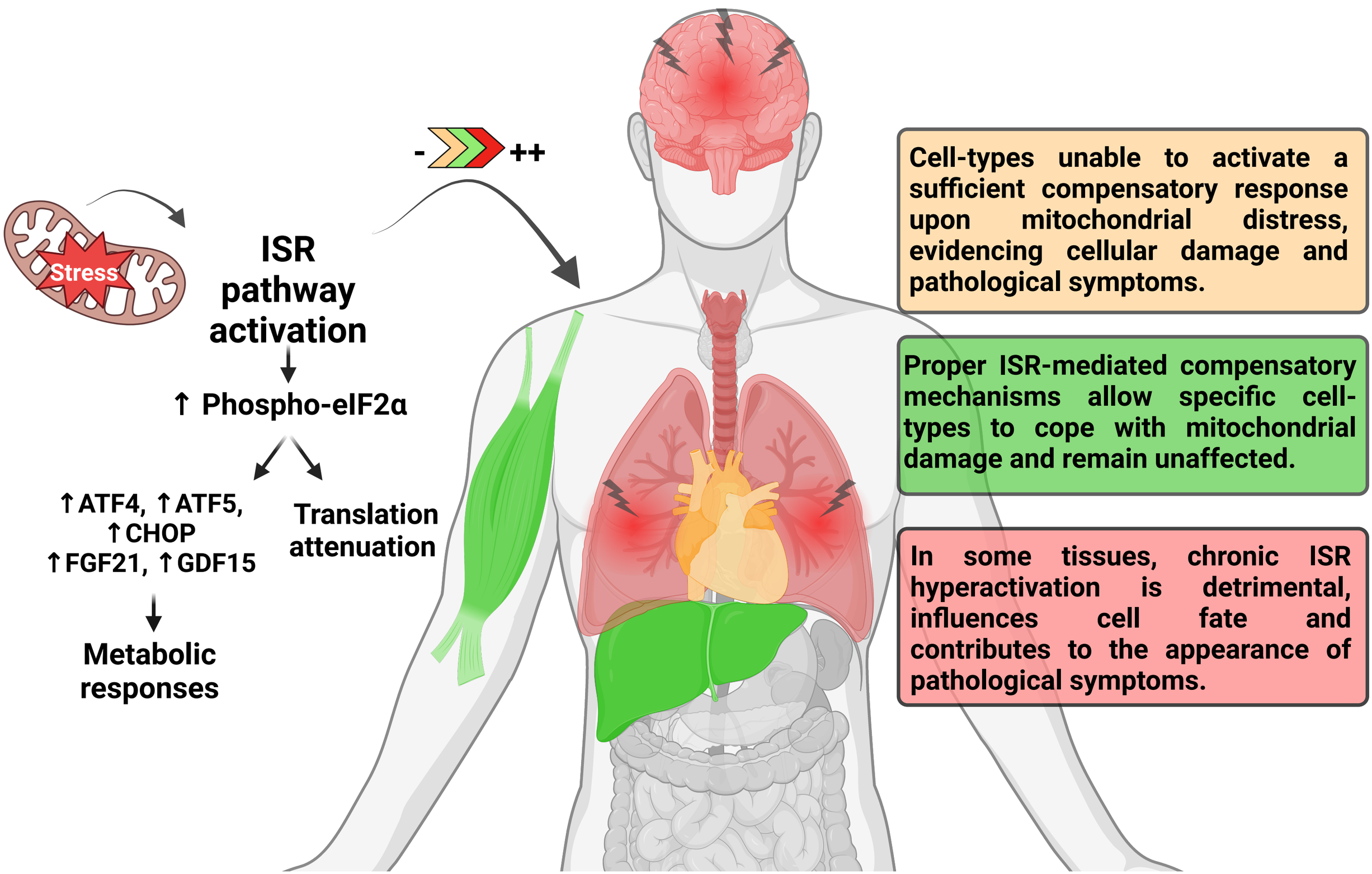
Figure


Stress
ISR pathway activation
↑ Phospho-eIF2α
↑ATF4, ↑ATF5, ↑CHOP
↑FGF21, ↑GDF15
Translation attenuation
Metabolic responses
-
++
Cell-types unable to activate a sufficient compensatory response upon mitochondrial distress, evidencing cellular damage and pathological symptoms.
Proper ISR-mediated compensatory mechanisms allow specific cell-types to cope with mitochondrial damage and remain unaffected.
In some tissues, chronic ISR hyperactivation is detrimental, influences cell fate and contributes to the appearance of pathological symptoms.

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