Stress and glucocorticoid receptor regulation of mitochondrial gene expression

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Abstract
Glucocorticoids have long been recognized for their role in regulating the availability of energetic resources, particularly during stress. Furthermore, bidirectional connections between glucocorticoids and the physiology and function of mitochondria have been discovered over the years. However, the precise mechanisms by which glucocorticoids act on mitochondria have only recently been explored. Glucocorticoids appear to regulate mitochondrial transcription via activation of glucocorticoid receptors (GRs) with elevated circulating glucocorticoid levels following stress. While several mechanistic questions remain, GR and other nuclear transcription factors appear to have the capacity to substantially alter mitochondrial transcript abundance. The regulation of mitochondrial transcripts by stress and glucocorticoids will likely prove functionally relevant in many stress-sensitive tissues including the brain.

Stress and mitochondria
The physiological stress response can be highly adaptive. It serves evolutionary purposes, including contributions to the ‘fight or flight’ response that direct energy sources to physiological processes that will increase immediate survival and facilitate memory of the stress event and associated stimuli so they can be avoided in the future. Stressors can be physical or psychological and depend largely on the individual’s attitude or perspective of the event. Controllable stressors, such as voluntary exercise, lead to stress tolerance, whereas uncontrollable stress exposure contributes to stressful helplessness (McEwen 2000). Stress follows an inverted ‘U’ shape, where moderate or tolerable stress, such as that induced by a controllable challenge, can enhance behavioral outcomes and cognitive performance. In contrast, excessive, chronic or uncontrollable stress results in a decrement in performance and can contribute to allostatic overload. Predictable, frequent and prolonged stressors increase species’ chance of learning avoidance on an intergenerational level, whereas shorter term avoidance is enabled through behavioral and physiological plasticity (Badyaev 2005). An appropriate response to stress requires a considerable amount of energy regardless of timescale or type of stress. Mitochondria, the powerhouse organelle of the cell, provide the energy to adapt to stress on every level: intracellular reactions (gene transcription and translation, epigenetic modifications), hormonal changes in the endocrine system, structural changes in tissue (synaptic changes in the brain) and behavioral and cognitive responses (Picard et al. 2018a).

Mitochondria are thought to be the result of a symbiotic relationship from fusion of a eukaryotic cell and aerobic bacterium that precedes complex multicellular life (Margulis & Bermudes 1985). The ability of mitochondria to generate energy (adenosine triphosphate) from food substrates and oxygen via the
electron transport chain made it valuable to eukaryotic cells and allowed for transcriptional regulation needed for development of different cell types, tissues and complex organisms (Lane & Martin 2010). Complex organisms have evolved to contain hundreds to thousands of copies of mitochondria per cell to provide the energy fundamental for development, repairing damage and responding to change. Over time, mitochondria have also become more integrated with their cellular environment, resulting in reciprocal exchange of information with the nucleus (Picard et al. 2018a). Mitochondria are essential to physiological processes fundamental in maintaining multicellular life including apoptosis, inflammation and thermogenesis (Picard et al. 2016).

Because of their endosymbiotic origin, mitochondria are the only metazoan organelles that have their own genome (the chloroplast in plants being the other significant eukaryotic organelle with its own genome). The circular mitochondrial genome has been described as a hybrid of the bacteria from which it is believed to be derived and the machinery of a bacteriophage containing eukaryotic polycistronic transcript regulation (Leigh-Brown et al. 2010). The mitochondrial genome is lean, containing only 37 genes (13 protein-coding genes related energy metabolism, 22 transfer RNAs and the small and large ribosomal subunits) in contrast to the approximately 20,000 protein-coding genes, which constitute only about 5% of the nuclear genome and a larger number of nuclear non-protein-coding RNA genes. Importantly, the nuclear genome has several orders of magnitude a range of promoter, enhancer and suppressor complexity, while the mitochondrial genome has only three well-characterized promoter regions all found along the D-loop control region (Montoya et al. 1982), though other potential sites of transcription factor interaction have been identified (Psarra & Sekeris 2009).

The physiological stress response begins with the brain, which receives and filters sensory input about stressful stimuli and integrates that information with contextual information, emotional state and previous experience. The hypothalamic–pituitary–adrenal (HPA) axis is the primary neuroendocrine pathway involved in stress response. When a physical or psychological stressor is experienced, the paraventricular nucleus of the hypothalamus is activated and releases AVP and corticotropin-releasing hormone (CRH). CRH and AVP travel to the anterior pituitary gland via portal blood and axonal transport respectively. CRH and AVP induce adrenocorticotropic hormone (ACTH) release into the blood stream, which is taken up by the adrenal gland. In the adrenal gland, ACTH stimulates synthesis of glucocorticoids from cholesterol. Ultimately, activation of the HPA pathway leads to increased circulating glucocorticoids (cortisol in humans or corticosterone in rodents), which can then regulate downstream processes including metabolism, immune function, emotional state and reproductive function. As the name suggests, glucocorticoids are critical in maintaining glucose and lipid homeostasis and a rise in glucocorticoids can elevate blood glucose in minutes (Kuo et al. 2015). As ligands, glucocorticoids elicit their effects via binding to the mineralocorticoid receptor (MR) and GR. These receptors are abundantly found in stress-sensitive regions of the brain (e.g. prefrontal cortex and hippocampus; Sánchez et al. 2000). Importantly, there are several negative feedback mechanisms in this system, including inhibition of the hypothalamus and pituitary induced by glucocorticoids.

Mitochondria are not only indispensable for providing the energy needed for an organized stress response, but are fundamentally integrated into the HPA axis themselves. Mitochondria are responsible for synthesizing all steroid hormones, including glucocorticoids (Bose et al. 2002). Upon ACTH activation in the adrenal gland, steroidogenic acute regulatory protein facilitates the movement of cholesterol across the mitochondrial membrane, which is the rate-limiting step in the production of glucocorticoids (Bose et al. 2002, Clark 2016). After conversion to pregnanolone in the mitochondrial matrix, pregnanolone is exported to the endoplasmic reticulum where it is converted to deoxycorticosterone (rodents) or 11-deoxycorticisol (humans). Finally, 11β-hydroxylase produces corticosterone or cortisol in the mitochondrial matrix for release into circulation (Picard et al. 2018a).

Elsewhere in the body, mitochondria must reallocate cellular resources to make stored energy available to meet the demand induced by stressful stimuli, especially in tissues required for stress adaptation, such as the brain. Mitochondria facilitate the cellular response in the body and the brain by attuning to local levels of glucocorticoids (Manoli et al. 2007, Picard et al. 2014). However, only GR (as opposed to MR) is found in mitochondria, suggesting that GRs drive the direct actions of glucocorticoids on mitochondria (though it should be noted that subcellular localization of MR has remained controversial, e.g. Fejes-Tóth et al. 1998, Psarra & Sekeris 2008, 2009, Du et al. 2009, Hernández-Díaz et al. 2010, Prager et al. 2010). While not fully understood, GR activation seems to drive the expression of genes encoded by the mtDNA via binding to the mtDNA D-loop (Psarra & Sekeris 2011).
Stress affects mitochondria physiology

Physiological processes affected by chronic stress and dysregulated in stress-related disorders overlap with processes governed by mitochondrial function, suggesting a role for mitochondria in facilitating some of the effects of chronic stress on the body (Picard & McEwen 2018). There is evidence from human and animal studies that mitochondrial function or mitochondrial copy number is associated with stress and aging (Picard 2011, Mengel-From et al. 2014). For example, a recent study demonstrated that mitochondrial health index, a measure that accounts for measures of mitochondrial biochemical function and copy number, in peripheral blood mononuclear cells (PBMCs) was associated with mood and was lower in individuals who experienced chronic caregiving stress compared to controls (Picard et al. 2018b). An earlier study found reduced mtDNA copy number in male veterans with PTSD (Bersani et al. 2016). Furthermore, early life adversity has been associated with increased mtDNA copy number (Tyurka et al. 2016), increased methylation near MTND-6 (the sole protein-coding gene located on the light inner strand; Lapp et al. 2018) and increased oxidative stress and mitochondrial function in PBMC in women (Boeck et al. 2016) in peripheral tissues. Mitochondrial function in the nucleus accumbens has been shown to mediate social dominance in high anxiety rats, demonstrating that mitochondrial function in the brain may play a role in emotionality and social status (Hollis et al. 2015). Social dominance has been widely described as a rodent model of social stress, and stress and anxiety involve many of the same neural substrates. Therefore, the findings by Hollis et al. (2015) suggest that mitochondrial function in the brain may underlie resilience to social stress and importantly may serve as a pharmacological target for intervention. Together, these studies provide evidence that stress history may induce long-lasting changes in mitochondrial function. However, more animal studies are needed to replicate these findings, evaluate tissue specificity and determine the mechanisms underlying these phenomena. From a translational perspective, whether peripheral mitochondrial measures in humans extend to the brain must be explored as this may have important implications for behavior and affective states underlying psychiatric illness.

Mitochondria also appear to be both regulators of and contributors to cellular allostatic load. For instance, defects in genes involved in mitochondrial function result in widespread alterations in stress physiology (Picard et al. 2015). In this landmark study, mitochondrial defects were associated with altered peripheral CORT and ACTH responses to acute stress. Additionally, large-scale changes in hippocampal transcription were observed for several metabolic, inflammatory and neuroendocrine-related genes. These findings provided direct evidence for mitochondrial regulation of the peripheral endocrine acute stress response as well as regulatory effects on efferent brain targets of stress (i.e. the hippocampus).

Conversely, changes in mitochondrial physiology have been observed following stress or corticosteroid administration (Picard et al. 2018a). Upon translocation of GR into mitochondria of cortical neurons, time- and dose-dependent effects were observed on mitochondrial oxidation, such that an acute or low-to-moderate dose increased mitochondrial oxidation and long-term or high-dose reduced mitochondrial oxidation. Similar effects were seen for membrane potential, calcium buffering capacity and GR–Bcl-2 complex assembly (Du et al. 2009). In vivo, chronic CORT produced comparable dose dependency for cortical mitochondrial Bcl-2. Functional relevance of this interaction has been described as chronic CORT appears to increase the generation of reactive oxygen species (ROS), which may help explain cellular degeneration observed following chronic CORT treatment (Du et al. 2009, Tang et al. 2013). Chronic stress also appears to be permissive for the accumulation of reactive species in numerous stress-sensitive brain regions (e.g. cortex and hippocampus; Madrigal et al. 2001, Rezin et al. 2008, Gong et al. 2011). Indirectly, GR-dependent regulation of available glucose may contribute to the production of ROS (Yu et al. 2006, Picard et al. 2014). Furthermore, GR interacts with numerous molecular co-factors (e.g. sirtuins) involved in metabolic homeostasis and therefore may have several undescribed circuitous effects on mitochondrial function (Amat et al. 2007, Nogueiras et al. 2012, Alageel et al. 2018).

GR as regulator of mitochondrial transcription

The basal transcriptional machinery in mammalian mitochondria consists of the mitochondrial RNA polymerase (POLRMT), mitochondrial transcription factor A (TFAM) and mitochondrial transcription factor B2 (TFB2M), all of which are encoded by the nuclear genome (Gustafsson et al. 2016). Beyond these core molecules, several mitochondrial proteins are known to act as regulators of mtRNA transcript abundance, including the mitochondrial termination factor (MTERF) family
of proteins, mitochondrial ribosomal protein (MRP)L12, leucine-rich pentatricopeptide repeat containing protein and TEFM (transcription elongation factor, mitochondrial) (Bestwick & Shadel 2013). However, our understanding of mitochondrial transcriptional regulation remains incomplete.

Studies showing that glucocorticoids are found in mitochondria and that glucocorticoid treatments impact mtRNA metabolism date to the 1960s (Beato et al. 1969, Mansour & Nass 1970, Yu & Feigelson 1970). The Sekeris group was among the first to suggest the potential role of steroid receptors in the transcriptional regulation of mtDNA by identifying a putative response element with partial sequence homology to the GR and estrogen response element consensus sequences (Sekeres 1990). More recently, studies have shown that nuclear transcription factors can translocate to the mitochondria, including CREB, NFKB, p53 and GR, among others (Psarra et al. 2006, Psarra & Sekeris 2008, Szczepanek et al. 2012). While some of these TFs have been shown to alter other aspects of mitochondrial physiology, such as electron transport chain function and apoptosis, several appear to have the capacity to regulate mtRNA expression (Psarra & Sekeris 2009, Bestwick & Shadel 2013, Hunter et al. 2016).

However, the mechanism for these effects is still unclear.

GR was shown to enter liver mitochondria in response to treatment with the synthetic GR agonist dexamethasone in the 1990s (Demonacos et al. 1993). Subsequent work showed that GR translocation in response to glucocorticoid administration occurs in several tissues, including the brain (Moutatsiou et al. 2001, Du et al. 2009, Psarra & Sekeris 2009). Studies using a variety of techniques showed that not only GR, but also other nuclear hormone receptors (including the estrogen, androgen and thyroid hormone receptors) appeared to translocate to mitochondria in response to hormone stimulation (Psarra & Sekeris 2008), though, as noted earlier, MR does not appear to do so in the cell types thus far examined. Interestingly, only the GRα isoform appears to be translocated to the mitochondria, as GRβ is exclusively targeted to the nucleus (Psarra et al. 2005). GR translocation to the mitochondria appears to be in association with Bcl-2 family proteins (Du et al. 2009, Prenek et al. 2017). It is also evident that GR translocation to the mitochondria may require proteolytic cleavage by a serine 9 like endoprotease (Boopathi et al. 2008, Avadhani et al. 2011), which appears to be involved in the translocation of other nuclear transcription factors, like p53, to mitochondria. More recent work has shown that histone deacetylase 6 (HDAC6) and heat shock protein 90 (Hsp90) are associated with the translocation of GR into mitochondria (Li et al. 2014). Hsp90 is a target of HDAC6, which regulates its function as a chaperone (Rao et al. 2012) and inhibition of HDAC6 with N-hydroxy-4-(2-[2-hydroxyethyl] (phenyl)amino]-2-oxoethyl) benzamide attenuated GR translocation to the mitochondria via interaction with the TOM/TIM complex mitochondrial translocation machinery (Li et al. 2016).

Because GR functions as a nuclear transcription factor, it is plausible to propose that it acted in a similar fashion in the mitochondrion. The thyroid hormone receptor was previously shown to regulate mtRNA transcript levels (Casas et al. 1999, Enriquez et al. 1999), and in vitro and in silico work has identified a number of potential glucocorticoid response elements in the mitochondrial genome, including sites in the D-loop control region and loci in the region of the mtND-1, mtCOX-I and mtCOX-III genes (Psarra & Sekeris 2009). Transfection of mitochondrial GRE constructs into LATK cells showed that they were dexamethasone responsive (Tsiriotis et al. 1997), and gel shift assays confirmed that both purified GR and GR containing mitochondrial extracts bound to the mitochondrial GREs (Demonacos et al. 1995).

Work in HepG2 hepatocarcinoma cells showed that GR binds to a site the mitochondrial D-loop and dexamethasone induces changes in the expression of mitochondrial genes, significantly in the case of the mtCOX-1 gene. Further, inhibition of nuclear RNA polymerase II with α-amanitin did not alter expression of mtDNA-derived transcripts in response to dexamethasone stimulation (Psarra & Sekeris 2011). This provided compelling evidence that GR acts as a transcriptional regulator. However, this study was performed in cancer cells, which are known to harbor a number of mitochondrial abnormalities (Lennon & Salgia 2014) and left open the question of the phenomenon in normal physiology. Using ChIP sequencing, we demonstrated that GR also bound to the mitochondrial D-loop in rat hippocampus following acute treatment with corticosterone. In the same study, PCR- and RNA-sequencing techniques confirmed that acute restraint stress altered expression of mtRNA and the diversity of mitochondrially derived transcripts was in a manner consistent with a role for GR as a transcriptional regulator under normal physiological conditions in the brain. We observed an overall downregulation of mtDNA-encoded genes with significant effects on mtND-1, mtND-3, mtND-6 and mtATP-6 expression (Hunter et al. 2016). In both studies, mitochondria gene transcription varied by treatment exposure (1-h vs 5-h dexamethasone treatment or single 30-min acute restraint stress and 1-h
1. Stress & elevated glucocorticoids via HPA axis or dexamethasone treatment

2. GR activation in cytosol

3. GRα translocation to mitochondria

4. GR regulation via binding to the D-loop control region and potentially other loci

5. Changes in mtRNA

<table>
<thead>
<tr>
<th>Tissue</th>
<th>HepG2 hepatocarcinoma cells (Psarra &amp; Sekeris, 2011)</th>
<th>Rat hippocampus (Hunter et al., 2016)</th>
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<tbody>
<tr>
<td>Treatment</td>
<td>Dexamethasone</td>
<td>Restraint stress</td>
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<td>Duration</td>
<td>1 h</td>
<td>5 h</td>
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<tr>
<td>mtRNA ↑</td>
<td>ND2, Cyt b, 16 S, ATP6, ATP8, Cox I, ND4, ND5</td>
<td>ND1, ND2, Cyt b, 16 S, ND4, ND5, ND6</td>
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<tr>
<td>mtRNA ↓</td>
<td>ND1</td>
<td>12 S, ATP6, ATP8, Cox I, Cox II, Cox III, ND3</td>
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Figure 1

GR-induced changes in mtRNA. Elevated levels of circulating glucocorticoids following stress or dexamethasone treatment activates GR in cytoplasm of cell in target tissues. Upon activation, GRα translocates to mitochondria where it influences mtDNA transcription, likely by binding to the D-loop control region and other loci. Effects on transcription depend on treatment, exposure duration and region of mtDNA. *Significantly downregulated following 300 µg corticosterone treatment in adrenalectomized rats.
recovery vs repeated restraint stress for 21 days) and the direction of mtrNA change varied by mitochondrial gene
(see Fig. 1 for a summary). Given the biphasic effects of glucocorticoids on mitochondrial GR and on GR binding
to the mitochondrial D-loop, under basal conditions, unliganded mitochondrial GR may serve as a regulator of
gene expression or specific GR isoforms may coordinate mtDNA gene transcriptional responses (Koufali et al. 2003,
Polman et al. 2013, Hunter et al. 2016, Morgan et al. 2016). While the above studies demonstrate a mitochondrial
transcriptional response to elevation of glucocorticoids and subsequent activation of GR, the specific downstream
effects of these changes in mtrNA are yet to be identified.

Future directions

Work using multiple technical approaches has made it clear that the GR binds to the mitochondrial genome
and that GR activation and mitochondrial translocation is associated with changes in levels of mtDNA derived
transcripts, but the mechanism by which GR-dependent regulation of mitochondrial gene transcription remains
unclear. It is unknown if GR interacts with the core mitochondrial transcriptional machinery (TFAM,
TFRB2M or POLRMT) or with other known regulators of mitochondrial transcription, such as the MTERF proteins.
While the most parsimonious explanation for the transcriptional effects of GR in mitochondria is that it acts
in the same fashion in the mitochondria as it does in the nucleus, experimental evidence remains inadequate. One
significant question is the mechanism of GR translocation to the mitochondria, and to address this question, future
studies could explore GR–mtDNA interactions using HPOB (Li et al. 2016) and serine protease-9 inhibition
(Boopathi et al. 2008). Examining GR effects on mtDNA transcription will benefit from emerging techniques such
as metabolomic labeling of mitochondrial-specific transcripts (Nguyen et al. 2018).

In addition to establishing the mechanism by which GR induces transcriptional effects, more work needs to
be done to elucidate the long-term impact of chronic stress and glucocorticoid dysregulation on mitochondria
function, mtDNA gene expression and mtDNA copy number in the brain and periphery. Specifically, more
studies on the long-term effects of chronic and early life stress in humans and animals on epigenetic regulation of
mtDNA, including mtDNA methylation (which appears to be a low frequency event at mitochondrial cytosines
relative to the methylation levels in the nucleus, likely due to the prokaryotic origin of the mitochondrial genome;
Mechta et al. 2017), will be instrumental in uncovering the role of mitochondria in stress and stress-related
psychopathology. Finally, more comprehensive measures of mitochondrial function (e.g. mitochondrial health
index described above) that incorporate measures of copy number, function, methylation status to fully understand
the effect of stress on mitochondrial physiology.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This paper did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Author contribution statement

All authors contributed equally to the writing and editing of this paper.

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