



## Estrogenic control of mitochondrial function

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### ABSTRACT

Sex-based differences in human disease are caused in part by the levels of endogenous sex steroid hormones which regulate mitochondrial metabolism. This review updates a previous review on how estrogens regulate metabolism and mitochondrial function that was published in 2017. Estrogens are produced by ovaries and adrenals, and in lesser amounts by adipose, breast stromal, and brain tissues. At the cellular level, the mechanisms by which estrogens regulate diverse cellular functions including reproduction and behavior is by binding to estrogen receptors  $\alpha$ ,  $\beta$  (ER $\alpha$  and ER $\beta$ ) and G-protein coupled ER (GPER1). ER $\alpha$  and ER $\beta$  are transcription factors that bind genomic and mitochondrial DNA to regulate gene transcription. A small proportion of ER $\alpha$  and ER $\beta$  interact with plasma membrane-associated signaling proteins to activate intracellular signaling cascades that ultimately alter transcriptional responses, including mitochondrial morphology and function. Although the mechanisms and targets by which estrogens act directly and indirectly to regulate mitochondrial function are not fully elucidated, it is clear that estradiol regulates mitochondrial metabolism and morphology via nuclear and mitochondrial-mediated events, including stimulation of nuclear respiratory factor-1 (NRF-1) transcription that will be reviewed here. NRF-1 is a transcription factor that interacts with coactivators including peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1 $\alpha$ ) to regulate nuclear-encoded mitochondrial genes. One NRF-1 target is TFAM that binds mtDNA to regulate its transcription. Nuclear-encoded miRNA and lncRNA regulate mtDNA-encoded and nuclear-encoded transcripts that regulate mitochondrial function, thus acting as anterograde signals. Other estrogen-regulated mitochondrial activities including bioenergetics, oxygen consumption rate (OCR), and extracellular acidification (ECAR), are reviewed.

### 1. Introduction

The history of the discovery and characterization of estradiol (E<sub>2</sub>) was recently published by Evan Simpson and Richard J. Santen [1]. Drs. Simpson [2] and Santen [3] are noted for their work on estrogens, reproduction, aromatase, and the clinical use of aromatase inhibitors for the treatment of postmenopausal breast cancer patients with estrogen receptor  $\alpha$  (ER $\alpha$ ) positive primary tumors. Estrone (E<sub>1</sub>) and estriol (E<sub>3</sub>) were first isolated in 1930-31 from the urine of pregnant women by Edward A. Doisy [4]. E<sub>2</sub> was later isolated by Dr. Doisy from pig follicular fluid [4]. Subsequent discoveries of E<sub>2</sub> metabolism, tissue-specific uptake, cellular activities, E<sub>2</sub>-dependent changes in the subcellular distribution of ER $\alpha$  [5], cloning of ER $\alpha$  from MCF-7 human breast cancer cells [6], and the discovery and cloning of ER $\beta$  [7] have enriched our understanding of estrogen action. Studies of the genomic binding distribution of ER $\alpha$  [8-13] and ER $\beta$  [14] in chromatin immunoprecipitation (ChIP) studies revealed the diversity of genes regulated by these receptors in response to E<sub>2</sub>. "Omics" approaches have identified cell-specific estrogen-regulated transcriptomes of mRNA and all the ncRNAs (reviewed in Ref. [15]).

At this point, astute readers will note that the use of the word estrogen is not synonymous with E<sub>2</sub>. That is because there are three primary estrogens with E<sub>2</sub> considered to be the most potent estrogen, *i.e.*, E<sub>2</sub> has the highest affinity for estrogen receptor  $\alpha$  (ER $\alpha$ ) [16] and is the predominant estrogen in circulation in premenopausal women (high pM to nM range). The lower ER $\alpha$  affinity estrogens are E<sub>1</sub> and E<sub>3</sub>. E<sub>2</sub> is synthesized in the ovary whereas E<sub>1</sub> is synthesized from androstenedione in the adrenal cortex and E<sub>3</sub> is primarily from the placenta, although each can be synthesized from androgenic precursors depending on the tissue expression of aromatase (CYP19) [17,18]. Obese and overweight postmenopausal women have higher levels of circulating estrogens produced by adipose tissue compared with lean women [19].

Estrogens bind ER $\alpha$  and ER $\beta$  that are conserved nuclear receptors (NR) showing high identity within the DNA binding and ligand binding domains, but differences in the amino acid (aa) composition of N terminus [20]. In addition to the full length ER $\alpha$  and ER $\beta$ , each subtype has numerous splice variants [21,22]. More recent studies identified a 'hot spot' within the ligand binding domain (LBD) of ER $\alpha$  where mutations activate the receptor independent of ligand binding to drive

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## Abbreviations

AF-2	Activation function-2	mtDNA	mitochondrial genome
AD	Alzheimer's disease	(MNX) mouse model	Mitochondrial-Nuclear-eXchange
AR	androgen receptor	UPR <sup>mt</sup>	mitochondrial unfolded protein response
AHR	arylhydrocarbon receptor	MERKO	muscle-specific Esr1 (ER $\alpha$ ) ER $\alpha$ knockout
BPA	Bisphenol A	NK	natural killer
ChIP	chromatin immunoprecipitation	NRF-1	nuclear respiratory factor-1
CREB	cAMP Response Element-Binding Protein	ncRNA	non-coding RNA
DILI	drug-induced liver injury	NB	neuroblastoma
EDC	endocrine disrupting chemical	OCR	oxygen consumption rate
EGFR	epidermal growth factor receptor	OXPHOS	oxidative phosphorylation
E <sub>2</sub>	estradiol	PPARA	peroxisome proliferator activated receptor $\alpha$
eRNA	enhancer RNA	PGC-1 $\alpha$	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha
E <sub>1</sub>	estrone	PHB	prohibitin
E <sub>3</sub>	estriol	PKA	protein kinase A
ER $\alpha$ and ER $\beta$	estrogen receptors $\alpha$ , $\beta$	PM	plasma membrane
ERE	estrogen response element	PKC	protein kinase C
ER	endoplasmic reticulum	PNPase	polynucleotide phosphorylase
ETC	electron transport chain	ROS	reactive oxygen species
EIF2AK2	eukaryotic translation initiation factor 2- $\alpha$ kinase 2	RTKs	receptor tyrosine kinases
ECAR	extracellular acidification rate	RAR $\alpha$ , RAR $\gamma$	retinoic acid receptors $\alpha$ and $\gamma$
FXR	farnesoid-X-receptor	RXR	retinoid X receptor
GPER1	G-protein coupled ER	4-OHT	4-hydroxytamoxifen
GR	glucocorticoid receptor	SERM	selective estrogen receptor modulator
JNK2	C-Jun N-Terminal Kinase 2	STAT	signal transducer and activator of transcription
LBD	ligand binding domain	SIRT1	sirtuin 1
ncRNA	long non-coding RNAs	SIRT3	sirtuin 3
MPA	Medroxyprogesterone acetate	SLE	systemic lupus erythematosus
miRNA	microRNAs	TFAM	transcription factor A, mitochondrial
MRE	miRNA response element	TR	thyroid receptor
		T1D and T2D	Type 1 and Type 2 diabetes

aromatase-resistant metastatic breast cancer [23,24].

E<sub>2</sub> forms hydrogen bonds within the ligand binding pocket in the LBD of both ER $\alpha$  and ER $\beta$  resulting in activation of activation function 2 (AF-2) [25]. E<sub>2</sub>-ER $\alpha$  and ER $\beta$  binding within the cytoplasm causing conformational changes in the receptor resulting in dissociation of the receptor from chaperone proteins, e.g., Hsp90, Hsp40, p23, hsp70, allowing receptor dimerization [26]. This also exposes the receptors' nuclear localization domain, thus allowing its nuclear localization, although some proportion of each receptor subtype is in the nucleus, and bound to DNA, in the absence of ligand [27,28]. ER $\alpha$  and ER $\beta$  bind directly with high affinity to a DNA sequence that is an inverted repeat called the estrogen response element (ERE), i.e., 5'-AGGTCAnnnT-GACCT-3' [29]. ERs are located throughout the genome with their accessibility regulated by chromatin structure. ER $\alpha$  and ER $\beta$  binding to ERs facilitates chromatin looping bringing DNA-bound proteins and their interacting proteins into proximity to increase RNA pol II recruitment to promoter sequences and stimulate its conversion to an elongation complex [30]. ER $\alpha$  and ER $\beta$  were identified in mitochondria of various cell types where they bind mtDNA (reviewed in Ref. [31]). ER $\alpha$  also interacts indirectly to nuclear DNA by its direct (protein-protein) interaction with other DNA-bound transcription factors, e.g., SP1 [32], AP-1 [33], and RUNX1 [9]. This mechanism of ER $\alpha$  activation of transcription is called "tethering". In addition to ligand binding, ER $\alpha$  is regulated by phosphorylation that can activate the receptor independent of ligand binding (reviewed in Refs. [34,35]). Recent studies show the cistrome of phospho-ser118-ER $\alpha$  is enriched for ERs and also for association with Grainyhead Like Transcription Factor 2 (GRHL2), a transcription factor best known as a tumor suppressor [36], which acts like a pioneer factor [37].

The conformational changes elicited upon E<sub>2</sub> binding to ER $\alpha$  facilitates the recruitment of coactivators and chromatin remodeling complexes that interact with the C-terminus of the receptors via helix 12 of

the LBD in a ligand-dependent manner [38]. ER $\alpha$  is a component of a pre-formed "MegaTrans complex" of 1–2 MDa which includes the pioneering factor FOXA1 that can remodel chromatin and retinoic acid receptors  $\alpha$  and  $\gamma$  (RAR $\alpha$ , RAR $\gamma$ ), STAT1, GATA3, and AP2 [39]. The MegaTrans complex is required for activation of enhancer RNA (eRNA) transcription and recruitment of coactivators, including p300 and Mediator (MED1), forming "super enhancers" that span large chromatin domains, are co-occupied by multiple transcription factors and Mediator, and drive high cell-type specific gene transcription programs [39,40]. Acute enhancer activation by E<sub>2</sub> results in eRNA-mediated ribonucleoprotein (RNP) assembly of the MegaTrans complex that has properties of phase separated condensates that are required for cooperative interactions to bring genomic loci into proximity which increases local cofactor retention time [41].

E<sub>2</sub> also activates GPER1 (previously called GPR30 and GPER) which is a seven *trans*-membrane G-protein coupled receptor (GPCR) that localizes to the plasma membrane (PM) and also shows intracellular localization in a tissue-specific manner [42]. GPER1 shares structural homology with chemokine GPCRs, but is not activated by cytokines [43]. Ligand binding and activation of GPER1 rapidly stimulates intracellular signaling pathways in a cell-specific manner including activation of adenylate cyclase, epidermal growth factor receptor (EGFR), mitogen-activated protein kinase/extracellular regulated protein kinase (MAPK/ERK), insulin-like growth factor receptor (IGFR), phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), and [Ca<sup>2+</sup>]<sub>i</sub> (reviewed in Refs. [44,45]). GPER1 (GPR30) was reported to localize to the nucleus of C2C12 murine skeletal muscle cells [46]. E<sub>2</sub>-GPER1 interaction activates adenyl cyclase thus increasing intracellular cAMP production and protein kinase A (PKA) signaling [47], and activates epidermal growth factor receptor (EGFR) [48]. GPER1 is also activated by 4-hydroxytamoxifen (4-OHT), an active metabolite of tamoxifen a selective estrogen receptor modulator (SERM) used in breast cancer

therapy [49]. Additional ligands that activate GPER1 include Bisphenol A (BPA), an endocrine disrupting chemical (EDC) [50] and  $E_3$  [42]. GPER1 is the target of synthetic agonists (G-1) and antagonists (G-15 and G-36) produced for experimental studies [51]. GPER1 has also been identified as an aldosterone receptor [52–55], although this is disputed [56]. A recent report identified the first  $ER\alpha/ER\beta$  selective ligand (AB-1) that does not activate GPER1 [57]. Although GPER1 knockout mice have no reproductive phenotype, they are insulin resistant, obese, and have cardiovascular dysfunction (reviewed in Refs. [44,47]). GPER1 knockout mice showed increased cardiac oxidative stress and damage, increased 4-hydroxynonenal and 8-hydroxy-2'-deoxyguanosine (8-oxo-DG) staining, and increased oxidative stress-related genes, e.g., *Gstk1*, *Ucp3*, and *Sod2* [58]. Recent studies in Wistar rats demonstrated a sexual dimorphic role of GPER1 in regulating body weight in early postnatal life [59]. GPER1 expression is widespread in the central nervous system (CNS) and contributes to spatial memory, anxiety, social memory, and lordosis behavior in mice (reviewed in Ref. [60]). These data indicate that estrogens regulate normal function in the nervous, immune, skeletal, and cardiovascular systems, adipocytes, liver, pancreas, and kidney by activating GPER1 (reviewed in Refs. [44,47]).

In addition to their genomic activity, ~5–10% of  $ER\alpha$  and  $ER\beta$  are localized to the PM [61].  $ER\alpha$  is palmitoylated and interacts with PM-associated caveolin-1 and signaling proteins to activate intracellular signaling cascades that ultimately alter transcriptional responses [62]. The small ubiquitous redox-active protein Memo (*MEMO1*) is overexpressed in breast tumors and conveys migratory signals from receptor tyrosine kinases (RTKs), e.g., epidermal growth factor receptor 2 (HER2), and promotes rapid  $ER\alpha$  and  $ER\beta$  phosphorylation [63]. This membrane-initiated  $E_2$ -signaling can occur within seconds (changes in calcium flux [64]) or minutes (altered kinase cascades, e.g., ERK1/2 [65]) and cooperates with nuclear  $ER\alpha$  and  $ER\beta$  transcriptional programming in a cell-specific manner [62]. The use of transgenic and knockout  $ER\alpha$  mouse models has revealed that the protective effect of  $E_2$  in tissues including adipose tissues (white and brown), heart, liver, pancreas, and skeletal muscle require nuclear  $ER\alpha$  whereas membrane- $ER\alpha$  mediates some of the endothelial/vascular effects of  $E_2$  [66].

EDCs can interfere with any aspect of hormone action including altering synthesis, release, transport, receptor binding, metabolism, or clearance [67,68]. A subclass of EDCs are termed “metabolism disrupting chemicals” (MDCs) that can alter any aspect of metabolism [69]. Further, there is crosstalk between EDCs and MDCs in terms of interaction with  $ER\alpha$ ,  $ER\beta$ , and GPER1 and their signaling pathways. In a study testing 8000 EDCs *in vitro*, ~11% disrupted the mitochondrial membrane potential, although further studies are clearly needed to understand potential impacts for human health and fundamental mechanisms [70]. The effects of some MDCs in mitochondrial bioenergetics was recently reviewed [71].

## 2. Sex differences in disease

A number of tissues, diseases, and cancers, in addition to those expected: gonads, breast, and prostate; show sex differences. There is currently great interest in understanding these sex-based differences in disease with the goal of personalizing treatment. The National Institutes of Health requires every grant application to address “sex as a biological variable”. The list of diseases showing sex differences is far too long to include here, but examples include hypertension [72], ischemic stroke and myocardial infarction [73], and neurodegenerative and neuropsychiatric diseases [74]. Each of these diseases are associated with mitochondrial dysfunction. Clinical and knockout mouse studies have provided evidence that the protective effects of estrogens in vascular and metabolic protection are mediated by  $ER\alpha$ 's AF-2 domain by both nuclear and PM-initiated signaling (reviewed in Ref. [66]). Other differences in disease prevalence that are associated with higher  $E_2$  levels in premenopausal women include both Type 1 and Type 2 diabetes

(T1D and T2D) where premenopausal women show lower incidence of both metabolic disorders whereas postmenopausal women with T1D and T2D show higher development of cardiovascular disease and end stage kidney disease than men [75]. Mitochondrial dysfunction is associated with insulin-resistance [76] and multiple endocrine disorders [77]. Mechanisms for sex differences include not only estrogens and androgens, but also sex chromosome-encoded genes (reviewed in [78,79]). Here, I will focus on estrogens and their role in metabolism and disease.

It is well established from rodent studies and observations in humans that  $E_2$  increases fat oxidation, inhibits lipogenesis, and has a host of regulatory functions on the cells of the immune system, e.g., B cells, T cells, natural killer (NK) cells, neutrophils, and macrophages [80]. Over 75% of autoimmune disorders show higher prevalence in females and recent studies demonstrated a direct role for T cell  $ER\alpha$  expression in the development of T-cell dependent colitis in mice and reduced T cell proliferation [81]. The role for estrogens and  $ER\alpha$  in systemic autoimmune disease, e.g., systemic lupus erythematosus (SLE) involves B cells and T cells has been reviewed [82].

$E_2$  acts centrally and systemically to regulate energy balance and metabolism. Sex differences in neurodegenerative diseases suggest a protective role for estrogens in some diseases, e.g., there is a higher incidence of Parkinson's disease in men and a role for loss of estrogen-protection at menopause is associated with a higher prevalence and severity of Parkinson's disease as well as Alzheimer's disease (AD) in women [83]. Mitochondrial dysfunction plays a role in these diseases [84,85].  $E_2$  is produced in brain, specifically in the hippocampus, amygdala, and preoptic area, in both female and male rats [86]. The mechanisms of action of  $E_2$  in the brain are identical to those in peripheral tissues including both genomic and non-genomic  $ER\alpha$  and  $ER\beta$  (reviewed in Ref. [87]).

A recent report examined mitochondrial function in peripheral mononuclear blood cells (PBMCs) from healthy male and female volunteers (avg. age 30 and 31, respectively) [88]. PBMCs from females showed higher mitochondrial mass, higher Complex I, I + II, IV, and electron transport compared to males; however, no difference in ATP production were detected. The mitochondrial brain metabolite N-acetylaspartate (NAA) was also significantly higher in females compared to males; however, there was no significant correlation between individual parameters in PBMCs and brain NAA. Nonetheless, the authors conclude that measuring mitochondrial function in PBMCs could be a useful surrogate marker to examine differences in mitochondrial function in older adults and determine if this could be an AD marker [88].

Hepatocellular cancer (HCC) has a higher incidence in males than females where estrogens have protective effects against the initiation and progression of HCC [89]. Sex-dependent differences in liver include the expression of hepatic cytochrome P450 enzymes as well as transcription factors (TF) including  $ER\alpha$ , arylhydrocarbon receptor (AHR), peroxisome proliferator activated receptor  $\alpha$  (PPARA), and farnesoid-X-receptor (FXR) leading to differences in drug responses and metabolism in men and women [19,90,91]. Non-alcoholic fatty liver disease (NAFLD) is higher in men than premenopausal women, but increases in postmenopausal women [91]. Drug-induced liver injury (DILI) also shows sex differences with 41 drugs showing female-dominant DILI only in premenopausal women [92]. Interestingly, drugs with female-biased DILI show a higher prevalence of mitochondrial liability, reactive metabolite formation and higher transporter inhibition potential [92]. An immune-mediated DILI model in BALB/c mice showed that females had higher production pro-inflammatory hepatic cytokines (IL-6) than males and had more severe hepatitis suggesting that  $E_2$  and IL-6 may be responsible for reducing protective regulatory T-cells [93]; however, no measure of mitochondrial function was included in this study.

### 3. Mitochondria: structure and function

The mitochondrion is a densely packed, dynamic organelle of bacterial ancestry and endosymbiotic origin [94]. Mitochondria sustain life by converting metabolites from dietary fuels to ATP, CO<sub>2</sub>, and H<sub>2</sub>O, producing heat in the process, and by enabling stress adaptation for survival. An average mitochondrion is ~1 μm and changes its shape constantly by fusion and fission to form a dynamic network that interacts with the endoplasmic reticulum (ER) at points called MAM (mitochondria-associated membranes) for the transfer of Ca<sup>++</sup>, lipids, and other signals [95]. Fission and fusion are regulated by nutrient availability and metabolic demand [96]. Mitochondrial fusion is required for removal of damaged mitochondria by autophagosomes (mitophagy) whereas fission is necessary for the distribution of mitochondrial DNA during cell division [97]. Mitochondria are commonly considered the cellular powerhouse because the enzyme complexes I–V of the electron transport chain (ETC), with complex V being T1F0 (ATPase) reside as supramolecular complexes in the inner mitochondrial membrane producing ATP by oxidative phosphorylation of reduced substrates (NADH and FADH<sub>2</sub>). In addition, mitochondrial metabolic multienzyme complexes (metabolons) are dynamically regulated [98,99]. Of the 80 proteins in complexes I–V, 13 are encoded by the mitochondrial genome (mtDNA) [100]. The OXPHOS-ETC complexes interact with each other forming supercomplexes referred to as ‘respirasome’ and ‘respiratory megacomplexes’, depending on the exact stoichiometry [101]. The regulation of this process is not yet completely elucidated.

Nuclear-encoded mitochondrial transcription factors TFAM (transcription factor A, mitochondrial) and TFB (mitochondrial transcription factor B, encoded by *TFB1M* and *TFB2M*), regulate mtDNA transcription [102] (Fig. 1). These and other nuclear-to-mitochondria anterograde signals regulate mitochondrial function including bioenergetics, biogenesis, mitophagy, and fission/fusion. Within each cell, mitochondria are heterogeneous and mitochondria from different tissues display diversity in terms of fuel preference, protein composition, and metabolic functions [94]. mtDNA is oocyte-derived, so inherited mt disorders follow maternal inheritance [94]. Paternal mitochondrial transmission is a rare occurrence [103].

During the production of ATP, the transport of electrons also generates reactive oxygen species (ROS) that damage macromolecules including mtDNA, proteins, and lipids. Mitochondrial dysfunction contributes to age-related degeneration and disease in tissues including neurons and vestibulocochlear hair cells resulting in hearing loss [104], retina leading to age-related macular degeneration (AMD) [105], muscle causing weakness and falls [106], heart [107], liver and adipose [108], and brain [109]. Estrogens and androgens protect mitochondria against degenerative effects of aging in a tissue-specific manner by activation of their respective receptors [108]. ROS contributes to mitochondrial stress and protein misfolding. Misfolded proteins and aggregates accumulate in the inner mitochondrial membrane space (IMS) and the mitochondrial matrix which leads to activation of the mitochondrial unfolded protein response (UPR<sup>mt</sup>). UPR<sup>mt</sup> responsive pathways include antioxidant response enzymes, oxidative phosphorylation (OXPHOS), mitophagy, and mitochondrial biogenesis (reviewed in Ref. [110]). Activation of UPR<sup>mt</sup> initiates mitochondrial-nuclear crosstalk (retrograde signaling) for the maintenance of cellular homeostasis, and some mtDNA mutations may perturb crosstalk signaling [111]. UPR<sup>mt</sup> stimulates dsRNA-activated protein kinase (PKR), eukaryotic translation initiation factor 2-α kinase 2 (EIF2AK2), and C-Jun N-Terminal Kinase 2 (JNK2) to activate JUN which increases the transcription CHOP/ATF4/5 which then increases UPR<sup>mt</sup> responsive genes [112]. In addition, activation of UPR<sup>mt</sup> activates AKT which phosphorylates ser167 of ERα that results in ligand-independent transcriptional ERα activation, thus increasing nuclear respiratory factor-1 (NRF-1, *NRF1*) [112]. E<sub>2</sub>-ERα also regulates UPR<sup>mt</sup> [110,112,113]. Recent studies demonstrate that breast cancer cells co-opt

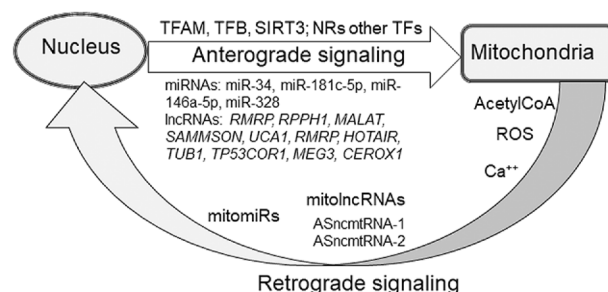
“mitohormesis”, a process of increased basal UPR<sup>mt</sup> and decreased oxidative stress leading to increased invasion and metastasis and worse survival of breast cancer patients with the UPR<sup>mt</sup> gene signature [114]. E<sub>2</sub>-ERα increases sirtuin 3 (SIRT3) transcription and SIRT3 localizes to mitochondria where it attenuates ROS by deacetylation of manganese superoxide dismutase (MnSOD, *SOD2*) and interacts with FOXO3A to activate its translocation to the nucleus to upregulate the expression of the genes encoding PGC-1α, a coregulator required for NRF-1 and PPAR-regulated transcription [115,116], and MnSOD [117].

Retrograde signals originating in the mitochondria in response to oxidative stress, ROS, increased intracellular calcium ([Ca<sup>++</sup>]), reduced ATP, acetyl CoA, and an imbalance in the NADH/NAD<sup>+</sup> ratio disrupt sirtuin 1 (SIRT1), protein kinase C (PKC), and NFκB signaling pathways leading to changes in the expression and activity of NRF-1, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1α), and TFAM [111] (Fig. 2). Mitoprotective outcomes elicited during UPR<sup>mt</sup> include increase in 26S proteasome, LC3B, Hsp10, Hsp90, CLPP, LonP, and activation of SOD1, SOD2, and catalase [118].

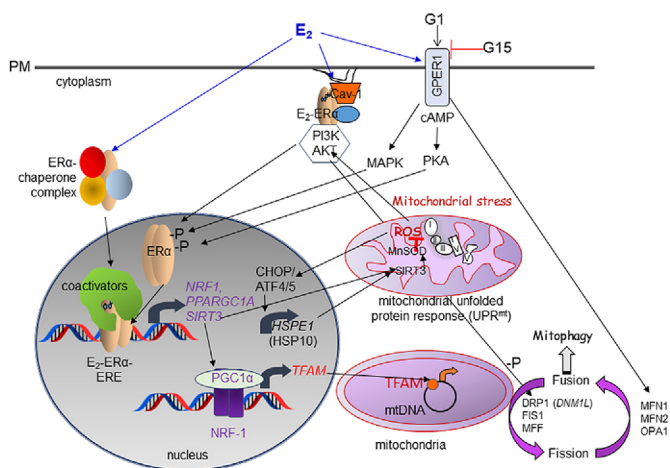
In addition to energy production, mitochondria are the site of synthesis for all steroid hormones [119], including E<sub>2</sub> in the ovarian granulosa cells [120]. Apoptotic signals result in permeabilization of the mitochondrial outer membrane and the release of cytochrome *c* initiating activation of a caspase cascade leading to cell death [121]. E<sub>2</sub> inhibits apoptosis by a variety of mechanisms (reviewed in Refs. [110,122]). Under mitochondrial stress, mitochondria also produce and secrete “mitokines”, e.g., humanin, an exercise-responsive peptide encoded by the *MT-RNR2* gene in mtDNA, and fibroblast growth factor 21 (FGF-21) that regulates energy metabolism [123].

The impact of mtDNA genome on metabolic function in mice has been examined using Mitochondrial-Nuclear-eXchange (MNX) mouse models in which C57BL/6J and C3H/HeN mouse strains have reciprocally exchanged mtDNA [124]. There are 2 non-synonymous mutations in protein coding subunits in the C57 versus C3H mtDNA (subunit 3 in complex I and subunit 3 in complex IV), with the C57 mtDNA linked to greater organelle economy and oxidant production relative to the C3H mtDNA [124]. Recent studies demonstrated that C57BL/6J mtDNA protects against high-fat diet-induced obesity and alters the expression of a higher number of genes in adipose tissue compared to C3H/HeN mtDNA, although the mechanisms is unknown [125].

Sex-specific differences in mitochondria and mitochondrial function in different organs, mostly from rodents, has been reviewed [126]. For example, human females have higher intracellular lipid content and show enhanced protection against oxidative stress in skeletal muscle and female rodents have higher ETC transport, ATP production, and



**Fig. 1. Examples of anterograde and retrograde signaling.** Anterograde signals from the nucleus to the mitochondria include the nuclear-encoded transcription factors (TFs), including TFAM and TFB that bind the mtDNA, and nuclear receptors (NRs). Other nuclear-encoded proteins such as SIRT3, a deacetylase that is important for regulating subunits of the ETC, among other targets are important for regulating mitochondrial metabolism. Nuclear encoded miRNAs and lncRNAs are also transported into mitochondria. Retrograde signals from mitochondria that regulate nuclear function include calcium and ROS. In addition, mtDNA-encoded lncRNAs have been reported in the nucleus.



**Fig. 2. Mechanisms of E2 regulation of cellular and mitochondrial responses.** Shown are ER $\alpha$  and GPER1. The mitochondrial unfolded protein response (UPR<sup>mt</sup>) includes retrograde signaling from mitochondria to the nucleus via CHOP and ROS. ROS activation of AKT results in phosphorylation and activation of ER $\alpha$ . ER $\alpha$  increases NRF-1 which, in turn, increases TFAM that stimulates mtDNA transcription. Mitochondria are in a dynamic network and, as described in the text, GPER1 was shown to increase MFN2 and MFN1 while reducing FIS1, suggesting a role of GPER1 in stimulating fusion.

mitochondrial biogenesis in the brain [126]. A recent study reported that the influence of sex on gene expression and mitochondrial metabolism in adipose tissue depended on the strain in a study of 100 inbred strains of mice [127].

Another recent review summarized sex differences in human skeletal muscle [128]. Interestingly, skeletal muscle is among the most sex-divergent tissues showing ~3000 gene expression differences between females and males at an average age of  $27 \pm 3$  yrs [129]. Many mitochondrial function genes were found to be differently expressed including *PPARGC1A* (PGC-1 $\alpha$ ), correlating with known sex difference in muscle fiber compositions with females having a higher percent of type I fibers with a more oxidative phenotype [129]. The differences in gene expression between the skeletal muscles of men and women is mediated in part by epigenetic changes including differences in DNA methylation, histone modification, and miRNA expression [128].

#### 4. Non-coding RNAs in mitochondrial function

Nuclear-encoded microRNAs (miRNA) and long noncoding RNAs (lncRNAs) can regulate anterograde signaling by translocation into mitochondria and the mtDNA genome encodes miRNA and lncRNAs that can act as retrograde signals [130] (Fig. 1). miRNAs generally inhibit their target gene's translation by binding to a miRNA response element (MRE) in the 3'UTR of the target mRNA transcript within the RNA induced silencing complex (RISC) [131,132]. lncRNAs are defined as non-coding RNAs of > 200 nucleotides that show tissue-specific expression and interact with DNA, RNA, and proteins to regulate gene expression, chromatin modification and dynamics, protein complex assembly, splicing, and translation [133]. Another type of non-coding RNA are circular-RNAs (circRNAs) that generally arise during splicing of the mRNA transcript and are localized to the cytoplasm where they, like lncRNAs, act as 'sponges' for miRNAs, blocking their function [15]. A recent study profiling the subcellular distribution of circRNAs did not find circRNAs in mitochondria from HepG2 cells [134]; thus, based on this and the lack of other studies on circRNA and mitochondria in PubMed, I will not discuss circRNAs.

Nuclear-encoded miRNA regulate mtDNA-encoded transcripts. The term 'mitomiRs' refers to miRNAs functioning in mitochondria, whether nuclear- or mtDNA-encoded [135]. For example miR-214 targets mtND6 and mtND4I in renal tubular cells [136]. miR-214 was

upregulated in response to insults that mimic chronic kidney disease in mice, e.g., ischemia-reperfusion injury, and resulted in a decrease in mtDNA copy number and OCR [136]. RNA seq of an isolated mitochondrial fraction from human tongue squamous cell carcinoma cells identified 57 mitochondria-enriched miRNAs, including miR-2392 that downregulated mtDNA-encoded ND2, ND4, ND5, CYTB, and COX1 and shifted cells to increased lactate production [137].

Examples of other nuclear-encoded miRNAs reported to be imported into mitochondria include miR-34, miR-181c-5p, miR-146a-5p, miR-1, miR-378, and miR-21 (reviewed in Refs. [138–140]). miRNAs are imported into mitochondria by interaction with AGO2 and polynucleotide phosphorylase (PNPase) [141]. PNPase is increased in arterial tissue from diabetic human patients and this increase was correlated with higher mitochondrial miR-378 in these samples, in db/db mice, and in cell culture studies [142]. The identity and roles of miRNAs in mitochondrial biology have recently been reviewed [139].

miRNAs also target nuclear and mtDNA-encoded transcripts for degradation. NRF1 is directly targeted by miR-504 [143] and mouse *Nrf1* is a direct target of miR-378 which is upregulated in fatty livers of mice and humans [144]. Other examples of proteins important in mitochondrial function that are regulated by miRNA include these paired examples: miR-494-TRAM; miR-696-PGC-1 $\alpha$ ; and miR-23-COX4 and PGC-1 $\alpha$  (reviewed in Ref. [140]). We reported that miR-29b-1 and miR-29a directly target *ATP5G1*, a subunits of ATP synthase (Complex V in OXPHOS), and *ATPIF1* that encodes ATPase Inhibitory Factor I that limits ATP depletion when the mitochondrial membrane potential falls below that required for ATP synthesis [145].

Mitochondrial-DNA encoded miRNAs, including miR-1974, miR-1977, and miR-1978 in humans, have been reported (reviewed in Ref. [135]).

Nuclear-encoded lncRNAs: *RMRP*, *RPPH1*, and *MALAT* were reported to act as anterograde signals between the nucleus and mitochondria in HepG2 cells [146]. Additional lncRNAs that regulate mitochondrial function include *SAMMSON*, *UCA1*, *RMRP*, *HOTAIR*, *TUG1*, *linc-p21* (*TP53COR1*), and *MEG3* were recently reviewed [147]. *MEG3* is a tumor suppressor that is downregulated in endocrine-related cancers, including breast prostate, and endometrial cancer [148]. There is cross-talk between mitochondrial function and the expression of nuclear-encoded lncRNAs as exemplified by the observation that various mitochondrial stressors, e.g., FCCP, oligomycin, rotenone, and doxycycline, increased expression of the lncRNA *NEAT1*, resulting in increased elongated nuclear paraspeckles and mRNA sequestration of nuclear-encoded mitochondrial proteins in HeLa cells [149]. The lncRNA *HOTAIR* is important for mitochondrial function in HeLa cells [150]. *NEAT1* is overexpressed in breast tumors and appears to play an oncogenic role in breast cancer (reviewed in Ref. [15]), but its involvement in mitochondrial dysfunction in breast tumorigenesis and metastasis is unknown. The lncRNA *CEROX1* sponges miR-488-3p resulting in increased transcript expression of 8 subunits of OXPHOS Complex I and increased mitochondrial respiration [151]. RNA binding proteins including HuR, GRSF1, PPR, PNPase, SLIRP, and SHARP may be involved in lncRNA transport into mitochondria [152]. One lncRNA, *LINC00116*, was recently reported to encode an evolutionarily conserved 56 aa peptide called MTLN that is detected in mitochondria where its deletion reduced OXPHOS Complex I activity [153].

Seven lncRNAs are derived from mtDNA [130] and are called "mitolncRNAs" [139]. Several are chimeric lncRNAs containing nucleotides of mtDNA: *LIPCAR*, *SncmtRNA*, *ASncmtRNA-1*, and *ASncmtRNA-2*. *ASncmtRNA-1* and *ASncmtRNA-2* are present in mitochondria and nuclei, suggesting a possible role in retrograde signaling [130].

#### 5. E2 stimulated NRF-1 transcription

Mitochondrial function can be regulated by E<sub>2</sub> activation of genomic ER $\alpha$  and ER $\beta$  which stimulate transcription of *NRF1* and by binding to mtDNA to promote mtDNA transcription (reviewed in Refs. [122,154])

(Fig. 2). NRF-1 promotes transcription of TFAM that binds and upregulates mtDNA-encoded genes [116,155]. The transcription of nuclear-encoded ETC proteins, e.g., mitochondrial ATP synthase subunit E and COVII, are increased by E<sub>2</sub> via NRF-1 (reviewed in Ref. [156]). NRF-1 binds to its DNA response element as a dimer and interacts with coactivators PGC-1 $\alpha$ , PGC-1 $\beta$ , and PRC to regulate target gene transcription depending on the cell type [115]. Whether mitochondrial ER $\alpha$  and ER $\beta$  play direct roles on mtDNA gene transcription and mitochondrial function appears to depend on the cell type, consistent with cell-type specific localization. Doris Germain's group has demonstrated that retrograde signaling via ROS-AKT pathway activation in response to UPR<sup>mt</sup> activates ER $\alpha$  and increases NRF-1 signaling (reviewed in Refs. [118,157–159]). The role of estrogens on mitochondria function has been reviewed ([122,154,160–163]). However the protective effects of estrogens in mitochondria remain to be fully elucidated (reviewed in Ref. [164]).

In addition to its role in regulating mitochondrial genes, NRF-1 has been demonstrated to have oncogenic activity in breast cancer cell lines and to increase breast cancer stem cell properties in stably transformed, NRF-1-overexpressing MCF-10A breast epithelial cells [165,166]. The NRF-1-overexpressing MCF-10A cells had higher mammosphere formation than the parental MCF-10A cells and had increased expression of pluripotency markers SOX2, NANOG, and OCT4 and the mesenchymal stem cell marker CXCR4 [165]. These authors did not examine the impact of NRF-1 overexpression on redox-sensitive events or mitochondrial bioenergetics in this paper. Knockdown of endogenous *NRF1* in MDA-MB-231 TNBC cells reduced spheroid formation and tumor growth in mammary fat pads and lung metastasis in immunodeficient mice [166]. Overexpression of NRF-1 in MCF-10A cells reduced basal OCR, but increased mitochondrial reserve whereas NRF-1 knockdown in MDA-MB-231 cells decreased basal OCR and mitochondrial reserve [166].

## 6. Nuclear transcription factors within mitochondria

Nuclear transcription factors also localize within mitochondria. TFs identified in mitochondria include the recently annotated JUNB [167] as well as NF $\kappa$ B, p53, cAMP Response Element-Binding Protein (CREB), signal transducer and activator of transcription (STAT)-1, STAT3, and STAT5, Interferon Regulatory Factor 3 (IRF3), and myocyte-specific enhancer factor-2D (MEF-2d) that may also regulate to mtDNA transcription (reviewed in Ref. [168]). ER $\alpha$  and ER $\beta$  were identified within mitochondria and were reported to regulate mtDNA transcription (reviewed in Ref. [31]). E<sub>2</sub> has been reported to increase redox signaling in MCF-7 cells containing ER $\alpha$  [169]. This process is considered part of the oncogenic process in breast cancer and involves E<sub>2</sub> activation of AKT signaling leading to NRF-1 activation [170]. E<sub>2</sub> rapidly increased transient ER $\alpha$  localization to mitochondria in MCF-7 breast cancer cells and stimulated ER $\alpha$ -MnSOD direct interaction as detected by confocal imaging and co-immunoprecipitation experiments [171]. The ER $\alpha$  mitochondrial localization and ER $\alpha$ -MnSOD interaction was blocked by fulvestrant, suggesting the ER $\alpha$  AF-2 conformation is important for these interactions. The rapid (increased by 15 min and gone by 60 min) E<sub>2</sub>-induced migration of ER $\alpha$  to mitochondria in MCF-7 cells is considered a non-genomic response E<sub>2</sub> increased MnSOD acetylation of K68, resulting in inhibition of MnSOD activity. The E<sub>2</sub>-ER $\alpha$ -MnSOD interaction was reported to block MnSOD-SIRT3 interaction, increasing superoxide and activating mTORC2 [171].

Mitochondria contain other nuclear receptors (NR), i.e., thyroid receptor (TR), androgen receptor (AR), retinoid X receptor (RXR), RARs, glucocorticoid receptor (GR), and peroxisome proliferator activated receptor gamma (*PPARG*, *PPAR* $\gamma$ 2) (reviewed in Ref. [172]). ER $\beta$  was identified in human heart mitochondrial proteins [173]; however, no independent confirmation of this finding has been reported. A recent study reported that low levels of mitochondrial ER $\beta$  (mitoER $\beta$ ) were associated with increased breast tumor recurrence [174]. Transfection

of MCF-7 breast cancer cells with GST-ER $\beta$  followed by GST-pulldown identified HSPA9 (heat shock 70 kDa protein, mitochondrial; also called GRP75) associated with ER $\beta$ . MALDI-TOF mass spectrometry identified ER $\beta$  and HSPA9 in a purified complex and knockdown and over-expression studies showed that HSPA9 translocates ER $\beta$  into MCF-7 mitochondria. Transfection of MDA-MB-231 triple negative breast cancer (TNBC) cells with a mitochondria-targeted ER $\beta$  expression vector reduced cell proliferation, invasion, and migration *in vitro* and tumor formation *in vivo*. Previous studies have implicated ER $\beta$  as a tumor suppressor in breast and other cancers [175–180]. Another group identified higher ER $\beta$  in mitochondrial fractions from ectopic endometrial tissues *versus* normal uterine myoma or non-lesion controls [181]. Given the uncertainty about the specificity of some ER $\beta$  antibodies [182,183], further studies of the localization and activity of ER $\beta$  in mitochondria are warranted.

The naturally-occurring ER $\alpha$  splice variants ER $\alpha$ 36 and ER $\alpha$ 46 result from differential promoter usage and splicing, resulting in truncated forms of ER $\alpha$  lacking the N-terminal A and B domains that constitute AF-1 [184]. ER $\alpha$ 36 also lacks the F-domain at the C-terminus of full-length ER $\alpha$ 66 and has a truncated LBD [184]. ER $\alpha$ 36 was reported to localize primarily in mitochondria in human uterine leiomyoma (UtLM) and smooth muscle cell lines and interacts with prohibitin (PHB) [185]. A recent report from the same investigators showed that BPA increased ER $\alpha$ 36 expression in the UtLM cells and that ER $\alpha$ 36 activated MAPK signaling, increased Src and EGFR phosphorylation and mitochondrial localization [186].

## 7. Estrogens regulate mitochondrial bioenergetics

The development of the Seahorse Bioscience Extracellular Flux Analyzer has allowed investigation of the effect of estrogens, SERMs, and other potential ER ligands on mitochondrial bioenergetics in live cells in real time and to define the impact of these perturbations on oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), a surrogate measure of lactate production and glycolysis [187,188]. We reported that E<sub>2</sub> (10 nM) stimulated baseline OCR and ECAR in MCF-7 and T47D luminal A (ER $\alpha$ +) breast cancer cells and stimulated ATP-linked OCR with no effect on maximal mitochondrial reserve capacity, suggesting that E<sub>2</sub> does not affect tolerance to cellular stress in these cell lines [189,190]. An earlier report demonstrated that 10 nM E<sub>2</sub> increased ECAR and decreased OCR in primary human stromal endometrial cells, but no mechanisms were examined [191]. Medroxyprogesterone acetate (MPA) inhibits E<sub>2</sub> potentiation of rat primary hippocampal neuron and glia mitochondrial respiratory reserve capacity *in vitro* [192], but no studies identifying mechanisms were included. A recent report demonstrated that knockout of ER $\alpha$  in CD4<sup>+</sup> T cells reduced the mitochondrial reserve capacity, suggesting that ER $\alpha$  regulates mitochondrial metabolism in T cells [81].

My search of PubMed and the Agilent Publications database for articles examining the effect of estrogens on mitochondrial bioenergetics identified relatively few reports. The ER $\beta$ -selective agonist DPN partially restored basal and maximum OCR in primary rat hippocampal neurons against synthetic amyloid  $\beta$  oligomer (A $\beta$ 1–42) treatment-induced suppression of mitochondrial OCR [193]. One group found that overexpression of ER $\alpha$  in SK-N-BE(2) MYCN-amplified (MNA) neuroblastoma (NB) cells repressed xenograft tumor growth downregulated many processes linked to NB tumorigenesis [194]. Glycolysis (measured as ECAR in the Seahorse Bioanalyzer), maximal glycolytic capacity, and the glycolytic reserve were all significantly reduced in cells overexpressing ER $\alpha$  and treatment with E<sub>2</sub> and nerve growth factor had no additional effect on any of these parameters in the NB cells [194]. Likewise, baseline OCR, ATP-linked OCR, and mitochondrial reserve were decreased in the ER $\alpha$  overexpressing SK-N-BE(2) MNA NB cells, mediated in part by suppression of utilization of fatty acids. Overexpression of mitochondrial-targeting sequence tagged ER $\beta$  in primary human endometriotic cells increased basal OCR and mitochondrial

reserve [181]. Knockdown of ER $\beta$  decreased the expression of NRF1, TFAM, MT-CO1, and MT-ATP6 transcripts in the endometriotic cells and increased anti-apoptotic protein BCL-2, thus “rescuing the cells from oxidative stress-induced mitochondrial-apoptosis” [181].

Studies in muscle-specific Esr1 (ER $\alpha$ ) knockout (MERKO) female mice showed that these mice had impaired glucose homeostasis, increased adiposity coupled with aberrant mitochondrial morphology, increased ROS, impaired mitochondrial fission, impaired calcium handling and ATP production, although difference in muscle size of maximum force [195]. These data implicate a critical role for ER $\alpha$  muscle mitochondrial function. ER $\alpha$  was reduced in the muscle of women with metabolic syndrome [195]. Transmission electron microscopy revealed elongated, hyperfused mitochondria with increased inactive DRP1 phosphorylated on the inhibitory SER 637 residue. They also observed an increase in *Rcan1*, an inhibitor of calcineurin which results in impaired mitophagy, increased ROS, inflammation, and insulin-resistance [195]. E<sub>2</sub>-ER $\alpha$  is also required for maintaining the satellite cell number (muscle stem cells) in the muscle of female rodents and humans [196]. Indeed, whole body ER $\alpha$  knockout (*Esr1* -/-) mice show decreased muscle fatty acid oxidation and increased overall adiposity while muscle-specific ER $\alpha$  knockout mice showed impaired mtDNA replication, mitophagy, autophagy, impaired insulin signaling (including glucose disposal), increased H<sub>2</sub>O<sub>2</sub>, superoxide, lipid accumulation and inflammation [197]. The authors noted the importance of identifying ways or therapeutics to modulate tissue-specific ER $\alpha$ -regulated pathways that will regulate energy balance and glucose homeostasis, specifically in postmenopausal women [197].

E<sub>2</sub> replacement in ovariectomized female mice improved mitochondrial complex I (CI) activity and decreased H<sub>2</sub>O<sub>2</sub> in skeletal muscle but increased CI-mediated H<sub>2</sub>O<sub>2</sub> production and decreased OXPHOS capacity in liver [198]. The authors stated that the “mechanism(s) behind tissue specificity of E<sub>2</sub> action on mitochondrial function remains unknown” [198]. While transcriptomic profiling has identified miRNAs regulating glycolysis and oxidative metabolism in male mouse muscle fibers [199], the role of estrogens in regulation of these miRNAs is not yet known. Interestingly, studies examining miRNAs in skeletal muscle of homozygotic twins with discordant use of hormone-replacement therapy (HRT) identified miR-182, miR-233, and miR-142-3p targeting IGF-1R and FOXO3A [200] and inflammatory signaling [201]. This group of investigators also identified E<sub>2</sub>-regulation of muscle energy pathways in HRT users [202,203].

## 8. GPER1 regulates mitochondrial respiration and function

When GPER1 was overexpressed in MCF-7 cells, it localized in the endoplasmic reticulum and inhibited cell growth while increasing intracellular cAMP and stimulating autophagy [204]. On the other hand, E<sub>2</sub> activation of GPER1 suppressed mitophagy of ATDC5 chondrocytes *in vitro* by stimulating the PI3K/AKT-mTOR pathway [205]. GPER1 overexpression decreased basal OCR and ECAR while increasing the maximal respiratory rate and reserve capacity. GPER1 overexpression increased the levels of mitofusion 1 (*MFN1*) and 2 (*MFN2*) and Parkin (*PRKN*) mRNA expression while reducing mitochondrial fission 1 protein (*FIS1*), implicating a role for GPER1 in regulating mitochondrial fission/fusion and thus increasing mitophagy [204]. Parkin associates with TFAM to increase mitochondrial transcription [206]. E<sub>2</sub> and receptor subtype-specific agonists (PPT, DPN, and G1) stimulated basal OCR and mitochondrial reserve capacity via activation of ER $\alpha$ , ER $\beta$ , and GPER1, respectively in 3T3-L1 adipocytes [207]. GPER1 agonist G1 increased ER $\alpha$  phosphorylation on ser 118, and indicator of receptor AF-1 activation, and both E<sub>2</sub> and G1 increased the expression of mouse *Ppargc1a*, *Ppargc1b*, and *Nrf1* in mouse white adipose tissue explants [207]. The authors proposed a model that E<sub>2</sub> activation of GPER1 in adipocytes activates adenylate cyclase PKA leading to CREB and ER $\alpha$  phosphorylation and activation of the expression of genes that stimulate mitochondrial function and protect adipocytes against IL6-induced

mitochondrial function [207]. However, gene knockout studies will be required to validate these conclusions that were based on the use of chemical inhibitors.

*In vivo* studies showed that *i.p.* injection of GPER1 agonist G1 (10  $\mu$ g/kg for two weeks) in 16-month old, ovariectomized, Sprague-Dawley rats had antidepressant- and anxiolytic-effects in the rats [208]. Examination of the hippocampal function in the rats showed that G1 increased mitochondrial function, SOD1, ER $\alpha$ , GPER1, and UCP2 protein levels, while reducing Bcl-2 (BCL2) protein expression [208]. The effect of estrogens on anxiety and depression in rodents and humans is considered to be mediated by ER $\alpha$ , ER $\beta$ , and GPER1 on neuronal signaling networks and pathways [209].

## 9. Estradiol regulates mitochondrial dynamics: fusion and fission

As stated previously, mitochondria form an interconnected network governed by signals regulating mitochondrial fusion and fission. DRP1 is a GTPase that is differentially regulated by phosphorylation at specific residues which regulate its movement from the cytoplasm to the mitochondria where GTP hydrolysis enables membrane constriction and scission [210]. E<sub>2</sub> increased the mRNA transcript levels of MFN1, MFN2, OPA1, and DRP1 while decreasing FIS1 with 4 h of treatment of MCF-7 cells and these transcriptional responses were inhibited by the antiestrogen fulvestrant (ICI 182,780) [211]. The authors reported that E<sub>2</sub> induced mitochondrial fusion in MCF-7 cells, decreased expression of OXPHOS complex proteins, and increased ATP levels. These investigators reported similar findings in E<sub>2</sub>-treated T47D cells and observed that overexpression of ER $\beta$  in T47D cells increased OXPHOS complex proteins and decreased fission while increasing fusion [212]. In contrast, E<sub>2</sub>-activation of ER $\alpha$  in MCF-7 cells increased Drp1 phosphorylation at ser616 to induce Drp1 activity resulting in mitochondrial fission [213]. The mechanism required ER $\alpha$ , since knockdown blocked E<sub>2</sub> induced Drp1 phosphorylation, but whether this was mediated by non-genomic activation of AKT or by upregulation of a gene that affected this response was not evaluated.

## 10. Conclusions

Sex differences in health and disease are mediated by the levels of endogenous estrogens and androgens as well as by genes encoded by sex chromosomes. Many of the sex-dependent differences in diseases include altered cellular metabolism. E<sub>2</sub> and other estrogens and synthetic GPER1 agonists regulate mitochondrial bioenergetics, fusion, and fission. Estrogens regulate the expression of genes, including miRNAs and lncRNAs, that regulate mitochondrial functions: metabolism, OXPHOS, apoptosis, UPR<sup>mt</sup>, fission, and fusion. The mechanism for these events involve binding of E<sub>2</sub> and other estrogens to ER $\alpha$  and ER $\beta$  to regulate nuclear gene transcription and PM-initiated signaling cascades. In addition, estrogens, as well as EDCs, activate GPER1 which also regulates intracellular signaling events, including by cross-talk with EGFR. NRs including ER $\alpha$  and ER $\beta$ , as well as AR, RXR, RARs, GR, and PPAR $\gamma$ 2, have been observed within mitochondria. NRF-1 is a key target of nuclear ER $\alpha$  and ER $\beta$ -mediated transcriptional activation in response to E<sub>2</sub>. NRF-1 is a regulator of nuclear-encoded mitochondrial genes including TFAM that binds mtDNA and regulates its transcription. Nuclear-encoded miRNA and lncRNA regulate mtDNA-encoded as well as nuclear-encoded transcripts thus functioning as anterograde signals. mtDNA-encoded lncRNAs may play a role in retrograde signaling. Since there are cell-specific pathways for each mechanism of estrogen regulation of mitochondrial function, further research is needed to delineate these mechanisms.

## Declaration of competing interest

The author declares no competing interests.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.redox.2020.101435>.

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