IUBMB *Life*, 60(4): 210–223, April 2008

Critical Review

Steroid and Thyroid Hormone Receptors in Mitochondria

Anna-Maria G. Psarra[1] and Constantine E. Sekeris[2]
[1]*Biomedical Research Foundation, Academy of Athens, Center for Basic Research, Athens, Greece*
[2]*Laboratory of Molecular Endocrinology, Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens, Greece*

Summary

Receptors for glucocorticoids, estrogens, androgens, and thyroid hormones have been detected in mitochondria of various cell types by Western blotting, immunofluorescence labeling, confocal microscopy, and immunogold electron microscopy. A role of these receptors in mitochondrial transcription, OXPHOS biosynthesis, and apoptosis is now being revealed. Steroid and thyroid hormones regulate energy production, inducing nuclear and mitochondrial OXPHOS genes by way of cognate receptors. In addition to the action of the nuclearly localized receptors on nuclear OXPHOS gene transcription, a parallel direct action of the mitochondrially localized receptors on mitochondrial transcription has been demonstrated. The coordination of transcription activation in nuclei and mitochondria by the respective receptors is in part realized by their binding to common trans acting elements in the two genomes. Recent evidence points to a role of the mitochondrial receptors in cell survival and apoptosis, exerted by genomic and nongenomic mechanisms. The identification of additional receptors of the superfamily of nuclear receptors and of other nuclear transcription factors in mitochondria increases their arsenal of regulatory molecules and further underlines the central role of these organelles in the integration of growth, metabolic, and cell survival signals.
IUBMB *Life*, 60(4): 210–223, 2008

Keywords steroid receptors; thyroid hormone receptors; mitochondria; transcription; OXPHOS; apoptosis.

INTRODUCTION

Steroid and thyroid hormones are major regulators of metabolic, growth, immune, and differentiation processes, exerting their biological effects by a variety of molecular strategies.

Received 5 December 2007; accepted 23 December 2007
Address correspondence to: C.E. Sekeris, Laboratory of Molecular Endocrinology, Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, 48 Vas Constantinou Avenue, 11635 Athens, Greece. Tel: +30 210 7273767. Fax: +30 210 7273677. E-mail: csekeris@eie.gr

ISSN 1521-6543 print/ISSN 1521-6551 online
DOI: 10.1002/iub.37

One major mechanism of action is by way of nuclear receptors and modulation of gene expression (*1*). The steroid/thyroid hormone receptors (TRs) are members of the superfamily of nuclear receptors (*2*). In the ligand unbound, unactivated state, the receptors are components of a macromolecular complex with heat-shock and immunophilin chaperones. Upon ligand binding (LBD), their conformation and dynamic behavior changes, they are released from the complex in an activated form, dimerize, and interact in the nucleus with respective DNA sequences, the hormone responsive elements (HREs). This leads to recruitment of several regulatory proteins, such as coactivators or corepressors, some containing intrinsic histone modifying enzymes, and to alteration of chromatin structure, thus facilitating or blocking the access of the transcriptional machinery to DNA (*3*). The receptors can also be activated by cross-talk with other regulatory agents, for example mitogens (*4*) and neurotransmitters (*5*), involving phosphorylation of the receptors by MAP-kinases or protein kinase A. Gene regulation by nuclear receptors is also achieved, not by direct binding to DNA, but by interaction with other DNA-binding transcription factors, resulting in enhancement or attenuation of transcription (*6*). The steroid and thyroid hormones also exert rapid effects by way of membrane bound receptors—classical, G-protein associated, or still unidentified molecules (*7–10*) resulting in modulation of membrane, cytoplasmic, and/or nuclear associated processes (*11*). The detection of steroid and TRs in mitochondria of a variety of cells raised the question as to the role of these agents in mitochondrial physiology and in the coordination of processes necessitating the involvement of both nuclear and mitochondrial actions (*12–27*).

STEROID AND THYROID HORMONE RECEPTOR STRUCTURE

Steroid and TRs are members of the superfamily of nuclear receptors, showing common structural characteristics (Fig. 1). They harbor well conserved domains for LBD and DNA binding (DBD). The amino-terminal regions of the receptors contain

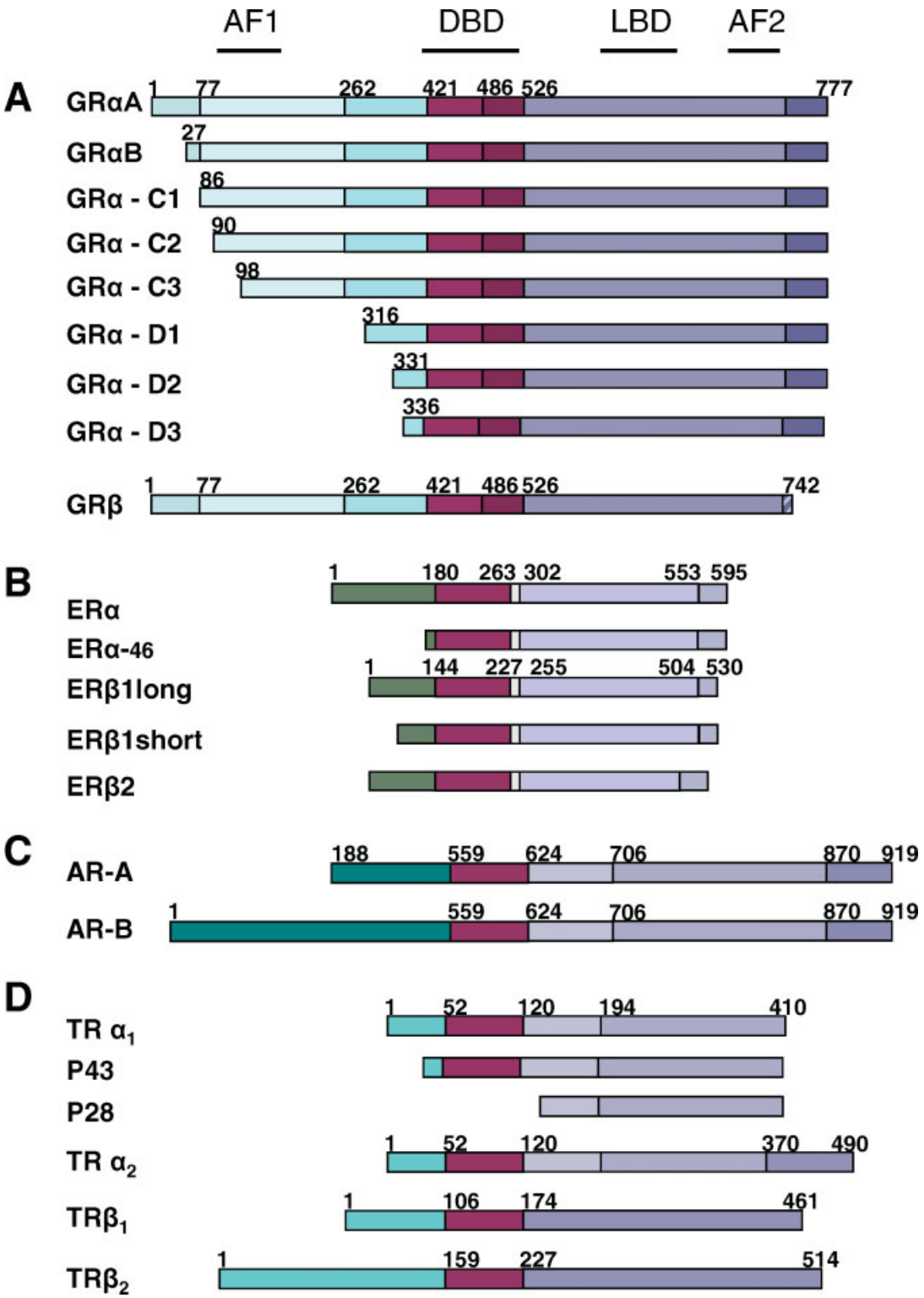

Figure 1. Structural features of glucocorticoid (A), estrogen (B), androgen (C), and thyroid hormone (D) receptors. Data from Refs. *8, 9, 28, 29, 30, 31, 32–37*.

a variable constitutive trans-activating domain (AF-1) and the C-terminal regions have a ligand dependent trans-activation domain (AF2), interacting with other transcription factors and regulatory molecules. Further regions have been identified, important for dimerization, for interaction with heat shock proteins, for nuclear translocation, and for posttranslational modification. Various receptor isoforms are generated from one gene as a result of differential splicing (*28, 29*) (Fig. 1). The catalog of receptor isoforms is increasing by the addition of receptor molecules produced by alternative translation initiation of the message (*28, 29*) (Fig. 1) and by posttranslational modification of the proteins by phosphorylation, acetylation, methylation, ubiquitination, and sumoylation (*38, 39*). The role of these various isoforms is now beginning to be revealed in connection to the increase of regulatory possibilities and of transcription specificity (*40*).

Two major glucocorticoid receptor isoforms, GRα and GRβ, have been detected, products of alternative splicing (*30, 31*) (Fig. 1A). The classical hGRα consists of 777 amino acids. In GRβ, the 50 carboxy-terminal amino acids of GRα have been replaced by 15 others encoded by exon 9β, resulting in a protein of 742 amino acids. Only GRα possesses hormone LBD activity and is the most abundant isoform. GRβ associates physically with GRα forming heterodimers, in this way hindering the formation of transcriptionally active GRα homodimers. Each GRα or GRβ mRNA produces additional isoforms by alternative translation initiation at seven internal AUG sites (*28*). Eight such GRα isoforms have been detected. They all are functional receptors, as they possess the intact LBD domain and demonstrate differences in LBD characteristics, tissue distribution, and gene expression patterns. GRα recognizes specific sequences on DNA (glucocorticoid responsive elements, GREs), either positive, dictating induction of transcription, or negative, directing transcription repression (*41*). The positive GREs represent palindromic sequences of the general type AGAACAxxxTGTTCT. In some genes only one half of the palindrome is sufficient to evoke glucocorticoid signaling. The negative GREs (nGREs) show varying nucleotide composition.

Two major estrogen receptors have been demonstrated, ERα and ERβ, encoded by two different genes (*32*) (Fig. 1B). Both are physiologically active, show differential tissue distribution and differing gene activation patterns. Differential splicing of the ERα and ERβ genes results in various splicing variants, lacking certain exons. Two major variants of ERβ, ERβ1 and ERβ2, differ from one another by the insertion of a 54 base pair alternatively spliced exon. The splicing variants are coexpressed in the various tissues and seem to be biologically active. ERα and ERβ bind to estrogen responsive elements (EREs), consisting either of palindromic sequences of TGACCT with a 3-bp spacer, or only of half palindromes.

The androgen receptor (AR-B) is a 110 kDa protein binding testosterone and dihydroxytestosterone (Fig. 1C) (*33*). The aminoterminal domain of AR represents 60% of the entire AR protein. A shorter isoform (AR-A) has been identified, a product of differential translation initiation of the gene. The two isoforms are identical except for the additional 165 amino acids found in the N-terminus of isoform B. The isoforms mediate their own genes and physiological effects with little overlap. A splicing variant of AR-B has been detected in prostate cancer, AR 23. This variant results from aberrant splicing of intron 2, resulting in the insertion of 23-amino acids between the two zinc-fingers of the DNA-binding domain. This AR-variant is exclusively localized in the cytoplasm (*34*). AR recognizes and binds to respective responsive elements (ARE) representing two hexameric direct repeats (AGAACA) separated by a three nucleotide spacer, with the half site repeated on the same strand.

The TRs are encoded in two genes, c-erbAa and c-erbAβ, localized in chromosomes 17 and 3, respectively (*35, 36*). Because of alternative splicing (Fig. 1D) three proteins are generated from the c-erbAa gene, a_1, a_2, and a_3, of which only a_1 binds thyroid hormone. Two additional TRa_1 forms are produced by alternative translational initiation of internal AUGs (Fig. 1D), proteins p28 and p43, detected specifically in mitochondria (*37*). Alternative splicing of c-erbAb leads to two proteins, β_1 and β_2, both binding T_3 with high affinity. These receptors show differential tissue distribution and are developmentally regulated. The TRs form homodimers, but also heterodimers, particularly with the retinoid X receptor (RXR), but also with the vitamin D receptor and the peroxisome proliferator-activated receptor (PPAR).

TRs bind to respective thyroid hormone responsive elements (TREs), consisting of a basic hexameric consensus sequence AGGT/ACA, in several different arrangements, such as direct repeats separated by a 4-bp spacer, everted repeats, separated by 4-bp, or other configurations.

THE DETECTION OF STEROID AND THYROID RECEPTORS IN MITOCHONDRIA

The presence of steroid and TRs in mitochondria was suggested on the basis of early studies using radiolabeled hormone ligands and binding experiments with mitochondrial extracts. The purification of receptor proteins and the availability of respective antibodies permitted the application of immune techniques, that is Western blotting, immunofluorescence, confocal and immunogold electron microscopy, for the identification of glucocorticoid (GR), estrogen (ER), androgen (AR), and thyroid hormone (TR) receptors in mitochondria (Fig. 2, Table 1) (*37, 42–60, 66–69*).

GR was the first receptor to be identified in mitochondria with this methodology (Fig. 2A, Table 1). Specifically, GR was found in rat liver mitochondria of adrenalectomized animals, 15 min after dexamethasone administration, whereas in noninduced animals the mitochondria showed only traces of the receptor (*45*). GR was detected in mitochondria of HeLa cells (*46*), in cytoplasmic and synaptosomal mitochondria of rat brain (*47*), in mitochondria of Mueller glia cells of the Salamander retina (*48*) and of rat C6 glioma cells (*49*). GR was also located in HepG2

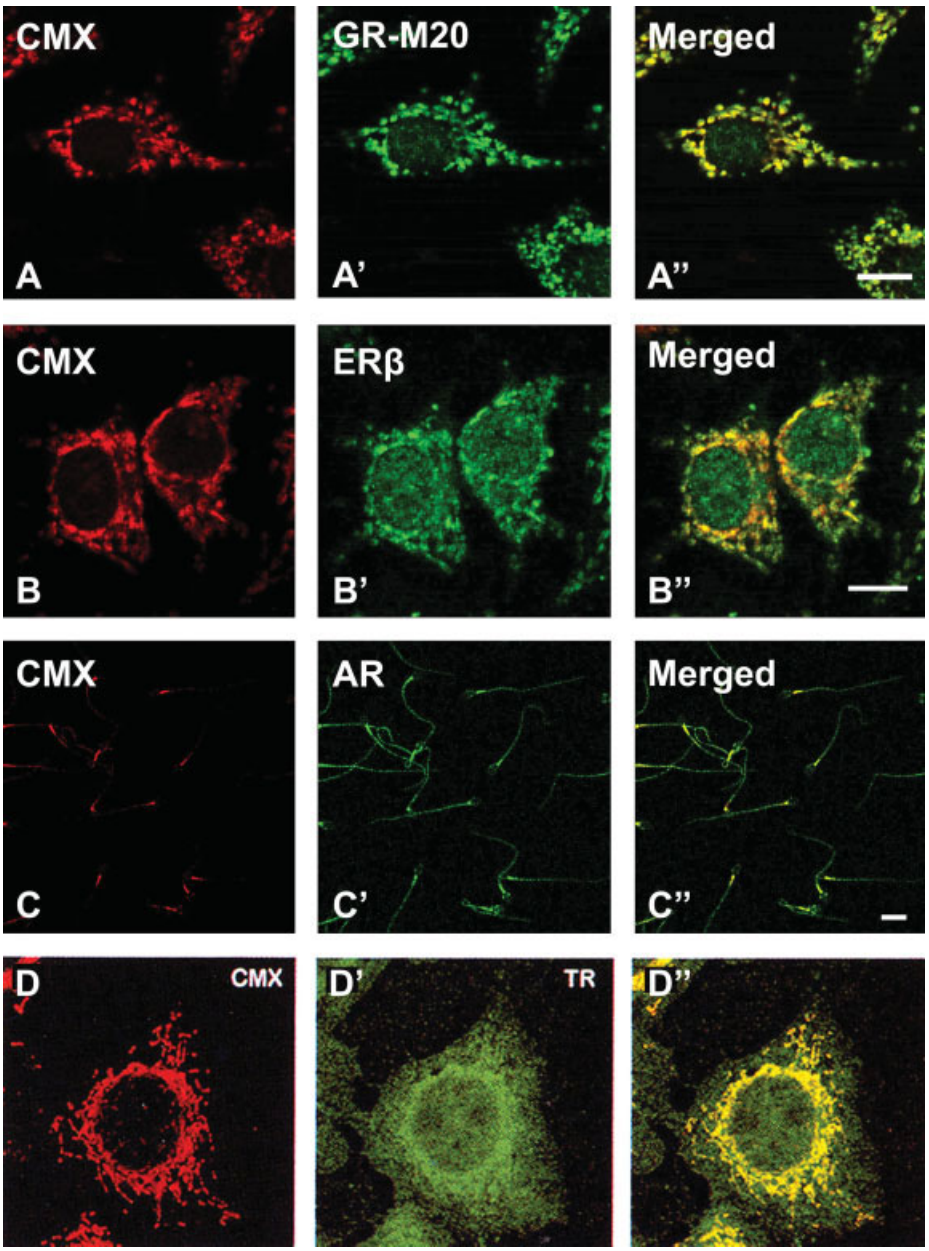

Figure 2. Localization of glucocorticoid (A) (*42*), estrogen (B) (*43*), androgen (C) (*44*), and thyroid hormone (D) receptors (*9*) in mitochondria of HepG2 human hepatocarcinoma cells (A, B), human sperm cells (C), and HeLa cells (D). Cells were treated with CMX-Mitotracker (A, B, C, D). Subsequently, the methanol-acetone fixed specimens were incubated with antibodies against antibodies to GRα (A′), ERβ (B′), AR (C′), and TR (D′), followed by FITC-conjugated secondary antibodies. Merged images A″, B″, C″, and D″.

hepatocarcinoma and SaOS-2 osteosarcoma cell lines (*42*). Using specific antibodies to GRα and GRβ, it was shown that GRα was the isoform detected in mitochondria, in addition to its presence in the cytoplasm and the nucleus, whereas GRβ was confined solely to the nucleus, accumulating preferentially in the nucleoli (*42*). Two main bands, reacting with anti-GRα, of molecular weight 95 and 90 K were observed in Western blots, as well as smaller proteins, which could represent GR degradation products. Mitochondrial GR was described by Sionov et al. (*50*) in T-lymphocytes, in relation to glucocorticoid induced apoptosis (see "Role of Mitochondrial Steroid Hormone Receptors in Apoptosis" section).

First reports for the presence of estrogen receptors in mitochondria were based on the distribution and binding of radioactively labeled ligands (*52, 66, 67*). Later, using immunotechniques, ER was detected in mitochondria of rat uterine and

Table 1
Receptors of the superfamily of nuclear receptors found in mitochondria

Receptor	Cell type	References
Glucocorticoid	Rat liver	45
	HeLa, Hep-2	46
	Rat brain	47
	Mueller	48
	C6-glioma	49
	HepG2, SaOS-2	42
	Thymic epithelial	50
	Periodontal ligament	51
Estrogen beta	HepG2	52
	Rabbit ovaries, uteri	53
	MCF-7	16
	Neurons, cardiomyocytes, hippocampal	54
	Human lens epithelial	55
	HepG2, SaOS-2	43
	Hippocampal	56
	Breast cancer	57
	Spermatocytes	44
Androgen	Spermatocytes, LNCaP	44
Thyroid	Rat liver	58
	Rat liver	59
	Rat liver	37
	Rat liver	60
	Cardiomyocytes	61
RXR	Rat liver	37
	Rat liver	60
RAR	Rat liver	62
Nur77/TR3	T cells, LNCaP	63
	Gastric cancer cell lines	64
PPARgamma2	Rat liver	65

ovarian cells (*53*), of MCF-7 breast cancer cells (*16, 57*), of cultured human lens epithelial cells (*55*), and of rat hippocampus and neuronal cells (*56*). Furthermore, ER was localized in mitochondria of cardiomyocytes (*54*), of endothelia (*17*), of HepG2 hepatocarcinoma and SaOS-2 osteosarcoma cells (*43*), of human sperm cells (*44*), and of human periodontal ligament cells (*51*). Although in some cell mitochondria the presence of both ERα and ERβ has been shown (*55, 57*), in most cases the predominant or sole receptor isoform is ERβ (*16, 43*). In HepG2 and SaOS-2 cells, ERβ is found solely in mitochondria, whereas ERα is confined to the nucleus, enriched in nucleoli (*43*). The molecular weights of the mitochondrial ERβs in the various cells and tissues span a range between 58 and 66 K, possibly reflecting among others, the presence of various receptor isoforms and posttranslational modifications of the receptors. The presence of ERβ in human heart mitochondria, as reported by Yang et al. (*54*), was contested by Scheyd and Gustafsson (*68*) on the basis of MALDI-TOF MS. However, Yang et al. (*69*) responded, attributing the negative results of the Gustafsson laboratory to the low concentration of ERβ in the mouse liver preparations assayed by this group.

Only scant information exists as to the mitochondrial localization of AR, and none at all as regards the progesterone receptor. AR has been detected in mitochondria of LNCaP cells (*44*) and of human sperm cells (*44*) in the midpiece, the region which harbors a high concentration of mitochondria. Two proteins reacting with AR antibodies were visualized on Western blots of human sperm extracts, one having a MW of 110 K conform to that of intact AR-B, and one of MW 90 K, which could represent AR-A, a specific mitochondrial AR or an AR degradation product.

TRs have been detected in rat liver mitochondria by biochemical (*37, 58–60*) and immunofluorescence techniques (*9, 37*). In a detailed analysis of mitochondrial TR, Wrutniak et al. (*37*) demonstrated the presence of two proteins with molecular weights of 43 K (p43) and 28 K (p28) reacting with antibodies to TRa, representing products of alternative translation initiation of the c-ErbAa gene (Fig. 1D). p43 is instrumental for mediating the transcriptional inducing effect of T3 in isolated mitochondria (*14, 37, 60*) (see the following section). An alternative translation initiation isoform of the c-ErbAa gene, TRa2, the dominant negative isoform of TR lacking the functional ligand-binding domain, has been detected in mitochondrial extracts of cardiomyocytes by Morrish et al. (*61*). Additionally, TRa1 and the isoforms p43 and p28 were also identified in these mitochondria by the same group (*61*). Neither of the nuclear receptors possesses a classical aminoterminal mitochondrial localization signal. However, Sionov et al. (*50*) experimentally defined internal mitochondrial localization signals in the glucocorticoid receptor and Psarra et al. (*42*), on the basis of computer analysis, also reported internal mitochondrial targeting sequences in the same receptor.

THE ROLE OF STEROID AND THYROID HORMONE RECEPTORS IN MITOCHONDRIA: REGULATION OF MITOCHONDRIAL TRANSCRIPTION AND OXPHOS BIOSYNTHESIS

The presence of steroid and TRs in mitochondria raises the question as to their role in mitochondrial physiology. Importantly, mitochondria are the key providers of the energy needs of the cell, generating by oxidative phosphorylation in the respiratory chain more than 90% of the total required ATP. The high rate of oxygen consumption in the aerobic ATP production unavoidably, as a consequence, leads to the formation of reactive oxygen species (ROS), which to a great extent can be inactivated by the mitochondrion with its appropriate enzymatic machinery. In case, however, this ROS inactivating process is compromised, ROS accumulate with deleterious effects on DNA (mutations) and on other macromolecules, leading to reduced ATP availability and increased oxidative stress, activation of the mitochondrial permeability transition pore and initiation

of apoptosis (*70, 71*). The effects of ROS on mitochondrial macromolecules have been etiologically correlated to various disease states (*72–74*), particularly neuromuscular and degenerative diseases (Alzheimer's, Parkinson's) (*75*), ageing (*76*), and cancer (*77*). Thus, the regulation of the energetic requirements of the cell is a crucial process, involving a pleiade of agents (*22*), necessitating a tight coordination of mitochondrial functions with those of other cell compartments (*21, 22, 26, 78–81*). The mitochondrion reacts to low ADP levels by mobilization of metabolically inactive mitochondria (*82*) and allosteric activation of enzymes of oxidative phosphorylation (OXPHOS) (*83*), the mitochondrial RNA polymerase being one of the sensors of the ADP/ATP ratio. However, higher energy requirements demand increased biosynthesis of OXPHOS, increased transcription of OXPHOS genes in the nucleus and in mitochondria, eventually an increase in mitochondrial gene dosage (*84, 85*).

Of the 82 subunit components of the mitochondrial respiratory complexes, 13 are encoded by mitochondrial genes, the rest by nuclear ones (Fig. 3). Under steady state conditions there is a clear relationship between levels of mRNAs for the OXPHOS encoded in the nucleus with those encoded in the mitochondrial genome and expression of nuclear and mitochondrial OXPHOS genes is coordinately regulated (*92, 93*). This is achieved mainly by pretranslational mechanisms, importantly by transcription control (*9*).

Steroid and thyroid hormones are major regulators of energy metabolism, acting in the nucleus and in the mitochondrion by inducing OXPHOS gene transcription and OXPHOS biosynthesis (*9*). Although a coordinated expression of nuclear and mitochondrial OXPHOS genes in the induced states is generally accepted, this does not seem to apply for all these genes, suggesting the existence of multiple control circuits (*94*). Parallel to inducing the cohort of genes involved in the hormones' phenotypic effects (*95–99*), these regulatory agents stimulate ATP generation required for these ongoing processes and for the replenishment of the cell's energy stores. Several publications refer to the stimulatory effects of glucocorticoids, estrogens, androgens, and thyroid hormones on transcription of nuclear and mitochondrial OXPHOS genes in various organs, such as heart, skeletal muscle, liver, kidney, and brain. Among others, well documented are the effects of glucocorticoids on the increase of COXI, COXIII, and 12S RNA transcripts in rat skeletal muscle (*85*), of COXI, II, III, and 16S RNA in rat distal colon (*100*), of COXII in rat GH4C1 pituitary cell (*101*), and of COXIII in rat hippocampus (*102*). Furthermore, the effects of estradiol on COXI and COXII mRNA in MCF-7 breast cancer cells (*103*), and of thyroxine on COXIII, COXIV, ND 1,4,5,6, and 16S RNA transcription in neonatal rats (*104*). Thus, the hormonal stimulus by way of the respective receptor must induce OXPHOS genes located in two different cell compartments. This can be accomplished by direct activation of nuclear OXPHOS genes containing HREs in their regulatory sites, or, indirectly, by induction of HRE-containing nuclear genes encoding transcription factors (*e.g.* NRF1 and NRF2), required for nuclear OXPHOS gene transcription. Furthermore, the same transcription factors can induce in the nucleus genes encoding mitochondrial transcription factors (TFAM, TFB1M, TFB2M) (Fig. 4), which, subsequently, can activate mitochondrial OXPHOS gene expression.

The presence of the mitochondrial genome sequences similar to nuclear HREs (Fig. 3) and the detection of steroid and TRs in these organelles suggested the possibility of an additional, direct mechanism for the induction of mitochondrial OXPHOS genes. Accordingly, the hormone-receptor complex can bind to the HREs of the mitochondrial genome and induce its transcription, parallel to the hormone's effect on nuclear OXPHOS genes, ensuring, thus, a coordinate expression of nuclear and mitochondrial OXPHOS genes (Fig. 4). Several findings demonstrate effects of steroid and thyroid hormones on mitochondrial transcription (reviewed in refs. *9, 18, 20, 23* and *107*). Experimental proof for a direct action of thyroid hormones on this process has been provided by Enriquez et al. (*15*) and of Casas et al. (*108*), using an in organello mitochondrial system.

Enriquez et al. (*15*) studied the influence of *in vivo* treatment and of *in vitro* addition of thyroid hormone on in organello mitochondrial DNA transcription. Hypothyroid rat liver mitochondria showed a 50% reduction of incorporation of radioactive precursor into RNA compared to that of euthyroid controls and a decrease in the mRNA/rRNA ratio. Administration of thyroid hormone to the hypothyroid animals restored the incorporation rate and the mRNA/rRNA ratio. *In vitro* addition of thyroid hormone to mitochondria form hypothyroid animals also restored the mRNA/rRNA ratio. Footprinting experiments showed that the hormonal effect is partially exerted by transcriptional action on the initiation step and selective modulation of the H-strand transcription initiation site and does not require previous activation of nuclear genes. Casas et al. (*108*) also using the in organello system targeted the mitochondrial specific TR p43 to mitochondria and observed that this lead to increased levels of precursor and mature RNA and of the mRNA to rRNA ratio, in a thyroid hormone dependent manner. These experiments demonstrate that the mitochondrial TR is a mitochondrial, thyroid hormone activated transcription factor, which in analogy to the action of the nuclear transcription factors would exert its modulatory effect by interaction with the mitochondrial transcriptosome (Fig. 5).

ROLE OF MITOCHONDRIAL STEROID HORMONE RECEPTORS IN APOPTOSIS

The mitochondria receive and integrate a series of exogenous and endogenous apoptotic and survival signals (*72, 78–81, 105, 110*). Among the major exogenous signals are steroid and thyroid hormones and accumulating findings demonstrate that some of the hormonal apoptotic/antiapoptotic effects are mediated by the respective mitochondrial receptors. Depending on the nature of the target cell, the same hormone can act as an apoptotic or as a survival factor, in part due to tissue specificity of the mitochondria. Proteomic analysis has shown differences in the com-

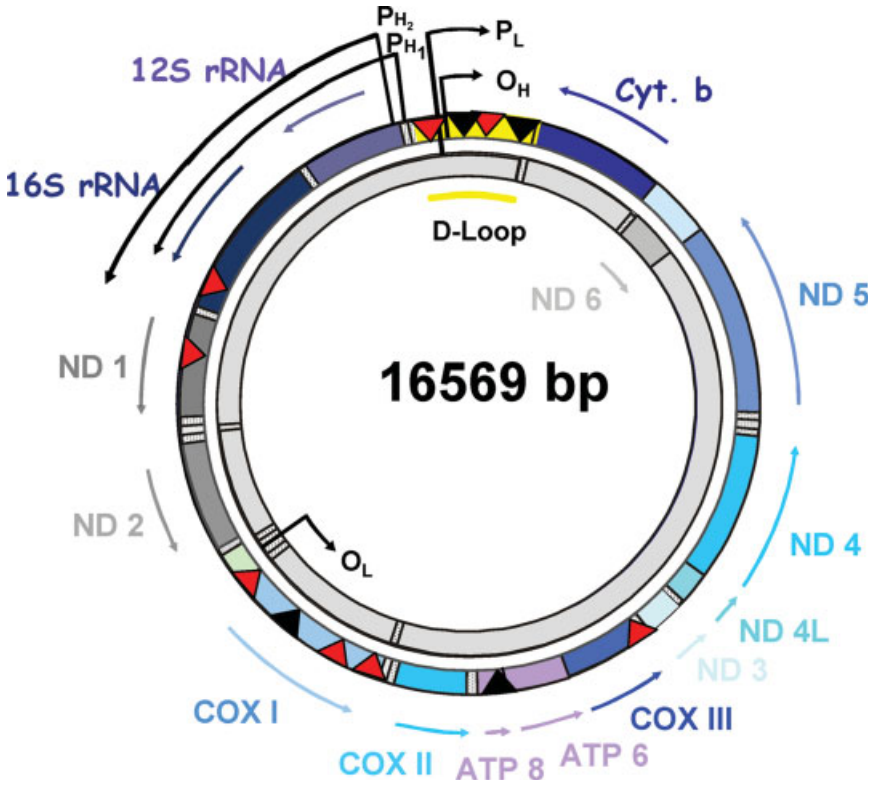

Figure 3. The mitochondrial genome and sites of positioning of putative HREs. The mammalian mitochondrial genome is a circular double stranded molecule, composed of one heavy (H) and one light (L) strand (*19, 86, 87*). The L strand is transcribed from one promoter (PL) and the H strand from two adjacent ones (PH1, PH2). All promoters are localized in the regulatory region, the D-loop. Modulation of gene expression is confined to the D-loop, however the presence of potential intragenic regulatory sequences (*e.g.* HREs) offers possibilities of regulation of other stages of the transcription process. Transcription from PH2 and PL generates long, polycistronic products, which are processed at the sites of transfer RNA coding genes, liberating mature mRNAs and tRNAs. Transcription from PHI produces a short message containing the two rRNAs. ND6 and the tRNAs for Gln, Ala, Asn, Cys, Tyr, Ser, Glu, and Pro are generated from transcription of the light strand, whereas COXI, II, III, NAD1-5, cytochrome b, ATP-synthase (ATP 6, 8), 12SRNA, 16SRNA, and the rest tRNAs from PH2. With the present level of knowledge, the transcription machinery of the mitochondrion seems rather simple, in comparison to the respective nuclear one. A single polypeptide, prokaryotic-type DNA-dependent RNA polymerase (POLRMT), displaying high sequence similarity to the C-terminal part of theT3/T7 bacteriophage family polymerases, is the sole RNA synthesizing enzyme in mitochondria (*19, 86, 87*). To interact with promoter elements, POLRMT requires the mediation of the mitochondrial transcription factor A (TFAM) and one of the two transcription factor B paralogues, TFBIM and TFB2M. POLRMT, TFAM, and TFBIM or TFB2M are sufficient to sustain transcription from a promoter containing DNA fragment. In addition to these transcription factors involved primarily in transcription initiation, a transcription factor, mTERF, has been isolated. mTERF binds in a sequence specific manner to the transcription termination site at the 3′ end of Leu-tRNA and to a region close to the transcription start site of PH1. It plays a role in termination of the H1 transcript and in blocking L-strand transcription, but also in facilitating reinitiation of PH1 transcription. A new transcription factor, MTERF3, acting as a negative regulator of mitochondrial transcription, has been added to the list of mitochondrial transcription factors. The sites on the genome of the predicted (*12, 13, 88*) and experimentally verified (*14, 16, 18, 89–91*) binding sites for steroid and thyroid hormone receptors are depicted: red triangles, HREs for class I receptors (consensus sequence, AGAACAxxxTGTTCT), black triangles, HREs for class II receptors (consensus sequence AGGTCAxxxTGACCT). Mutations not only of the structural genes but also of regulatory sites of the genome (D-loop), can be linked to disease states.

position of the mitochondrial proteins in different cell types, only a subset of proteins being common to all mitochondria (*111, 112*). Furthermore, the mitochondria vary in infrastructure and in their intrinsic oxidative phosphorylation capacity (*113*).

Glucocorticoids protect cells of epithelial origin, for example mammary gland cells, follicular cells, and hepatocytes, against apoptotic stimuli (*114*). However, glucocorticoids are apoptotic agents for cells of the hematopoietic system, such as monocytes,

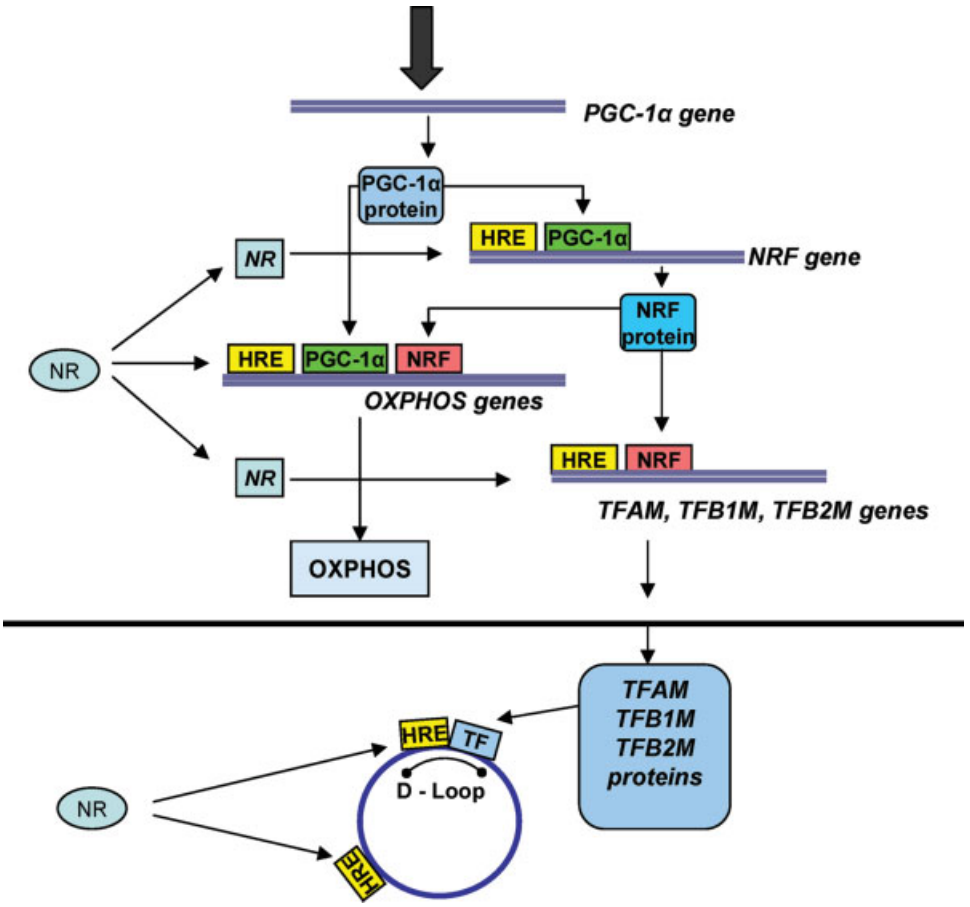

Figure 4. Coordination of transcription of nuclear and mitochondrial genes encoding OXPHOS by steroid and thyroid hormones. Nuclear receptors (NR) can directly induce in the nucleus genes having HREs in their regulatory regions, that is OXPHOS genes, nuclear transcription factor genes (NRF1/2), and mitochondrial transcription factor genes (TFAM, TFB1M, and TFB2M). NRF1/2 has a stimulatory effect on OXPHOS genes and on the mitochondrial transcription factor genes. The "master regulator" of mitochondrial biogenesis, peroxisome proliferator-activated receptor gamma-coactivator 1a (PGC-1a), acts directly on nuclear OXPHOS gene transcription and indirectly on nuclear and mitochondrial OXPHOS genes by way of induction of NRF1/2 and mitochondrial transcription factors, respectively. PGC-1alpha can be induced by various agents, among them steroid and thyroid hormones, IFN gamma and various stressors, by way signal transduction and Ca^{2+} dependent pathways (*9, 14–16, 21, 25, 26, 79, 85, 99, 105, 106*).

macrophages, thymocytes, and leukemic cells (*115, 116*). Several genes of the extrinsic and intrinsic death pathways are expressed in a proapoptotic manner in sensitive lymphoid cells treated with glucocorticoids, although additional signals are necessary to activate the apoptotic process. In this respect, Sionov et al. (*50, 117*) using T-lymphoid cell lines varying in sensitivity toward glucocorticoids have revealed a direct effect of the mitochondrial glucocorticoid receptor in the apoptotic process. In these cells, glucocorticoids induce a translocation of the cognate receptor from the cytoplasm to mitochondria, whereas in glucocorticoid resistant cells no such movement of GR is evident. Importantly, targeting of GR to mitochondria by linking the receptor to a mitochondrial localization signal resulted in an apoptotic effect, irrespective of the presence of glucocorticoids. Targeting to mitochondria of a GR devoid of its DBD domain also resulted in apoptosis, leading to the conclusion of a nongenomic action of GR in the process.

Estrogens are antiapoptotic factors for many cell types, such as breast cancer cells, endothelia, and brain cells. Stirone et al. (2005) (*17, 118*) showed that mitochondrial dysfunction is implicated in the etiology of vascular disease and that vasoprotection may involve hormonal effects on mitochondria. These researchers isolated mitochondria from cerebral blood vessels of ovariectomized rats with or without estrogen replacement, in which they detected ERα. Estrogen treatment increased the levels of nuclear encoded proteins, such as ERα, cytochrome c, COX IV, and manganese superoxide dismutase, and also of COX I, encoded in the mitochondrial genome. Furthermore, they showed an increase in the activity of citrate synthase and of complex IV. Incubation of cerebral vessels in the presence of

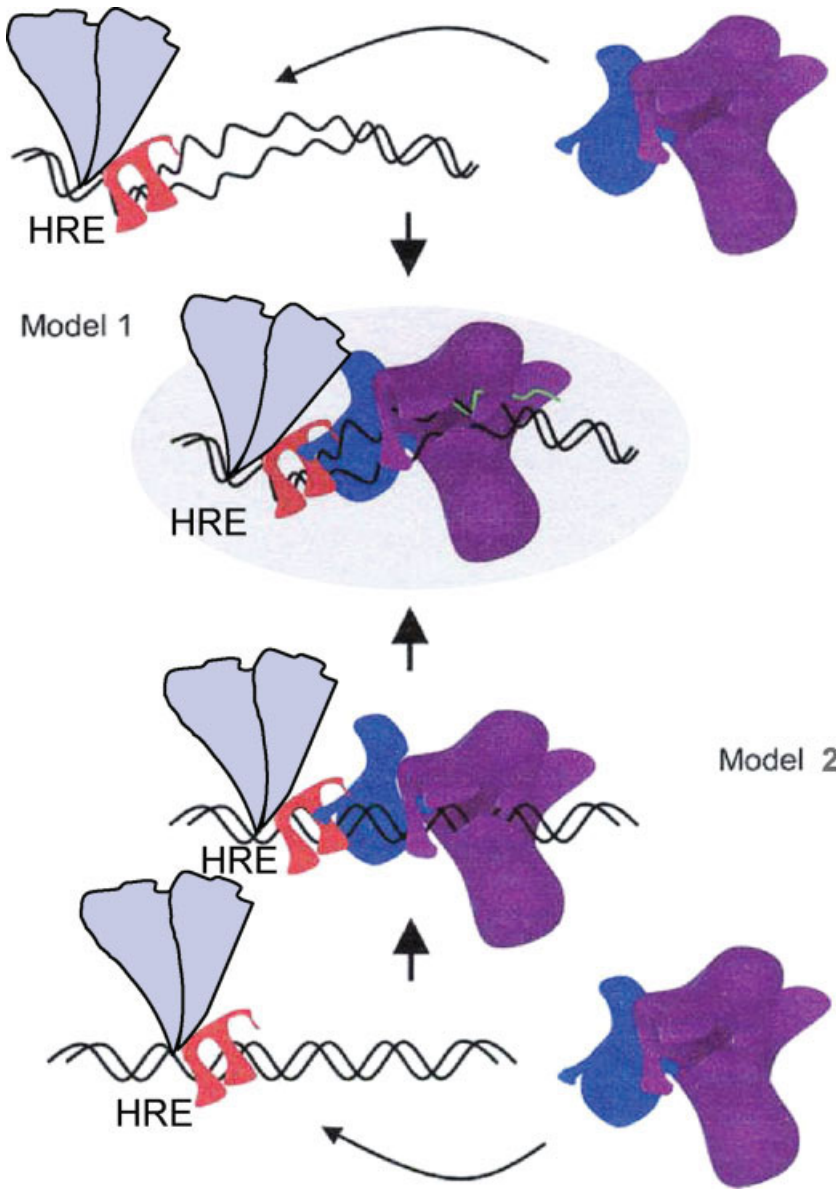

Figure 5. Mitochondrial transcription modulation by steroid/thyroid hormone receptors. On the basis of two models proposed by Bonawitz et al. (*86*), the role of the receptor on transcription initiation would be either to enhance the promoting action of MTFA on the formation and/or stabilization of an open DNA complex which is then recognized by POLRMT, either in complex or not with MTFB (1/2) (Model 1), or to promote promoter opening and/or stabilization of the core machinery after recruitment of POLRMT to the promoter and formation of the preinitiation complex (Model 2). Additionally, the hormone receptor could interact with the POLRMT/MTFB (1/2) complex. The characterization of MTERF3 as a negative effector of mitochondrial transcription offers additional possibilities of action of the nuclear receptors in the transcription process (*109*). In contrast to nuclear gene transcription involving a variety of genes serving diverse functions, each with its own promoter, the mitochondrial DNA possesses only three promoters, all with the sole role in the synthesis of OXPHOS and their translational machinery. Thus, the regulatory functions of the steroid/thyroid receptors or other relevant transcription factors on mitochondrial transcription, deal with the amount of the transcripts formed and their relative concentration, for example ratio of rRNA to mRNA. This implies that the regulatory agents acting on mitochondrial transcription, irrespective of their molecular structure, will show a uniform mode of action on the three mitochondrial promoters and will lack the versatility of the nuclear transcription factors acting on nuclear genes, as regards the choice of promoters. Accordingly, the number of mitochondrial transcription factors to be eventually discovered promises to be rather low. Some putative hormone response elements of steroid thyroid hormone receptors are placed within the structural genes (Fig. 3). As the effect of these hormones is not restricted to transcription initiation regulation, but also affects transcription termination, processing of the polycistronic message and stability of the transcripts, it is tempting to speculate that the HREs found outside the D-loop could be involved in such regulatory steps. One HRE found in the 16S RNA-Leu-tRNA transition area, overlapping the mTERF binding site, is of particular interest in this respect (*9*) (Reproduced from Ref. *86*, with permission from Elsevier).

estradiol elevated the levels of cytochrome c, an effect blocked by ER antagonists. The authors concluded that vascular protection by estrogens is in part mediated by modulation of mitochondrial functions, resulting in greater energy-producing capacity and decreased ROS production. Prolonged depression of cardiovascular functions, such as decreased cardiac output, occurs in rats subjected to trauma or hemorrhage, parallel to the decrease in cardiac mitochondrial ERβ (*119*). Restoration of cardiac function and of ERβ is achieved by the ERβ agonist DPN, but not by the agonist of ERα, PPT. Estradiol increased the binding of ERβ to mitochondrial DNA, the expression of COX I and COX II, and the activity of complex IV and ATP production, implicating ERβ mediated effects in the cardioprotective actions of estrogens. Lu et al. (*120*) have also demonstrated a protective effect of estrogens on cerebral blood vessels and cultured epithelial cells, supporting the involvement of mitochondrial estrogen receptors.

Applying gene expression profiling in mouse aorta in a program focused on the regulation of vascular tone, O'Lone et al. (*121*) observed that ERα and ERβ regulate distinct and nonoverlapping sets of genes and that ERα is essential for most of the observed estrogen-mediated decrease in gene expression. Nuclear genes encoding subunits of the major respiratory complexes are among the genes attenuated by direct binding of ERβ to the promoter of these genes. The down regulation of these genes in the aorta contrasts the stimulatory effects of ERβ in other cell types. Pedram et al. (*57*) applied the model system of UV irradiation of MCF-7 cancer cells to evaluate the role of estrogen receptors in cell survival. This treatment increases the mitochondrial generation of ROS and the translocation of the apoptotic factor Bax to mitochondria, decreases the mitochondrial membrane potential and leads to cytochrome c release, resulting in apoptosis. These effects are inhibited by estrogens. These researchers (*57*) then proceeded to show a direct antiapoptotic involvement of the mitochondrial estrogen receptors. They transfected ER-negative breast cancer derived HCC-1569 and CHO cells with the ligand-binding (E) domain of ER, specifically targeted either to the nucleus or to the mitochondria. No estrogen protection of the irradiated cells was observed by targeting the E-domain to the nucleus, however the cells were protected by its targeting to the mitochondria. As the estrogen receptor construct lacks the DNA-binding domain, its antiapoptotic effect appears to be mediated by a nongenomic action, in part due to activation of manganese superoxide dismutase, the mitochondrial enzyme catalyzing superoxide radical breakdown. Estrogen protection from oxidative stress was also demonstrated by Razmara et al. (*122*), involving activation of the same enzyme.

THE PRESENCE OF ADDITIONAL NUCLEAR RECEPTORS AND OTHER NUCLEAR TRANSCRIPTION FACTORS IN MITOCHONDRIA

Several publication report the presence of mitochondrial receptors of the nuclear receptor superfamily, in addition to the ones discussed above, and of other nuclear transcription factors (*123, 124*). Thus, the retinoic acid receptor RAR (*62*), the retinoid receptor alpha (RXRα) (*60*), the orphan receptor Nur 77/TR3 (*63, 64*), and the PPAR β- and γ2-coactivator (PPARβ-and γ_2) related proteins (*65*) have been localized in mitochondria (Table 1). Among the nuclear transcription factors found in mitochondria are NF-κB, AP-1, CREB, p53, c-myc, wnt 13, Dok-4, HMG-A1, and c-src (reviewed in refs. *123* and *124*). The role of these factors in mitochondrial functions is now being explored and their involvement in mitochondrial transcription regulation and in apoptosis/survival is now emerging (*123, 124*). These effects are realized both by genomic mechanisms-binding sites in the D-loop and in other regions of the mitochondrial genome for some of these factors and also their interaction with these regions has been revealed and by nongenomic effects by way of protein–protein interactions with apoptotic and survival proteins (*123, 124*).

CONCLUDING REMARKS

The recognition of the central role of mitochondria in apoptosis achieved in the last years had a stimulatory effect on research toward delineating the multiple functions of this organelle and the realization of its significance as a major integrator of cell signaling. In parallel, significant progress was achieved concerning the mechanisms of mitochondrial transcription, with the discovery of novel initiation and termination transcription factors (*109*), and in further understanding the functions of the mitochondrial RNA polymerase (*86, 87*). The detection of receptors of steroid and TRs in mitochondria, hormones that play an important role in metabolism, growth, development, and immunomodulation, stimulated research toward exploring the function and molecular mode of action of these receptors in mitochondria. Results stemming from many laboratories support the role of steroid and TRs as mitochondrial transcription factors, acting on mitochondrial transcription in a way similar to the action of the receptors on nuclear genes. A parallel action of the receptors on the OXPHOS genes in the two separate cell compartments is being revealed, an action aimed at the coordinate regulation of OXPHOS in response to hormonal stimulation, necessitating increase in energy yield. Furthermore, a role of the mitochondrial receptors on cell survival and apoptosis is being revealed. Both genomic and nongenomic actions of the mitochondrial receptors in this context are implicated. The detection in mitochondria of additional receptors of the superfamily of nuclear receptors and of nuclear transcription factors with well described nuclear actions expands the regulatory arsenal of the mitochondria. Some genomic and nongenomic actions of these regulatory factors in mitochondria have already being revealed. The future challenge in this rapidly evolving field is to further characterize and understand the role and mode of action and interaction of the host of nuclear receptors and transcription factors in mitochondria, and to elucidate the integration of their functions in mitochondria with the nuclear actions of these fac-

tors toward common regulatory goals. The new knowledge attained will be instrumental in understanding derangements in the mitochondrion-nuclear regulatory circuit and their role in the etiopathology of mitochondria related diseases.

ACKNOWLEDGEMENTS

Part of the work presented in this article from the authors' laboratory was supported by the Bodossaki Foundation, Athens, Greece, the Greek Secretariat of Science and Technology, the International Atomic Agency Commission, Vienna, Austria, and the University of Wuerzburg, Boveri Biocenter, Germany.

REFERENCES

1. Evans, R. (2004) A transcriptional basis for physiology. *Nat. Med.* **10**, 1022–1026.
2. Kumar, R., and Thompson, E. B. (1999) The structure of the nuclear hormone receptors. *Steroids* **64**, 310–319.
3. Lonard, D. M., and O'Malley, B. W. (2005) Expanding functional diversity of the coactivators. *Trends Biochem. Sci.* **30**, 126–132.
4. Papamichail, M., Ioannidis, C., Tsawdaroglou, N., and Sekeris, C. E. (1981) Translocation of glucocorticoid receptor from the cytoplasm into the nucleus of phytohemagglutinin-stimulated human lymphocytes in the absence of the hormone. *Exp. Cell Res.* **133**, 461–465.
5. Power, R. F., Mani, S. K., Codina, J., Conneely, O. M., and O'Malley, B. W. (1991) Dopaminergic and ligand-independent activation of steroid hormone receptors. *Science* **254**, 1636–1639.
6. Jonat, C., Rahmsdorf, H. J., Park, K. K., Cato, A. C., Gebel, S., Ponta, H., and Herrlich, P. (1990) Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell* **62**, 1189–1204.
7. Wehling, M., and Lösel, R. (2006) Non-genomic steroid hormone effects: membrane or intracellular receptors? *J. Steroid Biochem. Mol. Biol.* **102**, 180–183.
8. Kino, T., Tiulpakov, A., Ichijo, T., Chheng, L., Kozasa, T., and Chrousos, G. P. (2005) G protein beta interacts with the glucocorticoid receptor and suppresses its transcriptional activity in the nucleus. *J. Cell Biol.* **169**, 885–896.
9. Scheller, K., Seibel, P., and Sekeris, C. E. (2003) Glucocorticoid and thyroid hormone receptors in mitochondria of animal cells. *Int. Rev. Cytol.* **222**, 1–61.
10. Tasker, J. G., Di, S., and Malcher-Lopes R. (2006) Minireview: rapid glucocorticoid signaling via membrane-associated receptors. *Endocrinology* **147**, 5549–5556.
11. Nagy, L., Schüle, R., and Gronemeyer, H. (2006) Twenty years of nuclear receptors: conference on nuclear receptors—from chromatin to disease. *EMBO Rep.* **7**, 579–584.
12. Sekeris, C. E. (1990) The mitochondrial genome: a possible primary site of action of steroid hormones. *In Vivo* **4**, 317–320.
13. Demonacos, C. V., Karayanni, N., Hatzoglou, E., Tsiriyiotis, C., Spandidos, D. A., and Sekeris, C. E. (1996) Mitochondrial genes as sites of primary action of steroid hormones. *Steroids* **61**, 226–232.
14. Wrutniak, C., Rochard, P., Casas, F., Fraysse, A., Charrier, J., and Cabello, G. (1998) Physiological importance of the T3 mitochondrial pathway. *Ann. N. Y. Acad. Sci.* **839**, 93–100.
15. Enriquez, J. A., Fernandez-Silva, P., Garrido-Perez, N., Lopez-Perez, M. J., Perez-Martos, A., and Montoya, J. (1999) Direct regulation of mitochondrial RNA synthesis by thyroid hormone. *Mol. Cell Biol.* **19**, 657–670.
16. Chen, J. Q., Delannoy, M., Cooke, C., and Yager, J. D. (2004) Mitochondrial localization of ERalpha and ERbeta in human MCF7 cells. *Am. J. Physiol. Endocrinol. Metab.* **286**, E1011–E1022.
17. Duckles, S. P., Krause, D. N., Stirone, C., and Procaccio, V. (2006) Estrogen and mitochondria: a new paradigm for vascular protection? *Mol. Interv.* **6**, 26–35.
18. Gavrilova-Jordan, L. P., and Price T. M. (2007) Actions of steroids in mitochondria. *Semin. Reprod. Med.* **25**, 154–164.
19. Montoya, J., Lopez-Perez, M. J., and Ruiz-Pesini, E. (2006) Mitochondrial DNA transcription and diseases: past, present and future. *Biochim. Biophys. Acta* **1757**, 1179–1189.
20. Chen, J. Q., Brown, T., and Yager, J. D. Mechanism of hormone carcinogenesis: revolution of views and role of mitochondria. Innovative Endocrinol. Cancer (Berstein, L. M. and Santen, R. J. (edited) Eureka Bioscience Database) in press.
21. Scarpulla, R. C. (2006) Nuclear control of respiratory gene expression in mammalian cells. *J. Cell. Biochem.* **97**, 673–683.
22. Goffart, S., and Wiesner, R. J. (2003) Regulation and co-ordination of nuclear gene expression during mitochondrial biogenesis. *Exp. Physiol.* **88**, 33–40.
23. Weitzel, J. M., Iwen, K. A., and Seitz, H. J. (2003) Regulation of mitochondrial biogenesis by thyroid hormone. *Exp. Physiol.* **88**, 121–128.
24. Goglia, F., Moreno, M., and Lanni, A. (1999) Action of thyroid hormones at the cellular level: the mitochondrial target. *FEBS Lett.* **452**, 115–120.
25. Wrutniak-Cabello, C., Casas, F., and Cabello, G. (2001) Thyroid hormone action in mitochondria. *J. Mol. Endocrinol.* **26**, 67–77.
26. Scheller, K., and Sekeris, C. E. (2003) The effects of steroid hormones on the transcription of genes encoding enzymes of oxidative phosphorylation. *Exp. Physiol.* **88**, 129–140.
27. Chen, J. Q., Yager, J. D., and Russo, J. (2005) Regulation of mitochondrial respiratory chain structure and function by estrogens/estrogen receptors and potential physiological/pathophysiological implications. *Biochim. Biophys. Acta* **1746**, 1–17.
28. Lu, N. Z., and Cidlowski, J. A. (2005) Translational regulatory mechanisms generate N-terminal glucocorticoid receptor isoforms with unique transcriptional target genes. *Mol. Cell.* **18**, 331–342.
29. Russcher, H., Dalm, V. A., de Jong, F. H., Brinkmann, A. G., Hofland, L. J., Lamberts, S. W., and Koper, J. W. (2007) Associations between promoter usage and alternative splicing of the glucocorticoid receptor gene. *J. Mol. Endocrinol.* **38**, 91–98.
30. Bamberger, C. M., Bamberger, A. M., de Castro, M., and Chrousos, G. P. (1995) Glucocorticoid receptor beta, a potential endogenous inhibitor of glucocorticoid action in humans. *J. Clin. Invest.* **95**, 2435–2441.
31. Duma, D., Jewell, C. M., and Cidlowski, J. A. (2006) Multiple glucocorticoid receptor isoforms and mechanisms of post-translational modification. *J. Steroid Biochem. Mol. Biol.* **102**, 11–21.
32. Heldring, N., Pike, A., Andersson, S., Matthews, J., Cheng, G., Hartman, J., Tujague, M., Ström, A., Treuter, E., Warner, M., and Gustafsson, J. A. (2007) Estrogen receptors: how do they signal and what are their targets. *Physiol. Rev.* **87**, 905–931.
33. Dehm, S. M., and Tindall, D. J. (2007) Androgen receptor structural and functional elements: role and regulation in prostate cancer. *Mol. Endocrinol.* **21**, 2855–2863.
34. Jagla, M., Fève, M., Kessler, P., Lapouge, G., Erdmann, E., Serra, S., Bergerat, J. P., and Céraline, J. (2007) A splicing variant of the androgen receptor detected in a metastatic prostate cancer exhibits exclusively cytoplasmic actions. *Endocrinology* **148**, 4334–4343.
35. Lazar, M. A. (1993) Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocr. Rev.* **14**, 184–193.
36. Brent, G. A. (1994) The molecular basis of thyroid hormone action. *N. Engl. J. Med.* **331**, 847–853.
37. Wrutniak, C., Cassar-Malek, I., Marchal, S., Rascle, A., Heusser, S., Keller, J. M., Fléchon, J., Dauça, M., Samarut, J., Ghysdael, J., Cabello, G. (1995) A 43-kDa protein related to c-Erb A alpha 1 is located in the mitochondrial matrix of rat liver. *J. Biol. Chem.* **270**, 16347–16354.

38. Faus, H., and Haendler, B. (2006) Post-translational modifications of steroid receptors. *Biomed. Pharmacother.* **60**, 520–528.
39. Leader, J. E., Wang, C., Fu, M., and Pestell, R. G. (2006) Epigenetic regulation of nuclear steroid receptors. *Biochem. Pharmacol.* **72**, 1589–1596.
40. Lu, N. Z., and Cidlowski, J. A. (2006) Glucocorticoid receptor isoforms generate transcription specificity. *Trends Cell Biol.* **16**, 301–307.
41. Schoneveld, O. J., Gaemers, I. C., and Lamers, W. H. (2004) Mechanisms of glucocorticoid signaling. *Biochim. Biophys. Acta* **1680**, 114–128.
42. Psarra, A.-M. G., Solakidi, S., Trougakos, I. P., Margaritis, L. H., Spyrou, G., and Sekeris, C. E. (2005) Glucocorticoid receptor isoforms in human hepatocarcinoma HepG2 and SaOS-2 osteosarcoma cells: presence of glucocorticoid receptor alpha in mitochondria and of glucocorticoid receptor beta in nucleoli. *Int. J. Biochem. Cell Biol.* **37**, 2544–2558.
43. Solakidi, S., Psarra, A.-M. G., and Sekeris, C. E. (2005) Differential subcellular distribution of estrogen receptor isoforms: localization of ERalpha in the nucleoli and ERbeta in the mitochondria of human osteosarcoma SaOS-2 and hepatocarcinoma HepG2 cell lines. *Biochim. Biophys. Acta* **1745**, 382–392.
44. Solakidi, S., Psarra, A.-M. G., Nikolaropoulos, S., and Sekeris, C. E. (2005) Estrogen receptors alpha and beta (ERalpha and ERbeta) and androgen receptor (AR) in human sperm: localization of ERbeta and AR in mitochondria of the midpiece. *Hum. Reprod.* **20**, 3481–3487.
45. Demonacos, C., Tsawdaroglou, N. C., Djordjevic- Markovic, R., Papalopoulou, M., Galanopoulos, V., Papadogeorgaki, S., and Sekeris, C. E. (1993) Import of the glucocorticoid receptor into rat liver mitochondria in vivo and in vitro. *J. Steroid Biochem. Mol. Biol.* **46**, 401–413.
46. Scheller, K., Sekeris, C. E., Krohne, G., Hock, R., Hansen, I. A., and Scheer, U. (2000) Localization of glucocorticoid hormone receptors in mitochondria of human cells. *Eur. J. Cell Biol.* **79**, 299–307.
47. Moutsatsou, P., Psarra, A.-M. G., Tsiapara, A., Paraskevakou, H., Davaris, P., and Sekeris, C. E. (2001) Localization of the glucocorticoid receptor in rat brain mitochondria. *Arch. Biochem. Biophys.* **386**, 69–78.
48. Psarra, A.-M. G., Bochaton-Piallat, M. L., Gabbiani, G., Sekeris, C. E., and Tsacopoulos, M. (2003) Mitochondrial localization of glucocortocoid receptor in glial (Mueller) cells in the salamander retina. *Glia* **41**, 38–49.
49. Koufali, M. M., Moutsatsou, P., Sekeris, C. E., and Breen K. C. (2003) The dynamic localization of the glucocorticoid receptor in rat C6 glioma cell mitochondria. *Mol. Cell. Endocrinol.* **209**, 51–60.
50. Sionov, R. V., Cohen, O., Kfir, S., Zilberman, Y., and Yefenof, E. (2006) Role of mitochondrial glucocorticoid receptor in glucocorticoid-induced apoptosis. *J. Exp. Med.* **203**, 189–201.
51. Jönsson, D., Nilsson, J., Odenlund, M., Bratthall, G., Broman, J., Ekblad, E., Lydrup, M. L., and Nilsson, B. O. (2007) Demonstration of mitochondrial oestrogen receptor beta and oestrogen-induced attenuation of cytochrome c oxidase subunit I expression in human periodontal ligament cells. *Arch. Oral Biol.* **52**, 669–676.
52. Moats, R. K., II, and Ramirez, V. D. (2000) Electron microscopic visualization of membrane-mediated uptake and translocation of estrogen-BSA: colloidal gold by HepG2 cells. *J. Endocrinol.* **166**, 631–647.
53. Monje, P., and Boland, R. (2001) Subcellular distribution of native estrogen receptor alpha and beta isoforms in rabbit uterus and ovary. *J. Cell. Biochem.* **82**, 467–479.
54. Yang, S. H., Liu, R. Perez, E. J., Wen, Y., Stevens, S. M., Jr., Valencia, T., Brun-Zinkernagel, A. M., Prokai, L., Will, Y., Dykens, J., Koulen, P., and Simpkins, J. W. (2004) Mitochondrial localization of estrogen receptor beta. *Proc. Natl. Acad. Sci. USA.* **101**, 4130–4135.
55. Cammarata, P. R., Chu, S., Moor, A., Wang, Z., Yang, S. H., and Simpkins, J. W. (2004) Subcellular distribution of native estrogen receptor alpha and beta subtypes in cultured human lens epithelial cells. *Exp. Eye Res.* **78**, 861–871.
56. Milner, T. A., Ayoola, K., Drake, C. T., Herrick, S. P., Tabori, N. E., McEwen, B. S., Warrier, S., and Alves, S. E. (2005) Ultrastructural localization of estrogen receptor beta immunoreactivity in the rat hippocampal formation. *J. Comp. Neurol.* **491**, 81–95.
57. Pedram, A., Razandi, M., Wallace, D. C., and Levin, E. R. (2006) Functional estrogen receptors in the mitochondria of breast cancer cells. *Mol. Biol. Cell* **17**, 2125–2137.
58. Sterling, K., Campbell, G. A., and Brenner, M. A. (1984) Purification of the mitochondrial triiodothyronine (T3) receptor from rat liver. *Acta Endocrinol.* (Copenh) **105**, 391–397.
59. Ardail, D., Lerme, F., Puymirat, J., and Morel, G. (1993) Evidence for the presence of alpha and beta-related T3 receptors in rat liver mitochondria. *Eur. J. Cell Biol.* **62**, 105–113.
60. Casas, F., Daury, L., Grandemange, S., Busson, M., Seyer, P., Hatier, R., Carazo, A., Cabello, G., and Wrutniak-Cabello, C. (2003) Endocrine regulation of mitochondrial activity: involvement of truncated RXRalpha and c-Erb Aalpha1 proteins. *FASEB J.* **17**, 426–436.
61. Morrish, F., Buroker, N. E. Ge, M., Ning, X. H., Lopez-Guisa, J., Hockenbery, D., and Portman, M. A. (2006) Thyroid hormone receptor isoforms localize to cardiac mitochondrial matrix with potential for binding to receptor elements on mtDNA. *Mitochondrion* **6**, 143–148.
62. Berdanier, C. D., Everts, H. B., Hermoyian, C., and Mathews, C. E. (2001) Role of vitamin A in mitochondrial gene expression. *Diabetes Res. Clin. Pract.* **54**, S11–S27.
63. Li, H., Kolluri, S. K., Gu, J., Dawson, M. I., Cao, X., Hobbs, P. D., Lin, B., Chen, G., Lu, J., Lin, F., Xie, Z., Fontana, J. A., Reed, J. C., and Zhang, X. (2000) Cytochrome c release and apoptosis induced by mitochondrial targeting of nuclear orphan receptor TR3. *Science* **289**, 1159–1164.
64. Jeong, J. H., Park, J. S., Moon, B., Kim, M. C., Kim, J. K., Lee, S., Suh, H., Kim, N. D., Kim, J. M., Park, Y. C., and Yoo, Y. H. (2003) Orphan nuclear receptor Nur77 translocates to mitochondria in the early phase of apoptosis induced by synthetic chenodeoxycholic acid derivatives in human stomach cancer cell line SNU-1. *Ann. N. Y. Acad. Sci.* **1010**, 171–177.
65. Casas, F., Domenjoud, L., Rochard, P., Hatier, R., Rodier, A., Daury, L., Bianchi, A., Kremarik-Bouillaud, P., Becuwe, P., Keller, J., Schohn, H., Wrutniak-Cabello, C., Cabello, G., and Dauca, M. (2000) A 45 kDa protein related to PPARgamma2, induced by peroxisome proliferators, is located in the mitochondrial matrix. *FEBS Lett.* **478**, 4–8.
66. Noteboom, W. D., and Gorski, J. (1965) Stereospecific binding of estrogens in the rat uterus. *Arch. Biochem. Biophys.* **111**, 559–568.
67. Grossman, A., Oppenheim, J., Grondin, G., St. Jean, P., and Beaudoin, A. R. (1989) Immunocytochemical localization of the [3H]estradiol-binding protein in rat pancreatic acinar cells. *Endocrinology* **124**, 2857–2866.
68. Schwend, T., and Gustafsson, J. A. (2006) False positives in MALDI-TOF detection of ERbeta in mitochondria. *Biochem. Biophys. Res. Commun.* **343**, 707–711.
69. Yang, S. H., Prokai, L., and Simpkins, J. W. (2006) Correspondence regarding Schwend and Gustafsson. "False positives in MALDI-TOF detection of ERbeta in mitochondria." *Biochem. Biophys. Res. Commun.* **345**, 917–918.
70. Kroemer, G., Galluzzi, L., and Brenner, C. (2007) Mitochondrial membrane permeabilization in cell death. *Physiol. Rev.* **87**, 99–163.
71. Dawson, V. L., and Dawson, T. M. (2004) Deadly conversations: nuclear-mitochondrial cross-talk. *J. Bioenerg. Biomembr.* **36**, 287–294.
72. Onyango, I., Khan, S., Miller, B., Swerdlow, R., Trimmer P., and Bennett, P., Jr. (2006) Mitochondrial genomic contribution to mitochon-

drial dysfunction in Alzheimer's disease. *J. Alzheimers Dis.* **9**, 183–193.
73. Wallace, D. C. (1999) Mitochondrial diseases in man and mouse. *Science* **283**, 1482–1488.
74. Stark, R., and Roden, M. (2007) ESCI Award 2006. Mitochondrial function and endocrine diseases. *Eur. J. Clin. Invest.* **37**, 236–248.
75. Lin, M. T., and Beal, M. F. (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* **443**, 787–795.
76. Short, K. R., Bigelow, M. L., Kahl, J., Singh, R., Coenen-Schimke, J., Raghavakaimal, S., and Nair, K. S. (2005) Decline in skeletal muscle mitochondrial function with aging in humans. *Proc. Natl. Acad. Sci. USA.* **102**, 5618–5623.
77. Kroemer, G. (2006) Mitochondria in cancer. *Oncogene* **25**, 4630–4632.
78. Garesse, R., and Vallejo, C. G. (2001) Animal mitochondrial biogenesis and function: a regulatory cross-talk between two genomes. *Gene* **263**, 1–16.
79. Scarpulla, R. C. (2002) Nuclear activators and coactivators in mammalian mitochondrial biogenesis. *Biochim. Biophys. Acta* **1576**, 1–14.
80. Goldenthal, M. J., and Marin-Garcia, J. (2004) Mitochondrial signaling pathways: a receiver/integrator organelle. *Mol. Cell. Biochem.* **262**, 1–16.
81. Feige, J. N., and Auwerx, J. (2007) Transcriptional coregulators in the control of energy homeostasis. *Trends Cell Biol.* **17**, 292–301.
82. Faustin, B., Rossignol, R., Rocher, C., Bénard, G., Malgat, M., and Letellier, T. (2004) Mobilization of adenine nucleotide translocators as molecular bases of the biochemical threshold effect observed in mitochondrial diseases. *J. Biol. Chem.* **279**, 20411–20421.
83. Enriquez, J. A., Fernandez-Silva, P., and Montoya, J. (1999) Autonomous regulation in mammalian mitochondrial DNA transcription. *Biol. Chem.* **380**, 737–747.
84. Williams, R. S. (1986) Mitochondrial gene expression in mammalian striated muscle. Evidence that variation in gene dosage is the major regulatory event. *J. Biol. Chem.* **261**, 12390–12394.
85. Weber, K., Bruck, P., Mikes, Z., Kupper, J. H., Klingenspor, M., and Wiesner, R. J. (2002) Glucocorticoid hormone stimulates mitochondrial biogenesis specifically in skeletal muscle. *Endocrinology* **143**, 177–184.
86. Bonawitz, N. D., Clayton, D. A., and Shadel, G. S. (2006) Initiation and beyond: multiple functions of the human mitochondrial transcription machinery. *Mol. Cell.* **24**, 813–825.
87. Asin-Cayuela, J., and Gustafsson, C. M. (2007) Mitochondrial transcription and its regulation in mammalian cells. *Trends Biochem. Sci.* **32**, 111–117.
88. Ioannou, I. M., Tsawdaroglou, N., and Sekeris, C. E. (1988) Presence of glucocorticoid responsive elements in the mitochondrial genome. *Anticancer Res.* **8**, 1405–1409.
89. Chen, J. Q., Eshete, M., Alworth, W. L., and Yager, J. D. (2004) Binding of MCF-7 cell mitochondrial proteins and recombinant human estrogen receptors alpha and beta to human mitochondrial DNA estrogen response elements. *J. Cell Biochem.* **93**, 358–373.
90. Demonacos, C., Djordjevic-Markovic, R., Tsawdaroglou, N., and Sekeris, C. E. (1995) The mitochondrion as a primary site of action of glucocorticoids: the interaction of the glucocorticoid receptor with mitochondrial DNA sequences showing partial similarity to the nuclear glucocorticoid responsive elements. *J. Steroid Biochem. Mol. Biol.* **55**, 43–55.
91. Tsiriyotis, C., Spandidos, D. A., and Sekeris, C. E. (1997) The mitochondrion as a primary site of action of glucocorticoids: mitochondrial nucleotide sequences, showing similarity to hormone response elements, confer dexamethasone inducibility to chimaeric genes transfected in LATK- cells. *Biochem. Biophys. Res. Commun.* **235**, 349–354.
92. Gagnon, J., Kurowski, T. T., Wiesner, R. J., and Zak, R. (1991) Correlations between a nuclear and a mitochondrial mRNA of cytochrome c oxidase subunits, enzymatic activity and total mRNA content, in rat tissues. *Mol. Cell. Biochem.* **107**, 21–29.
93. Goldenthal, M. J., Ananthakrishnan, R., and Marín-García, J. (2005) Nuclear-mitochondrial cross-talk in cardiomyocyte T3 signaling: a time-course analysis. *J. Mol. Cell. Cardiol.* **39**, 319–326.
94. Nelson, B. D., Luciakova, K., Li, R., and Betina, S. (1995) The role of thyroid hormone and promoter diversity in the regulation of nuclear encoded mitochondrial proteins. *Biochim. Biophys. Acta* **1271**, 85–91.
95. Weitzel, J. M., Hamann, S., Jauk, M., Lacey, M., Filbry, A., Radtke, C., Iwen, K. A., Kutz, S., Harneit, A., Lizardi, P. M., and Seitz, H. J. (2003) Hepatic gene expression patterns in thyroid hormone-treated hypothyroid rats. *J. Mol. Endocrinol.* **31**, 291–303.
96. Fisher, I., Abraham, D., Bouri, K., Hoffman, E. P., Muntoni, F., and Morgan, J. (2005) Prednisolone-induced changes in dystrophic skeletal muscle. *FASEB J.* **19**, 834–846.
97. Yaylaoglu, M. B., Agbemafle, B. M., Oesterreicher, T. J., Finegold, M. J., Thaller, C., and Henning, S. J. (2006) Diverse patterns of cell-specific gene expression in response to glucocorticoid in the developing small intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* **291**, G1041–G1050.
98. Malorni, L., Cacace, G., Cuccurullo, M., Pocsfalvi, G., Chambery, A., Farina, A., Di Maro, A., Parente, A., and Malorni, A. (2006) Proteomic analysis of MCF-7 breast cancer cell line exposed to mitogenic concentration of 17beta-estradiol. *Proteomics* **6**, 5973–5982.
99. Hurson, C. J., Butler, J. S., Keating, D. T., Murray, D. W., Sadlier, D. M., O' Byrne, J. M., and Doran, P. P. (2007) Gene expression analysis in human osteoblasts exposed to dexamethosone identifies altered developmental pathways as putative drivers of osteoporosis. *BMC Musculoskelet. Disord.* **12**, 8–12.
100. Rachamin, N., Latter, H., Malinin, N., Asher, C., Wald, H., and Garty, H. (1995) Dexamethasone enhances expression of mitochondrial oxidative phosphorylation genes in rat distal colon. *Am. J. Physiol.* **269**, C1305–C1310.
101. Van Itallie, C. M., and Dannies, P. S. (1988) Estrogen induces accumulation of the mitochondrial ribonucleic acid for subunit II of cytochrome oxidase in pituitary tumor cells. *Mol. Endocrinol.* **2**, 332–337.
102. Bettini, E., and Maggi, A. (1992) Estrogen induction of cytochrome c oxidase subunit III in rat hippocampus. *J. Neurochem.* **58**, 1923–1929.
103. Felty, Q., and Roy, D. (2005) Estrogen, mitochondria, and growth of cancer and non-cancer cells. *J. Carcinog.* **4**, 1.
104. Mutvei, A., Kuzela, S., and Nelson, B. D. (1989) Control of mitochondrial transcription by thyroid hormone. *Eur. J. Biochem.* **180**, 235–240.
105. Puigserver, P., and Spiegelman, B. M. (2003) Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr. Rev.* **24**, 78–90.
106. Sonoda, J., Laganiere, J., Mehl, I. R., Barish, G. D., Chong, L. W., Li, X., Scheffler, I. E., Mock, D. C., Bataille, A. R., Robert, F., Lee, C. H., Giguere, V., and Evans, R. M. (2007) Nuclear receptor ERR alpha and coactivator PGC-1 beta are effectors of IFN-gamma-induced host defense. *Genes Dev.* **21**, 1909–1920.
107. Psarra, A.-M. G., Solakidi, S., and Sekeris, C. E. (2006) The mitochondrion as a primary site of action of steroid and thyroid hormones: presence and action of steroid and thyroid hormone receptors in mitochondria of animal cells. *Mol. Cell. Endocrinol.* **246**, 21–33.
108. Casas, F., Rochard, P., Rodier, A., Cassar-Malek, I., Marchal-Victorion, S., Wiesner, R. J., Cabello, G., and Wrutniak, C. (1999) A variant form of the nuclear triiodothyronine receptor c-ErbAalpha1 plays a direct role in regulation of mitochondrial RNA synthesis. *Mol. Cell. Biol.* **19**, 7913–7924.
109. Park, C. B., Asin-Cayuela, J., Cámara, Y., Shi, Y., Pellegrini, M., Gaspari, M., Wibom, R., Hultenby, K., Erdjument-Bromage, H., Tempst, P., Falkenberg, M., Gustafsson, C. M., and Larsson, N. G. (2007) MTERF3 is a negative regulator of mammalian mtDNA transcription. *Cell* **130**, 273–285.

110. Pinkoski, M. J., Waterhouse, N. J., and Green, D. R. (2006) Mitochondria, apoptosis and autoimmunity. *Curr. Dir. Autoimmun.* **9**, 55–73.
111. Mootha, V. K., Bunkenborg, J., Olsen, J. V., Hjerrild, M., Wisniewski, J. R., Stahl, E., Bolouri, M. S., Ray, H. N., Sihag, S., Kamal, M., Patterson, N., Lander, E. S., and Mann, M. (2003) Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. *Cell* **115**, 629–640.
112. Johnson, D. T., Harris, R. A., French, S., Blair, P. V., You, J., Bemis, K. G., Wang, M., and Balaban, R. S. (2007) Tissue heterogeneity of the mammalian mitochondrial proteome. *Am. J. Physiol. Cell Physiol.* **292**, C689–C697.
113. Benard, G., Faustin, B., Passerieux, E., Galinier, A., Rocher, C., Bellance, N., Delage, J. P., Casteilla, L., Letellier, T., and Rossignol, R. (2006) Physiological diversity of mitochondrial oxidative phosphorylation. *Am. J. Physiol. Cell Physiol.* **291**, C1172–C1182.
114. Evans-Storms, R. B., and Cidlowski, J. A. (1995) Regulation of apoptosis by steroid hormones. *J. Steroid Biochem. Mol. Biol.* **53**, 1–8.
115. Herr, I., Gassler, N., Friess, H., and Buchler, M. W. (2007) Regulation of differential pro- and anti-apoptotic signaling by glucocorticoids. *Apoptosis* **12**, 271–291.
116. Tuckermann, J. P., Kleiman, A., McPherson, K. G., and Reichardt, H. M. (2005) Molecular mechanisms of glucocorticoids in the control of inflammation and lymphocyte apoptosis. *Crit. Rev. Clin. Lab. Sci.* **42**, 71–104.
117. Sionov, R. V., Kfir, S., Zafrir, E. Cohen, O., Zilberman, Y., and Yefenof, E. (2006) Glucocorticoid-induced apoptosis revisited: a novel role for glucocorticoid receptor translocation to the mitochondria. *Cell Cycle* **5**, 1017–1026.
118. Stirone, C., Duckles, S. P., Krause, D. N., and Procaccio, V. (2005) Estrogen increases mitochondrial efficiency and reduces oxidative stress in cerebral blood vessels. *Mol. Pharmacol.* **68**, 959–965.
119. Hsieh, Y. C., Choudhry, M. A., Yu, H. P., Shimizu, T., Yang, S., Suzuki, T., Chen, J., Bland, K. I., and Chaudry, I. H. (2006) Inhibition of cardiac PGC-1alpha expression abolishes ERbeta agonist-mediated cardioprotection following trauma-hemorrhage. *FASEB J.* **20**, 1109–1117.
120. Lu, A., Frink, M., Choudhry, M. A., Hubbard, W. J., Rue, L. W., III, Bland, K. I., and Chaudry, I. H. (2007) Mitochondria play an important role in 17beta-estradiol attenuation of H(2)O(2)-induced rat endothelial cell apoptosis. *Am. J. Physiol. Endocrinol. Metab.* **292**, E585–E593.
121. O'Lone, R., Knorr, K., Jaffe, I. Z., Schaffer, M. E., Martini, P. G., Karas, R. H., Bienkowska, J., Mendelsohn, M. E., and Hansen, U. (2007) Estrogen receptors alpha and beta mediate distinct pathways of vascular gene expression, including genes involved in mitochondrial electron transport and generation of reactive oxygen species. *Mol. Endocrinol.* **21**, 1281–1296.
122. Razmara, A., Duckles, S. P., Krause, D. N., and Procaccio, V. (2007) Estrogen suppresses brain mitochondrial oxidative stress in female and male rats. *Brain Res.* **1176**, 71–81.
123. Psarra, A.-M. G., Solakidi, S., and Sekeris, C. E. (2006) The mitochondrion as a primary site of action of regulatory agents involved in neuroimmunomodulation. *Ann. N. Y. Acad. Sci.* **1088**, 12–22.
124. Psarra, A.-M. G. and Sekeris, C. E. (2008) Nuclear receptors and other nuclear transcription factors in mitochondria: regulatory molecules in a new environment. *Biochim. Biophys. Acta* **1783**, 1–11.