MINIREVIEW

Mitochondrial Gene Expression: Influence of Nutrients and Hormones

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Mitochondrial gene transcription research has exploded over the last decade. Nuclear-encoded proteins, nutrients, and hormones all work to regulate the transcription of this genome. To date, very few of the transcription factors have been shown to have negative effects on mitochondrial gene expression, although there are likely conditions where such downregulation may occur. Exp Biol Med 231:1593–1601, 2006

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Introduction

The existence of mitochondria in cells has been known for many years. These organelles are the main integrators of intermediary metabolism, as it is here that the high energy compound ATP is synthesized in large quantities. Its synthesis is coupled with the synthesis of water, and the whole process is called oxidative phosphorylation or OXPHOS. Metabolic regulation occurs through the management of the ratios of reduced to oxidized substrates and coenzymes and through the ratios of ATP to ADP, AMP, and Pi (1, 2). Metabolic regulation in response to changing hormonal conditions has also been observed with respect to mitochondrial function (2). For example, growth (and the hormones that regulate the growth process) affects mitochondrial function by increasing the efficiency of OXPHOS. In addition, it is now apparent that regulation can also occur at the level of the mitochondrial genome found in the matrix of the mitochondrion. The mitochondrial genome encodes thirteen gene products that are important to the appropriate functioning of OXPHOS as well as products essential to its own transcription and translation. It is the purpose of this review to summarize the transcription of this genome as it pertains to the responses of cells to nutritional and hormonal influences.

The Mitochondrial Genome

In contrast to the nuclear genome, the mitochondrial genome is circular (3–6). It consists of a light strand, a heavy strand, and a small fragment called the displacement loop or D-loop (Figure 1). Depending on how many mitochondria are present, hundreds to many thousand copies of the genome can be found in the cell, although cell types vary tremendously. Ova contain many more mitochondria and mitochondrial DNA copies than do liver cells or bone cells. The genome encodes 13 polypeptides that are constituents of the OXPHOS system. The remaining 63 polypeptides of OXPHOS are encoded by the nuclear genome and are imported into the mitochondrial compartment.

The map of the mitochondrial genome is fairly similar among mammals, although the size of the genome can vary. Many species have had their mitochondrial genome sequenced, and small differences in base sequence occur. In rats the genome is 16,298 bases, while in the human it is 16,569 bases. The human sequence was first published in 1981 (4) and corrected in 1999 (5). Its sequence can be found on the web at http://www.ncbi.nlm.nih.gov, file NC_001807 or http://www.mitomap.org. The base sequence of this genome can vary from individual to individual and even within the same individual, usually with no effect on the gene products or mitochondrial function (6, 7). This is called heteroplasmy, or the existence of different base sequences of the genome within the same cell; in some instances, an abnormal sequence, that is, a sequence which produces an abnormal gene product, can occur. When the

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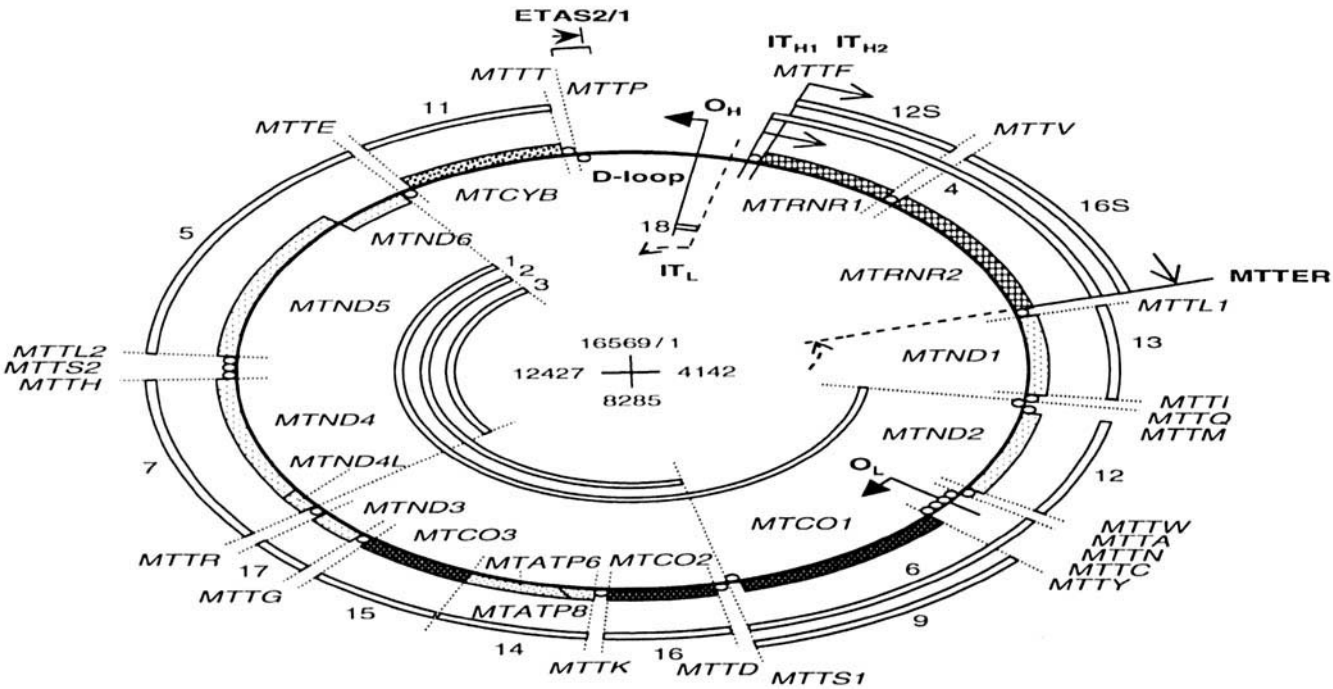

Figure 1. Map of the human mitochondrial genome showing D-loop, transcription start sites and the structural genes. The shaded boxes on the outside of the ring indicate the genes encoded by the heavy strand while the shaded boxes on the inner side of the ring represent the gene encoded by the light strand. It_L stands for the initiation of the light strand; O_L, origin of the light strand; O_H, origin of the heavy strand; transcripts are numbered according to size (1 to 18), except for the 12 S and 16S rRNAs; transcript 18 represents the RNA at the R-loop. The site where transcription is terminated by MTERF is indicated. Other abbreviations: MTCYB, mitochondrial cytochrome b; MTND6, NADH dehydrogenase (complex 1); MTND5, NADH ND1–6 = Complex I, NADH dehydrogenase genes; Cyt b = cytochrome b, Complex III, Ubiquinol: Cytochrome c oxidoreductase gene; COI-III = Complex IV, Cytochrome c oxidase genes; ATPase 6 & 8 = Complex V, ATP synthase genes; rRNA = ribosomal RNA; tRNA genes are listed by their single letter amino acid name. The displacement loop (D-loop) region is indicated. P_H and P_L = promoters for the heavy and light strands respectively. O_H and O_L = origins of replication for the heavy and light strands respectively. The figure is from Taanman (6).

mixture is such that the abnormal sequence predominates (exceeds 85%–90% of the mixture), abnormal function will be observed, especially in those tissues highly dependent on a ready supply of ATP, that is, neuronal tissue, renal tissue, and pancreatic tissue. A number of diseases associated with mutated mitochondrial DNA have been reported (8).

In addition to interspecies similarity, the mitochondrial genome is highly conserved. There are very few noncoding bases, and several of the genes overlap. That is, the start site of one gene occurs before the end site of the gene ahead of it in the base sequence. In some instances, the bases are not really shared. Half of the genes that share bases use bases in both strands such that the shared bases are at the 3′ ends of both genes. The other genes that share bases have a somewhat different arrangement. The shared bases are actually shifts in the reading frames. This allows the same bases to code for two different proteins. This is the sharing that occurs with the ATPase 6 and 8 genes. The ATPase 6 and ATPase 8 genes overlap, and the two genes share 52 bases. The initiator codon of the ATPase 8 gene occurs 52 bases before the 3′ termination sequence of the ATPase 6 gene.

In those genes that do not share bases, the initiator codon follows immediately after the termination sequence of its upstream neighbor. In a few instances there can be a couple of noncoding bases between genes, but most of the structural genes have none between them. The initiator codon of each reading frame can be either AUG, AUA, AUU, or AUC. These codons follow immediately or only a few bases after the termination sequence of the upstream gene.

The light and heavy strands of the DNA can be separated using a cesium chloride gradient (3). Structural genes are located on both strands; however, the heavy strand is the main coding strand. The light strand encodes eight tRNAs and one of the NADH dehydrogenases, while the heavy strand encodes 2 rRNAs, 14 tRNAs, and 12 structural genes. Most of the reading frames lack termination codons and code a T or a TA only after the last sense codon. Completion of the termination codon occurs at the time of RNA processing by polyadenylation. Transfer removal of the primary transcripts causes the production of both tRNA species themselves and the mature mRNAs.

Mitochondrial Gene Transcription

Common among species is the fact that there is a single promoter for all of the 13 structural genes. This is the displacement loop or D-loop (6, 7, 9–13). This single promoter is a three-stranded structure in which a short

nucleic acid strand, complementary to the light strand, displaces the heavy strand (14, 15). It is called 7S DNA, and in human DNA is about 650 bases long (16). The D-loop is sandwiched in between the genes for $tRNA^{Phe}$, which is downstream, and $tRNA^{Pro}$, which is upstream (16). It contains 97% of all of the noncoding bases in the mitochondrial genome. It contains the origin of replication of the heavy strand, the origins of both the heavy and light strand transcription, a number of transcription factor binding sites, and a termination factor binding sequence (12). The light strand promoter region serves as the major promoter for light strand transcription and is also the site of priming for leading strand mtDNA replication. Studies of the pattern of transcription of the rRNA genes indicate that there are two overlapping transcription units (12, 13). The two major transcription initiation sites for the H-strand and the L-strand are situated within 150 base pairs of one another in the D-loop (11, 13, 16–21). A promoter element with a pentadecamer consensus sequence motif of 5′-CAN-ACC(G)CC(A)AAAGAYA (N=A, C, G, or T; Y=C or T) encompasses the transcription start site (12, 17).

Initiation of Transcription

Both strands of the circular molecule are transcribed into polycistronic transcripts (6, 7). A nuclear-encoded RNA polymerase is required (11) for the transcripts, which are then processed, giving rise to 2 rRNAs, 13 mRNAs, and 22 tRNAs. Transcription is symmetrical and initiated within the D-loop. The heavy strand transcription begins at base position 561 located within the H-strand promoter, and the light strand transcription begins at position 407. Enhancer elements are located upstream and are required for optimal transcription. Two of these elements are binding sites for the transcription factor, TFAM. The binding sites are quite similar in sequence (12, 17, 20), yet they are functionally independent (12, 16, 17, 21, 22). In the heavy strand there is a second initiation start site located around position 638 in the MTTF gene, immediately adjacent to the MTRNR2 gene (12, 17, 18, 23). Its promoter region has limited similarity with the 15 base consensus sequence and is thought to be used less frequently than the first site for the transcription of the heavy strand. Initiation of transcription from each of the two human mitochondrial promoters requires unique nucleotides at the transcriptional start sites (12).

As mentioned above, the D-loop is regarded as the central promoter site for all 13 structural genes. In transcription, the light strand moves clockwise, while the heavy strand moves counterclockwise. The heavy strand is transcribed as two polycistrons. There are three promoters of transcription and two widely spaced origins of replication, one for each strand. Light strand transcription occurs faster than heavy strand transcription. It is 2–3 times greater, but the half-lives of the transcripts are significantly less than the half-lives of the heavy strand transcripts. Each strand has its own independent promoter. There is a third promoter coupled with a downstream transcription termination factor located downstream from the 16S rRNA. In addition to TFAM (24–30) and the above putative response elements and probable transcription agents, there are other binding proteins that affect mitochondrial gene transcription initiation: a relatively nonselective core RNA polymerase, mitochondrial polymerase (POLRMT), and a dissociable transcription factor (22, 31–33). The TFAM confers promoter selectivity on the polymerase, while the POLRMT binds to the promoter region. TFAM is an abundant 25-kDa nuclear-encoded mitochondrial protein. It has a bipartite structure consisting of a small initiation site (17, 31, 32) and a recognition domain located approximately −10 to −40 bp upstream from the RNA start site (22).

Another protein that regulates transcription is the mitochondrial termination factor (MTERF), which serves to terminate transcription (16, 23–37). This protein contains three leucine zipper motifs bracketed by two basic domains that are all critical for its specific DNA binding capacity. This binding protein can exist in two forms. One is a monomeric form exhibiting DNA binding and transcription termination activity, while the other is probably a homotrimeric form lacking these features. It has been proposed that the activity of the MTERF is modulated by the transition between the two forms. Studies of rat hepatocyte MTERF showed that the protein is phosphorylated; however, this phosphorylation may have nothing to do with the regulation of its activity. More studies are needed to clearly define the mechanism of action of this factor.

TFAM is a key activator of mitochondrial transcription in mammals. *In vivo* studies have shown that it binds to two promoter regions as well as to a region in between two of the conserved sequence blocks. The TFAM condenses, unwinds, and bends mitochondrial DNA and wraps around the D-loop region (24). *In vitro*, more TFAM is needed to promote transcription of the heavy strand than for the transcription of the light strand. The amount of TFAM correlates well with the amount of mitochondrial DNA and DNA copy number (34–37). If the TFAM gene is deleted, as in knockout mice, only the heterozygous mice can be studied. The mutation is lethal. Heterozygous mice have not only a reduced copy number but also less mitochondrial DNA than normal mice. Two additional human mitochondrial transcription factors (TFB1M and TFB2M) have been found recently (38–40). These two factors are quite similar with respect to amino acid sequence. Each factor can support heavy strand or light strand transcription when studied *in vitro* if the system also contains POLRMT and TFAM. TFB2M seems to be more active in this system than TFB1M (40).

Regulation of mitochondrial gene expression involves other nuclear gene products as well. The nuclear respiratory factors (NRF) 1 and 2 and a general transcription factor (Sp1) coordinate transcription by simultaneously regulating

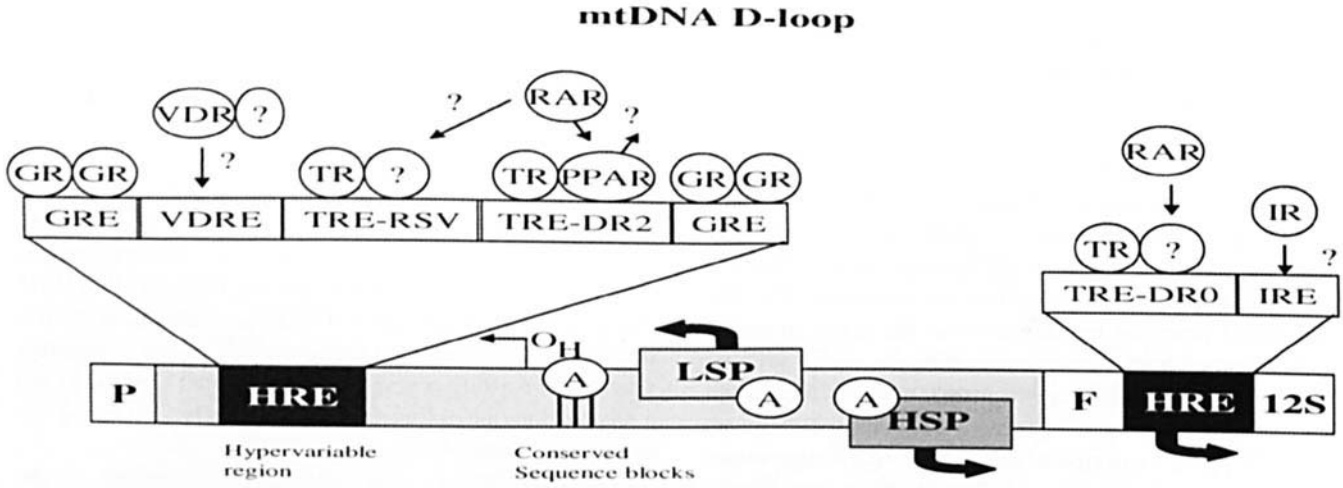

Figure 2. The region of the D-Loop that contains putative hormone response elements is shown as an expansion of the area upstream of the conserved sequence blocks. There are unknown elements as well as those thought to exist.

TFAM and the transcription of some of the nuclear-encoded OXPHOS genes (35, 39–49). A list of proteins involved in transcription and translation can be found at http://www.lab314.com/mitochondria/proteinas-dna.htm.

It has been suggested that the same transcription factor (or a small set of them) could act on the regulation of the different nuclear genes involved in mitochondrial biogenesis. In regenerating rat liver, increases in mitochondrial DNA binding proteins preceded increases in mitochondrial mRNAs (50). The expression of both nuclear and mitochondrial genes during biogenesis is developmentally regulated by post-transcriptional mechanisms (51). This may involve translational control of both genomes (51). However, *in vitro* studies have shown that mitochondrial DNA replication is independent of cell cycle, and, although mitochondrial gene expression is largely dependent on nuclear-encoded factors, some autonomy has remained. Mitochondrial replication persists for several hours in enucleated cells and it has been demonstrated that mitochondrial transcription and translation can be maintained in isolated mitochondria in the absence of the rest of the cell components (52–56). Mitochondrial function is, however, very dependent on the nucleus and the cytoplasmic translation apparatus. Nuclear genes encode most of the structural subunits of the OXPHOS complexes and mitochondrial DNA transcription factors. Functional NRF-1 sites have been found in genes encoding cytochrome C and at least one subunit each of respiratory complexes III, IV, and V (42, 48). NRF-1 and NRF-2 binding sites are also present at the regulatory region of the TFAM gene. These observations support the possibility of the subordination of mitochondrial DNA expression to a nuclear signal integration system. The activity of the proximal TFAM promoter is highly dependent on NRF-1 in both transfected cells and *in vitro* transcription assays (47). Similarly, the expression of the gene encoding 5′aminolevulinate synthase, the rate limiting enzyme in the biosynthesis of heme for the respiratory cytochromes, requires two NRF-1 recognition sites within its promoter region (49). These findings suggest an integrative role for NRF-1 in controlling nuclear-mitochondrial interactions in mammals. However, such coordination has not been found under all circumstances.

Some "local" transcription effects can also occur (57). Demonacos *et al.* (58), for example, have reported the existence of four steroid response elements in the CO I and CO III genes. A number of investigators have reported that the glucocorticoid treatment of rats increases hepatic OXPHOS (2). These observations, together with reports of increases in mitochondrial transcripts and the existence of glucorticoid response elements, indicate a role for this hormone in mitochondrial transcription regulation. Similarly, thyroid response elements have been identified in areas other than the D-loop. Vibasius and Scarpulla reported that there was NRF2 binding to a GGAA-rich region of the cytochrome c gene that served to activate the transcription of this particular gene *in vitro* (44).

Hormones and Nutrients that Affect Transcription

Computational analysis of the D-loop has suggested the presence of a number of response elements for proteins that bind vitamins and hormones. Shown in Figure 2 is a suggested arrangement of these binding sites. Included are response elements for the proteins that bind insulin, glucocorticoid, retinoic acid, thyroid hormone, and vitamin D, as well as some additional response elements as yet unknown. Likely there are other protein binding sites, as indicated by blocks and circles with question marks. There are a number of possibilities for these unknown binding proteins. Table 1 lists nutrients and hormones thought to have a role in mitochondrial transcription.

Among the nutrients that can affect mitochondrial transcription is retinoic acid. Ruff and Ong (59) reported on the presence of retinoic acid receptors in the mitochondrial compartment. Everts *et al.* (60) found retinoic acid receptors

Table 1. Factors That Affect Mitochondrial Transcription

Factor[a]	Effect[b]
MTFAM	↑
MTO1	↑
POLRMT	↑
MTERF	↑↓
TFB1M	↑
TFB2M	↑
TRNT1	↑
Retinoic acid	↑
Vitamin D	↑↓ Tissue dependent
Zinc	↑
Thyroid hormones	↑
Glucocorticoids	↑
Estrogen	↑
ACTH	↑
Insulin	↑

[a] MTFAM, mitochondrial transcription factor A; MTO1, mitochondrial translation homolog; POLRMT, mitochondrial RNA polymerase; MTERF, mitochondrial termination factor; TFB1M, mitochondrial factor B1; TFB2M, mitochondrial transcription factor B2; TRNT1, mitochondrial ATP(CTP):tRNA nucleotidyl-transferase.
[b] ↑increases transcription when bound to the DNA; ↑↓, can both upregulate or downregulate.

in the mitochondrial matrix and, because they also found increased mitochondrial gene transcripts in extracts of primary hepatocyte cultures cultured with graded (10^{-13}–10^{-5} *M*) amounts of retinoic acid and increased amounts of gene products in hepatic tissue extracts from vitamin A treated rats, they concluded that retinoic acid stimulates mitochondrial gene expression (60–63). Optimal expression occurred in primary hepatocytes cultured with 10^{-9} *M* retinoic acid. These findings correlated with an upregulation in OXPHOS by hepatic mitochondrial preparations (61). Retinoic acid has been shown to upregulate NADH dehydrogenase subunit 5 mRNA, as well as cytochrome c oxidase subunit I and 16 S rRNA (60). Others have also shown a role for retinoic acid in mitochondrial transcription (64). In addition, retinoid X receptor α knockout mice were shown to have alterations in mitochondrial gene expression (65). Everts and Berdanier reported that dietary vitamin A upregulates ATPase 6 gene expression and optimizes OXPHOS in diabetes prone BHE/Cdb rats (61). This particular strain of rats has two base substitutions in the mitochondrial ATPase 6 gene that phenotypes as a reduced ATP synthesizing efficiency, shortened lifespan and with age-impaired glucose tolerance as well as an impaired pancreatic insulin release in response to a glucose challenge (63). When vitamin A was restored to vitamin A depleted BHE/Cdb rats, there was an increase in mitochondrial number and an increase in TFAM. In feeding studies, a three-fold increase in vitamin A intake (12 IU/g diet) above that recommended (4 IU/g diet) for normal rats resulted in increased expression of mitochondrial ATPase 6 gene as well as an improvement in OXPHOS. This amount of vitamin A is the amount recommended by the National Research Council for pregnant rats, not for the maintenance of nonpregnant, nongrowing rats. Thus the dose used was within the normal range of intake for rats.

Retinoic acid probably affects mitochondrial transcription both directly and indirectly. Indirectly, it has an affect on the nuclear gene for TFAM, which in turn affects mitochondrial transcription. An alteration of mitochondrial TFAM gene expression would increase the steady-state levels of the protein in the cell. This would affect the total amount of transcriptional factor that is available for regulating the expression of the mitochondrial target genes. Because mitogenesis is linked to TFAM, it would also result in an increase in mitochondrial number. Studies with the BHE/Cdb rats have shown that this occurred. Not only was the level of TFAM elevated, so too was mitochondrial number. In addition to an effect on TFAM, retinoic acid had a direct effect on transcription. There were more transcripts, and the increased number of transcripts and gene products likely can explain the improved OXPHOS efficiency of mitochondria isolated from vitamin A supplemented rats. The conclusions drawn from these studies were that these rats needed more dietary vitamin A to maintain normal OXPHOS function, because without supplemental amounts the performance of the mitochondria was inadequate to sustain normal metabolism. Because their mitochondrial mutation affected OXPHOS modestly, this could be overcome with an increase in the number of mitochondria as well as an increase in the amount of mitochondrial gene products.

Is there an application of these findings to humans with mitochondrial mutation defects? If one could use a strategy that would increase the number of mitochondria as well as the number of DNA copies that would be translated into useful gene products, then the answer to this question is yes. Given that mitochondrial diseases include such problems as diabetes, Parkinson's disease, Alzheimer's disease, and other less common problems, one could hope that such strategies could be developed. Most mitochondrial diseases develop in humans who are heteroplasmic with respect to a mutation (8); that is, they have both normal and mutated mitochondrial DNA. If mitogenesis and DNA replication are stimulated, the chances are that both forms of the DNA will be replicated, and, as occurred in the rats, there is the possibility that some of the phenotype could be ameliorated. It should be noted, however, that the range of supplementation should be very carefully monitored. Too much vitamin A can be toxic, as could too much of any nutrient that is absorbed and stored in the body.

Other nutrients also may affect mitochondrial gene expression; however, some of these have yet to be identified. Vitamin D, through the actions of its metabolite 1α25-dihydroxyvitamin D, has been known to affect the transcription of a wide range of nuclear genes. It is bound by a vitamin D receptor that in turn binds to the promoter region of the gene in question. Not all cell types have this

receptor protein. Kidney and intestinal cells do, and Chou *et al.* (66) have reported that this nutrient affects both nuclear and mitochondrial gene expression in these cell types. Using subtractive hybridization analysis of complimentary DNA libraries prepared from messenger RNA, they found that in kidney, seven mitochondrial encoded transcripts were downregulated by vitamin D, while in intestinal cells six transcripts were upregulated. They concluded that vitamin D effects are tissue-specific with respect to its effect on mitochondrial transcription (66). Studies of hepatic tissue from vitamin D deficient and sufficient rats showed no effect of the vitamin on mitochondrial OXPHOS or mitochondrial gene expression (unpublished observations). This was not unexpected, as vitamin D receptors would not be expected to be present in hepatic tissue, even though the mitochondrial promoter region has vitamin D elements in its sequence.

Mitochondrial DNA is affected by vitamin B_{12}. Both DNA and RNA in mitochondria were reduced in lymphocytes from vitamin deficient humans (67). These observations however did not show a direct effect of this vitamin on transcription; rather, the data suggested that the lack of the vitamin affected overall nucleic acid synthesis rather than having a direct effect on the process of transcription or translation. During transcription and translation the nucleic acids are recycled, but if there is a low amount initially the process would not be affected except as a reflection of the low amount of newly synthesized nucleic acids. B_{12} is known to play an important role in nucleic acid synthesis.

Another nutrient known to affect gene expression is zinc (68). Primary hepatocyte cultures cultured with graded amounts of zinc had graded increases in mitochondrial transcripts and mitochondrial gene products (unpublished observations). Most likely this was due to the role zinc plays in the binding of receptor proteins to DNA. Those members of the steroid superfamily of receptors, for example, have zinc fingers that play an important part in receptor binding. Cells deficient in zinc would make fewer of these proteins, and as discussed above (and below), receptor proteins are an integral part of the effects of vitamins and hormones on gene expression in both the nucleus and the mitochondria. Again, as with vitamin A, these studies were *in vitro* studies, not *in vivo*. *In vivo* studies have yet to be conducted.

Hormonal effects on mitochondrial transcription have been reported (62, 69–79). For example, Casas *et al.* (70) reported the existence of a 45-kDa protein related to a peroxisome proliferator γ2 in the mitochondrial matrix. They found that this protein was bound to the D-loop complexed to the thyroid binding protein. Previously this group reported that a variant form of the nuclear triiodothyronine receptor was present in the mitochondrial matrix and that this receptor played an important role in mitochondrial RNA synthesis (71). It is well documented that the thyroid hormones stimulate mitochondrial activity as well as mitochondrial gene expression. They increase the number of mitochondria and mitochondrial protein synthesis (72, 73), increase mitochondrial RNA synthesis (69), increase the amount of gene product (62, 69, 72, 73), and increase OXPHOS efficiency when used within the normal range of thyroid hormone treatments (74). Thyroid hormones thus have both direct and indirect effects on mitochondrial respiratory activity (75).

As mentioned, the glucocorticoids have effects on mitochondrial gene expression (58, 76–79). This steroid can increase transcription and translation in the mitochondrial compartment. Mitochondrial biogenesis and mitochondrial DNA are increased with glucocorticoid treatment (76–79). Using primary cell cultures, Hermoyian showed that glucocorticoid as well as another steroid, dehydroepiandrosterone, increased mitochondrial gene expression (62). When the two hormones were used together, there were additive effects on mitochondrial gene expression.

ACTH stimulates the release of glucocorticoids by the adrenal cortex. However, it has been found to have another role as well: it stimulates the transcription of mitochondrial genes. Treatment of adrenocortical cells with ACTH resulted in increases in mitochondrial mRNA for the OXPHOS structural genes as well as increasing the activities of these enzymes (79). Another steroid, estrogen, has a similar effect. It should be noted that all of these hormones as well as vitamin A (as retinoic acid) bind to DNA via the group of receptors known as the hormone receptor superfamily of ligand-activated transcription factors. Although originally noted for their activity with respect to nuclear DNA, it is now apparent that they are active with respect to the mitochondrial genome as well.

Another hormone that appears to affect mitochondrial gene expression is insulin (80, 81). Mitochondrial gene expression has been shown to be increased during L6 cell myogenesis when these cells were cultured with insulin. In humans with diabetes, mitochondrial gene expression is compromised. In fact, some investigators believe that this may be a vicious cycle. Diabetes may cause some mutational events in the mitochondrial genome, and diabetes may develop because of some inherent mutation in this genome (82–84). A number of mutations have been reported that associate with the diabetic condition (83). Pancreatic β cell insulin release is dependent on mitochondrial function (82), as shown by the report of a study using a pancreatic β cell line. In this line mitochondrial transcription was inhibited by ethidium bromide, and glucose-stimulated insulin release was suppressed (85). Insulin has long been known to stimulate nuclear-encoded gene transcription. Now it is obvious that it has the same effect for mitochondrial transcription.

From the foregoing it is apparent that mitochondrial gene expression is similar to nuclear expression in several respects. Transcription is responsive to some of the same nutrients and hormones that affect nuclear transcription. Both genomes require binding proteins, but it seems that each compartment has its preferred binding form. Doubtless, as we learn more about the control of mitochondrial gene

expression, we will find that it is more complex than originally supposed. Future research will add to our knowledge about the mitochondrial DNA and its transcription.

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