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Role of Transcription Factors in mtDNA Biogenesis Mediated by Thyroid Hormones

M. V. Patrushev* and V. E. Patrusheva

I. Kant Russian State University, ul. A. Nevskogo 14, 236041 Kaliningrad, Russia; E-mail: maxpatrushev@gmail.com

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Abstract—Exogenous thyroid hormones are regulators of cellular metabolism that involves, along with other cell structures, mitochondria. Mechanisms of the influence of thyroid hormones on the biogenesis of mtDNA are not fully understood due to their pleiotropic nature. Different ways of regulation of mitochondrial biogenesis by thyroid hormones are discussed in literature, but thyroid receptors, localized in both the nucleus and mitochondria, are the main elements of most pathways. Data on events occurring after receptor activation are rather contradictory. We investigated the degree of involvement of mitochondrial transcription factors in the biogenesis of mtDNA induced by triiodothyronine. The contribution of TFAM, TFB2M, and helicase Twinkle in thyroid-induced mtDNA biogenesis was assessed. The activation of TFAM and TFB2M expression is shown to be required for the induction of mtDNA biogenesis. The role of helicase Twinkle, the expression induction of which is also observed after triiodothyronine addition, remains unclear. The analysis of factors that activate TFAM and TFB2M expression showed that NRF-1 is the determinative regulator: deficiency of this factor leads to complete collapse of mtDNA biogenesis. However, lack of transcriptional coactivator PGC-1α did not lead to significant reduction in thyroid-induced biogenesis, whereas literature data point to its key role in the biogenesis of mitochondria. Thus, in this study the role of key transcription factors in mtDNA biogenesis induced by triiodothyronine was demonstrated for the first time in a model system.

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Key words: biogenesis, mtDNA, thyroid hormones, transcription factors, triiodothyronine

Mitochondria of humans and other mammals have their own DNA (mtDNA) that encodes 13 polypeptides composing the multienzyme electron transport chain complex, 22 transfer RNAs, and two ribosomal RNAs. The biogenesis of mtDNA is independent of the cell cycle since all factors that ensure the transcription and replication of mtDNA are encoded in the nucleus. Regulation of mtDNA functioning is largely dependent on exogenous factors, the most important of which are hormones. The role of thyroid hormones requires special attention because they play a key role in energy metabolism. It was shown previously that hypothyroidism statistically reduces the level of mtRNA, particularly mRNA, thus altering the ratio of mRNA to rRNA [1]. This effect is achieved with low concentrations of hormones and is saturable [2]. A number of works have described a mitochondria-localized receptor that is capable of affecting the biogenesis of mtDNA after interacting with hormones [3, 4]. Mitochondrial T3 receptor, which has thyroid hormones as ligands, plays an important role in proton transport and is involved in the regulation of heat production by mitochondria, thus affecting the stability and functioning of mtDNA [5]. However, the main targets of thyroid hormones are transcription factors of nuclear DNA, some of which may directly or indirectly regulate the transcription and replication of mtDNA. Among these factors the nuclear T3 receptor that was proven to have a regulatory effect on a large number of transcriptional factors particularly stands out. Transcription and, consequently, mtDNA replication depends primarily on a few key factors: TFAM, TFB2M, POLG, Twinkle, mtRNApol. Among these factors, TFAM was shown to play a key role in the regulation of mtDNA transcription and its coordination with the expression of nuclear genes [6]. However, some observations confirm the central role of this protein in regulation of mtRNA level. TFAM is present in large quantities [6], and reducing its level in heterozygous knockout mice leads to a sharp drop in the level of mtDNA but not mtRNA in all tissues [7]. It is

Abbreviations: mtDNA, mitochondrial DNA; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1 alpha; siRNA, small interfering RNA; T3, triiodothyronine.
* To whom correspondence should be addressed.

obvious that regulation of mtDNA biogenesis by thyroid hormones can be done both via the mitochondrial T3 receptor and through the activation of nuclear transcription factors. In turn, the regulation of TFAM, TFB2M, and Twinkle expression can be implemented either directly by thyroid receptors after their interaction with ligands or through the activation of NRF-1 – a universal positive regulator of expression of genes involved in the functioning of mitochondria. In the literature, PGC-1α is considered to be a coactivator of transcription; it acts as a mediator between thyroid receptors and NRF-1 [8]. The aim of this work was to determine the cascade of protein factors involved in mtDNA biogenesis induced by triiodothyronine (T3), the most important of the thyroid hormones.

MATERIALS AND METHODS

Mouse fibroblast cell line was cultured in DMEM medium containing 6% serum in an atmosphere with 5% CO_2.

DNA isolation. Lysis solution (300 µl of 4 M guanidine-thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sarcosyl) were added to precipitated cells, the solution was stirred; after this we added 15 µl of 2 M Tris-HCl, pH 8.0, 30 µl of 4 M NaCl, 600 µl of phenol saturated with Tris-HCl, pH 8.0, 120 µl of chloroform; the solution was homogenized, kept in a refrigerator for 20 min, then precipitated for 3 min at 6000 rpm in an Eppendorf 5415 centrifuge. The upper phase was taken, two volumes of 96% ethanol were added, and the solution was kept at –20°C overnight. The precipitate obtained by centrifugation for 7 min at 13,000 rpm was washed twice with 70% alcohol, dried in air, and dissolved in 50 µl of TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

RNA isolation. We added to precipitated cells 10 volumes of lysis buffer containing 0.1 M β-mercaptoethanol. Then 1/10 volume of 3 M AcONa, pH 5.0, and one part of water-saturated (acidic) phenol were added to the lysate. The solution was stirred and incubated for 5-10 min at room temperature. One-fifth volume of chloroform–isoamyl alcohol mixture with 24 : 1 ratio was added. The solution was stirred and incubated for 15 min at 4°C. The solution was centrifuged for 5 min at 10,000*g*. The supernatant was transferred to a new tube, and one volume of chloroform–isoamyl alcohol was added. The solution was stirred and centrifuged for 5 min at 10,000*g*. The supernatant was transferred to a new tube, and 0.8 volume of isopropanol was added. The supernatant was incubated for 12 h at –20°C and then centrifuged for 10 min at 13,000*g* at 4°C. The RNA precipitate was washed three times with 70% ethanol. After drying, the RNA was dissolved in water treated with diethylpyrocarbonate.

The first strand of cDNA was synthesized according to the following protocol: 2 µl of mRNA and 1 µl of oligo-d(T)18 primer were mixed. After incubation for 5 min at 70°C, the mixture was placed on ice and then added to buffer containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.2 mM dNTPs, 1.6 mM Mg^{2+} and stirred. One microliter of RNasin (Fermentas, Lithuania) and 1 µl (200 units) of H-Minus M-MuLV reverse transcriptase (Fermentas) were added to the mixture. The mixture was incubated for 30 min at 40°C. The reaction was stopped by heating at 95°C for 10 min.

Real-time PCR. We used real-time PCR (for estimation of DNA amount) and real-time reverse-transcription PCR (RT-PCR) (for estimation of mRNA amount) for DNA and cDNA amplification. Real-time PCR and real-time RT-PCR was performed using a CFX96 system for real-time PCR product detection (Bio-Rad, USA). The real-time PCR and real-time RT-PCR systems used TaqMan technology based on the 5′-exonuclease activity of Taq polymerase. The probes were labeled with a fluorophore at the 5′-end and a quencher at the 3′-end. All real-time PCR reactions were performed in buffer containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.25 mM dNTPs, 2.5 mM Mg^{2+}, 300 nM of each primer, 150 nM of each probe, and 1.5 units of Taq polymerase (see table).

We used a calibration curve for quantification with intersection points of ΔCt values with concentration of pGEM-T Easy vector containing the analyzed fragment and the same vector with the amplified fragments of reference genes:

$$\Delta Ct = Ct \text{ of analyzed fragment} - \text{mean } Ct \text{ of reference genes};$$

$$\text{mean } Ct = \frac{\Sigma Ct\ (\beta\text{-actin, } \beta\text{-globin, gapdh})}{3}.$$

Small interfering RNA synthesis and translation inhibition. Amplified DNA fragments corresponding to 5′-UTR segments of target mRNAs were used as template for the synthesis of small interfering RNA (siRNA). The promoter for T7 polymerase was linked to amplicons after amplification with the use of partially hybridized oligonucleotides and Taq polymerase. Then siRNA was synthesized using the TranscriptAid T7 High Yield Transcription Kit (Fermentas) in accordance with the manufacturer's manual. The DNA was cleaved with DNase I (Fermentas). Next, siRNA was precipitated with 96% ethanol in the presence of 200 mM LiCl and centrifuged at 16,000*g* for 10 min at 4°C. The precipitate was washed three times with 70% ethanol and dissolved in 50 µl of deionized water.

Transfection of siRNA was performed with TurboFect (Fermentas) reagent in accordance with the manufacturer's manual.

Statistical significance of results was estimated by Student's *t*-test for independent samples with four replicates for each sample.

Primers and probes for quantitative analysis of mRNA

Gene name	Sequence from 5′ to 3′	
nd2	F	TTGCCATTATCTACTTCACAA
	R	GTTGCTTATAGTTGAGTACGA
	Probe	F-TCAGCCTACTAGCAATTATCCC-Q
nd4	F	ATGGCCTCACATCATCA
	R	GGCTTGCAATCAGTCAT
	Probe	F-TCGGGCCATAATTATAGTACGG-Q
coII	F	CTGCCAATAGAACTTCC
	R	TGGCCATAGAATAATCCT
	Probe	F-CAGGCCGACTAAATCAAGCAA-Q
nd5	F	CCCCTAATCTCCATTAACG
	R	GTGGGATATTATATGAGATGACAA
	Probe	F-CCTGCAAAGATGCTTCCG-Q
coI	F	CCCCAGATATAGCATTCCCA
	R	GCACCTAAAATAGACGACA
	Probe	F-CCGGAAATCTAGCCCATGCAG-Q
TFAM	F	GCCCTTAGAGAACTCATGG
	R	AGGCAAGTTTTCTACCCA
	Probe	F-ATGCGTTCTTCTGTTCCTACCTT-Q
TFB2M	F	CTGCCGTAGATCAATGGTA
	R	ACGTGTGCAAAAGAATCCTG
	Probe	F-CCAGCAAGAATGACGCCAC-Q
Twinkle helicase	F	ACGGTATCTGCAGGTGTCCAA
	R	GGCCACTAGTCCATTGTCGTCC
	Probe	F-ACCCAAGAGCAAAGCCCGACTCA-Q
NRF-1	F	CGCCACAGGAGGTTAATTCAGA
	R	CGACCTGTGGAATACTTGAGC
	Probe	F-CTGCCGCCTCTCACCATCG-Q
Actin beta	F	CGGGACCTGACAGACTACCTC
	R	AGCACAGCTTCTCTTTGATGTCAC
	Probe	F-ACCGAGCGTGGCTACAGCTT-Q
Globin beta	F	TGCTGGTTGTCTACCCTT
	R	GGCCTTCACTTTGGCATT
	Probe	F-CCCAGCGGTACTTTGATAGCTT-Q
gapdh	F	CCGCATCTTCTTGTGCAG
	R	TGTGCCGTTGAATTTGCC
	Probe	F-ACGACCCCTTCATTGACCT-Q

RESULTS AND DISCUSSION

Thyroid hormones (TH) play an important role in cell energy metabolism. The most important players in energy metabolism are mitochondria, which carry out the synthesis of ATP coupled with oxidative phosphorylation, the effectiveness of which depends on the timely synthesis of polypeptides necessary for this process. It was stated above that the immediate consequence of low TH concentration is a reduction in mtDNA transcription [2]. Based on this fact, one may suggest that high concentrations of TH observed under hyperthyroidism are able to induce the replication of mtDNA. Figure 1 shows the results of quantitative determination of mtDNA content in the presence of 15 nM triiodothyronine (T3).

The finding suggests the ability of T3 to induce replication of mtDNA. The figure shows that the amount of mtDNA increased more than 10-fold within 24 h after addition of T3. It should be noted that we amplified five different regions of mtDNA and then averaged the data to determine the number of copies (in this experiment). Specifically, some areas of the mtDNA showed a statistically significant more than 12-fold increase in the number of copies (figure not shown). Due to the fact that replication of the H-chain requires short RNA primers, coming from L-chain promoter transcript processing, we assumed that mtDNA transcription induction precedes replication. The quantitative assessment of mitochondrial mRNA is shown in Fig. 2.

An increase in the number of copies of mitochondrial mRNA is observed in the presence of 15 nM T3. The first 24 h are characterized by exponential growth. Growth rates decrease significantly after 24 h, but there is a slight rise through the time of measurement (72 h). However, there are no significant changes in medium containing no T3 except for minor variations in the number of mitochondrial mRNAs apparently associated with a clonal distribution of fibroblasts in culture.

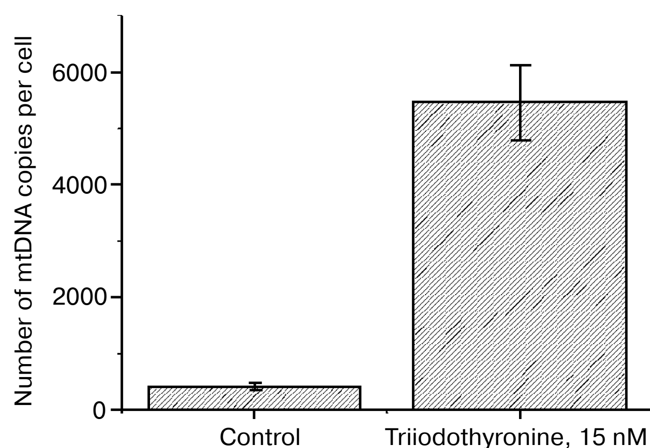

Fig. 1. Number of mtDNA copies in fibroblasts (per cell) before triiodothyronine treatment and 24 h after addition of 15 nM triiodothyronine.

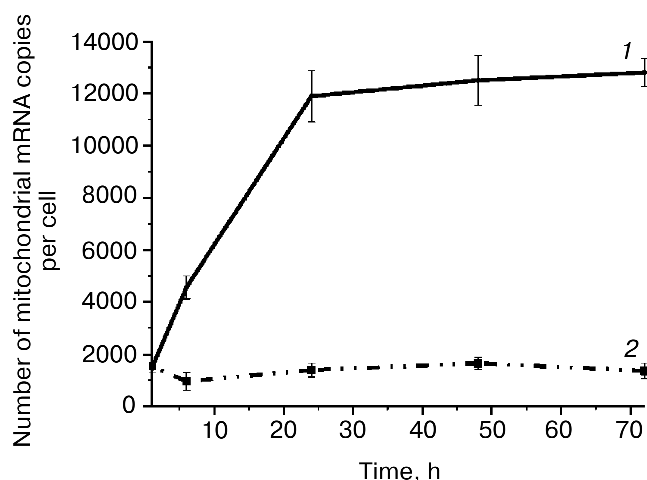

Fig. 2. Quantitative analysis of mitochondrial mRNA in the presence of 15 nM triiodothyronine. Average values of mRNA amount corresponding to five mitochondrial genes: *nd2*, *nd4*, *coII*, *nd5*, *coI*. Curves: *1*) in the presence of 15 nM triiodothyronine; *2*) control.

Thyroid hormone-mediated induction of mtDNA transcription can be implemented by the activation of several mechanisms: a) through interaction with the mitochondrial T3 receptor resulting in a change in the activity of oxidative phosphorylation; b) through interaction with nuclear T3 receptor signaling cascade that modulates the transcription of many genes, among which may be genes encoding subunits of multienzyme complexes of oxidative phosphorylation; c) induction which occurs as a result of metabolic imbalances caused by high concentrations of TH. Metabolic imbalance and activation of the mitochondrial T3 receptor, in our opinion, cannot lead to such dramatic and unidirectional changes in mtDNA biogenesis. Furthermore, mtDNA transcription inhibition in the presence of thyroid hormones was demonstrated in experiments with isolated yeast mitochondria [4]. The induction mediated by a nuclear T3 receptor-initiated signaling cascade is more likely in this case. The final targets of this cascade, in the aspect of the problem in point, may be the genes of mitochondrial DNA transcription factors. To test this hypothesis, we conducted studies that assessed the mRNA level of mtDNA basic transcription factors: TFAM, TFB2M, and mitochondrial helicase Twinkle (Fig. 3).

The figure shows that T3 induces the expression of all studied genes. Triiodothyronine increases the number of mRNA copies for all three investigated factors in the first hours after treatment. Maximal mRNA levels were reached in 24 h for Twinkle and TFAM, and in 48 h for TFB2M, after which no change was recorded. Significant differences were observed in the dynamics of mRNA lev-

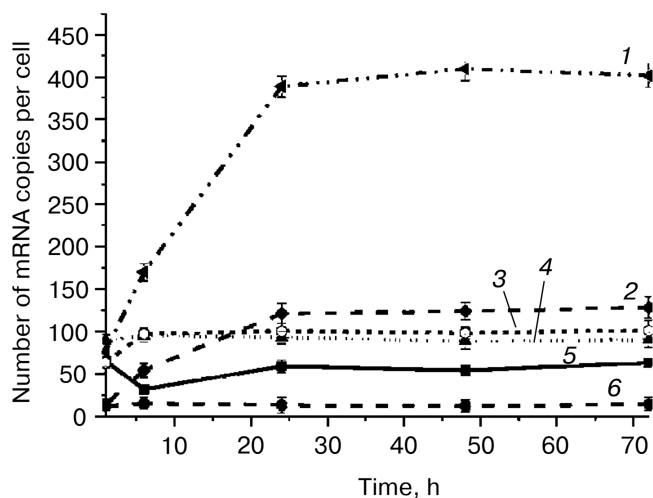

Fig. 3. Changes in expression of mtDNA transcription factors in the presence of 15 nM triiodothyronine (*1-3*) and without triiodothyronine (*4-6*); *1*, *4*) number of TB2M mRNA copies; *2*, *6*) number of TFAM mRNA copies; *3*, *5*) number of helicase Twinkle mRNA copies.

els. The least TH-susceptible gene is the one encoding Twinkle helicase (transcription was increased by not more than 30%). The number of TFAM mRNA copies increased about 5-fold and the number of TFB2M mRNA copies increased 4.5 times.

The analysis of the results showed that T3 affects mtDNA biogenesis via expression induction of mitochondrial transcription factors. It should be noted, however, that T3 receptor is also located in mitochondria and its activation may also lead to the induction of mtDNA biogenesis. To distinguish the parts of various pathways of mtDNA biogenesis activation, we investigated mtDNA transcription in the absence of each of the studied transcription factors. The translation of mtDNA transcription factors was suppressed with small interfering RNA (siRNA) complementary to 5′-untranslated regions of the target mRNAs. The degree of siRNA degradation in cells was assessed by hybridization with labeled complementary oligonucleotides after the transfer of the total RNA preparation onto nylon membrane.

Mitochondrial helicase Twinkle translation suppression did not lead to significant changes in the number of mitochondrial mRNA copies. Triiodothyronine activates the transcription of mtDNA in the same manner as in the system containing no siRNA (Fig. 4). The only difference is a slower increase in the number of mRNA copies. However, the absolute amount of mitochondrial mRNA per cell is roughly equal to that in cells without inhibitors of translation. A completely different picture can be seen in the case of TFAM translation suppression. The number of TFAM mRNA copies is reduced by half after the application of siRNA. The reduction of TFAM mRNA pool may lead to lower levels of the factor itself, which in turn affects the process of mtDNA transcription. As seen from Fig. 4, the amount of mitochondrial mRNA gradually decreased after addition of siRNA. The reduction of transcription under TFAM deficiency is caused by weakening of transcription activation normally implemented by this factor together with TFB2M and mtRNApol. However, TFAM can implement its main function (decondensation of supercoiled mtDNA structure before the start of transcription) without creating complexes with other factors.

Another factor whose absence, according to literature data, leads to reduction in mtDNA transcriptional activity is TFB2M [9]. We also used the suppression of translation with siRNA to assess the involvement of this factor in thyroid-mediated biogenesis of mtDNA. Transcriptional activity of mtDNA is reduced subsequently to the suppression of TFB2M translation. The number of mitochondrial mRNA copies decreased almost twofold (Fig. 5) 19 h after introduction of siRNA into the culture medium containing T3. Subsequently, the amount of mitochondrial mRNA gradually reduced and reached a lower than control level in 72 h.

These results suggest a direct dependence of mitochondrial transcriptional activity on the number of TFB2M. Comparing the change in the amount of mitochondrial mRNA while lowering concentrations of the three factors, we can see that TFB2M deficit makes the greatest impact on mitochondrial transcription.

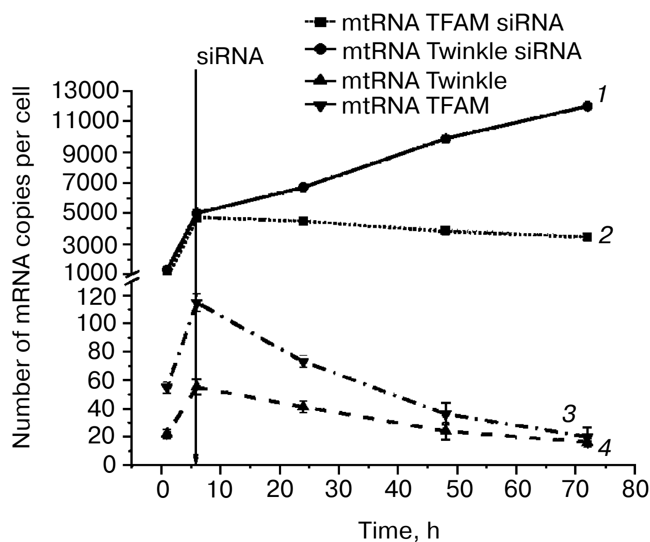

Fig. 4. Changes in number of mRNA copies of mitochondrial transcription factors TFAM and Twinkle in the presence of 15 nM triiodothyronine. siRNA complementary to the 3′-untranslated regions of TFAM and Twinkle were added every 5 h after the addition of triiodothyronine. The perpendicular arrow shows the time of first introduction of siRNA. Curves: *1*) number of copies of mitochondrial mRNAs in the presence of siRNA complementary to helicase Twinkle mRNA; *2*) number of copies of mitochondrial mRNAs in the presence of siRNA complementary to TFAM mRNA; *3*) number of copies of TFAM mRNA; *4*) number of copies of helicase Twinkle mRNA.

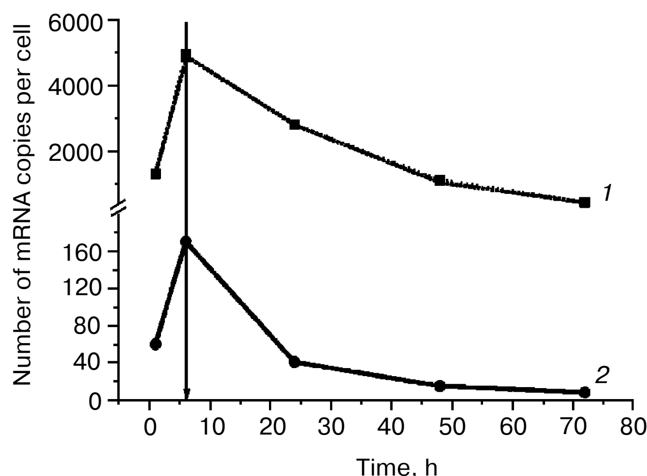

Fig. 5. Changes in level of mitochondrial mRNAs after translation suppression in the presence of 15 nM triiodothyronine. siRNA complementary to 3′-non-translated regions of TFB2M were added every 5 h after addition of triiodothyronine. The perpendicular arrow shows the time of the first siRNA introduction. Curves: *1*) number of mitochondrial mRNA copies; *2*) number of TFB2M mRNA copies.

Thus, the transcriptional profile analysis of three mitochondrial transcription factors conducted under T3 deficiency and under its presence in sub-physiological concentrations suggests that Twinkle translation inhibition does not cause slowing of biogenesis of mitochondria. Two reasons may explain this phenomenon: i) the level of Twinkle in the mitochondrial matrix is sufficient to maintain the biogenesis within a certain time, and ii) Twinkle is not a necessary component for the biogenesis of mtDNA. *In vitro* studies of mitochondrial biogenesis support the second cause as they showed the transcription of mtDNA under these conditions to require only two factors: TFAM and TFB2M [10]. On the other hand, model and association studies indicate certain pathologies are caused by lack of Twinkle [11]. However, based on our results it can be asserted that Twinkle helicase does not play an important role in the thyroid regulation of mitochondrial biogenesis.

As stated above, *in vitro* both TFAM and TFB2M factors are required for transcription of mitochondrial DNA. Translation inhibition of both factors leads to a dynamics change of T3-induced mtDNA biogenesis. It should be noted that TFAM expression deficit leads to stabilization of transcript level, which is the same as prior to addition of siRNA, whereas the result of TFB2M shortage is a gradual decline in mitochondrial biogenesis up to its complete termination. TFAM is a DNA-binding protein belonging to high-mobility proteins family. Its main activity is at the stage of transcription initiation where it acts as an activator of helicase and topoisomerase [12]. Therefore, it may be proposed that one molecule of this protein may participate in multiple rounds of initiation. Additionally, TFAM not only binds with the promoter region of mtDNA, but it covers the entire chain, forming the "skeleton" of the mitochondrial nucleoid in a complex with mtDNA [13]. Summarizing these facts, we conclude that mitochondria contain sufficient TFAM to maintain physiologically required level of expression of mitochondrial genes within a certain time.

In the case of TFB2M translation suppression, the number of mtDNA transcripts does not reach a plateau, as in the case of TFAM, but decreases in direct proportion to decrease in the factor. The differences in impacts of deficit of the two main factors on the transcription of mtDNA are caused by different mechanisms of their functioning. While TFAM is an independent functional unit, TFB2M, according to recent data, is a component of a multienzyme complex that ensures polymerase activity during transcription [14]. The basis of the complex is T7-like RNA polymerase. During the stage of initiation TFB2M interacts with the primed substrate, acting as an activating component of the catalytic site of the initiator complex. Each molecule of TFB2M is able to interact with the initiating complex only once, and then it is transformed into a nonfunctional state [14]. In addition, the amount of TFB2M in mitochondrial matrix is much less than the amount of TFAM [15].

Thus, the induction of transcription and mtDNA replication by thyroid hormones is a consequence of gene activation by key transcription factors: TFAM and TFB2M. Expression of TFAM and TFB2M is regulated in different ways. However, the most important positive regulator is NRF-1. The positive regulatory function of this factor was first demonstrated by the example of cytochrome *c*, which has a corresponding palindromic binding site for NRF-1 [16]. The same authors also showed that NRF-1 is able to induce the transcription of many genes, most of which are somehow related to the functioning of mitochondria. Figure 6 shows the main pathways for which the regulatory role of NRF-1 was demonstrated.

We hypothesized that thyroid hormones activate the expression of mitochondrial transcription factors not directly but through the activation of NRF-1. Using

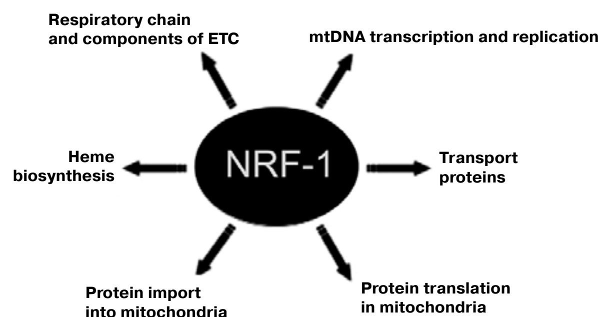

Fig. 6. NRF-1 regulates expression of genes involved in functioning of mitochondria [17].

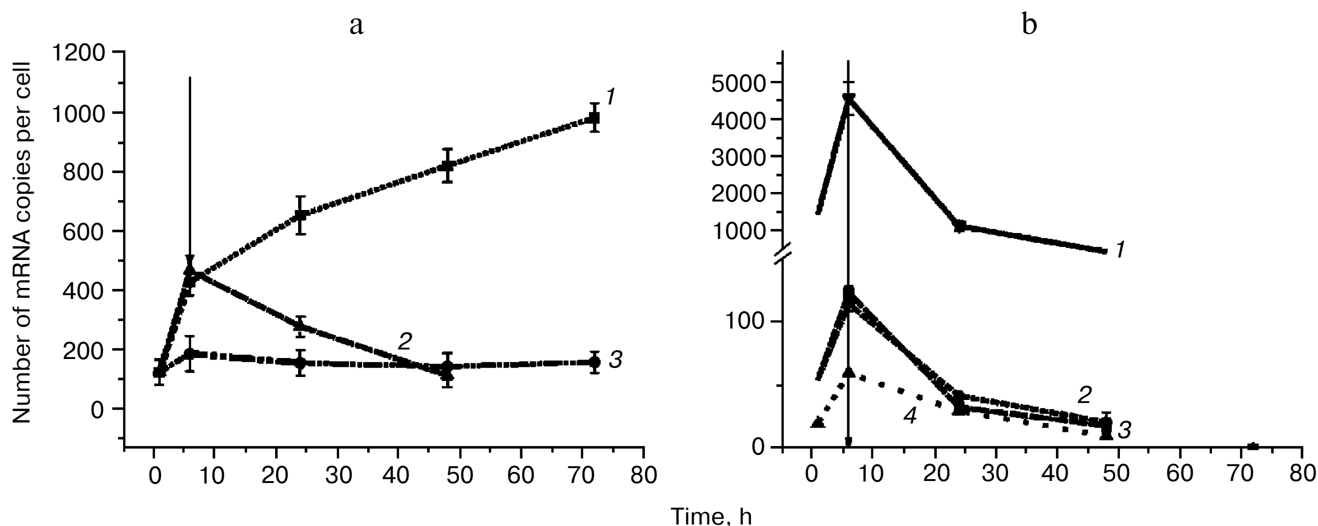

Fig. 7. a) Quantitative estimation of NRF-1 factor mRNA in control cells, in cells cultivated in a 15 nM triiodothyronine-containing medium, and in cells cultivated in a 15 nM triiodothyronine and anti-NRF-1 siRNA-containing medium. The perpendicular arrow shows the time of the first siRNA introduction. Curves: *1*) number of NRF-1 mRNA copies in the presence of 15 nM triiodothyronine; *2*) number of NRF-1 mRNA copies in the presence of siRNA inhibiting its translation; *3*) number of NRF-1 mRNA copies in control cells. b) Number of copies of mitochondrial mRNA and transcription factors after translation suppression of NRF-1 in the presence of 15 nM triiodothyronine: *1*) number of mitochondrial mRNA copies; *2*) number of TFB2M mRNA copies; *3*) number of helicase Twinkle mRNA copies; *4*) number of TFAM mRNA copies.

translation suppression with siRNA, we assessed the impact of NRF-1 deficit on the transcription level of mitochondrial transcription factors and the biogenesis of mtDNA (Fig. 7).

Indeed, application of 15 nM T3 leads to a sharp increase in the amount of NRF-1 mRNA: NRF-1 mRNA amount increases ~10 times in 72 h. Naturally, such dramatic increase in the amount of a universal positive transcription regulator leads to induction of mitochondrial biogenesis. An abrupt decrease in the number of mRNA copies of this factor was registered upon adding siRNA that inhibits translation of NRF-1 (Fig. 7a). The same cells demonstrated a simultaneous decrease in the number of copies of all studied mtDNA transcription factors and mitochondrial mRNAs (Fig. 7b). Moreover, NRF-1 translation inhibition is accompanied by suppression of proliferation and death of the vast majority of cells. There was insufficient number of cells for representative quantification by 72 h.

Based on these results, it can be concluded that the effect of thyroid hormones on mtDNA biogenesis is mediated by the key factors of mtDNA transcription: TFAM and TFB2M. They are in turn activated by a universal positive regulator of transcription – NRF-1. Direct regulation of the expression of mitochondrial transcription factors is confirmed by the presence of NRF-1 binding sites in the promoters of TFB2M and TFAM [18]. The role of NRF-1 in mitochondrial biogenesis has been demonstrated previously in experiments *in vivo*. The authors showed that even an incomplete knockout of the gene encoding NRF-1 leads to a total mortality of mouse embryos caused by an insufficient number of mitochondria in their cells [19].

Transcriptional activity is regulated not only by transcription factors, but also by so-called coactivators and corepressors of transcription acting as intermediaries between signaling molecules and transcription factors. In 2001, Knutti et al. analyzed the effect of ectopic overexpression of PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1 alpha) and drew some conclusions about its impact on mitochondria biogenesis. PGC-1α acts as coactivator for many nuclear receptors, including thyroid receptors as well as NRF-1 [20]. In our studies we assessed the contribution of PGC-1α in the biogenesis of mtDNA in response to triiodothyronine treatment. According to the data, thyroid hormones do not affect the number of copies of PGC-1α mRNA, and the suppression of its transcription does not lead to statistically significant changes in the biogenesis dynamics of mitochondrial mRNA and transcription of all investigated transcription factors. There is no doubt that PGC-1α is one of the key coactivators of transcription factors for mtDNA biogenesis. The absence of changes in PGC-1α expression is probably due to the fact that this coactivator is transcribed constitutively, and its quantity is sufficient to carry out the co-activation process even after triiodothyronine application.

Thyroid hormones play an important role in normal development of the organism, cell differentiation, and metabolism. The state of hypothyroidism is characterized by a decrease in metabolic activity and redox processes.

Thyroid hormone content increase, on the contrary, leads to increased metabolism, heat production, and accumulation of oxygen. Mitochondria are involved in all these processes that respond to changes in the concentration of thyroid hormones by correspondingly altering their own biogenesis. Functioning of mitochondria is directly dependent on their own genetic system to ensure the biosynthesis of polypeptides that are subunits of oxidative phosphorylation complexes. These complexes affect the processes of ATP synthesis, production of free radicals, and disposal of xenobiotics. Using the model system, we demonstrated that triiodothyronine – the most important factor among thyroid hormones – leads to a sharp increase in mtDNA biogenesis. The mechanism of T3-mediated biogenesis is based on the interaction of T3 with nuclear thyroid receptors. T3 receptor, which is a universal transcription factor [21], after binding to T3 activates the transcription of NRF-1, which, in turn is a positive modulator of the majority of genes whose products are involved in mitochondrial biogenesis. The results indicate that NRF-1 positively regulates at least three mtDNA transcription factors: TFAM, TFB2M, and helicase Twinkle. Deficiency of NRF-1 leads to transcription weakening of these factors, which in turn affects mtDNA biogenesis, the transcription of which also decreased dramatically.

According to our data, TFB2M plays the most important role in induction of mtDNA transcription, which is confirmed by numerous mtDNA biogenesis studies both *in vitro* and *in organelle* [13]. TFAM is of no less significance for the induction of mtDNA transcription. However, its pool in mitochondria is sufficient to compensate for translation suppression, and therefore mtDNA transcription decrease is not as sharp as in the case of TFB2M.

We registered an increase in transcription of mitochondrial helicase Twinkle after T3 treatment. However, suppression of its transmission had almost no effect on mtDNA transcription. These data are confirmed by studies on isolated mitochondria, which showed that only TFAM and TFB2M are necessary for mtDNA biogenesis. On the other hand, the induction of T3 of transcription of this factor displays its involvement in some processes. In our opinion, helicase Twinkle ensures functional accuracy of the transcription apparatus and has no effect on the number of transcription initiation acts. The question of the mitochondrial T3 receptor role remains open. Indirectly, our data suggests that it is not involved in mtDNA transcription induction by T3. On the other hand, it may act as a repressor of mtDNA transcription in case it exceeds the physiological norm. This hypothesis is consistent with observations made earlier [4].

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REFERENCES

1. Van Itallie, C. M. (1990) *Endocrinology*, **127**, 55-62.
2. Enriquez, J. A., Fernandez-Silva, P., and Montoya, J. (1999) *Biol. Chem.*, **380**, 737-747.
3. Wrutniak, C., Cassar-Malek, Marchal, S., Rascle, A., Heusser, S., Keller, J. M., Flechon, J., Dauca, M., Samarut, J., Ghysdael, J., et al. (1995) *J. Biol. Chem.*, **270**, 16347-16354.
4. Casas, F., Rochard, P., Rodier, A., Cassar-Malek, I., Marchal-Victorion, S., Wiesner, R. J., Cabello, G., and Wrutniak, C. (1999) *Mol. Cell. Biol.*, **12**, 7913-7924.
5. Gordon, J. W., Rungi, A. A., Inagaki, H., and Hood, D. A. (2001) *J. Appl. Physiol.*, **90**, 389-396.
6. Takamatsu, C., Umeda, S., Ohsato, T., Ohno, T., Abe, Y., Fukuoh, A., Shinagawa, H., Hamasaki, N., and Kang, D. (2002) *EMBO Rep.*, **3**, 451-456.
7. Larsson, N. G., Wang, J. M., Wilhelmsson, H., Oldfors, A., Rustin, P., Lewandoski, M., Barsh, G. S., and Clayton, D. A. (1998) *Nat. Genet.*, **1**, 231-236.
8. Weitzel, J. M., Iwen, K. A., and Seitz, H. J. (2003) *Exp. Physiol.*, **88**, 121-128.
9. Cotney, J., Wang, Z., and Shadel, G. S. (2007) *Nucleic Acids Res.*, **35**, 4042-4054.
10. Litonin, D., Sologub, M., Shi, Y., Savkina, M., Anikin, M., Falkenberg, M., Gustafsson, C. M., and Temiakov, D. (2010) *J. Biol. Chem.*, **285**, 18129-18133.
11. Bohlega, S., van Goethem, G., Al Semari, A., Lofgren, A., Al Hamed, M., van Broeckhoven, C., and Kambouris, M. (2009) *Neuromuscl. Disord.*, **19**, 845-848.
12. Shutt, T. E., Lodeiro, M. F., Cotney, J., Cameron, C. E., and Shadel, G. S. (2010) *Proc. Natl. Acad. Sci. USA*, June 18 (electronic version).
13. Norrbom, J., Wallman, S. E., Gustafsson, T., Rundqvist, H., Jansson, E., and Sundberg, C. J. (2010) *Acta Physiol. (Oxf.)*, **198**, 71-79.
14. Sologub, M., Litonin, D., Anikin, M., Mustaev, A., and Temiakov, D. (2010) *Cell*, **139**, 934-944.
15. Gaspari, M., Larsson, N. G., and Gustafsson, C. M. (2004) *Biochim. Biophys. Acta*, **1659**, 148-152.
16. Kelly, D. P., and Scarpulla, R. C. (2004) *Genes Dev.*, **18**, 357-368.
17. Scrapulla, R. C. (2006) *J. Cell. Biochem.*, **97**, 673-683.
18. Gleyzer, N., Vercauteren, K., and Scarpulla, R. C. (2005) *Mol. Cell. Biol.*, **25**, 1354-1366.
19. Huo, L., and Scarpulla, R. C. (2001) *Mol. Cell. Biol.*, **21**, 644-654.
20. Knutti, D., and Kralli, A. (2001) *Trends Endocrinol. Metab.*, **12**, 360-365.
21. Cheng, S. Y., Leonard, J. L., and Davis, P. J. (2010) *Endocr. Rev.*, **31**, 139-170.