Chapter 14

Radioactive Labeling of Mitochondrial Translation Products in Cultured Cells

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Abstract

The mammalian mitochondrial genome contains 37 genes, 13 of which encode polypeptide subunits in the enzyme complexes of the oxidative phosphorylation system. The other genes encode the rRNAs and tRNAs necessary for their translation. The mitochondrial translation machinery is located in the mitochondrial matrix, and is exclusively dedicated to the synthesis of these 13 enzyme subunits. Mitochondrial disease in humans is often associated with defects in mitochondrial translation. This can manifest as a global decrease in the rate of mitochondrial protein synthesis, a decrease in the synthesis of specific polypeptides, the synthesis of abnormal polypeptides, or in altered stability of specific translation products. All of these changes in the normal pattern of mitochondrial translation can be assessed by a straightforward technique that takes advantage of the insensitivity of the mitochondrial translation machinery to antibiotics that completely inhibit cytoplasmic translation. Thus, specific radioactive labeling of the mitochondrial translation products can be achieved in cultured cells, and the results can be visualized on gradient gels. The analysis of mitochondrial translation in cells cultured from patient biopsies is useful in the study of disease-causing mutations in both the mitochondrial and the nuclear genomes.

Key words: Mitochondria, Oxidative Phosphorylation (OXPHOS), Mitochondrial DNA (mtDNA), Pulse-chase labeling, Mitochondrial translation

1. Introduction

Pulse labeling of the mitochondrial translation products is useful for the assessment of both the individual and global rates of synthesis of the 13 proteins encoded by the mtDNA, and pulse-chase labeling permits the evaluation of the stability of the newly synthesized polypeptides. In both cases, cells are exposed to a mixture of radiolabeled methionine and cysteine in the presence of an inhibitor of cytoplasmic protein synthesis, which results in the specific radiolabeling of the mitochondrial translation products.

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Three main differences distinguish pulse, from pulse-chase labeling: first, the length of the chase, defined as the incubation time in regular, "cold" medium following removal of the radiolabel; second, the type of inhibition (i.e., irreversible versus reversible) of the cytoplasmic protein synthesis; and third, exposure to chloramphenicol, a reversible inhibitor of mitochondrial protein synthesis, which is used only in pulse-chase labeling. In pulse labeling, an irreversible inhibitor of cytoplasmic translation such as emetine can be used, given the short duration of the chase (10 min). The purpose of such short chases is to allow the ribosomes to finish translating any radiolabeled proteins; otherwise, any "hot" polypeptide shorter than the full-length species will be detected at a different size. By contrast, in pulse-chase labeling, when the rates of degradation of the radiolabeled proteins are assessed, cells need to be maintained in culture for longer periods of time (up to 17–18 h), which requires a reversible inhibitor of cytoplasmic translation. For this purpose, we use anisomycin, although other groups have used cycloheximide (1, 2). Finally, in pulse-chase labeling, cells are exposed to chloramphenicol prior to the incubation with radioisotope. This step allows the accumulation of a pool of nuclear-encoded structural subunits within mitochondria, which facilitates the assembly of radiolabeled mitochondrial subunits into nascent OXPHOS complexes following removal of the drug. It also results in increased labeling and preferential stabilization of the two subunits of Complex V that are encoded by the mtDNA (3, 4).

Changes in the normal pattern of mitochondrial protein synthesis, as revealed through radioactive labeling, have served to identify and confirm causal mutations, and to analyze the pathogenic mechanism of mitochondrial diseases caused by mutations in components of the mitochondrial translation system that are encoded by either the mitochondrial (5–10) or the nuclear genome (11–20).

2. Materials

2.1. Labeling of Mitochondrially Synthesized Proteins with Radioactive [^{35}S] Methionine and Cysteine

1. Labeling medium: Dulbecco's Modified Eagle's Medium (DMEM) without methionine and cysteine, supplemented with 10% dialyzed fetal bovine serum (FBS), 1× GlutaMax™-1, and 110 mg/L sodium pyruvate (see Note 1). Store at 4°C.
2. Regular DMEM supplemented with 10% FBS. Store at 4°C.
3. Phosphate buffered saline reconstituted from tablets according to supplier's instruction and sterilized by autoclaving. Store at room temperature.
4. Inhibitors of cytoplasmic translation: emetine for pulse labeling or anisomycin for pulse-chase labeling. In each case, prepare a

2 mg/ml solution in PBS, and sterilize by passing through a 0.2 μm syringe filter (Sarstedt, Nümbrect, Germany). Make fresh as required.

5. EasyTag Expre^{35}S^{35}S Protein Labeling Mix [^{35}S], >1,000 Ci/mmol (see Note 2). Store at 4°C. Observe handling and storage conditions required for this particular radioactive isotope.
6. Chloramphenicol (CAP) for pulse-chase labeling. Prepare a 1-mg/ml solution in regular DMEM without serum (see Note 3), then sterilize by passing through a 0.2 μm syringe filter. Stable at 4°C for up to 1 week.
7. Cell lifters (Corning, Inc. Life Sciences, Lowell, MA, USA).

2.2. Sample Preparation

1. Gel loading buffer (2×): 186-mM Tris–HCl, pH 6.7–6.8, 15% glycerol, 2% sodium dodecyl sulfate (SDS), 0.5-mg/ml bromophenol blue, 6% β-mercaptoethanol (β-ME). Store at room temperature. Add β-ME just before use.
2. Micro-BCA™ Protein Assay Kit (Thermo Scientific Pierce Protein Research Products, Rockford, IL, USA).
3. High Intensity Ultrasonic Processor (Sonics & Materials, Inc., Danbury, CT, USA).

2.3. SDS–PAGE

1. Separating buffer (4×): 1.5-M Tris–HCl (pH 8.8), 8-mM EDTA-Na_2, 0.4% SDS. Store at room temperature.
2. Stacking buffer (4×): 0.5-M Tris–HCl (pH 6.8), 8-mM EDTA-Na_2, 0.4% SDS. Store at room temperature.
3. Thirty percent acrylamide/bisacrylamide solution (37.5:1). Avoid exposure to unpolymerized solution as it is a neurotoxin. Store at 4°C.
4. *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine (TEMED). Store at 4°C.
5. Ammonium persulfate (APS): prepare a 10% solution in double-distilled water. Make fresh as required.
6. Running buffer (1×): to 3 L of double-distilled water (total volume required for one run), add 9.08-g Tris base, 43.25-g glycine, and 3.0-g SDS. Do not pH. Store at room temperature.
7. Molecular weight markers: Page Ruler™ Prestained Protein Ladder (Fermentas Canada, Burlington, ON, USA).
8. WIZ Peristaltic Pump (Teledyne Isco, Lincoln, NE, USA).

2.4. Generation and Analysis of the Data

1. SGD2000 Digital Slab Gel Dryer (Thermo Fisher Scientific, Waltham, MA, USA).
2. Storm 840 Gel and Blot Imaging System (GE Healthcare).

3. Methods

As outlined in this chapter, the pulse and pulse-chase labeling procedures can be applied to all types of adherent cells, independent of either the species of origin or their proliferative state (i.e., dividing versus terminally differentiated), regardless of whether they are transformed, primary or immortalized. We have also used this technique in our laboratory equally successfully for cells growing in suspension: in this case, the only change to the method presented hereafter is that at each wash, cells have to be spun down, then resuspended in the next wash solution or growing medium, as specified in the protocol.

It is important to recognize that the characteristic pattern of mitochondrial translation is unique to each individual species, even when the identical cell type is being considered. Variation across both individuals and tissues within a single species is also possible. This variation can be qualitative, with differences in the electrophoretic mobility of a specific protein, or quantitative, with differences in the overall abundance of mitochondrial translation products (see Fig. 1). Qualitative differences can be due to neutral polymorphisms or to pathogenic mutations, while quantitative differences may reflect different energetic requirements across cell types or a pathogenic event. It is therefore essential that all appropriate controls be included in each experiment.

3.1. Labeling of Mitochondrially Synthesized Proteins with Radioactive [^{35}S] Methionine and Cysteine

1. One 60 mm tissue culture plate between 75 and 90% confluent on the day of the experiment should provide sufficient material for analysis (see Note 4).
2. If cells will be pulse-chase labeled, prepare the CAP solution.
3. For pulse-chase labeling only, aspirate growth medium from each plate 22–24 h prior to the start of the labeling procedure and add 4.8 ml fresh growth medium and 200 μl CAP solution (total volume of 5 ml/plate, final CAP concentration of 40 μg/ml).
4. At least 30 min before the start of the labeling procedure, pipette the total volume of labeling medium (2 ml/plate) and of DMEM + 10% FBS (5 ml/plate) that are required for the entire experiment (see Note 5) into two separate tissue culture plates, and place the plates in the incubator. This step will allow the media to equilibrate to 5% CO_2 and 37°C.
5. For each plate to be labeled, aspirate the growth medium and wash twice with ~3-ml PBS each time.
6. Add 2-ml equilibrated labeling medium/plate, and incubate for 30 min (see Note 6). During this time, prepare and sterilize a 2-mg/ml solution of either emetine (pulse labeling) or anisomycin (pulse-chase labeling).

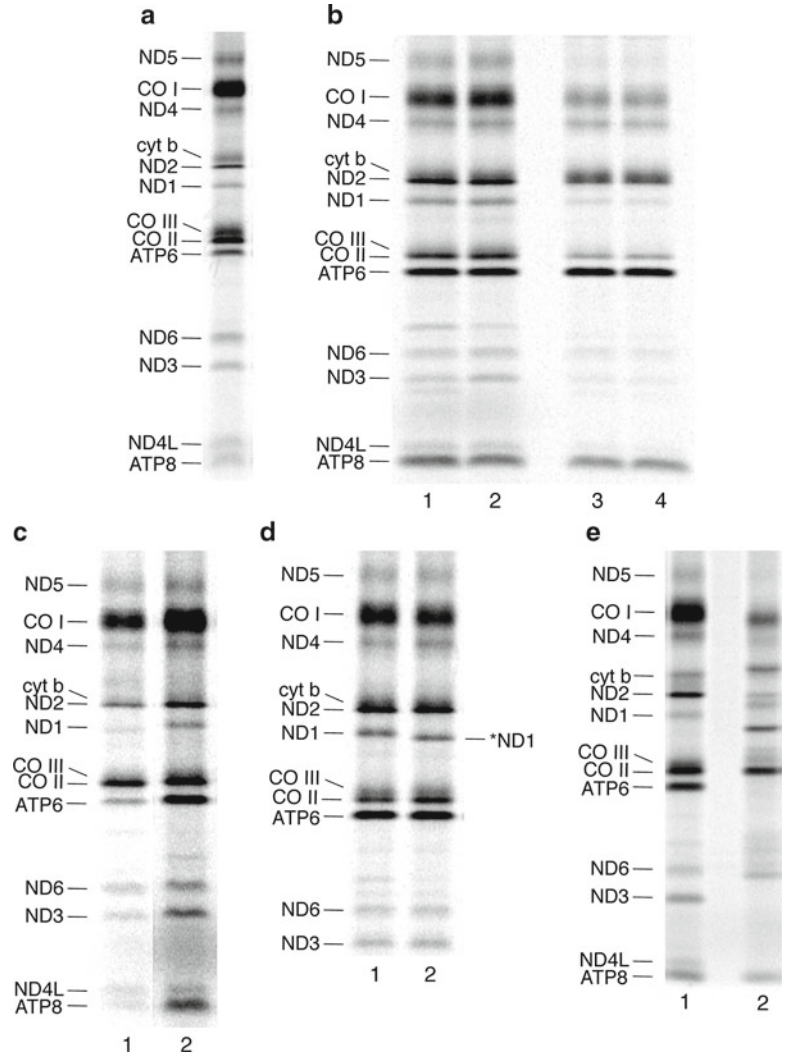

Fig. 1. In vivo analysis of mitochondrial translation by pulse and pulse-chase labeling. *Panel a*: Typical pattern of pulse-labeled mitochondrial translation products in human cultured cells, shown here for immortalized myoblasts. The 13 mitochondrially synthesized proteins are indicated at the *left* of the panel: *ND* subunits of Complex I, *CO* subunits of Complex IV, *ATP* subunits of Complex V, *cyt b* subunit of Complex III. *Panel b*: Pulse (1, 2) and pulse-chase (3, 4) labeling of two lines of immortalized human fibroblasts. Note that in pulse-chase labeling, the two Complex V subunits are preferentially stabilized, a characteristic event resulting from the addition of CAP. Each lane contains 50 μg of total cellular protein. *Panel c*: Increased levels of mitochondrial translation products in the transformed cell line HEK293 (2), when compared to a line of immortalized myotubes (1). Each lane contains 50 μg of total cellular protein. The two lanes are part of the same gel and have been placed side by side to facilitate comparison. *Panel d*: Different migration of the ND1 subunit (most likely due to a neutral polymorphism) in two different lines of immortalized human fibroblasts analyzed by pulse labeling. *Panel e*: Difference between human- and mouse-cultured cells in the overall pattern of pulse-labeled mitochondrial translation products, shown here for the human osteosarcoma line 143B (1) and the mouse myeloma line A9 (2).

7. Add 100 μl of the appropriate inhibitor of cytoplasmic translation (final concentration of 100 μg/ml) to each plate and incubate for 5 min.
8. Add 400 μCi of EasyTag labeling mixture to each plate (final concentration of 200 μCi/ml) and incubate for 60 min (see Note 7).
9. Remove labeling mixture from cells and dispose of it according to university guidelines for the handling of radioisotopes. For pulse labeling, add 5 ml of equilibrated DMEM + 10% FBS/plate and return plate(s) to the incubator for 10 min. For pulse-chase labeling, wash cells once with either DMEM + 10% FBS or with PBS, and chase in DMEM + 10% FBS (5 ml/plate) for up to 17–18 h (see Note 8).
10. Wash cells three times with PBS (see Note 9).
11. Using the cell lifter, scrape cells in 0.7- to 0.8-ml ice-cold PBS and then use a pipette to transfer the entire volume to an Eppendorf tube. Repeat with an additional 0.7- to 0.8-ml ice-cold PBS to collect cells remaining on the plate, and transfer to the same Eppendorf tube (total volume of ~1.5 ml) (see Note 10).
12. Collect cells by centrifugation at 1,500 × *g* for 10 min at 4°C.
13. Aspirate PBS and resuspend the pellet in 200 μl ice-cold PBS. From this point onward, keep cells on ice until they are resuspended in gel loading buffer. Samples may now be stored at −80°C for later use, or the procedure may be continued.

3.2. Sample Preparation

1. Use the Micro-BCA™ Protein Assay Kit to determine the protein concentration of each sample. Duplicates (5 and 10 or 3 and 6 μl) of each sample should be measured, and the calculated protein concentration of the duplicates must be within no more than 10–15% of each other, otherwise the measurement should be repeated.
2. For each sample, spin down the desired amount of protein (usually 50 μg) by centrifugation at >20,000 × *g* for 20 min at 4°C (see Note 11).
3. Resuspend each pellet in 10 μl of 2× gel loading buffer (room temperature) and then add 10 μl of double-distilled water.
4. Sonicate each sample for 3–8 s at an output control of 60.
5. Spin samples at room temperature for 10–15 min at >20,000 × *g*, or until the bubbles resulting from sonication have disappeared.

3.3. SDS–PAGE

1. These instructions assume the use of a PROTEAN II xi gel system from Bio-Rad Laboratories. Rinse the glass plates, spacers, combs, and casting stand gaskets several times with

deionized, then double-distilled water, followed by a final rinse in 70–95% ethanol. Air-dry.

2. Prepare 12 ml of a 15% gel mixture by combining 6 ml of acrylamide/bisacrylamide solution, 3 ml of 4× separating buffer, and 2.9 ml of double-distilled water, and then prepare 12 ml of a 20% gel mixture by combining 8 ml of acrylamide/bisacrylamide solution, 3 ml of 4× separating buffer, and 0.9 ml of double-distilled water. Just before pouring the gradient gel (see next step), add 60 µl of 10% APS and 6 µl of TEMED to each gel mixture.
3. Using the WIZ Peristaltic Pump at its maximum flow rate, pour a 1.0-mm-thick, 15–20% gradient gel by using the entire volume (24 ml) of the 15% and 20% gel solutions (see Fig. 2, for detailed instructions). Overlay the gradient gel with double-distilled water.
4. Once the gradient gel has polymerized (see Note 12), pour off the water overlay and dry the area above the gel with Whatman paper. Prepare the stacking gel by mixing 1.04 ml of acrylamide/bisacrylamide solution, 2.5 ml of 4× stacking buffer, 6.5 ml of double-distilled water, 50 µl of 10% APS, and 10 µl of TEMED, then pipette the mixture on top of the separating

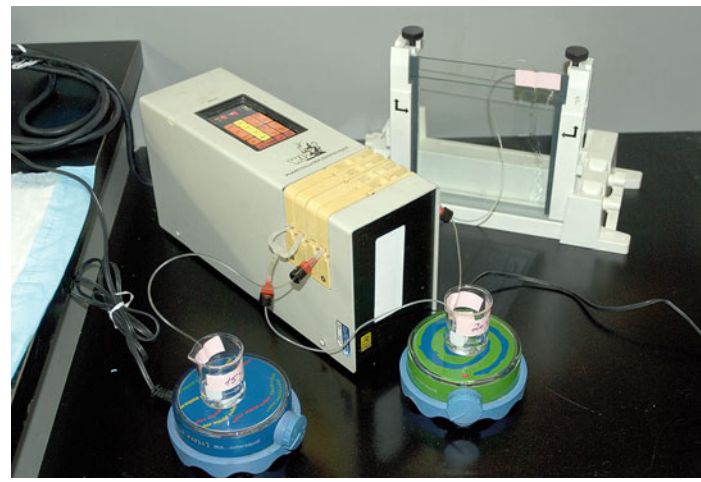

Fig. 2. Organization, assembly, and casting of gradient gels. Clean and assemble the components required to cast the gradient gel (see 3.3.1. for detailed instructions). Run double-distilled water through the *WIZ Peristaltic Pump* for roughly 5 min at maximum flow rate to ensure that the tubing is clean. Completely empty the tubing of all double-distilled water, and secure the relevant lines of tubing to the *beakers* containing the 15 and 20% acrylamide mixtures and to the gel casting setup using tape, as shown. Once the tubing is secured, add the TEMED and 10% APS solutions to the *two beakers* and turn on the *pump set* to a flow rate of maximum. Exhaust the entire volume of both solutions prior to stopping the pump. Overlay gently with water and allow 45 min to 1 h for polymerization.

gel until it begins to overflow. Insert the comb and allow the stacking gel to polymerize (see Note 13).

5. Once the stacking gel has set, remove the comb by pulling it straight up slowly and gently. Rinse the wells three times with double-distilled water.
6. Assemble the electrophoresis unit and add the running buffer to the inner and outer chambers of the unit. Load the whole 20 μl of each sample in an individual well. Reserve at least one well for the prestained molecular weight marker (15–20 μl/well).
7. Complete the assembly of the electrophoresis unit and connect to a power supply. Run the gel at constant current (8–10 mA) for 15–17 h or until the lowermost (usually 11 kDa) molecular weight marker is at ~1 cm from the bottom of the glass plates.

3.4. Generation and Analysis of the Data

1. At the end of the run, disconnect the electrophoresis unit from the power supply and disassemble it. Separate the glass plates sandwiching the gel by vigorously twisting one of the spacers. Remove and discard the stacking gel, and cut one corner of the separating gel for orientation.
2. Rinse the gel by submerging it in a vessel containing double-distilled water, and transfer it by hand to a piece of thick filter paper cut to the dimensions of the gel. Cover the gel with Saran wrap.
3. Dry gel under vacuum at 60°C for 1 h by using the SGD2000 Digital Slab Gel Dryer or equivalent.
4. Expose to a phosphorimager cassette for at least 3 days, then scan with the Storm 840 Gel and Blot Imaging System. Analyze the resultant image with the help of the ImageQuant TL Software. For characteristic patterns of mitochondrial translation analyzed by pulse and pulse-chase labeling and visualized by this method, see Fig. 1.

4. Notes

1. Certain formulations of DMEM without methionine and cysteine contain sodium pyruvate, while others do not. Check before adding.
2. Pure [^{35}S] methionine results in the strongest signal and the best signal/noise ratio. However, the EasyTag mixture of [^{35}S] methionine/cysteine is considerably less expensive and gives a comparable result (approximately 75% of the signal intensity compared with pure [^{35}S] methionine).

3. Add warm medium to the CAP powder and incubate in a water bath at 37°C with occasional vortexing to help dissolve the powder.
4. Depending on the cell type and cell size, starting with a plate less than ~75% confluent might result in insufficient protein for SDS–Polyacrylamide Gel Electrophoresis (PAGE) analysis. At the same time, cycling cells should be less than 100% confluent, as they should still be able to divide during radiolabeling.
5. Label a maximum of six plates at a time, otherwise it will be difficult to respect the required timing for several steps of the procedure.
6. Throughout the labeling procedure, it is important that individual plates be placed directly on the shelf of the incubator rather than stacked on top of each other. This ensures that during short incubation times, all the plates equilibrate in terms of temperature and CO_2 concentration.
7. Remember to dispose appropriately of all materials that come in contact with the radioisotope: pipettes, cell plates, Eppendorf tubes, pipette tips, cell lifters, etc.
8. While short chases can be universally done in DMEM + 10% FBS, the longer chases required in pulse-chase labeling should be done in cell-specific medium (e.g., chase myoblasts in myoblast-specific medium).
9. Be gentle when washing cells loosely attached to the plate, such as large myotubes or certain transformed cell lines.
10. Alternatively, in the case of myotubes, an enriched population of fused cells can be obtained by selective trypsinization: trypsinize cells for about 2 min or until fused cells start lifting (unfused myoblasts will take a minimum of 5 min to trypsinize). Dilute trypsin by adding 5-ml PBS to the plate and transfer trypsinized cells to a 15-ml Falcon tube. Rinse plate with another 5-ml PBS and add to the same 15-ml tube. Collect cells by centrifugation at $1{,}500 \times g$ for 5 min. Aspirate PBS, then resuspend pellet in ~1.5-ml cold PBS and transfer to an Eppendorf tube.
11. Sometimes the pellet is not easily visible; always be extra careful when removing the PBS to not disturb the pellet.
12. To save time, prepare the running buffer while the separating gel polymerizes. Likewise, start preparing the samples after pouring the stacking gel.
13. This gel system allows the use of 15- and 20-well combs. While the 20-well comb has the obvious advantage of a higher number of samples per run, the 15-well comb will result in better definition of the bands and a higher resolution between lanes, both of which allow easier quantification of the signal.

References

1. Chomyn, A. (1996) In vivo labeling and analysis of human mitochondrial translation products. *Methods Enzymol*, **264**, 197–211.
2. Fernandez-Silva, P., Acin-Perez, R., Fernandez-Vizarra, E., Perez-Martos, A. and Enriquez, J.A. (2007) In vivo and in organello analyses of mitochondrial translation. *Methods Cell Biol*, **80**, 571–588.
3. Costantino, P. and Attardi, G. (1977) Metabolic properties of the products of mitochondrial protein synthesis in HeLa cells. *J Biol Chem*, **252**, 1702–1711.
4. Mariottini, P., Chomyn, A., Doolittle, R.F. and Attardi, G. (1986) Antibodies against the COOH-terminal undecapeptide of subunit II, but not those against the NH2-terminal decapeptide, immunoprecipitate the whole human cytochrome c oxidase complex. *J Biol Chem*, **261**, 3355–3362.
5. Chomyn, A., Martinuzzi, A., Yoneda, M., Daga, A., Hurko, O., Johns, D., Lai, S.T., Nonaka, I., Angelini, C. and Attardi, G. (1992) MELAS mutation in mtDNA binding site for transcription termination factor causes defects in protein synthesis and in respiration but no change in levels of upstream and downstream mature transcripts. *Proc Natl Acad Sci USA*, **89**, 4221–4225.
6. Chomyn, A., Meola, G., Bresolin, N., Lai, S.T., Scarlato, G. and Attardi, G. (1991) In vitro genetic transfer of protein synthesis and respiration defects to mitochondrial DNA-less cells with myopathy-patient mitochondria. *Mol Cell Biol*, **11**, 2236–2244.
7. Enriquez, J.A., Chomyn, A. and Attardi, G. (1995) MtDNA mutation in MERRF syndrome causes defective aminoacylation of tRNA(Lys) and premature translation termination. *Nat Genet*, **10**, 47–55.
8. Hayashi, J., Ohta, S., Kikuchi, A., Takemitsu, M., Goto, Y. and Nonaka, I. (1991) Introduction of disease-related mitochondrial DNA deletions into HeLa cells lacking mitochondrial DNA results in mitochondrial dysfunction. *Proc Natl Acad Sci USA*, **88**, 10614–10618.
9. King, M.P., Koga, Y., Davidson, M. and Schon, E.A. (1992) Defects in mitochondrial protein synthesis and respiratory chain activity segregate with the tRNA(Leu(UUR)) mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes. *Mol Cell Biol*, **12**, 480–490.
10. Sasarman, F., Antonicka, H. and Shoubridge, E.A. (2008) The A3243G tRNALeu(UUR) MELAS mutation causes amino acid misincorporation and a combined respiratory chain assembly defect partially suppressed by overexpression of EFTu and EFG2. *Hum Mol Genet*, **17**, 3697–3707.
11. Antonicka, H., Ostergaard, E., Sasarman, F., Weraarpachai, W., Wibrand, F., Pedersen, A.M., Rodenburg, R.J., van der Knaap, M.S., Smeitink, J.A., Chrzanowska-Lightowlers, Z.M. *et al.* (2010) Mutations in C12orf65 in patients with encephalomyopathy and a mitochondrial translation defect. *Am J Hum Genet*, **87**, 115–122.
12. Antonicka, H., Sasarman, F., Kennaway, N.G. and Shoubridge, E.A. (2006) The molecular basis for tissue specificity of the oxidative phosphorylation deficiencies in patients with mutations in the mitochondrial translation factor EFG1. *Hum Mol Genet*, **15**, 1835–1846.
13. Coenen, M.J., Antonicka, H., Ugalde, C., Sasarman, F., Rossi, R., Heister, J.G., Newbold, R.F., Trijbels, F.J., van den Heuvel, L.P., Shoubridge, E.A. *et al.* (2004) Mutant mitochondrial elongation factor G1 and combined oxidative phosphorylation deficiency. *N Engl J Med*, **351**, 2080–2086.
14. Fernandez-Vizarra, E., Berardinelli, A., Valente, L., Tiranti, V. and Zeviani, M. (2007) Nonsense mutation in pseudouridylate synthase 1 (PUS1) in two brothers affected by myopathy, lactic acidosis and sideroblastic anaemia (MLASA). *J Med Genet*, **44**, 173–180.
15. Kemp, J.P., Smith, P.M., Pyle, A., Neeve, V.C., Tuppen, H.A., Schara, U., Talim, B., Topaloglu, H., Holinski-Feder, E., Abicht, A. *et al.* (2011) Nuclear factors involved in mitochondrial translation cause a subgroup of combined respiratory chain deficiency. *Brain*, **134**, 183–195.
16. Miller, C., Saada, A., Shaul, N., Shabtai, N., Ben-Shalom, E., Shaag, A., Hershkovitz, E. and Elpeleg, O. (2004) Defective mitochondrial translation caused by a ribosomal protein (MRPS16) mutation. *Ann Neurol*, **56**, 734–738.
17. Riley, L.G., Cooper, S., Hickey, P., Rudinger-Thirion, J., McKenzie, M., Compton, A., Lim, S.C., Thorburn, D., Ryan, M.T., Giege, R. *et al.* (2010) Mutation of the mitochondrial tyrosyl-tRNA synthetase gene, YARS2, causes myopathy, lactic acidosis, and sideroblastic anemia – MLASA syndrome. *Am J Hum Genet*, **87**, 52–59.
18. Smeitink, J.A., Elpeleg, O., Antonicka, H., Diepstra, H., Saada, A., Smits, P., Sasarman, F., Vriend, G., Jacob-Hirsch, J., Shaag, A. *et al.* (2006) Distinct clinical phenotypes associated

with a mutation in the mitochondrial translation elongation factor EFTs. *Am J Hum Genet*, **79**, 869–877.

19. Valente, L., Tiranti, V., Marsano, R.M., Malfatti, E., Fernandez-Vizarra, E., Donnini, C., Mereghetti, P., De Gioia, L., Burlina, A., Castellan, C. *et al.* (2007) Infantile encephalopathy and defective mitochondrial DNA translation in patients with mutations of mitochondrial elongation factors EFG1 and EFTu. *Am J Hum Genet*, **80**, 44–58.
20. Zeharia, A., Shaag, A., Pappo, O., Mager-Heckel, A.M., Saada, A., Beinat, M., Karicheva, O., Mandel, H., Ofek, N., Segel, R. *et al.* (2009) Acute infantile liver failure due to mutations in the TRMU gene. *Am J Hum Genet*, **85**, 401–407.