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# Localization of mRNAs encoding human mitochondrial oxidative phosphorylation proteins

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ABSTRACT

The mitochondrial oxidative phosphorylation (OXPHOS) proteins are encoded by both nuclear and mitochondrial DNA. The nuclear-encoded OXPHOS mRNAs have specific subcellular localizations, but little is known about which localize near mitochondria. Here, we compared mRNAs in mitochondria-bound polysome fractions with those in cytosolic, free polysome fractions. mRNAs encoding hydrophobic OXPHOS proteins, which insert into the inner membrane, were localized near mitochondria. Conversely, OXPHOS gene which mRNAs were predominantly localized in cytosol had less than one transmembrane domain. The RNA-binding protein Y-box binding protein-1 is localized at the mitochondrial outer membrane and bound to the OXPHOS mRNAs. Our findings offer new insight into mitochondrial co-translational import in human cells.



## 1. Introduction

Mitochondria are essential organelles that are present in virtually all eukaryotic cells and have fundamental functions in energy production and other metabolic pathways. The primary function of mitochondria is ATP production via the oxidative phosphorylation (OXPHOS) pathway, which is subject to complex regulation. The circular 16.5-kb human mitochondrial DNA (mtDNA) molecule encodes two rRNAs, 22 tRNAs, and 13 proteins that are members of the respiratory chain (Anderson et al., 1981; Kang and Hamasaki, 2005). The mitochondrial respiratory chain consists of five complexes, comprising more than 85 structural proteins (Poyton and McEwen, 1996). While the mitochondrial genome encodes a few proteins, almost 70 additional proteins are encoded by nuclear genes, translated in the cytoplasm, imported into the mitochondrial inner membrane, and assembled with the mtDNA-encoded proteins via a series of complex molecular mechanisms (Kumar et al., 2002; Neupert and Herrmann, 2007; Prokisch et al., 2004; Sickmann et al., 2003).

The combination of mitochondrial and nuclear DNA-encoded proteins reflects the evolutionary origin of mitochondria proposed by the endosymbiotic theory (Sagan, 1967). In this theory, the bacterial ancestors of mitochondria joined with a primitive eukaryotic cell to contribute OXPHOS to the cellular metabolism. During the evolution of the eukaryotic cell, most genes of the mitochondrial ancestor were either lost or transferred to the nuclear genome (Andersson et al., 1998).

Mitochondrial translation products in the matrix are almost exclusively highly hydrophobic polypeptides that are inserted into the inner membrane during their synthesis (Ott and Herrmann, 2010). Membrane insertion of these OXPHOS proteins occurs co-translationally and is connected to a sophisticated assembly process (Ott and Herrmann, 2010). In contrast, some cytosolic translation occurs in the cytoplasm near the mitochondrial outer membrane. Global transcriptome studies have demonstrated that approximately half of the mRNAs encoding mitochondrially localized proteins are asymmetrically localized in the vicinity of mitochondria (Marc et al., 2002; Saint-Georges et al., 2008).

OXPHOS proteins can be separated into two groups according to their protein hydrophobicity. For example, complex II (succinate dehydrogenase, SDH) is composed of four subunits: two hydrophilic and two hydrophobic, membrane-integrated subunits. These four proteins are translated in the cytosol and are imported into the inner membrane for assembly. We speculated that the mRNAs for the hydrophobic proteins SDHC and SDHD would be localized in the vicinity of the mitochondria and that they would be imported by a co-translational mechanism.

The localization of mRNA has emerged as important in cellular and developmental biology as a means to regulate gene expression spatially. The localization process is determined by *cis*-acting signals

*Abbreviations:* EDTA, ethylene diamine tetraacetic acid; G3BP1, Ras-GAP SH3 binding protein 1; mtDNA, mitochondrial DNA; OXPHOS, oxidative phosphorylation; PBS, phosphate-buffered saline; qRT-PCR, quantitative reverse transcription polymerase chain reaction; SDH, succinate dehydrogenase; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; UTR, untranslated region; YB-1, Y-box binding protein-1.

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that are usually within the 3′-untranslated region (UTR) of mRNAs and by *trans*-acting factors that bind to these *cis*-elements (Kloc et al., 2002). Several analyses of asymmetric mRNA localization using DNA microarrays and real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) assays have been reported in yeast (Garcia et al., 2007; Marc et al., 2002; Sylvestre et al., 2003b) and mammalian cells (Sylvestre et al., 2003a). Specific mRNA localization is now known to be common in somatic and germ cells, and the importance of mRNA sorting has been described in mitochondrial biogenesis in yeast and human cells. In yeast, a genome-wide analysis was used to characterize the cytosolic mRNAs enriched near mitochondria (Marc et al., 2002; Sylvestre et al., 2003b). It was shown that the targeting of mRNAs to mitochondria is mediated largely by signals in the 3′-UTR in both yeast and human cells, and that this is essential for mitochondrial biogenesis (Margeot et al., 2002). In cancer cells, the Ras-GAP SH3 binding protein 1 (G3BP1) interacts with the *ATP5B* mRNA and inhibits its translation at the initial step, suggesting a role for G3BP1 in the glycolytic switch in cancer cells (Ortega et al., 2010).

A number of mRNA-binding proteins participate in translational regulation (Dugre-Brisson et al., 2005; Skabkina et al., 2003). Among these, Y-box binding protein-1 (YB-1) is a unique multifunctional protein that can regulate both transcription and translation (Kohno et al., 2003; Matsumoto and Wolffe, 1998). Mammalian YB-1 is a member of the Y-box family proteins that contain a common structural feature, the cold shock domain, and control gene expression at the translational level through their recognition of RNAs (Ashizuka et al., 2002; Fukuda et al., 2004; Kohno et al., 2003). YB-1 associates with mRNA in polysomes or free messenger ribonucleoproteins (mRNPs), depending on the molar ratio of YB-1 to its target mRNA, and functions as either an activator or inhibitor of translation (Evdokimova and Ovchinnikov, 1999; Skabkin et al., 2004).

In this experiment, we hypothesized that mitochondria- and cytosol-localized OXPHOS mRNAs would encode more hydrophobic and more hydrophilic proteins, respectively. First, we investigated which mRNAs encoding OXPHOS proteins were localized in mitochondria-bound polysomes in mammalian cells. We then examined whether the major RNA-binding protein YB-1 serves as a mitochondria-bound mRNA translation regulator at the mitochondrial outer membrane. We showed that mRNAs encoding hydrophobic OXPHOS proteins associate with mitochondria.

## 2. Material and methods

### 2.1. Cell culture

Human cervical cancer HeLa cells were cultured in Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO) supplemented with 10% heat-inactivated fetal bovine serum (MP Biomedicals, Aurora, OH). Human T lymphoid Jurkat cells were grown in RPMI 1640 medium (Sigma) with 10% fetal bovine serum. The cells were incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$.

### 2.2. Immunoblot analysis

Immunoblot analysis were carried out as described previously using polyclonal or monoclonal antibodies against YB-1 (Shibahara et al., 2004; Uchiumi et al., 2006), histone H1 (Millipore, Bedford, MA), HKII (Cell Signaling Technology, Beverly, MA), BAP37, VDAC, ENDOG, and TFAM (all produced in our laboratory). The signals were visualized with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG and Enhanced Chemiluminescence reagents (GE Healthcare, Little Chalfont, UK). The chemiluminescence was recorded and quantified with a cooled charge-coupled device camera (LAS1000plus; Fuji Photo Film, Tokyo, Japan).

### 2.3. RNA extraction and qRT-PCR

Total RNA was extracted from polysome fractions and immunoprecipitates using an RNAeasy kit (Qiagen, Hilden, Germany). RT of 500 ng of the total RNA was performed with SuperScript III RT (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The RT reaction mixtures were diluted 1:5 with water. The expression levels of nuclear-encoded mitochondrial genes were detected by qRT-PCR with a thermal cycler (StepOne plus; Invitrogen). The primer pairs used in this experiment are listed in Table 1.

### 2.4. Purification of free and mitochondria-bound polysomes

We purified free and mitochondria-bound polysomes essentially as described previously (Ades and Butow, 1980; Suissa and Schatz, 1982) with slight modification. Jurkat cells ($1\times10^9$ cells) cultured in a 1-l spinner flask were precipitated by centrifugation and washed with homogenization buffer (10 mM HEPES-KOH pH 7.4, 0.25 M sucrose, 1 mM EDTA). The cells were then suspended in two volumes of the same buffer, homogenized with a Potter-Elvehjem homogenizer, and centrifuged at $1600\times g$ for 10 min. The supernatant fraction was centrifuged at $22{,}000\times g$ for 15 min to pellet the mitochondria. The post-mitochondrial supernatant fraction was used for preparing free polysomes. The mitochondrial pellet was washed twice. For each wash, the pellet was resuspended in homogenization buffer and centrifuged at $1600\times g$ for 5 min, and the supernatant was then centrifuged at $15{,}000\times g$ for 15 min. The final pellet including the mitochondria was suspended in homogenization buffer containing 1.5% Triton X-100, and polysomes bound to the mitochondria outer membrane and mitochondrial ribosome bound to inner membrane were released by incubation on ice for 20 min, followed by centrifugation at $22{,}000\times g$ for 15 min. The pellet was discarded and the supernatant was used to prepare released polysomes.

**Table 1**
Primer pairs used in this experiment.

| | Forward | bp | Reverse | bp |
|---|---|---|---|---|
| ND1 | ATGGCCAACCTCCTACTCCT | 20 | GCGGTGATGTAGAGGGTGAT | 20 |
| NDUFA1 | AAGGACCCAGAAGTAGGGTTTT | 22 | CAGTGATACCCAAAATGAGCAA | 22 |
| NDUFA9 | AAATCCTTTGCTTTCGTTGGT | 21 | TTGTTATCCAGGGCTCAAATG | 21 |
| NDUFA10 | GATCGGGGGAGGGTAAATAA | 20 | CAGCAGACCACTGTTTTCCA | 20 |
| NDUFB2 | ACTGCTGGAGATGGTGGAGT | 20 | GACCCAGCACCTCTTCTGAG | 20 |
| NDUFB8 | TACAACAGGAACCGTGTGGA | 20 | CTGGTTCTTTGGAGGGATCA | 20 |
| NDUFS7 | AGTTCTCTGTGGCCCATGAC | 20 | GGCATCTGGTCGTAGACCTT | 20 |
| NDUFV1 | AGCTTCATGAATAAGCCCTCAG | 22 | TAGAATTCCCCTCGGATGTAGA | 22 |
| NDUFV2 | AATGTTTAGGGGCCTGTGTG | 20 | AGCTGGCTCACAAGAGAAGC | 20 |
| SDHA | TCGCACTGTGCATAGAGGAC | 20 | ATGCCTGTAGGGTGGAACTG | 20 |
| SDHB | TAAATGTGGCCCCATGGTAT | 20 | AGGTTGGTGTCAATCCTTCG | 20 |
| SDHC | TGAGTGCAGGGGTCTCTCTT | 20 | GGAATCTTCAGGCCTTTTCC | 20 |
| SDHD | GTATGCCTCTTTGCCTCTGC | 20 | GAGGCAACCCCATTAACTCA | 20 |
| CYTB | TATCCGCCATCCCATACATT | 20 | GGTGATTCCTAGGGGGTTGT | 20 |
| CYC1 | CCAGCTACCATGTCCCAGAT | 20 | TATGCCAGCTTCCGACTCTT | 20 |
| UQCRC1 | TGTCTCGTGCAGACTTGACC | 20 | GAAGCGGCATGGAGTAAGAG | 20 |
| UQCRFS1 | GGAAATTGAGCAGGAAGCTG | 20 | GGCAAGGGCAGTAATAACCA | 20 |
| UQCRH | TTGCTGCTCGTGTTGAATCT | 20 | CTCGCATTGCTCTCTCACTG | 20 |
| COX1 | GGCCTGACTGGCATTGTATT | 20 | TGGCGTAGGTTTGGTCTAGG | 20 |
| COX5A | GCATGCAGACGGTTAAATGA | 20 | AGTTCCTCCGGAGTGGAGAT | 20 |
| COX6B2 | TCTGGAGACATTTTTGGTTGG | 21 | ATGGTGAGGGCGTTAGAAACT | 21 |
| COX15 | ATGGATGAAAGTTGCAGGAAGT | 22 | AAAGCAAAACAAAAACCCAGAA | 22 |
| COX17 | AGGAGAAGAAGCCGCTGAAG | 20 | ATTCACACAGCAGACCACCA | 20 |
| SCO1 | GGGGAGCTAAAACTGACAA | 20 | TGGGTCAATGCTGATGAAAA | 20 |
| ATP6 | TATTGATCCCCACCTCCAAA | 20 | GATGGCCATGGCTAGGTTTA | 20 |
| ATP5A1 | GTGGCTCCTTGACTGCTTTG | 20 | ACCTGCTTCATAGCCCTGGT | 20 |
| ATP5C1 | GCCAAGCTGTCATCACAAAA | 20 | GGACAAAGGCAGCAGTAAGC | 20 |
| ATP5D | GGAAGCTCCTCCTCAGCTTT | 20 | CAGGCTTCCGGGTCTTTAAT | 20 |
| ATP5G1 | ACATTGACACAGCAGCCAAG | 20 | GCCAAGAATGGCATAGGAGA | 20 |
| ATP5G2 | CAGAAAGCAGGACTCCCAAG | 20 | ACTGCGGTTTGGTCTGTACC | 20 |
| ATPAF2 | GAGATCAGCTCCTCCACCAG | 20 | TCAATCAGGCCCAAGGTTAG | 20 |
| β-actin | CCAACCGCGAGAAGTGAC | 19 | GGAAGGAAGGCTGGAAGAGT | 20 |
| LaminB1 | TAACGAGACCAGAAGGAAGCA | 21 | CATTCTCAAGTTTGGCATGGT | 21 |
| 18S rRNA | CATGGCCGTTCTTAGTTGGT | 20 | CGCTGAGCCAGTCAGTGTAG | 20 |