The International Journal of Biochemistry & Cell Biology 45 (2013) 845–849

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Organelles in focus

Mitochondria: Mitochondrial RNA metabolism and human disease

Thomas J. Nicholls, Joanna Rorbach, Michal Minczuk*

MRC Mitochondrial Biology Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 0XY, UK

ARTICLE INFO

Article history:
Received 30 November 2012
Accepted 8 January 2013
Available online 17 January 2013

Keywords:
Mitochondrial disease
RNA stability
RNA processing
RNA turnover
tRNA modification

ABSTRACT

Post-transcriptional control of RNA stability, processing, modification, and degradation is key to the regulation of gene expression in all living cells. In mitochondria, these post-transcriptional processes are also vital for proper expression of the thirteen proteins encoded by the mitochondrial genome, as well as mitochondrial tRNAs and rRNAs. Our knowledge on mitochondrial RNA (mt-RNA) metabolic pathways, however, is far from complete. All the proteins involved in mt-RNA metabolism are encoded by the nucleus, and must be imported into the organelle. Mutations in these nuclear genes can lead to perturbations in mitochondrial RNA processing, modification, stability and decay and thus are a cause of human mitochondrial disease. This review summarises the current knowledge on mt-RNA metabolism and its links with human mitochondrial pathologies.

Organelle facts

- Human mitochondria contain their own genome (mtDNA) that encodes mRNAs for 13 (out of ~90) subunits of the complexes performing oxidative phosporylation (OXPHOS) as well as 2 rRNAs and 22 tRNAs needed for translation of mtDNA-encoded proteins in the organelle.
- Apart from the 13 mtDNA-encoded subunits of OXPHOS, the remaining part of the mitochondrial proteome, including factors involved in mt-RNA metabolism, is encoded by the nucleus and must be imported into the organelle.
- Defects of the machinery involved in human mitochondrial RNA stability, modification and possibly decay are a known cause of respiratory chain disorders, yet details of how these processes are regulated remain to be unveiled.

1. Introduction

In human mitochondria, RNA is produced from a very compact, circular double stranded genome that encodes two ribosomal RNAs (mt-rRNA), 22 mt-tRNAs and 13 proteins. All the mitochondrially encoded proteins are vital subunits of the oxidative phosphorylation (OXPHOS) system, the end stage of aerobic cellular energy production. The rest of the mitochondrial proteome, including factors involved in mt-RNA metabolism, is encoded by the nucleus and must be imported into the organelle. Human mitochondrial transcripts are synthesised from both strands of mtDNA (denoted the heavy and light strands) as large polycistronic molecules that must be processed to generate mature rRNA, tRNA and mRNA. Transcription from the heavy strand results in a large precursor that encodes 2 rRNAs, 14 tRNAs and 12 proteins, whereas the light strand codes for 8 tRNAs and 1 mRNA. In principle, the processing of polycistronic molecules should result in stoichiometric amounts of all matured RNAs. However, there is significant variation in the final concentrations of matured transcripts, implying complex post-transcriptional regulation of individual RNAs, through processes that we still do not fully understand. Nonetheless, recent years have brought the identification and characterisation of several important players in RNA metabolism in mitochondria.

Mitochondrial dysfunction is increasingly being recognized as a major contributor in a number of metabolic and degenerative diseases, ageing and cancer. Pathological alterations of mitochondrial function fall into two main categories: those that are caused by mutations in the mitochondrial genome and defects of nuclear genes involved in mitochondrial function. Many diagnosed mitochondrial disorders are considered as isolated deficiencies, affecting one of the mitochondrial complexes either due to a defect in a specific subunit of the OXPHOS machinery (either mitochondrially or nuclearly encoded) or due to mutation of one of the factors facilitating assembly of the complex. In contrast, combined deficiencies exhibit decreased activities of multiple complexes. This phenotype is often associated with mutations of mitochondrial tRNAs or rRNAs, leading to a generalised defect in gene expression. In addition, the number of disease-causing mutations identified in nuclear genes required for mtDNA integrity, replication and expression has grown considerably over the past few years. Many important players in RNA metabolism in mitochondria have been

* Corresponding author. Tel.: +44 1223 252750; fax: +44 1223 252715.
E-mail addresses: michal.minczuk@mrc-mbu.cam.ac.uk, mam@mrc-mbu.cam.ac.uk (M. Minczuk).

1357-2725/$ – see front matter.
http://dx.doi.org/10.1016/j.biocel.2013.01.005

identified and implicated in human disease, indicating the functional importance of these pathways for mitochondrial activity. In this review article we focus on the critical factors that are involved in the mt-RNA life-cycle and review emerging evidence on the involvement of mt-RNA metabolism pathways in human disease.

2. Organelle function

Promoters for the transcription of heavy-strand mtDNA (HSP) and light strand (LSP) are both located in the mtDNA non-coding region, and transcription produces two long, almost genome-length, polycistronic transcripts of mRNAs and rRNAs interspersed with tRNAs. Endonucleolytic cleavage of tRNAs at the 5′ end (by RNase P (Holzmann et al., 2008)) and 3′ end (by ELAC2 (Brzezniak et al., 2011)) is required to release individual immature transcripts for further processing; this is known as the tRNA punctuation model (Anderson et al., 1981).

Processing and maturation of mt-mRNAs is relatively straightforward in comparison to their cytosolic counterparts. There are no introns in the human mitochondrial genome. Free 3′ ends of mRNA are stably polyadenylated by the enzyme mitochondrial poly(A) polymerase (hmtPAP) (Tomecki et al., 2004), and polyadenylation is required to complete a functional UAA stop codon in seven mt-mRNAs. However, the wider function of this modification in human mitochondria remains poorly understood, and variable effects on mRNA stability and translation have been observed when polyadenylation is perturbed (see (Rorbach and Minczuk, 2012) for a more detailed discussion). Mt-mRNAs are not 5′-capped (as cytosolic mRNAs are) and do not contain Shine-Dalgarno sequences (as bacterial mRNAs do), and so the mechanisms by which mitochondria regulate transcript stability and translation will provide an interesting basis for future study.

Only a few proteins involved in regulating mt-mRNA stability have been identified to date. LRPPRC, a member of the pentatricopeptide repeat (PPR) family of proteins, has been implicated in the stability and polyadenylation of mt-mRNAs, particularly COX mRNAs (Ruzzenente et al., 2011). LRPPRC interacts with another protein SLIRP (stem-loop interacting RNA binding protein) which together are proposed to bind and stabilise a pool of untranslated RNAs (Ruzzenente et al., 2011).

The decay mechanisms of mRNA in mitochondria are also a matter of current debate. Human Suv3 helicase is involved in the regulation of stability of mature mt-RNAs and for removal of the processing intermediates (Szczesny et al., 2010). The enzyme polynucleotide phosphorylase (PNPase), encoded by *PNPT1*, possesses both PAP and 3′ → 5′ exoribonuclease activities and was initially a candidate for mitochondrial poly(A) tail removal, and possibly RNA turnover in mitochondria (Piwowarski et al., 2003). However, a more recent study demonstrating an intermembrane space localisation of PNPase and a role in RNA import into mitochondria (Wang et al., 2010) has cast doubt upon the relevance of these findings. More work will be required to properly elucidate the function of PNPase in mitochondria. We recently identified PDE12 as a 3′ → 5′ exoribonuclease in the mitochondrial matrix that removes poly(A) tails from mt-mRNAs *in vitro* and *in vivo* (Rorbach et al., 2011).

The two RNA components of the mitoribosome, 12S and 16S, are encoded by mtDNA and require post-transcriptional modifications (base methylation, ribose methylation, and pseudouridylation) for their stability and assembly into the ribosome. Some of the enzymes responsible for these modifications have been identified in humans, and these have been recently described in detail elsewhere (Rorbach and Minczuk, 2012).

Mt-tRNAs also require extensive post-transcriptional modification for their proper folding and stability, as well as their accurate decoding capacity. The CCA trinucleotide is added to the 3′ end of newly synthesised tRNAs by ATP(CTP):tRNA nucleotidyltransferase, necessary for amino acid attachment and proper tRNA positioning at the ribosome (Nagaike et al., 2001). Mt-tRNAs also undergo a number of base modifications, and a detailed review of these has recently been published by Suzuki et al. (2011). As well as base modifications common to cytosolic and bacterial tRNAs (such as methylation, pseudouridylation and queuosine formation) certain tRNAs (Gln, Glu, Lys, LeuUUR and Trp) also carry a mitochondrial-specific modification of wobble-base uridines through incorporation of the amino acid taurine to form 5-taurinomethyluridine (τ-m^5U) (Suzuki et al., 2002). The mammalian enzymes thought to be responsible for this modification, MTO1 and GTPBP3, are homologs of the *Escherichia coli* proteins gidA and mnmE, which create the equivalent bacterial modification, 5-carboxymethylaminomethyluridine ($cmnm^5U$) (Moukadiri et al., 2009). The U34 base is also thiolated (in addition to the τ-m^5 modification) in the cases of the mt-tRNAs Gln, Glu and Lys, forming τ-m^5S^2U. In mammals, this is carried out by mitochondrial tRNA-specific 2-thiouridylase 1 (MTU1) (Umeda et al., 2005), also commonly (but inappropriately) known as tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase (TRMU).

Finally, tRNAs are attached ('charged') with their cognate amino acids by aminoacyl-tRNA synthetases (aaRSs). The human genome encodes 19 aaRSs, with one enzyme per amino acid except $tRNA^{Gln}$, which is charged by an indirect pathway (Nagao et al., 2009). A growing number of mt-aaRSs have been implicated in human disease, as will be discussed later (see also Table 1).

Mitochondria are also unusual in that they encode only a single $tRNA^{Met}$, rather than two separate tRNAs for initiation and elongation. A proportion of the mt-Met-$tRNA^{Met}$ pool is formylated by mitochondrial methionyl-$tRNA^{Met}$ transformylase (MTFMT), which is then tightly bound by $IF2_{mt}$ and directed to the ribosome P site for use in translation initiation (Tucker et al., 2011).

3. Cell physiology

Mutations affecting mt-RNA metabolism can perturb mitochondrial gene expression, resulting in a variety of pathologies due to defective OXPHOS. The obvious consequence of OXPHOS inhibition is an inability to supply sufficient ATP needed by a cell. However,

Table 1
Clinical phenotypes associated with mutations in genes coding for mitochondrial aminoacyl-tRNA synthetases.

Gene	tRNA	Clinical phenotype	Reference
AARS2	Ala	Infantile cardiomyopathy	Gotz et al. (2011)
DARS2	Asp	Leukoencephalopathy with brain stem and spinal cord involvement (LBSL)	Scheper et al. (2007)
EARS2	Glu	Leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL)	Steenweg et al. (2012)
FARS2	Phe	Alpers encephalopathy	Elo et al. (2012)
HARS2	His	Perrault syndrome	Pierce et al. (2011)
MARS2	Met	Autusomal recessive spastic ataxia with leukoencephalopathy (ARSAL)	Bayat et al. (2012)
RARS2	Arg	Pontocerebellar hypoplasia type 6	Edvardson et al. (2007)
SARS2	Ser	Hyperuricemia, pulmonary hypertension, renal failure in infancy and alkalosis (HUPRA syndrome)	Belostotsky et al. (2011)
YARS2	Tyr	Myopathy, lactic acidosis and sideroblastic anemia (MLASA)	Riley et al. (2010)