Pathways of tRNA turnover in eukaryotic cells

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Cell ability to control amount of a transfer RNA is one of the ways to regulate rate of protein synthesis. Because 80–90% dry mass of cells are proteins, the level of translation is determinant to the cell growth. Growth of cells is a key question in tumors therapy and biotechnology.

tRNA turnover consist of a three pathways described in a last few years: exosome and TRAMP complex dependent pathway in nucleus, directed to hypomodified or affected tRNA; rapid tRNA decay pathway involving two 5'–3' exonucleases Rat1 and Xrn1, proposed to occur in nucleus and cytoplasm; stress-activated endonucleolytic cleavage to tRNA halves pathway, founded in cytoplasm with a clear role to direct regulation of translation by tRNA half-molecules inhibition.

Keywords and phrases: tRNA turnover, rapid tRNA decay, polymerase III.

Introduction

Eukaryotic organisms have developed very sophisticated mechanism to control a proper amount of ribonucleic acids. Complex regulation of gene expression, transcription performed by three types of polymerases; specific and highly complex processing pathways; subcellular distributions of different RNA; and multiple turnover pathways. Mentioned systems provide cell a possibility to precise regulate a pool of each RNA main type: mRNA, rRNA, tRNA.

DNA is transcribed by RNA polymerases I, II and III (Pol I, Pol II and Pol III), although recent evidence suggest that plants evolved two adjacent RNA polymerases (Pol IV and Pol V) [1, 2]. Each polymerase synthese own class of genes (Fig. 1): Pol I — precursors of large ribosome subunit; Pol II — pre-messenger and small nuclear RNA; Pol III — mainly transfer RNA; Pol IV and V express non-coding RNAs probably involved in genes silencing [2]. The balance between each other is a key element to regulation cellular metabolism and in consequence, way to response to environmental factors.

tRNA genes contain in their structure unique internal promoter, which is recognized by Pol III transcription factor TFIIIC. The upstream region of class III genes is bound by TFIIIB, second part of preinitiation complex [3–5]. This complex allows Pol III to start RNA synthesis. To increase yield Pol III have possibility to recycle after one round of transcription [5].

New synthesized pre-tRNA undergo few steps of maturation [6, 7]. RNase P catalyzes endonucleolytic cutting of the 5' leader sequence, afterwards 3' trailing sequence are cleavaged. Introns contained by part of tRNA genes are spliced in cytoplasm, most likely at the external surface of mitochondria. Final step of tRNA maturation is nucleotides modification. Order of maturation steps are still discussed. Probably some steps occur cotranscriptionaly like in pre-mRNA maturation, though another are separated, also by localization.

tRNA modification

Great stability of tRNAs is obtained by large variety of modifications. About 100 different modification of nucleotides are known, however each type of tRNA carries own modification pattern. The diversity of modification is multiple, it could be on sugar or bases: methylations, isomerisation of uracil (U) to pseudouracil, conversion U to dihydrouracil, conversion adenosine to inosine, N-acetylation of cytidine, conversion guanosine to wybutosine or queuosine, isopentenylation of adeno-

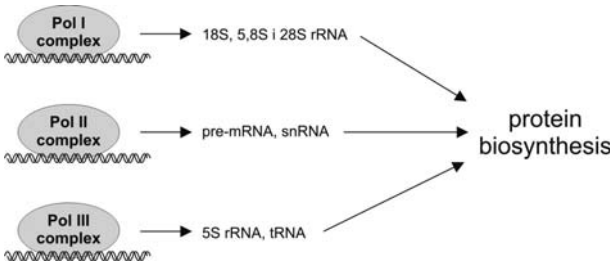

Fig. 1. Described in text.

sine, methoxycarbonylmethylation and thiolylation of uracil [6, 7]. Modification is necessary for stabilization of tRNA tertiary structure, changes thermodynamical features and increases stability by preventing from ribonucleases [8]. Generally, lack of genes coding enzymes involved in modification of the anticodon nucleotides gives more significant influence than modification of a different part of tRNA [6], but depletion of some nonessential enzymes modifying non-anticodon nucleotides reveal characteristic phenotypes i.e. lack of methyltransferases in *trm4Δ trm8Δ* gives thermosensitive or lack of methyltransferase Trm6 are lethal [9, 16]. Variety of significant modifications could be connected with large diversity, multiple modification of one tRNA particle and numbers of tRNAs transporting the same amino acid, but different codon recognizing.

tRNA turnover

Although tRNAs is very stable molecule and is necessary in a large amount in cell, recent findings distinguish three tRNA turnover pathways:

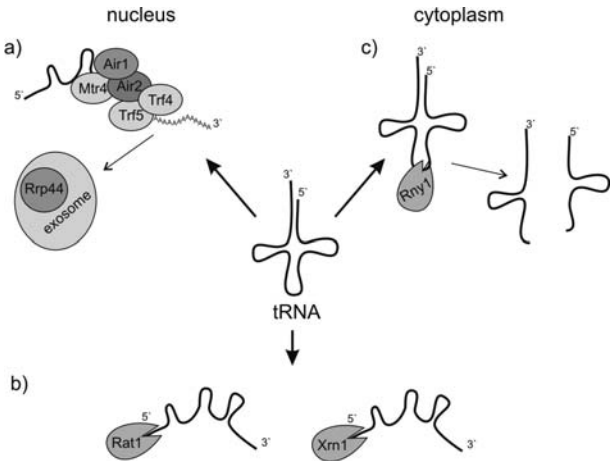

Fig. 2. Pathways of tRNA turnover. Described in text.

Degradation of damaged tRNA in nucleus by TRAMP complex and by the exosome

First tRNA turnover pathway described in year 2004, discovered by mutations in *RRP44* and *TRF4* genes found as a suppressors of *trm6-504* mutant. Trm6p is m^1A methylotransferase, lack of m^1A58 in $tRNA_i^{Met}$ results temperature-sensitive growth phenotype this is a reason of $tRNA_i^{Met}$ instability [7, 9] — altered interaction between loops of tRNA particle. In consequence, tRNA turnover pathway recognize this particle as defected and direct it to exosome degradation. Nuclear complex of proteins Trf4p, Air1/Air2 and Mtr4 recognizes improper tRNA, adds short poly(A) tail and activates nuclear exsosome degradation [10]. Air1/Air2 are RNA binding protein, Mtr4 is ATP-dependent 3'–5' helicase and Trf4/Trf5 are poly (A) polymerases that catalyze addition of short poly(A) tail [10, 11]. Poly(A) provide a recognition to degradation by exosome subunit Rrp44 (Fig. 2a) [12].

Moreover TRAMP complex with Rex1, a 3'–5' exonucleases, plays role in turnover of tRNAa with both unprocessed and processed 3' extension, but not spliced [13, 14].

Orthologues of TRAMP complex proteins were found (beyond in bakery yeasts) in *Schizosaccharomyces pombe*, Arechea and *Homo sapiens*, that indicate evolutionary conserved tRNA nuclear surveillance pathway by the TRAMP complex [15].

Rapid tRNA decay pathway

Second pathway was discovered by analysis of temperature-sensitive growth defect of strains with double deletion of nonessential tRNA modification enzymes [16]. Temperature-sensitive phenotype was an effect of a rapid degradation of hypomodified $tRNA^{Val(AAC)}$ in *trm4Δ trm8Δ* strain. In wild type strain Trm4p catalyzes m^5C modification of cytosine 34, 40, 48 and 49, additionally, Trm8p catalyzes m^7G modification of guanine 46. Only one enzyme absence have no effect to growth phenotype. Name of rapid tRNA decay (RTD) pathway fallowing decrease of $tRNA^{Val(AAC)}$ level observed since 4^{th} minute after shift to 37°C. Degradation is independent to the TRAMP complex and the nuclear exosome.

Two 5'–3' exonucleases, Rat1 and Xrn1 was identified as elements of RTD pathway (Fig. 2b). Rat1 is nuclear localized single-stranded exonuclease also involved in snRNA and pre-rRNA processing, moreover promotes transcription termination by Pol II [17, 18]. Xrn1 is component of P bodies — cytoplasmic structures engaged in mRNA decay; also plays role in rRNA maturation [19]. Surprising is localization of both exonucleases (Rat1 — nucleus, Xrn1 — cytoplasm) if we notice that deletion each one have additional effect to the other to rescue *trm4Δ trm8Δ* growth at 37°C [16].

Upon stress degradation by cleavage in cytoplasm

This pathway, firstly described in nutrient starved *Tetrahymena* and few years later in nutrient deprivation

bacteria and protozoa, oxidative stressed yeasts and stressed mammalian cells and tissues [7]. Enzymes that catalyzes tRNA cleavage have been identified in yeast and mammalian cells. In *Saccharomyces cerevisiae*, the tRNA anticodon is endonucleolytic cleavaged by Rny1; a member of RNase T2 family that residues in vacuole [20]. Upon oxidative stress condition Rny1 is released to cytoplasm and cut mature tRNA (Fig. 2c) [20]. In human cells cleavage is catalyzed by angiogenin [21, 22]. Angiogenin is a member of RNase A family, which is kept in inactive state by interaction with inhibitor RNH1 and activated by stress trough release to cleavage of tRNA.

Surprisingly, level of mature tRNA is relatively stable, however 5' half-molecules product of cleavage inhibit protein synthesis [21]. It indicates, that transport of amino acid to translation isn't only one role of tRNA in cell. Endonucleolytic cleavage pathway also shows that turnover pathways could play more important role than only decreasing of tRNA level.

Future prospects

In comparison to well known degradation of Pol II transcripts, the knowledge about degradation of tRNAs, main Pol III transcripts is poor. However general mechanisms are described and may constitute some basis to future investigation. One of the most interesting topic is coupling of transcription and turnover of transcripts. This question, well described for Pol II is some new way for Pol III researchers. Our team have some interesting data that suggest existing of coupling polymerase III transcription control and RTD pathway.

Furthermore, work about decrease of tRNA level in tumor cells is one of the most applicable question. Influence of additional amount of $tRNA_i^{Met}$ to tumor growth was well described [23]. Mouse fibroblasts with extra copies of gene coding initiator $tRNA^{Met}$ shows enhanced proliferation and oncogenic transformation. Obtain proteins or chemicals compounds to increase turnover pathway(s) only in tumor cells could be repressors for tumor growth.

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