Transfer RNA modifications and modifying enzymes in *Saccharomyces cerevisiae*

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Abstract

Transfer RNAs are adaptor molecules, which decode mRNA into protein and, thereby, play a central role in gene expression. During the maturation of a primary tRNA transcript, specific subsets of the four normal nucleosides adenosine, cytidine, guanosine, and uridine are modified. The formation of a modified nucleoside can require more than one gene product and may involve several enzymatic steps. In the last few years, the identification of gene products required for formation of modified nucleosides in tRNA has dramatically increased. In this review, proteins involved in modification of cytoplasmic tRNAs in *Saccharomyces cerevisiae* are described, emphasizing phenotypic characteristics of modification deficient strains and genetic approaches used to determine the *in vivo* role of modified nucleosides/ modifying enzymes.

1 Introduction

Transfer RNAs are characterized by a variety of different modified nucleosides, which are derivatives of adenosine (A), cytidine (C), guanosine (G), and uridine (U). In total, 86 different modified nucleosides have been described in tRNA from organisms within the three domains of life, Archaea, Bacteria, and Eukarya (Rozenski et al. 1999). Some modified nucleosides are found in all three domains and some are even found in identical positions of the tRNA, suggesting a conserved function (Björk 1986). In this review, we will discuss modified nucleosides and their modifying enzymes in the budding yeast *Saccharomyces cerevisiae*, but where appropriate, also include descriptions from other organisms. The discussion is focused on modification of cytoplasmic tRNAs.

2 tRNA maturation

In eukaryotic cells, tRNA genes are transcribed by RNA polymerase III generating precursor forms with a 5´ leader, a U-rich 3´ trailer, and sometimes an intervening sequence that undergo a series of processing events to yield mature functional tRNAs. The enzyme responsible for endonucleolytic cleavage of the 5´ leader is

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Ribonuclease P (RNase P), a ribonucleoprotein found in all domains of life (Altman et al. 1995; Xiao et al. 2002; Hartmann and Hartmann 2003). One tRNA species ($tRNA^{His}$) has an unusual 5′ end with an extra 5′ GMP residue (G_{-1}), which is added after transcription and RNase P cleavage by a guanylyltransferase (Cooley et al. 1982; Pande et al. 1991; Gu et al. 2003). The removal of the 3′ trailer of pre-tRNA in eukaryotes occurs by either an endo- or exonucleolytic mechanism (Papadimitriou and Gross 1996). A conserved endoribonuclease capable of processing the 3′ end has been identified in several organisms, including yeast (Schiffer et al. 2002; Takaku et al. 2003). The primary pathway of 3' processing is endonucleolytic due to the presence of the Lhp1 (La) protein, which binds to 3′-terminal U residues of the pre-tRNA and protects the end from exonucleolytic digestion (Yoo and Wolin 1994, 1997). The Lhp1 protein also promotes correct folding of pre-tRNAs (Chakshusmathi et al. 2003). The 3′ CCA end present in mature tRNA is not encoded in eukaryotic tRNA genes and has to be added posttranscriptionally by the ATP(CTP):tRNA nucleotidyl transferase (Aebi et al. 1990; Chen et al. 1990). In *S. cerevisiae*, genes for ten different tRNA species contain introns (Hani and Feldmann 1998) that are always located one nucleotide 3′ of the anticodon. Splicing of the pre-tRNAs requires three different enzymes, a tRNA splicing endonuclease that excises the intron, a tRNA ligase to join the two tRNA half molecules and a phosphotransferase to remove a 2′ phosphate at the splice junction (Abelson et al. 1998).

Steps in the maturation of tRNA are ordered. The removal of the 5′ leader normally precedes processing of the 3′-end and in *S. cerevisiae* splicing usually follows end maturation (O'Connor and Peebles 1991). However, a specific order of maturation events is not obligatory as removal of the 3′ trailer can occur before 5′-end processing (Kufel and Tollervey 2003) and some pre-tRNAs can be spliced before end maturation (O'Connor and Peebles 1991). RNase P and some pre-tRNAs are localized primarily to the nucleolus, indicating that 5′-end processing is performed in this compartment (Bertrand et al. 1998). In contrast, the tRNA splicing endonuclease is predominantly cytoplasmic and associated with the mitochondrial surface (Huh et al. 2003; Yoshihisa et al. 2003). Thus, the trafficking of pre-tRNA through various cellular compartments is likely to influence the order of processing events. A nuclear system that senses the status of tRNA processing and transmits a signal to the translational machinery has been proposed (Qiu et al. 2000). This model is based on the evidence that mutations or conditions that interfered with 5'- and 3'-end tRNA processing derepressed translation of the *GCN4* mRNA (Qiu et al. 2000). Recent evidence suggests the presence of a nuclear mechanism to degrade aberrant pre-tRNAs (Kadaba et al. 2004). For a detailed discussion about nuclear surveillance, see Chapter by Anderson and Droogmans in this volume.