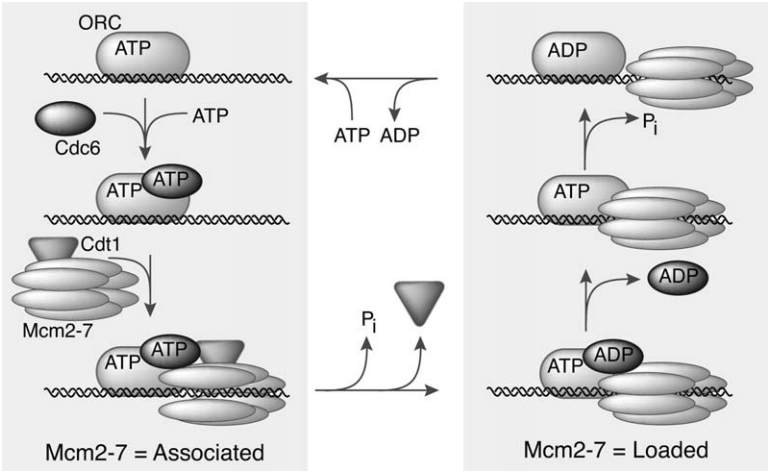

Figure 1. A Model for Mcm2-7 Loading by the ORC and Cdc6 ATPases

ATP bound ORC first binds origin DNA. Cdc6 then binds ORC and ATP. Cdt1 and Mcm2-7 (possibly as a complex) associate with ORC and Cdc6 at the origin. ATP hydrolysis by Cdc6 leads to the loading of Mcm2-7 complexes on DNA and the release of Cdt1 from the origin. Cdc6 association is destabilized by Cdc6 ATP hydrolysis. ATP hydrolysis by ORC completes the Mcm2-7 loading reaction allowing further rounds of Mcm2-7 loading. The figure and figure legend are reprinted from Randell et al. (2006).

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Modified View of tRNA: Stability amid Sequence Diversity

In a recent issue of *Molecular Cell*, a report from the Phizicky lab challenges two commonly held notions regarding tRNAs: (1) tRNAs are stable molecules shielded from the machinery that cause other RNAs to have short half-lives, and (2) the many modifications found on tRNAs serve only subtle biological functions. Alexandrov et al. (2006) show that tRNA modifications can play an important role in tRNA stability, as without particular modifications, tRNA half-life can be reduced from hours to minutes by a new turnover pathway.

RNA molecules from all forms of life have been found to contain chemical modifications of nucleosides that are added to the RNA posttranscriptionally—over a hundred different modifications in all (Grosjean and Benne, 1998, Grosjean, 2005). These modifications serve a variety of functions, often providing specialized recognition for use in cellular pathways. Eighty percent of these modifications are found in the transfer RNA (tRNA) molecules that bring specific amino acids into the peptidyl transferase centers of ribosomes, thus decoding the genetic code expressed in messenger RNAs.

A question that has long intrigued RNA researchers is why so many modifications of the tRNAs are retained. Genetic studies in bacteria and yeast have shown that the great majority of the tRNA modification enzymes are not essential for life and, indeed, that deletion of individual modification enzymes have no serious growth phenotypes. Although a small number of modifications near the termini and anticodons of tRNAs have been shown to affect aminoacyl charging and fine tuning of codon recognition (Hopper and Phizicky, 2003, Agris, 2004), many of the modifications occur at positions that would not obviously impact these functions. Two prominent explanations have been discussed for the frequency and diversity of tRNA modifications. The first is that the individual modifications provide additional options for highly selective molecular recognition of specific tRNAs. The second explanation is an answer to a unique structural problem faced by tRNA molecules. Transfer RNAs must all conform, within narrow parameters, to a specific tertiary structure to fit into the ribosomal active sites and also be recognized by common processing enzymes (e.g., RNase P). However, each tRNA must have unique sequence determinants to be recognized as the carrier of a different amino acid. In varying the sequences of the dozens to hundreds of different tRNAs needed by the cell, it is inevitable that certain sequences will be less likely to fold into the requisite structure or to maintain a single, stable structure once folded.

In yeast modification of tRNA at position G46 to 7-methyl-G (m^7G_{46}) is catalyzed by the collaboration of the yeast *TRM8* and *TRM82* gene products (Alexandrov et al., 2005). Alexandrov et al. (2006) show that cells lacking *TRM8* or *TRM82* and any one of seven tested other

tRNA modifications genes have growth defects and that $tRNA^{Val(AAC)}$ in these cells lacking a combination of m^7G_{46} and another modification moiety is degraded extremely rapidly, on the order of mRNAs. The reason for the turnover is presumably structural instability, because rapid turnover of presynthesized tRNAs is observed at elevated temperatures, but it is possible that loss of specific recognition by binding partners could also be responsible. The conclusions are likely general for other tRNAs and their modifications, because previous studies reported synthetic growth defects by the combination of a mutant $tRNA^{Ser(CGA)}$ and any one of several modification defects. In these strains, the levels of mature mutant $tRNA^{Ser(CGA)}$ lacking the modifications were highly reduced (Johansson and Byström [2004] and references therein). Moreover, precursor of initiator $tRNA^{Met}$ lacking an essential 1-methylation of the adenosine at tRNA position 58 (m^1A_{58}) is subject to degradation by a nuclear surveillance mechanism (Kadaba et al., 2004).

Alexandrov et al. (2006) is seminal not only because it demonstrates the need for the modifications on normal tRNAs but also for the very fact that the existence of a previously undetected pathway for rapid turnover of the existing tRNAs is demonstrated. The extreme stability of tRNAs and the ability to regenerate both the −CCA 3′ terminus and the charged form has historically left in doubt whether there is any specific salvage pathway that turns over mature tRNAs. The nuclear Trf4/Rrp6 surveillance pathway, discovered in yeast, that degrades the precursor of initiator $tRNA^{Met}$ lacking m^1A_{58} involves polyadenylation of the tRNA by the novel poly(A) polymerase (Trf4) followed by turnover catalyzed by the nuclear exosome (Kadaba et al., 2004). The turnover system (RTD) that degrades undermodified $tRNA^{Val(AAC)}$ discovered by Phizicky and colleagues is clearly distinct not only because of the rapid deaminoacylation and degradation of mature tRNAs but also because it is independent of the Trf4 and Rrp6 proteins involved in the tRNA nuclear surveillance system.

What is not yet clear is whether the RTD pathway for tRNA turnover is nuclear or cytoplasmic. Mature tRNAs usually reside in the cytoplasm, and therefore, one might have anticipated that their turnover would also occur there. However, it has recently been shown that amino acid deprivation or mutations that interfere with tRNA aminoacylation cause tRNAs to move retrograde from the cytoplasm to the nucleus (Shaheen and Hopper, 2005; Takano et al., 2005). Because the rapid loss of $tRNA^{Val(AAC)}$ occurs concomitantly with accumulation of uncharged $tRNA^{Val(AAC)}$, undermodified tRNA may move to the nucleus prior to its degradation in this subcellular compartment. The temperature-sensitive growth defect of cells lacking m^7G_{46} and another tRNA modification should provide a robust genetic system to identify this newly discovered RTD tRNA turnover machinery and to learn where in the cell tRNA degradation takes place.

This work by Phizicky and coworkers makes it clear that there is much to learn about the in vivo roles of RNA modifications and that there are RNA surveillance machineries that remain uncharacterized. The now proven role of RNA modifications in RNA structure should also serve as a precautionary note for those biochemical studies that employ naked RNAs obtained by in vitro transcription.

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Chaperoning through the Mitochondrial Intermembrane Space

The first high-resolution structure of a mitochondrial translocase complex, the Tim9-Tim10 chaperone, is reported by Webb et al. (2006) in a recent issue of *Molecular Cell*, providing important insight in the transport of hydrophobic proteins through the aqueous intermembrane space and the mechanisms of protein assembly.

Mitochondria contain about 1000 different proteins, 99% of which are synthesized as precursors in the cytosol. An elaborate system of transport machineries is