Editing and modification in trypanosomatids: the reshaping of non-coding RNAs

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Abstract

Trypanosomatids include a number of protozoan parasites that infect over 27 million people worldwide. Besides their medical importance, these organisms have also provided a wealth of novel biological discoveries including: RNA editing, mRNA trans-splicing, eukaryotic poly-cistronic transcription, and a mechanism for large-scale mitochondrial tRNA import. For many years, the study of RNA post-transcriptional modification in trypanosomatids has lagged behind when compared to bacterial, yeast, and animal systems. However, the discovery of editing in tRNA and 7SL RNAs has produced renewed interest in the processing of non-coding RNAs in these organisms. This chapter will compile what is currently known about RNA editing and modification in trypanosomatids, emphasizing the role these processes play in the structural reshaping of non-coding RNAs. Due to a number of substantive recent reviews, mRNA editing will not be the subject of this chapter. In addition, snoRNA-mediated modification of ribosomal RNAs will be covered in chapter 8 of this book.

1 Introduction

This chapter will address what is currently known about the editing and modification of non-coding RNAs in trypanosomatids. Despite their medical importance and the prospect of RNA processing events as therapeutic targets, research on editing and modification of non-coding RNAs in trypanosomatids has lagged behind when compared to other model systems such as *S. cerevisiae*, *E. coli*, and *Xenopus* (to name a few). It was the discovery of mRNA editing in 1988 by Rob Benne and colleagues that led to a renewed interest in RNA processing in these organisms. Since its discovery, much of the research on RNA editing in trypanosomatids has concentrated in the study of mRNA editing in mitochondria. Indeed great strides have been made towards understanding this important mechanism of mRNA processing and many of the factors involved have now been identified. Owing to the novelty and serendipitous nature of discovering editing, mRNA editing was widely accepted as the rule in trypanosomatids, perhaps to the exclusion of other types of editing. The more recent discovery of C to U editing in non-coding RNAs both in the cytosol and the mitochondria then expanded the variety of editing

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mechanisms in trypanosomatids, highlighting the connection between editing and modification in these medically important organisms.

Rather than concentrating on a particular editing mechanism, we will discuss a number of independent examples of editing and/or modification of various RNAs, highlighting how by affecting RNA structure both editing and modification may act alone or in concert to modulate RNA function. Unlike other systems (animal cells, yeast, and bacteria), little is known about how editing and modification is specified in trypanosomatids and neither the enzymes, the factors nor the actual mechanism(s) has been elucidated. To date, there are only two examples of editing of non-coding RNAs in trypanosomatids: editing of the 7SL RNA in the cytosol and the C to U editing of $tRNA^{Trp}$ in mitochondria. As we will further elaborate in this chapter, in the former example, the connection between editing and structural reshaping is clear, while in the latter example, editing directly affects decoding but may still impart subtle changes that affect tRNA anticodon structure.

This chapter will cover 4 specific examples in trypanosomatids where editing and/or modification may play a role in affecting structure and function of non-coding RNAs: 1) we will discuss the role of modifications in trans-splicing, 2) the possible role of editing in protein secretion, 3) the role of modification on tRNA trafficking, and 4) the role of editing and modification in mitochondrial tRNA function.

2 RNA modification and trans-splicing

In trypanosomatids, most, if not all, protein-coding genes in the nucleus are transcribed into long poly-cistronic pre-mRNAs that contain multiple open reading frames (Muhich and Boothroyd 1988). Individual mRNAs are then post-transcriptionally processed into defined units by polyadenylation and trans-splicing (Campbell et al. 1984; Parsons et al. 1984; Walder et al. 1986; Ullu and Tschudi 1990; Laird et al. 1985). In trans-splicing, a 39-41 nucleotides-long leader is attached to the 5' end of every nucleus-encoded mRNA creating a mature transcript (Parsons et al. 1984). Thus, every mature nucleus-encoded mRNA in these cells has precisely the same 5' end. Prior to trans-splicing, the 5' end of the spliced leader RNA (SL RNA) is heavily modified to form the cap structure (Laird et al. 1985; Lenardo et al. 1985; Freistadt et al. 1987; Perry et al. 1987). In this sense, trans-splicing serves two main functions: it provides a mature 5' UTR to mRNAs and it also adds the cap structure (Fig. 1), which is a trademark of translation for most eukaryotic mRNAs.

Among eukaryotes the structure of the 5' end cap is highly conserved. Synthesis of 7-methylguanosine (m^7G) is preceded by the non-templated addition of GMP to the 5' end of mRNAs followed by the methylation of the newly added guanosine, at the N7 position, to form m^7G. This process creates the minimal cap structure, m^7GpppN, where N is any nucleotide (Quiocho et al. 2000). Cap structure formation in trypanosomatids differs from that of other eukaryotes in both the number and type of post-transcriptional modifications it contains (Bangs et al. 1992). Like