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Transcripts in the *Plasmodium* Apicoplast Undergo Cleavage at tRNAs and Editing, and Include Antisense Sequences

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The apicoplast, an organelle found in *Plasmodium* and many other parasitic apicomplexan species, is a remnant chloroplast that is no longer able to carry out photosynthesis. Very little is known about primary transcripts and RNA processing in the *Plasmodium* apicoplast, although processing in chloroplasts of some related organisms (chromerids and dinoflagellate algae) shows a number of unusual features, including RNA editing and the addition of 3′ poly(U) tails. Here, we show that many apicoplast transcripts are polycistronic and that there is extensive RNA processing, often involving the excision of tRNA molecules. We have identified major RNA processing sites, and have shown that these are associated with a conserved sequence motif. We provide the first evidence for the presence of RNA editing in the *Plasmodium* apicoplast, which has evolved independently from editing in dinoflagellates. We also present evidence for long, polycistronic antisense transcripts, and show that in some cases these are processed at the same sites as sense transcripts. Together, this research has significantly enhanced our understanding of the evolution of chloroplast RNA processing in the Apicomplexa and dinoflagellate algae.

Key words: Apicoplast; chloroplast; RNA processing; *Plasmodium*; antisense RNA; RNA editing.

Introduction

Some of the major drugs used for combatting malaria, such as the antibiotics doxycycline and clindamycin, target gene expression in the apicoplast, an organelle found in *Plasmodium* and other members of the Apicomplexa group of parasitic eukaryotes. The apicoplast is a secondary plastid, resulting from an endosymbiosis event between the ancestor of the Apicomplexa and a member of the red algal lineage (Botté et al. 2013; Gardner et al. 1991b; Lemgruber et al. 2013; Wilson et al. 1996). The apicoplast has lost the ability to carry out photosynthesis, yet retains a circular genome of approximately 35 kbp, containing genes for numerous proteins, tRNAs and rRNAs (Fig. 1). Inhibition of apicoplast transcription and translation is lethal to the parasite, as shown by treatment by rifampicin (a transcription inhibitor), thiostrepton or doxycycline (translation inhibitors) (Goodman et al. 2007). Inhibition of apicoplast DNA replication is also lethal (Fichera and Roos 1997).

Despite the importance of antibiotics that target the apicoplast in the control of malaria, remarkably little is known about transcription,

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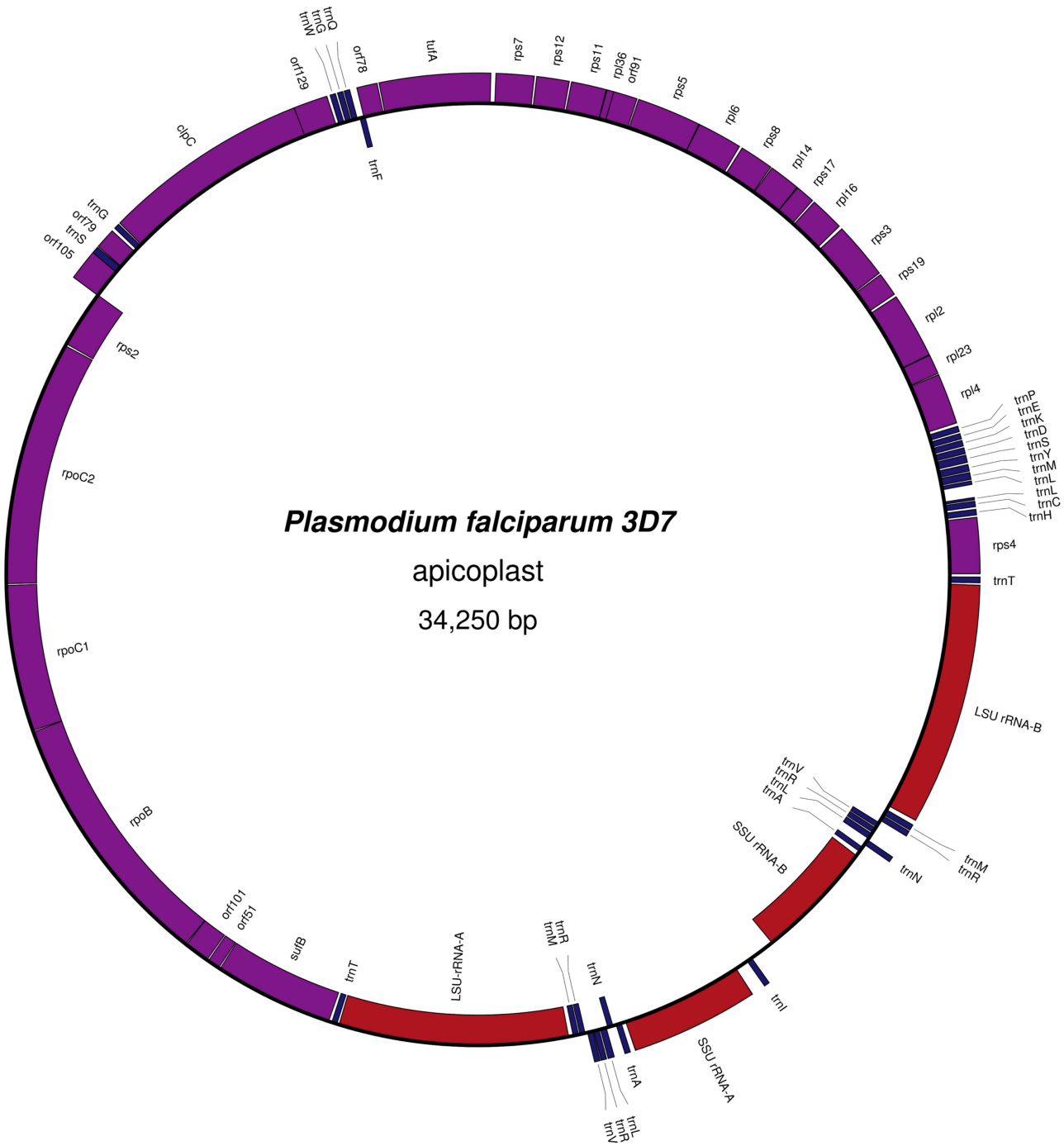

Figure 1. *Plasmodium falciparum* apicoplast genome. Purple indicates protein-coding genes, blue indicates tRNA genes and red indicates rRNA genes. Genome drawn using OrganellarGenomeDRAW (Lohse et al. 2013).

post-transcriptional processing or translation in the organelle. Northern blots using total *Plasmodium* RNA revealed that the transcription of at least some apicoplast genes is likely to be polycistronic, as the bands seen were larger than would be expected for a single-gene RNA molecule (Gardner et al. 1991a, b). RT-PCR carried out on two regions indicated that some ribosomal genes were transcribed as part of a polycistronic molecule (Wilson et al. 1996), and all tRNA molecules have been shown to be transcribed (Preiser et al. 1995). There are no recognisable eubacterial promoter elements