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Toxoplasma gondii Alba Proteins Are Involved in Translational Control of Gene Expression

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Abstract

Molecular mechanisms controlling gene expression in apicomplexan parasites remain poorly understood. Here, we report the characterization of two *Toxoplasma gondii* homologs of the ancient archeal Alba proteins named TgAlba1 and TgAlba2. The targeted disruption of TgAlba1 and TgAlba2 genes in both virulent type I and avirulent type II strains of *T. gondii* reveals that TgAlba proteins may have an important role in regulating stress response. We found that although the steady-state level of the *Tgalba2* transcript is increased in the ΔTgalba1 null mutant parasites, the cognate TgAlba2 protein is undetectable, suggesting that TgAlba1 is required for translation of TgAlba2. Using a tandem affinity purification tag strategy combined with proteomic analyses, we provide evidence that many factors known to be involved in the translation machinery are co-purified with TgAlba1 and TgAlba2. We further performed RNA pull-down and microarray analyses to show that TgAlba1 and TgAlba2 bind to more than 30 RNAs including their own transcripts. Moreover, we demonstrate that the tight translational regulation of the *TgAlba2* endogenous transcript relies on the presence of both its 3′ untranslated region and that of the TgAlba1 protein. Thus, our findings on TgAlba1 and TgAlba2 are consistent with a role in gene-specific translation.

Introduction

Toxoplasma gondii belongs to the apicomplexan phylum of eukaryotic parasites. *T. gondii* is responsible for encephalitis in immunocompromised individuals and birth defects in the offspring of infected mothers. The genetic tractability of *T. gondii* makes it a useful model for the study of other apicomplexan parasites.[1] The life cycle of *T. gondii* is complex with multiple differentiation steps that are critical to parasite survival in intermediate and final hosts.[1] It has been described that the gene expression profiles change dramatically during cell cycle and differentiation.[2,3] Nonetheless, the molecular mechanisms that control gene expression are still poorly understood in *T. gondii* and other apicomplexan parasites. The identification of numerous enzymes that modify or remodel chromatin and the discovery of a specific set of chromatin marks at the promoters of expressed genes suggest an important role for chromatin structure in *T. gondii* gene regulation.[4–6] However, the identification of a novel family of plant-like transcription factors together with DNA motifs required for the transcription of genes indicates that the control of transcription may also be important for the regulation of gene expression in apicomplexan parasites.[7,8] For instance, it has been described that *Plasmodium* sexual development relies on transcriptional and translational control of specific genes, indicating that both mechanisms are important

for protein expression.[9,10] Little is known about translational control of gene expression in *T. gondii* although several proteins deduced from the parasite genome are potential factors for translational regulation. Furthermore, discrepancies between the transcriptome and the proteome have been reported for *T. gondii* tachyzoites, suggesting that translational control is likely pertinent to gene expression in the parasite.[11] For instance, it has been described that even though the transcript level corresponding to *bsr4* gene was shown to be equally abundant in both rapidly replicating tachyzoites and dormant bradyzoites, the bsr4 protein is up-regulated only in bradyzoites.[12] It seems that *T. gondii* is able to shut down the global translation of proteins after stress-induced phosphorylation of the eukaryotic initiation factor eif2a.[13] It has been postulated that this mechanism of translational control through phosphorylation of eif2a may be particularly important for extracellular survival and parasite differentiation.[13] In addition, the recent discovery of a large repertoire of small RNA species in tachyzoites indicates that translational control may be underestimated as a mechanism that regulates gene expression in *T. gondii*.[14]

In an effort to discover proteins that are potentially involved in the regulation of gene expression in *T. gondii*, two proteins containing a domain similar to Alba proteins of archaebacteria were identified.[15] Proteins containing an Alba domain have been first described in the crenarchaeal genus but are also present in eukaryotes including apicomplexan parasites.[16] In archea, Alba proteins are major components of chromatin and mediate transcriptional repression through interactions with nucleic acids. This interaction requires lysine acetylation for protein dimerization.[17,18] However, Alba proteins in archea are also known to bind RNA *in vivo*.[19] Crystal structures of Alba proteins revealed that their fold is similar to prokaryotic translation initiation factor 3 and other RNA binding proteins.[16] In *Plasmodium berghei*, three proteins containing an Alba domain were co-purified with a translation repression complex that is crucial for the sexual cycle in mosquito.[20] Similarly, Alba proteins have also been reported to be involved in the control of translation initiation and differentiation in *Trypanosoma brucei*.[21,22] In contrast, *Plasmodium falciparum* Alba proteins have been shown to be able to bind both DNA and RNA.[23,24] Altogether, these studies reveal that Alba proteins may have evolved to differentially bind DNA and RNA. In this report, we provide evidence that *T. gondii* Alba proteins define a new eukaryotic RNA binding protein family that is involved in the translational regulation of specific transcripts and may also have an important role in parasite differentiation.

Results

Molecular characterization of TgAlba proteins

T. gondii Alba proteins were originally identified in a screen for candidate factors involved in the regulation of gene expression.[15] TgAlba2 (TGME49_018820) is a small 148-amino-acid protein while TgAlba1 (TGME49_021380) defines a longer protein (247 amino acids) containing the Alba domain at its N-terminus (Fig. S1). TgAlba2 belongs to the Rpp20/Pop7 subfamily, and TgAlba1, which possesses a long carboxy-terminal tail of ~100 amino acids composed of imperfect RGG repeats, belongs to the Rpp25/Pop6 subfamily.

Recombinant glutathione *S*-transferase fusion proteins were produced, purified, and used to raise polyclonal antibodies to better characterize TgAlba1 and TgAlba2 proteins. Using cytoplasmic and nuclear protein extracts from *T. gondii* tachyzoites and Western blots, we showed that two proteins of 15 and 35 kDa were recognized by the antibodies specific to TgAlba2 and TgAlba1, respectively (Fig. 1a). As expected, the glycolytic enzyme lactate dehydrogenase LDH1[25,26] was mostly detected in the cytoplasmic fraction of the parasite, and the *T. gondii* nuclear factor TgNF3[15] was mainly found in the nuclear fraction. In intracellular parasites, TgAlba1 and TgAlba2 were mainly detected in the cytoplasmic fractions (Fig. 1a, left and upper panels). In contrast, TgAlba1 and TgAlba2 are significantly present in the nuclear fraction of extracellular parasites (Fig. 1a, left and lower panels), suggesting that these proteins are enriched in the two subcellular compartments according to the extracellular or intracellular status of the parasites. We confirmed the nuclear/cytoplasmic localization for TgAlba1 and TgAlba2 by indirect immunofluorescence assays (IFAs) (Fig. 1b and c), with a predominant cytoplasmic *versus* nuclear signal in intracellular parasites. We noticed that TgAlba1 and TgAlba2 proteins showed a clear perinuclear labeling with granular signals around the nuclei of extracellular parasites (Fig. 1c).

It has been previously described that RNA granules were present in extracellular parasites upon induction by various stresses.[27] We found that both TgAlba1 and TgAlba2 proteins co-localized with the RNA granules (Fig. 1d and e). Interestingly, upon induction with high salt buffer, TgAlba1 (Fig. 1d) and TgAlba2 (Fig. 1e) signals are concentrated in discrete foci in extracellular parasites. We conclude that TgAlba proteins co-localize with RNA granules in extracellular parasites of *T. gondii* under stress conditions.

TgAlba1 affects response to alkaline stress *in vitro*

In order to characterize the biological function of these proteins, we performed a knock-out of each