Drug target for pathogenic amoebae

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Horizontal Gene Transfer of a Chlamydial tRNA-Guanine Transglycosylase Gene to Specific Algal and Protozoan Lineages: A Putative Drug Target for Pathogenic Amoebae

Abstract:

tRNA-guanine transglycosylases are found in all domains of life and mediate the base exchange of guanine with queuine in the anticodon loop of specific tRNAs. They are also known to regulate virulence in bacteria such as *Shigella flexneri,* which has prompted the development of drugs that inhibit the function of these enzymes *.* Here we report a group of tRNA-guanine transglycosylases in eukaryotes (algae and protozoa) which are more similar to their bacterial counterparts than previously characterized eukaryotic tRNA-guanine transglycosylases. *In silico* analysis of these bacterial-like tRNA-guanine transglycosylasesrevealed thatthe majority are predicted to be targeted to mitochondria, although some are likely to localize to chloroplasts, the secretory pathway or the cytosol. We provide evidence demonstrating that the gene encoding theseenzymes was acquired by these eukaryotic lineages via horizontal gene transfer which from the Chlamydiae. Given that the *S. flexneri* tRNA-guanine transglycosylase can be targeted by drugs, we propose that the bacterial-like tRNA-guanine transglycosylases could potentiallybe targeted in a similar fashion in pathogenic amoebae that possess these enzymes such as *Acanthamoeba castellanii.*

Keywords: mitochondria, tRNA-guanine transglycosylase, queuine tRNA-ribosyltransferase, horizontal gene transfer, tRNA, queuosine, Chlamydiae

Abbreviations:

TGTase: tRNA-guanine transglycosylase

E-TGTase: Eukaryotic tRNA-guanine transglycosylase

B-TGTase: Bacterial tRNA-guanine transglycosylase

BL-TGTase: Bacterial-like tRNA-guanine transglycosylase

HGT: Horizontal gene transfer

Introduction

Base modification of tRNAshas been implicated in tRNA structure, aminoacyl tRNA synthetase interaction andinfluencing codon-anticodon basepairing[1]. The function of the modification will depend on itstype and the position of the modified base. For example, most bases that are modified within the anticodon loop (positions 34-36) of tRNAsare important for accurate translation by facilitating interactions with their cognate codons in mRNAs [1]. One such modification that influences codon-anticodon basepairingis the incorporation of queuine within the anticodon loop.

Queuosine is a modified guanosine analogue found in tRNAs from all three domains of life. Despite its wide phylogenetic distribution, queuosine is only found in a select group of tRNAs ($tRNA^{His}$, $tRNA^{Asp}$, $tRNA^{Tyr}$ and $tRNA^{Asn}$) [2]. Reduced incorporation of queuosine in these tRNAs alters their codon recognition ability and has been linked to various cancers [3, 4].

tRNA-guanine transglycosylases

Queuosine modification of tRNA is mediated by tRNA-guanine transglycosylases (TGTases)(also known as queuine tRNA-ribosyltransferases). TGTases catalyze this modification via base exchange where the guanine at position 34 of the tRNA is post-transcriptionally

removed and substituted with queuine or a queuine precursor [5]. Eukaryotes are not capable of *de novo* queuine synthesis but acquire it through diet or their gastrointestinal microbiota [6]. After its acquisition, the eukaryotic TGTase (E-TGTase) mediates the replacement of guanine with queuine in the anticodon loop. In contrast, queuosine modification of bacterial tRNA is more complex. Prokaryotesuse GTP-cyclohydrolase-like enzymes tosynthesizea queuine precursor(e. g. preQ $_1$) from GTP. The bacterial TGTase (B-TGTase) then mediates the base exchange with guanine to incorporate preQ $_1$, unlike E-TGTases that use queuine itself as the substrate. This incorporatedpreQ $_1$ is then modified by *S*-adenosylmethionine tRNA ribosyltransferase to epoxyQ, which is further modified to form queuosine [6]. In addition to tRNA modification, B-TGTasesplay a role in regulating the expression of bacterial genes. TGTase mutants (*vacC*) in the bacterium *Shigella flexneri* exhibit reduced expression of the *virG* and *ipaBCD* genes, which encode virulence factors that facilitate the spread and invasion of the pathogen [7]. This is a result ofthe VacCTGTase beingrequired to modify a single base in *virF* mRNA, which encodes the transcriptional activator of *virG* and *ipaBCD* [8]. Thus, B-TGTases can modify substrates otherthan tRNA and are important mediators of bacterial virulence. As a result, B-TGTases have served as a targetfor the development of drugs that interfere with their function [9]. Here we report a new group of TGTases in eukaryotes that display significantly greater similarity to B-TGTases than E-TGTases. We hereby refer to these proteins as bacterial-like TGTases (BL-TGTases). *In silico* analysis identified 25 BL-TGTases in distinct protozoan and algal lineages and the reason for their similarity to B-TGTases is explored in this article.

Variation in the subcellular localization of bacterial-like tRNA-guanine transglycosylases

To investigate the putative subcellular localization of BL-TGTases, three bioinformatic programs were utilized: Mitoprot [10], Predotar [11] and Target P [12]. The putativelocalizationfor each BL-TGTase was supportedby predictions from at least two of the three programs. Most BL-TGTases possess N-terminal mitochondrial targeting signals (Table 1), suggesting a role in modification of mitochondrial tRNAs. Interestingly, the BL-TGTases from *Ostreococcus lucimarinus* and *Chondrus crispus* were predicted to localize to mitochondria with one program (Predotar) but to the plastid with another (Target P). While it is possible that these two proteins may localize to both organelles, further experimentation is required to elucidate their subcellular locations. The BL-TGTase from the diatom *Phaeodactylum tricornutum* was predicted to localize to the endoplasmic reticulum (ER) of the secretory pathway, indicating it maymodify other substrates in this organelle.

Bacterial-like tRNA-guanine transglycosylase genes originated from a Chlamydial gene acquired via horizontal gene transfer

While the localization of BL-TGTases varied, all 25 of the proteins exhibited higher levels of amino acid similarity to B-TGTases despite their existence in eukaryotes. A Bayesian analysis of phylogeny using MrBayes [13] withBL-TGTases, B-TGTases and E-TGTasesconfirmedthis similarity(Figure 1). The BL-TGTases were most similar to TGTases from members of the Chlamydiae. In fact, the Chlamydial TGTases were more similar to BL-TGTases than other B-TGTases. Given that Chlamydiaeare bacteria, the topology of the tree in the present study is incongruent with the universal tree of life. Instead, this

topology is consistent with a horizontal gene transfer (HGT) event. That is, the genes encoding BL-TGTases originated from a Chlamydial TGTase-encoding gene that was acquired via prokaryote-to-eukaryote HGT.

In addition to the strong statistical support for the BL-TGTase-Chlamydial TGTase sister group, there are several other factors that support this notion. The Chlamydiaeare known to be major contributors of genes to several eukaryotic genomes via HGT [14, 15]. This includes genes encoding tRNA modification enzymes such as the Chlamydial tRNA guanine methyltransferases found in protozoa, diatoms and algae[16, 17] and Chlamydial tRNA genes in vascular plants [18]. Similarly to the present study, sister groups were observed between the Chlamydial and the horizontally acquired eukaryotic genes in these cases. Lastly, the majority of eukaryotic lineages in which we identified BL-TGTases have previouslybeen reported to possess HGT-derived genes acquired from the Chlamydiae[16, 19]. Thus, the notion that BL-TGTases resulted from the acquisition of a B-TGTase from the Chlamydiaevia HGT in eukaryotes is highlyplausible.

Indirect acquisition of a Chlamydial tRNA-guanine transglycosylase in protozoa via anon-Chlamydialbacterial intermediate

Interestingly, a B-TGTase sequence from the δ-proteobacterium ‘ *Candidatus* Babela massiliensis’clustered with the BL-TGTases of protistsrather than the B-TGTases (Figure 1). Although the protozoan BL-TGTases displayed similarity to Chlamydial B-TGTases, the possibility of a HGT event from ‘ *Ca.* B. massiliensis’to protistswas present. SinceChlamydiae and δ-proteobacteria are not closely related, the phylogeny of their B-TGTases was investigated. Interestingly, the ‘ *Ca.* B. massiliensis’TGTase clustered with

the Chlamydial TGTase clade rather than other δ-proteobacterial (*Pelobacter, Geobacter, Myxococcus, Desulfobulbus*) B-TGTases (Figure 2).

‘ *Ca.* B. massiliensis’and members of the Chlamydiae are found as obligate intracellular symbionts of protists such as *Acanthamoeba* , *Dictyostelium* and *Naegleria* [20, 21]. The presence of both of these bacteria within the one eukaryotic cell would provide the ideal conditions for HGT between them. Therefore, it is likely that at least two independent HGT events have occurred: 1) The Chlamydiae donated a TGTase-encoding gene to an ancestral ‘ *Ca.* B. massiliensis’species; and 2) ‘ *Ca.* B. massiliensis’then donated this gene to theAmoebozoa and Heterolobosea. How the BL-TGTase genes were acquired in the algal lineagesremains to be elucidated, but may have occurred via additional HGT events (either prokaryote-to-eukaryote or eukaryote-to-eukaryote).

Bacterial-like tRNA-guanine transglycosylases as drug targets for pathogenic amoebae

In addition to their role in queuosine modification of tRNAs, TGTases are important for *S. flexneri* virulence [7, 8]. As a result, studies have focused on the development of TGTase inhibitors that specifically target the *S. flexneri* B-TGTase to treat shigellosis, while the E-TGTases of the human host remain unaffected. Some of these inhibitors, such as *lin-* benzoguanine, function by occupying the binding site for preQ $_1$ [22, 23]. While most eukaryotic species that possess BL-TGTases are non-pathogenic, we identified a BL-TGTase in *Acanthamoeba castellanii,* the causative agent of amoebic keratitis and encephalitis. *Naegleria gruberi* , whichalso has a BL-TGTase, is non-pathogenic, but is closely related to *Naegleria fowleri,* the etiologic agent of

primary amoebic meningoencephalitis, which may possess an unidentified BL-TGTase. Given the development of B-TGTase inhibitors has already been achieved, the BL-TGTasesin pathogenic eukaryotes could also potentially be targeted with the same drugs. Alternatively, new inhibitors could be developed following resolution of the BL-TGTase crystal structure. To confirm BL-TGTases as a putative drug target future research should attempt to characterize these proteins and determine if they have retained their prokaryotic functions and mechanism of action.

Concluding remarks

In this report, we have described a group of TGTases in algae and protozoa (BL-TGTases). Theseproteins are predicted to localize to various subcellular locations including mitochondria, chloroplasts, the ER and the cytosol, depending on the organism. Lastly, we showed that via multiple HGT events, BL-TGTases were originallyfrom bacteria of the Chlamydiae lineage. The bacterial origin of these proteins could be exploited in the development of drugs similar to those synthesized for the *S. flexneri* B-TGTase. Research into the identification and synthesis of BL-TGTase inhibitors may provide a novel treatment for infectious diseases which are caused by pathogenic amoebae that possess these proteins.