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Short Communication

Horizontal gene transfer of a Chlamydial tRNA-guanine transglycosylase gene to eukaryotic microbes ☆

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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 tRNAs. They can also 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 eukaryotic microbes (algae and protozoa) which are more similar to their bacterial counterparts than previously characterized eukaryotic tRNA-guanine transglycosylases. We provide evidence demonstrating that the genes encoding these enzymes were acquired by these eukaryotic lineages via horizontal gene transfer from the Chlamydiae group of bacteria. Given that the *S. flexneri* tRNA-guanine transglycosylase can be targeted by drugs, we propose that the bacterial-like tRNA-guanine transglycosylases could potentially be targeted in a similar fashion in pathogenic amoebae that possess these enzymes such as *Acanthamoeba castellanii*. This work also presents ancient prokaryote-to-eukaryote horizontal gene transfer events as an untapped resource of potential drug target identification in pathogenic eukaryotes.

1. Introduction

Base modification of tRNAs has been implicated in tRNA structure, aminoacyl tRNA synthetase interaction and influencing codon–anticodon base pairing (Jackman and Alfonzo, 2013). The function of the modification will depend on its type and the position of the modified base. For example, most bases that are modified within the anticodon loop (positions 34–36) of tRNAs are important for accurate translation by facilitating interactions with their cognate codons in mRNAs (Jackman and Alfonzo, 2013). One such modification that influences codon–anticodon base pairing is 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 $tRNA^{Asn}$) (Katze et al., 1982). Reduced incorporation of queuosine in these tRNAs alters their codon recognition ability and has been linked to various cancers (Emmerich et al., 1985; Meier et al., 1985).

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 (Garcia and Kittendorf, 2005). Eukaryotes are not capable of *de novo* queuine synthesis but acquire it through diet or their gastrointestinal microbiota (Vinayak and Pathak, 2010). 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. Prokaryotes use GTP-cyclohydrolase-like enzymes to synthesize a 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 incorporated $preQ_1$ is then modified by *S*-adenosylmethionine tRNA ribosyltransferase to epoxyQ, which is further modified to form queuosine (Vinayak and Pathak, 2010). In addition to tRNA modification, B-TGTases play 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

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.

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(Durand et al., 1994). This is a result of the VacC TGTase being required to modify a single base in *virF* mRNA, which encodes the transcriptional activator of *virG* and *ipaBCD* (Hurt et al., 2007). Thus, B-TGTases can modify substrates other than tRNA and are important mediators of bacterial virulence. As a result, B-TGTases have served as a target for the development of drugs that interfere with their function (Brenk et al., 2003). 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.

2. Materials and methods

2.1. BL-TGTase identification and analysis

All BL-TGTase proteins were identified using chlamydial B-TGTases as query sequences in the NCBI protein database (BLASTP). Candidate BL-TGT sequences were confirmed using NCBI BLASTP and InterProScan to search for TGTase domains. Similarity of BL-TGTases to B-TGTases and chlamydial-specific B-TGTases was determined using CLUSTAL W and MUSCLE to produce amino acid sequence alignments. Localization of BL-TGTases was determined using Mitoprot 1.101 (Claros and Vincens, 1996), Predotar 1.03 (Small et al., 2004) and Target P 1.1 (Emanuelsson et al., 2007).

2.2. Phylogenetic analysis

Phylogenetic analyses were performed using MrBayes 3.2.3 (Ronquist et al., 2012) for a Bayesian analysis of phylogeny and the calculation of posterior probabilities. Sequences of interest; either BL-TGTases, Clamydial B-TGTases and B-TGTase or Clamydial B-TGTases and B-TGTases were aligned using MUSCLE. In both phylogenetic analyses, E-TGTases were used as the outgroup. Both phylogenetic analyses were run using mixed amino acid rate matrices for the number of generations (from 6×10^5–3.3×10^6) required to reduce the standard deviation of split frequencies below 0.01. This was performed independently of burnin parameters. Statistical support for the branches was ascertained via bootstrapping. The topologies of the trees were visualized using Tree Graph 2.

3. Results and discussion

3.1. 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 1.101 (Claros and Vincens, 1996), Predotar 1.03 (Small et al., 2004) and Target P 1.1 (Emanuelsson et al., 2007). The putative localization for each BL-TGTase was supported by predictions from at least two of the three programs. Most BL-TGTases possess putative 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 that it may modify other substrates in this organelle.

Table 1
Complete list of all BL-TGTases identified in this study.

Organism	NCBI protein Accession No.	Localization[a]
Amoebozoa		
Dictyostelium discoideum	XP_643448	Mito.
Dictyostelium purpureum	XP_003292241	N/A
Dictyostelium fasciculatum	XP_004360613	Mito.
Polysphondylium pallidum	EFA80165	Mito.
Acanthamoeba castellanii	XP_004337524	Mito.
Heterolobosea		
Naegleria gruberi	XP_002681039	Mito.
Stramenopiles		
Aureococcus anophagefferens	EGB11651	Cyt.
Thalassiosira pseudonana	XP_002286144	Mito.
Thalassiosira oceanica	EJK76959	N/A
Phaeodactylum tricornutum	XP_002177321	ER
Saprolegnia diclina	XP_008610966	Mito.
Saprolegnia parasitica	KDO24421	Cyt.
Aphanomyces invadans	XP_008880281	Cyt.
Aphanomyces astaci	XP_009845718	Mito.
Nannochloropsis gaditana	EWM28513	Mito.
Viridiplantae		
Micromonas pusilla	XP_003058508	Cyt.
Bathycoccus prasinos	CCO14599	Cyt.
Ostreococcus tauri	XP_003080748	Mito.
Ostreococcus lucimarinus	XP_001419335	Mito./Pla.
Chlorella variabilis	EFN58624	Mito.
Auxenochlorella protothecoides	KFM22519	Mito.
Haptophyceae		
Emiliania huxleyi	EOD28566	Pla.
Rhodophyta		
Cyanidioschyzon merolae	BAM80913	Mito.
Galdieria sulphuraria	EME26237	Mito.
Chondrus crispus	CDF35656	Mito./Pla.

[a] Mito: Mitochondria; Cyt: Cytosol; ER: Endoplasmic reticulum; Pla: Plastid/Chloroplast; N/A: Localization could not be determined as only a partial sequence was available.

3.2. 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 similarity to B-TGTases despite their existence in eukaryotes (mean amino acid similarity of BL-TGTases with B-TGTases and E-TGTases was 48.9% and 41.6%, respectively, as inferred from three representative sequences from each family). A Bayesian analysis of phylogeny using MrBayes 3.2.3 (Ronquist et al., 2012) (for the calculation of posterior probabilites) with BL-TGTases, B-TGTases and E-TGTases confirmed this similarity (Fig. 1). The BL-TGTases were most similar to TGTases from members of the Chlamydiae (mean amino similarity of 54.6%). In fact, the Chlamydial TGTases were more similar to BL-TGTases than other B-TGTases. Given that Chlamydiae are 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 (Gupta and Griffiths, 2006; Manna and Barth, 2013; Moustafa et al., 2008). 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 Chlamydiae are known to be major contributors of genes to several eukaryotic genomes via HGT (Gupta and Griffiths, 2006; Moustafa et al., 2008). This includes genes encoding tRNA modification enzymes such as the Chlamydial tRNA