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Incomplete nonsense-mediated mRNA decay in *Giardia lamblia*

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Abstract

Messenger RNAs containing premature translation stop codons are degraded by a nonsense-mediated mRNA decay (NMD) system. The NMD pathway is present in yeast, plants and mammals and is thought to protect cells from production of nonfunctional proteins by rapidly degrading mutant mRNAs. There is little understanding of the biology of the origins of eukaryotes, particularly of the NMD pathway. Searches using the BLAST program revealed that the protozoan *Giardia lamblia* has only some of the components of the NMD pathway. We developed a luciferase reporter system with a nonsense mutation to monitor NMD in *Giardia*. The nonsense mutation triggered a decrease in luciferase mRNA levels and stability, suggesting that the NMD phenomenon could be present in *Giardia*. We also found a significant reduction of the mRNA levels of another system containing *Giardia* its own *cyst wall protein 3* gene with a nonsense mutation. However, the reduction levels observed in these two systems are lower than that in late-branching eukaryotes, suggesting that the NMD system in *Giardia* may be less functional. Interestingly, the effect of G418 in promoting read-through of the nonsense mutation and inhibiting NMD in *Giardia* is similar to that in late-branching eukaryotes. We also characterised the giardial homologue of a conserved NMD factor, UPF1. Immunofluorescence assays revealed that giardial UPF1, like yeast UPF1, is expressed in the cytoplasm, but not in the nucleus. In addition, overexpression of UPF1 resulted in a reduction of the levels of nonsense-containing transcripts and enhanced translation termination at a nonsense codon. These results suggest that *Giardia* may have an incomplete NMD pathway and giardial UPF1 may be functionally conserved, involved in NMD and in preventing nonsense suppression.

Keywords: UPF1; G418; NMD; Cyst wall protein; Encystation; *Giardia*

1. Introduction

Giardia lamblia is a common intestinal protozoan parasite responsible for outbreaks of waterborne diarrhoea (Adam, 2001). It has two stages in the life cycle that adapt well to different environments – a pathogenic trophozoite form and a resistant infectious cyst form (Gillin et al., 1996; Eichinger, 2001). Trophozoites that colonise the upper intestinal tract differentiate into cysts when they are carried to the lower intestine. During this differentiation, *G. lamblia* synthesizes a resistant extracellular wall that encases the trophozoite, sequestering it from host defenses.

Giardia lamblia is of biological interest in understanding the progress of eukaryotic evolution (Sogin et al., 1989; Hashimoto et al., 1994, 1995; Morrison et al., 2007). *Giardia lamblia* has fewer cellular components for DNA synthesis, transcription and RNA processing, possibly due to their divergence or their functional redundancy with other proteins in some pathways (Morrison et al., 2007). There is little understanding of the biology of eukaryotes, particularly of regulation of RNA stability.

In late-branching eukaryotes, either a frameshift or a nonsense mutation often leads to rapid degradation of the gene's mRNA by a nonsense-mediated mRNA decay (NMD) pathway (Nagy and Maquat, 1998; Gonzalez et al., 2001; Wilkinson, 2005). This surveillance system protects cells from the production of nonfunctional proteins

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by eliminating mutant mRNAs. The NMD pathway is present in yeast, plants, *Caenorhabditis elegans* and mammals (Nagy and Maquat, 1998; Gonzalez et al., 2001; Wilkinson, 2005). NMD is a translation-dependent event since its mechanism depends on the recognition of the nonsense mutations by the translational machinery (Wilkinson, 2005). Aminoglycosides and puromycin that have been used extensively to select stably transfected *Giardia* (Singer et al., 1998; Sun et al., 1998) can increase the stability of mRNAs with nonsense mutations by suppressing NMD in late-branching eukaryotes (Burke and Mogg, 1985; Koeller et al., 1991; Lim et al., 1992). Both kinds of drugs can facilitate the detection of nonsense mutations in disease-related genes by suppressing NMD (Koeller et al., 1991; Lim et al., 1992; Andreutti-Zaugg et al., 1997; Bedwell et al., 1997). Aminoglycosides not only interfere with NMD, they may also cause the ribosome to translate through the nonsense codon and to introduce missense mutations (Eustice and Wilhelm, 1984; Burke and Mogg, 1985; Bedwell et al., 1997; Schroeder et al., 2000). Puromycin is a translation inhibitor that causes premature releases of polypeptide chains (Nathans, 1964; Kirillov et al., 1997).

NMD factors such as up-frameshift 1 (UPF1), UPF2 and UPF3 have been identified in yeast, *C. elegans*, mice and humans (Perlick et al., 1996; Applequist et al., 1997; Page et al., 1999; Lykke-Andersen et al., 2000; Mendell et al., 2000; Serin et al., 2001). These have been shown to interact with each other and form a complex (Weng et al., 1996; He et al., 1997). Mutations in *upf* genes stabilise mRNAs with nonsense mutations (Leeds et al., 1991, 1992). Other NMD-related factors include translation termination factors eRF1 and eRF3 which interact with UPF1, UPF2 and UPF3 to trigger translation termination (Culbertson and Neeno-Eckwall, 2005). Decapping factors DCP1 and DCP2, and 5′–3′ exoribonuclease XRN1 are required for the next step, 5′–3′ mRNA decay (Gonzalez et al., 2001). SKI7p can interact with UPF1 to mediate exosomal 3′–5′ mRNA decay (Takahashi et al., 2003). HRP1 is a marker protein that binds to a *cis*-acting sequence involved in NMD (downstream sequence element) and interacts with UPF1 (Gonzalez et al., 2001). SMG1 is a kinase that phosphorylates UPF1 (Yamashita et al., 2001). SMG5, SMG6 and SMG7 are involved in dephosphorylation of UPF1 (Chiu et al., 2003).

UPF1 is one of the most conserved NMD factors (Nagy and Maquat, 1998; Gonzalez et al., 2001; Wilkinson, 2005). The yeast *Saccharomyces cerevisiae upf1* gene encodes a protein of 971 amino acids with a deduced molecular mass of 109 kDa (Leeds et al., 1992). It contains cysteine–histidine-rich zinc binding motifs near the N terminus and group I RNA helicase motifs (DEAD box) (Leeds et al., 1992). These motifs are flanked by divergent sequences in UPF1s of other organisms (Applequist et al., 1997; Page et al., 1999). The cysteine–histidine-rich motifs in *S. cerevisiae* UPF1 contain two modules similar to RING or U-box domains of ubiquitin ligases (Kadlec et al., 2006). UPF1 has RNA binding, RNA-dependent ATPase and RNA helicase activities (Czaplinski et al., 1995; Weng et al., 1996; Bhattacharya et al., 2000). Mutations of the *upf1* gene not only stabilised nonsense-containing mRNAs but also resulted in a nonsense suppression phenotype (Maderazo et al., 2000), indicating that UPF1 functions in translation termination at a nonsense codon. Studies have shown that UPF1 enhances translation termination at a nonsense codon through interaction with the termination release factors (Weng et al., 1996; Czaplinski et al., 1998). The presence of the *upf1* gene in the *Giardia* genome database has also been reported recently (Kadlec et al., 2006).

In this study, we found that *Giardia* has only some components of the NMD pathway. We made a luciferase mutant with a nonsense mutation to test whether *Giardia* has an NMD phenomenon. We found that the nonsense mutation triggered a decrease in luciferase mRNA levels and a decrease in mRNA stability. In addition, G418 can suppress the nonsense mutation by both translation read-through and inhibition of NMD in *Giardia*. We also characterised the giardial homologue of the *upf1* gene and demonstrated that UPF1 was preferentially expressed in vegetative trophozoites and localised to cytoplasm, as is yeast UPF1. We also found that overexpression of UPF1 reduced the levels of nonsense-containing transcripts and enhanced translation termination at a nonsense codon. Our findings provide new insights into the evolution of the eukaryotic NMD pathway.

2. Materials and methods

2.1. Giardia culture

Trophozoites of *G. lamblia* WB (ATCC 30957) clone C6 were cultured in modified TYI-S33 medium (Keister, 1983) and encysted as previously described (Sun et al., 2003). In experiments exposing *Giardia* vegetative trophozoites to different drugs, trophozoites were cultured in medium with 150 μg/ml G418 or 45 μg/ml actinomycin D (in PBS) for indicated times (see Fig. 4).

2.2. RNA extraction and Northern blot analysis

Total RNA was extracted from *Giardia* clones C6 at the indicated differentiation stages (see Figs. 3, 5, 6 and 8) using TRIzol reagent (Invitrogen). For Northern blot analysis, 10 μg total RNA was fractionated and transferred to charged Nylon membranes (Biodyne B membrane, Pall). Full-length coding region probes of luciferase, *cwp1* (GenBank Accession No. U09330), *cwp2* (GenBank Accession No. U28965), *cwp3* (GenBank Accession No. AY061927), *ran* (GenBank accession no. U02589) and *upf1* (GenBank Accession No. DQ861427) genes were prepared by PCR amplification of genomic DNA using primers lucF (ATGG AAGACGCCAAAAAC) and lucR (TTACACGGCGA TCTTTCC), cwp1F (ATGATGCTCGCTCTCCTT) and cwp1R (TCAAGGCGGGGTGAGGCA), cwp2F (ATGA TCGCAGCCCTTGTT) and cwp2R (TCACCTTCTGC