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High-level expression of the *Toxoplasma gondii STT3* gene is required for suppression of the yeast *STT3* gene mutation☆

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Abstract

N-linked glycosylation is the most frequent modification of secretory proteins. The central reaction of this process in eukaryotic cells is catalyzed by the hetero-oligomeric protein complex oligosaccharyltransferase (OST). The gene *STT3* gene encodes a protein, which is the most conserved among the components of the OST. In this report, we describe the isolation and functional characterization of a *STT3* homologue from *Toxoplasma gondii*. The topology of the *Tg*Stt3p is similar to that of the yeast Stt3p with 47% identity. We demonstrate that high level expression of the homologues gene is required to completely suppress the defect caused by a stt3 mutation in yeast, suggesting that homologous Stt3 proteins can serve analogous functions in distantly related eukaryotic cells regardless of their degree of conservation.

Keywords: Oligosaccharyltransferase (OST); *N*-glycosylation; *Toxoplasma gondii*; GAL promoter; Conditional lethal mutant; Complementation; *STT3*

1. Introduction

Toxoplasma gondii is an apicomplexan protozoan parasite with a worldwide distribution, infecting many kinds of cells, including fibroblasts, epithelial and endothelial cells, macrophages and cells of the central nervous system [1]. The infection is generally asymptomatic, however, coinfection with HIV often leads to fatal encephalitis [2]. Developmental switching in *Toxoplasma gondii*, from the virulent tachyzoite to the relatively quiescent bradyzoite stage, is responsible for disease propagation and reactivation. The redifferentiation event is characterized by an overexpression of a tachyzoite specific set of surface proteins, most notably the major tachyzoite surface antigen P30/SAG1 [3]. The SAG1 posses one potential *N*-glycosylation site [4], which remains unoccupied except when the protein was expressed by the baculovirus system or in mammalian cells [5,6]. The first clear evidence of *N*-glycosylation in *Toxoplasma* has been shown by structural characterization of four *N*-linked structures on the tachyzoite glycoprotein gp23 [4]. *N*-linked glycosylation is the most frequent modification of secretory proteins in eukaryotic cells [7]. A core oligosaccharide is assembled on dolichol pyrophosphate in a complex conserved pathway and transferred to selected asparagine residues within the consensus motif Asn-X-Ser/Thr of nascent proteins [8]. Proteins entering the secretory pathway may be glycosylated by transfer of an oligosaccharide ($Glc_3Man_9GlcNAc_2$) from a dolichol-P-P derivative to nascent polypeptide chains in the lumen of the endoplasmic reticulum (ER). In eukaryotic cells, the central reaction of this process is catalyzed by the hetero-oligomeric protein complex oligosaccharyltransferase (OST) [9]. In yeast and higher eukaryotes besides $Glc_3Man_9GlcNAc_2$ incomplete core oligosaccharides can also be transferred to the proteins [10]. So far, eight genes have been identified in

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Saccharomyces cerevisiae as members of the OST complex. Enzymatically active OST preparations from yeast were shown to be composed of four (Ost1p, Wbp1p, Ost3p and Swp1p) or six subunits (Ost2p and Ost5p in addition to the four listed). Recently, genetic studies have disclosed Stt3p and Ost4p as additional proteins needed for *N*-glycosylation [11]. Yeast Stt3p regulates the substrate specificity and assembly of the OTase complex [10]. The *STT3* gene encodes a protein, which is the most conserved among components of the OST [12]. Stt3p is more than 50% identical to eukaryotic proteins of similar topology from human, mouse and *Caenorhabditis elegans* [12,13]. ORFs encoding homologous proteins were also found in archaea, such as *Methanococcus janaschii* and *Archaeoglobus fulgidus*, with a lower identity (23%), but similar topology. Depletion of the Stt3p causes loss of OST activity in vivo and in vitro as well as a deficiency in the assembly of the complex [13]. Neither the fission yeast *Schizosaccharomyces pombe* nor the mouse *STT3* genes that are highly homologous to the *ScSTT3* were capable to complement a stt3 *S. cerevisiae* mutant [14]. So far, this phenomenon remains to be elucidated. In this report, we describe the isolation and functional characterization of the *STT3* homologue from the parasite *Toxoplasma gondii*. The *Tg*Stt3p shows a topology similar to that of the yeast Stt3p with 47% identity. Here, we demonstrate that high level expression of the homologous gene is required to completely suppress the defect caused by a stt3 mutation in yeast, suggesting that homologous Stt3 proteins serve analogous function in distantly related eukaryotic cells regardless of their degree of conservation.

2. Material and methods

2.1. Parasites and host cells

RH strain *T. gondii* tachyzoites were grown in Vero cells. Confluent cell cultures (75 cm^2) were infected with 5×10^7 tachyzoites in Dulbecco's modified Eagle's medium, supplemented with 1% (v/v) fetal calf serum and 2 mM L-glutamine. Cell line and parasites were routinely tested for *Mycoplasma* contamination.

2.2. Recombinant DNA methods

Restriction endonucleases and other DNA modifying enzymes used in recombinant DNA experiments were from Roche, New England Biolabs, or Stratagene, and used according to the manufacturer's instructions. Individual restriction fragments were isolated with the QIAquick Gel extraction kit (Qiagen). Plasmid isolation from *Escherichia coli* was done using the E.Z.N.A plasmid Miniprep Kit I (Peqlab, Germany). Sequencing was performed by PCR-cycle sequencing using the ABI PRISM Dye Terminator Cycle Sequencing kit from the Perkin-Elmer Gene Amp 2400 PCR system. Sequence analysis was performed with the ABI Prism 377 DNA sequencer.

2.3. Computer analysis

Searches of the GenBank were performed using the *Sanger Information BLAST* server (http://www.sanger.ac.uk) as well as the *Toxoplasma* Genome Sequencing Consortium (http://www.toxodb.org/). Analysis of nucleotide and deduced amino acid sequence was done with the Heidelberg Unix Sequence Analysis Resource (HUSAR).

2.4. Strains and media

The *S. cerevisiae* and *E. coli* strains used in this work were: *YPH* 499 [Mat a; *ura* 3-52; *lys* 2-801*amber*; *ade* 2-101*ochre*; *trp* 1-63; *his* 3-200; *leu* 2-1] (Stratagene), was used as a control, and *E. coli* strain *XL1-blue* (Stratagene) for subcloning and other standard recombinant DNA procedures. *S. cerevisiae* strains were grown in YPAD medium [1% (w/v) Bacto yeast extract, 2% (w/v) Bactopeptone, 2% (w/v) dextrose, 4 mg l^{-1} adenine] or SD medium (0.17% Bacto yeast nitrogen base, 0.5% ammonium sulphate and 2% dextrose) containing the nutritional supplements necessary to complement strain auxotrophs or allow selection of transformants. *YPH* 499–*HIS–GAL–STT3* was maintained on an SGR medium (4% galactose, 2% raffinose, 0.17% Bacto yeast nitrogen base, 0.5% ammonium sulphate) in which dextrose is replaced by galactose/raffinose as the carbohydrate source. *E. coli* strains were grown in LB medium.

2.5. Identification of the STT3 gene

The TgESTzy23f09.rl clone, which contains a 420 bp insert and deduced amino acids with about 42% identity to the baker's yeast and human stt3p, was identified using a database search for the Sanger Information BLAST server. The cDNA fragment encoding the protein was obtained by screening of *T. gondii* cDNA library with the TgESTzy23f09.rl clone. The full-length cDNA was amplified by the 3′-RACE method and sequenced.

2.6. Construction of the YPH 499–HIS–GAL–STT3 S. cerevisiae strain

YPH 499–*HIS–GAL–STT3 S. cerevisiae* strain was constructed by bringing the expression of the yeast *STT3* gene under the control of the *GAL*1 promoter [15], which is repressed in the presence of glucose, as described before [16,17]. The *STT3* promoter in the yeast genome was exchanged by a selection marker/promoter *HIS*/*GAL*-cassette [18]. The primer STTgalS (5′-GCT TTC TTT TAC TTC TCT TCG CCT CTG CTA AAT GGT CAC CAT CGA CGG TTG GGC GAA TTG GAG CTC CAC-3′) and STTgalAS (5′-GAT GAC GAG CTT GAG GAT GGT CTG AAA CCA CAC GAC CGG TCG GAT CCC ATG GGG ATC CAC TAG TTC TAG-3′) were used for amplification of the *HIS–GAL* cassette. The transformation into the haploid *YPH* 499 strain, the selection on a minimal