Chapter 8
Trypanosoma cruzi Trans-Sialidase: Structural Features and Biological Implications

Isadora A. Oliveira, Leonardo Freire-de-Lima, Luciana L. Penha, Wagner B. Dias, and Adriane R. Todeschini

Abstract *Trypanosoma cruzi trans*-sialidase (TcTS) has intrigued researchers all over the world since it was shown that *T. cruzi* incorporates sialic acid through a mechanism independent of sialyltransferases. The enzyme has being involved in a vast myriad of functions in the biology of the parasite and in the pathology of Chagas' disease. At the structural level experiments trapping the intermediate with fluorosugars followed by peptide mapping, X-ray crystallography, molecular modeling and magnetic nuclear resonance have opened up a three-dimensional understanding of the way this enzyme works. Herein we review the multiple biological roles of TcTS and the structural studies that are slowly revealing the secrets underlining an efficient sugar transfer activity rather than simple hydrolysis by TcTS.

Abbreviations

4-MUNeu5Ac	4-methylumbelliferyl-N-acetyl neuraminic acid
Gal*f*	Galactofuranose
Gal*p*	Galactopyranose
GlcNAc	*N*-acetylglucosamine
Neu5Ac	*N*-acetylneuraminic
Neu5Gc	*N*-glycolylneuraminic acid
NGF	Nerve growth factor receptor
pNPNeu5Ac	p-nitrophenyl-N-acetyl-neuraminic acid
SAPA	Shed acute phase antigen

I.A. Oliveira • L. Freire-de-Lima • L.L. Penha • W.B. Dias • A.R. Todeschini (✉)
Laboratório de Glicobiologia Estrutural e Funcional, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Avenida Carlos Chagas Filho, 373, Centro de Ciências da Saúde-Bloco D-3, Cidade Universitária, 21941-902 Rio de Janeiro, Brazil
e-mail: adrianet@biof.ufrj.br

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Sias	Sialic acids
Siglecs	Sia-binding Ig-like lectin
TcTS	*Trypanosoma cruzi trans*-sialidase
TSs	*Trans*-sialidase family
UTR	Untranslated regions

1 Introduction

The initiation of communication between *Trypanosoma cruzi*, the etiologic agent of Chagas' disease, and mammalian cells requires contact between parasite molecules and host ligands. The parasite membrane is covered with a dense coat formed mainly of molecules of the *trans*-sialidase (TSs) family and of sialic acids (Sias) containing glycoproteins; this coat assures an interface with the host environment (Freitas et al. 2011; De Pablos and Osuna 2012). Sias are acidic monosaccharides found at the outermost ends of the sugar chains of glycoconjugates involved in a myriad of functions ranging from cell recognition to cell life and death (Varki 2006). *T. cruzi* is not able to synthesize Sias by the well-known route in which cytidine monophosphate-sialic acid is an intermediate. Instead, *T. cruzi* is part of a restrict group of parasites evolutionarily adapted to incorporate sialic acid from exogenous sialoglycoconjugates by means of a glycoside hydrolase known as *trans*-sialidase (TcTS) (Previato et al. 1985). Further evidences suggest that TcTS activity play several functions in the course of *T. cruzi* infection ensuring a life-long parasitism in humans. The multifunctional role of TcTS is due to its ability to dialogue with different cells from the mammalian host (Fig. 8.1) (Mendonça-Previato et al. 2010; dC-Rubin and Schenkman 2011; Chuenkova and Pereiraperrin 2011; Schauer and Kamerling 2011; Freire-de-Lima et al. 2012). Alongside, structural works are deciphering the way this enzyme works. TcTS was the first example of a retaining glycosidase utilizing an aryl glycoside intermediate (Watts et al. 2003; Amaya et al. 2004). Such finding had shed light in the catalytic mechanisms of other sialidases of medical importance (Kim et al. 2013; Vavricka et al. 2013).

Herein, we discuss the importance of this unique enzyme in curse of infection of mammalian host by *T. cruzi*, highlighting the studies that are deciphering the mechanism of TcTS catalysis.

2 Structure and Catalytic Mechanism of TcTS

TcTS is a retaining glycoside hydrolase (Todeschini et al. 2000) member of the family number 33 (GH-33) (http://www.cazy.org/GH33.html) that preferentially transfers sialic acid units to β-galactopyranosyl (β-Gal*p*)-containing molecules and exclusively synthesizes α2-3-linkages (Fig. 8.2a). In the absence of a galactoside, TcTS catalyzes sialoside hydrolysis (Fig. 8.2b) with retention of configuration (Todeschini et al. 2000).

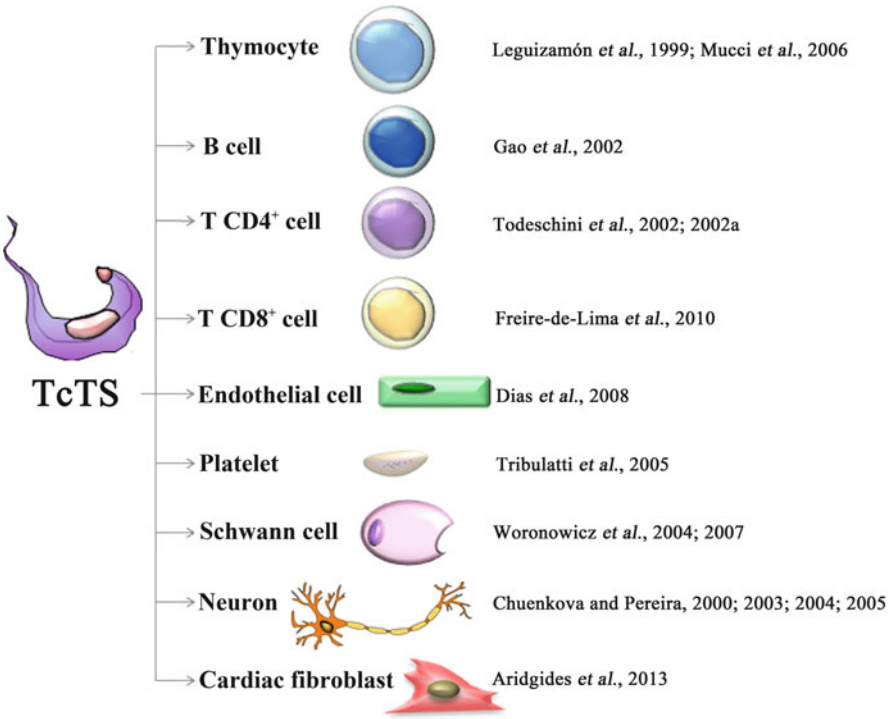

Fig. 8.1 Possible interactions of TcTS with different cells. The multifunctional role of TcTS during *T. cruzi* infection is due to its ability to dialogue with different cells from the mammalian host

The TcTS structure consists of three domains (Fig. 8.3a): (i) an N-terminal domain containing a binding site (green in Fig. 8.3a) that folds into a six-bladed β propeller-structure (Buschiazzo et al. 2002), (ii) a globular C-domain with a lectin-like organization (blue in Fig. 8.3a) that is not required for TcTS activity, and (iii) a C-terminal and unfolded domain that is formed by 12-amino-acid repeats, known as a 'shed acute phase antigen' (SAPA) (Pollevick et al. 1991).

TcTS is linked to the surface of blood-derived trypomastigotes by a glycosylphosphatidylinositol-anchor; the lipid portion of this anchor consists of ceramide and hexadecylglycerol (Agusti et al. 1997), while in metacyclic trypomastigotes, ceramide is the sole constituent lipid (Agusti et al. 1998). The presence of ceramide in the glycosylphosphatidylinositol-anchor allows the enzyme to be actively cleaved from the surface of *T. cruzi* (Pollevick et al. 1991) by the action of a phospholipase C (Rosenberg et al. 1991). These data explain the presence of SAPA antigens (Parodi et al. 1992) and *trans*-sialidase activity in the serum of patients in the acute phase of Chagas' disease (De Titto and Araújo 1988, Mallimaci et al. 2010; Gil et al. 2011). The SAPA repeats cause enzyme oligomerization and induce the production of antibodies (Cazzulo and Frasch 1992). The insect-derived epimastigote forms express a monomeric and transmembrane TcTS lacking the SAPA portion (Chaves et al. 1993).

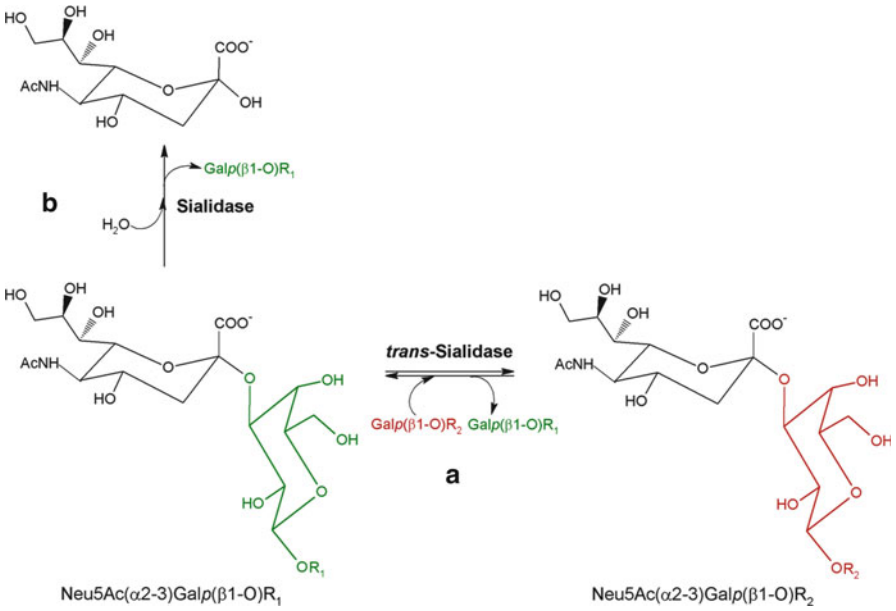

Fig. 8.2 (**a**) Transfer of Neu5Ac from Neu5Ac **α**-2-3Gal**β**1-x-containing linkage donors to terminal **β**-galactopyranosyl (**β**-Gal*p*) catalyzed by TcTS. (**b**) Sialoside hydrolysis catalyzed by TcTS

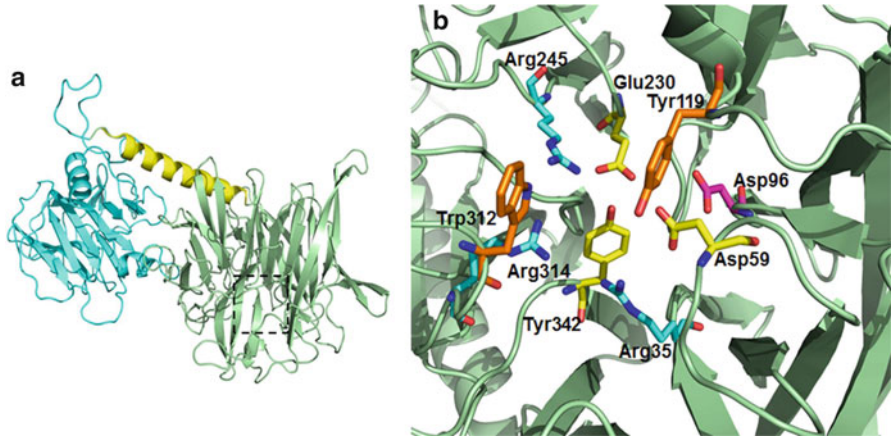

Fig. 8.3 (**a**) Overall structure of TcTS. The *square* shows the catalytic site position. (**b**) The active site of TcTS. The catalytic amino acid residues Tyr342, Glu230, and Asp59 (*yellow*), the Asp96 residue (*magenta*), the triad of arginines Arg35, Arg245, Arg314 (*blue*) and the gatekeepers Trp312 and Tyr119 (*orange*) are highlighted

The C-terminal globular domain is formed by two antiparallel β-sheets in a β-sandwich-like structure (Buschiazzo et al. 2002); this structure is linked to the N-terminal domain by a long α-helix (yellow in Fig. 8.2a), the largest of the few reported in the TcTS (Buschiazzo et al. 2002). The N-terminal domain comprises approximately 680 amino acids (Schenkman et al. 1992; Campetella et al. 1994) folded into a six-bladed β propeller, similar to the crooked β-barrel structure characteristic of microbial sialidases (Taylor 1996). This domain contains all the amino acid residues that are involved in sialic acid binding: (i) the motif S-x-D-x-G-x-T-W (also called Asp-box), repeated three to five times in the sequences of bacterial and mammalian sialidases (Roggentin et al. 1989), (ii) the x-R-x-P (or FRIP) region found at the N-terminal domain of the Asp-box, and (iii) three arginines known to bind the carboxylate group of sialic acid (Gaskell et al. 1995). Such a structure forms a deep catalytic pocket with hydrophobicity produced by residues such as Met95, Phe115, Trp120 and Val176 (Buschiazzo et al. 2000); these residues are suitable for the transfer reaction because they may contribute to water exclusion from the cleft, thus reducing the hydrolytic potential of the enzyme. The hydrophobic residues Trp312 and Tyr119 (Fig. 8.2b) are at the protein surface in close contact with water, thus acting as "gatekeepers" of the cleft (Carvalho et al. 2010). The loops carrying these residues move according to the incoming or outgoing of reagents (Demir and Roitberg 2009). The crystal structure of TcTS shows that Tyr119 adopts different positions in the absence or presence of sialoside (Buschiazzo et al. 2002). The loop carrying the Trp312 residue is at the opposite side of the catalytic cleft. This residue stabilizes the galactoside moiety of the substrate in the enzyme pocket through CH/π interactions (Nesmelova et al. 2010). The W312A mutation changes the substrate specificity, resulting in a mutant capable of hydrolyzing both α2-3- and α2-6-linked sialosides and leading to the loss of *trans*-sialidase activity (Paris et al. 2001).

The catalytic mechanism of TcTS represented in Fig. 8.4 shows that, as the sialoside approaches Tyr119 and Trp312 move away to allow the entry of the substrate into the catalytic cleft. Within the catalytic site, the carboxyl group of the sialoside binds to the arginine triad composed of Arg35, Arg245 and Arg314 (blue in Fig. 8.2b), while its acetamido group interacts with Asp96, forcing the ring to adopt a $^{4}H_{5}$ conformation. The hydroxyl group of Tyr342 reacts as a nucleophile, assisted by the nearby Glu230, and forms a covalent intermediate with the sialic acid ring. X-ray structures and experiments trapping the intermediate with fluorosugars followed by peptide mapping and crystallography support this mechanism (Watts et al. 2003; Amaya et al. 2004). The covalent intermediate assumes a $^{2}C_{5}$ conformation, and Asp59 donates its proton to the substrate aglycone. TcTS was the first enzyme described to undergo acid/base catalysis and to have a tyrosine (Tyr342) as the catalytic nucleophile (Watts et al. 2003). The use of a tyrosine as a nucleophile presents a distinct advantage over the use of a negatively charged carboxylate because the anomeric center of Sias is itself negatively charged and could therefore be subject to interfering charge repulsion. The single mutation from Tyr342 to His in the naturally occurring $TcTS_{Y342H}$ causes enzymatic inactivation (Cremona, et al. 1995) but conserves binding to Sia and β-Gal*p*-containing glycans (Todeschini et al. 2002a, 2004).

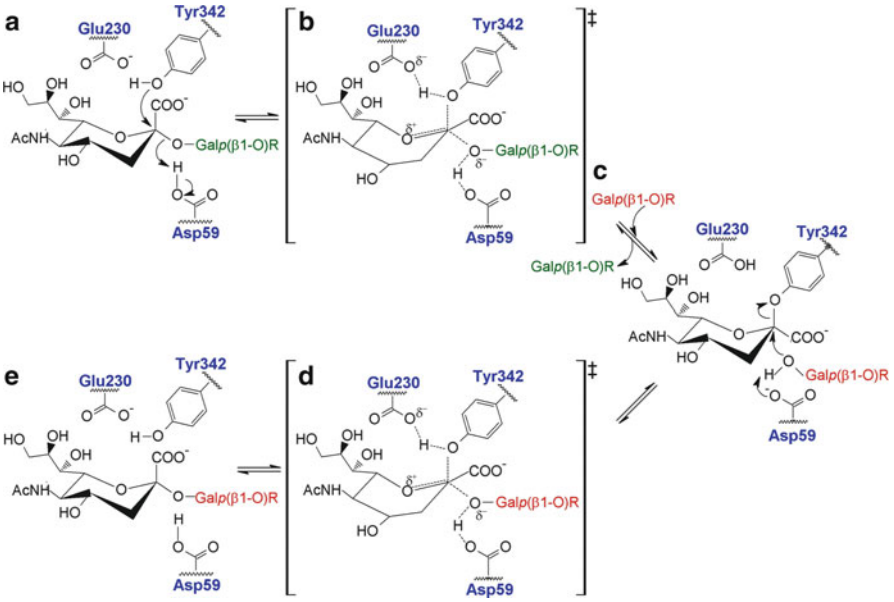

Fig. 8.4 Proposed reaction mechanism for TcTS. (**a**) Upon binding a sialic acid donor, the hydroxyl group of Tyr342 reacts as a nucleophile, assisted by the nearby Glu230 acting as a general base catalyst, and Asp59 protonates the leaving group. (**b**) Tyr342 attacks C2 from the sialyl moiety, and the reaction reaches its transition state. (**c**) The reaction proceeds with the complete breakdown of the glycosidic linkage, releasing the aglycone. At this time, the covalent intermediate of sialic acid bound to Tyr342 is formed. Such a state is perturbed by the entrance of an acceptor (*red*) in the active site of TS. (**d**) Asp59 acts as a basic catalyst by deprotonating the hydroxyl from C3 of terminal Gal, which, in turn, attacks the covalent intermediate, promoting a new transition state. (**e**) Gal completes the nucleophilic attack, and the reaction ends with the retention of the configuration of the sialic acid moiety

According to a ping-pong mechanism (Fig. 8.4), the aglycone leaves the pocket to enable the sialic acid acceptor substrate to bind to the enzyme. The transfer to the acceptor occurs through the attack of the 3-OH group of a lactose moiety or of water (as in other sialidases) deprotonated by the Asp59 residue acting as an acid/base catalyst (Damager et al. 2008) on the C2 of the sialyl-enzyme intermediate.

Further works have helped to elucidate the structural features that underlie efficient sugar transfer activity rather than simple hydrolysis by TcTS. Important evidences reveal that the $TcTS_{Y342H}$ binding site undergoes large conformational changes upon sialoside engagement, thus triggering the opening of a second binding pocket that accommodates a β-Gal*p* moiety in a ternary complex (Todeschini et al. 2004; Haselhorst et al. 2004). The incubation of $TcTS_{Y342H}$ with α2-6-sialyllactose in the presence of lacto-N-tetraose has shown that the incorrect positioning of sialoside into the binding site of TcTS does not trigger β-Gal*p* binding. Moreover, surface plasmon resonance results showed that lactose binds to an inactive mutant ($TcTS_{D59N}$) in the presence of α2-3-sialyllactose (Buschiazzo et al. 2002). Other key

residues that contribute to the plasticity of the binding site were identified by mutagenesis studies (Paris et al. 2001; Carvalho et al. 2010), by hybrid quantum mechanics/molecular mechanics simulations and by molecular dynamic simulation (Demir and Roitberg 2009; Mitchell et al. 2010; Pierdominici-Sottile and Roitberg 2011; Pierdominici-Sottile et al. 2011).

2.1 TcTS Substrate Specificity

TcTS catalyzes the transfer of Sias N-acetylneuraminic (Neu5Ac) and its derivative the N-glycolylneuraminic acid (Neu5Gc) from Siaα-2-3Galβ1-x-containing donors to terminal β-galactopyranosyl (β-Gal*p*)-containing acceptors and attaches them in α2-3-linkage (Vandekerckhove et al. 1992).

Natural sialic acid acceptors for TcTS on the surface of *T. cruzi* consist mainly of a family of highly *O*-glycosylated, threonine-rich mucin-like glycoproteins (Buscaglia et al. 2006; Mendonça-Previato et al. 2008) that are glycosylphosphatidylinositol-anchored to the parasite membrane (Previato et al. 1995). The Tc-mucins are the most expressed components of *T. cruzi* (2×10^6 copies per parasite) and compose the third most widely expanded gene family in the genome, comprising more than 1,000 genes (El-Sayed et al. 2005a, b; De Pablos and Osuna 2012). Carrying up to 60 % of their total mass in carbohydrates, mucins form an elaborate and highly decorated glycocalyx that allows the parasite to interact with and respond to its external environment. The structures of the sialic acid acceptors of non-infective epimastigote forms were described (Previato et al. 1994, 1995; Todeschini et al. 2001, 2009; Agrellos et al. 2003; Jones et al. 2004) as *O*-linked oligosaccharides attached to the peptide backbone through an *N*-acetylglucosamine (α-GlcNAc) residue (Previato et al. 1995, Mendonça-Previato et al. 2013) further substituted by β-Gal residues on O-4 and O-6. The major sialylated oligosaccharides reported thus far comprise a Neu5Acα2-3Gal*p*β1-4GlcNAc sialoside (Jones et al. 2004), a Gal*f*β1-4(Neu5Acα2-3Gal*p*β1-6) GlcNAc sialoside (Agrellos et al. 2003), a Gal*p*β1-4(Neu5Acα2-3Gal*p*β1-6)GlcNAc sialoside, and a Galpβ1-6(Neu5Acα2-3Gal*p*β1-4) GlcNAc sialoside (Previato et al. 1995; Todeschini et al. 2001). The Neu5Ac residue was distributed approximately equally between the digalactosylated species of the 4-arm and 6-arm. This observation suggests that the addition of the first Neu5Ac residue hinders the addition of a second residue (Previato et al. 1995), as disialylated forms were not observed. Neither the terminal β-galactofuranose- (β-Gal*f*)-linked residues found in *O*-glycans from G (Previato et al 1994), DM28c (Agrellos et al. 2003), Tulahuen (Jones et al. 2004) or Colombiana (Todeschini et al. 2009) strains nor the α-galactosyl residues found in the mucin glycans of infective trypomastigotes (Almeida et al. 1994) were found to be acceptors for TcTS.

A wide variety of molecules containing a terminal β-Gal*p*-unit are suitable acceptors for *trans*-sialidase activity *in vitro* (Vandekerckhove et al. 1992; Scudder et al. 1993). The natural acceptor N-acetyllactosamine (Galβ1-4GlcNAc) is a better substrate for the TcTS reaction than is lactose (Lac, Gal*p*β1-4Glc)

(Vandekerckhove et al. 1992). In addition, TcTS shows higher transfer rates for the Galβ1–4-linkage than for the Galβ1–3-linkage. The lactose open-chain derivatives lactitol and lactobionic acid and the products generated by the addition of Gal*p*, Gal*f* or benzyl residues to the lactitol molecule were found to be good acceptors of sialic acid (Agustí et al. 2004, 2007). Recently, the reactions of a series of octyl galactosides and octyl *N*-acetyllactosamines with TcTS were tested. The results showed that the TcTS acceptor binding site does not tolerate the substitution of Gal*p* at positions 2 and 4, while substitutions at position 6 of the Gal ring are well accepted (Harrison et al. 2011).

The tolerance of TcTS to modifications in the C-6 position of the acceptor Gal moiety makes the 6-deoxy-galactose (D-Fuc) derivative D-Fucβ1-6GlcNAc-α-benzyl an interesting acceptor. As the disaccharide is not a substrate for galactose oxidase, it was reported to be an acceptor substrate for TcTS activity in a quantification assay (Sartor et al. 2010).

In terms of donor substrates, neither thiosialosides (Harrison et al. 2001) nor 2-6-, 2-8-, or 2-9-linked sialic acids are substrates for TcTS and $TcTS_{Y342H}$ (Vandekerckhove et al. 1992). Both proteins recognize α2-3-linked sialic acid and its 7-carbon analog (Previato et al. 1985; Todeschini et al. 2002a). Binding can be abolished by either fucosylation or carboxyl reduction (Vandekerckhove et al. 1992; Todeschini et al. 2002a). Therefore, the ligands of the selectins sialyl $Lewis^x$ and sialyl $Lewis^a$ are not ligands for TcTS or $TcTS_{Y342H}$.

Given that the pocket around the glycerol moiety of sialoside (comprising the amino acids W120, T121, Q195, V203) is too small to accommodate bulky groups, acetylation at C7 and C8 prevents enzyme to bind, so does modification at the C4 position (Vandekerckhove et al. 1992). Interestingly, incorporation of aryl groups such as umbelliferyl and benzamide at the C9 position of the 2,3-difluorosialic acid produced selective and potent inhibitors of TcTS (Buchini et al. 2008).

The synthetic donors 4-methylumbelliferyl-*N*-acetyl neuraminic acid (4-MUNeu5Ac) and p-nitrophenyl-N-acetyl-neuraminic acid (pNPNeu5Ac) are poorer sialic acid donors to the enzyme than are α2-3sialyllactose (Neu5Acα2-3Galβ1-4Glc) or α2-3sialyllactosamine (Neu5Acα2-3Galβ1-4GlcNAc) (Ribeirão et al. 1997; Todeschini et al. 2000). In fact, natural sialosides would fit correctly in the TcTS catalytic pocket, thus inducing the acceptor donor to bind and increasing transfer rates, while synthetic donors such as 4-MUNeu5Ac and pNPNeu5Ac would not be able to trigger a sufficient shifting in the enzyme framework to allow acceptor binding and would thus be better substrates for hydrolysis reaction (Harrison et al. 2001).

Other sialosides such as 2-difluoromethyl-4-nitrophenyl-*N*-acetyl neuraminic acid and 5-acetamido-2-(4-N-5-dimethylaminonaphthalene-1-sulfonyl-2-difluoromethylphenyl)-*N*-acetyl neuraminic acid were suicide substrates for TcTS (Carvalho et al. 2010). The 2-difluoromethylphenyl aglycone released upon sialoside hydrolysis irreversibly inactivates TcTS. Recently, the synthesis of 1,2,3-triazole-linked sialic acid-6-O-galactose and the sialic acid-galactopyranoside were reported as a prototype for further design of new neoglycoconjugates as TcTS substrates (Campo et al. 2012).

Unlike free sialic acid, the synthetic monosaccharide 2,3-difluorosialic acid is recognized as a donor by the enzyme (Watts et al. 2003). The molecule α2,3-difluorosialic acid temporarily inactivates TcTS through covalent binding with the hydroxyl group of Tyr342, thus opening new avenues for the design of irreversible TcTS inhibitors (Watts et al. 2003).

Besides, recognition of a wide variety of substrates, glycoproteins, glycolipids, and oligosaccharides recognized by TcTS makes it an appropriate tool for enzymatic glycosylation of glycans (Šardzík et al. 2011).

3 *Trypanosoma cruzi Trans*-Sialidase Superfamily (TSs)

TcTS is part of the *trans*-sialidase-like superfamily (TSs), a large and highly polymorphic gene family comprising 1,430 gene members and 693 pseudogenes (Freitas et al. 2011) divided into eight groups. Group I contains active TcTS and inactive $TcTS_{Y342H}$ proteins, expressed in trypomastigote (tTS) and epimastigote (eTS) forms. Although the primary sequences of eTS and tTS are highly conserved (Chaves et al. 1993; Briones et al. 1995, Jäger et al. 2008), their 3′ untranslated regions (3′UTR) are entirely different (Jager et al. 2007). The 3′UTRs of the region regulate the expression of several genes in *T. cruzi* (Nozaki and Cross 1995; Weston et al. 1999; Di Noia et al. 2000) and are thought to play a role in the coordinated modulation of TSs stage-specific expression (Jager et al. 2007, 2008). Genes encoding $TcTS_{Y342H}$ members in the *T. cruzi* genome are found in the same number of copies (60–80 per haploid genome) as those encoding TcTS (Cremona et al. 1999). Group II comprises members of TSs proteins that have no *trans*-sialidase activity, including members of the family of gp85 surface glycoproteins gp82, TSA-1, SA85, gp90 and ASP-2. These proteins bind to β-galactose (Yoshida 2008), laminin (Giordano et al. 1994), fibronectin (Ouaissi et al. 1988), collagen (Velge et al. 1988; Santana et al. 1997), and cytokeratin (Magdesian et al. 2001) and are implicated in host cell attachment and invasion. Recently, it was demonstrated that regulatory elements in the 3′UTR of the GP82 are responsible for its stage-specific expression in *T. cruzi* metacyclic trypomastigotes (Bayer-Santos et al. 2012). FL-160, a representative of group III, is a regulatory protein that inhibits the alternative and classical complement pathways (Mathieu-Daudé et al. 2008). Tc13 is representative of group IV with an unknown function (García et al. 2008).

Various groups of the TSs family present motifs common to bacterial and mammalian sialidases, including FRIP (xRxP) and Asp box (Freitas et al. 2011), suggesting that other inactive members of TcTS might have lectinic properties. For instance, evidences have shown that the insect vector-derived metacyclic trypomastigote uses its stage-specific surface molecule gp82 to bind to gastric mucin and establish *T. cruzi* infection via an oral route (Neira et al. 2003; Yoshida 2008; Staquicini et al. 2010; Cortez et al. 2012a, b).

The significant sequence variability observed thus far suggests a strong selective pressure on the TSs gene family to diversify. This pressure may be provided in part

by the mammalian immune response because TSs proteins are targets of both humoral and cell-mediated immune responses (Frasch 2000). The TSs family is much smaller in *T. brucei* than in *T. cruzi*, and it is absent in *L. major* (El-Sayed et al. 2005a, b).

Studies show that all groups of the TSs are represented in the subtelomeric regions. Most of the sequences are members of group II (GP82, GP85, TC85), which includes 22 complete genes (Moraes Barros et al. 2012). To confirm that TSs and other subtelomeric genes were translated, the authors searched for peptides in the database of proteins expressed by *T. cruzi* (TriTrypDB). The results of this search suggest that *T. cruzi* subtelomeric regions can contain expression sites. The abundance of surface protein genes in the subtelomeric regions suggests that these regions may have acted as sites for DNA recombination and expansion and for the generation of new variants of surface proteins (Moraes Barros et al. 2012).

3.1 TcTS and Its Role in Host Parasite Interaction

Immune evasion is particularly important for organisms that target long-lived hosts. The sialylation of parasite glycoconjugates by TcTS activity plays a major role in protecting the infective agent from the host's innate immune response, thus favoring parasite survival. Evidence suggests that the sialylation of trypomastigote glycans confers resistance to killing by lytic antibodies (Almeida et al. 1994) directed to terminal α-galactosyl residues (Pereira-Chioccola et al. 2000). Indeed, the presence of Sia groups on the parasite mucins protects the blood forms of the parasite against complement-induced lysis and macrophage uptake (Tomlinson et al. 1994).

T. cruzi is an intracellular parasite and invasion of host cell is necessary to establish the infection. Infection of mammalian host cells by *T. cruzi* is a multi-step process that requires activation of multiple signal transduction pathways in both the host and the parasite that lead to parasite entry (Caradonna and Burleigh 2011). The host cells contain macromolecules such as laminin, thrombospondin, fibronectin and glycoconjugates that cover the surface of the host cells. The TSs family and sialic acids containing glycoproteins present on parasite surface assure an interface with host environment. In this scenario, the importance of Sia on the parasite surface during host cell infection is still not elucidated. While some studies have shown that the Sia-containing epitopes on parasite augment *T. cruzi* infection (Piras et al. 1987; Schenkman et al. 1991), other groups suggest that the presence of Sia is not required for the invasion of host cells (Araújo-Jorge and De Souza 1988; Yoshida et al. 1997). The finding that $TcTS_{Y342H}$, a natural mutant of TcTS, has two carbohydrate binding sites may explain these apparently ambiguous results. Although Schenkman et al. (1991) showed that the sialylation of the Ssp-3 epitope of mammalian cell-derived trypomastigotes is required for target cell recognition, Yoshida et al. (1997) reported that the removal of Sia from the surface of insect-derived metacyclic trypomastigotes enhances parasite-host interactions. The removal of Sia from *T. cruzi* glycoproteins and the concomitant

exposure of cryptic β-Gal*p* residues would favor $TcTS_{Y342H}$ interaction with both host sialoglycoconjugates and terminal β-Gal*p*-containing glycoproteins on the parasite surface, thus enhancing *T. cruzi*/host adhesion by the removal of Sia and the concomitant exposure of β-Gal*p* residues from host cell glycans. This phenomenon was well characterized for CD22, a mammalian Sia-binding lectin (Varki and Gagneux 2012). Thus, the removal of Sia and the concomitant exposure of β-Gal*p* residues from host cell glycans can be physiologically significant by promoting parasite adherence and penetration of host cells.

Another hypothesis is that TcTS sialylates the parasite glycomolecules, generating ligands for Sia-binding Ig-like lectin (Siglecs). Recent studies have shown that *T. cruzi* sialylglycoproteins binds to siglecs on the host cell surface (Erdmann et al. 2009; Jacobs et al. 2010). Siglecs are a family of sialic-acid-binding immunoglobulin-like lectins that promote cell–cell interactions and regulate the functions of cells in the innate and adaptive immune systems through glycan recognition (Varki and Gagneux 2012). *T. cruzi* mucin engagement with the Sia-binding protein Siglec-E promotes the immunosuppression of dendritic cells (Erdmann et al. 2009).

Concerning host cells it has being shown that TcTS promotes parasite attachment and entry into host cells through sialyl receptors (Souza et al. 2010). Experiments with Sia-deficient mutants of Chinese Hamster Ovaries (CHO) (Ciavaglia et al. 1993; Ming et al. 1993) first supported this premise. Sia-deficient cells were less infective than wild type cells, suggesting that the sialylation of glycoconjugates on CHO cell surfaces is necessary during *T. cruzi* invasion. The role of TcTS in parasite adhesion to and invasion of host cells is supported by results showing that the treatment of cells with modified Sia precursors N-acylmannosamines (Lieke et al. 2011) or with an irreversible inhibitor of the enzyme decreased cell invasion by *T. cruzi* (Carvalho et al. 2010). Studies with endothelial cells support the importance of TcTS and Sia-containing molecules on the first steps of parasite interaction and penetration of host cells. However, transfer reaction does not seem to be involved in this process as the inactive mutant $TcTS_{Y342H}$ also up regulates parasite entry into endothelial cells. The data show that $TcTS_{Y342H}$ binds α2-3-sialic acid containing molecules on endothelial cells resulting in NF-κB activation, expression of cell adhesion molecules E-selectin, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and rescue from apoptosis (Dias et al. 2008). Other evidences show that TcTS promotes trypanosome-host cell interaction independent of sialidase/*trans*-sialidase activities. TcTS mediates invasion of neural, epithelial, and phagocytic cells via nerve growth factor receptor (NGF) TrkA (Melo-Jorge and PereiraPerrin 2004, 2007). The effect is reproduced by TcTS mutants lacking catalytic activity (Chuenkova et al. 1999) and a ~22mer synthetic peptide (and thus without enzymatic activity) reproduces biological activities of the TcTS (Chuenkova and PereiraPerrin 2005).

Nevertheless, studies suggest that a major function of TcTS in host cell infection is to facilitate the escape of the trypomastigote forms from the parasitophorous vacuole into the cytosol and their subsequent differentiation into amastigotes (Hall et al. 1992; Hall 1993; Hall and Joiner 1993; Lopez et al. 2002; Rubin-de-Celis et al. 2006). The exit of trypomastigotes from the parasitophorous vacuole in sialic

acid-deficient cells occurs earlier than in wild-type cells suggesting that Sia may act as a barrier for parasite to escape (Hall et al. 1992; Hall and Joiner 1993; Rubin-de-Celis et al. 2006). Likewise, parasites over expressing TcTS on the surface escaped earlier from the vacuole than non-transfected parasites indicating that TcTS may help parasites to enter the cytosol (Rubin-de-Celis et al. 2006). TcTS seems to metacyclics over remove sialic acid from lysosomal membranes, which fuse with *T. cruzi* containing phagolysosomes after parasite invasion (Andrews 2002; Andrade and Andrews 2004). This appears to facilitate phagosomal membrane disruption by TcTox, the parasite pore forming molecule (Andrews et al. 1990).

In addition to its role in mammalian cell invasion, TcTS is involved in the pathogenicity of *T. cruzi*; indeed, the *in vivo* injection of small amounts of purified native TcTS activity increased parasitemia and mortality in *T. cruzi*-infected mice (Chuenkova and Pereira 1995; Freire-de-Lima et al. 2010). The effect observed was specific to the transfer activity of TcTS, as the same effect did not occur in mice primed with viral or bacterial sialidases. Nevertheless, the injection of TcTS into deficient SCID mice had no effect on parasitemia or mortality, suggesting that the mechanisms responsible for the observed effects involve host B and T lymphocytes (Chuenkova and Pereira 1995). These findings suggest that the soluble form of TcTS is a virulence determinant molecule with relevant biological effects on the host immune system. Consistent with TcTS functioning as a virulence factor, the heterologous expression of TcTS in *Leishmania major* enhances parasite virulence (Belen-Carrillo et al. 2000).

Multiple effects of TcTS on host T- and B-lymphocyte function were demonstrated (Fig. 8.1). The SAPA repeats induce the production of antibodies (Cazzulo and Frasch 1992; Buscaglia et al. 1998). The high immunogenicity of SAPA antigens might play a role *in vivo* by increasing the half-life of the protein in the blood and by delaying the formation of inhibitory antibodies against the catalytic site of TcTS (Buscaglia et al. 1999; Pitcovsky et al. 2002), which correlates with control of parasite levels (Risso et al. 2007). The crystal structure of an inhibitory antibody fragment in complex with the globular region of TcTS was recently determined (Buschiazzo et al. 2012). The structure showed that the antibody does not occlude the catalytic site enzyme, instead, the antibody performs a delicate action by inhibiting the movement of an assisting Tyr119, whose mobility is known to play a key role in the catalyze reaction. Moreover, the C-terminal region of TcTS activates B cells, inducing the production of nonspecific antibodies independent of the activity of T cells (Gao et al. 2002).

Furthermore, the engagement of TcTS and its inactive analog $TcTS_{Y342H}$ with epitopes containing α2-3-Sia on CD43 from host $CD4^+$ T cells, triggers a co-stimulatory response through the mitogen-activated protein kinase ERK1/2 cascade inducing mitogenesis (Todeschini et al. 2002b). These results suggest that TcTS is responsible for host polyclonal lymphocyte activation, a condition underlying the induction of immunopathology and preventing effective vaccination (Reina-San-Martin et al. 2000; Minoprio 2001) in the course of *T. cruzi* infection; these observations also corroborate the hypothesis that $TcTS_{Y342H}$ promotes glycan cross-linking.

Studies on the effect of TcTS on $CD8^+$ T cells show that TcTS resialylates $CD8^+$ T cell surface, thereby dampening the Ag-specific response and favoring parasite persistence in the mammalian host (Freire-de-Lima et al. 2010). TcTS-mediated resialylation *in vitro* and *in vivo* decreases the cytotoxic activity of antigen-experienced $CD8^+$ T cells against the immunodominant synthetic peptide IYNVGQVSI (Freire-de-Lima et al. 2010). These results demonstrate that *T. cruzi* subverts sialylation to attenuate $CD8^+$ T cell interactions with peptide/MHC class I complexes. $CD8^+$ T cell resialylation may represent a sophisticated strategy to ensure lifetime host parasitism. In an attempt to establish the nature of the Sia acceptor for TcTS on the $CD8^+$ T cell surface, $CD8^+$ T cells from mice lacking the ST3Gal-I sialyltransferase, an enzyme required for sialylation of core 1 *O*-glycans (Priatel et al. 2000), were infected with *T. cruzi*. The loss of ST3Gal-I sialyltransferase exposes the Gal1-3GalNAc-Ser/Thr moiety, creating an attractive model to establish CD43 as a natural receptor for native TcTS during *T. cruzi* infection. Indeed, the infection of mice lacking ST3-Gal-I sialyltransferase restores, at least in part, the binding of anti-CD43 S7 mAbs that recognize Sia-containing epitopes on CD43 of $CD8^+$ T cells. These findings indicate that CD43 is a target receptor for TS on the $CD8^+$ T cell surfaces. However, the resialylation by TcTS was also observed on $CD8^+$ T cells from CD43 KO mice, suggesting that, in the absence of CD43, other molecules are substrates for TcTS. Other studies using azido-modified unnatural Sia revealed that CD45 isoforms are Sia acceptors for TcTS activity as well (Muiá et al. 2010). Moreover, the sialylation of thymocytes by TcTS activity is crucial to deciding the outcome of the cells during interaction with thymic lectins. The alteration of the surface sialylation by TcTS (Mucci et al 2006) leads to *in vivo* depletion of the $CD4^+CD8^+$ double-positive thymocytes inside the "nurse cell complex" (Leguizamón et al. 1999).

TcTS activity can also compromises host cell homeostasis. Tribulatti and co-authors (2005) demonstrated that the administration of TcTS to uninfected mice reduces the Sia content of platelets (Fig. 8.1), thus exposing terminal galactose residues, which may explain the severe thrombocytopenia observed in *T. cruzi*-infected individuals. The recognition of the terminal galactose moiety exposed on the platelet surface accelerates platelet clearance by asialoglycoprotein receptor-expressing scavenger cells (Sørensen et al. 2009). The effect of TcTS on the lifetime of other cell types and plasma glycoproteins should be further verified.

Another interesting example of how TcTS activity can modulate host responses is the effect of the sialylation of host cell receptors. The desialylation of sialyl TOLL-like receptor 4 (TLR4) by TcTS induces receptor dimerization and facilitates MyD88/TLR4 complex formation and NF-kappaB activation in a manner similar to the responses observed with LPS (Amith et al. 2010). Likewise, TcTS alters the sialylation status of the tyrosine kinase receptor-A (TrkA) in PC12 cells, inducing receptor internalization, activation, neuronal differentiation and rescue from apoptosis (Woronowicz et al. 2004, 2007). The observed effects are triggered by the hydrolysis of Sia residues of TrkA by TcTS because a purified recombinant α2-3-neuraminidase, but not a catalytically inactive mutant of TcTS, induces the receptor phosphorylation.

Due its role in neuronal differentiation, neural repair and neuron protection against apoptosis upon Trk receptor binding, TcTS was termed a "parasitokine" or

"parasite-derived neurotrophic factor (PDNF)" (Chuenkova and Pereira 2000, 2003; Chuenkova and Pereiraperrin 2005, 2011). Trk receptors are a family of tyrosine kinases that regulate synaptic strength and plasticity in the mammalian nervous system (Fig. 8.1). TcTS binds to the TrK through the globular C-domain corresponding to the amino acid sequence 425–445 (Chuenkova and Pereiraperrin 2005), thus independently of any enzymatic activity. Following binding to Trk, TcTS causes receptor dimerization, the phosphorylation of tyrosine residues in the cytoplasmic domain and the generation of cell signals critical for neuronal survival (Chuenkova and Pereiraperrin 2011). More recently, it was demonstrated that TcTS-TrkA interaction in cardiac fibroblast induces an increased production of NGF, enabling, in a paracrine fashion, myocytes to resist oxidative stress (Aridgides et al. 2013). Thus, TcTS-elicited regenerative responses likely prolong parasite persistence in infected tissues.

4 Conclusions

On the basis of the above observations it would be reasonable to compare the apparently contradictory effects of TcTS during *T. cruzi* infection outlined in this review to the Chinese concept of yin-yang used to describe how seemingly opposite or contrary forces are interconnected, interacting to form a whole greater than either separate part. Thus, it would be more appropriate to say that TcTS effects are actually complementary, not opposing, effects, sometimes favoring the host, sometimes favoring the parasite ensuring a parasitism that last for host whole life.

Beyond the urgency of alternative drugs to treat the illness, to pursuit of TcTS inhibitors might clarify the role of TcTS in the pathogenesis of Chagas' disease. Although effective TcTS inhibitors have not yet been reached, efforts made in this area have found interesting lead compounds.

Furthermore, recognition of a wide variety of substrates recognized by TcTS makes it the proper tool for direct enzymatic glycosylation of sialyloligosaccharides in glycans synthesis. Finally the TcTS may be used as therapeutic agents to treat not only infectious diseases but also unrelated disorders.

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