Organization and Expression of a Multigene Family Encoding the Surface Glycoproteins of *Trypanosoma cruzi* Metacyclic Trypomastigotes Involved in the Cell Invasion

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The infective forms of *Trypanosoma cruzi* are the trypomastigote stages found in the bloodstream of mammalian hosts or the metacyclic trypomastigotes present in the digestive tract of the insect. Metacyclic trypomastigotes express two stage-specific glycoproteins (gp90 and gp82) that have no counterpart in blood trypomastigotes (Yoshida 1983, Teixeira & Yoshida 1986). The gp90 and gp82 are involved in the penetration of the parasite into host cells (Araguth et al. 1988, Yoshida et al. 1993, Ramirez et al. 1993, Santori et al. 1996a). Gp82 can induce Ca^{2+} signal in target cells (Dorta et al. 1995, Ruiz et al. 1998), an event essential for *T. cruzi* internalization (Dorta et al. 1995). Gp90 and gp82 are also relevant immunologically. Immunization with gp90 or gp82 protects mice against acute infection by *T. cruzi* (Araguth et al. 1988, Gonzalez et al. 1991, Yoshida et al. 1993, Santori et al. 1996b). Here we present studies on the genomic organization and expression of genes encoding gp90 and gp82.

STRUCTURE OF *gp90* AND *gp82* GENES

cDNA clones encoding gp82 and gp90 were isolated from expression libraries using specific monoclonal antibodies (Franco et al. 1993, Araya et al. 1994). Analysis of cDNA clones encoding gp82 and gp90 revealed the existence of several post-translational modifications such as *N*-glycosylation and addition of a GPI anchor (Franco et al. 1993, Araya et al. 1994). The open reading frames (ORF) of gp82 cDNA clones encode polypeptides of 516 to 595 amino acids with molecular masses of 55.6 to 64 kDa, which are lower than the 70-kDa polypeptide devoid of *N*-linked oligosaccharides, which is the precursor to the gp82. Structurally, gp82 and gp90 are surface glycoproteins containing *N*-linked oligosaccharide side chains and anchored to the membrane through a glycosylphosphatidylinositol (GPI) moiety (Schenkman et al. 1989, Yoshida et al. 1990, Ramirez et al. 1993, Cardoso de Almeida & N Heise 1993, Araya et al. 1994, Ramirez et al. 1998).

Heterologous expression of gp82 in mammalian cells indicates that the requirements for translocation of the nascent polypeptide across the endoplasmic reticulum and membrane glycosylphosphatidyl-inositol anchoring are distinct in mammals and *T. cruzi* (Ramirez et al. 1998, 1999). In the carboxy-terminal domain of gp82 and gp90 was identified a cleavage/attachment site for GPI anchor addition (ω site) composed by the following residues: aspartic, glycine and serine (Ramirez et al. 1999). The ω site (ω=Asp, ω+1=Glyc, ω+2=Ser) of gp82 and gp90 differs from that found in GPI linked proteins of mammalian cells in which serine is at site w and alanine or glycine are found at site ω+2 (Udenfriend & Kodukula 1995). The presence of aspartic acid and serine at sites ω and ω+2 in the gp90 and gp82 suggests that transamidase of these organisms are different from that of mammalian cells. We could speculate that the binding pocket of mammalian transamidase is not large

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enough to accomodate two slightly larger residues as aspartic acid and serine at sites ω and ω+2 present in trypanosome GPI signal. The differences found between trypanosomes and mammalian host cells could be useful for the development of specific antiparasite drugs.

Comparison of sequences of gp90 and gp82 showed 40% identity at amino acid level, with homologous regions separated by sequences displaying significant amino acid differences (Franco et al. 1993, Araya et al. 1994). Sequence analysis of gp90 and gp82 also revealed 40-60% identity at amino acid level with members of *T. cruzi* gp85/sialidase family (Cross & Takle 1993, Colli 1993). Camptella et al. (1992) suggested that the *T. cruzi* surface antigens may have originated from an ascentral (neuraminidase) gene, and proposed the grouping of these gene families in a superfamily of *T. cruzi* surface antigens. Based on these structural features, *gp90* and *gp82* genes could be considered as members of gp85/sialidase family. The gene family encoding metacyclic surface antigens could be included in the group II of the gp85/sialidase family together with the glycoproteins of 85 kDa (gp85, Tc85, TSA-1, Tt34c1, SA85) expressed in bloodstream trypomastigotes.

GENOMIC ORGANIZATION OF *gp90* AND *gp82* GENES

The genes *gp90* and gp90 are organized in subsets spread out in the genome. To study the organization of these genes several distinct YACs were isolated from a library constructed with DNA from the *T. cruzi* clone CL Brener (Ferrari et al. 1997). The *T. cruzi* YAC library containing about 3,000 recombinant clones with a mean insert size of 365 kb and representing more than 10 genome equivalents, was screened by hybridization and PCR with a sequence that encodes the C-terminal domain of gp90. Twenty four YACs (mean insert size = 150 to 450 kb) were isolated and characterized. Restriction mapping and hybridization analysis showed that several YACs may contain at least two copies of gene gp*90*. A common feature of the YACs was the presence of other members of gp85/sialidase family such as *gp85* and *gp90* indicating that genes *gp90* and genes *gp82* and/or *gp85* are linked at multiple sites of the genome.

Gp90 and gp82 are present in multiple copies, distributed in several chromosomes, and this gene family can be divided into subsets on the basis of hybridization patterns obtained with probes derived from different regions of gp90 and gp82 genes (Araya et al. 1994, Cano et al. 1995, Santos et al. 1997). Many members of gp90 and gp82 gene family are closely linked to members of gp85/sialidase family at multiple sites in the genome of different *T. cruzi* strains. Hybridization patterns of *gp90*, *gp82* and *gp85* genes with *T. cruzi* chromosomal bands separated by pulsed field gel electrophoresis are very similar, suggesting that many of these genes could be linked in different chromosomal *loci*. This was confirmed by isolation of genomic DNA clones from YAC and cosmid libraries as described above.

It is interesting to note that some several subtelomeric regions are made of sequences associated to the gp90 and gp85 (Chiurillo et al. 1999). The presence of gp90 and gp85 at *T. cruzi* telomeres suggests that new variants of the gp85/sialidase family can continously be arising by duplication, mutation, and recombination of copies that have been transposed to the telomeres.

TRANSCRIPTION OF *gp90* AND *gp82* GENES

Northern blot and western blot analyses showed that gp90 and gp82 are preferentially transcribed and expressed in the metacyclic trypomastigote stage (Franco et al. 1993, Araya et al. 1994). Further studies on the transcription of these genes using "run on" and RNA-PCR assays showed the presence of gp82 and gp90 transcripts in epimastigotes and blood trypomastigotes. Taken together these results suggest that the expression of genes gp90 and gp82 is constitutive and may be regulated at post-transcriptional level, for instance, at translational level and/or mRNA stabilization.

IDENTIFICATION OF *gp82* AND *gp90* HOMOLOGUES IN *TRYPANOSOMA RANGELI*

The non pathogenic protozoon *T. rangeli* and *T. cruzi* share several characteristics, including the minicircle structure and several antigenic determinants, suggesting that both parasites are closely related. A comparative analysis of the *T. cruzi* gp82/gp90 and *T. rangeli* related sequences may aid in the determination of the features of gp82 and gp90 proteins that contribute to its function in host cell interactions.

The presence of multiple copies of genes in *T. rangeli* enconding products related to *T. cruzi* gp82, gp90 and gp85 was revealed when genomic *T. rangeli* DNA was hybridized at moderate and high stringencies with *gp82*, *gp90* and *gp85* genes. Even at high stringency conditions, both probes hybridized with several genomic fragments suggesting that gp82, gp90 and gp85 related sequences are interspersed in the genome rather than arranged in tandem repeats. Sequence analysis showed that many of hybridizing fragments contain sequences associated to the gp85/sialidase gene family. These results suggested that gp90, gp82 and gp85 genes have been originated from a common ancestral gene present in several member of *Trypanosoma* genus.

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