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Gene Discovery through Expressed Sequence Tag Sequencing in *Trypanosoma cruzi*

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Analysis of expressed sequence tags (ESTs) constitutes a useful approach for gene identification that, in the case of human pathogens, might result in the identification of new targets for chemotherapy and vaccine development. As part of the *Trypanosoma cruzi* genome project, we have partially sequenced the 5′ ends of 1,949 clones to generate ESTs. The clones were randomly selected from a normalized CL Brener epimastigote cDNA library. A total of 14.6% of the clones were homologous to previously identified *T. cruzi* genes, while 18.4% had significant matches to genes from other organisms in the database. A total of 67% of the ESTs had no matches in the database, and thus, some of them might be *T. cruzi*-specific genes. Functional groups of those sequences with matches in the database were constructed according to their putative biological functions. The two largest categories were protein synthesis (23.3%) and cell surface molecules (10.8%). The information reported in this paper should be useful for researchers in the field to analyze genes and proteins of their own interest.

Partial cDNA sequencing to generate expressed sequence tags (ESTs) is being used at present for the fast and efficient obtainment of a detailed profile of genes expressed in various tissues, cell types, or developmental stages (1). Genome projects have taken advantage of EST studies because ESTs represent a particular type of sequence-tagged sites useful for the physical mapping of genomes (24). ESTs can serve the same purpose as sequence-tagged sites, with the additional bonus of pointing directly to expressed genes.

One of the most interesting applications of the EST database (dbEST) is gene discovery (6). A significant development with important implications in this field has been the enormous growth of the dbEST (5). Novel genes can be found by querying the dbEST with a protein or DNA sequence. Among a number of recent examples of findings made by following this approach, a new member of the human Ly-6 family was detected (10) and 66 human ESTs were identified and mapped based on their resemblance to 66 *Drosophila* genes (3).

In 1994, the Special Programme for Research and Training in Tropical Diseases of the World Health Organization launched an initiative to analyze the genomes of the parasites *Filaria*, *Schistosoma*, *Leishmania*, *Trypanosoma brucei*, and *Trypanosoma cruzi*. Five networks were established, with the aims of (i) gaining significant knowledge on the molecular biology of these parasites; (ii) identifying new genes and their products which could be used to design new drugs, to speed up vaccine development, and to improve diagnosis; and (iii) sharing material and expertise and providing an information system that is accessible globally to researchers in the field (32).

T. cruzi is the agent of the American trypanosomiasis, Chagas' disease, for which there is neither a definitive chemotherapeutic treatment nor a vaccine being tested at present. This parasite has a complex life cycle in the Triatomine insect vector (epimastigote and metacyclic trypomastigote parasite stages) and in the mammalian host (the bloodstream trypomastigote and the intracellular amastigote stages). Thus, the expression of a number of stage-specific genes might be related to the different environments and requirements of each parasite stage. Given these facts, and as part of the *T. cruzi* genome project (32), we have started a project on gene discovery through EST sequencing. A total of 1,949 ESTs were sequenced from a normalized epimastigote cDNA library of the parasite clone (CL Brener) selected for this genome project (31). Their analysis revealed that the putative functions of about 18.4% of the ESTs might be deduced by sequence comparison with genes from other organisms, while about 67% have no sequence homologies in the databases and thus might represent some *T. cruzi*-specific sequences.

MATERIALS AND METHODS

cDNA library. Poly(A)$^+$ RNA isolated from CL Brener epimastigotes was used to construct a directional cDNA library in the plasmid vector pT7T318D with a modified polylinker, which consists of the restriction sites for *Sfi*I, *Eco*RI, *Sna*BI, *Bam*HI, *Pac*I, *Not*I, and *Hin*dIII placed between the T7 and T3 promoters (7). This reduced polylinker was necessary for the efficiency of the subsequent normalization procedure. Normalization was done by partial reassociation kinetics and hydroxyapatite chromatography, whereby the excess of abundant cDNA clones was removed (7). Further details of the construction and characterization of the normalized library will be described elsewhere. Around 23,040 clones were randomly picked and plated in 384-well microplates in the laboratory of Ulf Pettersson (Uppsala, Sweden).

Nucleotide sequencing. Aliquots (1 to 2 μl) of each clone from 384-well microplates were grown overnight at 37°C in 3 ml of 2xTY containing 100 μg of ampicillin per ml (26). The template DNA for the sequencing reaction was prepared from 1.5 ml of culture by an alkaline lysis method with minor modifications (26), followed by a polyethylene glycol 8000 precipitation. The amount of isolated DNA template was estimated on a 1.0% agarose gel by comparison to serial dilutions of pBluescript II KS(+) (Stratagene). Sequencing reactions were performed in a Genius thermal cycler (Techne) by using a Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase (FS enzyme) (Applied Biosystems) according to the protocols supplied by the manufacturer and were analyzed in an ABI prism 377 sequencer (Applied Biosystems). Single-pass sequencing was performed on each template with T7 primer, and sequences longer than 100 bases were further analyzed. The ESTs were edited to remove

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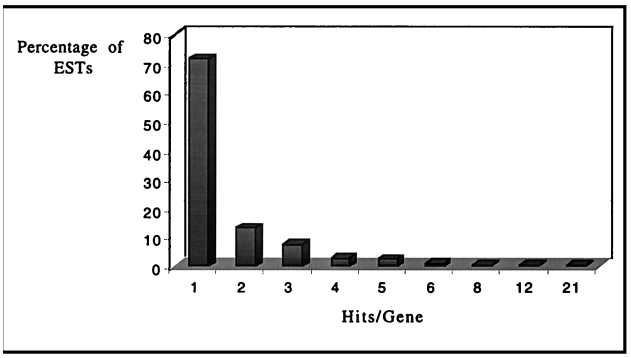
A



B

Redundancy	Gene
5	Heat Shock Protein 70
5	Gene for Putative Ribosomal Protein S31
5	mRNA For Ribosomal P1 Type Protein
5	Antigen Tyrosine Aminotransferase
5	CL Hexose Transporter Gene
5	Hypothetical Protein
5	Histone H4
5	Heat Shock Protein 70
5	Probable 40S Ribosomal Protein S9
5	Histone H2B
6	60s Ribosomal Protein L13a
6	40S Ribosomal Protein S19
6	40S Ribosomal Protein S3
8	Ribosomal Protein L28
8	Mitochondrial HSP70
12	Histone H3
21	Histone H2A

FIG. 1. Level of redundancy of ESTs that matched sequences in the NCBI nonredundant databases. (A) Percentage of ESTs with the indicated number of matches to the same gene. (B) Genes with five or more hits. The analysis was performed on a total of 644 ESTs.

vector sequences from 5′ ends and to remove unreliable data from the 3′ ends by using the program Factura (Perkin-Elmer).

Sequence analysis. The sequences were compared against the National Center for Biotechnology Information (NCBI) nonredundant protein database by using the program BLASTx (2) on the BLAST network service at NCBI. Sequences that did not match sequences in the protein databases were further analyzed by searching for similarities at the nucleotide level by using the BLASTn program against the nonredundant nucleotide sequence database.

Nucleotide sequence accession numbers. EST sequence data has been deposited in the dbEST with the following accession numbers: AA867894 to AA867980, AA882519 to AA883010, AA890742 to AA891021, AA908031 to AA908158, AA926379 to AA926628, AA952317 to AA952754, AA958023 to AA958272, and AA960728 to AA960749.

RESULTS AND DISCUSSION

A normalized cDNA library was used to reduce considerably the number of high- and intermediate-abundance sequences and to maximize the chances of finding new genes through random sequencing (28). A total of 1,994 clones were randomly selected, and the 5′ ends of the inserts were sequenced. After deletion of vector sequences and unreliable data, an average length of 420 bases per clone was obtained and used for database searches. Sequence similarities identified by the BLAST programs were considered statistically significant with a Poisson P value of $\leq 10^{-5}$. Among the 1,994 sequences, 31 contained no insert and 14 exhibited homology with rRNA and were excluded from further analysis.

We first estimated the redundancy of our data on the basis of the redundancy of homology with sequences in the databases. A total of 644 ESTs were identified by homology with 398 different genes in the databases, representing a calculated level of redundancy of 27.9%. As shown in Fig. 1, data were classified according to the number of matches (hits) per gene. Among the 644 ESTs, 357 appeared more than once (redundant EST group), representing 111 putative genes, and 287 appeared only once. The most frequently represented genes in the library were those encoding histone H2A (accession no. gnl|PID|e290647) and histone H3 (gi|442456), which appeared 21 and 12 times, respectively (Fig. 1B). In contrast to the case for other organisms, histone transcripts in trypanosomatids are polyadenylated (19). Since the clones were picked from a normalized library, the redundancy of a cDNA clone should not be thought to represent the expression level of the gene.

TABLE 1. Database match categories of ESTs sequenced in *T. cruzi*

EST category	No. of ESTs	% of ESTs
Total	1,949	100
Database matches to:		
Total	644	33
T. cruzi	285	14.6
Other trypanosomatids	80	4.1
Other organisms	279	14.3
No database match[a]	1,305	67

[a] ESTs without significant matches ($P > 10^{-5}$) to database sequences.

On the basis of database searches, the 1,949 EST sequences were classified into four groups, as shown in Table 1. About 18.7 and 14.3% matched sequences from trypanosomatids and from other organisms, respectively. About 67% did not have a database match and thus might represent *T. cruzi*-specific genes. The percentage of ESTs with matches was somewhat higher (33%) than that obtained in other EST studies of protozoan parasites (11, 16, 20).

Further analyses of our data were performed by taking into account only nonredundant ESTs. That is, when more than one EST showed homology to a gene annotated in the databases, only one EST was considered in the analysis.

ESTs with predicted or known functions were classified into putative cellular roles (4). The proportion of ESTs in each role category is shown in Fig. 2. Of the 398 nonredundant ESTs

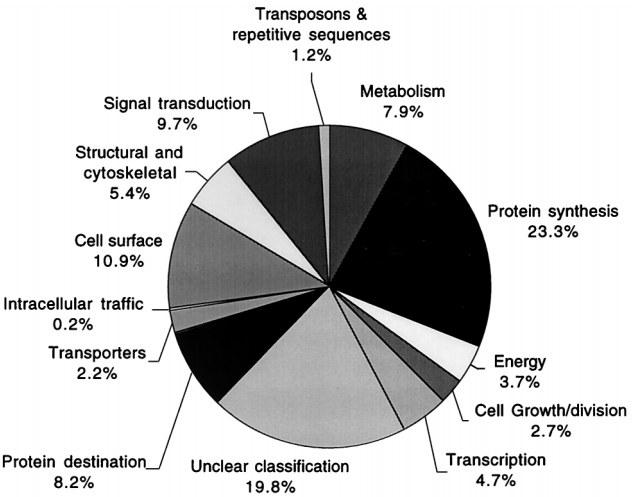


FIG. 2. Functional classification of *T. cruzi* ESTs, showing the proportions of predicted genes according to their putative biological functions. A total of 398 nonredundant ESTs having a P value of $\leq 10^{-5}$ were classified into 13 categories.

TABLE 2. *T. cruzi* EST matches to known sequences from trypanosomatids (not *T. cruzi*) and other organisms in NCBI databases[a]

EST (TENS no.)[b]	Putative identification[c]	Accession no.	BLAST[d]
Other trypanosomatids			
1273	40S ribosomal protein L14	sp|P55842|	X
0051	40S ribosomal protein S12	sp|Q03253|	X
0057	40S ribosomal protein S14	sp|P19800|	X
1630	60S ribosomal protein L18	sp|P50885|	X
1451	60S ribosomal protein L30	sp|P49153|	X
1271	Activated protein kinase C receptor homolog mRNA	gb|U72205|	N
1408	Activated protein kinase C receptor homolog TRACK	gi|2952301|	X
0472	Cyclophilin A	gi|1532210|	X
1314	Cytochrome *c* oxidase polypeptide I	sp|P04371|	X
1285	Fructose-bisphosphate aldolase	pir||A54500 |	X
1354	GP63-3 surface protease homolog	gi|2196917 |	X
1942	GP63-3 surface protease homolog	gi|2196917	X
0362	H^+-transporting ATPase (EC 3.6.1.35)	pir||A45598|	X
1233	Hypothetical protein 2	pir||A05123 |	X
1614	Intergenic region from the EF-1alpha upstream-associated gene-1 to the EF-1alpha gene	gb|U52680|	N
1421	Kinetoplastid membrane protein 11	gnl|PID|e225864	X
0020	mRNA for S12-like ribosomal protein	emb|Z15031|	N
1636	mRNA, clone Q14R1	emb|Z86119|	X
1943	Nucleic acid-binding protein	gi|1841864	X
0506	ORF 1	gnl|PID|e37082	X
1439	Phosphoglycerate kinase	sp|P41760|	X
1204	Phosphoglycerate kinase, glycosomal	sp|P41762|	X
0072	Probable 40S ribosomal protein S9	sp|P17959|	X
1291	Putative serine/threonine protein kinase	sp|Q08942|	X
0021	Ribosomal protein L27a	gb|U96757|	N
1345	Thioredoxin peroxidase	sp|Q26695|	X
Other organisms			
1260	1,5-Heptosyltransferase I (Rfac) and Flax genes, complete Cds	gb|U40862|	N
0451	10-kDa heat shock protein, mitochondrial (Hsp10)	pir||S47532|	X
1352	14-3-3-Like protein	gi|1773328|	X
1290	2-Oxoglutarate dehydrogenase E1 component precursor	sp|P20967|	X
1838	3,2-*trans*-Enoyl-coenzyme A isomerase	sp|P42125|	X
1264	31.1-kDa protein In Dcm-Seru intergenic region	sp|P31658|	X
1485	40S ribosomal protein	sp|Q06559|	X
0047	40S ribosomal protein S10	sp|Q07254|	X
1750	40S ribosomal protein S13	sp|Q05761|	X
0904	40S ribosomal protein S15	sp|P20342|	X
0046	40S ribosomal protein S16	sp|P46294|	X
0084	40S ribosomal protein S17	sp|O01692|	X
0037	40S ribosomal protein S19	sp|P40978|	X
0012	40S ribosomal protein S2	sp|P25444|	X
1725	40S ribosomal protein S23	sp|P39028|	X
0063	40S ribosomal protein S25	sp|P46301|	X
0079	40S ribosomal protein S26e	sp|P21772|	X
0053	40S ribosomal protein S3	sp|Q06559|	X
0045	40S ribosomal protein S4	sp|P47961|	X
0038	40S ribosomal protein S6	sp|P02365|	X
0056	40S ribosomal protein Sa	sp|P38981|	X
0077	50S ribosomal protein L13	sp|O06260|	X
0949	55.2-kDa protein in Hxt8 5′ region	sp|P39976|	X
0028	60S ribosomal protein L10	sp|Q09127|	X
0027	60S ribosomal protein L11	sp|P42922|	X
0075	60S ribosomal protein L12	sp|P30050|	X
0954	60S ribosomal protein L13a	sp|P35427|	X
1482	60S ribosomal protein L17	sp|P24049|	X
1794	60S ribosomal protein L18a	sp|P41093|	X
0054	60S ribosomal protein L2	sp|P29766|	X
1589	60S ribosomal protein L21	sp|Q43291|	X
1003	60S ribosomal protein L22	sp|P13732|	X
1923	60S ribosomal protein L24	sp|P38663|	X
0049	60S ribosomal protein L26	sp|P47832|	X
0003	60S ribosomal protein L26-B	sp|P53221|	X
0008	60S ribosomal protein L3	sp|P35684|	N
0043	60S ribosomal protein L31	sp|P46290|	X
1875	60S ribosomal protein L32	sp|Q94460|	X
0081	60S ribosomal protein L35	sp|P42766|	X
0085	60S ribosomal protein L37a	sp|P32046|	X
0953	60S ribosomal protein L5	sp|Q26481|	X
0061	60S ribosomal protein L7	sp|P11874|	X
1925	60S ribosomal protein L7b	sp|P25457|	X
0033	60S ribosomal protein L9	sp|P49209|	X
1917	Acidic ribosomal protein P1	gi|2865615|	X
1468	Actin-interacting protein 2	sp|P46681|	X
1830	Acyl carrier protein	sp|P53665|	X
1801	Adenosylhomocysteinase	pir||A45569|	X
1946	ADP-ribosylation factor 1	sp|P35676|	X
0459	Af-9 Protein	sp|P42568|	X
1326	Alpha NAC/1.9.2. protein	gi|1142653|	X
1289	Alpha proteasome	gnl|PID|e321980	X
1374	Alpha-adaptin	gnl|PID|d1022258	X
1381	Alpha-enolase/tau-crystallin	gi|213085|	X
1520	Alpha-gliadin storage protein pseudogene	gb|U51305|	N
1944	TBP-interacting protein (TIP 49)	gnl|PID| d1029109	X
1301	Alternative oxidase	dbj||AB003176_1	X
1358	Arg kinase	prf||2020435A	X
1329	ATP synthase delta′ chain, mitochondrial precursor	sp|Q41000|	X
1582	ATP synthase F1 subunit alpha	gi|2258360|	X
1242	ATP-dependent RNA helicase, DEAD family (Dead)	gi|2648271|	X
1300	B0025.2 gene product	gi|1938574|	X
0281	BAC-146N21 chromosome X contains iduronate-2-sulfatase gene	gb|AC002315|	N
0265	BBC1 protein	gnl|PID|d1024629	X
1303	Bop1	gi|1679772|	X
1322	C25a1.6	gnl|PID|e275630	X
1635	CAGH26 mRNA	gb|U80739|	N
0644	Calmodulin	gi|167676|	X
0259	Caltractin	gb|U03270|	X
1184	Cctalpha chaperonin subunit	gi|2231589|	X
1281	Cell binding factor 2	sp|Q46105|	X
0416	Chaperonin containing T complex polypeptide 1, beta subunit; CCT-beta	gi|2559012|	X
1227	Chromosome 21q22.2 PAC clone P169K17, complete sequence	gb|AF015720|	N
1599	Cnjb	gi|161752|	X
1331	Contains similarity to enoly-coenzyme A hydratases	gi|2854202|	X
1592	Contains similarity to human spliceosome-associated protein	gi|2384908|	X
1862	Cyclophylin	gnl|PID|e267528	X
1294	Cytochrome b_5	gi|2062405|	X
1856	Cytochrome P450-like TBP	gnl|PID|d1011583	X
1304	Cytoplasmic malate dehydrogenase	gi|2286153|	X
1272	Deoxyhypusine synthase mRNA	gb|U40579|	N
1435	Dihydrolipoamide acetyltransferase component (E2) of pyruvate dehydrogenase complex (Pdc-E2)	sp|P08461|	X
1279	Dihyroorotate dehydrogenase	sp|P28272|	X
1851	DNA polymerase delta small subunit	gnl|PID|e243837	X
1376	DNA-directed DNA polymerase	pir||A55874|	X
1338	Dnaj protein	sp|P35515|	X
1293	Drome Pelota protein	sp|P48612|	X
1406	Dynein beta chain, flagellar outer arm	sp|Q39565|	X
1274	Enolase 1	P51555	X
1320	Enoyl-coenzyme A Hydratase, mitochondrial precursor	sp|P14604|	X
0438	Estb = esterase II	gb|S79600|	N
0501	Eukaryotic translation initiation factor 1a	sp|P38912|	X
1633	Excision repair protein Ercc-6	sp|Q03468|	X
1602	F21b7.26	gi|2809257|	X
1313	F421: this 421-aa ORF is 31% identical (3 gaps) to 91 residues of an approximately 864-aa protein, LOX3_SOYBN SW: P09186	gi|1787042|	X
1699	F44g4.1	gnl|PID|e236517	X
0581	Fast tropomyosin isoform	gi|2660868|	X
1284	G10 protein homolog	sp|P34313|	
0002	Gene for putative ribosomal protein S31	emb|X14247|	N
0351	Genes for ORF1, ORF2, ORF3, ORF4, and Srb, partial and complete Cds	dbj|D64116|	N
1356	Glucosamine-6-phosphate isomerase	sp|P44538|	X
1722	Glycine cleavage system H protein precursor	sp|P23434|	X
1308	GTP-binding protein Ypt3	sp|P17610|	X
1400	Guanine nucleotide-binding protein alpha subunit	sp|P43151|	X
1327	H protein subunit of glycine decarboxylase mRNA, complete Cds	gb|AF022731|	X
1687	Heat shock protein 10	gi|2623879|	X
1493	Heat shock protein 75	gi|2865466|	X
0670	Heat shock protein HSLV	sp|P31059|	X
1437	Helicase	gi|780410|	X

Continued on following page

TABLE 2—*Continued*

EST (TENS no.)[b]	Putative identification[c]	Accession no.	BLAST[d]
0088	Histone H3	sp|P40285|	X
0094	Histone H4	gnl|PID|e324304	X
1192	Hit family protein 1	sp|Q04344|	X
0448	Homologous to acyl-coenzyme A dehydrogenase	gi|436861|	X
0421	Hydroproline-rich protein mRNA	gb|J03625|	X
1380	Hypothetical 20.8-kDa protein in Fgf-Vubi intergenic region	sp|P21286|	X
1341	Hypothetical 22.6-kDa protein F46c5.8 in chromosome Ii	sp|P52879|	X
1328	Hypothetical 23.5-kDa protein in Rfa2-Stb1 intergenic region	sp|P42844|	X
1910	Hypothetical 24.9-kDa protein in Sura-Hepa intergenic region	sp|P39219|	X
1330	Hypothetical 31.9-kDa protein in Gog5-Clg1 intergenic region	sp|P53081|	X
1302	Hypothetical 39.3-kDa protein in Gcn4-Wbp1 intergenic region	sp|P40004|	X
1364	Hypothetical 41.9-kDa protein in Sds3-Ths1 intergenic region	sp|P40506|	X
1177	Hypothetical 44.5-kDa protein in Pgpb-Pyrf intergenic region precursor	sp|P45576|	X
1824	Hypothetical 47.3-kDa protein in Ompx-Moeb	sp|P38821|	X
1585	Hypothetical 54.2-kDa protein in Cdc12-Orc6 intergenic region	sp|P38821|	X
0386	Hypothetical 90.8-kDa protein T05h10.7 in chromosome Ii	sp|Q10003|	X
1298	Hypothetical protein	gnl|PID|e326877	X
1385	Hypothetical protein	pir||S57550	X
1323	Hypothetical protein	gnl|PID|e339926	X
1618	Hypothetical protein	gnl|PID|e276614	X
1360	Hypothetical protein	gnl|PID|d1018647	X
1185	Hypothetical protein and to PIR:C48583 stress-inducible protein ST11	gi|1213541|	X
1186	Hypothetical protein YDR531w	pir||S69586	X
1812	Hypothetical protein YPL235w	pir||S61029	X
1476	Initiation factor 5a (Eif-5a) (Eif-4d)	sp|P56332|	X
1741	Insulinase	pir||SNHUIN	X
1369	Isocitrate dehydrogenase	gi|1277203|	X
1431	JC8.C	gnl|PID|e1247056	X
1580	KIAA0107-like protein	gi|2982297|	X
1805	Kiaa0305	gnl|PID|d1021601	X
1317	L1231-38	gi|2194152|	X
1315	L1231-6d	gi|2194149|	X
1609	L1439-18	gi|2266918|	X
1407	L4 protein (aa 1–256)	gi|4396(X17204)	X
0069	Large ribosomal subunit protein L13	sp|P38014|	X
1392	Male sterility 2-like protein	gnl|PID|e258459	X
1395	Meiotic spindle formation Protein Mei-1	sp|P34808|	X
0287	Mel-13a transcript	gb|U35309|	N
1889	Membrane-associated diazepam-binding inhibitor	prf||1911410A	X
1692	Mex-1	gi|1899062|	X
1399	Mitochondrial trifunctional enzyme beta subunit precursor	sp|Q60587|	X
1375	Mitotic centromere-associated kinesin	prf||2103270A	X
1515	mRNA for ribosomal protein L12	emb|X53504|	N
0018	mRNA for ribosomal protein S17	emb|X07257|	N
1443	mRNA for surface antigen P2	emb|X56810|	N
1336	No definition line found	gi|2384956|	X
1900	No definition line found	gi|2570931|	X
1391	Novel serine/threonine protein kinase	gnl|PID|d1006875	X
1335	N-terminal acetyltransferase complex Ard1 subunit homolog	sp|Q05885|	X
1941	NUC-1 negative regulatory protein PREG	sp|Q06712|	X
1505	Nucleoside diphosphate kinase	sp|P27950|	X
0667	Peptidase T (aminotripeptidase) (tripeptidase)	sp|P29745|	X
1311	Peptidylprolyl Isomerase	pir||S50141|	X
1905	Peroxisome targeting signal 2 receptor	gi|1907315|	X
1373	Phosphoglucomutase isoform 1 (glucose phosphomutase)	sp|P00949|	X
1347	Phosphoinositide-specific phospholipase C	prf||2123392A	X
1945	Phosphorylation regulatory protein HP-10	pir||A61382	X
1353	Phosphotyrosyl phosphatase activator	gi|974837|	X
1762	Potential Caax prenyl protease 1 (prenyl protein-specific endoprotease 1)	sp|Q10071|	X
0055	Probable 60S ribosomal protein L35	sp|P49180|	X
1370	Probable cell division control protein P55cdc	pir||A56021	X
1382	Probable membrane protein	pir||S51473	X
1412	Probable reductase protein	pir||A32950	X
1844	Proteasome iota chain (macropain iota chain)	sp|P34062|	X
1377	Proteasome subunit P112	gnl|PID|d1008506	X
1581	Protein kinase isolog	gi|2347199|	X
1359	Protein transport protein Sec61 alpha subunit	sp|P79088|	X
1393	Putative dimethyladenosine transferase	gi|2529685|	X
1390	Putative mevalonate kinase	sp|Q09780|	X
1371	Putative protein	gnl|PID|1253348	X
0016	Putative ribosomal protein L7A	gi|2529665|	X
1250	Pyruvate dehydrogenase E1 component, beta subunit precursor	sp|Q09171|	X
1947	RAS homolog GTPase rab28 isoform S	sp|P51157|	X
1948	RAS-related protein RAB-2	sp|Q05975|	X
0394	RAS-related protein Rab-23 (Rab-15)	sp|P35288|	N
1240	Red-1	gnl|PID|e209012	X
0062	Rer1 protein	sp|P25560|	X
1612	Ribonucleoprotein La	pir||A53781	X
0026	Ribosomal protein	gnl|PID|d1019682	X
0010	Ribosomal protein (Rp112)	gb|L04280|	N
0022	Ribosomal protein 15a (40S subunit)	emb|Z21673|	N
1882	Ribosomal protein L10, cytosolic	pir||JN0273	X
0065	Ribosomal protein L13.E, fruit fly	pir||S42877	X
0078	Ribosomal protein L15.E	sp|P30736|	X
0004	Ribosomal protein L3	sp|P39023|	X
1207	Ribosomal protein S11 homolog	pir||A48583	X
1526	Ribosomal protein S30	gnl|PID|e1173009	X
1332	SC2 = synaptic glycoprotein	pir||I56573	X
1318	Serine/threonine protein phosphatase 2b catalytic subunit, beta isoform	sp|P20651|	X
1297	Seryl-tRNA synthetase	pir||S71293	X
1758	Similar to acetyltransferases	gi|1825778|	X
1256	Similar to mammalian ZFP36 proteins in zinc finger regions	gi|1255428|	X
1819	Similar to pig tubulin-tyrosine ligase	gnl|PID|d1012156	X
1387	Similar to *Saccharomyces cerevisiae* BCS1 Protein, SWISS-PROT Accession no. P32839	dbj||D89136_1	X
1720	Similar to *S. cerevisiae* unknown, EMBL Accession no. Z68195	gnl|PID|d1014559	X
0319	Spermidine synthase mRNA	gnl|PID|e267359	X
1253	Succinate dehydrogenase	gnl|PID|e341165	X
1191	Succinyl coenzyme A synthetase alpha Subunit mRNA	gb|U23408|	N
1193	Succinyl-Coa ligase (Gdp-forming)	sp|P13086|	X
1278	Sulfated surface glycoprotein SSG185	prf||1604369	X
1397	Symbiosis-related protein	gi|2072023|	X
1684	T-complex protein 1, alpha subunit	sp|O15891|	X
1309	Thermostable carboxypeptidase 1	sp|P42663|	X
1288	Thyroid receptor-interacting protein 12	sp|Q14669|	X
1949	Translation initiation factor 5A	gnl|PID|e266087	X
1416	Triacylglycerol lipase	sp|P21811|	X
1368	Ubiquinol--cytochrome *C* reductase	pir||A44033	X
1389	UDP-glucose 4-epimerase (Gale-2)	gi|2648515|	X
1405	Unknown	gnl|PID|e223630	X
1436	Vacuolar aminopeptidase I precursor	gi|699234|	X
1307	Wd40 repeat protein 2	sp|P54686|	X
1182	Weak similarity to SP:YAD5_CLOAB (P33746) hypothetical protein and to PIR:C48583 stress-inducible protein STI1	gi|1213541	X
1325	White	gi|2182784	X
0449	Yeast probable phosphatidylinositol-4-phosphate 5-kinase	sp|P34756|	X
1324	ZK795.D	gnl|PID|e1188511	X

[a] All significant similarities ($P \leq 10^{-5}$) of nonredundant ESTs against non-*T. cruzi* entries in NCBI nonredundant databases are listed, together with the accession numbers and the program used for the search. Matches are sorted according to the "Other trypanosomatids" and "Other organisms" categories. A complete (including matches to *T. cruzi*) and more detailed table is available at http://www.iib.unsam.edu.ar/genomelab/tcruzi/5ests.html.

[b] EST names in the dbEST are the four-digit numbers given here preceded by TENS.

[c] ORF, open reading frame; aa, amino acids.

[d] N, BLASTn; X, BLASTx.

analyzed, the largest number (23.3%) was related to protein synthesis; other categories include sequences related to metabolism (7.9%), protein destination (8.2%), transcription (4.7%), and energy (3.7%). Interestingly sequences related to cell surface proteins accounted for 10.9% of the analyzed ESTs (the second-largest category of known functions). It is well known that *T. cruzi* has a large number of surface proteins belonging to at least two main families: the mucin gene family and the superfamily of surface antigens.

The mucin gene family, for which a minimum of 484 genes has been estimated (15), is composed of two groups of genes, as defined by their central domains. One group contains genes having a variable number of tandem repeats, whereas genes in the second group have nonrepetitive sequences (14). Six ESTs matched members of the mucin gene family; one matched members belonging to the former group (TENS0234), whereas the other five ESTs matched different members belonging to the second group of genes (TENS0206, TENS0592, TENS1868, TENS0163, and TENS1740).

The superfamily of surface antigens is composed of hundreds of members that can be grouped into four families (groups I to IV) based on their similarities (9, 13).

Several ESTs showed significant matches to members belonging to group II, which comprises the so-called GP85 surface glycoproteins (TENS0211, TENS0203, TENS0196, TENS0182, TENS0142, TENS0215, TENS1365, TENS0190, TENS0229, TENS1292, and TENS0222). Interestingly, the top-ranking sequences of the BLAST searches corresponding to the last two ESTs matched the sequences coding for amastigote surface protein-2 and -1, respectively, which have recently been described as the first *trans*-sialidase (TS) superfamily members preferentially expressed in the amastigote stage (21, 27). In contrast, members of group I (which contains some members that express TS activity), group III, and group IV were hit by only one EST each (TENS0149, TENS0779, and TENS1235, respectively).

The results reported above show that several ESTs have significant matches to trypomastigote- and amastigote-expressed members of the TS superfamily. Although these molecules are stage-specific proteins not present at detectable levels in the epimastigote stage, this result might be expected for trypanosomatids. Unlike transcriptional gene regulation in other organisms, gene regulation in these parasites takes place mainly by posttranscriptional mechanisms (23), even for the expression of stage-specific proteins (29). Thus, it is possible that a low level of trypomastigote- and amastigote-specific mature mRNAs coding for these proteins is present at the epimastigote stage, even though the encoded proteins are absent. Another possibility is that these cDNAs are derived from contaminating metacyclic trypomastigote forms (estimated to be at about 1%) present in the epimastigote culture.

We next organized the EST data set according to matches to the NCBI nonredundant databases. Table 2 lists all significant matches to non-*T. cruzi* entries in GenBank sorted according to matches to the "other trypanosomatids" and "other organisms" categories. In cases where several entries from various species had significant scores, only the top-ranking score is given. A complete (including matches to *T. cruzi*) and updated listing of matches to known sequences present in GenBank can be found at our laboratory home page (http://www.iib.unsam.edu.ar/genomelab/tcruzi/5ests.html). A detailed analysis of the putative genes identified is not within the scope of this work and will certainly be done by interested researchers in the field. However, a number of interesting matches with sequences from other organisms were observed. Among them are several proteins identified in other trypanomatids, including several metabolic enzymes (TENS1285, TENS1439, TENS1345, and TENS1204); a homolog to a recently described TRACK (receptor for activated C kinase) in *T. brucei rhodesiense* (TENS 1408); a cyclophilin A (TENS0472); a nucleic acid-binding protein (homolog to the universal minicircle binding protein) (TENS1943); and a homolog to GP63-3 (TENS1942), a metalloprotease originally found in *Leishmania* and recently described for *T. brucei rhodesiense* (17). This protein seems to play an important role in the invasion (30) and survival (12) of the leishmanial parasites within the macrophage and has not been detected previously in *T. cruzi*. This result emphasizes the efficacy of the EST approach, which has allowed us to identify a gene potentially important in the host-parasite interplay.

Other ESTs matched known proteins in other organisms, including TATA-binding protein-interacting protein 49 (TENS 1944), serine/threonine protein kinase (TENS1391), serine/threonine protein phosphatase 2b catalytic subunit (calcineurin) (TENS1318), phosphorylation-regulatory protein HP-10 (TENS1945), meiotic spindle formation proteins (TENS1395, and TENS1293), mitotic centromere-associated kinesin (TENS 1375), α and p112 proteosome subunits (TENS1289 and TENS 1377), DNAJ protein (TENS1338), ADP-ribosylation factor (TENS1946), a probable cell division control protein (TENS 1370), several RAS-related proteins (TENS1644, -1947, -1948, and -0394), translation initiation factor 5A (TENS1949), a negative regulatory factor of a transcriptional activator (TENS 1941), enolases (TENS1381 and -1274), and a phosphoinositide-specific phospholipase C (TENS1347). Interestingly this last EST showed significant matches to phosphatidylinositol-specific phospholipases C from different organisms and did not show any significant match either to an already-reported *T. cruzi* glycosylphosphatidylinositol-specific phospholipase C (PID|e329378) or to glycosylphosphatidylinositol-specific phospholipases from other trypanosomatids, suggesting the presence of at least two different enzymes in *T. cruzi*. Some of the sequences mentioned above have also been identified in a recently published paper (8).

Several ESTs had strong matches with hypothetical, probable, or putative proteins (Table 2), many of them derived from genome sequencing projects for different organisms (mouse, human, *Drosophila*, yeast, and *Arabidopsis*, etc.). Although statistically significant similarities do not necessarily mean that these putative proteins actually exist, some of the highly significant matches might indicate that they are indeed real proteins conserved during evolution. Obviously, further sequence analysis and biochemical work are needed to distinguish among these and other possible alternatives.

Until the budget for the complete sequencing of the *T. cruzi* genome is available, a reasonable accomplishment will be the identification of a large proportion of the gene content in *T. cruzi*. This might be done by EST or genomic sequencing (18) in the near future. The next step in the short run would be the analysis of the data and the development of new approaches both for the identification of targets for chemotherapy and for vaccine development. Given the difficulties in the treatment of parasitic diseases and the frequent appearance of mutants resistant to chemotherapeutic agents among some protozoa such as *Plasmodium* and *Leishmania* (22, 25), gene discovery might be a cost-efficient way to contribute to the eradication of these diseases, which mostly affect developing countries.

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