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A transcriptome analysis of the *Aedes aegypti* vitellogenic fat body

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Abstract

Aedes (Stegomyia) aegypti is an important dengue vector in tropical and subtropical zones throughout the world. A transcriptome of *Ae. aegypti* vitellogenic fat bodies is described here. The fat body is a dynamic tissue that participates in multiple biochemical functions of intermediate metabolism. A total of 589 randomly selected cDNAs were assembled into 262 clusters based on their primary sequence similarities. The putative translated proteins were classified into categories based on their function in accordance with significant similarity using the BlastX at NCBI FTP site and Pfam (Bateman et al. 2000) and SMART (Schultz et al. 2000) databases. The characterization of transcripts expressed in the fat body of *Ae. aegypti* at 24 hours post blood meal provides a basic tool for understanding the processes occurring in this organ and could identify putative new genes whose promoters can be used to specifically express transgenes in the fat bodies of *Ae. aegypti*.

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Introduction

Insect vectors are responsible for transmitting human pathogens such as dengue viruses and malaria parasites that account for millions of disease cases every year with associated high mortality and morbidity (TDR, 2004). While research devoted to alleviate the burden of these diseases on human populations focuses on the development of vaccines, preventive and therapeutic drugs, pioneering avenues for the control of diseases may also be opened from the studies of vector biology. The *Anopheles gambiae* genome project has provided the scientific community with a powerful tool for the analyses of gene expression in this malaria vector species (Holt et al. 2002). The 280-Mb *An. gambiae* genome and the tens of thousands of ESTs generated from RNA extracted from specific tissues such as midgut, fat body, salivary gland, hemocytes and antennae have made it possible to better understand mosquito biology and its interactions with pathogenic organisms. As expected, taking advantage of all the sequence information, and gene search and annotation, new types of experiments are possible that likely will result in novel observations and discoveries (Marinotti et al. 2005; Tabachnick 2003; Toure et al. 2004).

The genome of the mosquito, *Aedes aegypti*, the major vector of dengue viruses, is currently being sequenced (Severson et al. 2004), and once completed it will be a fundamental tool for studies on vector biology. The characterization of the trancriptomes of mosquitoes at various physiological stages as well as gene expression patterns in isolated tissues are fundamental additions to the complete genome description. The focus here is on the description of the transcriptome of *Ae. aegypti* vitellogenic fat bodies, which are dynamic tissues that participate in multiple biochemical functions of intermediate metabolism, including protein, amino acid, lipid and carbohydrate synthesis and storage, xenobiotic detoxification, and immune response (Bartholomay et al. 2004). Because dengue fever is the most prevalent mosquito-borne viral disease among human populations, causing 50 million infections, 500,000 cases of dengue hemorrhagic fever and least 12,000 deaths per year (Gubler 2002), the transcriptome of *Ae. aegypti* could prove useful in control of dengue.

A total of 800 randomly-picked cDNAs were sequenced and after quality validation, 589 cDNAs were assembled in 262 clusters, annotated and assigned gene ontology terminology.

Materials and Methods

Animals

Aedes aegypti (Rockefeller strain) were reared in a local facility at the Institute of Biomedical Sciences, University of São Paulo, Brazil. Temperature was maintained at 26°C, humidity at 80% and a 12/12 photoperiod. Larvae were fed on powdered rat food. Adult mosquitoes were given continuous access to a 10% sucrose solution and five-day old females were fed on anesthetized mice when required.

Fat body cDNA library construction

Fat body of adult female mosquitoes, not including the body-wall integument, were dissected at 24 hrs post blood meal (PBM). Adult females were anesthetized on ice and dissected with a stereoscopic microscope in 0.15 M sodium chloride. Fat bodies were transferred to 500 µl of the TRIZOL reagent (Invitrogen) and mRNA extracted using the Micro-FastTrack mRNA isolation kit (Invitrogen). A PCR-based cDNA library was made following the instructions for the SMART cDNA library construction kit (Clontech). Four hundred nanograms of fat body mRNA were reverse-transcribed to cDNA using Superscript II RNase H-reverse transcriptase (Invitrogen) and the CDS III/3' PCR primer (Clontech) for 1 h at 42°C. Second-strand synthesis was performed through a PCR-based protocol using the SMART III primer (Clontech) as the sense primer and the CDS III/3' primer as antisense primer. These two primers create *Sfi* IA and B sites at the ends of nascent cDNA. Double-strand cDNA synthesis was carried out on a MJ Research Thermal cycler using the Platinum *Pfx* DNA polymerase. Amplification conditions were the following: 94°C for 2 min; 19 cycles of 94°C for 10 s and 68°C for 6 min. Double-stranded cDNA was treated immediately with proteinase K (0.8 µg/µl) for 20 min at 45°C. The double-stranded cDNA was digested with *Sfi* I for 2 h at 50°C. The cDNA then was fractionated using columns provide by the manufacturer (Clontech). Fractions containing cDNA of more than 400 base pairs (bp) in length were pooled and concentrated to a volume of 7 µl. The concentrated cDNA was ligated into a Lambda TriplEx2 vector

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(Clontech), and the resulting ligation reaction packaged using the Gigapack Gold III from Stratagene/Biocrest. The library was plated by infecting log-phase XL1-Blue cells (Clontech).

Sequencing and analysis of the *Ae. aegypti* cDNA library

Randomly-picked cDNA clones from the cDNA library were sequenced, assembled and analyzed (Durham *et al.* 2005; Calvo et al. 2004), using the CAP 3 program (Huang and Madan 1999). BLASTX searches were done locally from programs obtained at the NCBI FTP site (ftp://ftp.ncbi.nih.gov/blast/executables/) (Altschul et al. 1997). All the ESTs were deposited in dbEST at NCBI. Accession numbers for sequences originating from the *Ae. aegypti* cDNA library are given as DT366744 to DT367332 corresponds to the referenced gene product.

Results and Discussion

Organization of the transcriptome information

A total of 589 cDNA inserts were assembled into 262 clusters. Thirty-one clusters (237 cDNAs) were identified as corresponding to nuclear or mitochondrial rRNAs and were not investigated further. The 351 remaining cDNAs were grouped in 231 clusters and classified in 21 categories, according to function or putative assigned functions (Tables 1 and 2, and a supplementary table where cDNA is classified in categories: http://lineu.icb.usp.br/~mcapurro/aafatbody.zip).

The known or putative functional assignments were based on similarity of the deduced protein sequences to previously-described molecules deposited in the NR protein database at NCBI. Translated sequences also were screened with RPSBlast for protein motifs of the combined set of Pfam (Bateman et al. 2000) and SMART (Schultz et al. 2000) databases (also known as the Conserved Domains Database [CDD]) (Table 1). The sequences were compared to those available through the *Ae. aegypti* genome-sequencing project, at the TIGR web site (09/22/2004).

The vast majority of identified ESTs were represented only once in the database, indicating the high complexity of the fat body tissue. Fifty-seven contigs correspond to cDNAs that occur more than once in the database, may be indicative of a higher level of gene expression. Transcripts encoding proteins of the unknown group (68 ESTs), which did not show significant similarity with known proteins, could represent novel proteins unique to *Ae. aegypti* fat bodies and require further investigation.

Description of the transcriptome

All of the cDNAs reported here were divided into 21 groups according to their predicted function by gene ontology (Ashburner et al. 2000), (Figure 1 and Table 2). In total, 142 sequences distributed in 75 clusters, representing 40.46% of the total of fat body ESTs database, correspond to genes whose products are associated with protein synthesis. This is consistent with the synthetic capability of the fat bodies. The majority of these cDNAs encode ribosomal proteins (67 clusters). The presence of transcripts encoding an elongation factor and a translation initiation factor supports further the conclusion of an abundant protein synthetic activity of this organ.

Six transcripts encoding molecules associated with post-translational modification of proteins and protein transport pathways were found. Furthermore, transcripts of mitochondrial components are among the most represented in the database and this is consistent with the energy requirements for protein synthesis during vitellogenesis. Sequences related to energy metabolism (28 sequences distributed in 24 clusters) correspond largely to cytochrome c oxidase, ATP synthase and NADH dehydrogenase. Two transcripts involved in oxidative metabolism were identified as members of the cytochrome P450 family.

Transcripts involved with a wide variety of functions such as carbohydrate, lipid and nucleotide metabolism, transport, storage, signal transduction, lysosomal and proteosome activities and, nuclear regulation, were found among the sequenced cDNAs. All of these were represented only once or twice in the database, except for a transferrin and an odorant binding protein-like transcript, which were represented three and four times, respectively. The function of *Ae. aegypti* transferrin has not been determined, but these molecules may be involved in iron transport, oogenesis and innate immunity against parasites and pathogens (Harizanova et al. 2005). Insect odorant binding proteins and pheromone binding

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Table 1. *Aedes aegypti* vitelogenic fat body cDNA cluster encoding proteins

GenBank Accession number	ID sequence	Number of sequences	Best match of AEGY-TIGR to NR protein database	E value	% identity	Best match of AEGY-TIGR to PFAM database	E value	Best match of AEGY_ TIGR to GO database	E value	Comments	class
DT366820	F990	7	vitellogenin-C [Aedes aegypti]	1E-161	99	Podocalyxin	2E-006	nucleus	0.000	vitellogenin-C [Aedes aegypti]	oogenesis
DT366849	F715	1	vitellogenin-C [Aedes aegypti]	0.0	98	Vitellogenin_N	3E-069	nucleocytoplasmic transport	0.0	vitellogenin-C [Aedes aegypti]	oogenesis
DT366800	F782	9	vitellogenin-B [Aedes aegypti]	0.0	96	Candida_ALS	5E-026	extracellular space	0.0	vitellogenin-B [Aedes aegypti] Partial clone	oogenesis
DT366845	F1436-D02	2	Vitellogenin A1 precursor (VG)	0.0	99	Vitellogenin_N	5E-040	nucleocytoplasmic transport	0.0	vitellogenin-B	oogenesis
DT366844	F1404-A06	1	Vitellogenin A1 precursor (VG)	0.0	99	Vitellogenin_N	5E-040	nucleocytoplasmic transport	0.0	vitellogenin-B	oogenesis
DT366847	f28	1	vitellogenin - yellow fever mosquito	0.0	99	Candida_ALS	9E-011	cell wall (sensu Fungi)	0.0	Vitellogenin A1 precursor	oogenesis
DT366836	F1513-B07	4	vitellogenin - yellow fever mosquito	0.0	99	Candida_ALS	9E-011	cell wall (sensu Fungi)	0.0	vitellogenin	oogenesis
DT366811	F665	9	vitellogenic cathepsin-B like protease	0.0	99	Peptidase_C1	5E-064	cathepsin B activity - lysosome	0.0	vitellogenic cathepsin-B like protease	oogenesis
DT366842	F1125	1	vitellogenic cathepsin-B like protease	0.0	99	Peptidase_C1	5E-064	cathepsin B activity - lysosome	0.0	vitellogenic cathepsin-B like protease	oogenesis
DT366838	F1204-D10	1	vitellogenic cathepsin-B like protease	0.0	99	Peptidase_C1	5E-064	cathepsin B activity- lysosome	0.0	vitellogenic cathepsin-B like protease	oogenesis
DT366852	F1213-E07	1	vitellogenic cathepsin-B like protease	0.0	99	Peptidase_C1	5E-064	cathepsin B activity - lysosome	0.0	vitellogenic cathepsin-B like protease	oogenesis
DT366830	F269	3	Vitellogenic carboxypeptidase pre	0.0	99	serine_carbpept	1E-104	mitochondrion - protein binding	0.0	Vitellogenic carboxypeptidase pre	oogenesis
DT366809	F690	2	Vitellogenic carboxypeptidase pre	0.0	99	serine_carbpept	1E-104	mitochondrion - protein binding	0.0	vitellogenic carboxypeptidase	oogenesis
DT366839	F273	1	Vitellogenic carboxypeptidase pre	0.0	99	serine_carbpept	1E-104	mitochondrion - protein binding	0.0	vitellogenic carboxypeptidase	oogenesis
DT366843	f17	1	ENSANGP00000014169 Anopheles gambi	1E-168	67	serine_carbpept	1E-098	mitochondrion - protein binding	0.0	Vitellogenic carboxypeptidase	oogenesis
DT366827	F666	3	cathepsin B-like thiol protease	0.0	99	Peptidase_C1	5E-066	cathepsin B activity - lysosome	0.0	cathepsin B-like thiol protease	oogenesis
DT366837	F278	1	cathepsin B-like thiol protease	0.0	99	Peptidase_C1	5E-066	cathepsin B activity - lysosome	0.0	cathepsin B-like thiol protease	oogenesis
DT366840	F528	2	cathepsin B-like thiol protease	1E-122	98	Peptidase_C1	1E-045	cathepsin B activity - lysosome	0.0	cathepsin B-like thiol protease	oogenesis
DT366850	F535	1	cathepsin B-like thiol protease	0.0	99	Peptidase_C1	5E-066	cathepsin B activity - lysosome	0.0	cathepsin B-like thiol protease	oogenesis

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DT366851	F559	1	cathepsin B-like thiol protease	1E-149	100	Peptidase_C1	2E-062	cathepsin B activity - lysosome	0.0	cathepsin B-like thiol protease	oogenesis
DT366853	F1033	1	ENSANGP00000021680 Anopheles gambi	3E-037	37					similar to 27k hemolymph protein from Galleria	oogenesis?
DT366793	F640	1	similar to Paramecium tetraurelia	3E-009	23	Candida_ALS	1E-004	molecular_function unknown	0.000	possible Cyclophilin-RNA interacting protein	nuclear regulation
		54									
protein export											
DT366858	F825	1	ENSANGP00000020919 Anopheles gambi	1E-118	94	SNF7	2E-047	biological_process unknown	0.0	Vacuolar assembly/sorting protein DID4	protein export
DT366856	F1246-H04	1	ENSANGP00000012186 Anopheles gambi	1E-121	78	TRAP_alpha	7E-086	signal sequence binding	0.0	Translocon-associated complex TRAP,	protein export
DT366857	F623	1	ENSANGP00000019007 Anopheles gambi	1E-030	95	SecE	2E-013	protein transporter activity	0.0	Protein transport protein SEC61 gamma sub	protein export
		3									
carbohydrate metabolism											
DT366745	F694	1	ENSANGP00000018537 Anopheles gambi	6E-069	59	UDPGT	3E-033	xenobiotic metabolism	0.0	UDP-glucuronosyltransferase 8 - extracellular	carbohydrate metabolism
DT366744	F1128	1	ENSANGP00000018537 Anopheles gambi	6E-069	59	UDPGT	3E-033	xenobiotic metabolism	0.0	UDP-glucuronosyltransferase 2B15 precursor	carbohydrate metabolism
		2									
energy metabolism											
DT366779	F1450-E04	1	similar to Drosophila melanogaster	9E-010	50			mitochondrion	0.000	ubiquinol-cytochrome c reductase	energy metabolism
DT366778	F438	1	ENSANGP00000018542 Anopheles gambi	5E-091	90	mito_carr	2E-021	carrier activity - calcium ion binding	0.0	possible mitochondrial transporter	energy metabolism
DT366777	F354	1	LD14731p [Drosophila melanogaster	8E-014	52	COX7C	2E-012	integral to membrane - mitochondrion	0.0	cytochrome c oxidase, subunit VIIc	energy metabolism
DT366766	F603	1	ENSANGP00000010310 Anopheles gambi	4E-048	80	COX6A	5E-032	energy pathways	0.0	Cytochrome c oxidase polypeptide VIa-liver	energy metabolism
DT366772	F993	2	Cytochrome c oxidase polypeptide	7E-074	92	COX2	3E-061	cytochrome-c oxidase activity	0.0	Cytochrome c oxidase polypeptide	energy metabolism
DT366759	F1079	1	Cytochrome c oxidase polypeptide	1E-114	91	COX2	4E-061	cytochrome-c oxidase activity	0.0	Cytochrome c oxidase polypeptide	energy metabolism

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DT366781	F709	1	Cytochrome c oxidase polypeptide	7E-074	92	COX2	3E-061	cytochrome-c oxidase activity	0.0	mitochondrial product	energy metabolism
DT366776	F1373-F11	1	cytochrome oxidase subunit 3 An. g	1E-116	82	COX3	1E-116	cytochrome-c oxidase activity	0.0	cytochrome c oxidase subun	energy metabolism
DT366774	f19	1	ENSANGP00000020278 Anopheles g	1E-166	88	mito_carr	2E-022	molecular_function unknown	0	ADP/ATP translocase [Apis mellifera	energy metabolism
DT366760	F1313-A11	3	ENSANGP00000020278 Anopheles g	1E-166	88	mito_carr	2E-022	molecular_function unknown	0	ADP/ATP translocase [Anopheles gambiae]	energy metabolism
DT366769	F1064	1	cytochrome c oxidase subunit I Aedes	0.0	91	COX1	0.0	mitochondrion	0	cytochrome c oxidase subunit I [Aedes	energy metabolism
DT366763	F314	1	cytochrome c oxidase subunit I Aedes	0.0	91	COX1	0.0	mitochondrion	0	cytochrome c oxidase subunit I [Aedes	energy metabolism
DT366767	F249	2	cytochrome c oxidase subunit I Aedes	0.0	91	COX1	0.0	mitochondrion	0	cytochrome oxidase subunit I [Aedes c	energy metabolism
DT366780	F637	1	ENSANGP00000012587 Anopheles	6E-098	95	NAD_kinase	3E-033	NAD+ kinase activity phosphorylation	0.0	putative inorganic polyphosphate/ATP-NAD	energy metabolism
DT366758	F364	1	ENSANGP00000015816 Anopheles	2E-079	80	COX4	7E-049	growth - reproduction	0.0	Cytochrome c oxidase, subunit IV/COX5b	energy metabolism
DT366784	F507	1	ENSANGP00000019912 Anopheles	1E-030	70	COX7a	3E-005	cytochrome-c oxidase activity	0.000	Probable cytochrome c oxidase polypeptide	energy metabolism
DT366771	F3	1	ENSANGP00000011122 Anopheles	1E-062	87	COX5B	2E-017	determination of adult life span	0.0	Cytochrome c oxidase, subunit Vb/COX4	energy metabolism
DT366764	F1094	1	adenosine triphosphatase subunit 6	1E-104	85	ATP-synt_A	2E-047	ATP synthesis coupled proton transport	0.0	ATP synthase F0 subunit 6	energy metabolism
DT366770	F460	1	adenosine triphosphatase subunit 6	1E-104	85	ATP-synt_A	2E-047	ATP synthesis coupled proton transport	0.0	ATP synthase F0 subunit 6	energy metabolism
DT366783	F1289-F05	1	ENSANGP00000022850 Anopheles	1E-033	73	TT_ORF1	0.008	mitochondrion - NADH dehydrogenase	0.0	NADH dehydrogenase	energy metabolism
DT366749	F517	1	ENSANGP00000019428 Anopheles	1E-105	97	oxidored_q6	4E-038	mitochondrial electron transport, NADH	0.0	NADH-ubiquinone oxidoreductase 20 kDa sub	energy metabolism
DT366748	F1217-E11	1	ENSANGP00000019428 Anopheles	1E-105	97	oxidored_q6	4E-038	mitochondrial electron transport	0.0	NADH-ubiquinone oxidoreductase 20 kDa sub	energy metabolism
DT366765	F619	1	NADH dehydrogenase subunit 6	1E-039	59	oxidored_q3	0.001	mitochondrial electron transport	0.015	NADH dehydrogenase subunit 6	energy metabolism
DT366775	F589	1								NADH-ubiquinone oxidoreductase c	energy metabolism
		28									
cytoskeletal											
DT366747	F1024	1	ENSANGP00000021085 Anopheles	3E-089	93	TCTP	5E-057	positive regulation of growth rate	0.0	translationally controlled tumor protein	cytoskeletal

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DT366746	F376	1	ENSANGP00000021085 Anopheles	3E-089	93	TCTP	5E-057	positive regulation of growth rate	0.0	translationally controlled tumor protein	cytoskeletal
		2									
transcription machinery											
DT367256	F1203	1	ENSANGP00000017886 Anopheles	1E-112	57	Keratin_B2	0.035	DNA binding - transcription from Pol II	0.0	similar to small nuclear RNA	transcription machinery
		1									
protease											
DT366865	F916	1	ENSANGP00000024673 Anopheles	1E-101	51	trypsin	6E-046	serine-type endopeptidase activity	0.0	serine protease	protease
DT366864	F810	1	ENSANGP00000016930 Anopheles	1E-095	72	trypsin	7E-036	chymotrypsin activity	0.0	Testisin precursor - membrane fraction	protease
DT366863	F592	1	ENSANGP00000019999 Anopheles	2E-089	45	trypsin	3E-039	proteolysis and peptidolysis	0.0	similar to A. gambiae serine protease 14D	protease
		3									
protein synthesis											
DT367296	F995	1	ENSANGP00000012554 Anopheles	1E-124	68	Tymo_45kd_70kd	3E-016	positive regulation of growth rate	0.0	unknown	unknown
DT367289	F1334-C08	1	60S acidic ribosomal protein P1	6E-033	69	60s_ribosomal	7E-026	RNA binding	0.0	unknown	unknown
DT367224	F578	1	ENSANGP00000022149 Anopheles	7E-064	76	Ribosomal_ L35Ae	7E-031	structural constituent of ribosome	0.0	similar to ribosomal protein L35A	protein synthesis
DT367200	F590	1	similar to D. melanogaster R protein	5E-080	68	Ribosomal_L13e	1E-076	structural constituent of ribosome	0.0	possible 60S Ribosomal protein L13	protein synthesis
DT367223	F686	1	ENSANGP00000021108 Anopheles	6E-066	95	Ribosomal_S8	2E-037	embryonic development (sensu Animalia)	0.0	possible 40S ribosomal protein S15A	protein synthesis
DT366886	F349	1	elongation factor 1 beta Aedes aegypti	1E-125	100	EF1BD	4E-035	cytosol	0	elongation factor 1 beta	protein synthesis
DT367201	F197	1	ENSANGP00000011566 Anopheles	4E-077	63	Mpp10	4E-005	translation initiation factor activity - regulation of translational initiation - eukaryotic translation initiation factor 3 complex	0.0	Adam-PA similar to eukaryotic translation initiation factor 3	protein synthesis
DT367058	F546	1	ENSANGP00000025329 Anopheles	1E-129	84	Ribosomal_L7Ae	4E-020	hermaphrodite genital morphogenesis - physiological process - reproduction	0.0	60S ribosomal protein L7a	protein synthesis

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DT366877	F551	6	ENSANGP00000017987 Anopheles	8E-070	81	L15	8E-006	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	60S ribosomal protein L27a	protein synthesis
DT367214	F764	1	ENSANGP00000012554 Anopheles	1E-124	68	Tymo_45kd_70kd	3E-016	positive regulation of growth rate	0.0	60s ribosomal protein L23	protein synthesis
DT367222	F526	1	ENSANGP00000016619 Anopheles	3E-091	94	Ribosomal_L18ae	7E-085	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	60S ribosomal protein L18a	protein synthesis
DT367221	F695	1	ENSANGP00000022049 Anopheles	7E-096	94	Ribosomal_L5_C	4E-040	protein binding	0.0	60S ribosomal protein L11	protein synthesis
DT366799	F410	1	ENSANGP00000013781 Anopheles	1E-086	56	PBP_GOBP	0.005	extracellular space	0.086	5'UTR of D7 family member	odorant binding protein family
DT367219	F729	1	ENSANGP00000013302 Anopheles	1E-138	92	Ribosomal_S4e	8E-024	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya)	0	40S ribosomal protein S4	protein synthesis
DT367209	F468	1	40S ribosomal protein S16 Aedes	5E-078	100	Ribosomal_S9	8E-044	larval development (sensu Nematoda) - growth - physiological process - reproduction	0.0	40S ribosomal protein S16	protein synthesis
DT367091	F544	2	ENSANGP00000020171 Anopheles	1E-125	77	Ribosomal_S2	4E-058	laminin receptor activity - regulation of translation - cell adhesion - cell surface receptor linked signal transduction - cytosolic small ribosomal subunit (sensu Eukarya) - integrin complex - integral to plasma membrane	0.0	40S ribosomal protein SA (P40)/Laminin receptor 1	protein synthesis
DT367231	F1088	1	ENSANGP00000023607 Anopheles	7E-035	96	Ribosomal_S4	4E-015	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya)	0.0	S9e ribosomal protein	protein synthesis
DT367220	F747	1	ENSANGP00000014951 Anopheles	1E-107	91	Ribosomal_S8e	6E-054	larval development (sensu Nematoda) - growth - physiological process - reproduction	0.0	ribosomal protein S8	protein synthesis

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DT367215	F612	1	ribosomal protein S6 Aedes aegypti	0.0	99	Ribosomal_S6e	7E-054	protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya) - structural constituent of ribosome - cytoplasm	0.0	ribosomal protein S6	protein synthesis
DT367198	F1418-B08	1	ENSANGP00000025326 Anopheles	1E-108	88	Ribosomal_S7	1E-047	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein S5	protein synthesis
DT367063	F378	1	ribosomal protein S24 Aedes aegypti	1E-069	100	Ribosomal_S24e	1E-026	larval development (sensu Nematoda) - growth - physiological process - reproduction	0.0	ribosomal protein S24	protein synthesis
DT366883	F231	1	ENSANGP00000015322 Anopheles	1E-145	93	Ribosomal_S5	4E-021	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya)	0	ribosomal protein S2	protein synthesis
DT367194	F752	2	ENSANGP00000012543 Anopheles	5E-073	76	Ribosomal_S19e	2E-058	intracellular - ribosome - protein biosynthesis	0.0	ribosomal protein S19	protein synthesis
DT367093	F263	2	putative ribosomal protein S13 Anopheles	1E-078	98	Ribosomal_S15	1E-017	protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya) - structural constituent of ribosome	0.0	RIBOSOMAL PROTEIN S13	protein synthesis
DT367177	F584	1	ENSANGP00000017313 Anopheles	2E-039	70	Ribosomal_S17	3E-006	embryonic development (sensu Animalia) - physiological process - reproduction	0.000	ribosomal protein S11	protein synthesis
DT367216	F658	3	RE33114p Drosophila melanogaster	1E-140	81	Ribosomal_L18p	3E-033	structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya) - RNA binding	0	ribosomal protein L5	protein synthesis
DT366943	F1061	1	ENSANGP00000011028 Anopheles	0.0	93	Ribosomal_L3	1E-057	RNA binding - structural constituent of ribosome - protein biosynthesis - nucleolus - cytosolic large ribosomal subunit (sensu Eukarya)	0	ribosomal protein L3 - RNA binding	protein synthesis

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DT366935	F431	2	ENSANGP00000022122 Anopheles	6E-077	92	KOW	2E-005	intracellular - ribosome - cytosolic large ribosomal subunit (sensu Eukarya) - RNA binding - protein biosynthesis	0.0	ribosomal protein L26	protein synthesis
DT367061	F1449-E03	2	ENSANGP00000014421 Anopheles	1E-107	77	Ribosomal_L13	4E-029	structural constituent of ribosome - cytosolic large ribosomal subunit (sensu Eukarya) - protein biosynthesis	0.0	ribosomal protein L13A	protein synthesis
DT367106	F434	18	60S ribosomal protein	2E-006	100	Ribosomal_L41	2E-008	protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya) - structural constituent of ribosome	0.000	ribosomal protein	protein synthesis
DT366905	F633	5	60S acidic ribosomal protein	2E-044	84	60s_ribosomal	2E-026	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT366914	F348	4	ENSANGP00000021108 Anopheles	6E-066	95	Ribosomal_S8	2E-037	embryonic development (sensu Animalia) - positive regulation of growth rate - larval development (sensu Nematoda) - growth - physiological process - reproduction - gametogenesis	0.0	ribosomal protein	protein synthesis
DT366894	F730	4	ENSANGP00000018909 Anopheles	8E-053	93	Ribosomal_L7Ae	7E-021	peripheral nervous system development	0.0	ribosomal protein	protein synthesis
DT366889	F696	3	ENSANGP00000011144 Anopheles	1E-057	94	Ribosomal_L36e	3E-039	structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT366923	F207	3	ENSANGP00000014054 Anopheles	2E-074	81	Ribosomal_L21e	4E-042	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis

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DT367187	F908	3	ribsomal protein S29 Culex pipiens	3E-029	100	Ribosomal_S14	1E-008	intracellular - ribosome - cytosolic small ribosomal subunit (sensu Eukarya) - structural constituent of ribosome - zinc ion binding - RNA binding - regulation of cell shape - protein biosynthesis	0.0	ribosomal protein	protein synthesis
DT366940	F1329-C03	3	40S ribosomal protein S28	1E-025	92	Ribosomal_S28e	2E-024	positive regulation of growth rate - physiological process - reproduction	0.0	ribosomal protein	protein synthesis
DT366918	F237	3	ENSANGP00000018631 Anopheles	3E-037	93	Ribosomal_S21e	3E-029	embryonic development (sensu Animalia) - larval development (sensu Nematoda) - growth - physiological process - reproduction	0.0	ribosomal protein	protein synthesis
DT366872	F406	3	ENSANGP00000021870 Anopheles	1E-104	96	Ribosomal_S4	7E-024	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT366910	F1386-G12	2	ENSANGP00000010955 Anopheles	7E-092	82	Ribosomal_L18e	6E-072	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT367192	F424	2	ENSANGP00000021862 Anopheles	7E-063	80	Ribosomal_L22e	1E-049	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT366884	F1002	2	60S ribosomal protein L31	7E-065	100	Ribosomal_L31e	4E-036	structural constituent of ribosome - cytosolic large ribosomal subunit (sensu Eukarya) - protein biosynthesis	0.0	ribosomal protein	protein synthesis
DT366932	F1021	2	ENSANGP00000011018 Anopheles	1E-085	85	Ribosomal_L6	3E-011	RNA binding - structural constituent of ribosome - protein biosynthesis - ribosome	0.0	ribosomal protein	protein synthesis

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DT367196	F1098	2	ENSANGP00000014265 Anopheles	3E-051	68	Ribosomal_L28e	5E-034	structural constituent of ribosome - cytosolic large ribosomal subunit (sensu Eukarya) - protein biosynthesis	0.0	ribosomal protein	protein synthesis
DT366937	F1316-B02	2	ENSANGP00000017702 Anopheles	3E-065	91	Ribosomal_L32e	1E-047	protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya) - structural constituent of ribosome	0.0	ribosomal protein	protein synthesis
DT366887	F850	2	ENSANGP00000016601 Anopheles	2E-059	96	Ribosomal_S26e	5E-056	positive regulation of body size - embryonic development (sensu Animalia) - embryonic morphogenesis - larval development (sensu Nematoda) - growth - physiological process - reproduction - locomotory behavior	0.0	ribosomal protein	protein synthesis
DT366870	F374	2	ENSANGP00000019214 Anopheles	7E-086	94	Ribosomal_L11	9E-016	RNA binding - structural constituent of ribosome - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT367060	F784	2	Ribosomal protein L39	9E-022	94	Ribosomal_L39	5E-019	embryonic development (sensu Animalia) - positive regulation of growth rate - larval development (sensu Nematoda) - growth - gametogenesis	0.0	ribosomal protein	protein synthesis
DT366929	F271	2	ENSANGP00000012302 Anopheles	3E-085	99	ubiquitin	2E-028	embryonic development (sensu Animalia) - reproduction	0.0	ribosomal protein	protein synthesis
DT367225	F854	2	ribosomal protein S17 Anopheles	2E-062	93	Ribosomal_S17e	1E-053	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT366921	F388	2	ENSANGP00000017618 Anopheles	1E-059	90	Ribosomal_S25	8E-042	positive regulation of growth rate	0.0	ribosomal protein	protein synthesis
DT367097	F581	2	ENSANGP00000022149 Anopheles	7E-064	76	Ribosomal_ L35Ae	7E-031	structural constituent of ribosome - cytosolic large ribosomal subunit (sensu Eukarya) - RNA binding	0.0	ribosomal protein	protein synthesis

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DT366892	F661	2	putative large subunit ribosomal prote	3E-056	99	Ribosomal_L44	1E-029	embryonic development (sensu Animalia) - larval development (sensu Nematoda) - growth - reproduction	0.0	ribosomal protein	protein synthesis
DT367088	F395	1	ENSANGP00000021108 Anopheles	1E-053	94	Ribosomal_S8	4E-032	embryonic development (sensu Animalia) - positive regulation of growth rate - larval development (sensu Nematoda) - growth - physiological process - reproduction - gametogenesis	0.0	ribosomal protein	protein synthesis
DT366899	F1287-F03	1	ENSANGP00000010955 Anopheles	7E-092	82	Ribosomal_L18e	6E-072	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT366939	F236	1	ENSANGP00000021862 Anopheles	7E-063	80	Ribosomal_L22e	1E-049	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT367228	F1055	1	ENSANGP00000013363 Anopheles	1E-044	98	Ribosomal_L37ae	2E-038	embryonic development (sensu Animalia) - positive regulation of growth rate - larval development (sensu Nematoda) - growth	0.0	ribosomal protein	protein synthesis
DT366898	F1097	1	ENSANGP00000017558 Anopheles	3E-058	94	Ribosomal_L29	8E-011	mRNA binding - structural constituent of ribosome - protein biosynthesis - nucleolus - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT366912	F1245-H03	1	ENSANGP00000021358 Anopheles	1E-110	92	Ribosomal_L15e	5E-089	positive regulation of growth rate - physiological process - reproduction - gametogenesis	0.0	ribosomal protein	protein synthesis
DT366926	F1376-G02	1	LP04958p Drosophila melanogaster	2E-065	78	S10_plectin	6E-049	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT366900	F1411-B01	1	ENSANGP00000023750 Anopheles	1E-122	95	Ribosomal_L10e	8E-091	cytosolic large ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis

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DT367086	F1446-D12	1	ENSANGP00000019074 Anopheles	9E-077	97	Ribosomal_S11	3E-035	intracellular - ribosome - cytosolic small ribosomal subunit (sensu Eukarya) - RNA binding - protein biosynthesis	0.0	ribosomal protein	protein synthesis
DT367227	F1458-E12	1	ribosomal protein S6 Aedes aegypti	3E-061	84	TT_ORF1	4E-011	cellular_component unknown	0.000	ribosomal protein	protein synthesis
DT366917	F243	1	ENSANGP00000015019 Anopheles	1E-114	92	Ribosomal_L1	1E-043	embryonic development (sensu Animalia) - positive regulation of growth rate - larval development (sensu Nematoda) - growth - reproduction	0.0	ribosomal protein	protein synthesis
DT366902	F276	1	S7 ribosomal protein Aedes aegypti	1E-103	99	Ribosomal_S7e	6E-095	intracellular - ribosome - cytosolic small ribosomal subunit (sensu Eukarya) - structural constituent of ribosome - RNA binding - protein biosynthesis	0.0	ribosomal protein	protein synthesis
DT366927	F471	1	ENSANGP00000023750 Anopheles	1E-122	95	Ribosomal_L10e	8E-091	cytosolic large ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT366931	F555	1	ENSANGP00000014147 Anopheles	7E-032	53	Ribosomal_L36	5E-005	protein biosynthesis - mitochondrial large ribosomal subunit	0.0	ribosomal protein	protein synthesis
DT366930	F597	1	ENSANGP00000022284 Anopheles	9E-065	93	Ribosomal_L7Ae	5E-025	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic small ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT367064	F667	1	ribosomal protein L31 - forest day mosq	4E-067	97	Ribosomal_L34e	6E-042	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT366934	F701	1	ENSANGP00000012582 Anopheles	3E-031	95	Ribosomal_L38e	9E-028	positive regulation of growth rate - gametogenesis	0.0	ribosomal protein	protein synthesis
DT366901	F746	1	ENSANGP00000021358 Anopheles	1E-110	92	Ribosomal_L15e	5E-089	positive regulation of growth rate - physiological process - reproduction - gametogenesis	0.0	ribosomal protein	protein synthesis
DT367202	F762	1	ENSANGP00000010500 Anopheles	5E-062	96	Ribosomal_L7Ae	3E-023	neurogenesis	0.0	ribosomal protein	protein synthesis

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DT367203	F862	1	ENSANGP00000016601 Anopheles	2E-059	96	Ribosomal_S26e	5E-056	positive regulation of body size - embryonic development (sensu Animalia) - embryonic morphogenesis - larval development (sensu Nematoda) - growth - physiological process - reproduction - locomotory behavior	0.0	ribosomal protein	protein synthesis
DT366903	F972	1	ENSANGP00000013957 Anopheles	1E-069	83	Ribosomal_S19	1E-030	embryonic development (sensu Animalia) - positive regulation of growth rate - larval development (sensu Nematoda) - growth - physiological process - reproduction	0.0	ribosomal protein	protein synthesis
DT366909	F976	1	ENSANGP00000019453 Anopheles	1E-044	97	Ribosomal_S27e	3E-022	RNA binding - structural constituent of ribosome - zinc ion binding - protein biosynthesis - signal transduction - cell proliferation - cytosolic small ribosomal subunit (sensu Eukarya)	0.0	ribosomal protein	protein synthesis
DT367084	F1181-B11	2	elongation factor 1 beta Aedes aegypti	1E-125	100	EF1BD	4E-035	cytosol	0	Probable elongation factor 1-beta - cytosol	protein synthesis
		142									
transporter/ storage											
DT367262	F308	1	ENSANGP00000011786 Anopheles	7E-024	30	zf-C3HC4	5E-007	biological_process unknown - cellular_ component unknown	0.000	possible potassium channel modulatory factor	transporter/ storage
DT367257	F855	3	transferrin precursor Aedes aegypti	0.0	99	transferrin	3E-012	integral to plasma membrane - iron ion binding	0.0	transferrin precursor [Aedes aegypti]	transporter/ storage
DT366848	F588	1	ENSANGP00000018348 Anopheles	1E-135	54	vwd	0.005	microtubule binding - heme binding	0.0	lipophorin - fruit fly	transporter/ storage
DT367261	F553	1	LD22509p Drosophila melanogaster	4E-081	74	sugar_tr	1E-010	inorganic phosphate transporter activity - phosphate transport - integral to membrane	0.0	Putative inorganic phosphate cotransporter	transporter/ storage

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		6									
odorant biding protein family											
DT366796	F192	1	odorant-binding protein OBP	6E-010	34	PBP_GOBP	3E-007	salivary gland cell death - autophagic cell death	0.000	odorant-binding protein AgamOBP9	odorant binding protein family
DT367249	F437	1	putative salivary odorant binding pro	8E-018	34	PBP_GOBP	2E-007	salivary gland cell death - autophagic cell death	0.003	putative salivary odorant binding pro	odorant binding protein family
DT366797	F1353-E03	2	odorant-binding protein AgamOBP9	5E-054	69	PBP_GOBP	3E-018	salivary gland cell death - autophagic cell death	0.0	odorant-binding protein AgamOBP9	odorant binding protein family
DT366794	F1018	2	odorant-binding protein AgamOBP9	5E-054	69	PBP_GOBP	3E-018	salivary gland cell death - autophagic cell death	0.0	odorant-binding protein AgamOBP9	odorant binding protein family
DT366799	F410	1	ENSANGP00000013781 Anopheles	1E-086	56	PBP_GOBP	0.005	extracellular space	0.086	5'UTR of D7 family member	odorant binding protein family
		7									
extracellular matrix											
DT366750	F1119	1	ENSANGP00000021083 Anopheles	1E-088	43	Podocalyxin	3E-008	extracellular space	0.0	mucin-like protein	extracellular matrix
		1									
protein modification											
DT366862	F771	1	ENSANGP00000019887 Anopheles	1E-131	95	HSP70	4E-075	heat shock protein activity - neurotransmitter secretion - vesicle-mediated transport	0	Molecular chaperones HSP70/HSC70	protein modification
DT366861	F1138	1	ENSANGP00000024752 Anopheles	0.0	78	Peptidase_M2	0.0	peptidyl-dipeptidase A activity - metamorphosis (sensu Insecta)	0	peptidyl-dipeptidase A activity (based on TIGR data)	protein modification
DT366860	F1340-D02	1	ENSANGP00000024752 Anopheles	0.0	78	Peptidase_M2	0.0	peptidyl-dipeptidase A activity - metamorphosis (sensu Insecta)	0	peptidyl-dipeptidase A activity (based on TIGR data)	protein modification
DT366859	F558	1	ENSANGP00000011976 Anopheles	1E-066	83	OST3_OST6	2E-045	mitochondrion	0.0	Oligosaccharyltransferase, gamma subunit	protein modification
		4									
signal transduction											

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DT367250	F977	1	ENSANGP00000014018 Anopheles	1E-107	92	pkinase	2E-015	MAPKKK cascade - protein serine/threonine kinase activity - nucleus - wing morphogenesis - SAP kinase activity - regulation of BMP signaling pathway - regulation of innate immune response	0.0	Mitogen-activated protein kinase 14B	signal transduction
DT367252	F447	1	ENSANGP00000010163 Anopheles	2E-090	54			neurogenesis	0.047	Fibrillins and related proteins containing Ca2+-binding EGF-like domains	signal transduction
DT367251	F574	1								Sex-regulated protein janus-A	signal transduction
DT367255	fF344	1	cAMP generating peptide precursor	3E-009	63			mitochondrion	0.000	cAMP generating peptide precursor	signal transduction
DT367254	F1416-B11	1	ENSANGP00000014526 Anopheles	3E-078	70	Fe_acq	0.006	RNA polymerase II transcription factor activity - transcription corepressor activity - regulation of transcription from Pol II promoter - development - cell proliferation	0.0	similar to cellular repressor of E1	signal transduction
DT367253	F448	1	ENSANGP00000018569 Anopheles	4E-070	59	pkinase	3E-036	plasma membrane - protein amino acid phosphorylation - terminal region determination - eggshell pattern formation (sensu Insecta)	0.0	Tyrosine-protein kinase receptor torso precursor	signal transduction
		6									
lipid metabolism											
DT366785	F427	1	ENSANGP00000010825 Anopheles	1E-103	55	lipase	2E-048	extracellular space	0.0	lipase	lipid metabolism
DT366786	F736	1	ENSANGP00000012410 Anopheles	0.0	74	FA_desaturase	1E-016	mitochondrion	0.0	Delta-5 fatty acid desaturase	lipid metabolism
		2									
nuclear regulation											
DT366789	F586	1	ENSANGP00000012210 Anopheles	1E-164	78	Peptidase_C1	2E-059	nuclear export signal receptor activity - protein-nucleus export - nucleus - nuclear pore - cytoplasm	0	Exportin	nuclear regulation

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DT366792	F291	1	ENSANGP00000012210 Anopheles	1E-164	78	Peptidase_C1	2E-059	nuclear export signal receptor activity - protein-nucleus export - nucleus - nuclear pore - cytoplasm	0	Exportin	nuclear regulation
DT366791	F267	2	Tamozhennic protein	7E-059	48			molecular_function unknown - spermatogenesis - cytoplasm	0.000	homolog of Dm Tamozhennic protein - nuclear import	nuclear regulation
		4									
oxidant metabolism											
DT366855	F416	1	ENSANGP00000001916 Anopheles	1E-113	84	p450	6E-055	embryonic development (sensu Animalia)	0.0	Cytochrome P450 CYP4/ CYP19/CYP26 subfamilies	oxidant metabolism
DT366854	F828	1	ENSANGP00000022709 Anopheles	4E-030	66			binding - vitamin A metabolism - visual perception - soluble fraction	0.000	possible cytochrome P450 28a5	oxidant metabolism
DT367260	F613	1	ENSANGP00000022709 Anopheles	4E-030	66			binding - vitamin A metabolism - visual perception - soluble fraction	0.000	possible CRALBP	transporter/ storage
		3									
immunity											
DT366753	F599	1								cecropin A precursor [Aedes aegypti]	immunity
DT366754	F572	3	RE15268p Drosophila melanogaster	2E-014	35	lectin_c	1E-010	galactose binding	0.000	C-type lectin	immunity
DT366875	F837	2	ENSANGP00000012229 Anopheles	2E-081	96	Ribosomal_S12	1E-036	intracellular - ribosome - cytosolic small ribosomal subunit (sensu Eukarya) - structural constituent of ribosome - RNA binding - protein biosynthesis	0.0	ribosomal protein	protein synthesis
DT366751	F861	2	defensin A4 Aedes aegypti	2E-052	100	Arthro_defensin	6E-011	antibacterial humoral response (sensu Protostomia) - defense response to bacteria - defense response to Gram-positive bacteria	0.0	defensin	immunity
		8									
lysosomal											

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Table 1. *Aedes aegypti* vitelogenic fat body cDNA cluster encoding proteins

GenBank Accession number	ID sequence	Number of sequences	Best match of AEGY-TIGR to NR protein database	E value	% identity	Best match of AEGY-TIGR to PFAM database	E value	Best match of AEGY_ TIGR to GO database	E value	Comments	class
DT366757	F538	1	ENSANGP00000020002 Anopheles	1E-127	90	Peptidase_C1	1E-091	salivary gland cell death - autophagic cell death	0	Cathepsin L precursor - cathepsin L activity - lysosome	lysosomal
		1									
nucleotide metabolism											
DT366788	F1092	1	ENSANGP00000010003 Anopheles	0.0	84	5_nucleotidase	1E-117	5'-nucleotidase activity - cytosol	0	5'-nucleotidase, cytosolic	nucleotide metabolism
DT366787	F1537-D07	1	ENSANGP00000024036 Anopheles	0.0	86	FA_desaturase	1E-044	integral to membrane - fatty acid biosynthesis	0.0	fatty acid desaturase	nucleotide metabolism
		2									
proteasome											
DT366866	F409	2	ENSANGP00000010608 Anopheles	1E-123	78	Nin1_C	4E-057	endopeptidase activity - proteasome regulatory particle (sensu Eukarya) - proteolysis and peptidolysis	0.0	26S proteasome regulatory complex,	proteasome
DT366868	F823	1	similar to ENSANGP00000007022 Apis	1E-121	85	proteasome	2E-048	proteasome complex (sensu Eukarya)	0	Proteasome subunit alpha type 7 - 26S proteasome	proteasome
DT367248	F288	1	similar to Drosophila melanogaster Rp	3E-069	100	ubiquitin	2E-028	embryonic development (sensu Animalia) - reproduction	0.0	ubiquitin	ps proteasome
		4									
Unknown											
DT367265	F1410-A12	1	ENSANGP00000004798 Anopheles	3E-076	75	DUF852	2E-054	mitochondrion	0.0	unknown conserved	unknown
DT367263	F362	1	ENSANGP00000011689 Anopheles	1E-036	68					unknown conserved	unknown
DT367264	F567	1	ENSANGP00000009630 Anopheles	4E-018	57			structural molecule activity - epidermis development - intermediate filament	0.000	unknown conserved	unknown
DT367291	F824	4	ENSANGP00000025118 Anopheles	4E-047	83	60s_ribosomal	4E-019	RNA binding - structural constituent of ribosome - protein biosynthesis - cytosolic large ribosomal subunit (sensu Eukarya)	0.0	unknown	unknown
DT367313	F185	4								unknown	unknown
DT367268	F1347-D09	3	CpCOWP2; cryptosporidium	0.023	32			larval development (sensu Nematoda) - growth - locomotory behavior	0.058	unknown	unknown

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DT367270	F1223-F05	2	Ab2-057 [Rattus norvegicus]	2E-020	58			positive regulation of cell proliferation - hemopoiesis	0.001	unknown	unknown
DT367278	F1001	1	ENSANGP00000024365 Anopheles	0.83	29	DUF216	0.002			unknown	unknown
DT367290	F1016	1								unknown	unknown
DT367316	F1026	1								unknown	unknown
DT367317	F1038	1								unknown	unknown
DT367306	F1051	1	similar to Y55B1BR.3.p Caenorhabditi	6E-006	28	DUF164	0.031	biological_process unknown - cellular_ component unknown	0.000	unknown	unknown
DT367288	F1056	1	hypothetical protein	0.40	23					unknown	unknown
DT367318	F1058	1								unknown	unknown
DT367319	F1115	1								unknown	unknown
DT367320	F1143	1								unknown	unknown
DT367281	F1190-C08	1	hypothetical protein	0.022	58	Keratin_B2	0.001	structural constituent of epidermis - biological_ process unknown - extracellular	0.021	unknown	unknown
DT367284	F1225-F07	1	ENSANGP00000014861 Anopheles	0.002	34					unknown	unknown
DT367272	F1298-H02	1	hypothetical protein Plasmodium fa	5.8	37					unknown	unknown
DT367286	F1320-B06	1								unknown	unknown
DT367273	F1360-E10	1	coatomer alpha subunit	7.2	24					unknown	unknown
DT367321	F1361-E11	1								unknown	unknown
DT367322	F1362-E12	1								unknown	unknown
DT367279	F1483-H01	1	similar to Dictyostelium discoideum	4E-028	39	SKN1	5E-004	regulation of protein activity, epigenetic	0.0	unknown	unknown
DT367305	F1497-A03	1	ENSANGP00000016160 Anopheles	4E-020	65					unknown	unknown
DT367277	F205	1	bA99E24.1.1 protocadherin 19	3.6	36					unknown	unknown
DT367302	F219	1	ENSANGP00000016495 Anopheles	1E-026	60	DUF227	6E-014	protein binding	0.000	unknown	unknown
DT367285	F225	1	NTPase/helicase maize rayado fino	8.2	41					unknown	unknown
DT367323	F261	1								unknown	unknown

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GenBank Accession number	ID sequence	Number of sequences	Best match of AEGY-TIGR to NR protein database	E value	% identity	Best match of AEGY-TIGR to PFAM database	E value	Best match of AEGY_ TIGR to GO database	E value	Comments	class
DT367282	F306	1								unknown	unknown
DT367324	F350	1								unknown	unknown
DT367311	F380	1	hypothetical protein Plasmodium fa	8.8	36					unknown	unknown
DT367297	F402	1	ENSANGP00000014951 Anopheles	1E-107	91	Ribosomal_S8e	6E-054	larval development (sensu Nematoda) - growth - physiological process - reproduction	0.0	unknown	unknown
DT367298	F403	1	ENSANGP00000014951 Anopheles	1E-107	91	Ribosomal_S8e	6E-054	larval development (sensu Nematoda) - growth - physiological process - reproduction	0.0	unknown	unknown
DT367325	F417	1								unknown	unknown
DT367326	F420	1								unknown	unknown
DT367300	F445	1	ENSANGP00000012302 Anopheles	3E-085	99	ubiquitin	2E-028	embryonic development (sensu Animalia) - reproduction	0.0	unknown	unknown
DT367327	F450	1								unknown	unknown
DT367304	F533	1	GM14559p [Drosophila melanogaster]	7E-021	94	GTP_EFTU_D3	7E-006	cytoplasm	0.0	unknown	unknown
DT367276	F547	1	seven transmembrane helix receptor	9E-007	29	Sre	0.021			unknown	unknown
DT367308	F591	1	maturase [Narcissus tazetta]	0.82	26					unknown	unknown
DT367328	F604	1								unknown	unknown
DT367287	F609	1	ENSANGP00000018748 Anopheles	3E-047	54	TP2	0.028			unknown	unknown
DT367299	F638	1	ENSANGP00000011784 Anopheles	3E-098	93	Ribosomal_L22	4E-026	RNA binding - structural constituent of ribosome - ribosome	0.0	unknown	unknown
DT367329	F662	1								unknown	unknown
DT367266	F697	1	ENSANGP00000023084 Anopheles	6E-006	38					unknown	unknown
DT367330	F738	1								unknown	unknown
DT367303	F754	1	ENSANGP00000014492 Anopheles	2E-023	59					unknown	unknown
DT367331	F760	1								unknown	unknown
DT367275	F763	1								unknown	unknown
DT367332	F768	1								unknown	unknown

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DT367293	F1045	1	ENSANGP00000014492 Anopheles	2E-023	59					unknown	unknown
DT367310	F863	1	hypothetical protein cgd8_140	4.8	48					unknown	unknown
DT367279	F1483-H01	1								unknown	unknown
DT367309	F970	1	100 kDa heme:hemopexin-binding prot	2.3	20					unknown	unknown
DT367295	F210	1	ENSANGP00000002975 Anopheles	1E-137	64	TMS_TDE	1E-122	plasma membrane - integral to membrane	0.0	membrane protein TMS1d	unknown
DT367301	F1368-F06	1	ENSANGP00000020785 Anopheles	1E-074	64	Somatomedin_B	0.001	extracellular matrix structural constituent - endosome transport - extracellular	0.0	similar to tubulointerstitial nephritis antigen	unknown
DT366869	F575	1	ribosomal protein P0 [Aedes albopictus]	1E-171	98	Ribosomal_L10	1E-030	structural constituent of ribosome - DNA-(apurinic or apyrimidinic site) lyase activity - cytosolic ribosome (sensu Eukarya) - protein biosynthesis	0	ribosomal protein	protein synthesis
DT367274	F338	1	ribosomal protein P0 [Aedes albopictus]	1E-171	98	Ribosomal_L10	1E-030	structural constituent of ribosome - DNA-(apurinic or apyrimidinic site) lyase activity - cytosolic ribosome (sensu Eukarya) - protein biosynthesis	0	unknown	unknown
		68									

The function were based on similarity of the deduced *Ae. aegypti* protein sequences deposited in the NR protein database at NCBI. Translated sequences deposited in the NR protein database at NCBI. Translated sequences were also screened with RPSBlast for protein motifs of the combined set of Pfam (Bateman et al. 2000) and SMART (Schultz et al. 2000) databases.

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Table 2. Division of the transcriptis according to their functions

Category	Number of transcripts	Percentege %
carbohydrate metabolism	2	0.57
cytoskeletal	2	0.57
energy metabolism	28	7.98
extracellular matrix	1	0.28
immunity	8	2.28
lipid metabolism	2	0.57
lysosomal	1	0.28
nuclear regulation	4	1.14
nucleotide metabolism	2	0.57
odorant binding protein family	7	1.99
oxidant metabolism	3	0.85
protease	3	0.85
proteasome	4	1.14
protein export	3	0.85
protein modification	4	1.14
protein synthesis	142	40.46
signal transduction	6	1.71
transcription machinery	1	0.28
transporter/storage	6	1.71
oogenesis	54	15.38
unknown	68	19.37
total	351	100.00

The function were based on similarity of the deduced *Ae. aegypti* protein sequences deposited in the NR protein database at NCBI. Translated sequences were also screened with RPSBlast for protein motifs of the combined set of Pfam (Bateman et al. 2000) and SMART (Schultz et al. 2000) databases.

proteins (Hekmat-Scafe et al. 2000) are expressed mainly in the insect antennae and they are proposed to bind small hydrophobic odorant molecules, carry and present them to the olfactory receptors. Proteins structurally related to odorant binding proteins have been identified in non-sensory organs and in the hemolymph of insects, where they might carry hydrophobic ligands related to a variety of functions (Biessmann et al. 2002; Calvo et al. 2004). A hemolymph protein with sequence and structural characteristics similar to odorant binding proteins, designated THP12, was described in *Tenebrio molitor* as a carrier protein, associated with the transport of small hydrophobic ligands through the hemolymph (Graham et al. 2001; Rothemund et al. 1999). These *Ae. aegypti* cDNAs may correspond to novel mosquito proteins yet to be described.

Three immunity-related transcripts were found, corresponding to molecules with high amino acid similarity (93%) to the C-type lectin found in *Drosophila melanogaster* (Haq et al. 1996), defensin and cecropin of *Ae. aegypti*. One sequence was identified as a cecropin precursor, also from *Ae. aegypti*. The cecropins have antibacterial and antifungal activity (Zheng and Zheng, 2002) while defensins are antimicrobial peptides that are activated in the presence of Gram-positive or negative bacteria. These peptides also are activated by infection with filarial worms (Bartholomay et al. 2004).

Transcripts related to vitellogenesis (Table 1 and Table 2), represent 15.38% of the fat body transcriptome (54 sequences, 22 clusters) at 24 h PBM, and encode vitellogenins, vitellogenic cathepsin-B and vitellogenic carboxypeptidase. These data are in agreement with the previously-described increase of vitellogenic proteins expression in mosquitoes following a blood meal (Kokoza et al., 2001). Transcripts representing three distinct vitellogenins, three cathepsin Bs and two vitellogenic carboxypeptidases were observed in the analyzed set of cDNAs. Multiple vitellogenin genes in the genome of *Ae. aegypti* have been described (Hamblin et al. 1987) while the multiple transcripts with similarity to cathepsin B and vitellogenic carboxypeptidase indicate that these proteins may be encoded by more than one gene as well. It has been already established that vitellogenin, vitellogenic carboxypeptidase and cathepsin B are synthesized by the fat bodies in response to a blood meal, secreted into the hemolymph and accumulate in ovaries, where they are deposited in the oocytes during development (Raikhel, 1987; Raikhel, et al., 2002; Raikhel and Lea, 1983; Ribeiro, 2003).

Final remarks

The sequencing of 800 *Ae. aegypti* fat body-derived cDNAs, and their annotation and organization provides a general picture of the metabolic state of the tissue at 24 h PBM. The insect fat body is a complex tissue where many indispensable processes of intermediate metabolism, storage and immunity occur, however its major function is synthesis and export of proteins (ex. hexamerins, transport proteins and vitellogenic proteins, etc).

This survey revealed strongly-expressed genes in the fat bodies of *Ae. aegypti* and is useful for the purpose of identifying genes whose promoters can be used for driving the expression of anti-pathogen molecules in transgenic mosquitoes, (James et al. 1999). In addition, the use of transgenic mosquito strains in which a female-specific promoter controls the expression of a lethal gene has been proposed as a powerful technique to generate a male-only population useful for a variation of the Sterile Insect Release technique (Thomas et al.

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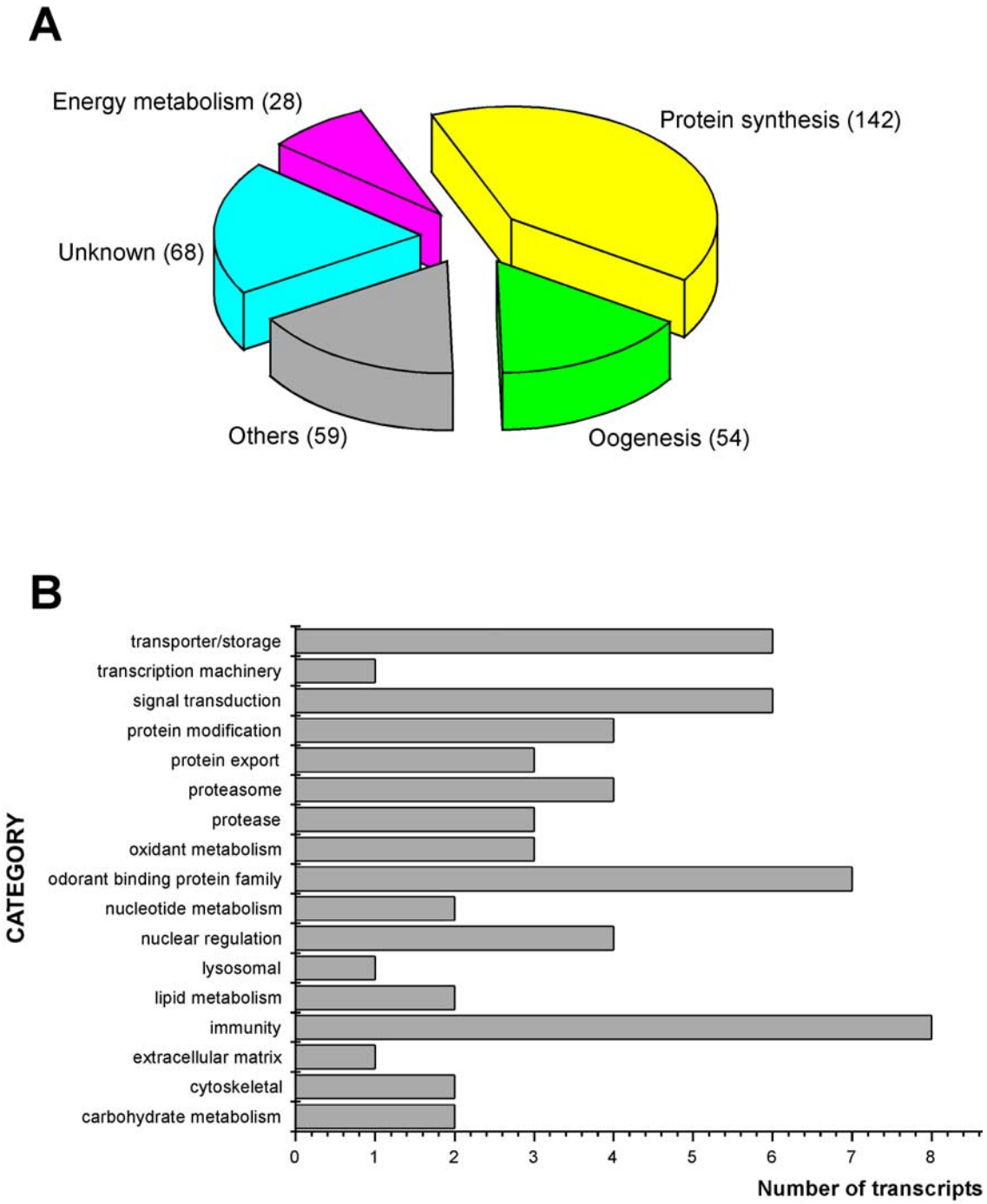


Figure 1. Number of sequences of the *Aedes aegypti* fat body cDNA library. The transcript categories are more represented (A) and less represented (B - Others). The sequences were classified in accordance with function based on similarity of the deduced Ae. aegypti protein sequences deposited in the NR protein database at NCBI. Translated sequences were also screened with RPSBlast for protein motifs of the combined set of Pfam (Bateman et al. 2000) and SMART (Schultz et al 2000) databases. See also Table 2.

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2000). This requires that a strain of the target organism carry a dominant, sex-specific lethal gene whose expression can be repressed in the laboratory or insectary under controlled conditions. Some of these genes may be suitable targets for new insecticides or strategies to block parasite development within the vector. Other advances could result from the comparative analysis of regulatory *cis*-acting elements found in the promoters of selected genes. For example, searches for conserved motifs may identify common regulatory motifs in the promoters of genes strongly-expressed in the fat bodies of mosquitoes. It is anticipated that genes expressed exclusively in the fat body, mostly those regulated coordinately, may share transcription factor-binding sites in their promoters and other regulatory sequences in the respective UTRs.

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