Published in final edited form as:

Insect Mol Biol. 2005 October ; 14(5): . doi:10.1111/j.1365-2583.2005.00578.x.

Modulation of *Anopheles gambiae* gene expression in response to o'nyong-nyong virus infection

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Abstract

To determine if gene expression of *An. gambiae* is modulated in response to o'nyong-nyong virus (ONNV) infection, we utilized cDNA microarrays including about 20 000 cDNAs. Gene expression levels of ONNV-infected female mosquitoes were compared to that of the uninfected control females harvested at 14 days postinfection. In response to ONNV infection, expression levels of 18 genes were significantly modulated, being at least two-fold up- or down-regulated. Quantitative real-time PCR analysis (qRT-PCR) further substantiated the differential expression of six of these genes in response to ONNV infection. These genes have similarity to a putative heat shock protein 70, DAN4, agglutinin attachment subunit, elongation factor 1 alpha and ribosomal protein L35. One gene, with sequence similarity to mitochondrial ribosomal protein L7, was down-regulated in infected mosquitoes. The expression levels and annotation of the differentially expressed genes are discussed in the context of host/virus interaction including host translation/replication factors, and intracellular transport pathways.

Keywords

Anopheles gambiae; o'nyong-nyong virus; gene expression; cDNA microarray

Introduction

Anopheles gambiae is the primary vector of malaria parasites in sub-Saharan Africa, where almost 90% of the world's malaria cases occur (WHO, 1998). However, *An. gambiae* is also the primary vector of the alphavirus o'nyong-nyong (ONNV) (Williams *et al.*, 1965; Powers *et al.*, 2000). ONNV epidemic data have implicated *An. gambiae* and *Anopheles funestus* as important vector species (Williams *et al.*, 1965). This is an unusual vector-virus relationship because mosquito-borne viruses are typically transmitted by Culicine mosquitoes. ONNV is the only arbo-virus commonly transmitted by *An. gambiae* and the molecular basis of this unique relationship is unknown. ONNV is an alphavirus which is an enveloped, single-stranded (+) RNA virus with a genome of approximately 12 kb and serologically classified in the Semliki Forest virus complex (Karabatsos, 1975; Levinson *et al.*, 1990). ONNV was first identified during an epidemic in Uganda in 1959, which ultimately infected over 2

million patients across East Africa from 1959 to 1961 (Johnson, 1988). ONNV symptoms in human include fever, severe back and joint pain, lymphadenopathy and an itchy rash (Corbet *et al.*, 1961). After a 35-year absence, an ONNV epidemic occurred in Southern Uganda (Rwaguma *et al.*, 1997).

Alphaviruses including Sindbis (SINV) and ONNV infect mosquitoes after ingestion of blood from a viremic vertebrate, replicate in midgut epithelial cells, and then disseminate through the haemocoel to the salivary glands and other target tissues, e.g. fat body and thoracic muscle (Pierro *et al.*, 2003). The tissue tropism of SINV differs in *Aedes albopictus* and *Aedes aegypti* mosquitoes (Jackson *et al.*, 1993; Bowers *et al.*, 1995), suggesting that the susceptibility of mosquitoes to alphavirus infection may be based on the existence of tissue-specific factors such as cellular proteins. The infection and replication events for alphaviruses are well understood (Strauss & Strauss, 1994). Following binding to the cell membrane and endocytosis, the RNA genome is released into the cytoplasm, and is translated into the four non-structural proteins that form the viral RNA-dependent RNA polymerase. This RNA polymerase may interact with host protein subunits which construct a replication complex and replicate the RNA genome into (–) RNA genome. (–) RNA genome produces both the (+) RNA genome and the 26S subgenome. By modulating the host translation machinery, 26S subgenomic RNAs are translated into the capsid and envelope proteins (Lemm & Rice, 1993; Strauss & Strauss, 1994). Cellular proteins are therefore critical factors for the susceptibility of mosquitoes to alphaviruses, but factors such as the modulation of host protein subunits required for viral transcription/replication/translation in the mosquito might also be essential for successful viral replication. For successful replication, the alphavirus needs effectively to modulate gene expression of host cells. We hypothesized that by comparing the expression profile of genes of ONNV-infected and uninfected *An. gambiae*, candidate genes that are critical for susceptibility of *An. gambiae* to ONNV would be identified.

To investigate the gene expression profile for *An. gambiae* resulting from ONNV infection, we conducted cDNA microarray experiments utilizing about 20 000 cDNAs prepared from a normalized library of *An. gambiae* and two immune competent cell lines (G. K. Christophides, unpublished data; <http://www.komar.embl-heidelberg.de/>). As cDNA microarray experiments by their nature are combined with the many sources of error associated with the microarray technology itself (Chuaqui *et al.*, 2002), new RNA samples were used to verify the universality of the cDNA microarray results. All candidate genes from cDNA microarray experiment were further examined by qRT-PCR analysis, which has been demonstrated as a reliable technique to quantify mRNA over a broad range of mRNA expression levels (Freeman *et al.*, 1999; Walker, 2001).

Consequently, we have identified seven transcripts that are differentially expressed in ONNV-infected female *An. gambiae* compared to non-infected control mosquitoes at 14 days postinfection (p.i.). Using *An. gambiae* and ONNV as a model, this study provides the first genome-wide molecular identification of mosquito genes, associated with arboviral infection and replication in the arthropod vector.

Results and discussion

Genome-wide screening of differentially expressed transcripts of ONNV-infected blood-fed female *An. gambiae* relative to naive blood-fed females was conducted at 14 days p.i. At 14 days p.i. ONNV is disseminated into the haemocoel from the midgut, infecting other tissues in the mosquito such as fat body, salivary gland and ovaries. The disseminated ONNV would therefore be able to be transmitted to humans (Vanlandingham *et al.*, 2005).

After signal intensities for hybridized cDNAs were filtered, a total of 10 116 cDNAs from the four slides with signal intensity values less than 300 pixels were excluded from further analysis. From cDNA microarray analysis using approximately 18 640 cDNAs, 64 genes were identified as being at least two-fold up-regulated in ONNV-infected compared to uninfected mosquitoes at 14 days p.i. In addition, 38 genes displayed at least two-fold down-regulation in ONNV-infected compared to non-infected mosquitoes at 14 days p.i. (Fig. 1).

Among the two-fold up- and down-regulated genes, differential expression of individual genes was determined using a paired Student's *t*-test comparing the signals derived from the ONNV-infected vs. uninfected mosquitoes across replicates. Of the 102 unique cDNA clones that the statistical preliminary screening procedure showed to be differentially expressed in response to ONNV infection, 18 genes were associated with significant signal intensity differences at the 95% confidence level. Following further screening by qRT-PCR analysis, this infection-induced effect at the 95% confidence level was confirmed in seven of the 18 genes. The elimination of 11 genes from our data set may reflect the differential sensitivities of the techniques or sample variation. The conformation that seven genes were differentially expressed using two analytical methods provides confidence that this is an infection-specific phenomenon. As methodology improves, the correlation between the techniques may be increased.

The ribosomal protein S4 (RpS4) was chosen as an internal control for two reasons. Firstly, expression levels in our cDNA microarray experiment were relatively consistent. Secondly, when analysed by qRT-PCR, Student's *t*-test determined that the mRNA expression levels of RpS4 in uninfected and infected mosquitoes collected at 24 h, 48 h and 14 days p.i. were not significantly different (data not shown). We therefore concluded that the RpS4 gene can be used as an internal control at early and late stages of ONNV. As shown in Table 1 and Fig. 2, qRT-PCR analysis of RpS4 transcript levels at 14 days p.i. detected no significant difference between the relative transcript levels derived from the ONNV-infected vs. uninfected mosquitoes. We are therefore confident that the differential expression observed for other genes was related to ONNV infection and not attributed to variation in sample loading.

By combining a high-density cDNA microarray and qRT-PCR analysis we identified seven genes whose expression is modulated by ONNV infection. To verify the amplification of our qRT-PCR primers, the amplification efficiency values of the primers of each cDNA and the regression coefficient of its standard curve were evaluated (Table 1). Amplification efficiency values of the primers were all within 1 ± 0.1 , the range of manufacturer's recommendation, and the regression coefficient of the standard curve of each primers were all greater than 0.99. Sequence analyses of the seven unique transcripts determined that they all shared sequence homology with genes predicted in the *An. gambiae* genome (Table 2). The results of BlastN, BlastX and BlastP against Nr, EST sequence database and the Universal Protein database, respectively, are shown in Table 2. The products of the seven genes are seemingly involved in modulation of translation/replication factors and intracellular transport pathways. As discussed below, these biological processes may be critical for successful ONNV infection in *An. gambiae*.

Initially, the qRT-PCR data showed a 4.8-fold higher expression of EF-1 in ONNV infected females at 14 days p.i. (Table 1, Fig. 2). Host protein subunits are frequently used for replication and transcription of viral RNAs by RNA viruses (Lai, 1998). EF-1 binds to the 3' UTRs of West Nile virus (WNV) and a range of RNA viruses (Joshi *et al.*, 1986; Barrera *et al.*, 1993; Blackwell & Brinton, 1997). The sequence of EF-1 is highly conserved between different organisms, and the WNV 3' (+) RNA binds to EF-1 in mammalian, chicken and mosquito cell extracts, suggesting that EF-1 is used by

flaviviruses in divergent host species (Brinton, 2001). As alphaviruses and flaviviruses are both (+) RNA viruses, and relatively closely related (Schlesinger & Schlesinger, 2001), the implication is that functional roles of EF-1 may be highly conserved between ONNV and WNV in different host species.

The 26S RNA of alphaviruses, e.g. ONNV, Semliki Forest Virus (SFV) and Sindbis (SIN) is translated to produce a structural polyprotein consisting of capsid, E3, E2, 6K and E1 proteins (Kaariainen & Ahola, 2002). The capsid protein associates transiently with the 60S subunit of the translating ribosome, and is believed to be transferred to the RNA genome during the assembly of the nucleocapsid (Ranki *et al.*, 1979; Ulmanen *et al.*, 1979). From the qRT-PCR analysis the RpL35 shows a 3.5-fold up-regulation in gene expression in ONNV infection at 14 days p.i. (Table 1, Fig. 2). The up-regulated expression of RpL35 gene suggests that RpL35 may interact with capsid protein in order to transfer the RNA genome and assemble the nucleocapsid.

From the qRT-PCR data, HSP70 shows a 29.2-fold increase in gene expression in ONNV infection at 14 days p.i. (Table 1, Fig. 2). As ONNV infection in vertebrate cells are cytopathic, the elevated gene expression of heat shock protein that we observed may protect the vector host cell from ONNV-induced molecular damage (Morimoto & Santoro, 1998). HSP70 may also play a role in ONNV entry. ONNV (like other alphaviruses) is suggested to use an active clathrin-dependent endocytic pathway for infection (DeTulleo & Kirchhausen, 1998). HSP70 causes dissociation of clathrin from the vesicle surface, and enables it to recycle onto membranes (Newmyer & Schmid, 2001; Young *et al.*, 2003).

DAN4 and agglutinin also displayed significantly higher transcript levels, 6.0- and 6.4-fold changes in ONNV infection at 14 days p.i., respectively (Table 1, Fig. 2). Similarity of DAN4 and agglutinin could not be detected by BlastX and BlastN searches against the Nr and EST database, respectively. However, significant sequence similarities were detected against the Universal protein database. Despite this limitation, the gene ontology of DAN4 and agglutinin suggest that they may interact with endosome and lysosome membranes. As the four non-structural proteins and RNAs of alphaviruses are associated with the membranes of modified endosomes and lysosomes of the replication complex (Kaariainen & Ahola, 2002), DAN4 and agglutinin may therefore be involved in a membrane attachment of the replication complex of ONNV. Further analyses, including functional studies, are necessary to determine the role of each protein in ONNV replication.

Conclusion

We have presented data from cDNA microarray and qRT-PCR analyses as a first attempt to evaluate gene expression profiles in the mosquito vector *An. gambiae* during infection with ONNV. Gene expression studies of *An. gambiae* in response to ONNV infection may provide new insight into potential mechanisms through which specific host proteins of the arthropod vector can influence the outcome of arboviral infection. Identification of these proteins may be important in determining the host range and virulence of the virus (Strauss & Strauss, 1999). The understanding of host determinants of vector competence with respect to ONNV infection in *An. gambiae* may be broadly applicable to other vector-virus relationships, and may identify molecular methods to intervene in the transmission of ONNV and other arboviruses in nature. From a practical viewpoint, the identification of the binding domains of host and virus proteins, for example, might be utilized to empirically design a vaccine to block the essential interactions between virus and host proteins, and may represent possible drug targets. A characterized binding domain may also be a good target for effector molecules which may be introduced into the mosquito genome to block virus replication. Furthermore, given the development of ONNV as an expression vector in *An.*

gambiae (Brault *et al.*, 2004; Keene *et al.*, 2004), the elucidation of molecular interactions between *An. gambiae* and ONNV may provide new approaches by which infection efficiency and expression levels can be optimized. This will eventually help us to better perform *in vivo* gene expression studies in *An. gambiae* and other Anopheline mosquitoes.

Experimental procedures

Virus

The SG650 strain of ONNV was obtained from the World Reference Center for Arboviruses at the University of Texas Medical Branch (Galveston, TX). Strain SG650 was isolated from human serum in Uganda in 1996 (Lanciotti *et al.*, 1998) and has been passed once in Vero cells (GenBank Accession Number AF079456). Stock virus was produced following a single passage in Vero cells maintained at 37 °C in Leibovitz L-15 medium with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin. Cell supernatant was harvested when 75% of the cells showed cytopathic effect (3 + CPE), aliquoted, and stored at –80 °C for use in all experiments.

Mosquitoes

The G3 strain of *An. gambiae* was reared at 27 °C and 80% relative humidity under a 16 h light: 8 h dark photoperiod, as previously described (Higgs 2004). Adults were supplied with a cotton wool pad soaked in a 10% sucrose solution *ad libitum* and fed on anaesthetized hamsters once per week for egg production.

Virus infections of mosquitoes

Protocols to infect *An. gambiae* with ONNV SG650 have been optimized to give infection rates of greater than 90% (Vanlandingham *et al.*, 2005). Four-day-old adult female *An. gambiae* mosquitoes were divided into two groups, one fed an infectious blood meal, the second fed a meal prepared with uninfected tissue culture supernatant. Fresh virus was grown from stock and harvested from Vero cells when 75% of the cells showed CPE. The viral supernatant was mixed with an equal volume of defibrinated sheep blood (Colorado Serum Company, Denver, CO). As a phagostimulant, adenosine triphosphate at a final concentration of 2 mM was added to the blood meal. Mosquitoes were fed in a Biosafety Level 2 insectary. Blood meals were heated to 37 °C and placed in a Hemotek feeding apparatus (Discovery Workshops, Accrington, UK) and mosquitoes were allowed to feed for 1 h (Cosgrove *et al.*, 1994). Fully engorged females were separated from unfed females and were placed into new cartons. Cartons were placed in a sealed, humidified plastic box and maintained with 10% sucrose in a Precision model 818 environmental chamber (Precision, Winchester, VA) at 27 °C, with a 12 h light: 12 h dark cycle.

Mosquito sampling

Based on previous data (Vanlandingham *et al.*, 2005), samples of approximately 50 mosquitoes were harvested and stored at –80 °C for three time points postinfection (p.i.) representing different phases in the infection cycle: 24 h (early and restricted midgut infection), 48 h (established midgut infection with more active viral replication), and 14 days (disseminated infection with multiple tissue involvement). Identical numbers of uninfected mosquitoes were harvested simultaneously. After titration of three experimental mosquitoes collected at 14 days p.i. had confirmed that all were infected with ONNV (Vanlandingham *et al.*, 2005), RNA was extracted as described below.

Mosquito Microarray Consortium-1 (MMC-1) microarray construction

Inserts of the 16 000 NAP1 cDNA clones were amplified by a two-step PCR amplification using T3 and T7 primers and bacterial cultures as primary templates. The resulting PCR products were purified through Millipore, dried and resuspended in 3× SSC. The amplicons were arrayed on aminosilane-coated glass slides with the Omnigrid microarray spotter (GeneMachines, San Carlos, CA). The slides were then incubated at 60 °C for 3 h and 100 °C for 10 min to cross-link spotted DNAs. MMC-1 microarray also includes the 4000 cDNA clones from two immune competent *An. gambiae* cell lines (Dimopoulos *et al.*, 2002).

cDNA microarray target preparation and hybridization

Total RNA samples were extracted from three batches of approximately 50 ONNV-infected adult female mosquitoes 14 days p.i. and from non-infected female mosquitoes, which were used as control, using the Trizol Reagent (Invitrogen, Carlsbad, CA). To remove genomic DNA contamination, RNA samples were treated with 1.0 µl DNase I following the manufacturer's instruction (50–375 units/µl; Invitrogen). RNA labelling and hybridization were performed in triplicate using the 3DNA Array 50 kit according to the methods of Dana *et al.* (2005). Briefly, to make the first strand of cDNA, the RNA from the non-infected mosquitoes was labelled with Cy5 specific primer and the ONNV-infected with Cy3 specific primer. Competitive hybridizations between infected and non-infected samples were carried out following the two-step hybridization: (1) cDNA hybridization to the amplified DNA probes spotted on the slides; (2) hybridization of fluorescent dendrimers (Cy3 and Cy5) onto cDNA (Genisphere, Hatfield, PA). The cDNA hybridizations were performed at 45 °C overnight. The slides were washed after hybridization. For postwashing, the array was air dried via centrifugation for 3 min at 156 g. The 3DNA hybridizations were performed at 53 °C for 2 h and 0.5 mM DTT was added to the first two wash solutions to protect the fluorochromes from oxidation.

Microarray data acquisition and statistical analysis

Following hybridization and washing, microarray slides were scanned successively at two wavelengths, 532 and 635 nm, using the Affymetrix 428 Array Scanner (Affymetrix, Santa Clara, CA). Raw signal intensities were acquired using the adaptive circle algorithm and spot intensities were quantified using JAGUAR 2.0 software (Affymetrix). Average signal intensities were normalized using the Lowess curve for intensity-dependent normalization followed by a per-gene median normalization using the GENESPRING software (version 5.1; Silicon Genetics, Redwood City, CA). Signal intensities for hybridized cDNAs were filtered and only gene products with raw signal intensity values greater than 300 pixels were included. In addition, genes with more than two-fold expression difference between the ONNV-infected and non-infected samples were utilized in further analysis. Statistical significance of differences in the expression of individual genes was determined by using a paired Student's *t*-test between the signals derived from the ONNV-infected and non-infected mosquitoes across three replicates for each gene queried, and a *P*-value less than 0.05 was considered to be a significant gene expression change.

Sequence analysis

Seven confirmed sequences of significant gene expression changes were analysed using BLASTX, BLASTN and BLASTP (Altschul *et al.*, 1997) against the nr (non-redundant), EST database (<http://www.ncbi.nlm.nih.gov/BLAST/>), and Universal protein database (<http://www.pir.uniprot.org/>), respectively. Gene Ontology (GO) (Ashburner, 2000) annotation was recorded to each sequence on the basis of highest BLAST hit with an *E*-value 10^{-6} .

Quantitative real-time PCR Analysis (qRT-PCR)

To validate the cDNA microarray results, qRT-PCR was performed using an ABI 7700 Sequence Detection System (Applied Biosystems, Foster City, CA). Standard curves were generated for each transcript tested using 10-fold serial dilutions of *An. gambiae* genomic DNA ranging from 116 to 0.0116 ng per reaction. All reactions were performed in triplicate in a total volume of 25 μ l containing 12.5 μ l of SYBR Green PCR Master Mix, 300 nmol of each primer at the following conditions: 50 °C for 2 min, 95 °C for 10 min followed by 50 cycles of denaturation at 95 °C for 15 s, annealing and extension at 60 °C for 1 min. To enhance universality of the results, RNA samples were extracted from mosquitoes at 14 days p.i. which were not used in cDNA microarray experiments. Sequences of gene-specific primer sets are given in Table 2. Statistical significance of differences in the expression of individual genes was determined by using a Student's *t*-test between the relative transcript values derived from the ONNV-infected and non-infected mosquitoes across three replicates for each gene, and a *P*-value less than 0.05 was considered to be a significant gene expression change.

Acknowledgments

This project was supported by the U01-AI48846 from NIH/ NIAID to F.H.C. and by NIH grant number AI47877 and DARPA grant cooperative agreement N00178-02-2-9002 with the Chemical Biological Radiological Sciences & Technology Defense Branch of the Naval Surface Warfare Center, Dahlgren, VA, awarded to S.H. We thank J. Huang, C. Hong and K. Tsatsarkin for technical assistance.

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Figure 1.

Scatterplot analysis of variation in gene expression. The RNA from the non-infected was labelled with Cy5 and the ONNV-infected with Cy3. Red and green spots represent up- and down-regulated gene expression in ONNV-infective compared to non-infected control mosquitoes, respectively. The middle diagonal line indicates one-fold (no change) and the upper and lower diagonal lines show either two-fold up- or down-regulated gene expression, respectively. The *x*- and *y*-axes are in logarithmic scales.

Figure 2. qRT-PCR analysis of differentially expressed genes of *An. gambiae* in ONNV infection. For cDNA construction total RNA was isolated from ONNV-infected and non-infected control mosquitoes. Biological replicates were used to generate fold changes and error bars for qRT-PCR experiments ($n = 3$). Each bar represents standard deviation. The fold changes were calculated to the average transcript level of ONNV infected mosquitoes divided by that of non-infected control mosquitoes. However, there was a fold change decrease in the value of MRpL7 (white bar), therefore, the value was negatively inversed. RpS4 gene (black bar) shows no significant loading variation of the samples.

Table 1qRT-PCR analysis of differentially expressed genes of *An. gambiae* in ONNV infection

Gene ID (GenBank Accession No.)	Primer sequence (5' to 3')	Fold change (P- value, SD)	Amplicon size (bp)	Amplification efficiency ^a (R ^b)
RpS4 (AJ283756)	Forward: GCTGCCGCTGGTGATCTT			
	Reverse: TCGTCACCTCGCTGTTGGT	1.2 (0.259, 0.42)	65	0.90 (0.99)
AdeL (AL692204)	Forward: CGTGCAGAATGGCCGAA			
	Reverse: CGTGCTCAGCGGCGA	5.4 (0.031, 3.0)	59	0.90 (0.99)
RpL35 (BX466552)	Forward: GCAACCAGCAGAGAAGCA			
	Reverse: GAACAGGACGTTCTTGC GGT	3.5 (0.002, 0.96)	68	1.1 (0.99)
Hsp 70 (AL930714)	Forward: GCGATCCAGGCCGACAT			
	Reverse: TCTTTGGCTTGCCCTCGAT	29.2 (0.02, 16.4)	64	0.97 (0.99)
Dan4 (AL694117)	Forward: GGTAGGAAAAATGCGGGACAA			
	Reverse: TTATCGTGCAGCCGCAATC	6.0 (0.004, 3.24)	66	1.1 (0.99)
EF-1 (N/D) ^c	Forward: GGACAGATCAGCAACGGATACA			
	Reverse: TCGGAGAACTTGACGCA	4.8 (0.01, 0.10)	73	1.1 (0.99)
Agglutinin (N/D)	Forward: CGCTGACCGCTACGGTGT			
	Reverse: AATCGGCTGTTCTCGTTGGA	6.4 (0.01, 1.14)	67	0.91 (0.99)
MRpL7 (N/D)	Forward: GGTGGTGAAGACGACGTTCA			
	Reverse: CCTTGATCAGGGCCACCTT	-2.0 (0.04, 0.09)	71	1.1 (0.99)

^a Amplification efficiencies were calculated from the slope of standard curves as $E = 10^{[-1/\text{slope}]} - 1$. 100% PCR efficiency corresponds to an amplification efficiency of 1 (Applied Biosystems Application Note);

^b Regression coefficient of linear standard curve.

^c Accession number has not yet been designated.

Table 2
Confirmed list of differentially expressed genes in ONNV-infected *An. gambiae* identified by cDNA microarray experiments

Gene ID	Ensembl Gene ID	BlastN (Nr)	BlastX (dbEST)	BlastP (dbUniprot)	Gene ontology	P-value (Mean ^a , SD ^b)	Fold change
2× Up-regulated genes							
AdoL	ENSANGG000000012419	No description	Adenylosuccinase (8e-39)	Adenylosuccinase (8.1e-213)	purine biosynthesis	0.014 (814344, 412362)	2.2
Rpl35	ENSANGG000000015069	Ribosomal protein L35 (3e-59)	Ribosomal protein L35 (1e-12)	Ribosomal protein L35 (3.4e-45)	protein biosynthesis	0.041 (1993477, 1545856)	2.2
HSP 70	ENSANGG000000017398	No description	Molecular chaperone Hsc70-4 (6e-38)	Heat Shock Protein 70 (0)	protein folding	0.014 (1067897, 527168)	2.6
2× Down-regulated genes							
DAN4	ENSANGG000000014767	No description	No description	Yeast, DAN4 precursor (8.8e-08)	integral to membrane	0.039 (291445, 149264)	2.4
EF-1	ENSANGG000000015883	EF-1 alpha (0)	EF-1 alpha (0)	EF-1 alpha (0)	translational elongation	0.022 (1146203, 685794)	2.0
Agglutinin	ENSANGG000000011280	No description	No description	A-agglutinin attachment subunit (4.6e-07)	membrane adhesion	0.004 (1046330, 335650)	2.0
MRPL7	ENSANGG000000012624	No description	Mitochondrial ribosomal protein L7/L12 (3e-24)	Mitochondrial ribosomal protein L7/L12 (4.6e-34)	mitochondrion	0.035 (-293774, 314742)	-2.2

^aMeans of differences of signal intensities;

^bSD: Standard deviations of differences of signal intensities.