SUPPLEMENTAL DATA

Supplemental Experimental Procedures:

cDNA library construction

Total RNAs were obtained from the whole body of third instar larvae 0 h (hydrated and active larvae), 12 h and 36 h after the beginning of the desiccation process (Fig. 1). Purification of Poly(A)$^+$ RNAs was followed by cDNA synthesis, using oligo-dT primers. Independent cDNA libraries for the three groups (named pvD00, pvD12 and pvD36, respectively) were made by directional cloning in the lambda ZAP vector (Lambda ZAP cDNA cloning kit; Stratagene) at EcoRI and XhoI sites for 5´ and 3´ ends, respectively. *In vivo* mass excision of the bluescript SK$^-$ phagemid from the ZAP vector was performed to obtain bacterial colonies.

EST sequencing

Sequencing templates were obtained from bacterial colonies picked up randomly from agar plates of each library by colony PCR using M13-20 and reverse primers. The amplified products were purified with a spin column of sephacryl S-300 HR (Amersham Biosciences AB, Sweden). The sequencing reactions were performed using Big Dye Terminator v3.1 (Applied Biosystems, USA) on a DNA Sequence System (model 377, PE Applied Biosystems) or a DNA analyzer (model 3700, PE Applied Biosystems). Each clone was sequenced in both 5' and 3' directions with T3 and T7 primers.

Sequence processing and assembly

The sequences obtained were first processed to remove vector sequences, using the VecScreen and cross_match softwares. Remaining vector sequences were eliminated manually. After trimming of under QV20 sequences and removal of *E. coli* sequences, ESTs with a length of less than 300 bp were discarded. The resulting ESTs were grouped into clusters with two clustering algorithms, as described in detail in a previous work (1). Briefly, the first method exploits BLAST similarity (CLOBB) (2), using Perl script parameters of 95% identity and 50 bp coverage. The second is a method combining BLAST and PhredPhrap (CBP) (1) with identity value and coverage length of over 97% and 120 bp, respectively. The coverage length and identity values were increased to achieve the final consensus result. Contig members were tested for a 95% identity and 90% cover ratio after the clustering process.

Gene annotation

Genes were identified by sequence similarity comparison against amino acid databases, using the customized program BLASTx 1.5.6 Paracel. The BLASTX analyses were performed independently against the databases: the non-redundant protein sequence database at NCBI, FlyBase

(dmel-all-translation-r4.2.1) from the fruit fly *Drosophila melanogaster* and WormBase (wormpep 152) from the nematode *Caenorhabditis elegans*. The degree of sequence homology was defined as follows: ESTs with an E-value of 1e-10 or less, amino acid homologous regions greater than 100 bp, and identity exceeding 30% were termed 'high homology', while 'low homology' ESTs had an E-value of 1e-10 or less, but did not fulfil the two other conditions. Finally, 'no homology' ESTs fulfilled none of the conditions described above.

Gene ontology was obtained by scanning EST sequences with the InterPro software (iprscan_4.1_10.0).

EST database analysis

Assuming that the number of ESTs for each gene cluster is representative of its expression level, gene clusters were classified as a function of their expression pattern in the three time point specific libraries (pvD00, pvD12 and pvD36), using the Macro function Visual Basic Editor of Excel software (Microsoft Corp., USA). As the number of ESTs analyzed was different in each library, the number of ESTs in each cluster was normalized, so that all three libraries contained the same total number of ESTs. Expression patterns were defined as follows: (0) stable expression over the three time points; (1) expression increasing over the three time points; (2) expression increasing between 0 h and 12 h, then remaining almost stable; (3) expression almost stable, then increasing between 12 h and 36 h; (4) expression increasing between 0 h and 12 h and subsequently decreasing, with a difference between 0 h and 36 h less than 2-fold; (5) expression decreasing over the three time points; (6) expression decreasing between 0 h and 12 h, then remaining almost stable; (7) expression almost stable, then decreasing between 12 h and 36 h; (8) expression decreasing between 0 h and 12 h and subsequently increasing, with a difference between 0 h and 36 h less than 2-fold.

Real-time quantitative PCR

Messenger RNAs from wet larvae (desiccation 0 h) and from larvae desiccated for 8 h, 16 h, 24 h and 48 h (third instar, 10-50 individual each) were extracted with TRIzol® (Invitrogen, Carlsbad, CA) and subsequently reverse-transcribed to cDNA, using a Ready-To-Go T-primed First-Strand kit (Amersham Biosciences, Piscataway, NJ). Primer sets were designed with LightCycler Probe Design software 2.0 (Roche Diagnostics GmbH, Mannheim, Germany).

For *PvLea1*, *PvTps*, *PvAqp1* and *PvTret1*, quantitative PCR analyses were performed with the QuantiTect™ SYBR® Green PCR kit (Qiagen, Hilden, Germany) on a LightCycler 2.0 system (Roche Diagnostics GmbH, Mannheim, Germany). PCR thermal cycling conditions were as follows: 95ºC for 10 min, then 45 cycles of 95ºC, 60ºC and 72ºC every 10 s. Data were analyzed with LightCycler software 4.0 (Roche Diagnostics GmbH, Mannheim, Germany). Results were corrected with an

internal control, *P. vanderplanki* ribosomal protein L32 (*PvRpl32*; Accession No. AB244986).

For *PvGlobin2*, *PvHbCTT6*, *PvTrx2* and *PvDip1*, quantitative PCR analyses were performed on a StepOnePlus™ system (Applied Biosystems, Foster City, CA), using the Power SYBR® Green Master Mix (Applied Biosystems, Foster City, CA). PCR thermal cycling conditions were as follows: 95ºC for 10 min, then 45 cycles of 95ºC for 15 s and 60ºC for 1 min. Data were analyzed with the StepOne™ software V2.1 (Applied Biosystems, Foster City, CA) and results were corrected with *P. vanderplanki* Elongation factor 1-alpha (PvEf1-alpha; accession No. AB490338) as an internal control. Each category was quantified in triplicate. The primers sequences used for each gene are listed in supplemental table S2.

REFERENCES

1. Suetsugu, Y., Minami, H., Shimomura, M., Sasanuma, S., Narukawa, J., Mita, K., and Yamamoto, K. (2007) *BMC genomics* **8**, 314
2. Parkinson, J., Guiliano, D. B., and Blaxter, M. (2002) *BMC bioinformatics* **3**, 31

SUPPLEMENTAL FILE LEGENDS

Figure S1:

Size distribution of ESTs in the database

Figure S2:

Expression profiles of some groups of clusters, related to selected housekeeping genes

X-axis: cluster numbers. Y-axis: number of ESTs in each cluster. Z-axis: expression pattern, represented by the number of ESTs at 0 h (yellow bars), 12 h (orange bars) and 36 h (red bars) after the beginning of the desiccation treatment. The total number of clusters in each group is indicated in brackets. Statistical significance (Chi-square test) of the proportion changes in the three libraries is shown on the right (*: $P<0.05$; ***: $P<0.0001$).

Table S1:

Lists of the clusters showed in figures 4 and S2

For each functional group of clusters, the following data are provided: name of the cluster (representative clone), homology value obtained by InterProScan, definition of the gene ontology or

of the hit against NCBI database, number of ESTs corresponding to this cluster in each library.

Table S2:

List of the genes investigated by Real-Time Quantitative PCR, with the corresponding primer sequences