Quantitative transcript imaging in normal and heat-shocked *Drosophila* embryos by using high-density oligonucleotide arrays

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Embryonic development in *Drosophila* is characterized by an early phase during which a cellular blastoderm is formed and gastrulation takes place, and by a later postgastrulation phase in which key morphogenetic processes such as segmentation and organogenesis occur. We have focused on this later phase in embryogenesis with the goal of obtaining a comprehensive analysis of the zygotic gene expression that occurs during development under normal and altered environmental conditions. For this, a functional genomic approach to embryogenesis has been developed that uses high-density oligonucleotide arrays for large-scale detection and quantification of gene expression. These oligonucleotide arrays were used for quantitative transcript imaging of embryonically expressed genes under standard conditions and in response to heat shock. In embryos raised under standard conditions, transcripts were detected for 37% of the 1,519 identified genes represented on the arrays, and highly reproducible quantification of gene expression was achieved in all cases. Analysis of differential gene expression after heat shock revealed substantial expression level changes for known heat-shock genes and identified numerous heat shock-inducible genes. These results demonstrate that high-density oligonucleotide arrays are sensitive, efficient, and quantitative instruments for the analysis of large scale gene expression in *Drosophila* embryos.

Recently the genome of the first multicellular eukaryote *Caenorhabditis elegans* was completely elucidated (1). Sequencing of the *Drosophila melanogaster* genome has now also been carried out, and currently the corresponding putative open reading frames are being defined and verified (2). On the basis of this complete genomic information, it will now be important to determine the complex expression of all encoded genes and to analyze physiological as well as pathological phenomena from a global genetic perspective. Large-scale transcript analysis is made possible by DNA micro- or oligonucleotide arrays (3, 4), both of which allow the simultaneous monitoring of hundreds of mRNA expression profiles (5, 6). In this study, we used *Drosophila* high-density oligonucleotide arrays to monitor the simultaneous expression of zygotically active genes during the later postgastrulation stages of embryonic development (7–9). We analyzed the relative abundance levels of hundreds of embryonically expressed genes under normal physiological conditions and in response to heat shock (10). In embryos raised under normal conditions, we obtained highly reproducible quantification for 563 expressed genes corresponding to different functional classes. After a 36°C heat shock, we detected increases in expression levels for known heat-shock genes and identified numerous heat-shock-inducible genes.

Materials and Methods

Embryos. *D. melanogaster* Oregon R stocks were kept on standard cornmeal/yeast/agar medium at 25°C. Embryos were collected overnight on grape-juice plates for 12 h and were kept for a further 5 h at 25°C before RNA isolation. Therefore, at the time of RNA isolation, these embryos were at embryonic stages 10–17 (9). In heat-shock experiments, embryos were collected overnight in the same way, kept for a further 4 h at 25°C, and then subjected to a 36°C heat shock for 25 min followed by a recovery period of 25 min at 25°C before RNA isolation. Embryos younger than embryonic stage 10 were not used, because heat shock in these earlier stages results in lethality (11). Embryos used for *in situ* hybridization studies were collected and heat shock treated in the same way.

Preparation of Biotinylated cRNA. Initial experiments designed to determine the sensitivity and reproducibility of hybridization showed that the use of total RNA vs. poly(A)$^+$ RNA as template for cDNA synthesis and subsequent amplification (synthesis of cRNA) gave comparable results, despite the fact that we consistently detected 5S RNA and histone genes present on the array with cRNA derived from total RNA. On the basis of these findings, all experiments were carried out by using a total RNA protocol (12, 13).

Total RNA was isolated from 200 mg of embryonic tissue by using guanidinium isothiocyanate in combination with acidic phenol (pH 4.0) (fast RNA tube green kit from BIO101) in a fast-prep homogenizer FP120 (Bio 101). After precipitation, the RNA was dissolved in diethyl pyrocarbonate-treated water (Ambion, Austin, TX) and spectrophotometrically quantified by using a GENEQUANT RNA/DNA calculator (Pharmacia Biotech). cDNA was synthesized on total RNA as a template by using the SuperScript Choice System for cDNA synthesis (GIBCO/BRL) with a T7-(T)24 DNA primer: 5′GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGG(T)24VN-3′). For first-strand cDNA synthesis, a typical 40-μl reaction contained 25 μg RNA, 200 pmols T7-(T)24 primer, 500 μM of each dNTP, and 800 units of reverse transcriptase (AMV Superscript II) (GIBCO/BRL). The reaction was incubated for 1 hour at 42°C. Second-strand cDNA synthesis was carried out at 18°C for 2 hours in a total volume of 340 μl by using 20 units *Escherichia coli* DNA ligase, 80 units *E. coli* DNA polymerase I, and 4 units RNase H in the presence of 250 μM of each dNTP. After second-strand cDNA synthesis, 0.5 μl of RNase A (100 mg/ml) (Qiagen, Chatsworth, CA) was added, and the samples were incubated at 37°C for one-half hour. Thereafter 7.5 μl proteinase K (10 mg/ml) (Sigma) was added and the samples were further incubated at 37°C for another half-hour. After

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Abbreviations: RT-PCR, reverse transcriptase–PCR; Avg Diff, average difference value.

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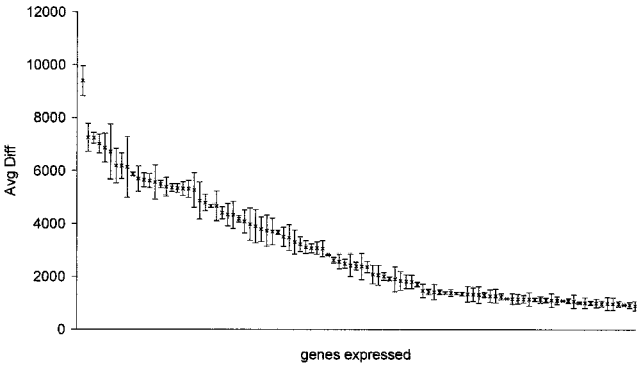

Fig. 1. Gene expression monitoring of stage 10–17 wild-type embryos raised under standard conditions (25°C). Compilation of the 100 genes expressed with the highest Avg Diff values (for details, see *Materials and Methods*) and the corresponding SD (indicated by bars) over four experimental replicates.

cDNA synthesis was completed, samples were phenol chloroform extracted (three times) by using Phase Lock Gel (Eppendorf-5Prime, Boulder, CO) and precipitated overnight at −20°C with 2.5 volumes of 100% ethanol. After precipitation, the samples were stored at −20°C. Biotinylated antisense cRNA was synthesized from the double-stranded DNA template, by using T7 RNA polymerase (MEGAscript T7 Kit, Ambion). A 20-μl reaction volume contained 0.3–1.5 μg of cDNA, 7.5 mM of both ATP and GTP, 5.6 mM of both UTP and CTP, and 1.8 mM of both biotinylated Bio-16-UTP and Bio-11-CTP (Enzo Diagnostics), and 2 μl 10× T7 enzyme mix. The reaction was incubated at 37°C for 8 h. Thereafter, the unincorporated NTPs were removed by putting the sample over an RNeasy spin column (Qiagen). Samples were precipitated overnight at −20°C, taken up in 20 μl DEPC-treated water, and spectrophotometrically quantified. Thereafter, 40 μg of the biotinylated antisense cRNA was fragmented by heating the sample to 95°C for 35 min in a volume of 25 μl, containing 40 mM Tris·acetate (pH 8.1), 100 mM KOAc, and 30 mM MgOAc.

High-Density Oligonucleotide Arrays. In this study, a custom-designed *Drosophila* oligonucleotide array (ROEZ003A, Affymetrix, Santa Clara, CA) was used. The genes represented on the array correspond to 1,519 sequenced *Drosophila* genes encoding open reading frames deposited in SwissProt/Tr EMBL databases as of spring 1998. Each gene is represented on the array by a set of 20 oligonucleotide probes (25 mers) matching the gene sequence. To control the specificity of hybridization, the same probes are synthesized with a single nucleotide mismatch in a central position. As such, each gene is represented by 20 probe pairs comprised of a perfect match and a mismatch oligo. The difference between the perfect match hybridization signal and the mismatch signal is proportional to the abundance of a given transcript (4). *Drosophila* genes that were not unambiguously represented by a probe set of 20 probe pairs on the array were excluded from further analysis (23 probe sets were not used). The oligonucleotide probe selection corresponding to each *Drosophila* gene and the array fabrication was performed by Affymetrix.

Hybridization and Scanning. Gene chips were prehybridized with 220 μl hybridization buffer (1× Mes (pH 6.7)/1 M NaCl/0.01% triton/0.5 μg/μl acetylated BSA/0.5 μg/μl sonicated herring sperm DNA) for 15 min at 45°C on a REAX 2 rotisserie at 60 rpm (Heidolph, Swabach, Germany). Hybridization was done in a final volume of 220 μl hybridization buffer, containing 40 μg fragmented biotinylated cRNA. The samples were heated to 95°C for 5 min and briefly spun down. Hybridizations were carried out for 16 h at 45°C with mixing on a rotisserie at 60 rpm. After hybridization, the solutions were removed, arrays were briefly rinsed with 6× SSPE-T buffer (0.9 M NaCl/0.06 M NaH_2PO_4/6 mM EDTA/0.01% triton) and washed on a Fluidics station (Affymetrix). Hybridized arrays were stained with 220 μl detection solution (1× Mes buffer containing 2.5 μl streptavidin-R phycoerythrin conjugate (1 mg/ml) (Molecular Probes) and 2.0 mg/ml acetylated BSA (Sigma) at 40°C for 15 min and washed again (13).

Data Analysis. Probe arrays were scanned with a commercial confocal laser scanner (Hewlett–Packard). Pixel intensities were measured, and expression signals were analyzed with commercial software (GENECHIP 3.1, Affymetrix). Detailed data analysis was carried out by using RACE-A (F. Hoffmann–La Roche), ACCESS 97, and EXCEL 97 (Microsoft) software. For quantification

Table 1. *Drosophila* oligonucleotide array: Expression data for wild-type embryos

Functional class	Number of genes on the chip (*N*)	Number of transcripts detected (*n*)	$n/N \times 100$ (%)	Transcripts detected (%)
Metabolism	315	112	35.5	19.8
Transcriptional regulation	268	74	27.6	13.1
Cell surface receptors/CAMs/ion channels	181	63	34.8	11.1
Translation	60	52	86.6	9.2
Cytoskeleton/structural proteins	149	48	32.2	8.5
Signal transduction	107	41	38.3	7.2
RNA binding	59	29	49.1	5.1
Transcription/replication/repair	73	28	38.3	4.9
Unknown function	85	23	27.0	4.0
Proteolytic systems/apoptosis	62	22	35.4	3.9
Cell cycle	37	18	48.6	3.1
Transposable elements	35	18	51.4	3.1
Chromatin structure	36	18	50.0	3.1
Heat-shock proteins	18	10	55.5	1.7
Secreted proteins	34	7	20.5	1.2
	$\Sigma N = 1519$	$\Sigma n = 563$		

Genes expressed in stage 10–17 wild-type embryos raised under standard conditions (25°C), grouped according to functional classes. Number of genes within a functional group present on the chip (*N*); total number of genes represented on the chip, $\Sigma N = 1{,}519$. Number of genes expressed within a functional group (*n*); total number of transcripts detected $\Sigma n = 563$. ($n/N \times 100$ in %) Distribution of genes expressed within a functional group in relation to the total number of identified genes in this group present on the chip. Distribution of genes expressed within a functional group, given as percentage of the total number of genes expressed.

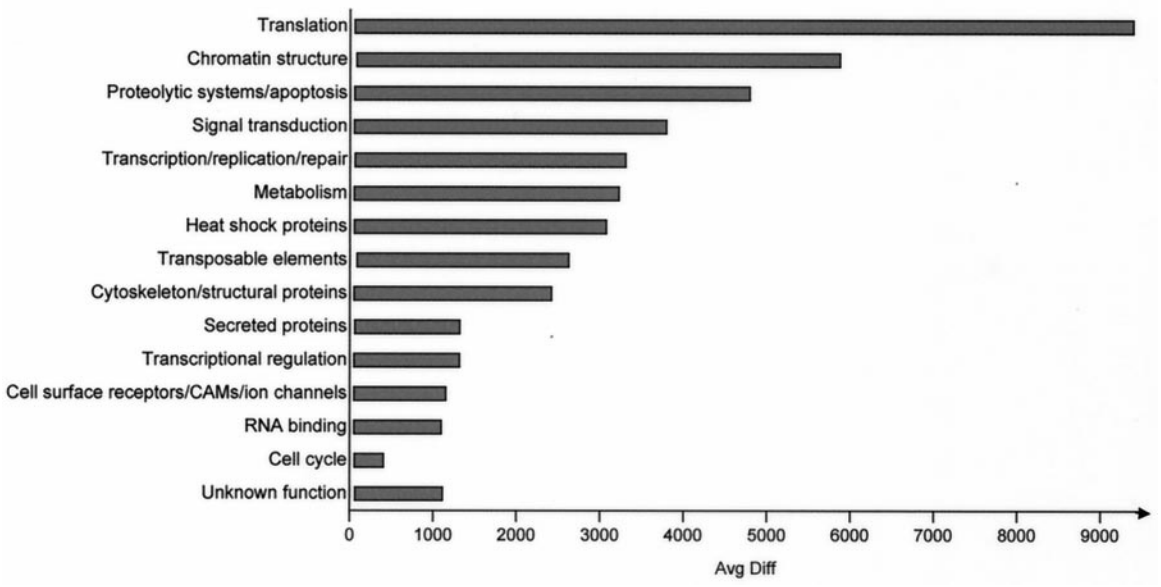

Fig. 2. Range of Avg Diff values for expressed genes, grouped according to their functional classes. Translation (min Avg Diff 56, max Avg Diff 9,394), chromatin structure (78–5,873), proteolytic systems/apoptosis (53–4,792), signal transduction (52–3,791), transcription/replication/repair (59–3,303), metabolism (51–3223), heat-shock proteins (55–3,073), transposable elements (87–2,624), cytoskeleton/structural proteins (52–2,419), secreted proteins (59–1,317), transcriptional regulation (51–1,315), cell-surface receptors/cell adhesion molecules/ion channels (51–1,152), RNA binding (52–1,095), cell cycle (56–405), and unknown function (61–1,114).

of relative transcript abundance, the average difference value (Avg Diff) was used. Four replicates for wild type (condition 1) as well as heat-shock-treated wild type (condition 2) embryos were carried out. All chips were normalized against the mean of the total sums of Avg Diff values across all eight chips. For the analysis of expression profiling of condition 1 embryos, two filter operations were combined. First, all genes with a mean Avg Diff over the four replicate chips that was below 50 were excluded from further analysis. Second, a transcript was judged as present only if the standard deviation of its mean Avg Diff value over the four replicate chips was below 25% of its mean Avg Diff. For differential transcript imaging, only genes with a change factor quality above 1 were considered in this analysis, meaning that the difference of the means of the Avg Diff values over the four replicates between condition 1 and condition 2 was larger than the sum of the standard deviations of the mean Avg Diff values of condition 1 and condition 2 (RACE-A software, M. Neeb and C. Broger, personal communication). In addition, for down-regulation, the mean Avg Diff value of a gene had to be above or equal to 50 in condition 1; for up-regulation, the mean Avg Diff value of a gene had to be above or equal to 50 in condition 2.

Whole-Mount *in Situ* Hybridization. Digoxigenin (DIG)-labeled sense and antisense RNA probes were generated *in vitro* with a DIG labeling kit (Roche Diagnostics), by using commercially available templates (Research Genetics, Huntsville, AL) and hybridized to *Drosophila* whole-mount embryos following standard procedures (14). Hybridized transcripts were detected with an alkaline phosphatase-conjugated anti-DIG Fab fragment (Roche Diagnostics) by using Nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Sigma) as chromogenic substrates. Embryos were mounted in Canada balsam (Serva) and photographed with a Prog/Res/3008 digital camera (Kontron, Zurich) on a Zeiss Axioskop microscope with differential interference contrast optics.

Reverse Transcriptase–PCR (RT-PCR). Three hundred nanograms of poly(A)$^+$ RNA, isolated from heat-shocked embryos and from embryos that were raised under standard conditions (mRNA isolation kit; Roche Diagnostics), was reverse transcribed with AMV-RT and random hexamers (first-strand cDNA synthesis kit for RT-PCR; Roche Diagnostics). PCR was performed with 100 pg template DNA and gene-specific primers (SEQ WEB, Wisconsin Package Ver. 10.0, GCG) on a light cycler (LightCycler, Roche Diagnostics). Continuous fluorescence observation of amplifying DNA was possible by using SYBR Green I (LightCycler-FastStart DNA master SYBR Green I; Roche Diagnostics). After cycling, a melting curve was produced by slow denaturation of the PCR end products to validate the specificity of amplification. To compare the relative amounts of PCR products, we monitored the amplification profile on a graph, displaying the log of the fluorescence against the number of cycles. Relative change folds for a given gene under both conditions (standard vs. heat shock) were calculated by using the fit point method (LightCycler operator's manual Ver. 3.0; Roche Diagnostics).

Functional Classification and Data Presentation. The *Drosophila* genes represented on the high-density oligonucleotide array were classified into 14 functional classes according to the function of the gene product and currently available genetic data. For this, notations in Flybase, Interactive Fly, and SwissProt/Tr EMBL databases were used. These functional classes are signal transduction, transcriptional regulation, cell cycle, cytoskeleton/structural proteins, metabolism, translation, heat-shock proteins, transcription/replication/repair, proteolytic systems/apoptosis, cell surface receptors/cell adhesion molecules/ion channels, transposable elements, chromatin structure, RNA-binding proteins and secreted proteins. A comprehensive presentation of all the genes represented on the oligonucleotide array as well as their attribution to the 14 functional classes is given in the supplemental data (www.pnas.org). This web site also presents all of the original expression data from the experiments on which this report is based. For each gene characterized, Avg Diff values, change fold, and change fold quality are given.

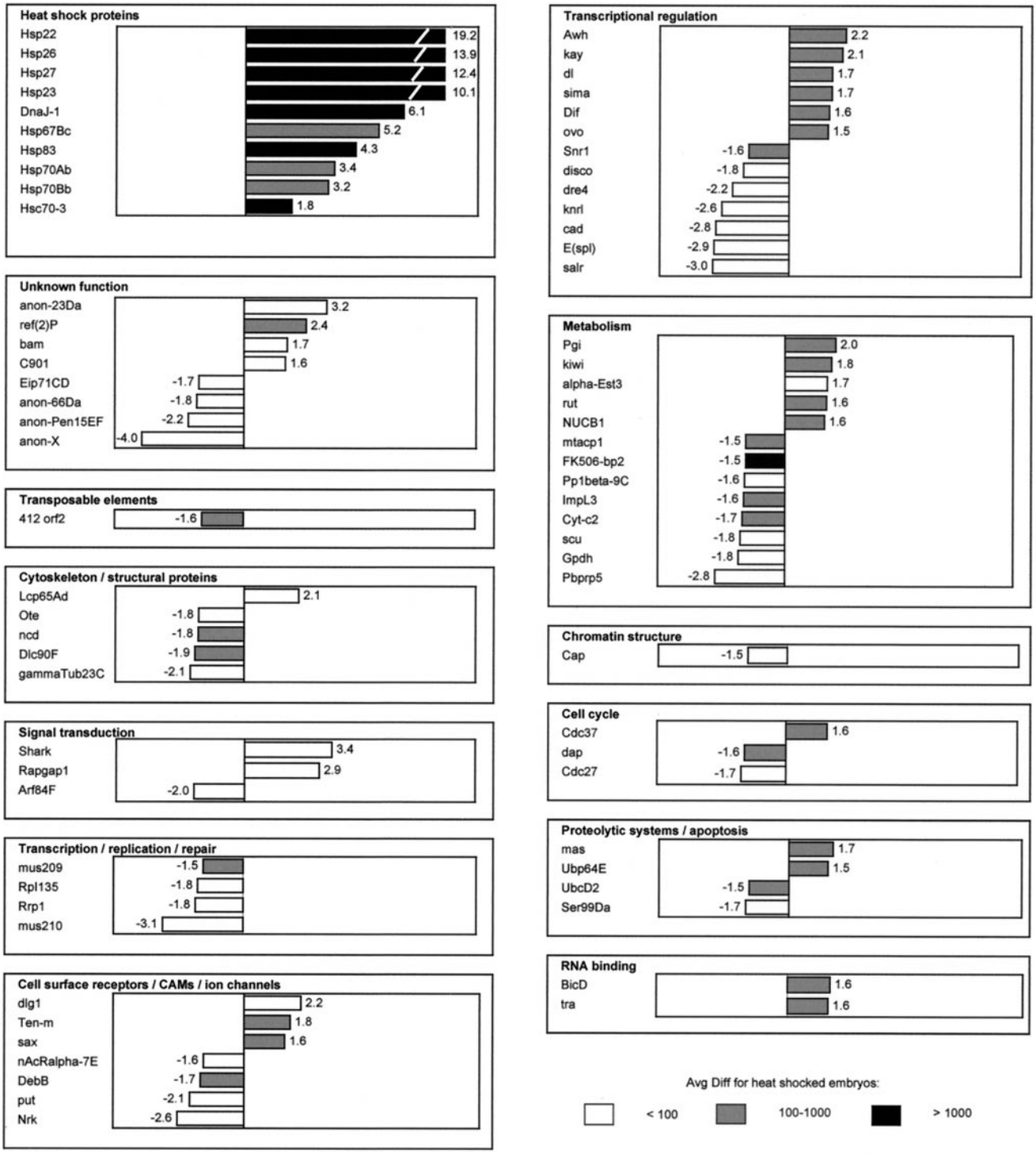

Fig. 3. Differentially expressed genes observed in heat-shocked vs. non-heat-shocked stage 10–17 wild-type embryos, grouped according to functional classes. Bars represent the fold change of differentially expressed genes in the heat-shock vs. standard condition. Positive values indicate that the relative expression level of a gene is increased after heat shock, and negative values indicate a decrease. Avg Diff values are given for the heat-shocked condition as follows: white bars represent Avg Diff < 100, gray bars represent Avg Diff ranging from 100 to 1,000, and black bars represent Avg Diff $> 1,000$.

Results and Discussion

Quantitative Transcript Imaging of Genes Expressed in Postgastrulation Embryogenesis Under Standard Conditions. The oligonucleotide array used contains probe sets that are complementary to 1,519 identified sequenced *Drosophila* genes. Most of these genes (96%) can be grouped into 14 functional categories according to the nature of the encoded protein. In a first set of experiments, we used this oligonucleotide array to identify transcripts expressed in wild-type embryos raised under standard conditions (25°C). Transcript imaging revealed a total of 563 (37%) of the 1,519 *Drosophila* genes as expressed in embryonic stages 10–17. To document the quantitative reproducibility of the relative

expression levels, Avg Diff (see *Materials and Methods*) and corresponding standard deviations for the detected transcripts were determined over four experimental replicates (Fig. 1).

Over two-thirds of the detected transcript types encode proteins involved in metabolism (19.8%), transcriptional regulation (13.1%), cell-surface receptors/cell adhesion molecules/ion channels (11.1%), translation (9.2%) cytoskeleton/cell structure (8.5%), or signal transduction (7.2%) (Table 1). Marked differences were observed in the range of relative expression levels for the different functional categories (Fig. 2). Highest expression levels were seen for specific genes encoding proteins involved in translation. Thus, of the 21 transcripts with Avg Diff >5,000, 18 encode ribosomal proteins. High expression levels with Avg Diff >4,000 are also seen for specific individual transcripts encoding proteins involved in chromatin structure and protein degradation. For example, the highest Avg Diff in the functional class protein degradation/apoptosis is the transcript encoding the Cystatin-like protein (Avg Diff 4,792). Some transcripts for proteins involved in signal transduction, DNA transcription/replication/repair, metabolism, as well as the transcript encoding the heat-shock cognate protein 70–4 have maximal Avg Diff in the 3,000–4,000 range. Surprisingly elevated expression levels are observed for transcripts encoded by specific transposable elements; in three cases, Avg Diff were above 2,000, namely for two open reading frames encoded by the transposon I element and for a putative reverse transcriptase encoded by an F element. Remarkably elevated expression levels are also seen for the transcription factor Box B-binding factor 1 (1,315); for other genes encoding transcription factors such as *snail* (Avg Diff 394), *glial cells missing* (237), *islet* (136), and *paired* (64) transcript levels were in the intermediate to low range (Avg Diff <550).

Quantitative Transcript Imaging of Heat-Shocked Compared with Non-Heat-Shocked Embryos. Oligonucleotide arrays were next used to determine transcript profile changes after heat-shock exposure. For this, transcript imaging was carried out on stage 10–17 embryos subjected to a 36°C heat shock for 25 min (see *Materials and Methods*). The expression profile from embryonically expressed genes after heat shock was quantitatively compared with the expression profile from embryos raised under standard conditions. Comparative transcript imaging identified 74 genes, distributed among 12 functional classes, whose relative expression level changed in response to heat shock; 36 genes had increased and 38 genes had decreased expression levels (Fig. 3).

Heat shock is known to induce the expression of an evolutionary conserved family of genes encoding the heat-shock proteins (Hsps) (10, 15, 16). Accordingly, in our comparative screen, we observed a prominent increase in relative transcript abundances for all genes encoding Hsps represented on the chip and that have been reported to be highly up-regulated by heat shock. Transcript imaging detected increases above 3-fold in relative expression levels for 9 genes encoding *Drosophila* heat-shock proteins: *Hsp22, Hsp26, Hsp27, Hsp23, DnaJ-1, Hsp67Bc, Hsp83, Hsp70Ab,* and *Hsp70Bb* (17, 18). The largest changes (>10-fold) were observed for *Hsp22*, *Hsp26*, *Hsp27*, and *Hsp23*, in accordance with several studies that report that these four small Hsps are expressed during normal fly development and are up-regulated under heat shock (19, 20). For five other genes known to encode heat-shock proteins, *DnaJ-1, Hsp67Bc, Hsp83, Hsp70Ab,* and *Hsp70Bb*, we detect an increase in expression in the 3- to 6-fold range. All of these genes are known to be responsive to heat shock (20). The heat-shock cognate genes (Hsc) have been reported to be expressed at normal temperatures but are not further induced by heat shock (21, 22). In accordance with this, we observed no marked change in expression level for *Hsc70–1, Hsc70–4*, and *Hsc70–5*. We did, however, detect a small increase in expression level for *Hsc70–3*.

Two other genes with increases in relative expression levels above 3-fold are *Shark,* involved in a signaling pathway for epithelial cell polarity (23), and *anon-23Da,* encoding a protein with currently unknown function. Twenty-five other genes show increased expression levels in the 1.5- to 3-fold range. Heat-shock-induced expression of these genes in *Drosophila* has not been reported before. However, *Cdc37* is known to interact genetically with *Hsp83* in a common signaling pathway in *Drosophila* (24), and in several other cases, homologous genes in other eukaryotes are known to be stress inducible. The gene *kayak* (*kay*), for example, is the *Drosophila* homologue of the mammalian *c-fos. c-fos* mRNA is induced after exposure to noxious stimuli such as heat, arsenite, and heavy metals, and recently it has been reported that the human and rodent *c-fos* promoters contain heat-shock element consensus sequences that enhance transcription in response to heat (25). A second example is *Tenascin major* (*Ten-m*), encoding a protein implicated in patterning the early fly embryo. The mammalian homologue of *Tenascin major* is the gene *DOC4*, known to act *d*ownstream *o*f *C*HOP, a small nuclear protein that mediates changes in cell phenotype in response to stress (26).

Heat-shock-induced decreases in relative expression levels greater than 3-fold are seen for *mus210*, the *Drosophila* homologue of the xeroderma pigmentosum complementation group C gene, which is involved in DNA repair, and for *anon-X,* which encodes a novel WD repeat protein of unknown function (27, 28). The remaining 36 genes with decreased relative expression levels are in the 1.5- to 3-fold range. A decrease in relative expression in response to heat shock has not been reported previously for any of these genes in *Drosophila*.

For most of the 74 identified genes, which show differential expression levels in response to heat shock, changes are in the 1.5- to 3-fold range. It was not possible to unambiguously reveal these small quantitative changes by using qualitative detection techniques such as *in situ* hybridization. Changes in gene expression that are in higher ranges can, however, be detected with *in situ* hybridization. To document this, whole-mount *in situ* hybridization was carried out for transcripts of *Hsp22* (19-fold

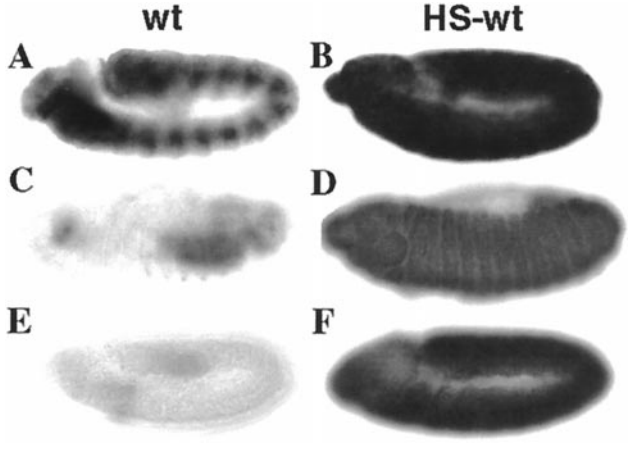

Fig. 4. Comparison of whole-mount *in situ* hybridizations between non-heat-shocked and heat-shocked wild-type embryos. (*A–F*) Lateral views, anterior to the left. (*A, C,* and *E*) Non-heat-shocked wild type. (*B, D,* and *F*) Heat-shocked wild-type embryos. (*A* and *B*) At stage 11, *Hsp22* expression is confined to metameric ectodermal patches in non-heat-shocked wild-type embryos (*A*), whereas *Hsp22* is ubiquitously expressed in the ectoderm of heat-shocked wild-type embryos (*B*). (*C* and *D*) At stage 12, there is no expression of *Hsp26* in the ectoderm of non-heat-shocked wild-type embryos (*C*, gut staining out of focal plane), whereas *Hsp26* is expressed in all ectodermal cells of heat-shocked wild-type embryos (*D*). (*E* and *F*) At stage 11, *DnaJ-1* is not detected in non-heat-shocked wild-type embryos (*E*), whereas heat-shocked wild-type embryos show strong expression in all ectodermal cells (*F*).

Table 2. Comparison of change folds between oligonucleotide arrays and RT-PCR

	Avg diff (array)		Change fold	
Gene	wt	HS-wt	Array	RT-PCR
Hsp27	347	4,646	12.4	20.0
Hsp67Bc	183	944	5.2	8.0
anon-23Da	6	64	3.2	2.6
kay	74	153	2.1	1.4
Ten-m	92	162	1.8	2.1
kiwi	108	199	1.8	4.0
Cdc37	179	286	1.6	3.4
Rac2	424	425	1.0	1.1
FK506-bp2	1,918	1,248	−1.5	−2.0

RT-PCR was performed on cDNA derived from heat-shocked embryos and embryos raised under standard conditions. Change folds determined by RT-PCR are represented as the mean value of eight independent replicates, derived from two different cDNA preparations. wt, wild type; HS, heat shock.

increase*)*, *Hsp26* (14-fold increase), and *DnaJ-1* (6-fold increase) (Fig. 4). In all three cases, *in situ* hybridization revealed clear increases in hybridization signal after heat shock.

To verify the differential expression levels in response to heat shock and also to confirm differential expression values in the 1.5- to 3-fold range, semiquantitative RT-PCR was performed on selected genes. Changes in expression levels were determined for eight genes that showed differences in expression level after heat shock, namely *Hsp67Bc*, *Hsp27*, *anon-23Da*, *kay*, *Ten-m*, *Cdc37*, *kiwi*, and *FK506-bp2* and also, as a control, for the gene *Rac2*, which is not heat shock regulated. These experiments show that the changes in relative expression level as measured by RT-PCR are comparable to the data obtained with oligonucleotide arrays (Table 2).

Taken together, these results demonstrate that oligonucleotide arrays have the potential to analyze the relative expression levels of hundreds of known genes in a complex RNA sample of the multicellular *Drosophila* embryo. In addition, they allow a quantitative assessment of differential gene expression under normal vs. heat-shock conditions. Thus, the oligonucleotide probe arrays used in our study establish highly reproducible transcript images of *Drosophila* embryos and allow accurate comparisons of changes in gene expression under different environmental conditions. In this respect, they complement the DNA microarray technique that has recently been used to study gene expression during metamorphosis in *Drosophila* (29). With the completion of whole genome sequence data for *Drosophila* (2), it will now be possible to expand quantitative transcript imaging to include all functional genes and set the stage for a complete genomic analysis of expression profiles in normal and environmentally or genetically manipulated *Drosophila* embryos.

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