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The Number of the Genes in a Functional Category Matters During Rat Liver Regeneration After Partial Hepatectomy

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ABSTRACT

Rat liver regeneration after partial hepatectomy (PH) is a good model to study the regulation of cell proliferation. We isolated hepatocytes from regenerating liver at different time points after PH and used microarray Rat Genome 230 2.0 chip to analyze the functional profiles of all up- or down-regulated genes manually and with automatic gene ontological tools. We found that the transcript expressions of PH and sham operation group were apparently different. For PH group, in the priming phase (2–12 h), signaling, transcription, response to stimulus genes predominated in up-regulated genes; in the proliferation phase (24–72 h), cell proliferation genes predominated; in the termination phase (120–168 h), differentiation and translation genes predominated; while metabolism genes predominated in the down-regulated genes at all time points (2–168 h). These functional profiles are consistent with the cellular and molecular phenomenon observed during liver regeneration, and can be closely connected with the biological process. Moreover, the results indicated that not only the quantity of specific genes but also the number of the genes in the specific functional category was regulated during liver regeneration, which means the number of similar genes in a specific functional category matters as well as the regulation of the genes. The changes of the number of the regulated cell proliferation genes and metabolism genes during liver regeneration were similar to the expression patterns of some cell division genes and metabolism genes. J. Cell. Biochem. 112: 3194–3205, 2011.

KEY WORDS: LIVER REGENERATION; MICROARRAY; PARTIAL HEPATECTOMY; GENE ENRICHMENT; GENE FUNCTIONAL PROFILE

The liver of mammal has prodigious ability to regenerate after injury. After 2/3 partial hepatectomy (PH), the remaining liver of rat proliferates to restore the mass of organ within 7–10 days. Rat liver regeneration after PH is a very good model to study cell proliferation and regulation. This phenomenon has attracted the attentions of researchers for decades, and the ideas pertaining to the mechanisms of liver regeneration have been evolving. At the beginning, it was thought that a single humoral agent could function as a key, capable of unlocking all of the events required for liver regeneration. Then, it was speculated that the activation of one pathway involving multiple components could be responsible for regeneration. The recent idea is that the activity of multiple pathways is required for liver regeneration [Fausto et al., 2006].

Microarray is a powerful tool to study genome wide expression pattern of tens of thousands of genes simultaneously, wherefore the expression changes of a large number of genes activated or suppressed under various biological conditions can be monitored. Microarray has been used to study rat liver regeneration after PH [Fukuhara et al., 2003; Otu et al., 2007; Guo and Xu, 2008; Li et al., 2009; Wang et al., 2009; Xu et al., 2009]. The regulated genes were clustered based on the expression patterns; the functional profiles of clustered genes and the functions of some genes were analyzed. However, the functional profiles of all up- or down-regulated genes have not yet been studied and connected with the cellular mechanisms of liver regeneration. Moreover, all those experiments were conducted using intact liver, whereas the liver is composed of various differentiated cell types, which have different expression profiles as they play different roles during liver regeneration.

On the other hand, liver regeneration is a complex process involving thousands of genes up- or down-regulated. The challenge faced by the researchers is to translate such lists of differentially regulated genes into a better understanding of the biological phenomena. A first step in this direction can be the translation of the list of the genes into a functional profile able to offer insight into the cellular mechanisms relevant in the given condition [Khatri and Draghici, 2005]. Many automatic ontological analysis tools using

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Gene Ontology (GO) have been developed since 2002 for this purpose [Khatri and Draghici, 2005; Huang et al., 2009]. However, the usefulness of these tools to solve real biological problems has not been evaluated.

We isolated hepatocytes from regenerating liver at different time point after PH, to check cell-specific expression profiles using microarray. We analyzed the functional profiles of the newly expressed or closed genes manually and compared the results with that of many automatic gene ontological analysis tools. Then, we used the chosen best tool to analyze the functional profiles of all up- or down-regulated genes in the regenerating hepatocytes.

MATERIALS AND METHODS

RAT 2/3 PARTIAL HEPATECTOMY

Healthy 12-week-old Sprague-Dawley (SD) rats, 230 ± 20 g, were obtained from the Experimental Animal Center of Henan Normal University. The animal experiments were conducted in strict compliance with animal welfare regulations approved by Institutional Animal Care and Use Committee of Henan Normal University in China. The rats were bred in $21 \pm 2^{\circ}$C, relative humidity $60 \pm 10\%$, illumination time 12 h/d (8:00–20:00), and free access to water and food. In the experiment, a total of 114 rats were randomly divided into 19 groups, and 6 rats in each. Among these rats, 9 groups, total 54 rats for 2/3 PH, 9 groups, total 54 rats for sham operation (SO), and 1 group, total 6 rats for the control. PH was performed on the rats according to the procedure originally described by Higgins et al. [Higgins and Anderson, 1931]. Briefly, the upper abdomen was opened along the abdomen midline from xiphisternum down to 3 cm, and the left and median lateral liver lobes (about 68% of the whole liver weight) were surgically removed. Finally, incision was sutured and sulfanilamide was sprinkled on it. For SO, surgical operation of rats was done as did for the PH, but no liver lobes dissection. After that, the rats were bred in above-mentioned conditions, and their regenerating livers were taken for isolating liver cells, respectively, at 0, 2, 6, 12, 24, 30, 36, 72, 120, and 168 h after PH. Zero hour means that when the liver lobes were removed, the remaining liver was immediately used for cell isolation.

ISOLATION OF HEPATOCYTES

At different time points, the recovering rats were recruited. The rats were anesthetized by aether and sterilized with 75% alcohol, and the upper abdomen of the rat was opened to expose liver. The liver was turned over to expose the hepatic portal vein. After a tube was inserted into the hepatic portal vein, D-Hank's solution at 37°C was perfused into the liver at the rate of 10–20 ml/min [Selgen, 1976]. When the liver surface turned slight yellow, 30 ml 0.05% collagenase IV replaced D-Hank's solution to perfuse continuously into the liver at the rate of 1–2 ml/min. The perfused liver was removed and incubated in a flask with 0.05% collagenase IV at 37°C for 15 min. Then 4°C PBS was added and the liver was combed with glass needle to disperse cells. The dispersed cells were collected and filtered through 400-mesh nylon net. The cells were suspended in cold PBS buffer and centrifugated at 200 g 1 min for three times to wash off blood cells and non-parenchymal cells. The pellet was harvested and resuspended in cold PBS. Cell concentration was adjusted to 1×10^8 cells/ml for use. Ninety-five percent of the cells were live hepatocytes.

RNA EXTRACTION AND MICROARRAY ANALYSIS

1×10^6 isolated cells were taken, and their total RNA was extracted according to the manual of Trizol reagent (Invitrogen Corporation, Carlsbad, CA) [Norton, 1992], and purified following the RNeasy mini protocol (Qiagen, Inc, Valencia, CA) [Scott, 1995]. The quality of total RNA was assessed by optical density measurement at 260/280 nm and agarose electrophoresis (180 V, 0.5 h). It was regarded as qualified sample when 28S RNA to 18S RNA is equal to 2:1. T7-oligo dT(24) (W. M. Keck Foundation, New Haven, CT) SuperScript II RT (Invitrogen Corporation, Carlsbad, CA) and 5 μg of total RNA was used to synthesize the first strand of cDNA. The second strand synthesis was performed using the Affymetrix cDNA single-stranded cDNA synthesis kit. The cDNA product was purified following the cDNA purify protocol. The 12 μl purified cDNA and the reagents from the GeneChip In Vitro Transcript Labeling Kit (ENZO Biochemical, New York, NY) were used to synthesize biotin-labeled cRNA. The labeled cRNA was purified using the RNeasy Mini Kit columns (Qiagen, Valencia, CA). Their concentration, purity and quality were assessed as above "RNA extraction". Fifteen microliter cRNA (1 μg/μl) was incubated with 6 μl 5× fragmentation buffer and 9 μl RNase free water for 35 min at 94°C, and digested into 35–200 bp cRNA fragments. The prehybridized Rat Genome 230 2.0 Array was put into a hybridization buffer, which was prepared following the Affymetrix protocol [Affymetrix: Expression Analysis Technical Manual], and hybridized in a rotating chamber (60 rpm, 16 h, 45°C). The hybridized arrays were washed by wash buffer to remove the hybridization buffer, and stained in GeneChip fluidics station 450 (Affymetrix Inc., Santa Clara, CA). Then, the arrays were scanned and imaged with a GeneChip scanner 3000 (Affymetrix Inc, Santa Clara, CA) [Guo et al., 2008].

DATA ANALYSIS AND NORMALIZATION

The images were converted into normalized signal values, signal detection *p*, and experiment/control change *P*-values using GCOS 1.4 software (Affymetrix) [Affymetrix: Data Analysis Fundamentals]. The data of each array were initially normalized through scaling all signals to a target intensity of 200. Gene expression was analyzed using the defaults parameter settings. When *detection P*-value was <0.05, it meant the gene was present (*P*); when $P > 0.05$, it meant the gene was marked absent (A). On the other hand, the ratio of normalized signal values of PH group to that of control were used to calculate the fold change, but the regulation of gene expression was determined by *change P*-value. When *change P*-value was <0.002, it meant the gene expression was increased compared with control; when $0.002 < P < 0.998$, gene expression had no change; when $P > 0.998$, gene expression was decreased. To minimize errors from microarray analysis, each sample was analyzed at least three times using Rat Genome 230 2.0 Array. The averages of *P*-values were used. If the average of three *detection* $P < 0.05$, it means the gene is expressed. Otherwise, the gene is not expressed. For the *change P*-value, the average of 9 *change P*-values (three experimental data versus three control data) was calculated.

When the average *change* $P < 0.002$, it means the gene expression has increased; when the average *change* $P > 0.998$, it means the gene expression has decreased.

The processed microarray data are available online as Supporting Information files. The data file is an Excel file. All transcripts expressed in hepatocytes were marked pink with the addition of *detection* *P*-value lower than 0.15 (average *detection* $P < 0.05$). All transcripts up regulated at different time points were marked yellow with the addition of *change* *P*-value lower than 0.018 (average *change* $P < 0.002$) in PH and SO group. All transcripts down regulated were marked blue with the addition of *change* *P*-value higher than 8.982 (average *change* $P > 0.998$).

FUNCTIONAL PROFILING

The gene lists of up- and down-regulated genes or newly opened and closed genes at different time points after PH were obtained through above data analysis and used to conduct functional profiling.

Basically, GO data from Affymetrix annotation file was used to determine the function of a gene manually. If we were not sure about the functions, GeneCards database (http://www.genecards.org/) was also used. The functions of regulated genes were classified into several categories: cell growth (cell proliferation, cell division, cell cycle, mitosis, DNA replication, cell development, cell migration, etc.), cell differentiation (cell apoptosis, cell differentiation, cell adhesion, cell junction, etc.), metabolism, transport, signaling (cell surface receptor linked signal transduction, intercellular signaling cascade, signaling molecules, etc.), transcription (transcription factors, regulation of transcription), cell components (extracellular matrix, membrane, cytoplasm, nucleus, etc.), protein modification (phosphorylation, glycosylation, etc.), immune response (inflammatory response, acute-phase response, antigen representation), response to wounding, and others (angiogenesis, gamete generation, calcium ion binding, etc.). A gene may have many functions and sometimes the specific function of a gene in liver regeneration is hard to determine. We had to determine the function of a gene based on our knowledge about liver regeneration. The classification of functions was exclusive. If we were completely unsure about the specific function of a gene, we assigned a function randomly from the GO list. This might cause bias. Because of the complexity of gene functions, bias is inevitable. Careful analysis of those functions could reduce the bias to minimum.

For automatic gene ontological analysis, the enriched gene categories with similar functions were also classified together.

REAL-TIME POLYMERASE CHAIN REACTION (RT-PCR)

The mRNA sequences for selected housekeeping genes *b2m*, *gapdh* *ubc*, hepatic specific genes *g6pc*, *jun*, *myc*, *trim24*, and *β-actin* were retrieved and the primers were designed for each transcript by primer express 2.0 software and synthesized in Beijing Sunbiotech Co. Ltd. RT-PCR was performed with total RNA using SYBR Green on a 214 Rotor-Gene 300 (Corbett Robotics). Threshold cycle numbers for each gene were normalized to that of *β-actin* as described earlier [Wang and Xu, 2010]. All RT-PCR experiments were done in triplicate. After Ct values were generated, relative quantity of the target genes were calculated according to the standard curves. In the general controls, water was used to replace template (NTC). In the no reverse transcription control, reverse transcription enzyme (AMV) was omitted in the RT steps.

RESULTS

Healthy adult rats were performed 2/3 PH, and then their hepatocytes were isolated with two-step perfusion method at different time points (0–168 h) after PH to conduct RNA extraction, cRNA synthesis, and microarray analysis using Rat Genome 230 2.0 Array. The data were normalized and analyzed using GCOS 1.4 software (Affymetrix) to get the expression pattern of 31,099 transcripts, including 25,020 rat genes (11,789 known).

There were 15,152 transcripts (48.7% of all 31,099 transcripts) expressed in normal rat hepatocytes (average detection $P < 0.05$), which included 8,470 known genes (71.8% of all known genes), while the number of unexpressed transcripts was 15,947(51.3%), which included 7,119 known genes (60.3%). So almost half transcripts were expressed in normal hepatocytes and there was an overlap between the number of expressed genes (8,470, 71.8%) and unexpressed genes (7,119, 60.3%), i.e., there were 3,800 known genes, of which some transcripts were expressed while some were not in normal hepatocytes. Because there exists alternative splicing, different transcripts of the same gene may have different functions. In this paper, when the transcript expression patterns of one gene were different, we did not average the signal values of these transcripts but treated each transcript individually.

THE TRANSCRIPT EXPRESSION PROFILES AFTER PH

The transcript expression profiles of PH group and SO group were different and could be easily distinguished by dot plot of signal values against the control (Fig. 1a). The yellow dots are the signal values of one sample of the control, which align with the diagonal very well. This indicated the three samples of the control group were consistent and repeated well. If the scanning results of the three samples were not very consistent, the dots would deviate the diagonal. The pink dots are from SO group. Because SO was not supposed to cause much changes in the transcript expression pattern of hepatocyes, the dots scattered around the diagonal. The blue dots are from PH group, which caused huge changes in the transcript expression profiles. The dot plot of expression profile is better than any other methods to manifest the reliability of the microarray data, because if the data were not reliable, the dots from SO group would be indistinguishable with the dots from PH group. Any errors could be found in this way.

We used the *P*-values provided by Affymetrix software rather than the fold change in signal intensity to identify the up- or down-regulation of gene expression. This method is theoretically better than fold change according to Affmetrix's manual because the signal intensity is meaningless when the *P*-values are not right. Usually twofold change is used to identify regulated gene expression, but the fold change of 2 is quite arbitrary. The statistic methods used to calculate the *P*-values are not described in the manual, but the *P*-value method seems to be more accurate. More transcripts were identified as regulated by *P*-value method than by

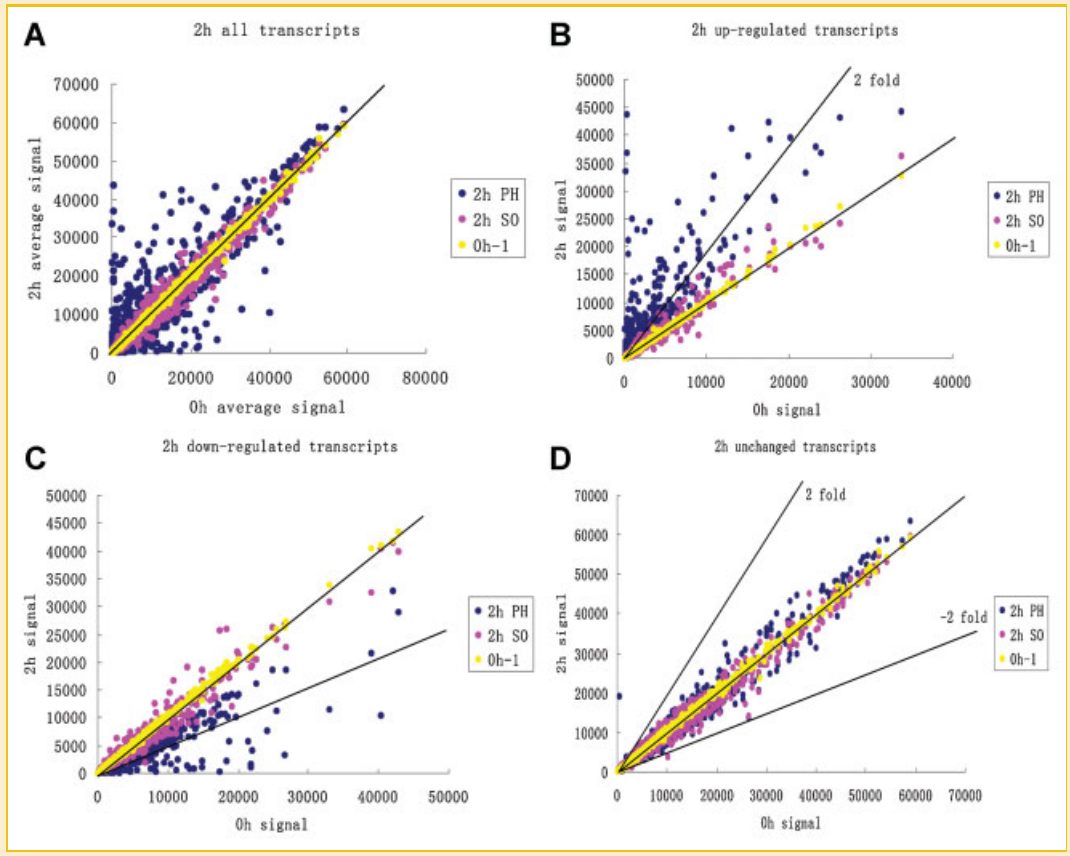

Fig. 1. A: Comparison of transcript expression profiles of PH and SO group at 2 h after PH: scatter plots of the average signal value against that of the control group (0 h). Yellow dots are from one sample of the control group (0 h). Pink dots are from SO group. Blue dots are from PH group. B, C, D: comparison of *P*-value and fold change methods. B: The transcripts up-regulated at 2 h (*change* $P > 0.998$). Some blue dots are within twofold change. C: The transcripts down-regulated at 2 h (*change* $P < 0.002$). Some blue dots are within −2-fold change. D: The transcripts unchanged at 2 h (*change* $0.002 < P < 0.998$). Almost all blue dots are within twofold change. The results indicated *change P*-value method was more sensitive than twofold change method to identify gene expression change.

fold change method (Fig. 1), and most dots of unchanged transcripts by *P*-value were within the range of twofold change.

The comparison of expression patterns of microarray and RT-PCR for housekeeping genes *b2m*, *gapdh ubc*, and hepatic specific genes *g6pc*, *jun*, *myc*, *trim24* are shown in Figure 2. Although the expression patterns of some genes detected by RT-PCR were a little different from the microarray data, the expression trends of these genes from both techniques were generally similar.

GENE FUNCTIONAL PROFILE

The numbers of up- or down-regulated transcripts increased after PH and reached the first peak at 12 h and the second peak at 72 h (Fig. 3), and then decreased dramatically. The numbers of regulated genes in SO group were very few (data not shown). Because there were thousands of genes regulated, we first focused on functional profiling of newly expressed genes and closed genes manually. These genes were especially important in transforming the normal

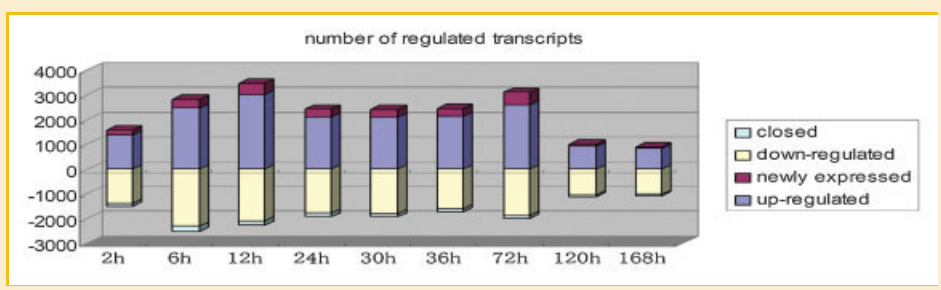

Fig. 2. The number of regulated transcripts during liver regeneration. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

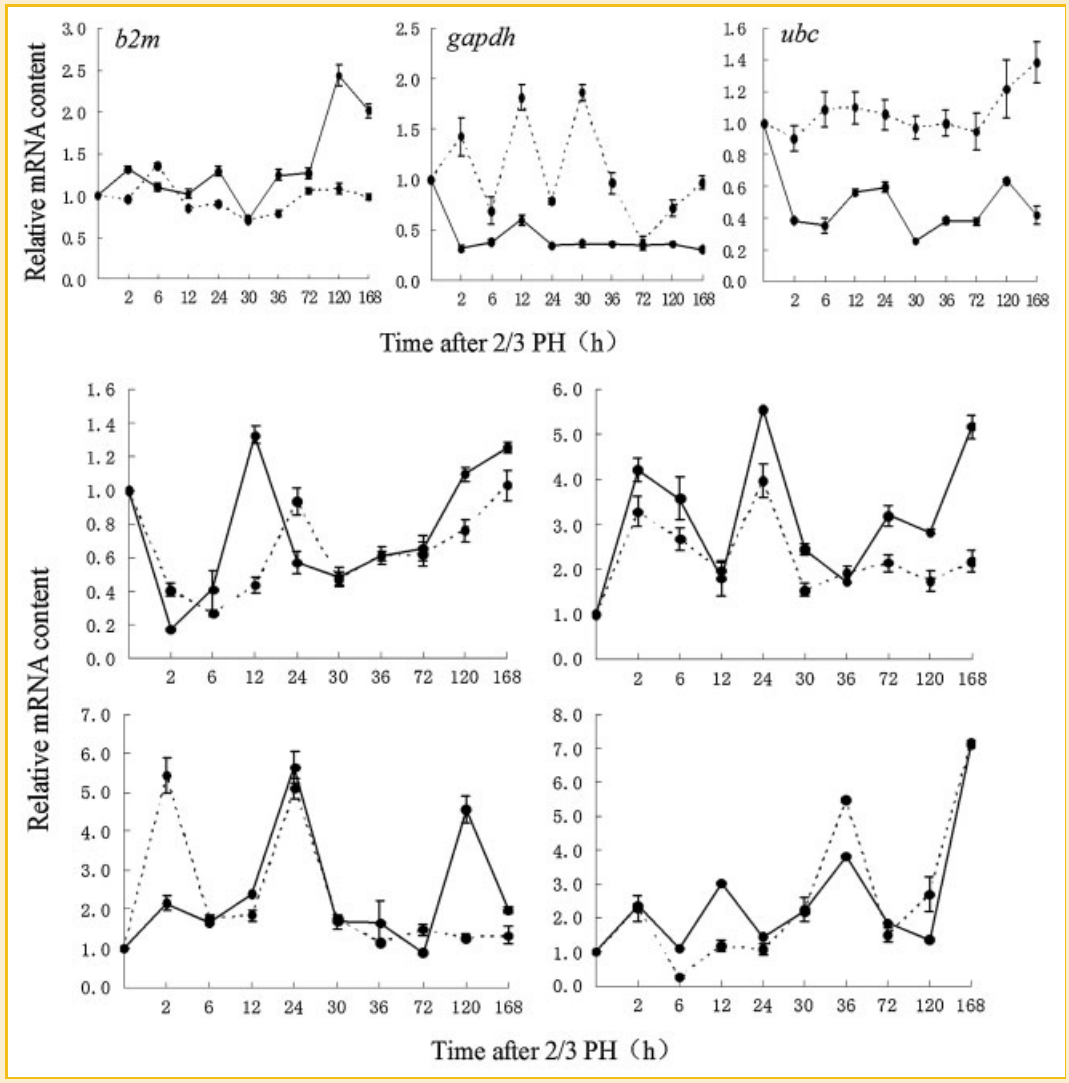

Fig. 3. The comparisons of micaroarray and RT-PCR: the real lines denote the results of RT-PCR; the virtual lines denote the results of micrarray.

liver to regenerating liver and the numbers were relatively small. The newly expressed genes were those not expressed in normal liver (*detection* $P > 0.05$) but up regulated (*change* $P < 0.002$) and significantly expressed in regenerating liver (*detection* $P < 0.05$) and not expressed in SO group. The closed genes were those expressed in normal liver but down regulated (*change* $P > 0.998$) and not expressed in regenerating liver and not closed in SO group. The numbers of these newly expressed or closed transcripts are shown in Table I. The numbers of newly expressed or closed transcripts were roughly proportional to the numbers of up- or down-regulated transcripts. About half of the transcripts were from known genes.

We did functional profiling manually using GO terms and only analyzed the terms in "biological process" because biological process is the most important and meaningful. We also tried many web-based automatic gene enrichment tools, including DAVID [Huang et al., 2007], GOEAST, ProfCom [Antonov et al., 2008], eGOn, FuncAssociate, G-SESAME, GFINDer, Genecodis [Nogales-Cadenas et al., 2009], GOrilla, GOstat, GO Term Finder, GOTM, EASE[Hosack et al., 2003], etc, and the latest published tool GARNeT [Rho et al., 2011]. Some tools gave irrelevant meaningless results, difficult to be connected with the cellular or molecular process of liver regeneration. Some tools were not user friendly or didn't work at all (Onto-express). Some were very slow. GARNeT worked well with KEGG, but worked poorly with GO. The old EASE was good [Hosack et al., 2003; Otu et al., 2007], but updated EASE did not work so well. Finally, we found ProfCom best. There are three classes of gene enrichment tool [Xu et al., 2009]: singular enrichment analysis (SEA), gene set enrichment analysis (GSEA), and modular enrichment analysis (MEA). ProfCom belongs to MEA, which considers the inter-relationship of GO terms in enrichment calculation.

We conducted gene functional profiling based on the up- or down-regulated gene lists at each time point rather than on clustering genes according to expression pattern change, because we did not want to trace how gene expressions changed during liver regeneration. We did not cluster genes at all. What we wanted to know was, at each time point after PH, what kinds of genes were

TABLE I. The Number of Transcripts Up- or Down-Regulated during Liver Regeneration

	2 h	6 h	12 h	24 h	30 h	36 h	72 h	120 h	168 h
Up-regulated genes	1,397	2,477	3,020	2,118	2,086	2,138	2,598	917	845
Newly expressed (known)	177(91)	351(197)	460(189)	319(231)	331(221)	307(200)	509(366)	87(64)	61(32)
Down-regulated genes	−1,378	−2,303	−2,105	−1,777	−1,793	−1,607	−1,867	−1,043	−1,020
Closed (known genes)	−110(54)	−202(107)	−154(101)	−154(56)	−137(77)	−103(44)	−123(35)	−71(16)	−55(22)

regulated, or what were the functions of those genes regulated during liver regeneration?

It is generally accepted that the liver regeneration process after the loss of functional mass consists of three fundamental phases: (a) initiation or priming phase (0–12 h after PH), (b) proliferation phase (12–72 h), (c) termination phase (72–168 h) [Rychtrmoc et al., 2009; Fausto, 2000]. In these three phases, different functional profiles were identified (Fig. 4).

From 2 to 12 h after PH, signaling genes predominated in the newly expressed genes. At this stage, the number of cell growth genes was equal to the number of cell differentiation genes. This is consistent with that the signaling process involving various hormones, cytokines, and growth factors initiates liver regeneration. The profiles of manual and ProfCom were similar but less numbers of genes were included in ProfCom.

From 24 to 72 h after PH, the number of cell growth and cell division genes accounted for the majority. This is consistent with that regenerating liver reaches the peak of mitosis at the time from 24 to 36 h post-PH. Both manual and ProfCom showed the highest level of cell growth genes.

From 72 to 168 h after PH, signaling genes predominated again and the number of cell differentiation genes increased. The number of cell components genes, including extracellular matrix and collagen, also increased. It has been reported that, in vivo, the extracellular matrix is of critical importance both in maintaining growth arrest in the adult liver and regulating liver regeneration [Steer, 1995]. Complex preparations of extracellular matrix inhibited cell proliferation and enhance differentiation of hepatocytes in culture [Michalopoulos, 2007]. Our results were in favor that the reassembly of the extracellular matrix facilitates termination of the regenerative process. From 120 h, the number of newly expressed genes dropped dramatically. ProfCom failed to give meaningful results at this time point probably because this kind of tools works better with larger data set.

The signaling genes newly expressed in priming phase and termination phase were different. Eighty-nine percent of the signaling genes at 168 h were the same as 120 h; 56% of signaling genes at 120 h were the same as 72 h; about half of the signaling genes were the same within priming phase (2–12 h) and proliferation phase (24–36 h); but the similarity between 36 and 72 h was only 35%, between 12 and 24 h was 25%, between 12 and 72 h was 30%. These prompt that different signaling genes in different phases responded to different signals.

The functional profiles of the closed genes during liver regeneration are shown in Figure 5. The genes that predominated in all phases were metabolism genes and cell differentiation genes. Among the metabolism genes, most were protein metabolism, lipid metabolism, organic acid metabolism, and carbonhydrate metabolism, but no nucleic acids metabolism. There were also signaling genes closed, which had no similarity with the newly expressed genes. This indicated that different signaling pathways were switched off and on. The profiles of manual and ProfCom were basically similar.

After the comparison of the gene functional profiles of manual and ProfCom, we applied ProfCom to profile all up- and down-regulated genes during liver regeneration because this was a heavy task for manual work to analyze thousands of genes. We could see clearly how hepatocytes reacted at transcriptional level during liver regeneration (Figs. 6, 7).

At 2 h after PH, the genes of response to various stimuli, transcription, and signaling were up regulated. At 6 h, the number of cell growth began to increase. At 12 h, cell growth genes began to predominate the up-regulated genes. From 24 to 36 h, cell division and transport genes predominated absolutely. From 72 to 168 h, genes of response to stimulus predominate again, and the number of translation genes also increased (Fig. 6).

For the down-regulated genes, most were of metabolic process and response to stimulus (Fig. 7). This is consistent with the functional profiles of closed genes. The reason why genes of response to stimulus predominated in termination phase in both up- and down-regulated genes is unknown. In fact, the functional category ''response to stimulus'' is not strictly a function, and should be clarified by how to response to stimulus in detail at molecular level.

THE RELATIONSHIP OF GENE EXPRESSION PATTERN AND THE NUMBER OF THE GENES IN THE FUNCTIONAL CATEGORY

During liver regeneration, not only the expression of genes but also the number of the regulated genes in the functional category was regulated. For example, the number of regulated cell proliferation genes changed during liver regeneration (Fig. 8), and the change was coincidently consistent with the expression pattern of some cell division genes, such as *Cdc2a* and *Cdc45l*. This indicated that not only the regulation of some genes but also the number of this kind of genes participating in the process, such as cell proliferation, was important. For a complex biological process, the regulation of one or a few genes was not enough; more genes needed to be involved in the process. And the expression patterns of some key genes were similar with the quantity change of the genes involved. The same situation applies to metabolism genes. The temporal functional profile of metabolism genes was similar to the expression patterns of the metabolism genes *Ephx2* and *Ddhd1* (Fig. 8).

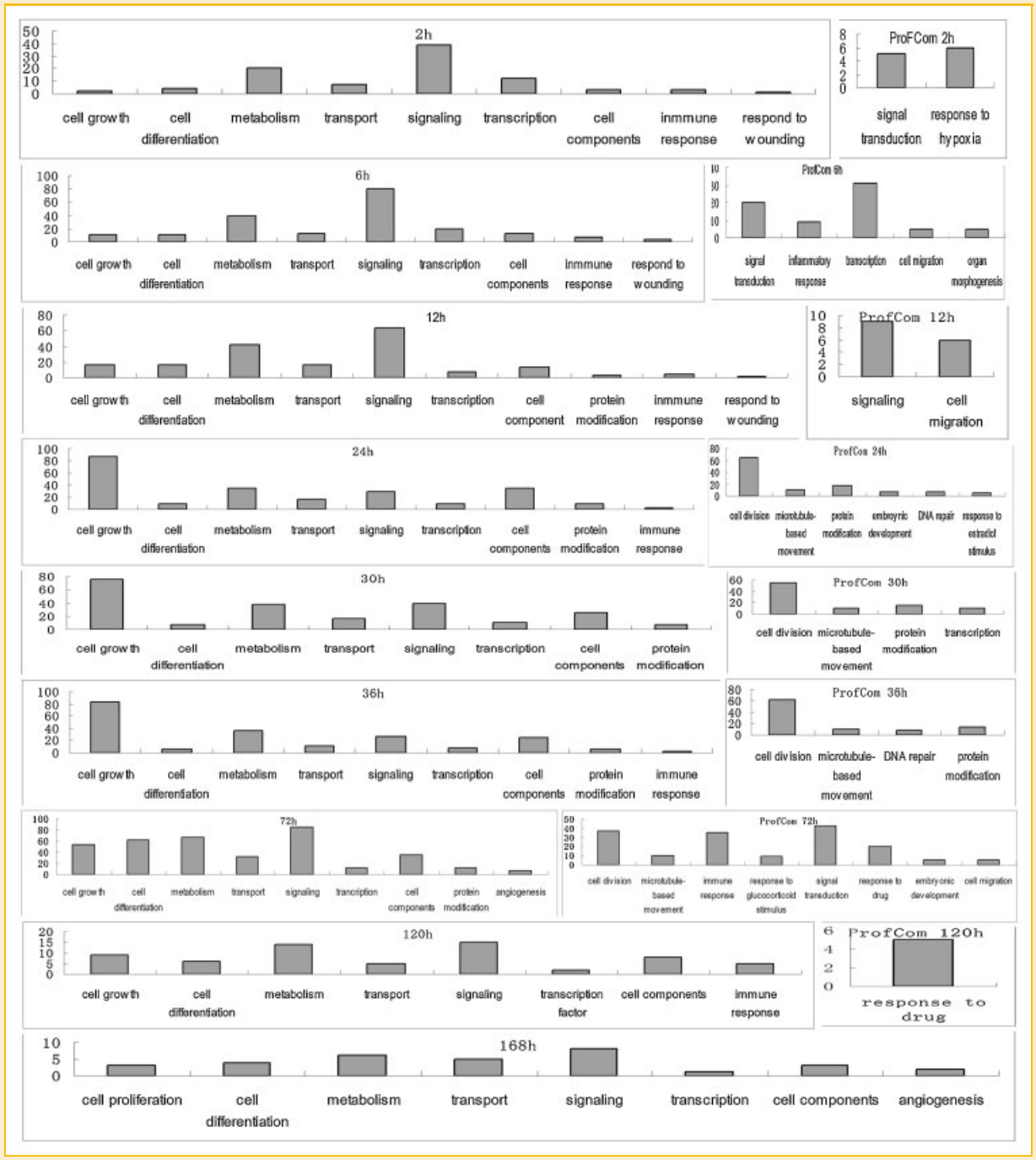

Fig. 4. The functional profiles of the newly expressed genes: the left is the numbers of genes manually classified; the right is numbers of genes enriched by ProCom.

DISCUSSION

The results demonstrated that the functional profiles of regulated genes, especially the genes that predominated in the profiles, could be very closely related with the biological process. In priming phase, signaling, transcription [Juskeviciute and Vadigepalli Hoek, 2008], response to stimulus genes predominated in up-regulated genes; in the proliferation phase, cell proliferation genes predominated; in the termination phase, differentiation and translation genes predominated; while metabolism genes predominated in the down-regulated genes at all time points. The predominant functional categories and the numbers of genes inside can be used as an index of a biological process.

It is not only the specific functions of a gene and the quantity of the gene expression but also the number of that kind of genes regulated in the functional category that play an important role in a biological process. A biological process is not completed by a few

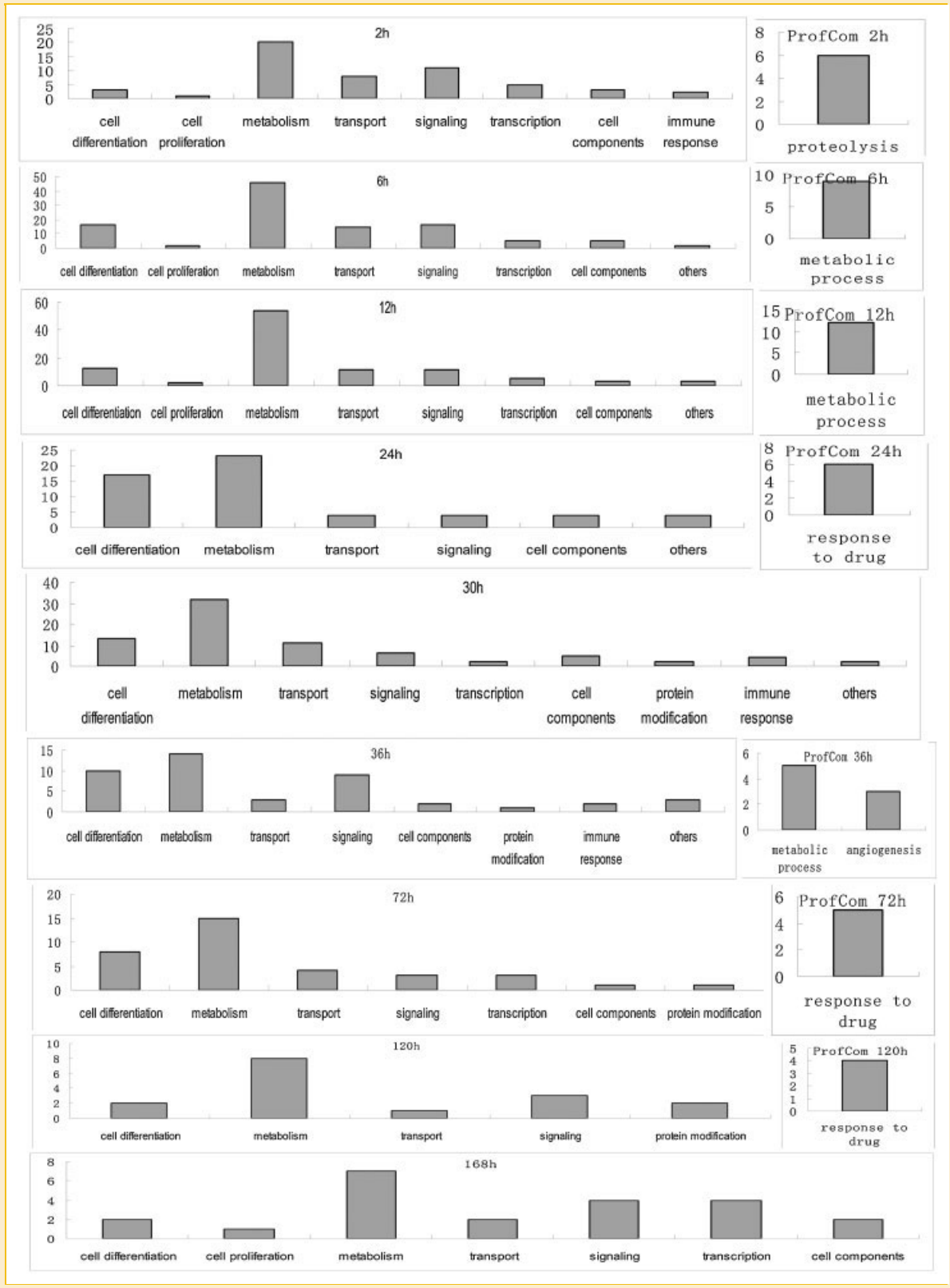

Fig. 5. The functional profiles of the closed genes: the left is the numbers of genes manually classified; the right is numbers of genes enriched by ProCom.

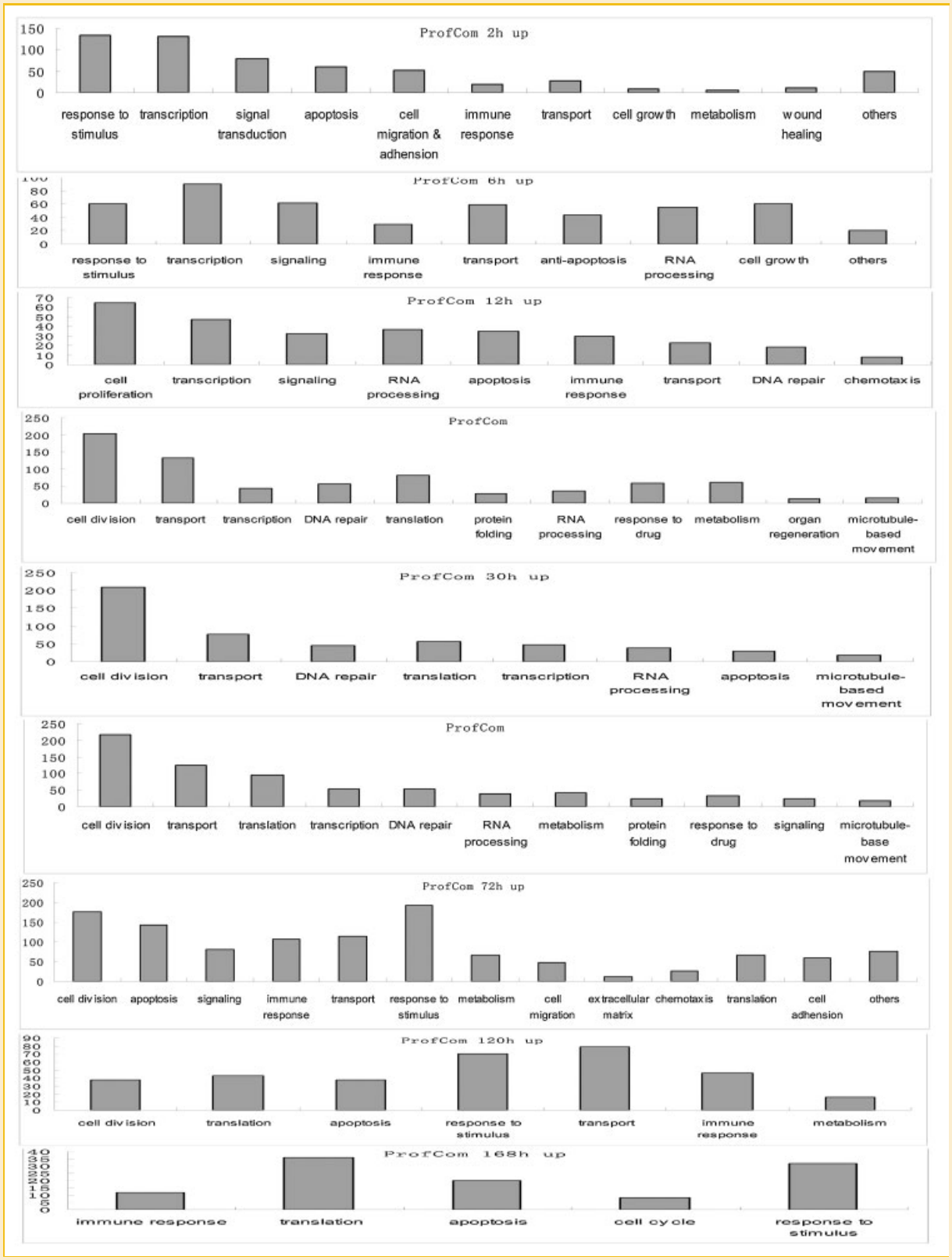

Fig. 6. The functional profiles of all up-regulated genes enriched by ProCom. The Y-axis is the number of genes enriched in a functional category.

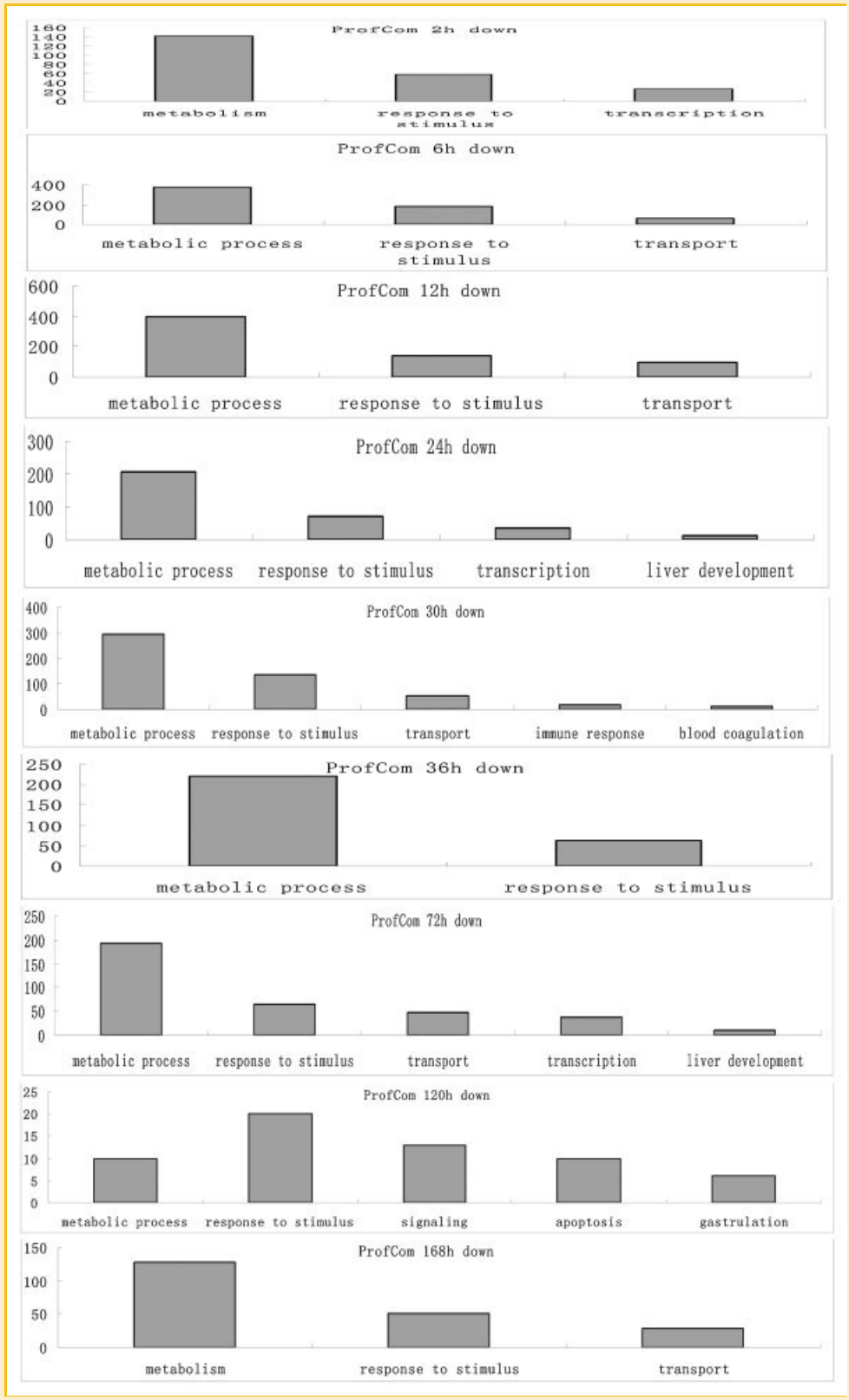

Fig. 7. The functional profiles of all down-regulated genes enriched by ProCom. The Y-axis is the number of genes enriched in a functional category.

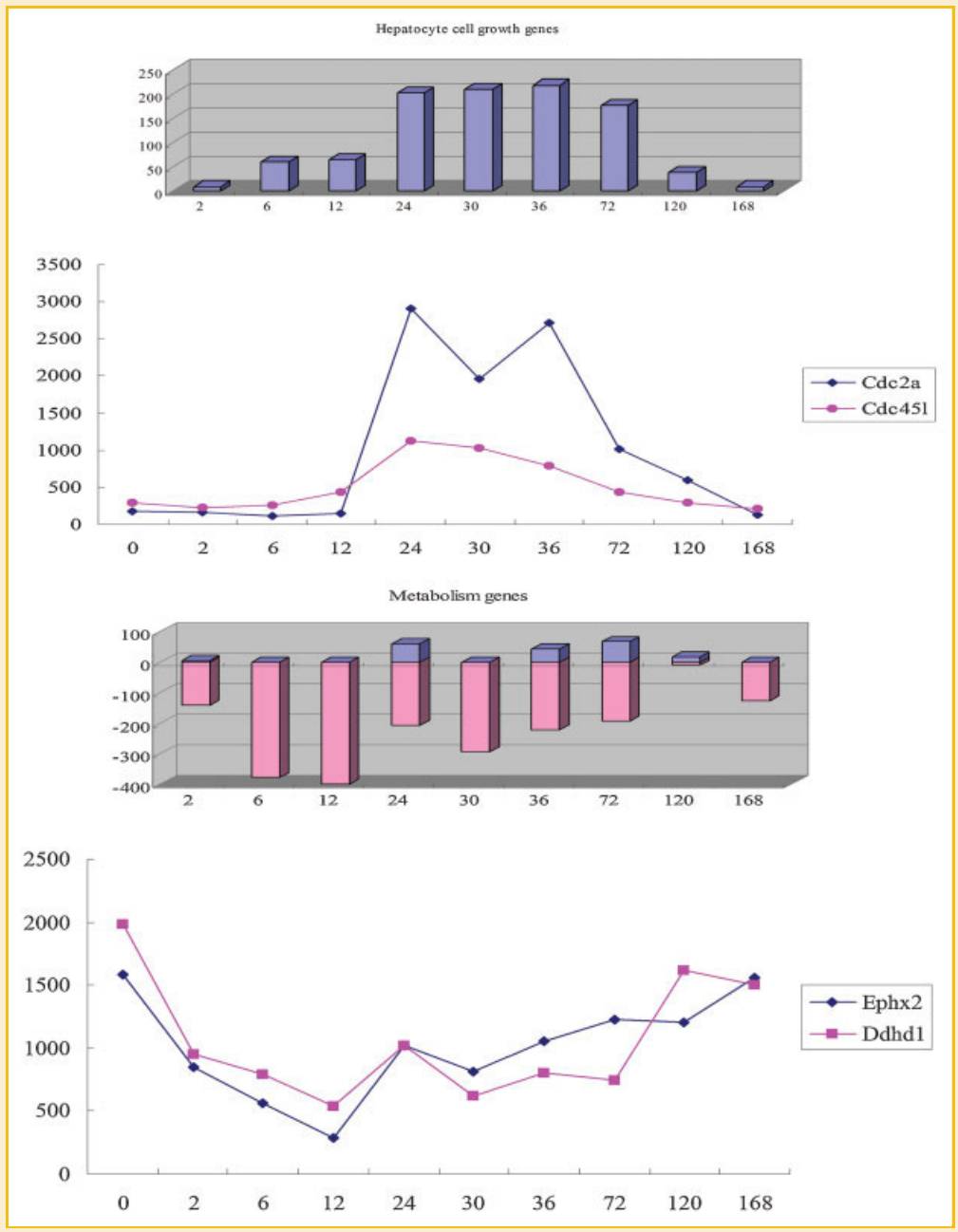

Fig. 8. Comparison of the temporal functional profile of cell growth genes and the expression patterns of cell division genes *Cdc2a* and *Cdc45l*; and comparison of the temporal functional profile of metabolism genes and the expression patterns of metabolism genes *Ephx2* and *Ddhd1*. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

genes but by a group of genes, and the size of the group matters. Perhaps how many genes regulated in a functional category involved in a process is more important than how a gene is regulated. How a gene is regulated can be seen as to describe the intensity of a process, while how many genes regulated can be seen as to describe the complexity of the process.

The temporal functional profiles of cell proliferation genes and metabolism genes are consistent with the expression patterns of some of these genes. This is understandable. Some genes only participated in part of a process, but some genes participated in the whole process. The expression patterns of those genes participating in the whole process must be similar to the temporal functional profile of that functional category. Because these genes participated in the whole process, when the temporal functional profile indicated increased number of up-regulated genes, the expressions of these genes must have been up regulated; when the number of down-regulated genes increased, the expression of these genes must have been down regulated. However, the mechanisms that regulate synchronously the gene expression and the number of this kind of genes are still unknown.

This is the first time report of this kind of phenomenon to the best of our knowledge.

It was thought that hepatocytes proliferated while simultaneously performing all essential functions during liver regeneration [Michalopoulos, 1997]. With so many metabolic genes closed and down regulated in hepatocytes after PH, at least some metabolic activities in hepatocytes decreased. Although some essential functions, such as glucose regulation, synthesis of blood proteins, secretion of bile, biodegradation of toxic compounds, are maintained for homeostasis in regenerating liver, down-regulation of many metabolic genes indicated that hepatocytes were half dedifferentiated during liver regeneration.

Although many automatic gene functional profiling tools have been developed (68 in 2008) [Huang et al., 2009], most are not satisfying. Even our chosen ProfCom gave much less numbers of enriched genes than manual. Manual work can classify every known gene exclusively, while most profiling tools could only group some genes from the gene list. The tools need to be improved by increasing the sensitivity, enriching more genes in a functional category. The GO terms are also imperfect. There are a lot of redundancy and also incompleteness in the GO terms of a gene, which may mislead an automatic gene enrichment tool. The hierarchy structures of GO terms are also problematic. Some tools used different level of terms and gave totally different results. When the terms of lower level was enriched, the higher levels should be automatically enriched. For the purpose of term enrichment, we feel simple nonredundant keywords may be better.

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