Restoration of Liver Mass after Injury Requires Proliferative and Not Embryonic Transcriptional Patterns*5

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Normal adult liver is uniquely capable of renewal and repair after injury. Whether this response represents simple hyperplasia of various liver elements or requires recapitulation of the genetic program of the developing liver is not known. To study these possibilities, we examined transcriptional programs of adult liver after partial hepatectomy and contrasted these with developing embryonic liver. Principal component analysis demonstrated that the time series of gene expression during liver regeneration does not segregate according to developmental transcription patterns. Gene ontology analysis revealed that liver restoration after hepatectomy and liver development differ dramatically with regard to transcription factors and chromatin structure modification. In contrast, the tissues are similar with regard to proliferation-associated genes. Consistent with these findings, real-time polymerase chain reaction showed transcription factors known to be important in liver development are not induced during liver regeneration. These three lines of evidence suggest that at a transcriptional level restoration of liver mass after injury is best described as hepatocyte hyperplasia and not true regeneration. We speculate this novel pattern of gene expression may underlie the unique capacity of the liver to repair itself after injury.

The ability of the adult mammalian liver to restore its function and mass after injury is unique among organs. In tissues with only limited repair potential, including the bone and kidney, developmentally important genetic pathways are co-opted during healing (1, 2). What distinguishes the liver from these tissues? Possibilities include that liver repair does not recapitulate pathways of embryonic morphogenesis or that the liver can reproduce embryonic signaling pathways more effectively than other tissues. Transcriptional profiling and advances in quantitative biology can distinguish these possibilities and shed light on the mechanism of liver repair. Transcription factors play central roles in liver repair and development. Reconstitution of liver mass after injury requires c-Jun, signal transducer and activator of transcription 3 (STAT3),4 CAAT enhancer-binding protein β, and cAMP-response element modulator, widely distributed factors that modulate a variety of cellular processes (3–6). In contrast, many transcription factors required for liver development are more tissue- and role-specific. Homeobox factors, including Hlx and prospero-related homeobox 1, are required for normal hepatic growth and hepatoblast migration into the septum transversum mesenchyme, respectively (7, 8). Bile duct formation requires hepatocyte nuclear factor-1β (HNF-1β) (9). Loss of Forkhead Box (Fox) transcription factors A1 and A2 results in failure of hepatic specification (10). Hepatoblast mitosis and intrahepatic bile duct formation require Fox M1 (11). Finally, liver development depends on transcription factors thought to modulate large-scale transcriptional programs, such as hairy and enhancer of split 1 (Hes1) (12).

To better understand the transcriptional mechanisms at work during these processes, multiple groups have presented array analysis on both regenerating and developing liver. Interestingly, these studies have identified relatively few transcription factors regulated during either process. During the first 4 h after hepatectomy in the mouse, only 11 transcription factors were up-regulated during regeneration among 6500 genes examined (13). In the rat, regeneration was associated with induction of only one transcription factor, hepatocyte nuclear factor 4 (14). Another extensive microarray analysis examining networks active during liver regeneration revealed a dramatic shift toward genes involved in cytoskeleton assembly and DNA synthesis; in contrast, no shift toward genes involving transcription was identified (15). Similar studies of embryonic liver identified relatively few transcription factors specific to each stage (16–18). In studies designed to censor genes not specific to hepatocytes, plated embryonic liver tissue demonstrated only 281 up-regulated genes on an array of nearly 9000 tran-
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scripts as hepatocytes matured (19). Of these, only 12 were related to transcription or regulation of transcription.

To elucidate the molecular similarities between regenerating and developing liver, we performed high density microarray analysis of ~45,000 targets at 1, 2, 6, 12, 18, 24, 30, 48, and 72 h after partial hepatectomy and at 10.5, 11.5, 12.5, 13.5, 14.5, and 16.5 days post-conception (dpc). Combining principal component analysis, gene ontology (GO) assignments, and quantitative real-time polymerase chain reaction (QRT-PCR) of selected genes, a picture of liver regeneration at the molecular level emerges and allows determination of the extent to which regeneration recapitulates embryogenesis.

EXPERIMENTAL PROCEDURES

Mice—All experiments used CD-1 mice obtained from Charles River Laboratories (Wilmington, MA) or our own breeding colony. Animals were maintained in a pathogen-free facility administered by Harvard Medical School in accordance with the institutional guidelines of Harvard Medical School and Beth Israel Deaconess Medical Center.

2/3 Hepatectomy—Mouse protocols were consistent with National Institutes of Health guidelines and approved by the Animal Care and Use Committee of Harvard Medical School. Female CD-1 mice were operated on at 10 weeks of age. After anesthesia with 100 mg/kg ketamine and 10 mg/kg xylazine, the abdomen was opened and alcohol and betadine. After midline laparotomy, the tip of the xiphoid was resected and the liver exteriorized. A silk tie was placed around the three anterior lobes of the liver, including the gallbladder, and the 2/3 hepatectomy with cholecystectomy was completed. The liver was returned to the abdominal cavity and the abdomen closed in two layers. Post-operative analgesia consisted of buprenorphine 0.05–0.1 mg/kg twice daily for 48 h or until sacrifice. At harvest, mice were sacrificed with carbon dioxide asphyxiation followed by cervical dislocation.

Embryo Harvest—Pregnant CD-1 females were sacrificed with carbon dioxide asphyxiation followed by cervical dislocation. The abdomen was opened and the uterus removed. After removal of the uterine musculature, embryos were removed from the yolk sacs and livers micro-dissected, taking care to remove all extraneous tissue including the retrohepatic vena cava. A maximum of 5 min was allowed to elapse between sacrifice and liver harvest to prevent RNA degradation and transcriptional changes.

RNA Isolation—All tissue samples were placed into an RNA stabilization reagent (RNA later; Qiagen Inc., Valencia, CA). Total RNA was isolated from tissue homogenate with a commercial kit (RNeasy Protect mini kit; Qiagen Inc.). Quantitation and assessment of RNA purity was determined by UV absorbance and electrophoresis. Samples were discarded if they did not have a strong 28 S band at least twice the intensity of the 18 S band or if there was significant smearing of the sample.

Microarray Analysis—Transcription profiles were determined using Affymetrix MG 430 2.0 chips according to previously described protocols for total RNA extraction and purification, cDNA synthesis, in vitro transcription to produce biotin-labeled cRNA, hybridization of cRNA to the chips, and scanning of image output files (20). Each experimental time point is represented by two separate samples, each consisting of at least three pooled tissues from different animals. For example, six hepatectomies were performed for the 1-h post-hepatectomy time point. The first three were pooled into one sample, and the remaining three were pooled into a second sample. Each sample was run on a separate Affymetrix MG 430 2.0 chip containing over 45,000 transcripts using the two-cycle cDNA synthesis method that contains one round of in vitro transcription cRNA amplification according to the manufacturer’s instructions.

Scanned array images were analyzed by dChip (21) (www.dchip.org), as it is more robust than Affymetrix software Microarray Analysis Suite 5.0 and Robust Multiarray Average in signal calculation (22). Raw probe-level data were normalized using the smoothing spline invariant set method (21). Signal values that represent the expression levels of the transcripts were then obtained using the model-based expression algorithm following the perfect match (PM)-mismatch (MM) difference model described in dChip (21). Briefly, a single signal value is obtained for each transcript based on the difference between the intensity of the PM and MM signals. This value is normalized to the overall intensity of the chip determined by an iterative algorithm that identifies transcripts that are not differentially expressed as opposed to defining them a priori. In this way, the signal value corresponds to an absolute level of expression of the transcript. Analysis of this oligo-based array is therefore different from the analysis of a cDNA array. These normalized and modeled signal values were used in subsequent clustering and differential expression analysis.

During the calculation of model-based expression values, single, array, and probe outliers are interrogated, and image spikes are treated as single outliers. If the absolute residual (the difference between the model-fitted value and observed value) of a probe exceeds three times the 95th percentile of absolute residual values of all probes on a chip, it is called a single outlier. Array outliers are the probe pair sets on a chip whose values are different from the consensus probe response pattern (for that particular probe pair set) seen in most chips. If the model-based standard error of a probe pair is three times larger than the median standard error of all probe pairs, it is called a probe-outlier. In the dChip analysis, array outliers are not treated as missing expression values and no truncation on signal values is applied. A chip is marked as an “outlier” if probe, single, or array outlier percentage exceeds a preset threshold. Using the default threshold values (5%) defined in dChip, no outlier chips were identified, so all samples were carried on for subsequent analysis. The data are available both at NCBI’s minimum information about a microarray experiment (MIAME)-compliant Gene Expression Omnibus (GEO) data base and in the supplemental material.

Principal Component Analysis (PCA)—PCA projects multivariate data objects onto a lower dimensional space while retaining as much of the original variance as possible. Replicates were averaged to obtain one vector of expression values for each time point in development and regeneration. The 10.5-time point was omitted because of technical concerns with the array but did not influence the conclusions. We used PCA to project the five samples of the liver development time series,
each consisting of 45101 variables (probe sets), into a two-dimensional plane. Each sample was normalized to mean zero and S.D. 1. \( \text{dev}_{2D} \), a matrix of developmental time points in two dimensions, was obtained through the transformation \( \text{dev}_{2D} = \phi_{\text{dev}} \times \text{dev}_{45101D} \). \( \phi_{\text{dev}} \) is a 45101 \times 2 matrix consisting of the first two principal components of the original data set, \( \text{dev}_{45101D} \).

Global similarity between liver development and regeneration samples was assessed using the principal components of the development data to transform the regeneration time series (23). Specifically, we obtain \( \text{reg}_{2D} \), a matrix of regeneration time points in two dimensions through \( \text{reg}_{2D} = \phi_{\text{dev}} \times \text{reg}_{45101D} \). Again, \( \text{reg}_{45101D} \) was normalized to mean zero and S.D. 1 before transformation.

**Analysis of Differential Gene Expression**—dChip assigns an S.E. associated with the expression level of each transcript. This S.E. is implied by the fitted model during expression index calculation and reflects the measurement accuracy for the transcript. When calculating the -fold change of a transcript between two groups of chips, standard errors are used to assign confidence intervals to the -fold change. For example, consider a simple model where the expression level of a transcript is compared in two chips. Assume the model-based expression indices for the transcript are \( e_1 \) and \( e_2 \), and the associated standard errors are \( s_1 \) and \( s_2 \), respectively. Defining \( f \) as \( e_1/e_2 \), an inference on \( f \) can be made using the quantity \( Q = (e_1 - f e_2)^2/(s_1^2 + s_2^2 f^2) \). \( Q \) can be used to construct inverted fixed level tests to define a confidence interval on \( f \), the -fold change.

It has been shown that the lower confidence bound (LCB) of -fold change is a stringent estimate of the -fold change and the better ranking statistic for differential gene expression (24). In the dChip analysis, if the 90% LCB of the -fold change between the expression level of the gene in the control 10-week adult liver versus the experimental time point was above 1.5, the corresponding gene was considered differentially expressed.

dChip’s LCB method for assessing differentially expressed genes is superior to other commonly used approaches, such as Microarray Analysis Suite 5.0- and Robust Multiarray Average-based methods (24, 25). Using custom arrays and QRT-PCR, it has been suggested that Affymetrix chips may underestimate differences in gene expression; selecting genes that have a LCB >1.5 most likely corresponds to genes with an “actual” -fold change of at least 3 in gene expression (26).

**Hierarchical Clustering and Heat Map Generation**—A hierarchical clustering technique was used to construct an Unweighted Pair Group Method with Arithmetic-mean tree using Pearson’s correlation as the distance measure (27). Samples are clustered using the normalized and modeled expression values obtained using dChip analysis. The expression data matrix was row-normalized for each gene prior to the application of average linkage clustering.

Selected transcripts that are involved in chromatin modifications and induced in development or regeneration time points compared with the control 10-week adult liver (LCB >1.5) were used to generate heat maps to follow expression levels over time. Normalized and modeled signal values obtained by dChip analysis were used. These are single values that correspond to the intensity of the hybridization signal. Each transcript has a 0 time point that corresponds to normal adult liver and serves as a reference value for each experimental time point (time after hepatectomy or embryological time) to determine differential expression. Because dChip assigns an absolute intensity for each value, the 0 time reference value varies with each transcript. Expression levels of each transcript are used to scale the samples across the heat map to the [-3,3] interval, where 3, 0, and 3 on the scale correspond to green, black, and red, respectively.

**Gene Ontology Analysis**—Expression Analysis Systematic Explorer (EASE) identifies biologically relevant categories that are over-represented in the set and therefore may be of further interest (28). EASE maps each probe to an Entrez Gene identifier that is associated with a GO category (29, 30). The transcripts on the chip were mapped to 33,229 unique genes used in the downstream EASE analysis. GO Consortium (www.geneontology.org) assigns each gene (where applicable) to a class of biological function. EASE identifies GO categories in the input gene list that are over-represented using jackknife iterative resampling of Fisher exact probabilities, with Bonferroni multiple testing correction. The “EASE score” is the upper bound of the distribution of Jackknife Fisher exact probabilities. Categories containing low numbers of genes are underestimated so that the EASE score is more robust than the Fisher exact test. The EASE score is a significance level with smaller EASE scores indicating increasing confidence in overrepresentation. We picked GO categories that have EASE scores of 0.05 or lower as significantly over-represented.

**Quantification of Gene Expression by QRT-PCR**—QRT-PCR was performed using the ABI 7700 sequence detector (Applied Biosystems). Reverse transcription of 1 \( \mu \)g of RNA was performed using murine leukemia virus reverse transcriptase (GeneAmp RNA PCR; Applied Biosystems, Foster City, CA). The resulting cDNA product was diluted 1:5. PCR amplification was performed in a total volume of 25 \( \mu \)l containing 12.5 \( \mu \)l of 2 \( \times \) TaqMan Universal PCR Master Mix (no Amperase UNG; Applied Biosystems), 6.25 \( \mu \)l of nuclelease-free water, 5 \( \mu \)l of cDNA, and 1.25 \( \mu \)l of the appropriate 20 \( \times \) probe (Applied Biosystems). To quantify the levels of mRNA we normalized expression of the target genes to 18 S ribosomal RNA and glyceraldehyde-3-phosphate dehydrogenase. Data were analyzed using ABI sequence detector software (Applied Biosystems).

**RESULTS**

**Comprehensive Assessment of Differential Gene Expression during Liver Development and Regeneration**—Using a microarray of more than 45,000 probes we generated a nearly comprehensive data base of gene expression in the adult liver after 2/3 hepatectomy and during embryonic development. A complete list of gene expression, up- and down-regulated genes, and functional and correlation analysis are provided at www.bidmcgenomics.org/LiverDevReg/index.html. Regeneration time points examined were 1, 2, 6, 12, 18, 24, 30, 48, and 72 h after hepatectomy. Embryonic time points examined were 10.5, 11.5, 12.5, 13.5, 14.5, and 16.5 dpc. Control mice were normal 10-week-old females. Each time point is represented by two chips with at least three hepatectomy samples/chip. Replicate chips resembled each other with a correlation coefficient of
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0.96 ± 0.03 for regeneration and 0.99 ± 0.01 for development (average ± S.D.).

Cluster analysis demonstrated high similarity within hepatectomy time points and within embryonic days, but not between the two groups. Complete array expression values produced a cladogram (Fig. 1) reflecting global expression similarities across the samples. Hierarchical clustering of all samples demonstrated a clear distinction based on the post-hepatectomy or development samples. Correlation values close to 1 were common when comparing two time points within the regeneration group or comparing two points within the development group, whereas comparing any sample in one group with any sample in the other group did not yield a correlation of >0.67 (see supplemental data). Replicate time points were not always closest but were always very highly correlated with each other. Compared with normal adult liver, 2111 were up-regulated at least once during the regeneration time course, 9761 genes were up-regulated at least once during the developmental time course, and 885 genes were up-regulated at least once during both regeneration and development.

Principal component analysis demonstrates development and regeneration are distinct.

Analysis of the set of regenerating genes identified groups including acute phase reactants and genes involved in cell proliferation as over-represented. Individual genes known to be important for liver regeneration, including STAT3, GATA6, and C/EPBβ, were all identified by this analysis.

During development, the large number of genes with LCB >1.5 are preferentially distributed into EASE categories representing chromatin organization, chromosome organization and biogenesis, and transcription. Reflecting the high rate of cell division in the embryonic liver, categories of cell cycle, DNA replication, and mitosis have significant EASE scores, as do genes involved in heme biosynthesis.

EASE analysis applied to categories of genes with LCB >1.5 in both regeneration and development revealed significant scores for cell division, including cell cycle, DNA replication, mitosis, and regulation...
of cell cycle, and genes involved in cytokinesis. Categories conspicuously not represented in this set include transcription factors and genes involved in large-scale chromatin structure and remodeling.

**Genes Involved in Large-scale Chromosome Structure of the Developing Liver Are Not Induced during Liver Regeneration**—We next performed EASE analysis on groups related to transcription and chromatin modification and remodeling. In all examined categories related to transcriptional control, there were dramatically more genes with an LCB >1.5 in the developing liver compared with the regenerating liver (Table 1). For example, among the genes with LCB >1.5 during development, 744 were identified in the GO category regulation of transcription, whereas only 136 genes in this category were identified in the regeneration set. These included genes involved in pathways already known to be important for regeneration, including Jun and Jun-related factors, GATA6, general transcription factors, cAMP-responsive genes, and hypoxia-inducible protein 1.

To better understand the temporal progression of the regulation of chromatin structure, we identified genes involved in chromosome modification from the set of genes with LCB >1.5 in development, regeneration, or both. We generated heat maps for these genes using normalized and modeled signal values obtained by dChip analysis (Fig. 3). Four separate heat maps demonstrate the regulation of 34 genes uniquely induced in development time points, 10 genes induced both at development and regeneration time points, and 4 genes uniquely induced in regeneration time points. Samples are denoted in columns and genes are denoted in rows. The expression level of each gene across the samples used to generate the heat map is scaled to the [-3,3] interval. These mapped expression levels are depicted using a color scale as shown at the bottom of the figure where red indicates high expression and green indicates low expression. Note that because each gene has an absolute expression, the color of the 0 reference points are not equivalent. Expression of genes that influence chromatin structure decreases dramatically as development progresses. In contrast, the few genes that demonstrate high expression during regeneration were scattered throughout the time course.

**Transcription Factors Critical for Liver Development Are Not Induced during Regeneration**—Using QRT-PCR, we investigated the transcription pattern during regeneration of genes known to be important for normal liver development. Functional data exist demonstrating that many transcription factors play seminal roles in liver development, including Hlx, Prox-1, transcription factor 2 (TCF2, also HNF1α), FoxA2 (HNF3β) and FoxM1, genes involved in transforming growth factor β signaling, SMAD (mothers against DPP homologue) 2 and SMAD3 and Jumonji, HNF6, Hes1, GATA-4, and X-box-binding protein 1 (XBP-1) (7–12, 31–35). Ten of these twelve genes were not induced at least 2-fold during regeneration, whereas only two were up-regulated in a limited manner, GATA-4 1 h after regeneration, and Fox M1 48 and 72 h after regeneration (data not shown).

**DISCUSSION**

Microarray analysis presented here represents the most complete data set to date looking at transcription during liver regeneration and development. Applying powerful computational techniques and the immense and growing data base of known gene function to this dataset allows new insights into how similar these processes are on a global scale. Determining whether regenerating liver recapitulates hepatogenesis at the transcriptional level may be important for understanding the unique regenerative capacity of the liver and for developing or enhancing liver replacement therapies.

Overall, we noted the transcriptional profiles of the developing and regenerating liver are dissimilar. To analyze these data in a non-biased fashion, correlation coefficients between each pair of samples were generated using all genes; within the regeneration group and within the development group, correlation values were often close to 1, whereas between groups, correlation values were never greater than 0.67 (see supplemental data). Replicate chips showed high reproducibility with aver-
S.D. correlation coefficient values of 0.96 ± 0.03 for regeneration and 0.99 ± 0.01 for development time points.

In the cladogram generated using the complete transcriptional profile of the samples, duplicates are generally but not always branched immediately next to each other. Importantly, the high correlation coefficients between duplicates suggest this lack of juxtaposition is due to the large number of probes interrogated (>45,000) and the relatively low number of genes that change over time. Furthermore, there is variability in the extent of hepatectomy and variation in actual ages within a litter and of the time of day of plugging between litters. Selecting only genes that are differentially expressed restores the expected closeness of duplicates (see supplemental data).

Hierarchical clustering analysis successfully separated development samples from regeneration samples and further divided regeneration samples into early and late time points by forming a subgroup of 0-, 1-, 2-, and 6-hour samples.

Two possible explanations for the segregation of regeneration from developing liver are the presence of hematopoietic precursors in the developing liver and the evolution of the liver cell population over time. The first explanation is unlikely, based on the similarity of the 10.5-dpc point, which includes immature red blood cells, the 12.5-dpc point, which includes mature red blood cells, and the 16.5-dpc time point, when hematopoiesis involutes (36, 37). Similarly, it is unlikely differences in cell type explain transcriptional differences, as all major cell types present in the mature liver are present in 16.5-dpc livers, including hepatocytes, cholangiocytes, stellate cells, endothelial cells, and macrophage-type cells (38).

Evaluating regeneration profiles through the lens of developmental gene expression dynamics, we next asked how the overall gene expression profile of the liver changes as development progresses and whether the characteristics of this change are observed during liver regeneration. PCA outlines the major trajectories of liver development by simplifying the high-dimensional data into a comprehensible form and exposing its dynamic structure. Most of the 45,101 probes on the microarray are not necessary to distinguish one tissue from another, because many probes will be correlated or not exhibit variability. PCA transforms this high-dimensional data set into a lower-dimensional data set consisting of independent variables. Although the first dimension PC1 captures as much variance as possible, in most cases, the data will also exhibit variability explained by other orthogonal (linearly independent) axes that can be used to display the data in 2, 3, or more dimensions.

We used PCA to represent liver development time points in a two-dimensional space spanned by PC1 and PC2; together these axes represent more than 90% of the variance of the data (Fig. 2). Regenerating liver was projected onto the same space using the principal components derived from development. Regeneration time points segregate distinctly from all times of development. Regenerating liver is similar to later stage development, almost certainly representing the maturation of elements in the developing liver as they take on adult gene expres-

**FIGURE 3. Most genes involved in chromatin modification induced during development are not induced during regeneration.** Heat maps were generated for all genes involved in chromatin modification induced during regeneration and development. There is little overlap between the two categories. Columns represent samples, and rows represent genes. Signal values used to generate the colorgram are mapped to the [-3,3] interval for visual purposes. This scaling and corresponding color code is shown at the bottom of the figure. Red denotes high expression, and green denotes low expression. Numbers above the figures with .5 represent days post-conception. Whole numbers are hours after hepatectomy. Genes involved in embryonic chromatin structure become more similar to adult over time, suggesting a large-scale transcriptional pattern.
sion patterns. The clustering of the regenerating adult liver when projected in the development space suggests that the variance of development is different from the variance observed during regeneration. Movement of the 18-h regeneration time point toward earlier embryogenesis was investigated by determining the 100 genes that most contributed to the shift. No patterns based on molecular function or biological relevance could be elucidated (data not shown).

To characterize genes with LCB >1.5 after liver resection or during development compared with control adult liver, genes were curated according to GO categories and EASE scores calculated. For the regenerating liver, preferentially transcribed categories included genes involved in the acute phase response and cell proliferation (Table 1). Importantly, individual genes known to be involved in liver regeneration, including STAT3, GATA6, and cAMP-response element binding protein, were identified by this analysis, validating the accuracy of the approach.

In contrast, categories during development with significant EASE scores include families involved in modification of large-scale DNA structure and transcription, as well as cell proliferation and heme biosynthesis (Table 1). The majority of induced genes common to both regeneration and development were primarily involved in cell proliferation and not transcription (Table 1), suggesting that developing and regenerating liver are primarily similar in genes necessary for cell proliferation, not for transcriptional control.

To further elucidate the similarities of the regenerating and developing liver we focused on genes involved in transcription, including the factors themselves, binders, activators, coactivators, cofactors, regulators, repressors, and corepressors (Table 1). EASE scores identified all these categories as being preferentially transcribed in developing liver. Importantly, the number in developing liver was on the order of 5- to 10-fold above those common to both tissues, confirming liver regeneration does not recapitulate most of the transcriptional program of the developing liver.

We next examined how genes involved in chromatin structure change over time during development and regeneration. Most of the genes with an LCB >1.5 during development were not induced during regeneration. Interestingly, as development progressed, expression levels of these genes decreased, shifting away from an embryonic and toward an adult pattern. This most likely represents a developmental paradigm indicating a transition toward a very different chromatin structure after 48 to 72 h after hepatectomy (data not shown), consistent with the conclusion that transcriptional paradigms of development are not reproduced during regeneration.

Taken in total, these results suggest that the regenerating liver recapitulates the proliferative machinery of the developing liver but not its large-scale transcriptional regulation. Liver regeneration is therefore more correctly termed hepatocyte hyperplasia at the transcriptional level. This may be in contrast to the situation in highly ordered tissues, including bone and kidney, which recapitulate developmental signaling processes during repair. After fracture of a bone, the parathyroid hormone/parathyroid hormone-related binding peptide receptor, indian hedgehog, patched, bone morphogenetic proteins, and the transcription factor Gli are expressed in similar spatial domains they occupy during embryonic development (1). During kidney regeneration after experimentally induced acute tubular necrosis, Paired box gene 2, a transcription factor critical for development of the excretory system, is expressed in regenerating proximal tubular epithelial cells (2).

We speculate that the re-establishment of liver mass is a relatively simple process at the transcriptional level and that this underlies the prodigious ability of the liver to repair itself. Though there are major differences in transcription patterns among mammals during liver regeneration (41), we nevertheless believe the broad conclusions of the similarities and differences between regeneration and embryonic development hold across species. At this time, little is known about how the complicated local cell-cell architecture is re-established after liver injury. This process may be a simpler one than in other tissues in which spatial requirements may be more stringent.

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