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A Microarray Analysis of the Temporal Response of Liver to Methylprednisolone: A Comparative Analysis of Two Dosing Regimens

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Abstract

Microarray analyses were performed on livers from adrenalectomized male Wistar rats chronically infused with methylprednisolone (MPL) (0.3 mg/kg·h) using Alzet mini-osmotic pumps for periods ranging from 6 h to 7 d. Four control and 40 drug-treated animals were killed at 10 different times during drug infusion. Total RNA preparations from the livers of these animals were hybridized to 44 individual Affymetrix REA230A gene chips, generating data for 15,967 different probe sets for each chip. A series of three filters were applied sequentially. These filters were designed to eliminate probe sets that were not expressed in the tissue, were not regulated by the drug, or did not meet defined quality control standards. These filters eliminated 13,978 probe sets (87.5%) leaving a remainder of 1989 probe sets for further consideration. We previously described a similar dataset obtained from animals after administration of a single dose of MPL (50 mg/kg given iv). That study involved 16 time points over a 72-h period. A similar filtering schema applied to the single-bolus-dose data-set identified 1519 probe sets as being regulated by MPL. A comparison of datasets from the two different dosing regimens identified 358 genes that were regulated by MPL in response to both dosing regimens. Regulated genes were grouped into 13 categories, mainly on gene product function. The temporal profiles of these common genes were subjected to detailed scrutiny. Examination of temporal profiles demonstrates that current perspectives on the mechanism of glucocorticoid action cannot entirely explain the temporal profiles of these regulated genes.

Glucocorticoids are a class of steroid hormones that play a central role in regulating the production, storage, and distribution of substrates for systemic energy metabolism. Most tissues are targets for glucocorticoid action and contribute in some way to their wide-ranging physiological effects. Synthetic glucocorticoids (corticosteroids) are used therapeutically for a wide variety of conditions that require immune and/or inflammatory modulation. Because corticosteroids pharmacologically magnify the physiological actions of endogenous glucocorticoids, therapeutic use of this class of drugs is accompanied by a wide range of adverse effects that include hyperglycemia, dyslipidemia, muscle wasting, hypertension,

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nephropathy, fatty liver, and an increased risk of arteriosclerosis (1–5). The physiological and pharmacological effects of these drugs are complex and involve changes in the expression of many genes in multiple tissues.

Microarrays can provide a method of high-throughput data collection that is necessary for constructing comprehensive information on the transcriptional basis of such complex systemic polygenic phenomena. When microarrays are used in a rich *in vivo* time series, they yield temporal patterns of changes in gene expression that illustrate the cascade of molecular events that cause broad systemic responses. However, the magnitude of data produced in such studies provides challenges of data mining and analysis.

Previously, we described the mining and analysis of microarray time series illustrating the responses of liver, skeletal muscle, and kidney taken from the same set of animals to a single bolus dose of the corticosteroid methylprednisolone (MPL) (6 – 8). These time series included individual chips from multiple control animals as well as multiple animals at each of 16 times over a 72-h period after bolus dosing with MPL. Because these experiments were initiated using adrenalectomized animals, the drug in essence acts as a stimulus that perturbs the homeostatic balance of the system, and the experiment monitored the deviation of the system and its return to the original state. Mining such time series datasets presents uniquely different problems from those encountered when microarrays are used to distinguish one group from another (*e.g.* cancerous *vs.* noncancerous tissues) (9 –11). For this type of application, one attempts to define a pattern or fingerprint that distinguishes such groups with very high probability, and need not include all differentially regulated genes. In those cases, it is identifying a distinguishing pattern of gene expression rather than the relationship between the genes that is the important focus. In mining a time series microarray dataset, the problem is sorting through the vast amount of data to identify probe sets with temporal patterns of change in expression that indicate that the gene is regulated over time. In this case, the mechanistic relationships between the genes whose expression is changing in response to the stimulus are of paramount importance. For example, the stimulus may change the expression of a particular transcription factor that in turn alters the expression of downstream genes. For this application, the goal of the initial data mining is to avoid discarding valuable data. This is of particular importance because in our hands, each gene that is identified as being potentially regulated becomes the subject of extensive literature searches to allow placement into a temporal context of all other regulated genes.

Although very useful, a single time series only provides a one-dimensional view of the dynamics of the system in response to the stimulus. A pharmacological time series is different from most time series studies (for example those assessing developmental changes) in that it can be repeated using a different dosing regimen. A second dosing regimen is valuable in two regards. First, it can serve to corroborate results of the first dosing regimen. When considering gene array results, this can be useful. Second, the results can be used to group genes into clusters with common mechanisms of regulation. If two or more genes have a common mechanism of regulation, then their response profiles should be the same regardless of the dosing regimen. In the present report, we describe the use of microarrays to broadly characterize the response of liver to a second dosing regimen that entailed chronic infusion of MPL. Here the drug essentially was used as an unbalancing stimulus, and the

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experiment evaluated the capacity of the system to rebalance in the continuous presence of the drug. This dataset was mined using a similar filtration approach as was applied to the acute dosing dataset, and results from both datasets were compared. Probe sets common to both analyses were identified, allowing the coincidental evaluation of the two profiles for each gene.

Materials and Methods

Animals

Adrenalectomized male Wistar rats with body weights of 339 ± 28 (SD) g were used in the study. All animals were housed in our University Laboratory Animal Facility maintained under constant temperature (22 C) and humidity with a controlled 12-h light, 12-h dark cycle. A time period of at least 2 wk was allowed before they were prepared for surgery. Rats had free access to rat chow and 0.9% NaCl drinking water. This research adheres to Principles of Laboratory Animal Care (National Institutes of Health publication 85-23, revised 1985) and was approved by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo.

Forty rats were administered 0.3 mg/kg·h infusions of MPL sodium succinate (Solu-Medrol; The Upjohn Co., Kalamazoo, MI) reconstituted in supplied diluent. The infusions were administered via Alzet osmotic pumps (model 2001, flow rate 1 μl/h; Alza, Palo Alto, CA). The pump drug solutions were prepared for each rat based on its predose body weight. On the day of implantation, rats were anesthetized using 60 – 80 mg/kg ketamine and 8 –10 mg/kg xylazine im. Pumps were sc implanted between the shoulder blades on the back. Rats were killed at various times up to 7 d. The time points included were 6, 10, 13, 18, 24, 36, 48, 72, 96, and 168 h. A control group of four animals was implanted with a saline-filled pump and killed at various times throughout the 7-d study period. A more detailed description of the experiment can be found in previous reports (12, 13).

Microarrays

Liver samples from each animal were ground into a fine powder in a mortar cooled by liquid nitrogen and 100 mg was added to 1 ml prechilled Trizol reagent (Invitrogen, Carlsbad CA). Total RNA extractions were carried out according to manufacturer's directions and were further purified by passage through RNeasy mini-columns (QIAGEN, Valencia, CA) according to manufacturer's protocols for RNA clean-up. Final RNA preparations were resuspended in RNase-free water and stored at −80 C. The RNAs were quantified spectrophotometrically, and purity and integrity were assessed by agarose gel electrophoresis. All samples exhibited 260/280 absorbance ratios of approximately 2.0, and all showed intact ribosomal 28S and 18S RNA bands in an approximate ratio of 2:1 as visualized by ethidium bromide staining. Isolated RNA from each liver sample was used to prepare target according to manufacturer's protocols. The biotinylated cRNAs were hybridized to 44 individual Affymetrix GeneChips Rat Genome 230A (Affymetrix, Inc., Santa Clara, CA), which contained 15,967 probe sets. These gene chips contain over 7000 more probe sets than the ones used (U34A) in our previous liver bolus-dose MPL study (6 –

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8). The high reproducibility of *in situ* synthesis of oligonucleotide chips allows accurate comparison of signals generated by samples hybridized to separate arrays.

Data analysis

Affymetrix Microarray Suite 5.0 (Affymetrix) was used for initial data acquisition and analysis. The signal intensities were normalized for each chip using a distribution of all genes around the 50th percentile. The dataset was then loaded into a data mining program, GeneSpring 7.0 (Silicon Genetics, Redwood City, CA), for further analysis. The generated dataset has been submitted to the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/projects/geo/) database (GDS972). Before screening for probe sets with altered expression levels, the dataset was normalized again to the mean values of control samples so that all probe sets from control samples had a mean value of 1, and probe sets from treated animals had a value of either greater than, less than, or equal to 1, representing up-regulation, down-regulation, or no change. These normalized probe sets were then filtered with a series of predetermined screening criteria to identify probe sets with appreciable expression levels, expression changes, and acceptable data quality. This set of filters is approximately the same as used to analyze the acute bolus dosing datasets with minor modifications to accommodate the different number of samples in the two experiments (6 – 8).

The process of data mining was performed in the GeneSpring program, and the progress after each step was visualized using a cluster feature of the program. This cluster feature could rearrange the order of the probe sets and group them based on the similarity (Pearson’s correlation) of their expression dynamics. Then the probe sets that were not eliminated by the filter were displayed vertically as a gene tree, and their expression dynamics over time were displayed horizontally in colors with yellow in the graph representing an expression ratio around 1, or no change. The color progressing toward red indicates a normalized value greater than 1, or up-regulation, and the color toward blue indicates a value less than 1, or down-regulation from control levels. The brightness of the colors reflects the original signal intensities or expression levels before normalization. The more abundantly expressed mRNAs exhibit brighter colors. Figure 1 shows the gene tree of the entire dataset (15,967 probe sets). The x-axis represents the 11 time points including vehicle controls (nominally time 0). The y-axis represents the list of the probe sets in order of similarity. Figure 2 (*top*) shows a magnification of five probe sets on the tree with an apparent response of enhanced expression. It should be noted that two of these probe sets directly adjacent to each other, 1370200 and 1387878, are both for the enzyme glutamate dehydrogenase 1 (Glud1). This enzyme is involved in ammonia detoxification, which is necessary when amino acid carbon is used for gluconeogenesis (14). A significant deficit of the gene tree representation is that all time intervals are represented as equal, and therefore to some degree temporal patterns are misrepresented in the gene tree presentations. This is illustrated by comparing the *top* and *bottom panels* in Fig. 2. The *bottom panel* shows a linear plot of the data for the two probe sets for Glud1 presented in the *top panel*. As illustrated by this example, it is possible to visually identify genes under regulation using gene trees. However, this approach does not entail objective criteria for selection of probes for further consideration.

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To screen for the probe sets objectively, the entire dataset was filtered with criteria similar to the ones applied to the dataset from a bolus-dose MPL experiment (6 – 8). This approach does not select for probe sets but rather eliminates those probe sets that do not meet certain criteria, leaving the remainder for further consideration. The first filter was designed to eliminate probe sets for genes that are not expressed in the liver. This filter employed a function in the Affymetrix Microarray Suite 5.0. During initial data analysis, a call of present (P), absent (A), or marginal (M) for each probe set on each chip was determined based on the intensity comparison of the matched and mismatched probe sequence pairs. The first filter eliminated all probe set that did not have a call of P on at least four of the 44 chips. This filter eliminated 6668 probe sets, leaving a remainder of 9299 probe sets for further consideration. These genes are more likely to be expressed in rat liver than those that were eliminated.

The second level of filtering that we applied was designed to eliminate probe sets that could not meet the basic criterion of a regulated probe. Specifically, this filtering approach was designed to eliminate probe sets whose average did not deviate from baseline by a certain value for a reasonable number of time points and employed two filters that were designed to eliminate probe sets that were neither down- nor up-regulated. The first of these filters eliminated probe sets that could not meet a minimal criterion for down-regulation. Starting with the 4P filtered list, we eliminated all probe sets that did not have average values less than 0.65 in at least two conditions (time points). Those probe sets that were not eliminated by this filter were retained as potentially down-regulated probe sets. The next filter was designed to eliminate probe sets that could not meet a minimal criterion for up-regulation. Starting with the 4P filtered list, we eliminated all probe sets that did not have average values above 1.5 in at least two conditions (time points). Those probe sets that were not eliminated by this filter were retained as potentially up-regulated probe sets. However, there were a small number of probe sets that were not eliminated by either filter. Using a Venn diagram, we removed these from both lists and created a list of probe sets with potential complex regulation. Probe sets not eliminated by this filtering approach included 1581 potential down-regulated probe sets, 1212 potential up-regulated probe sets, and a group of 85 probe sets that met both criteria.

The last filter we applied addressed the quality of the data. For this quality control filter, we eliminated probe sets that did not meet two conditions. The first condition focused on the control chips. As indicated above, our initial operation was to divide the value of each individual probe set on each chip by the mean of the values for that probe set on the four control chips. Therefore, the quality of the control data for each particular probe set is of unique importance in defining regulation by the drug. This filter eliminated probe sets whose control values exhibited coefficients of variation of greater than 50%. The second condition focused on the remaining 10 time points. This filter also eliminated probe sets with coefficients of variation for more than five of the remaining 10 time points exceeded 50%. After the application of this filter, 1989 probe sets remained for consideration. Of the 1989, 1049 were in the up-regulated list, 922 were in the down-regulated list, and 18 were in the list that met both criteria. Figure 3 shows a gene tree of all 1989 remaining probe sets. The three lists are published as supplemental data on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org.

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Results

Figure 4 (left) presents concentrations of MPL in plasma of animals receiving chronic glucocorticoid administration through Alzet pumps. The infusion dose (0.3 mg/kg·h) was chosen for dose equivalency at 168 h (the final time point in this study) with the single 50 mg/kg dose employed in our acute studies. By 6 h, MPL levels reach a stable steady state that is maintained throughout the 7-d infusion period. Preliminary experiments (data not shown) demonstrated that steady-state drug levels were attained by 6 h after pump implantation. Therefore, 6 h was chosen as the initial time point for these studies. In contrast, single bolus dose administration (*right*) results in drug levels that dissipate in a biexponential fashion and reach below the level of detection by 7 h after drug administration. Analyses of MPL kinetics for both acute and chronic dosing have been described previously (12, 15). In addition, pharmacokinetic/pharmacodynamic relationships for the expression of both tyrosine aminotransferase (TAT) and phosphoenolpyruvate carboxykinase (PEPCK) activities and mRNA levels assessed by Northern hybridization have been previously published for both the acute and chronic dose regimens (15, 16).

Data mining of gene arrays from this chronic MPL treatment series identified 1989 MPL-regulated probe sets on the R230A gene chips used in this experiment. We also previously obtained liver samples from a population of animals after administration of a single dose of MPL. Liver samples from those animals were collected in a time series that involved 16 time points over a 72-h period. RNAs from those livers were applied to the older Affymetrix RU34A chip. A similar filtering schema as applied to that dataset identified 1519 probe sets as being regulated by MPL. Using Affymetrix homology tables and Blast searches, we identified 464 probe sets of the 1519 on the U34A chip that corresponded to 417 of the 1989 identified probe sets on the U230A chip. Because both chips in some cases contain multiple probe sets for the same gene, and because there is a higher degree of probe set redundancy on the older U34 chip, the number of corresponding probe sets common to the two chips are not equal. Likewise, the number of genes actually represented in this common set is less than the number of probe sets. We identified 358 genes that were regulated by MPL in response to both dosing regimens. Most likely this list does not contain all genes regulated by MPL in liver. A perusal of both data-sets indicates that there were many probe sets that failed the quality control filter on one of the two chips and were thus eliminated. Nonetheless, these 358 genes have a very high degree of certainty of being regulated by MPL in the liver. In addition, the two profiles taken together provide an important foundation for understanding the mechanisms underlying the drug's regulation of genes in the liver.

Response profiles

Of the 358 genes, profiles for 109 showed enhanced regulation after both dosing regimens. A reasonable hypothesis is that if different genes are responding by the same mechanism of regulation, then their profiles should be the same in response to these two different dosing regimens. However, examination of individual profiles demonstrates that this is not always the case. As an example, Fig. 5 shows response profile exemplars of two different genes with enhanced expression to the two dosing regimens but that differ from each other in

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response to MPL. The first gene (Fig. 5, *left*) is ornithine decarboxylase 1 (Odc1), the first enzyme in polyamine biosynthesis (17). This gene exhibits tolerance, a phenomenon we have earlier described for the enzyme TAT in liver (12, 18). Specifically, this gene almost recovers to its baseline after a period of time in response to chronic MPL infusion despite continuous presence of the drug (12). In various earlier reports, we presented data demonstrating that the glucocorticoid receptor (GR) is strongly down-regulated in response to MPL (12, 13, 15, 18, 19). Because GR mediates the effect of the drug, the large reduction in this effector molecule should greatly reduce the driving force for changes in gene expression, thus reducing the effect of the drug. The second gene (Fig. 5, *right*) showing enhanced expression is tryptophan 2,3-dioxygenase (Tdo2), the first enzyme in the kynurenine pathway (20). For this gene, the enhanced expression in response to the single bolus dose is more sustained than Odc1. However, the chronic time profile shows a second and higher period of enhanced expression that continues throughout the entire 168-h infusion period. These data illustrate that enhanced expression to corticosteroids probably involves multiple mechanisms and that our initial classification of enhanced regulation is too simplistic.

Of the 358 genes, the profiles of 104 showed down-regulation after both dosing regimens. As with enhanced regulation, this classification is inadequate to capture the multiple patterns extant within this group. As an example, Fig. 6 shows the expression profiles of two genes that exhibit reduced expression in response to both dosing regimens. The first gene (Fig. 6, *left*) is progestin and adipoQ receptor family member VII (paqr7). One endogenous ligand for this receptor is adiponectin, a protein hormone produced and secreted by adipocytes and which regulates the metabolism of lipids and glucose (21). The response of this gene to acute dosing is a transient reduced expression. The first part of the response profile to chronic infusion also suggests a transient down-regulation with return to baseline. However, a second influence seems to then cause a slow decline in expression throughout the remainder of the infusion period. The second exemplar of a gene responding to both dosing regimens with decreased expression (Fig. 6, *right*) is C-type lectin, superfamily member 13, also known as Kupffer cell receptor (Kclr), which is involved in the cellular immune response (22). The response of this gene to acute dosing is a longer lasting transient period of decreased expression than is seen with paqr7. However, the response to chronic dosing is a decreased expression that is sustained throughout the entire infusion period.

The remaining 145 of the 358 genes showed profiles suggesting complex regulation involving both enhanced expression and down-regulated expression. In the initial analysis of the acute response dataset, we identified two clusters of genes with what we referred to as biphasic regulation in which there was an initial down-regulation followed by enhanced expression. Figure 7 shows the acute and chronic profiles of two genes that show initial down-regulation in the acute profile followed by a period of enhanced regulation. The first gene (Fig. 7, *left*) is G0/G1 switch gene 2 (GOS2), which is involved in cell cycle regulation (23). The acute profile shows down-regulation followed by enhanced expression. In the response to chronic infusion, the initial down-regulation phase is only suggested by one point and is followed by enhanced expression that continues throughout the entire 168 h. However, the pattern suggests that a third factor may be influencing expression beyond 48 h.

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In preliminary experiments, we determined that the release from the pump required about 6 h to reach steady-state blood MPL concentrations. This governed our choice of 6 h as the first sampling point. The second exemplar of down-regulation followed by enhanced regulation (Fig. 7, *right*) is arginase 1 (Arg1), which is involved in ammonia detoxification (24). Acutely the profile shows a sharp down-regulation followed by a period of enhanced regulation and a return to baseline by 48 h. However, in the response to chronic infusion, the initial down-regulation was missed due to our sampling schema. The profile does suggest that a second influence may be intervening after 24 h to maintain the enhanced expression throughout the remainder of the infusion period.

Figure 8 provides examples of two genes in which the profile suggests a period of enhanced expression followed by down-regulation. The first exemplar (Fig. 8, *left*) is heterogeneous nuclear ribonucleoproteins methyltransferase-like 2 (Hrmt1L2), which is involved in the regulation of transcription (25). This enzyme shows a period of enhanced regulation followed by down-regulation in both profiles. The second exemplar in this group is nuclear receptor superfamily 0, group B, member 2 (NR0b2), also known as small heterodimer partner (Fig. 8, *right*). Proteins in this superfamily bind and are activated by small hydrophobic hormones such as retinoic acid, thyroid hormone, and steroids. This superfamily also includes orphan nuclear receptors (26). The acute profile for this gene shows a rapid and short-lived transient increase followed by a relatively long-lasting period of down-regulation. The chronic profile misses the initial up-regulation but captures what appears to be a two-phase down-regulation.

Gene groupings and expression profiles

We also searched all 358 genes primarily using the NCBI "search across databases" feature. Based on this information and domain knowledge, we grouped the 358 genes primarily on function with two additional groups based on subcellular localization (mitochondrial and plasma membrane). These groups are presented in Tables 1–13 and list identifying criteria as well as temporal responses after both acute and chronic treatments. Although not perfect, these groupings together with the expression profiles provide insight into the global impact of corticosteroids on the liver. The most highly populated group is termed transcription-translation, which contains 54 genes (Table 1). The majority of genes in this group are transcription factors, indicating that a major influence of corticosteroids derives from their ability to alter the effect of other influences of transcription. Because alterations in the amount of messages have an impact only once they are reflected as changes in protein, the consequence of these changes should be delayed in time. Such a time delay may therefore explain the significant number of genes that express complex regulation. Also included in this group are a number of genes such as Nap1l1, Ddx3x, Nopp140, IMP3, EIF2C1, and EIF3 whose enhanced expression indicate increased translational activity, which is consistent with the generally anabolic effect of corticosteroids on liver.

The second most populated group is termed signaling with 53 members (Table 2). Eighteen members of this group show up-regulation in both the acute and chronic profiles, and 15 show down-regulation in both acute and chronic. What is unusual about this group is the large number of genes, 21, that show complex regulation. As might be expected, this group

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is dominated by kinases and phosphatases. We have also included in this group several membrane receptors that could have been included in the membrane group but were included in this group because of their involvement in signaling. Of particular interest is paqr7 whose endogenous ligand is adiponectin, a hormone produced by adipose tissue that is involved in the regulation of systemic energy metabolism (21). The down-regulation of this receptor after both acute and chronic dosing may provide additional insight into the metabolic dysregulation attendant on corticosteroid treatment.

The third most populated group is termed small molecule metabolism and contains 38 genes (Table 3). The large numbers of genes in this group reflect both the major role of the liver in small molecule metabolism and the impact of corticosteroids on this function. This group is also unusual in the large number of genes showing complex regulation.

The next most populated group is classified as plasma membrane localized with 33 members (Table 4). Because the surface membrane of a cell mediates its interaction with the external environment, this group by necessity is difficult to distinguish from the signaling group. Pharmacologically, corticosteroids are used for their antiinflammatory and immunomodulatory effects. The liver plays a major role in immune protection both directly and indirectly. The direct effects are the production and secretion of a variety of proteins involved in immune responses such as complement proteins. Indirectly, the liver harbors the Kupffer cell population that provides a defense barrier between the hepatic portal system and general circulation. The immune related group contains 31 genes (Table 5). These 31 genes illustrate the broad impact of corticosteroids on both immune and inflammatory processes. In addition, these results illustrate the type of coordinated effects corticosteroids have on the immune system. For example, complement protein C1q is down-regulated in both the acute and chronic profiles, whereas complement protein C1q binding protein, which binds and inhibits C1q activation, is up-regulated in both (27, 28).

The next group are 29 genes related to protein and amino acid metabolism (Table 6). The liver both produces and degrades serum proteins (29). In addition, the liver is only one of two tissues that can synthesize glucose (30). Amino acid carbon from the musculature is a major substrate for gluconeogenesis, which requires that the resulting ammonia be detoxified. We have included proteosome genes, aminotransferases, and chaperonins as well as a variety of genes involved in the metabolism and synthesis of several amino acids.

The next group of genes, containing 23 members, is termed nuclear encoded mitochondrial genes (Table 7). In this group, all but four genes have chronic profiles indicating enhanced expression. Interestingly, a significant number of these genes show an initial down-regulation followed by up-regulation, giving the acute profiles a biphasic characteristic. Of particular interest in this group is the up-regulation of pyruvate dehydrogenase kinase 1 (Pdk) and the down-regulation of pyruvate dehydrogenase phosphatase isoenzyme 2 (Pdp). The pyruvate dehydrogenase complex is a matrix multienzyme complex that provides the primary link between glycolysis and the tricarboxylic acid (TCA) cycle. This complex is inactivated when it is phosphorylated by Pdk and activated when it is dephosphorylated by Pdp (31). The coordinated opposite regulation of these two enzymes in effect breaks the link

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between glycolysis and the TCA cycle, which would contribute strongly to the insulin resistance caused by corticosteroids.

The liver also is centrally involved in systemic lipid distribution and metabolism. We identified 21 genes associated with lipid metabolism (Table 8). The regulation of these genes may provide insight into the dyslipidemia caused by corticosteroids. For example, the up-regulation of apolipoprotein C-IV suggests the increased production of very-low-density lipoproteins. Very-low-density lipoproteins transport triacylglycerols from liver to extrahepatic tissues (32). Corticosteroids also influence the expression of 16 transporters (Table 9). Because many of these transporters are located on the sinusoidal membranes of hepatocytes, they may influence a variety of functions such as small molecule metabolism. For example, Slc22a7 is involved in the hepatic uptake of organic anions such as salicylate (33). The down-regulation of this gene could alter the metabolism of such drugs.

The next 10 genes are grouped because they are involved in cellular fates, proliferation, differentiation, and apoptosis (Table 10). We have included ornithine decarboxylase 1 (Odc1) and ornithine decarboxylase antizyme inhibitor (oa-zin) because polyamines are important to cell proliferation. Odc, the first enzyme in polyamine synthesis, is regulated by a destabilizing antizyme that both inhibits its activity and accelerates it degradation. Oazin binds and traps the anti-zyme, thus promoting Odc activity (34, 35). The fact that both are up-regulated illustrates that corticosteroid treatment promotes polyamine synthesis in a coordinated fashion.

The two profiles also identified 10 genes involved in carbohydrate metabolism (Table 11). Some of these genes are involved in both glycolysis and gluconeogenesis. For example, aldolase B is a reversible enzyme that is necessary for gluconeogenesis, so its up-regulation with chronic corticosteroid treatment is reasonable. In contrast, malic enzyme 1 catalyzes the decarboxylation of malate to form pyruvate and provides a link between glycolysis and the TCA cycle (36). The down-regulation of malic enzyme is consistent with the inactivation of the pyruvate dehydrogenase complex discussed above.

We found 19 genes whose expression was regulated but were difficult to put into the above categories and were characterized as other (Table 12). For the most part, these are genes involved in secretory functions of the liver. The accession numbers for every one of the identified probe sets on both chips were submitted to the NCBI Blast program. However, there still remained 16 probe sets that Blasted to the same sequence(s) but could not be identified (Table 13). We have maintained them because identification is an ongoing process.

Discussion

This report describes the mining of a microarray dataset obtained from the analysis of livers from a population of adrenalectomized animals treated with a chronic infusion of MPL for up to 1 wk. Liver RNA from four control animals and four animals killed at each of 10 time points over a 168-h period were applied to individual Affymetrix R230A chips. The dataset was mined using a filtering approach similar to the one applied to datasets developed from

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the liver, skeletal muscle, and kidneys of animals treated with a single bolus dose of MPL where animals were killed at 16 time points after dosing and compared with untreated controls (6 – 8). This filtration approach does not select for probes but rather eliminates probe sets that do not meet explicit requirements. Those probe sets not eliminated are retained for analysis. The filtration yielded a remainder of 1989 probe sets for further consideration. These probe sets were compared with 1518 probe sets that remained after filtering of the dataset obtained after bolus dosing with MPL (6). This comparison yielded 464 probe sets in the bolus dosing dataset that corresponded to 417 probe sets in the chronic infusion dataset. The results identified 358 different genes regulated by both dosing regimens. Because the filtering process is quite stringent, these 358 genes most likely do not include all genes regulated by MPL in the tissue. However, they do provide a basis for evaluating the global effects of corticosteroids on the liver.

The objective of obtaining two time series profiles for each gene is to identify genes with common mechanisms of regulation. The hypothesis is that if two or more genes have a common mechanism of regulation then they should have the same temporal profile in response to all dosing regimens. There are available a variety of clustering methods designed to group genes based on their profiles. However, at present, there is no analytical method available to cluster using two profiles, bi-clustering. Our filtering approach crudely identifies genes that meet minimal criteria for up- and down-regulation based on deviation from baseline. In both the acute (bolus dose) and chronic (infusion) datasets, some profiles met both criteria, suggesting complex regulation. We visually inspected all profiles (464 acute and 417 chronic) and categorized each based on up or down deviation from baseline. In many cases, because of probe set redundancy, more than one profile was available for acute, chronic, or both. These instances are noted on the tables. With this information we proceeded to evaluate acute and chronic profiles together. Based solely on this crude classification, we identified four basic patterns. For more than 60% of the genes, the profile was either up or down in both the acute and chronic profiles. In the remaining cases, the profiles were complex involving both up- and down-regulation.

The value of expression profiling using a rich *in vivo* time course such as this, particularly when two different dosing regimens are examined, is that patterns of transcriptional changes become apparent. Examination of expression patterns, as illustrated by examples presented in Figs. 5– 8, indicate that our crude classification into four basic categories is too simplistic. Even when the more than 60% of the genes where the profiles from the two dosing regimens were either both up or down were examined, the mechanism of regulation is generally not straightforward. Single time point studies allow one to determine only magnitude of change at a single instant, which may not be indicative of either the actual extent or in some cases even the direction of change. This is particularly evident in cases of genes that express a biphasic pattern, where at different points in time the gene may show enhanced as opposed to reduced expression. Furthermore, temporal patterns may provide insight as to the mechanisms involved in regulation of transcriptional activity of a particular gene or genes.

The generally accepted mechanism for most glucocorticoid effects involves binding of free steroid to a cytoplasmically localized receptor, translocation of ligand-bound receptor to the nucleus, binding of a ligand receptor dimer to a specific DNA site [glucocorticoid response

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element (GRE)], and modulation of the amount of selective mRNAs (37). Although some effects on mRNA stability have been noted, a common mechanism involves increasing or decreasing the rate of transcription of particular genes. Previously, studying the enzyme TAT in liver and using both a repeated dosing and a chronic dosing paradigm, we described the phenomena of steroid tolerance (12, 13, 18). In those reports, we demonstrated that MPL treatment caused a rather long-lived down-regulation of the GR (mRNA and protein) and that when a second dose was administered before full recovery of receptor, the enhanced expression of TAT (mRNA and protein) was reduced proportional to the reduced concentration of receptor (18). In subsequent studies, we found that chronic infusion of MPL caused a sustained down-regulation of the receptor (mRNA and protein) and that the expression of TAT (mRNA and protein) returned back toward the baseline in the continuous presence of the drug (12, 13). If the receptor mediates the effect of the drug, then this is a rational result. Figure 5 (*left*) shows that the acute and chronic profiles of Odc1 come close to approximating those for TAT. Similarly, Fig. 6 (*left*) to some degree approximates this result for down-regulation. However, the major question posed by a perusal of the remaining profiles is: how can the drug continue to have sustained high-level effects when the receptor is greatly diminished to the point of almost being gone?

There are a number of possible explanations for more complex expression profiles that do not show tolerance. One rational possibility is that our concept of the structure and function of the GRE is entirely too simplistic. If multiple GREs with greatly different affinities for the drug receptor complex exist, then the type of result seen for Kclr in Fig. 6 (*right*) could be explained. A second and related possibility is that the GR is not a single entity. This possibility is supported by the recent report by Lu and Cidlowski (38), who showed the existence of multiple GR isoforms with different trafficking and transcriptional activities. It is possible that different isoforms may have different GRE binding affinities. Another possibility is that our concept that only the GR can mediate the effects of corticosteroids may be simplistic and that some other receptor can mediate the effects of these drugs. Alternatively, secondary or tertiary effects may involve glucocorticoid modulation of a secondary biosignal (which likely could be a different transcription factor) that in turn would modulate transcriptional activity. In addition, changes in the physiological states of the animal (such as altered glucose or lipid levels) with chronic treatment could also act as an additional biosignal that leads to secondary and tertiary changes in gene expression. This possibility is suggested by the chronic profile for Tdo seen in Fig. 5. Initially, it appears as if Tdo is going to respond with a pattern similar to Odc1 and return to baseline, but the expression is enhanced again to even a higher level that is maintained throughout the remainder of the infusion period. In any case, the results demonstrate that at present there is much we do not know about how corticosteroids influence gene expression.

We attempted to use domain knowledge to sort the 358 genes into groups, which are presented in Tables 1–13. In particular, Tables 1 and 2 demonstrate the broad impact these drugs have on transcription and translation as well as signaling. These results provide interesting examples of the type of physiological coordination that exists. Corticosteroids cause both insulin resistance and gluconeogenesis. The enhanced expression of Pdk along with the down-regulation of Pdp should maintain the pyruvate dehydrogenase complex in an

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inactive state, preventing pyruvate from entering the mitochondrial TCA cycle. The down-regulation of malic enzyme would also contribute to the cellular depletion of pyruvate. In contrast, the expression of aldolase B is enhanced, contributing to gluconeogenesis. Likewise, the down-regulation of complement protein C1q along with the enhanced expression of C1q binding protein demonstrates similar coordination, as does the enhanced expression of both Odc and Oazin.

Tables 1–13 present lists of genes regulated by both acute and chronic corticosteroid treatment. In these tables, we qualitatively list the response of each gene as down, up, down/up, or up/down to both acute and chronic treatments. We have not attempted to include a quantitative measure of magnitude of change for each gene in the two treatments. We feel that to choose a single time point to define a maximum or minimum would be potentially misleading, because it is the response pattern over time that is a true measure of magnitude of response. For example, one probe set response may show a sharp peak that rises and declines over a 4-h period, whereas a different gene may respond with a broader but shallower peak over an extended time range. In such a case, presenting magnitude of change at a single time is not a valid comparison of expression levels of the two. The situation becomes more complicated when one considers complex patterns of regulation (*i.e.* initial up-regulation followed by later down-regulation or vice versa).

All datasets described in this and related cited publications are available online in GEO. In addition, all data are available online at the Public Expression Profiling Resource site (http://pepr.cnmcresearch.org) developed and maintained by the Hoffman laboratory at Children's National Medical Center (39). These data are available to all researchers in a user-friendly format, where individual temporal profiles are searchable, and all data can be obtained and used without requirement for any additional specialized software.

Acknowledgments

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Abbreviations

GR Glucocorticoid receptor

GRE glucocorticoid response element

MPL methylprednisolone

TAT tyrosine aminotransferase

TCA tricarboxylic acid

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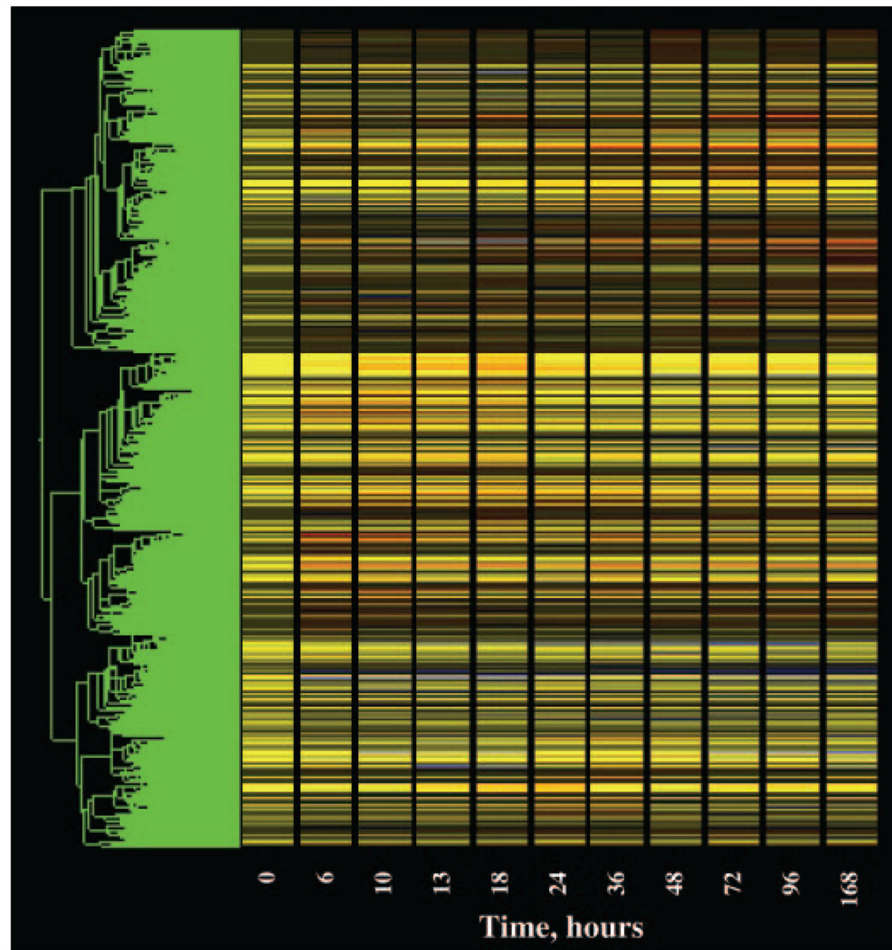

Fig. 1.

Gene tree representation of all probe sets (15,967) on individual Affymetrix R230A gene chips hybridized to total RNA prepared from livers taken from animals treated chronically with MPL (0.3 mg/kg·h) for periods ranging from 6 –168 h. The values for each individual probe set at each time point were normalized to the mean value of that probe set for time zero controls. The x-axis represents the 11 time points, including time zero controls. The y-axis presents the list of probe sets grouped by similarity using Pearson's correlation. *Yellow* indicates no change from controls, *red* indicates probe sets with enhanced expression relative to controls, and *blue* indicates suppressed expression relative to controls.

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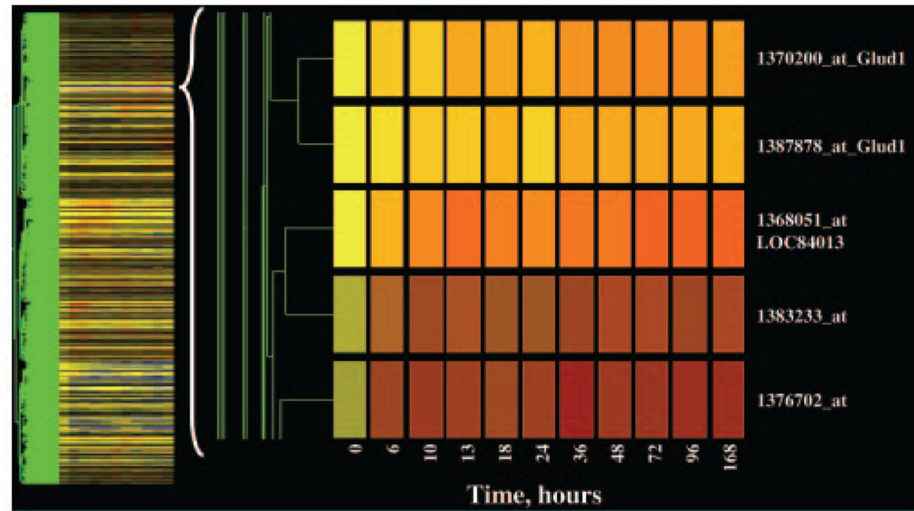

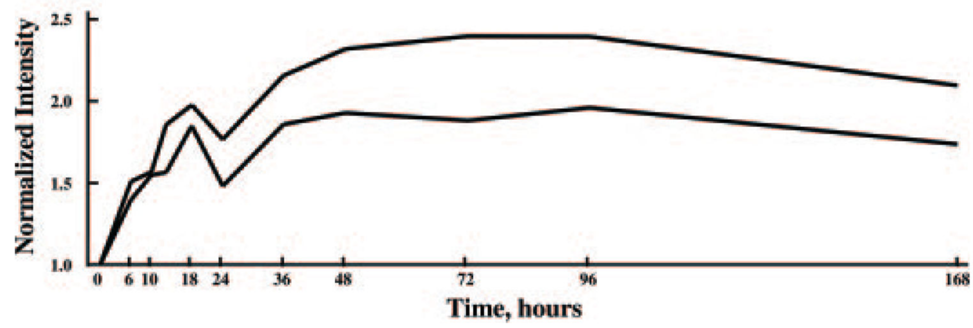

Fig. 2.
The *top panel* provides a magnification of five probe sets selected from Fig. 1 that show apparent enhanced regulation by MPL. Two of these probe sets represent the same gene (Glud1). The linear plots for both Glud1 probe sets are presented in the *lower panel*.

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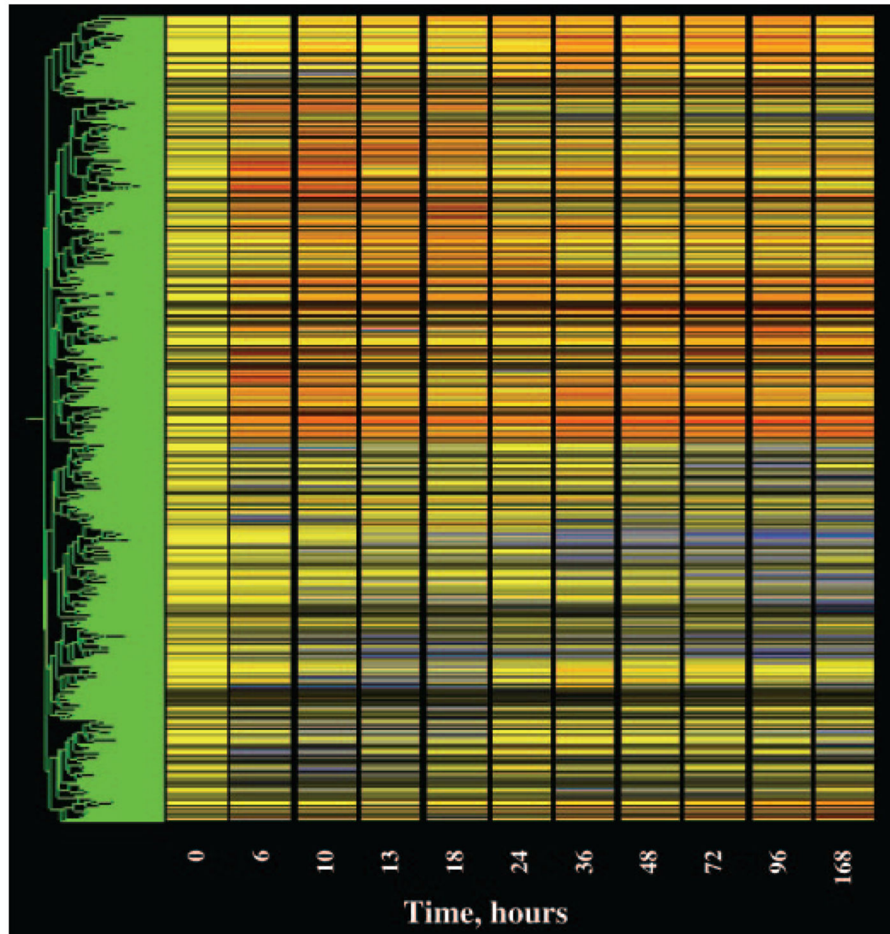

Fig. 3.
Gene tree representation of probe sets remaining (1989) after filtering.

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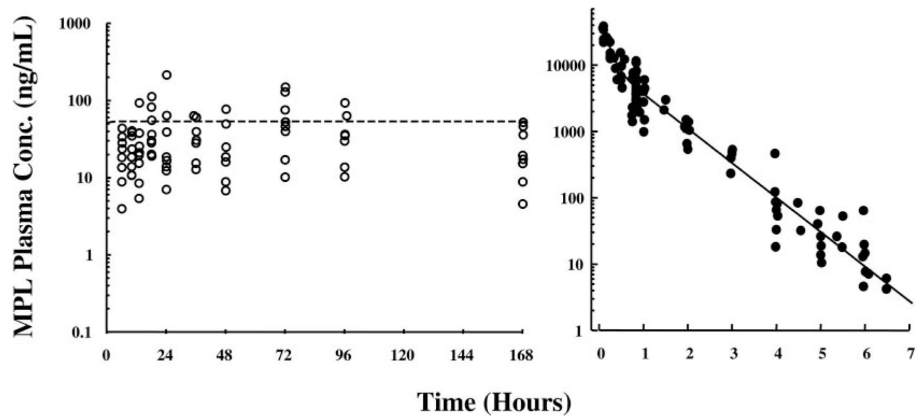

Fig. 4.

MPL concentrations in rat plasma after chronic (*left*) and acute (*right*) administration of drug. MPL concentrations were determined by normal-phase HPLC analysis of plasma samples obtained from individual animals.

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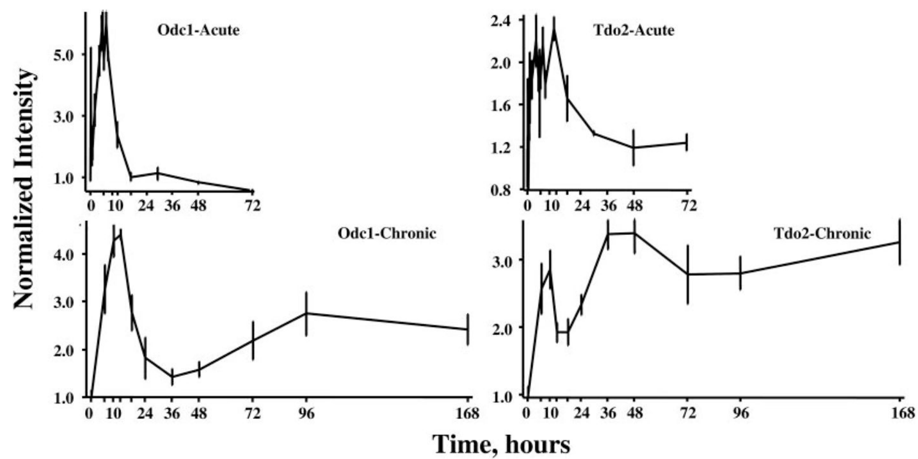

Fig. 5.

Response profiles of two genes showing enhanced expression after acute and chronic MPL dosing. Ornithine decarboxylase (Odc1) expression is shown in the two *left panels* and exhibits a similar pattern of enhanced expression with acute and chronic dosing. Tryptophan 2,3-dioxygenase (Tdo2) is presented in the two *right panels* and exhibit dissimilar patterns with acute and chronic dosing.

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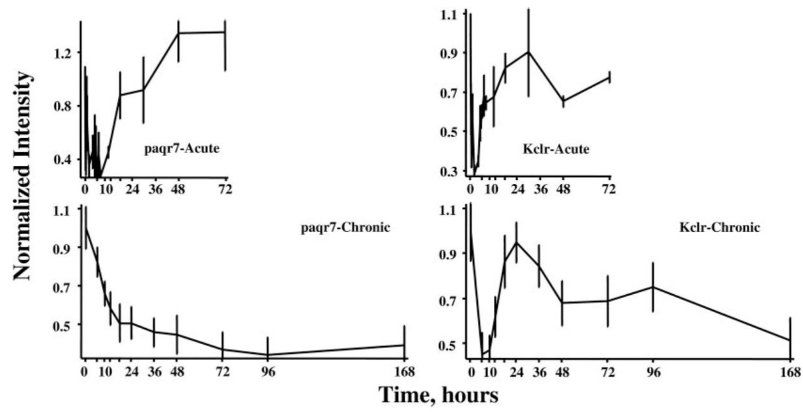

Fig. 6.

Response profiles of two genes showing reduced expression after acute and chronic MPL dosing. Progestin and adipoQ receptor family member VII (paqr 7) expression is shown in the two *left panels* and exhibits a similar pattern of reduced expression in the two dosing regimens. Kupffer cell receptor (Kclr), which exhibits dissimilar patterns of regulation with acute and chronic dosing, is presented in the *two right panels*.

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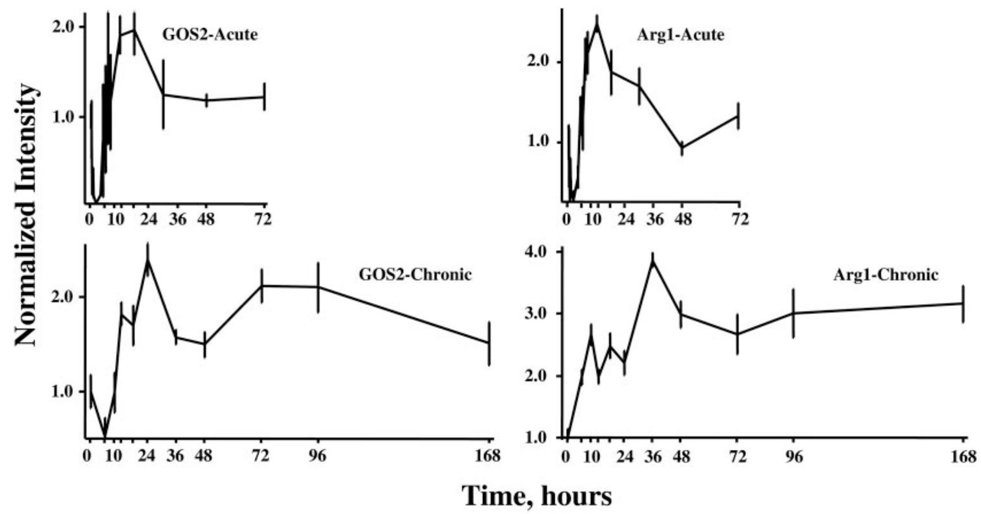

Fig. 7.

Response profiles of two genes showing complex regulation of expression after acute and chronic MPL dosing. G9/G1 switch gene 2 (GOS2) and Arginase (Arg1) both exhibit initial down-regulation followed by a period of enhanced regulation.

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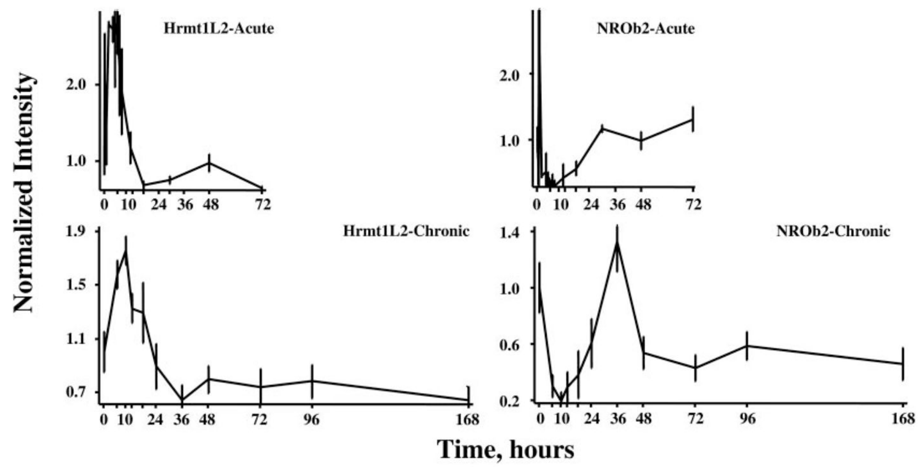

Fig. 8.

Response profiles of two genes showing complex regulation of expression after acute and chronic MPL dosing. In this case, both heterogenous nuclear ribonucleoprotein methyltransferase (Hrm1L2) and nuclear receptor superfamily 0, group B member 2 (NROb2) show initial up-regulation followed by a later down-regulation.

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TABLE 1

MPL-regulated probe sets related to transcription-translation

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1387947	U56241	U56241	Mafb	Transcription factor Maf1	Up	Up
1387870	AB025017	X63369	TIS11	Transcription factor (immediate early gene)	Up	Up
1386910	AF311054	D44495	Apex	Apurnic Endonuclease (DNA repair)	Up	Up
1371987	BI274697	AA891891	DNA repair	Polymerase (DNA directed) sigma (DNA repair)	Up	Up
1367601, 1367602	NM_053698	AA900476 (2), AI014091	Cited2	Cbp/p300-interacting transactivator	Up	Up
1387779	NM_031668	AI237258, AI229637	Mybbp1a	MYB binding protein (P160) 1a	Up	Up
1368308	NM_012603	Y00396 (2)	Myc	Myelocytomatosis proto-oncogene	Up	Up
1367831	NM_030989	X13058	Tp53	Tumor protein p53, p53 tumor sUppressor	Up	Up
1389508, 1374404	BI288619	AA944014		v-jun Sarcoma virus 17 oncogene	Up	Up
1389521	AI408553	AA799539	IVNS1ABP	Influenza virus NS1A binding protein	Up	Up
1392633	BE105480	AA946439	H4	Histone H4	Up	Up
1371872, 1370826, 1373473	BM386384	AF062594 (2), AA859920, AA866472	Nap1l1	Nucleosome assembly protein 1-like 1	Up	Up
1372242, 1375901	AI169598	U21719	Ddx3x	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, helicase (RNA)	Up	Up
1368031, 1368032, 1368033	NM_022869	AA998882, M94287	Nopp140, Nolc1	Nucleolar phosphoprotein 140, interacts with RNA pol	Up	Up
1388321	BG378108	AA799369	IMP3	U3 small nucleolar ribonucleoprotein (ribosome biogenesis)	Up	Up
1368867	BF281131	H31692	EIF2C1, GERP95	Translation initiation factor 2C	Up	Up
1388576	BF281368	AA875205 (2)	EIF3	Translation initiation factor 3	Up	Up
1372090	AI231566	D14448	Max	Myc family DNA-binding protein, leucine zipper	Up	Up
1388779, 1387962	U41164, BG381516	U41164	rKr1, Zfp180	Cys2/His2 zinc finger protein (Kruppel family)	Up	Up
1373012, 1373011	BE109520 (2)	AA894086	Zfp622	Zinc finger protein 622	Up	Up
1371864, 1370209	BE101336, AW524563	D12769	Bteb1	Klf9, Basic transcription element binding protein 1	Up	Up
1387714, 1369737, 1369738	AB031423, NM_017334 (2)	S66024	CREM	Transcriptional repressor, cAMP-responsive element	Up	Up

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230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1371781	BI285863	AI639141, X91810	Stat3	Signal transducer and activator of transcription 3	Up	Up
1371714	BG378760	AA893885	Foxk2	Forkhead box K2	Up	Up
1371489	AI011748	AF022081	Rnf4	Small nuclear RING finger protein	Up	Up
1370510	AB012600	AF015953	ARNTL, BMAL1b	BMAL1b, aryl hydrocarbon receptor nuclear translocator-like	Up	Up
1370309	AJ238854	AB016536	alf-c1	Heterogeneous nuclear ribonucleoprotein type A/B	Up/down	Up
1370474, 1370474	J03933	J03819	Thrb, (c-erbA-β)	Rat thyroid receptor hormone β	Down/up	Up
1387365	NM_031627	U11685	Nr1h3	Liver X receptor, α	Down/up	Up
1372876	AA799700	AA799700	SPS2	Selenophosphate synthetase 2	Down/up	Up
1369679	AB060652	D78018, × 13167	NFI-A	Transcription factor nuclear factor1-A1	Down/up	Up
1369834	NM_012742	X55955	Hnf3a, Foxa1	Hepatocyte nuclear factor 3-α Forkhead box A1	Down	Up
1375428	BE099979	AA858607	Creg1	Repressor of E1A-stimulated genes (cell growth and differentiation)	Down	Up
1376196	BG375059	AA800290		D receptor interacting protein	Down	Down
1389601, 1373644	BI293610	AA859994	Nfib	Nuclear factor I/B	Down	Down
1387769	AF000942	AI171268, AF000942	Id3	DNA binding protein inhibitor 3 BHLH	Down	Down
1368712	NM_019620	U67082	Kzf1	Zinc finger protein 386 (Kruppel-like)	Down	Down
1369959	NM_017172	AI136891, AI112516	Zfp36l1	Zinc finger protein 36, C^3H type-like 1, Kruppel Type 18	Down	Down
1368711	NM_012743	L09647	Hnf3b, Foxa2	Forkhead box A2	Down	Down
1373837	BI296633	AI227715	Rbl2	Retinoblastoma-like 2 (p130)	Down	Down
1369063	NM_012903	AA875495, D32209,	Anp32	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	Down	Down
1367826	NM_031789	AI177161	NF-E2-related factor 2	Nuclear factor erythroid 2-like 2	Down	Down
1388650	BM385445	AA899854	Top2a	Topoisomerase (DNA) 2α	Down	Down
1372889	AI407489	M64862, AA800797		DNA-binding protein; matrin F/G	Down	Down
1367759	NM_012578	AI232374	H1f0	Histone H1–0	Down	Down
1370381	U61729	U61729	Prol2	Proline rich 2	Up/down	Down
1368376	NM_057133	D86580	Nr0b2	Small heterodimer partner; SHP; SHP1	Up/down	Down
1374752	AI408734	AI639149	Mdfic	MyoD family inhibitor domain containing	Up/down	Down

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1398756,1398757, 1398758	NM_012992	J03969, J04943	Npm1	Nucleophosmin 1, nuclear protein B23	Up	Down
1398832	NM_012749	M55015, M55017	Ncl	Nucleolin	Up	Down
1370711	AF000900	U63839, AF000899, AF000901	p58/p45	Nucleoporin p58	Up	Down
1387028	M86708	L23148	Id1	DNA-binding protein inhibitor 1 BHLH	Up	Down
1372757, 1368835, 1387354	BM386875	AA892553	Stat1	Activator of transcription 1	Up	Down
1386897	NM_024363	U60882	Hrmt1l2	Heterogeneous nuclear ribonucleoproteins methyltransferase-like 2	Up/down	Up/down
1368303	NM_031678	AB016532	Per2	Period homolog 2	Oscillating	Oscillating

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration and categorized as relating to either transcription or translation. Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification.

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TABLE 2

MPL-Regulated probe sets related to cell signaling

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1371081	U78517	U78517	Cgef2, Rapgef4	Rap guanine nucleotide exchange factor (GEF) 4	Up	Up
1367960	NM_019186	X77235	Arl4	ADP-ribosylation factor-like 4	Up	Up
1398790	NM_017039	AI012595	Ppp2ca	Protein phosphatase 2a, catalytic sub-unit, α isoform	Up	Up
1368147, 1368146	U02553, BE110108	U02553	Ptpn16, Dusp1	MAPK phosphatase-1, protein tyrosine phosphatase non-receptor-type 16	Up	Up
1387078	NM_031002	U26397	Inpp4a	Inositol polyphosphate-4-phosphatase, type 1	Up	Up
1398847, 1370180	AA891213, BG376935	U95001	Nudt4	Diphosphoinositol polyphosphate phosphohydrolase 2	Up	Up
1377136, 1367697	NM_031020, AW254190	AA924542, U73142 (2), AI171630, U91847, AI137862	Mapk14, p38	MAPK 14	Up	Up
1367802	NM_019232	L01624	Sgk	Serum/glucocorticoid-regulated kinase, serine/threonine protein kinase SGK	Up	Up
1367725	NM_022602	AF086624	Pim3	Serine/threonine-protein kinase pim-3	Up	Up
1380262	AA893436	AA893436	sgk	Serum/glucocorticoid regulated kinase	Up	Up
1368947	NM_024127	L32591 (2), AI070295, AI070295	Gadd45a	Growth arrest and DNA-damage-inducible 45α	Up	Up
1369065	NM_017290	J04024	Atp2a2, Serca2	ATPase, Ca^{2+} transporting	Up	Up
1387154	NM_012614	M15880	Npy	Neuropeptide Y (Npy)	Up	Up
1372558	AI177404	AI177404		NMDA receptor-regulated gene 1	Up	Up
1389974	BF555171	L15618	CK2	Casein kinase II α subunit	Up	Up
1387907	J05510	J05510	InsP3R1 I145TR	Inositol 1,4,5-triphosphate receptor	Up	Up
1373306	BM386212	M81639	Snn	Stannin	Up	Up
1372355	BE109242	U09793	c-Ki-ras	Kirsten rat sarcoma viral oncogene homolog 2	Up	Up
1373082	AA893743	AA893743		Protein kinase inhibitor, α	Up	Up/down
1372770	BF281357	X13933	CaMI	Calmodulin 1	Up	Down/up
1390240	BM389611	AA800456	CKLiK	Calcium-calmodulin-dependent kinase I-like kinase (CKLiK)	Down	Up

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230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1377417	BE099931	AA800678	Ticam1	Toll-like receptor adaptor molecule 1	Down	Up
1370522	L04796	M96674	Gcgr	Glucagon receptor	Down	Up
1387981	AF079864	AF079864	Olfr78	G-protein coupled receptor RA1c, olfactory receptor 59	Down	Up
1368675	NM_032084	AI232194	CHN2	Chimerin (chimaerin) 2	Down	Up
1368289	AA944965	M12450	Vdbp, DBP02	Group-specific component (vitamin D-binding protein)	Down	Up
1390798	BF288130	M10072	L-CA	Leukocyte common antigen	Down	Down
1387024	NM_053883	X94185	mkp-3	MAPK phosphatase	Down	Down
1368871	NM_053887	U48596	Map3k1	MAPK kinase kinase 1	Down	Down
1368646	NM_017322	L27112, AI231354	Mapk9	MAPK 9	Down	Down
1387024	NM_053883	X94185, U42627	Dusp6	Dual specificity phosphatase 6	Down	Down
1368646	NM_017322	AI231354	SAPK	Stress-activated protein kinase α II	Down	Down
1372844, 1398273	AW531877, NM_053599	AA892417, D38056	Efna1, B61	Eph-related receptor tyrosine kinase ligand 1	Down	Down
1389779	AA800626	AA800626	Sh2d4a	SH2 domain containing 4A	Down	Down
1375879	BE111762	AF061443	Gpr48	G protein-coupled receptor 48	Down	Down
1367644	L01115	L01115	Adcy6	Adenylyl cyclase type VI	Down	Down
1369644	NM_134408	AF063102	CIRL-2	Calcium-independent α-latrotoxin receptor homolog 2, latrophilin 2	Down	Down
1368924	NM_017094	Z83757 (2)	Ghr	GH receptor	Down	Down
1377966	BI275560	AA894316	paqr7	Progestin and adipoQ receptor family member VII	Down	Down
1367745	NM_031143	D78588	Dgkz	Diacylglycerol kinase ζ	Down	Down
1371969	BI291848	AI180288	Cald1	Caldesmon 1	Down	Down
1368202	NM_024159	U95178	Dab2	Disabled homolog 2	Up/down	Down
1367881	NM_013016	D85183	SHPS-1	Protein tyrosine phosphatase, non-receptor-type substrate 1	Up/down	Down
1370949, 1370948, 1373432	BE111604, M59859	AA859896, AA925762, AA899253	Macs	Myristoylated alanine-rich C-kinase substrate	Up	Down
1368856	NM_031514	U13396, AJ000557, U13396	Jak2	Janus kinase 2	Up	Down
1368693	NM_024145	X57018	Fgr	Gardner-Rasheed feline sarcoma viral oncogene homolog (kinase)	Up	Down
1367844	M12672	AA875225	G-α-i2	GTP-binding protein	Up	Down

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1368975, 6, 1390325	NM_013127, D30795, BI289418	D29646	Cd38	ADP-ribosyl cyclase; CD38	Up	Down
1368821, 1368822, 1372331	BG665037, BI290885, NM_024369	AA891233	Fstl1	Follistatin-related protein	Up	Down
1370288	AF372216	X02412, M60666	TP-3	Tropomyosin α-isoform	Up	Down
1388140	AW253722	M83678	Rab13	RAB13, member RAS oncogene family	Down/up	Down
1370414	M94043	M94043	Rab38	Rab38, member of RAS oncogene family	Down/up	Down
1368821	NM_024369	AA859885, AA891233	Frp, Fstl1	Follistatin-like 1, activin-binding protein	Down/up	Down
1368536	NM_057104	D28560	NPH-type III	Phosphodiesterase I/nucleotide pyrophosphatase 2	Down	Down/up/down

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration and categorized as relating to cell signaling. Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification.

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TABLE 3

MPL-regulated probe sets related to small molecule metabolism

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1387109	NM_031576	AI137856, M10068	Por	P450 (cytochrome) oxidoreductase	Up	Up
1371021, 1375412	AI101331	D49434	Arsb	Arylsulfatase B, lysosomal Glycosaminoglycan degradation	Up	Up
1370370	AF034218	AF034218 (2)	Hyal2	Hyaluronidase 2, lysosomal glycosaminoglycan degradation	Up	Up
1387963	M24396	J03959, × 13098		Uricase, urate oxidase 2	Up	Up
1387740, 1379361	NM_053487	AA892128, AJ224120	Pex11a	Peroxisome biogenesis factor 11A	Down/up	Up
1368180	NM_017013	AI235747	Gsta2	Glutathione-S-transferase, α-type 2	Down/up	Up
1369986	NM_033349	AI012802	Hagh	Hydroxyacyl glutathione hydrolase, both cytosol and M	Down/up	Up
1367798	NM_017201	M15185	Ahcy	S-adenosylhomocysteine hydrolase, cytosol	Down/up	Up
1370698	M13506	M13506	UDPGTR-2	Liver UDP-glucuronosyltransferase, phenobarbital-inducible, microsomal	Down/ up	Up
1398307	D38381	D38381	Cyp3a18	P450 6β-2	Down/up	Up
1387314	NM_022513	D89375	Sult1b1	Sulfotransferase family 1B, member 1 cytosol	Down/up	Up
1387825	NM_031533	J02589		Androsterone UDP-glucuronosyltransferase	Down/up	Up
1368226	NM_133525	U82591	Rcl, C6orf108	Nucleoside 2-deoxyribosyltransferase domain (c-Myc-responsive)	Down/up	Up
1387659	AF245172	AA859837 (2)	Gda	Guanine deaminase	Up	Down/up
1387973	U39206	U39206	CYP4F4	CYP4F4	Down	Up
1387243	K02422	K03241	Cyp1a2	Cyp1a2	Down	Up
1370496	AB008424	AB008424	Cyp2d3	Cyp2d3	Down	Up
1367843	NM_134407	AA892821 (2)	Akr7a2, Aiar	Aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase), perox	Down	Up
1388300	AA892234	AA892234	Gst3	Glutathione S-transferase 3, microsomal	Down	Up
1368717	NM_024132	U72497	Faah	Fatty acid amide hydrolase (degrades bioactive fatty acid amides)	Down	Up
1387958	L32601	L32601		20 α-Hydroxysteroid dehydrogenase	Down	Up
1387672	NM_017084	X06150, AA893219	Gnmt	Glycine methyltransferase	Down/up/down	Up
1371076	AI454613	K01721	Cyp2b15	Cyp2b15	Down/up	Down/up

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230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1368497	NM_012833	D86086	Abcc2	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	Down/up	Down
1370397	M33936	AA924591	Cyp4A3	Cytochrome P450 4A3	Up	Down
1370080	NM_012580	J02722	HEOXG Heox Hmox	Heme oxygenas	Up	Up/down
1387916	U39208	U39208	U39208	CYP4F6	Down	Down
1369424	NM_012693	J04187	Cyp2a2	Cyp2a2	Down	Down
1367988	U04733	U04733	cyp 2C23	Cytochrome P450 arachidonic acid epoxygenase	Down	Down
1387669	NM_012844	M26125	Ephx1	Epoxide hydrolase 1, microsomal	Down	Down
1387214	NM_031565	X81395	Ces1	Carboxylesterase 1	Down	Down
1387022	NM_022407	AF001898	Aldh1a1	Aldehyde dehydrogenase family 1, member A1 cytosolic	Down	Down
1369296	NM_031732	L22339 (2)	Sult1a2	Sulfotransferase family, cytosolic, 1C, member 1, *N*-hydroxy-2-acetylaminofluorene; sulfotransferase	Down	Down
1387221	NM_024356	AI639457	Gch	GTP cyclohydrolase 1	Down	Down
1378753	AI638971	AI638971	Tpmt	Thiopurine methyltransferase	Down	Down
1368409	NM_012796	AI138143, D10026	Gstt2	Glutathione S-transferase, $\theta 2$	Down	Down
1370688, 1372523, 1370030	J05181, AA892770, NM_017305	J05181 (3), S65555	Glclc	Glutamate-cysteine ligase, catalytic subunit	Down	Down
1368826	NM_012531	M93257, M60753	Comt	Catechol-*O*-methyltransferase	Up/down/up	Down

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration and categorized as relating to small molecule metabolism. Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification.

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TABLE 4

MPL-regulated probe sets localized to plasma membranes

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1371113	M58040	M58040	Tfrc, CD71	Transferrin receptor	Up	Up
1387027	U72741	U72741	Lgals9	β-Galactoside binding lectin 9	Up	Up
1369716	NM_012976	L21711	Lgals5	β-Galactoside binding protein 5	Up	Up
1386937, 1367814	M14137, AI232036	AI112173	Atp1b1	ATPase Na^+/K^+ transporting β1	Up	Up
1367585, 1371108	M74494, M28647	M74494	Nkaa1b	Na,K-ATPase α-1 subunit	Up	Up
1370262_at	AI706785	AF100421	Lyric	Metadherin, metastasis	Up	Up
1367568_a_at	NM_012862	AI012030	Mgp	Matrix Gla protein	Up	Up
1367579_a_at	BI285434	AA892333	Tuba1	Tubulin, α6	Up	Up
1371542_at	BI284599	J00797		Tubulin, α4	Up	Up
1367669	AI233190	U05784	MPL3	Light chain 3 subunit of microtubule-associated proteins 1A and 1B	Up	Up
1367721	NM_012649	S61868	SYND4	Syndecan 4, ryudocan	Up	Up
1369976_at	NM_053319	AI009806	MGC94628	Dynein, cytoplasmic, light chain 1	Up	Up
1376098_a_at	BF282304	AA875126 (2)	Myo1g	Myosin IG	Up	Up
1369720_at	NM_053986	X68199	Myo1b	Myosin Ib	Up	Up
1374171, 1387287	D83598, AI170507	AF019628	Sur2	Sulfonylurea receptor 2	Up	Up
1373842	BM390718	AA858620	N-WASP	Wiskott-Aldrich syndrome gene-like	Up/down	Up
1370526_at	AF020045	AF020046	Itgae	Adhesion receptor, integrin α E1	Down/up	Up
1369953	BI285141	U49062 (2)	Cd24	CD24 antigen	Up	Down
1373932	BE098739	AA894029	Cybb	Endothelial type gp91-phox	Up	Down
1390659	BI302830	M61875	HAMM CD44A METAA	Cell surface glycoprotein (hyaluronate binding protein)	Up	Down
1368419	AF202115	AI639438	glypican 1	GPI-anchored ceruloplasmin	Up	Down
1387856_at	BI274457	AA944422	Cnn3	Calponin 3	Up/down	Down
1387206_at	NM_031740	AF048687	B4galt6	UDP-Gal:βGlcNAc-β1,4-galactosyltransferase, polypeptide 6	Up/down	Down
1372087	BG666916	AJ012603	TACE	TNF-α converting enzyme	Up/down	Down
1367849_at	NM_013026	X60651, S61865	Synd1	Syndecan 1	Down	Down
1368642, 1387259	AF097593, NM_031333	AF097593 (2)	Cdh2	N-cadherin	Down	Down
1388441_at	BG379987	AA892773		Thymosin, β4	Down	Down

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1386938_at	NM_031012	M25073	Anpep, CD13	Alanyl (membrane) aminopeptidase	Down	Down
1372780_at	BM391310	AA892353		Transmembrane protein 53	Down	Down
1368115_at	NM_031700	M74067	Cldn3	Claudin 3	Down	Down
1387061_at	NM_031047	1387061_at	Jup	Junction plakoglobin, γ-catenin	Down	Down
1367812_at	NM_019167	AB001347	Spnb3	β-Spectrin 3	Down	Down
1383606_at	BI302544	AI639417		Membrane targeting (tandem) C2 domain containing 1	Down	Down

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration and identified as localized to plasma membranes. Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification.

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TABLE 5

Immune-related MPL-regulated probe sets

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1398784	NM_019259	AI178135	C1qbp	C1q binding, inhibits C1 activation	Up	Up
1390104	BI296551	AI010580	Irgq	Immunity-related GTPase family, Q	Up	Up
1388331	BG057543	AA685903		Tumor rejection antigen gp96	Up	Up
1375686	AI706907	AA892680, AA892298	Ppil3	Peptidylprolyl isomerase (cyclophilin)-like 3	Up	Up
1367657	NM_017258	L26268 (2)	Btg1	B-cell translocation gene 1, antiproliferative	Up	Up
1368668	NM_053866	U17901	Plaa	Phospholipase A2, activating protein	Up	Up
1370750, 1369255	NM_013123	M95578 (2), U14010 (2)	Il1r1	IL-1 receptor, type I	Up	Up
1371926, 1373140, 1370957	BM383427	M92340	Il6st	IL-6 signal transducer	Up	Up
1386987	NM_017020	M58587	Il6r	IL-6 receptor	Up	Up
1372926	AI009159	U27201	TIMP-3	Tissue inhibitor of metalloproteinase 3	Up	Up
1370855	BG666933	AI231292	Cst3	Cystatin C (inhibitor of cysteine proteinases) cathepsins	Down	Up
1387127	NM_031351	AA859645	Atrn, DPPT-L	Attractin	Down	Up
1398256	NM_031512	M98820	Il1b	IL-1β	Down	Down
1374334	AI412189	AI234828		Ig heavy chain V-III region VH26 precursor	Down	Down
1371100	AA859049	D00362, M20629	Es2	Esterase 2	Down	Down
1368755	NM_053753	M55532	Kclr	C-type (calcium dependent) lectin, superfamily member 13	Down	Down
1368741	NM_057146	U52948	C9	Complement component C9	Down	Down
1373025	AI411618	AA891576		Complement protein C1q β chain	Down	Down
1370027, 1388229	M22359, NM_023103	M22360, M22359	Mug1	Murinoglobulin 1 homolog, plasma proteinase inhibitor α-1-inhibitor III	Down	Down
1371015	X52711	X52711	Mx1	Myxovirus (influenza virus) resistance 1	Up/down	Down
1370056	NM_020103	M30691	Ly6c	Ly6-C antigen	Up/down	Down
1368332	NM_133624	M80367	Gbp2	Guanylate nucleotide binding protein 2	Up/down	Down
1367614	NM_012904	S57478, AI171962	Anxa1	Annexin A1, lipocortin I	Up/down	Down
1368073	NM_012591	M34253 (2)	Irf1	Interferon regulatory factor 1	Up/down	Down
1368592, 1371170	AJ245643, NM_017019	D00403	Il1a	IL-1α (Il1a)	Up/down	Down
1367679	NM_013069	X13044	INVG34	Histocompatibility: class II antigens, γ-chain of	Up/down	Down
1383564	BF411036	AA799861(2)	Irf7	Interferon regulatory factor 7	Up	Down

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230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1398246	NM_053843	M32062 (2)	Fcgr3	Fc receptor, IgG, low affinity III	Up	Down
1387687	NM_133542	AJ223184	Igsf6	Ig superfamily, member 6	Up	Down
1367574	NM_031140	X62952	Vim	Vimentin	Up	Down
1376151	AI407953	AA891944		Interferon-γ-induced GTPase	Down/up	Down

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration and categorized as immune related. Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification.

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TABLE 6

MPL-regulated probe sets related to protein or amino acid metabolism

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1386923, 1375642	M62388	M62388 (2), AA799612		Ubiquitin-conjugating protein	Up	Up
1371814	BI282191	AA859722	Ube2g2	Ubiquitin-conjugating enzyme E2G 2	Up	Up
1382059	BI289529	AI639506	Fbxo30	F-box protein 30 (Fbxo30), protein-ubiquitin ligases	Up	Up
1392633	AI045724	AI639312	Fbxo32	F-box only protein 32 (Fbxo32), protein-ubiquitin ligases	Up	Up
1370964	BF283456	X12459	Ass	Argininosuccinate synthetase (ammonia detoxification)	Up	Up
1368720	NM_022403	AA945143	Tdo2	Tryptophan 2,3-dioxygenase (kynurenine pathway)	Up	Up
1368247	NM_031971	AI170613 (2)	Hspa1a, Hsp10	mitochondrial chaperonin	Up	Up
1398960	AI172328	AA875047	TCP20	chaperonin subunit 6a (ζ)	Up	Up
1388698	AI236601	AI236601	HSP105	Heat shock protein 105	Up	Up
1389021	BF284746	AA799531	ASNS	Asparagine synthetase domain containing 1	Up	Up
1368188	NM_017233	AA866302	Hpd	4-Hydroxyphenylpyruvic acid dioxygenase	Down/up	Up
1367695	NM_022390	J03481	Qdpr	Quinoid dihydropteridine reductase (PKU)	Down/up	Up
1368794	NM_020076	D28339, D44494	Haao	3-Hydroxyanthranilate 3,4-dioxygenase (synthesis of quinolinic acid)	Down/up	Up
1368266	NM_017134	J02720	Arg1	Arginase 1 (ammonia detoxification)	Down/up	Up
1370200, 1387878	AI179613, AI233216	BI284411	Glud1	Glutamate dehydrogenase 1(ammonia detoxification)	Down	Up
1368085	NM_133595	U85512	Gchfr	GTP cyclohydrolase I feedback regulator (phenylalanine, tyrosine, and tryptophan hydroxylases)	Down	Up
1368092	NM_017181	M77694	Fah	Fumarylacetoacetate hydrolase (tyrosinemia type I)	Down	Up
1367627	NM_031031	U07971	Gatm	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	Down	Down
1387307	NM_017159	M58308, AB002393	Hal	Histidine ammonia lyase	Down	Down
1373686	AA893495	AA893495	Serpina6	Serine (or cysteine) proteinase inhibitor, clade A (α-1 antiproteinase, antitrypsin), member 6	Down	Down
1387193, 1368447, 1368446	NM_012674	M35299	Spink1	Serine protease inhibitor, Kazal type 1	Down	Down
1368280	NM_017097	D90404 (2)	Ctsc	Cathepsin C	Down	Down
1370386	AB002406	AB002406	Ruvbl1	Ribosomal protein s25	Up/down	Down
1374255	BI281789	AA875602		Phenylalanine-tRNA synthetase-like, α-subunit	Up/down	Down
1373592	AI407094	AA875037		Serine proteinase inhibitor mBM2A, serine (or cysteine) peptidase inhibitor, clade B, member 9	Up/down	Down
1373263, 1376737	AW523737	H31976	SUMO	Sentrin specific protease 5	Up	Down

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230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1367710	NM_017257	D45250	Psme2	Proteasome (prosome, macropain) 28 subunit, β	Up	Down
1375421	AI600019	AA894089	Neurodap1	Neurodegeneration associated protein 1	Up	Down
1372665	AI230228	AI230228		Phosphoserine aminotransferase	Up	Down

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration and categorized as relating to protein or amino acid metabolism. Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification.

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TABLE 7

Nuclear-encoded mitochondrial genes regulated by MPL

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1398870, 1370785	D63411	U21871, D63411	TOM20	Outer mitochondrial membrane receptor rTOM20	Up	Up
1387773	NM_012839	K00750 (2)	Cycs	Cytochrome c, apoptosis	Up	Up
1398763, 1368051	NM_032066	U81186 (2)	Hsd17b12	Translocase of inner mitochondrial membrane 23	Up	Up
1371795, 1370005	NM_030586	Y12517, AI232256	omb5	Cytochrome b5, outer mitochondrial membrane isoform	Up	Up
1367982	NM_024484	J03190 (2)	Alas1	Aminolevulinic acid synthase 1, heme biosynthetic	Up	Up
1375504	BM390747	AA892950		Polymerase (DNA), $\gamma 2$, mitochondrial DNA	Up	Up
1370918	BI275939	L19927	Atp5c1	ATP synthase, H^+ transporting, mitochondrial F1 complex	Down/up	Up
1386917	NM_012744	U32314 (2)	Pc	Pyruvate carboxylase (pyruvate to oxaloacetate)	Down/up	Up
1377758	BF415386	AA893658		Short-chain dehydrogenase/reductase 9	Down/up	Up
1389021	BF284746	AA891785	Idh2	Isocitrate dehydrogenase 2	Down/up	Up
1368514	NM_013198	M23601	Maob	Monoamine oxidase B, mitochondria	Down/up	Up
1369799	U29701	D87839 (2)	Abat	4-Aminobutyrate aminotransferase, GABA amino-transferase mitochondria	Down/up	Up
1370151	NM_017072	M11710,	Cps1	Carbamoyl-phosphate synthetase 1, mitochondrial (ammonia detoxification)	Down/up	Up
1369671	K03040	M11266	Otc	Ornithine transcarbamylase mitochondria (ammonia detoxification)	Down/up	Up
1370592	AB019693	AA893035	HP33, Keg1	Kidney expressed gene 1	Down	Up
1369023	NM_031052	M96633	Mipep	Mitochondrial intermediate peptidase	Down	Up
1368566	AA964381	AB000098	Mipp65	NADH dehydrogenase (ubiquinone) flavoprotein 3-like	Down	Up
1370232	AI102838	J05031	Ivd	Isovaleryl coenzyme A dehydrogenase	Down	Up
1368079	NM_053826	L22294	Pdk1	Pyruvate dehydrogenase kinase 1	Down	Up
1370509	AF062741	AF062741(2)	Pdp2	Pyruvate dehydrogenase phosphatase isoenzyme 2	Down	Down
1368387, 1374765	BI288055, NM_053995	AA817846	BDH	3-Hydroxybutyrate dehydrogenase mitochondrial	Down	Down
1371824	AA891949	AA891949	Ak4	Adenylate kinase 4	Down	Down
1369588, 1370350	AF368860, NM_012915	D13122	IF1PA	ATPase inhibitor (mitochondrial IF1 protein)	Down	Down

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration and identified as nuclear-encoded mitochondrial genes. Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification.

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TABLE 8

MPL-regulated probe sets related to lipid metabolism

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1398272	NM_022860	D17809	Galgt1	(*N*-Acetylneuraminyl)-galactosylglucosylceramide *N*-acetylgalactosaminyltransferase	Up	Up
1369656, 1389299	M36071	AA925887	Pcyt1a	Phosphate cytidylyltransferase 1, choline, α (phosphatidylcholine synthesis)	Up	Up
1368890	NM_053410	AA799779 (2)	Gnpat	Glyceronephosphate *O*-acyltransferase (bio-synthesis of ether phospholipids, peroxisomes)	Up	Up
1384417	AA998783	AA945171	Apoc4	Apolipoprotein C-IV	Down	Up
1369465	NM_012584	M67465	Hsd3b	Steroid δ-isomerase, 3β (biosynthesis hormonal steroids)	Down	Up
1368239	NM_053541	AB009463	rLRp105	Low-density lipoprotein receptor-related protein 3	Down/up	Up
1382680	AA874941	AA874941, AA893280	ADRP	Adipose differentiation-related protein,	Down/up	Up
1367662	NM_031682	AA945583	Hsd17b10	Hydroxyacyl-coenzyme A dehydrogenase type II (β-oxidation of fatty acids)	Down/up	Up
1368232	NM_031063	AA924198, M29472	Mvk	Mevalonate kinase, biosynthesis of cholesterol	Down/up	Down/up
1387183	J02844	U26033, J02844	CROT	Carnitine octanoyltransferase	Down	Down
1370909, 1388153	D90109, BI277523	D90109, AI044900, AA893242 (2)	ACS COAA	Long-chain acyl-CoA synthetase	Down	Down
1392604	AA997187	AA893032	Nsdhl	NAD(P)-dependent steroid dehydrogenase-like	Down	Down
1389906, 1367839	AW530769	H33426, M95591	Fdft1	Farnesyl diphosphate farnesyl transferase 1	Down	Down
1367668	NM_031841	AA875269	SCD	Stearoyl-coenzyme A desaturase 2, δ-9-desaturase	Down	Down
1387058	NM_017225	AF040261	Pctp	Phosphatidylcholine transfer protein	Down	Down
1388348, 1387630	NM_134382	AA892832	ELO1	ELOVL family member 5, elongation of long chain fatty acids	Down	Down
1368075	NM_012732	AA874784, S81497 (2)	Lipa	Lysosomal acid lipase 1	Down	Down
1370024	NM_030832	U02096	Fabp7	Fatty acid binding protein 7	Down	Down
1370583, 1370465, 1370364, 1369161	NM_012690	L15079	Abcb4	ATP-binding cassette, sub-family B (MDR/TAP), member 4	Down	Down
1398310	D17309	S80431, D17309	Akr1d1	Aldo-keto reductase family 1, member D1	Down/up	Down
1386965	NM_012598	AI237731, L03294	Lpl	Lipoprotein lipase	Up	Down

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration and categorized as relating to lipid metabolism. Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification.

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TABLE 9

MPL-regulated probe sets related to transport

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1379739	BF408431	AA892414	SLC4A7	Solute carrier family 4, sodium bicarbonate co- transporter, member 7	Up	Up
1369460	NM_022619	U53927	Slc7a2	Solute carrier family 7 (cationic amino acid transporter, y+ system), member 2	Up	Up
1376267, 1390036	AA859652	AA859652	Slc16a6, MCT6	Monocarboxylate Transporter 6	Up/down	Up
1368600	NM_022287	L23413	sat-1, Slc26a1	Solute carrier family 26 (sulfate transporter), member 1	Down	Up
1398295	NM_031684	AF015304	Slc29a1	Equilibrative nucleoside transporter 1; ent1	Down	Up
1387228	NM_012879	NM_012879	GLUT2	Facilitated glucose transporter	Down	Up
1387896, 1370296	M62763	M62763, M58287	Scp2	Nonspecific lipid transfer protein	Down	Up
1368745	NM_017222	U07183	Slc10a2	Solute carrier family 10, member 2 (sodium/bile acid cotransporter family)	Up	Down
1375823	BF392130	AF004017	SLC4A4, NBC	Electrogenic Na^+ bicarbonate cotransporter	Up/down	Down
1392929	BF416678	AA800202	Slc4A11	Solute carrier family 4, sodium bicarbonate transporter-like, member 11	Down	Down
1371525	BI277550	AA799691	KCC3	Solute carrier family 12 (potassium/chloride transporters), member 7	Down	Down
1368047	NM_022866	AA892616	Slc13a3	Solute carrier family 13 (sodium-dependent dicarboxylate transporter), member 3	Down	Down
1398267	NM_053537	L27651 (2)	Slc22a7	Solute carrier family 22 (organic anion transporter), member 7	Down	Down
1390416	H35736	AA892522	Slc25a30, KMCP1	Solute carrier family 25, member 30, mitochondrial carrier protein-1	Down	Down
1390412, 1376972, 1387130	NM_133315	U76714 (2)	Slc39a1	Solute carrier family 39 (iron-regulated transporter), member 1	Down	Down
1368316	NM_019158	AB005547	Aqp8	Aquaporin 8	Down	Down

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration and categorized as relating to transport. Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification.

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TABLE 10

MPL-regulated probe sets related to cell fates

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1374595, 1376999	BF389721	AA892570	Tnks2	Tankyrase, telomeric repeat-binding factor 1	Up	Up
1388953	AA892598	AA800679, AA892598 (2)	GNL3	Guanine nucleotide binding protein-like 3 (nucleolar), stem cell proliferation	Up	Up
1370163	BF281299	J04791	Odc1	Ornithine decarboxylase 1	Up	Up
1370575	D50734	AI043631	Oazin	Ornithine decarboxylase antizyme inhibitor	Up	Up
1370807	AF411216	AA859954		Vacuole membrane protein 1 (apoptosis)	Up	Up
1367764	NM_012923	X70871	Ccng1	Cyclin G1	Up	Up
1388395	AI406939	AA893235	GOS2	G0/G1 switch gene 2	Down/up	Up
1367847	NM_053611	NM_053611	Nupr1, p8	Nuclear protein 1 (cell proliferation)	Down/up	Up
1389179	BF284899	AA800243	DFF	Cell death-inducing DNA fragmentation factor	Down/up	Up
1371643	AW143798	X75207	CCND1	CCND1 gene; cyclin D1	Down	Down
1368311	NM_012861	M76704	MGMT	O6-Methylguanine-DNA methyltransferase (DNA repair)	Down	Down
1371684	AA799330	AA799330		Pelota homolog (cell division)	Up	Down

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration and categorized as relating to cell fates (proliferation, differentiation, or apoptosis). Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification.

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TABLE 11

MPL-regulated probe sets related to carbohydrate metabolism

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1386864, 1369473	NM_053290	AI169417, U20195	Pgam1	Phosphoglycerate mutase 1 (glycolytic)	Up	Up
1387052	NM_031039	D10354	Gpt	Glutamic pyruvic transaminase 1, soluble	Down/up	Up
1369560, 1371363	NM_022215	AB002558	Gpd3	Glycerol-3-phosphate dehydrogenase 1 (soluble)	Down	Up
1370299	M10149	AA892395	Aldob	Aldolase B	Down	Up
1369635	NM_017052	X74593, AI030175	Sord	Sorbitol dehydrogenase	Down	Down
1373337	AI412065	AA892799, AA892799, AA892799		Glyoxylate reductase/hydroxypyruvate reductase	Down	Down
1370870, 1370067	NM_012600	AI171506, M26594, AI171506, AI008020	Me1	Malic enzyme 1 (cytosolic)	Down	Down
1369954	NM_031510	AA892314	IDH1	Isocitrate dehydrogenase 1, soluble	Down	Down
1370725, 1386944	NM_013098	L37333	G6pc	Glucose-6-phosphatase, catalytic	Down	Down/up
1387312	NM_012565	X53588	Gck	Glucokinase	Down/up	Down/up

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration and categorized as relating to carbohydrate metabolism. Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification.

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TABLE 12

Other MPL-regulated probe sets

230A probe set	230A accession	U34A probe set/accession	Symbol	Identification	Response	
					Acute	Chronic
1398784	NM_019259	AI178135	C1qbp	C1q binding, inhibits C1 activation	Up	Up
1367794	NM_012488	AA900582, M22670, M22670, AI113046	A2m	α2-Macroglobulin	Up	Up
1367801	NM_053596	D29683, AA956930	Ece1	Endothelin converting enzyme 1	Up	Up
1369065	NM_017290	J04024	Atp2a2, Serca2	ATPase, Ca^{2+} transporting	Up	Up
1371345	AI008699	AI073164	Scamp1	Secretory carrier membrane protein	Up	Up
1369982	NM_031008	AA800186		Adaptor protein complex AP-2	Up	Up
1386983	NM_013168	X06827	PBGD	Hydroxymethylbilane synthase PBGD	Down/up	Up
1368224	NM_031531	D00753	Spin2c	Serine protease inhibitor	Down	Up
1367647	NM_022519	X16273	Serpina1	Serine protease inhibitor α1	Down	Up
1387819	NM_012552	L00117	Ela1	Elastase 1, serine protease	Down	Up
1371237	AF411318	AI102562	Mt1a	Metallothionein 1	Down	Up
1387323	NM_012725	M30282	Klk3	Kallikrein B, plasma 1	Down	Down
1374524	BM384384	AA893080		Selenocysteine lyase	Down	Down
1368322	NM_012880	Z24721	ECSODPT	Superoxide dismutase 3 (secreted)	Down	Down
1367720	NM_012899	AA800745	Alad	Aminolevulinate, δ- dehydratase	Down	Down
1367980	NM_019124	D85844	RABPT5	Rabaptin 5	Down	Down
1387146	X57764	S65355, AA818970	Ednrb	Endothelin receptor type B	Up/down	Down
1367800	NM_013151	M23697	Plat	Plasminogen activator, tissue	Up/down	Down
1370080	NM_012580	AI179610, J02722	Hmox1	Heme oxygenase (decycling) 1	Up	Up/down

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration but uncategorized. Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification.

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TABLE 13

ESTs regulated by MPL

230A probe set	230A accession	U34A probe set/accession	Symbol	Response	
				Acute	Chronic
1394127	AA943800	AA892541	EST	Up	Up
1382516	AI170660	AA859725	EST	Up	Up
1376965	BM389656	AA893603	EST	Up	Up
1367543	BG377443	AA894234	EST	Up	Up
1383233	BI289032	AA891749	EST	Down/up	Up
1393061	AI030103	AA891739	EST	Down/up	Up
1385889	AA893212	AA893212	EST	Down	Up
1393751	AA859029	AA892778	EST	Down	Down
1372261	AI409067	AA891737	EST	Down	Down
1373970	AI716248	AA892986	EST	Down	Down
1389561	BE110624	AA891950	EST	Down	Down
1376792	AW251313	AA892027	EST	Down	Down
1374478	AA819329	AA892861	EST	Down	Down
1374767	AI105450	AA799396(2)	EST	Down	Down
1376098	BF282304	AA875126	EST	Up/down	Up/down

Probe sets regulated by MPL in rat liver after both acute and chronic drug administration but at present unidentified. Affymetrix 230A arrays use a probe set number distinct from GenBank accession numbers, whereas U34A chips use a common probe set/GenBank accession number for identification. EST, Expressed sequence tag.