

High Mobility Group N Proteins Modulate the Fidelity of the Cellular Transcriptional Profile in a Tissue- and Variant-specific Manner^{*[S]}

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Background: The ubiquitously expressed high mobility group N (HMGN) protein variants affect chromatin structure and transcription.

Results: *Hmgn*^{tm1/tm1} mice, which may express mutant proteins defective in nucleosome binding, display variant-specific phenotypes and tissue-specific altered transcription profiles.

Conclusion: HMGN variants fine tune the fidelity of the cellular transcription profile.

Significance: HMGN proteins impact the cellular phenotype by modulating the transcription levels of numerous genes.

The nuclei of most vertebrate cells contain members of the high mobility group N (HMGN) protein family, which bind specifically to nucleosome core particles and affect chromatin structure and function, including transcription. Here, we study the biological role of this protein family by systematic analysis of phenotypes and

tissue transcription profiles in mice lacking functional HMGN variants. Phenotypic analysis of *Hmgn1*^{tm1/tm1}, *Hmgn3*^{tm1/tm1}, and *Hmgn5*^{tm1/tm1} mice and their wild type littermates with a battery of standardized tests uncovered variant-specific abnormalities. Gene expression analysis of four different tissues in each of the *Hmgn*^{tm1/tm1} lines reveals very little overlap between genes affected by specific variants in different tissues. Pathway analysis reveals that loss of an HMGN variant subtly affects expression of numerous genes in specific biological processes. We conclude that within the biological framework of an entire organism, HMGNs modulate the fidelity of the cellular transcriptional profile in a tissue- and HMGN variant-specific manner.

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[S] This article contains supplemental Tables 1–3.

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The structure and function of chromatin are regulated by the action of numerous nuclear proteins, including that of nucleosome-binding proteins, such as the linker histone H1, and of the

various members of the high mobility group (HMG)⁴ protein superfamily. Histone H1 is a family of closely related proteins each encoded by a distinct gene, which are expressed in a species- and tissue-specific manner (1–3). The HMG protein superfamily consists of three distinct families denoted HMGA, HMGB, and HMGN, each of which consists of several protein variants (4–7). As a group, H1 and HMG variants are chromatin architectural proteins; all are intrinsically disordered structural proteins that bind to nucleosomes without any known specificity for the underlying DNA sequence and affect chromatin functions by altering either the global or local chromatin structure.

Numerous studies revealed that H1 and HMG protein variants affect gene regulation and impact the cellular phenotype (1, 5, 8–10), yet the cellular function and mechanism of action of these proteins remains one of the least understood aspects of chromatin biology. A major unanswered question is the extent of functional specificity of the various H1 and HMG variants. This question is particularly complicated because all HMGs affect the chromatin binding of all the H1 variants, and within each protein family, the various members compete with each other for nucleosome-binding sites (11, 12). Thus, all these architectural proteins function within a dynamic network in which changes in the levels of any protein trigger compensatory adjustments, perhaps aimed at minimizing deleterious biological effects (12, 13). Nevertheless, the presence of the proteins in most vertebrate cells, and a variety of studies, including analysis of genetically altered mice, suggest that most HMG variants have specific biological functions (5, 14). Here, we focus on the HMGN protein family, and we use genetically altered mice to examine whether HMGN variants have specific effects on the transcription profile in the biological context of an entire organism.

The HMGN protein family consists of five members, named HMGN1–5, which are encoded by distinct genes that have been detected only in vertebrates (15). Each HMGN is composed of the same basic structure as follows: a bipartite nuclear localization signal, an evolutionarily conserved nucleosome binding domain, and a divergent C-terminal region. HMGNs specifically recognize the generic structure of the nucleosome core particle: they do not bind specifically to either purified DNA or isolated histones. All HMGNs contain a highly conserved region through which they bind specifically to an acidic patch formed by the H2A and H2B dimer when it is folded into its nucleosomal conformation (16, 17).

Studies on the cellular function and mechanism of action of HMGN proteins indicate that these proteins affect the cellular transcription profile and cellular differentiation and enhance the ability of a cell to repair damaged DNA (5). The ability of HMGN to affect these processes is contingent on their binding to chromatin; mutants that do not bind to nucleosomes do not affect chromatin structure and function. Significantly, genome-wide analysis reveals that HMGN1 preferentially binds to chromatin regulatory sites such as DNase I-hypersensitive sites, enhancers, and promoters (18). The interaction of HMGN with

chromatin has been shown to affect chromatin “compaction” and the levels of histone modifications, two processes that play a major role in epigenetic regulation (15). Taken together, the emerging data indicate that HMGN proteins bind dynamically to chromatin, compete among themselves and with other nuclear proteins for chromatin-binding sites, and affect chromatin function, including transcription chromatin compaction and histone modifications.

Although several types of experiments provided insights into the mechanisms whereby HMGN proteins can affect chromatin functions, the extent of functional redundancy among the HMGN variants is still not fully understood. The structural similarities of all HMGNs, taken together with the similarity in their nucleosomal binding constants and their competition for nucleosome-binding sites, raised the possibility that the HMGN variants are fully functionally redundant. However, the presence of all HMGN variants in most tissues, and the phenotypes seen in *Hmgn^{tm1/tm1}* mice, argues for functional specificity. Thus, *Hmgn1^{tm1/tm1}* mice exhibit behavioral abnormalities (19) and impaired repair of damaged DNA (20, 21), whereas *Hmgn3^{tm1/tm1}* mice are mildly diabetic (22). Likewise, transcriptional analysis of mouse embryonic fibroblasts derived from *Hmgn1^{tm1/tm1}*, *Hmgn3^{tm1/tm1}*, and *Hmgn5^{tm1/tm1}* mice revealed that in these cells HMGN variants are not fully redundant (8).

Experiments with cells grown in tissue culture do not always reflect the true biological function of a protein. To gain insights into the function of HMGN variants in the biological setting of an entire organism, we first subjected *Hmgn1^{tm1/tm1}*, *Hmgn3^{tm1/tm1}*, and *Hmgn5^{tm1/tm1}* mice and their wild type littermate controls to a battery of standardized tests designed to reveal abnormalities in several physiological properties (23, 24). The various tests identified specific abnormalities in each of the mouse lines, reinforcing the general notion that HMGN variants are not fully redundant and raising the possibility that each variant has specific effects on the transcription profile of a tissue. To examine this possibility, we analyzed the expression profile of four different tissues in each of the *Hmgn^{tm1/tm1}* lines. We find that each HMGN variant significantly affects the expression of a limited number of genes, with minimal overlap between the genes affected, and that each tissue analyzed had a distinct set of genes affected by each HMGN. Furthermore, examination of the effect of HMGN loss on whole biological pathways, rather than focusing on the changes in expression levels of individual genes, reveals that each HMGN variant has distinct and significant effects on specific biological processes, perhaps rendering them prone to further disruption by subsequent genetic events or extracellular stress. Our studies demonstrate that within the framework of an entire organism, HMGNs modulate the fidelity of the cellular transcription profile, in a tissue- and variant-specific manner.

EXPERIMENTAL PROCEDURES

Mice—All HMGN mutants derived thus far are targeted mutations of their nucleosomal binding domain and are denoted as “tm1” mutants by the mouse nomenclature committee of The Jackson Laboratory. The generation of *Hmgn1^{tm1/tm1}* (21) and conditional *Hmgn3^{tm1/tm1}* (22), in

⁴ The abbreviations used are: HMG, high mobility group; HMGN, high mobility group N; GO, Gene Ontology.

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which the native gene was replaced with a genomic fragment that lacks the exons coding for the nucleosomal binding domain of these proteins (Fig. 1), was previously described. The same strategy was used to derive *Hmgn*^{tm1/tm1} mice.⁵ Mice were bred in a specific pathogen-free facility with food and water *ad libitum*.

Phenotype Analysis—A battery of tests was used to analyze the phenotype of the *Hmgn*^{tm1/tm1} mice at the German Mouse Clinic (24) for the study. A detailed description of the methods can be found on line, at the German Mouse Clinic website.

RNA Isolation—Eight mice (four mutants and four controls) for each HMGN at 17 weeks of age were killed and organs prepared as described recently (25). Total RNA of brain, liver, spleen, and thymus was prepared according to the manufacturer's protocol using RNeasy mini kits (Qiagen). RNA integrity was controlled on a formaldehyde-agarose gel, and the concentration was calculated from *A*_{260/280} measurements. 500 ng of this high quality total RNA was amplified using the Illumina TotalPrep RNA amplification kit (Ambion).

Illumina Bead Arrays—The amplified cRNA was hybridized to MouseRef-8 Version 2 Expression BeadChips (Illumina, San Diego) and after a 16-h incubation, staining and scanning were done according to the Illumina expression protocol. Data were normalized using the GenomeStudio Version 2011.1 software and processed using the quantile normalization, background subtraction option, and introduction of an offset to remove remaining negative expression values.

Identification of Up- and Down-regulated Genes—The identification of differentially expressed genes was performed using significant analysis of microarrays (25, 26) included in the TM4 software package (27). To estimate the false discovery rate, nonsense genes were identified using 1000 permutations of the measurements. Top differentially expressed genes were selected using the false discovery rate of 0.1 and fold change of >1.4. Array data are available in the GEO database under accession number GSE39062.

In addition, based on gene expression profiles in mutant and wild type mice, we identified significantly up- and down-regulated genes in mutant mice across the panel of available tissues. With four tissues under consideration (*i.e.* brain, liver, spleen, and thymus) and three HMGN variants (*i.e.* HMGN1, HMGN3, and HMGN5), we generated 12 differentially expressed gene lists, each list being specific to a tissue and protein variant. The gene lists contain from 17 to 97 genes. Five gene lists (HMGN3^{tm1/tm1} brain and liver tissues, HMGN1^{tm1/tm1} liver and spleen tissues, and HMGN5^{tm1/tm1} thymus) do not contain any up-regulated genes.

Principal Component Analysis of Gene Expression—Principal component analysis, which is a popular tool for dimension reduction and data visualization, linearly transforms a set of correlated features into a set of orthogonal features, known as principal components, so that they optimally describe data variability (28, 29).

To visualize gene expression data in a reliable manner, we focused on the genes featuring large expression changes,

because small expression changes may be caused by experimental noise. Only the genes with the S.D./ μ ratio higher than 0.1 (where S.D. and μ are the standard deviation and average level of gene expression, respectively) were selected for the study, and the expression of these genes was normalized so that the expression of each gene has the unit standard deviation and zero mean. Based on the normalized data, we then evaluated the discriminating capability of each gene by using an unpaired Student's *t* test. We used the top 100 genes with the largest discriminating capabilities for Principal component analysis.

Functional Analysis of Gene Lists—To evaluate the function of a gene list, we used gene functional annotations from the Gene Ontology (GO) database (30, 31). The enrichment of a gene annotation category in a given gene list was estimated according to Equation 1,

$$\Pr(x \leq m) = \sum_{0 \leq k \leq m} \frac{\binom{M}{k} \binom{N-M}{n-k}}{\binom{N}{n}} \quad (\text{Eq. 1})$$

where n is the number of genes in the tested gene list, and m is the number of genes in the tested gene list that have the investigated function. Furthermore, M is the number of all genes in the investigated functional category, and N is the number of all mouse genes. To account for multiple testing, we adopted the method of Benjamini and Hochberg (32) with the adjusted p value <0.05.

Pathway Analysis Based on Gene Expression—Using a set of pathways (each being represented by a group of the genes comprising it) and genome-wide gene expression profiles from samples belonging to two classes (labeled as wild type and mutation in this study) as input, we used a computational method. In contrast to traditional pathway analysis methods, such as gene set enrichment analysis (33) and significant analysis of microarray-GS (34), our significant pathway analysis method evaluates the activity of all pathways in each given sample. More specifically, significant pathway analysis includes three main steps as follows: normalization of gene expression, evaluation of the expression significance of each pathway, and estimation of the significance of the difference of pathway expression between wild type and mutation samples.

Given gene g_i , we normalized its expression in sample j as fold change relative to the expression in wild type samples, *i.e.* the expression of g_i in sample j , z_{ij} , is normalized as $x_{ij} = z_{ij}/z_{i,0}$, where z_{ij} is the original expression of g_i in sample j , and $z_{i,0}$ is the average expression of g_i in the wild type samples. Based on the normalized gene expressions (x_i), we measured the expression significance of the pathway pw_i in sample j as shown in Equation 2,

$$e_{ij} = \text{sign}(m_{ij} - m_j) \times (-\log_{10}(\text{sig}(pw_{ij}))) \quad (\text{Eq. 2})$$

where m_{ij} and m_j are the expression median of the genes in the pathway pw_i and among all genes in sample j , respectively. $\text{sig}(pw_{ij})$, the expression significance p value of pw_i in sample j , is evaluated through comparing the expression levels of the genes in pw_i with the entire gene expression profile under Wilcoxon rank-sum test, the nonparametric alternative to the two-sam-

⁵ M. Rochman, T. Furusawa, and M. Bustin, unpublished data.

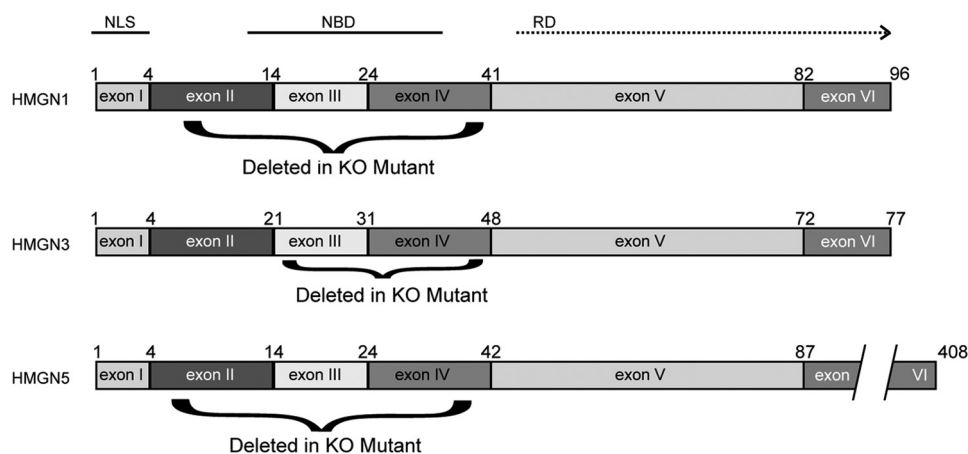


FIGURE 1. **Overview of the genotype of *Hmgntm1/tm1* mice.** Diagrams detailing structural domains of HMGN proteins and the mutations in them used in this study are shown. Each *rectangle* represents an exon from the coding sequence of the gene in question; the amino acids at the boundaries of the exons are marked *above* the diagrams. The exons deleted in creating the functional knock-outs for each HMGN variant are denoted by the *black curved line* below the diagrams, and the amino acid positions at the boundary of the excised domains, which are missing in the respective *Hmgntm1/tm1* mouse, are marked on either side of the *curved line*. NLS, nuclear localization sequence; NBD, nucleosome binding domain. RD, regulatory domain.

ple t test. The positive/negative e_{ij} indicates that the pathway pw_i is up-/down-regulated in the sample j . The greater the absolute value of e_{ij} , the significantly higher pw_i is expressed in sample j . Also, based on the normalized gene expression, we estimate the significance of the difference of the expression of pw_i between wild type and mutation samples. Wilcoxon rank-sum test is used for this purpose. pw_i is considered as up-regulated after mutation when the median of the expression of the genes in pw_i in all mutation samples is larger than that in wild type samples, otherwise pw_i is considered as down-regulated after mutation.

RESULTS

Genetically Altered HMGN Mice—All the members of the HMGN protein family contain distinct functional domains, encoded by six distinct exons (Fig. 1). Exon 1, which is absolutely conserved among all the variants, encodes for the N-terminal segment of a bipartite nuclear localization signal (35). Exon 2 codes for a variable region; its function has not yet been clearly defined. Exon 3 codes for the most highly conserved protein region, an 11-amino acid segment with the sequence P(X)RRSARLSAK, which is the signature of the HMGN protein family and is responsible for the specific binding of the HMGNs to nucleosome core particles (16, 17). This domain, together with the domain encoded by exon 4, constitutes the main nucleosome binding domain of the protein. Exons 5 and 6 encode a negatively charged region that has very little sequence conservation. This region is considered to be the “regulatory domain” of the proteins, because through this domain HMGNs affect chromatin compaction and levels of histone modification (36–39). These epigenetic activities of HMGN are absolutely dependent on the ability of the protein to bind to nucleosome core particles. Mutants that do not bind to nucleosomes do not affect the levels of histone modification, do not decompact chromatin, and do not noticeably affect chromatin related activities such as the repair of damaged DNA (21, 36–39).

The mouse genome encodes four HMGN proteins as follows: HMGN1, HMGN2, HMGN3, and HMGN5 (14). We generated

genetically altered mice for *Hmgn1*, *Hmgn3*, and *Hmgn5*; genetically altered *Hmgn2* mice are not yet available. We aimed to elucidate the biological significance of the interaction of each HMGN with chromatin; therefore, in all the *Hmgn^{tm1/tm1}* mice, we deleted only the nucleosome binding domain of the respective HMGN variant, thereby abolishing its ability to bind specifically to chromatin. In *Hmgn1^{tm1/tm1}* (21), *Hmgn3^{tm1/tm1}* (22), and *Hmgn5^{tm1/tm1}* mice we deleted, respectively, exons II–IV, III–IV, and II–IV (Fig. 1). Immunofluorescence and Western analysis of *Hmgn1^{tm1/tm1}* (21) and *Hmgn3^{tm1/tm1}* (22) indicate that the wild type proteins are not expressed in these mutants; however, we note that Western analysis of cellular extracts prepared from *Hmgn^{tm1/tm1}* cells occasionally reveals the presence of a truncated protein, an indication that the mutated genes can be transcribed.

All the *Hmgn^{tm1/tm1}* mice survived and did not display an obvious gross phenotype. However, upon more detailed analysis, the *Hmgn1^{tm1/tm1}* mice showed behavioral abnormalities, increased tumorigenicity, and impaired repair of damaged DNA (19–21). HMGN3 is highly expressed in pancreatic beta cells, and the *Hmgn3^{tm1/tm1}* mice are mildly diabetic (22). These studies suggest that loss of even a single HMGN variant can result in a phenotype detectable at the level of an entire organism.

To gain additional insights into the biological function of the HMGN variants in the context of a whole organism, we subjected the three HMGN knock-out mouse strains to a standardized battery of tests performed at the German Mouse Clinic (24, 41). For these tests, we bred sufficient heterozygous mice to obtain 20 males and 20 females from both wild type and *Hmgn* knock-out mice. Thus, for each strain a total of 80 mice, born within 1 week, were shipped for analysis from the National Institutes of Health, Bethesda, to the German Mouse Clinic. Table 1 summarizes the phenotypes detected in the mice, *i.e.* the tests that show distinct differences between the cohort of wild type mice and the cohort of their knock-out littermates, which lack the nucleosomal domain of a specific HMGN variant. Additional information, including the tests that do not

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TABLE 1

Phenotypes observed in *Hmgn*^{tm1/tm1} mice

The abbreviations used are as follows: IpGTT, intraperitoneal glucose tolerance test; IC, inspiratory capacity; ERV, expiratory reserve volume; VC, vital capacity; TLC, total lung capacity; FVC, forced vital capacity.

Screens	Test	Phenotype ^a		
		<i>Hmgn1</i> ^{tm1/tm1}	<i>Hmgn3</i> ^{tm1/tm1}	<i>Hmgn5</i> ^{tm1/tm1}
Behavior	Open field	Decreased rearing activity	No	No
	Acoustic startle and prepulse inhibition	Lower acoustic startle activity	Decreased PPI	Decreased PPI
Neurology	Modified SHIRPA protocol	No	No	No
	Grip strength	No	Reduced	No
Nociception	Hot plate assay	No	Hypoalgesia	No
	Clinical chemistry and hematology	Clinical chemistry	No	See website
Hematology		No	No	Changes in the red blood cell count
Steroid metabolism	Simplified IpGTT	No	Increased fasting-induced body mass loss, increased peak glucose	Sex-specific effects on basal glucose level and glucose tolerance
	Corticosterone level	Decreased in females	Decreased in females	No
Immunology	Testosterone level	No	Increased in males	No
	FACS, leukocyte proportions	No	No	Higher frequency of CD8 ⁺ T cells and lower frequency of granulocytes
Allergy	Bioplex: immunoglobulin levels	No	No	Higher IgA
	IgE level	No	No	Low IgE
Lung function	Pulmonary function test	No	No	Sign differences in lung volumes (IC, ERV, VC, TLC, and FVC), flow rates (FEV100), mechanical properties (Cchord); emphysema-like phenotype
Molecular phenotyping	Genome-wide transcriptome analysis	Differential gene expression in liver, spleen, thymus and brain		

^a See the Mouse Clinic website for additional data.

show differences between wild type and mutant mice, is available at the German Mouse Clinic website.

The phenotypic screen of the three *Hmgn*^{tm1/tm1} lines identified specific abnormalities in each of the mouse lines; however, the various phenotypes detected did not represent a major functional loss of any of the properties investigated (Table 1). Nevertheless, taken together with the previous studies on *Hmgn1*^{tm1/tm1} and *Hmgn3*^{tm1/tm1} mice (14, 19–21, 42, 43), the data presented in Table 1 indicate that loss of a specific HMGN variant leads to distinct phenotypes, an indication that the proteins are not fully functionally redundant.

HMGN Variant-specific Effects on Transcription—The phenotypes seen in the *Hmgn*^{tm1/tm1} mice suggest HMGN variant-specific effects on transcription. To test this possibility and to examine effects of HMGN variants on the transcription profile of a tissue, we compared the transcription profiles in the brain, liver, thymus, and spleen of wild type mice and their *Hmgn*^{tm1/tm1} littermates. RNA was prepared from tissues that were isolated from four male knock-out mice and four littermate controls for each HMGN variant and analyzed using Illumina bead arrays.

We found only small differences between the HMGN variants in their effect on the total number of genes whose expression was significantly changed in the four tissues examined; it ranged from 134 genes in the tissues taken from *Hmgn1*^{tm1/tm1} mice to 236 genes changed in *Hmgn5*^{tm1/tm1} tissues (Fig. 2A). Significantly more genes were down-regulated than up-regulated as a result of HMGN loss; 76% of genes regulated by HMGN1, 94% of genes regulated by HMGN3, and 81% of genes regulated by HMGN5 were down-regulated upon loss of the

respective HMGN variant (Fig. 2B). Notably, there was little overlap between the genes affected by the deletion of a specific HMGN variant. Within the genes up-regulated in the four tissues, there was no overlap at all, and within the down-regulated genes, only two genes, *Csn2*, an isoform of β -casein (44), and *Azgp1*, zinc- α 2-glycoprotein, a tumor suppressor involved in obesity and metabolism (45), were targets of all three HMGN variants. Within the cohort of the down-regulated genes, just 21 targets, *i.e.* only 6% of the total down-regulated genes, were altered by the loss of two HMGN variants (Fig. 2B). The genes whose expression was altered by the loss of more than one HMGN variant are listed in Table 2. GO analysis failed to reveal specific pathways associated with these genes (Table 3). These results indicate that HMGN proteins affect the transcription profile in a variant-specific manner. Our finding that most of the genes affected were down-regulated is consistent with the general notion that HMGNs localize to chromatin regulatory regions and reduce chromatin compaction, perhaps enhancing the ability of positive regulators to access their cognate binding sites.

Effects of HMGN Variant Loss on the Transcriptional Profiles of Specific Tissues—To examine the effect of HMGN variants on tissue-specific gene expression, we organized all the genes that were affected by HMGNs into 24 variant- and tissue-specific lists as follows: one per tissue (*i.e.* total of 4), per HMGN variant (*i.e.* total of 3), and per either up- or down-regulation of the genes in question. Principal component analysis of these gene lists (Fig. 2C) revealed that the tissues themselves tended to cluster according to tissue type, regardless of genotype. However, within each tissue, the four wild type mice clustered sep-

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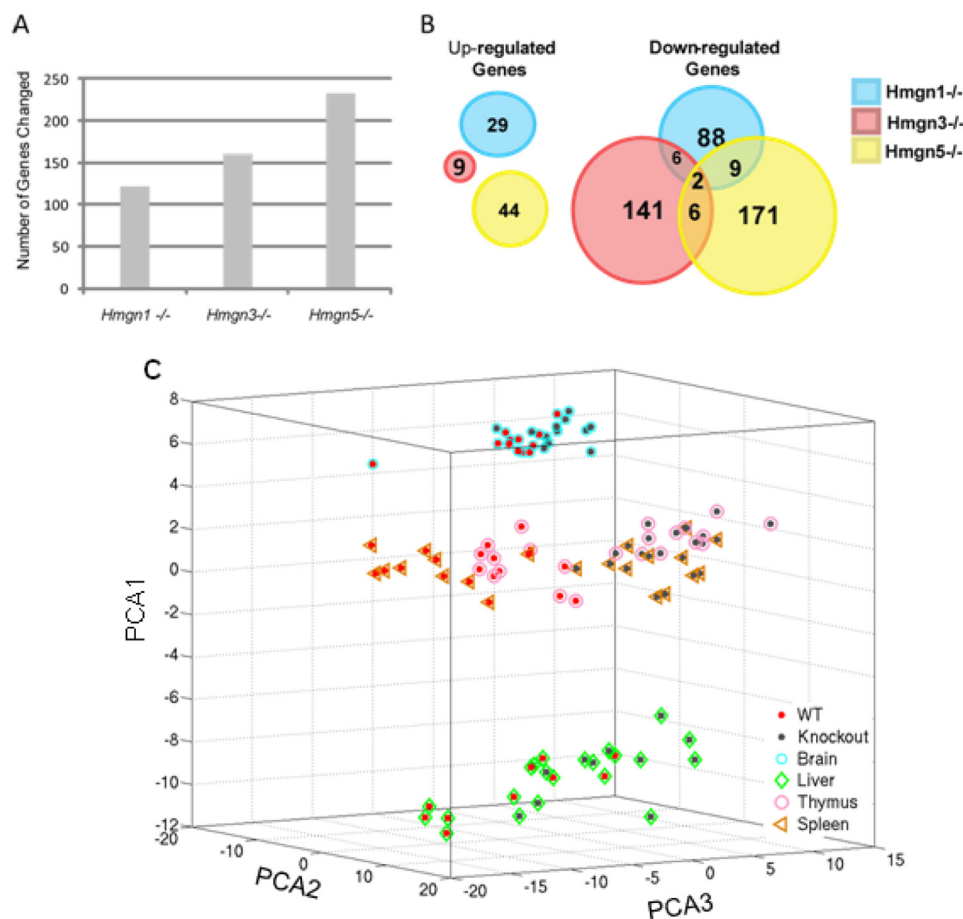


FIGURE 2. **Effects of HMGN variant loss on global gene expression in mouse tissues.** *A*, number of genes affected by each HMGN variant. *B*, HMGN variant-specific effects on the transcription profile of mouse tissues. *C*, principal component analysis of HMGN variant-specific transcriptional profile changes in mouse tissues.

TABLE 2
Overlapping genes regulated by HMGN1, HMGN3, and/or HMGN5

HMGN variants	Gene	Function
HMGN1 and HMGN3	<i>Mup2</i>	Regulator of glucose and lipid metabolism
	<i>Acaa1b</i>	Acetyl-coenzyme A acyltransferase 1B
	<i>H2-M2</i>	Histocompatibility 2, M region locus 2
	<i>Fga</i>	Fibrinogen α -chain
	<i>Knq1</i>	Kininogen 1, essential for blood coagulation and assembly of the kallikrein-kinin system
	<i>Ttr</i>	Transthyretin, a carrier protein for thyroid hormone
HMGN1 and HMGN5	<i>Sfrs5</i>	Serine/arginine-rich splicing factor 5, part of the spliceosome
	<i>Shisa2</i>	Protein controlling head formation during development
	<i>Sumo1</i>	Small ubiquitin-like molecule 1, a signal for protein trafficking
	<i>Uchl5</i>	Ubiquitin C-terminal hydrolase L5
	<i>Atp6ap2</i>	Lysosomal ATPase H ⁺ transporter
	<i>Csf2rb2</i>	Colony-stimulating factor 2 receptor, β 2
	<i>Ccl21a</i>	Chemokine (C-C motif) ligand 21A
	<i>Gp2</i>	Pancreatic zymogen granule membrane-associated protein
	<i>Ii4i1</i>	Involved in catalysis and binding of flavin adenine dinucleotide (FAD) cofactor
HMGN3 and HMGN5	<i>PomC</i>	Polypeptide hormone precursor
	<i>Tank</i>	Blocks TNF signaling
	<i>Mup1</i>	Regulator of glucose and lipid metabolism
	<i>Abl1</i>	Nonreceptor tyrosine kinase involved in cell division
HMGN1, HMGN3, and HMGN5	<i>Abpb</i>	Androgen binding protein β
	<i>Mup5</i>	Regulator of glucose and lipid metabolism
	<i>Csn2</i>	β -Casein
	<i>Azgp1</i>	Tumor suppressor, involved in obesity and metabolism

arately from the four knock-out mice (Fig. 2C). These results are consistent with the relatively small number of genes affected by each HMGN variant; in addition, they also indicate that functional loss of an HMGN variant does not alter the tissue-specific pattern of gene expression. Thus, HMGNs do

not act as specific regulators of gene expression; they fine tune the pre-existing tissue-specific transcription profile.

Examining the overall trends in gene regulation in HMGN knock-out mouse tissues, we observed that the largest number of genes changed in liver (186 down-regulated and 21 up-regu-

TABLE 3
GO functional categories affected by Hmgn knockdown
 The table lists the two GO categories most affected in the various tissues by the functional loss of an HMGN variant. Full list of all the GO categories is presented in supplemental Table 2. Tissues not listed contained no significant GO categories.

HMGN variant/tissue/ regulation	GO ID	GO category, biological process	Total no. of genes in GO category	No. of hits	P	q	Genes
<i>Hmgn3^{tm1/tm1}</i> brain down	GO:0016486	Peptide_hormone_processing	20	5	1.28E-10	6.31E-07	<i>Tshb, Lhb, Cga, Pomc, Fshb</i>
<i>Hmgn3^{tm1/tm1}</i> brain down	GO:0051604	Protein_maturation	75	6	2.60E-09	6.41E-06	<i>Tshb, Fshb, Serpinh1, Lhb, Cga, Pomc</i>
<i>Hmgn3^{tm1/tm1}</i> spleen down	GO:0051346	Negative_regulation_of_hydrolyase_activity	241	8	2.50E-11	1.23E-07	<i>Serpina1e, Apoa1, Ambp, Kng1, Apoc3, Apoa2, Serpina1c, Ahsg</i>
<i>Hmgn5^{tm1/tm1}</i> spleen down	GO:0043086	Negative_regulation_of_catalytic_activity	540	8	1.16E-08	2.85E-05	<i>Ahsg, Apoa1, Ambp, Kng1, Apoc3, Serpina1c, Apoa2, Serpinatc</i>
<i>Hmgn5^{tm1/tm1}</i> spleen down	GO:0006397	mRNA_processing	284	6	2.88E-06	7.10E-03	<i>Sfrs5, Hnrnpk, Ddx20, Celf1, Dhx15, Hnrnpa2b1</i>
<i>Hmgn5^{tm1/tm1}</i> spleen down	GO:0008380	RNA_splicing	269	6	2.11E-06	1.04E-02	<i>Sfrs5, Hnrnpk, Ddx20, Celf1, Dhx15, Hnrnpa2b1</i>
<i>Hmgn5^{tm1/tm1}</i> thymus down	GO:0003012	Muscle_system_process	121	7	8.94E-08	4.41E-04	<i>Tnnc2, Myl3, Tpm2, Acta1, Tnni2, Tnni3, Atp2a1</i>
<i>Hmgn5^{tm1/tm1}</i> thymus down	GO:0003009	Skeletal_muscle_contraction	9	3	2.29E-06	5.65E-03	<i>Tnnc2, Myl3, Tpm2, Acta1, Tnni2, Tnni3, Atp2a1</i>

lated), and the smallest number of genes (a total of 89) changed in brain tissues (Fig. 3, A and B). In all tissues, the majority of changed genes were down-regulated; the largest effect of a single variant was observed in the liver of *Hmgn3^{tm1/tm1}* mice where the expression of 91 genes was down-regulated (Fig. 3A). The highest percentage of up-regulated genes, 37%, was in the brain (Fig. 3, A and B). Whether up- or down-regulated, there was very little overlap between the genes affected in the various tissues by all the HMGN variants, as a group (Fig. 3B). Thus, only two genes overlapped between tissues among the up-regulated genes, and 14 genes among the down-regulated genes, i.e. 3% of the genes so regulated in either case (Fig. 3B). Spleen tissue had the highest amount of overlapping genes as follows: six genes were down-regulated in both *Hmgn1^{tm1/tm1}* and *Hmgn3^{tm1/tm1}* spleens, and another set of four genes were down-regulated in both *Hmgn1^{tm1/tm1}* and *Hmgn5^{tm1/tm1}* spleens, for a total of 10% overlap in spleen-specific genes. These data indicate that each tissue has a unique set of HMGN target genes.

Analysis of the effects of a single HMGN variant on the tissue-specific transcription profiles reveals very little overlap among the four tissues examined (Fig. 3C). None of the genes affected by any of the three HMGN variants are shared among all the four tissues examined. Only six of the 134 genes affected by the loss of HMGN1, i.e. 4.5%, are shared among two tissues. In *Hmgn3^{tm1/tm1}* and *Hmgn5^{tm1/tm1}* mice, respectively, only 4.8 and 1.2% of their target genes are shared among more than one tissue (Fig. 3C). Thus, each set of target genes is essentially both HMGN variant-specific and tissue-specific.

The magnitude of the changes in transcript levels does not vary significantly among the tissues or among the HMGN variants (Table 4). Interestingly, the effects seen in the various tissues obtained from the mice are smaller than those previously detected in mouse embryonic fibroblasts (8), cells which are not yet fully differentiated, and are grown in tissue culture. It is also possible that the effects are smaller due to the more stringent guidelines used to analyze the tissues as compared with the ones used for the mouse embryonic fibroblasts (8). It remains to be determined whether these differences reflect the changes in cellular differentiation stage or differences between cells grown in tissue culture and whole tissues obtained directly from the mice.

Further demonstrating the unique effects of each HMGN variant on the transcription profile of a specific tissue, we note that these effects form clear variant-dependent patterns (Fig. 4 and supplemental Table S1). Within each tissue, the target genes can be organized into distinct groups according to which HMGN variant is functionally deleted. In brain tissue, there are five distinct groups defined by HMGN variant loss as follows: genes down-regulated by HMGN5 loss (group A); genes down-regulated by HMGN3 loss (group B); genes down-regulated by HMGN1 loss (group C); genes up-regulated by HMGN1 loss (group D), and genes up-regulated by HMGN5 loss (group E) (Fig. 4A). In liver tissue, there are eight distinct groups defined by HMGN variant loss as follows: genes down-regulated by HMGN3 and HMGN5 loss and slightly up-regulated by HMGN1 loss (group F); genes down-regulated by HMGN1 loss (group G); genes down-regulated by HMGN1 loss and up-reg-

Transcriptional Specificity of HMGN Variants in Mouse Tissue

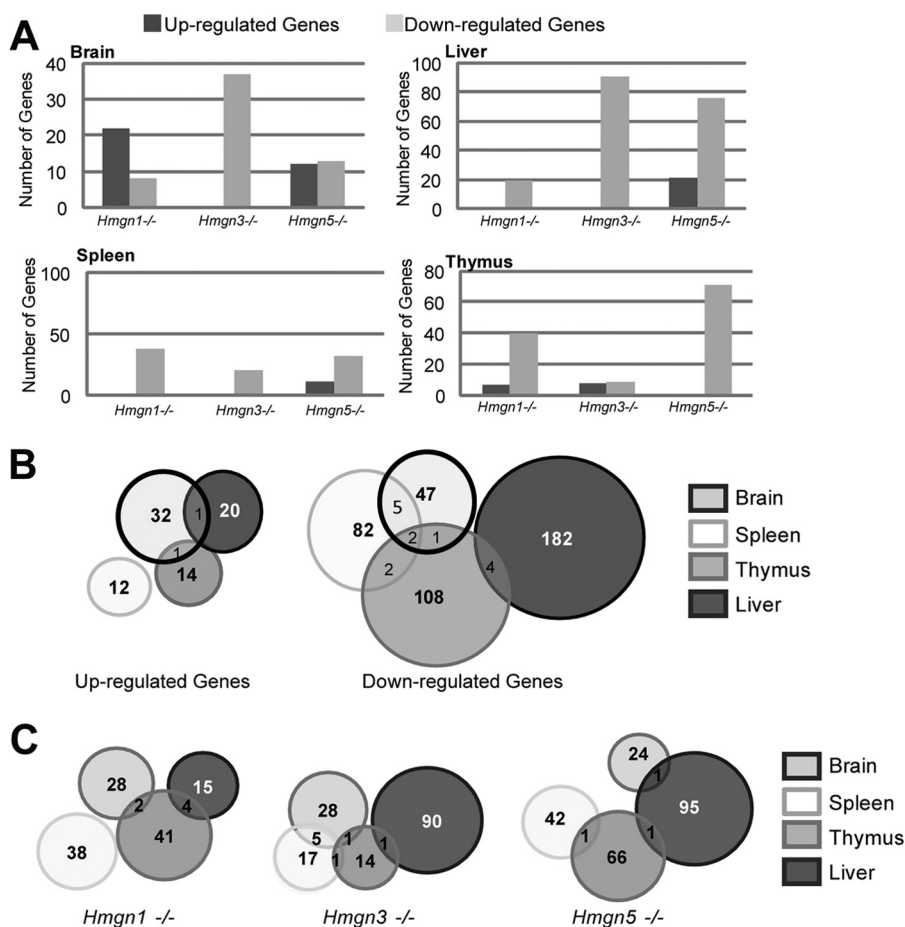


FIGURE 3. HMGN variants affect the transcription profile in a tissue specific-manner. *A*, number of genes affected in various tissues. *B*, Venn diagrams depicting the number of overlapping genes up- or down-regulated in various tissues from HMGN knock-out mice. *C*, Venn diagrams depicting the number of overlapping genes regulated by a specific HMGN variant in various tissues from HMGN knock-out mice.

TABLE 4

Magnitude of transcriptional changes in tissues and mouse embryonic fibroblasts from *Hmgn1/tm1* mice

	Genotype	Tissue	Magnitude of fold change	Total no. of genes changed	
Tissues	N1 KO vs. WT	Brain	-1.79 to 1.82	30	
	N1 KO vs. WT	Liver	-4.01 to -1.55	19	
	N1 KO vs. WT	Thymus	-5.48 to 3.24	47	
	N1 KO vs. WT	Spleen	-2.63 to -1.34	38	
	N3 KO vs. WT	Brain	-8.07 to -1.48	34	
	N3 KO vs. WT	Liver	-8.24 to -1.50	91	
	N3 KO vs. WT	Thymus	-2.73 to 2.43	17	
	N3 KO vs. WT	Spleen	-7.24 to 1.71	23	
	N5 KO vs. WT	Brain	-7.81 to 2.27	25	
	N5 KO vs. WT	Liver	-4.06 to 3.35	97	
	N5 KO vs. WT	Thymus	-10.11 to -1.70	68	
	N5 KO vs. WT	Spleen	-2.14 to 3.01	43	
	Mouse embryonic fibroblasts ^a	N1 KO vs. WT		-11.22 to 47.86 ^a	47 ^a
		N3 KO vs. WT		-28.8 to 59.24 ^a	82 ^a
N5 KO vs. WT			-12.88 to 43.95 ^a	16 ^a	

^a See Ref. 8.

ulated by HMGN3 or HMGN5 loss (group H); genes very mildly up-regulated by HMGN3 loss or HMGN5 loss (group I); genes up-regulated by HMGN5 loss (group J); genes down-regulated by HMGN3 loss or HMGN5 loss (group K); genes down-regulated only by HMGN3 loss (group L), and genes down-regulated only by HMGN5 loss (group M) (Fig. 4*B*). In spleen tissue, there are three distinct groups defined by HMGN variant loss as follows: genes up-regulated by HMGN1 loss and HMGN3 loss (group N); genes down-regulated by HMGN1 loss and HMGN3 loss (group O), and genes down-regulated only by

HMGN1 loss (group P) (Fig. 4*C*). In thymus tissue, there are five distinct groups defined by HMGN variant loss as follows: genes up-regulated by HMGN5 loss (group Q); genes down-regulated by HMGN1 loss (group R); genes down-regulated by HMGN3 loss (group S); genes up-regulated by HMGN1 loss (group T), and genes down-regulated by HMGN5 loss (group U) (Fig. 4*D*).

Notably, most of the genes in brain, liver, and thymus tissues respond specifically to the functional loss of a specific HMGN variant and remain relatively unchanged in response to the loss

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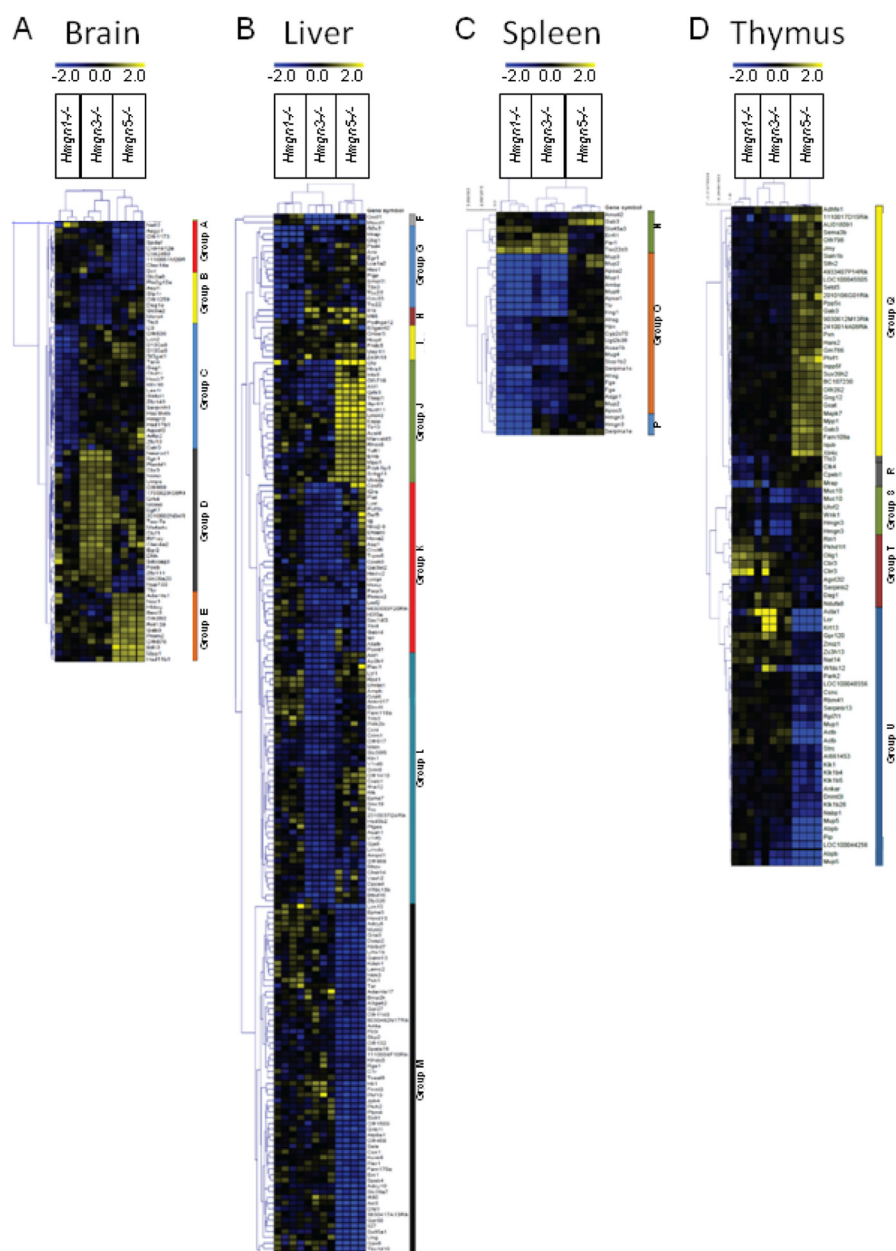


FIGURE 4. Heat plots describing general trends of changes in the transcription profile of mouse tissues obtained from mice lacking specific HMGN variants. A, brain. B, liver. C, spleen. D, thymus. Fold change, represented by the blue-yellow color scale, was calculated by dividing the signal intensity of an individual mutant mouse by the average signal intensity of the four appropriate control mice (mutant/over mean control) for the HMGN variant in question. The expression values used to generate the heat maps are available in the eight worksheets presented in supplemental Table 1.

of another HMGN variant (groups A–E, Fig. 4A; groups G, J, L, and M, Fig. 4B; and groups Q–U, Fig. 4D; also group P in Fig. 4C). Interestingly, group H in the liver consists of genes that are down-regulated by HMGN1 loss but up-regulated by HMGN3 loss as well as HMGN5 loss (Fig. 4B). Spleen tissue is unique, as the effects of HMGN1 and HMGN3 loss appear the same in all groupings, although HMGN5 loss appears to have very little effect (groups N–P, Fig. 4C); similar groupings can be seen in liver groups F, I, and K, where HMGN3 and HMGN5 loss appears to have comparable effects (Fig. 4B). Nevertheless, the majority of the groupings remain HMGN variant-specific, reinforcing the principle that each HMGN variant affects the expression of a distinct set of genes, and in some cases may regulate any shared targets in opposing ways.

HMGN Variants Fine Tune the Fidelity of the Cellular Transcription Profile—In addition to examining the genes with significant changes in expression level, we investigated the effect of HMGN variant loss on the entire transcriptional pathways, regardless of the magnitude of change in individual gene expression levels. Using a form of gene set enrichment analysis for pathway analysis of each sample (see “Experimental Procedures”), biological pathways were identified, in which an expression change was observed for the most of the associated genes (with potentially small expression changes of individual genes comprising the pathway) in each of the four mice examined in the three *Hmgn*^{tm1/tm1} mice lines (Table 5 and supplemental Table S3). In total, we identified 282 biological pathways that were significantly affected by the loss of an HMGN variant

(supplemental Table S3). The two pathways that were either most up- or down-regulated in the four tissues of the three mice lines examined are listed in Table 5. In each of these pathways, the majority of the involved genes are expressed coordinately (up- or down-regulated at the same time). Although the expression level of the individual genes comprising these pathways did not change significantly, the number of differentially expressed genes is significant, and as a group, the entire biological pathway is affected in each of the four *Hmgn^{tm1/tm1}* mice in the three mouse lines. These results indicate that the loss of any HMGN variant affects the fidelity to the transcriptional program in a subtle but significant manner in each of the tissues examined. The small changes in the fidelity to the transcriptional program do not lead to drastic phenotypes under normal conditions (Table 1); however, when the mice are placed under stress, the lack of fidelity takes its toll, and the mice develop symptoms (22). Such a mechanism would explain why HMGNs have been conserved and are present in all vertebrate cells. It is likely that their presence enhances the fidelity of the cellular transcription profile and the subtle changes resulting from their loss are evolutionarily unfavorable, especially when the organism is exposed to environmental stress.

Functional Relevance of Transcriptional Changes in *Hmgn^{tm1/tm1}* Mice—Although small changes in the expression of many genes within a pathway can affect an entire biological process, we note that the individual genes that were significantly affected by the loss of more than one HMGN variant do not fall into any one particular functional category (Table 2), indicating that as a protein family the HMGN variants do not target specific biological processes. GO analysis of the effects of single HMGN variants on genes expressed in the various tissues (Table 3 and supplemental Table 2) reveals that affected genes associated with specific biological processes were overrepresented in only four cases; the loss of HMGN3 affected several processes in the brain and in the spleen, whereas the loss of HMGN5 affected biological processes in the spleen and thymus. Examination of the genes listed revealed a significant overlap between the GO categories identified. The five genes from the GO category 0016486 in the brains of *Hmgn3^{tm1/tm1}* mice are also part of the three other GO categories affected in the brains of *Hmgn3^{tm1/tm1}* mice (Table 3 and supplemental Table 2). Likewise, all the sets of genes down-regulated in the *Hmgn3tm1/tm1* spleen contain *Apa1*, -2, or -3 genes, and in the spleen of *Hmgn5^{tm1/tm1}* an identical set of genes is affected in all the three categories listed.

Thus, our analyses do not reveal unique biological pathways that are markedly affected by the three HMGN variants in the tissues studied here when analyzed using only significantly changed genes, and they suggest that the phenotypes observed so far in the *Hmgn^{tm1/tm1}* mice are the consequence of the effects of HMGN variants on the expression levels of a few genes within a tissue. However, as elaborated below, some of these affected genes are indeed linked to the phenotypes observed in *Hmgn^{tm1/tm1}* mice and could provide insights into the molecular mechanisms leading to these phenotypes.

The gene coding for HMGN1 is located in the Down syndrome critical region (46); HMGN1 protein is overexpressed in patients suffering from this syndrome, and mice with altered

HMGN1 expression display behavioral abnormalities (19). Several of the HMGN1 targets identified here are consistent with these phenotypes. *Ttc3*, an E3 ubiquitin ligase, prevents proper neuronal differentiation when up-regulated in Down syndrome (47) and is accordingly down-regulated upon loss of HMGN1 function. Overexpression of the transcription factor *Neurod1*, which we detected in *Hmgn1^{tm1/tm1}* brain, could lead to increased neuronal survival at the expense of proper patterning (48). The transcription factor *Egfl7* inhibits Notch signaling and thus neuronal differentiation (49); the up-regulation of *Egfl7* in *Hmgn1^{tm1/tm1}* brain tissue therefore might lead to the differentiation of fewer neurons than normal. The potentially altered neuronal differentiation could be directly connected to the behavioral phenotype of the *Hmgn1^{tm1/tm1}* mice shown in Table 1. It has recently been demonstrated that modified levels of neuronal progenitor differentiation can be associated with modified levels of exploratory activity akin to the rearing/exploration phenotype observed here (50). Both alterations, up-regulation of transcription factors involved in neuronal differentiation and the altered rearing phenotype, are only present in *Hmgn1^{tm1/tm1}* mice and not in *Hmgn3^{tm1/tm1}* or *Hmgn5^{tm1/tm1}* mice.

In addition, *Hmgn1^{tm1/tm1}* mice are deficient in the repair of damaged DNA leading to hypersensitivity to both UV and ionizing radiation and to an increased incidence of certain tumors (20, 21). Several of the HMGN1 targets we found here could be involved in these processes. *Chtf8*, a component of the Ctf18 replication factor C complex, is down-regulated in *Hmgn1^{tm1/tm1}* spleen tissue; similar down-regulation has been observed in prostate and renal tumors (51). *Cbr3*, a member of the carbonyl reductase family, which is known to increase cellular sensitivity to ionizing radiation (52), is up-regulated in *Hmgn1^{tm1/tm1}* thymus tissue. Likewise, down-regulation of *Slfn1*, a regulatory gene affecting thymocyte development (53) in the thymus of *Hmgn1^{tm1/tm1}* mice, up-regulation of *Fosb*, a subunit of the AP1 transcription factor complex (54) in *Hmgn1^{tm1/tm1}* brain, and down-regulation of *G0s2*, a protein expressed during the G₀/G₁ switch in mitosis (55) in *Hmgn1^{tm1/tm1}* liver provide hints for future research on the possible role of HMGN1 in carcinogenesis.

HMGN3 is strongly expressed in brain (56, 57) and in pancreatic beta cells, and *Hmgn3^{tm1/tm1}* mice are mildly diabetic (22). HMGN3 is indirectly regulated by thyroid hormone and interacts with it during frog metamorphosis (58). Several of the HMGN3 targets identified in this study provide support for the known phenotypes and suggest additional genes of interest. Thus, the thyroid hormone receptor *Tshr* and *PomC*, a polypeptide hormone precursor that controls feeding and body weight (59), are down-regulated in *Hmgn3^{tm1/tm1}* brain tissue, and *Trib3*, a putative protein kinase, implicated in hyperglycemia and glucose intolerance (60, 61) is down-regulated in the *Hmgn3^{tm1/tm1}* liver. Muscle tissues were not analyzed in *Hmgn3^{tm1/tm1}* mice in this study, and only weak expression was previously reported in skeletal muscle (50). The reduced grip strength detected only in *Hmgn3tm1/tm1* mouse mutants could therefore be a secondary effect to differences in energy metabolism found in these mutants and not in *Hmgn1^{tm1/tm1}* or *Hmgn5^{tm1/tm1}* mice.

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TABLE 5

Transcriptional effects of HMGN variant loss on biological processes

	HMGN variant	Biological process	# of Genes in Category	p-value	Relative Expression (ranksum)			
					Mouse 1	Mouse 2	Mouse 3	Mouse 4
Brain	<i>Hmgn1^{tm1/t}</i>	BP:translation	198	0.000921	-0.695	-2.941	-15.044	-3.07
		BP:collagen catabolic process	18	0.001211	-3.67	-4.678	-2.292	-4.046
		BP:response to drug	108	0.00166	4.965	7.074	9.473	4.654
		BP:hindbrain development	18	0.004147	4.027	2.322	3.273	7.53
	<i>Hmgn3^{tm1/t}</i>	BP:translation	198	0.000138	-8.224	-17.94	-13.475	0.92
		BP:RNA splicing	176	0.000322	-2.57	-2.928	-10.384	-11.555
		BP:negative regulation of inflammatory response	28	0.002291	4.028	-0.421	8.902	7.738
	<i>Hmgn5^{tm1/t}</i>	BP:nervous system development	225	0.000187	-3.82	-10.558	-7.064	-16.084
		BP:cell morphogenesis	44	0.000331	-2.654	-1.848	-10.374	-8.398
		BP:translation	198	0	4.782	1.897	25.93	18.919
		BP:negative regulation of ubiquitin-protein ligase activity during mitotic cell cycle	62	0	0.958	9.22	16.1	17.779
	Liver	<i>Hmgn1^{tm1/t}</i>	BP:cell adhesion	410	0.000222	-1.281	-11.669	-2.493
BP:response to protein stimulus			35	0.000247	-2.507	-8.287	-7.592	-11.767
BP:ATP catabolic process			222	0.000116	14.259	6.127	8.38	1.961
BP:neural tube formation			10	0.000245	9.201	7.748	5.051	5.512
<i>Hmgn3^{tm1/t}</i>		BP:translation	198	0.000535	-6.392	-3.07	-10.65	-8.691
		BP:sterol biosynthetic process	21	0.000645	-0.043	-8.901	-28.009	-6.382
		BP:positive regulation of phagocytosis	25	0.000003	9.195	4.859	11.102	10.625
		BP:antigen processing and presentation of exogenous peptide antigen via MHC class II	14	0.000004	7.555	9.556	8.934	11.647
<i>Hmgn5^{tm1/t}</i>		BP:neuromuscular process	15	0.000216	-3.18	-6.867		
		BP:proton transport	82	0.000409	-5.054	-11.532		
		BP:chromatin modification	169	0.000078	8.596	7.33		
		BP:retrograde transport, endosome to Golgi	15	0.000109	7.284	9.393		
Spleen	<i>Hmgn1^{tm1/t}</i>	BP:protein metabolic process	15	0.000009	-6.231	-6.326	-4.915	-9.62
		BP:lipoprotein transport	10	0.000314	-3.823	-3.966	-5.636	-6.992
		BP:mRNA export from nucleus	15	0.00004	7.158	4.491	4.325	5.88
		BP:Wnt receptor signaling pathway through beta-catenin	59	0.000138	1.493	8.627	5.066	7.942
	<i>Hmgn3^{tm1/t}</i>	BP:post-translational protein modification	28	0.000266	-12.553	-3.139	-6.876	-4.18
		BP:leukocyte migration	12	0.00072	-0.31	-6.762	-7.095	-6.409
		BP:integrin-mediated signaling pathway	67	0.000025	0.851	11.411	6.536	9.167
		BP:cartilage development	57	0.000105	2.388	7.713	2.279	10.152
	<i>Hmgn5^{tm1/t}</i>	BP:positive regulation of interleukin-1 beta secretion	10	0.000153	-4.484	-5.762	-7.18	-3.486
		BP:protein sumoylation	12	0.000565	-2.257	-9.247	-2.764	-10.296
		BP:negative regulation of transcription from RNA polymerase II	244	0.000057	7.746	4.862	10.587	2.691
		BP:protein homooligomerization	82	0.000295	4.471	-1.426	7.796	7.147
Thymus	<i>Hmgn1^{tm1/t}</i>	BP:protein deubiquitination	27	0.000123	-5.508	-8.188	-3.166	-4.954
		BP:DNA damage response, signal transduction by p53 class mediator resulting in transcription of p21 class mediator	10	0.00027	-2.445	-8.881	-3.819	-5.143
		BP:ectoderm development	10	0.00067	5.023	2.739	5.82	9.641
		BP:melanocyte differentiation	19	0.001569	9.25	0.802	4.962	7.524
	<i>Hmgn3^{tm1/t}</i>	BP:N-acetylglucosamine metabolic process	13	0.000016	-4.679	-4.325	-7.909	-4.423
		BP:cell cycle	400	0.00037	-3.711	1.401	-10.989	-0.851
		BP:thermoregulation	15	0.000227	8.501	5.853	4.441	5.295
	<i>Hmgn5^{tm1/t}</i>	BP:phospholipase C activation	10	0.002178	5.201	4.079	5.27	6.081
		BP:sensory perception of light stimulus	10	0.001793	-1.369	-3.498	-7.419	-3.049
		BP:protein metabolic process	15	0.00515	-2.922	-4.609	-1.886	-4.457

The newly characterized HMGN variant, HMGN5, has not yet been implicated in any published phenotypes. Targets of potential biological interest include the members of the *Lce3* family, which are involved in the etiology of autoimmune disease (62); the mitogens *Eapp* and *Abl1* (63, 64), which are up-regulated in *Hmgn5^{tm1/tm1}* liver; and centrosomal protein *Cep57*, which is down-regulated in the *Hmgn5^{tm1/tm1}* spleen, and this suggests that HMGN5 mutant cells might be prone to aneuploidy (65).

DISCUSSION

Elucidation of the role of HMGN proteins in transcriptional regulation is central to understanding their biological function and mechanism of action. This study aims to provide insights into this question by systematic analysis of phenotypes in mice lacking functional HMGN variants and through the biological context of whole tissue transcription profile analysis. In some cases the transcriptional effects may be also due to the expression of the HMGN mutant proteins that further impact the HMGN-regulated gene expression. Analysis of the transcription profiles of the four different tissues derived from each of the three *Hmgn^{tm1/tm1}* mouse lines provides several new insights into their effect on transcription.

First, phenotypic analysis of the mice indicates that each HMGN variant affects specific cellular functions, suggesting specific effects on gene expression. Second, each HMGN variant significantly affects the expression of a limited number of genes in a variant-specific manner. Third, each variant affects gene expression in a tissue-specific manner. Fourth, functional loss of HMGN variants preferentially leads to down-regulation of gene expression levels. Fifth, HMGN variant loss leads to small changes in the expression levels of numerous genes in a particular pathway, thereby affecting of the transcriptional program in a cell type-specific manner.

In considering possible mechanisms involved in the transcriptional specificity, we note that although the nucleosome binding domains of all HMGNs are quite similar, their C-terminal domains are variable, and thus may interact with different regulators to enact their transcriptional effects (15). Furthermore, previous studies demonstrated that all HMGN proteins bind to nucleosomes and that this interaction affects the higher order chromatin structure and the levels of histone modification, in some cases in a variant-specific manner. Thus, HMGN1 and HMGN2 form specific complexes with nucleosome core particles, in which either two molecules of HMGN1 or two molecules of HMGN2 are associated with one core particle; nucleosome complexes with one molecule of each type of HMGN variant have not been detected (66). Likewise, it has been demonstrated that HMGN1 and HMGN2 affect the acetylation and phosphorylation of histone H3 in a variant-specific manner (38). In addition, both HMGN1 and HMGN2 form several types of multiprotein complexes and can associate with specific protein partners (67, 68). Recent work suggests that classic transcription factors may display cell type-specific behavior (40); whether HMGNs contribute to this specificity or may themselves be affected by it remains to be seen. As HMGN proteins bind to DNA in a nonsequence-specific manner, they may rely on specific protein partners to guide them to their

appropriate sites. We note that our studies do not exclude the remote possibility that in some cases the expression of mutant proteins, lacking the nucleosome binding domain may also impact the cellular transcription profile.

Our findings do not exclude the possibility of functional redundancy among HMGN variants. It is still possible that by and large HMGN variants are functionally redundant for most cellular genes, but that each variant has a more pronounced effect on a subset of genes.

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