A comparative encyclopedia of DNA elements in the mouse genome

The laboratory mouse shares the majority of its protein-coding genes with humans, making it the premier model organism in biomedical research, yet the two mammals differ in significant ways. To gain greater insights into both shared and species-specific transcriptional and cellular regulatory programs in the mouse, the Mouse ENCODE Consortium has mapped transcription, DNase I hypersensitivity, transcription factor binding, chromatin modifications and replication domains throughout the mouse genome in diverse cell and tissue types. By comparing with the human genome, we not only confirm substantial conservation in the newly annotated potential functional sequences, but also find a large degree of divergence of sequences involved in transcriptional regulation, chromatin state and higher order chromatin organization. Our results illuminate the wide range of evolutionary forces acting on genes and their regulatory regions, and provide a general resource for research into mammalian biology and mechanisms of human diseases.

Overview of data production and initial processing

To annotate potential functional sequences in the mouse genome, we used ChiP-seq, RNA-sequencing and DNase-seq to profile transcription factor binding, chromatin modification, transcriptome and chromatin accessibility in a collection of 123 mouse cell types and primary tissues (Fig. 1a, Supplementary Tables 1–3). Additionally, to interrogate large-scale chromatin organization across different cell types, we also used a microarray-based technique to generate replication-timing profiles in 18 mouse tissues and cell types (Supplementary Table 3). Altogether, we produced over 1,000 data sets. The list of the data sets and all the supporting material for this manuscript are also available at website http://mouseencode.org. Below we briefly outline the experimental approach and initial processing for each class of sequence features.

RNA transcriptome

To comprehensively identify the genomic regions that produce transcripts in the mouse genome, we performed RNA-seq experiments in 69 different mouse tissues and cell types with two biological replicates each (Supplementary Table 3, Supplementary Information) and uncovered 436,410 contigs (Supplementary Table 4). Confirming previous reports and similar to the human genome, the mouse genome is pervasively transcribed (Fig. 1b), with 46% capable of producing polyadenylated...

Despite the widespread use of mouse models in biomedical research, the genetic and genomic differences between mice and humans remain to be fully characterized. At the sequence level, the two species have diverged substantially: approximately one half of human genomic DNA can be aligned to mouse genomic DNA, and only a small fraction (3–8%) is estimated to be under purifying selection across mammals. At the cellular level, a systematic comparison is still lacking. Recent studies have revealed divergent DNA binding patterns for a limited number of transcription factors across multiple related mammals, suggesting potentially wide-ranging differences in cellular functions and regulatory mechanisms. To fully understand how DNA sequences contribute to the unique molecular and cellular traits in mouse, it is crucial to have a comprehensive catalogue of the genes and non-coding functional sequences in the mouse genome.

Advances in DNA sequencing technologies have led to the development of RNA-seq (RNA sequencing), DNase-seq (DNase I hypersensitive sites sequencing), ChiP-seq (chromatin immunoprecipitation followed by DNA sequencing), and other methods that allow rapid and genome-wide analysis of transcription, replication, chromatin accessibility, chromatin modifications and transcription factor binding in cells. Using these large-scale approaches, the ENCODE consortium has produced a catalogue of potential functional elements in the human genome. Notably, 62% of the human genome is transcribed in one or more cell types, and 20% of human DNA is associated with biochemical signatures typical of functional elements, including transcription factor binding, chromatin modification and DNase hypersensitivity. The results support the notion that nucleotides outside the mammalian-conserved genomic regions could contribute to species-specific traits.

We have applied the same high-throughput approaches to over 100 mouse cell types and tissues, producing a coordinated group of data sets for annotating the mouse genome. Integrative analyses of these data sets uncovered widespread transcriptional activities, dynamic gene expression and chromatin modification patterns, abundant cis-regulatory elements, and remarkably stable chromosome domains in the mouse genome. The generation of these data sets also allowed an unprecedented level of comparison of genomic features of mouse and human. Described in the current manuscript and companion works, these comparisons revealed both conserved sequence features and widespread divergence in transcription and regulation. Some of the key findings are:

- Although much conservation exists, the expression profiles of many mouse genes involved in distinct biological pathways show considerable divergence from their human orthologues.
- A large portion of the cis-regulatory landscape has diverged between mouse and human, although the magnitude of regulatory DNA divergence varies widely between different classes of elements active in different tissue contexts.
- Mouse and human transcription factor networks are substantially more conserved than cis-regulatory DNA.
- Species-specific candidate regulatory sequences are significantly enriched for particular classes of repetitive DNA elements.
- Chromatin state landscape in a cell lineage is relatively stable in both human and mouse.
- Chromatin domains, interrogated through genome-wide analysis of DNA replication timing, are developmentally stable and evolutionarily conserved.
De novo mapping delineated 8.9 million distinct transcription factor footprints. Genomic footprinting analysis in a subset (25) of these cell types further revealed a recognition repertoire nearly identical to that of the human, including both known and novel recognition factor footprints. The sensitivity of the approach. However, a higher percentage of intronic sequences were detected as transcribed in the mouse, and this might be owing to a greater sequencing depth and broader spectrum of histone modification patterns. All of these approaches have previously been shown to uncover cis-regulatory elements with high accuracy and sensitivity.

Candidate cis-regulatory sequences

To identify potential cis-regulatory regions in the mouse genome, we used three complementary approaches that involved mapping of chromatin accessibility, specific transcription factor occupancy sites and histone modification patterns. All of these approaches have previously been shown to uncover cis regulatory elements with high accuracy and sensitivity.

By mapping DNase I hypersensitive sites (DHSs) in 55 mouse cell and tissue types, we identified a combined total of ∼1.5 million distinct DHSs at a false discovery rate (FDR) of 1% (Supplementary Table 5). Genomic footprinting analysis in a subset (25) of these cell types further delineated 8.9 million distinct transcription factor footprints. De novo derivation of a cis-regulatory lexicon from mouse transcription factor footprints revealed a recognition repertoire nearly identical with that of the human, including both known and novel recognition motifs.

We used ChIP-seq to determine the binding sites for a total of 37 transcription factors in various subsets of 33 cell/tissue types. Of these 37 transcription factors, 24 were also extensively mapped in the murine and human erythroid cell models (MEL and K562) and B-lymphoid cell lines (CH12 and GM12878). In total we defined 2,107,950 discrete ChIP-seq peaks, representing differential cell/tissue occupancy patterns of 280,396 distinct transcription factor binding sites (Supplementary Methods and Supplementary Table 6).

We also performed ChIP-seq for as many as nine histone H3 modifications (H3K4me1, H3K4me2, H3K4me3, H3K27ac, H3K27me3, H3K36me3, H3K36me2 and H3K79me3) in up to 23 mouse tissues and cell types per mark. We applied a supervised machine learning technique, random-forest based enhancer prediction from chromatin state (RFECS), to three histone modifications (H3K4me1, H3K4me3 and H3K27ac) identifying a total of 82,853 candidate promoters and 291,200 candidate enhancers in the mouse genome (Supplementary Tables 7 and 8). To functionally validate the predictions, we randomly selected 76 candidate promoter elements (average size 1,000 bp, Supplementary Table 9) and 183 candidate enhancer elements (average size 1,000 bp, Supplementary Table 10) and performed luciferase reporter assays via transient transfection in pertinent mouse cell lines. For candidate promoter elements, we selected 76 candidate promoter elements (average size 1,000 bp, Supplementary Table 9) and 183 candidate enhancer elements (average size 1,000 bp, Supplementary Table 10). For candidate promoter elements, we cloned these previously unannotated sequences into reporter constructs, and performed luciferase reporter assays via transient transfection in pertinent mouse cell lines. For candidate enhancer elements, we performed functional validation assay using a high throughput method (see Supplementary Methods). Overall, 66/76 (87%) candidate promoters and 129/183 (70.5%) candidate enhancers showed significant activity in these assays, compared to 2/30 randomly selected negative controls (Supplementary Fig. 1c).

Collectively, our studies assigned potential regulatory function to 12.6% of the mouse genome (Fig. 1c).
Transcription factor networks
We explored the transcription factor networks and combinatorial transcription factor binding patterns in the mouse samples in two companion papers, and compared these networks to regulatory circuitry models generated for the human genome22,23. From genomic footprints, we constructed transcription-factor-to-transcription-factor cross-regulatory network in each of 25 cell/tissue types for a total of ~500 transcription factors with known recognition sequences. Analyses of these networks revealed regulatory relationships between transcription factor genes that are strongly preserved in human and mouse, in spite of the extensive plasticity of the cis-regulatory landscape (detailed below). Whereas only 22% of transcription factor footprints are conserved, nearly 50% of cross-regulatory connections between mouse transcription factors are conserved in human through the innovation of novel binding sites. Moreover, analysis of network motifs shows that larger-scale architectural features of mouse and human transcription factor networks are strikingly similar25.

Chromatin states
We produced integrative maps of chromatin states in 15 mouse tissue and cell types and six human cell lines (Supplementary Table 11), using a hidden Markov model (chromHMM)26,27 that allowed us to segment the genome in each cell type into seven distinct combination of chromatin modification marks (or chromatin states). One state is characterized by the absence of any chromatin marks, while every other state features either predominantly one modification or a combination of two modifications (Extended Data Table 1, Supplementary Information). The portion of the genome in each chromatin state varied with cell type (Fig. 1d, Supplementary Fig. 2). Similar proportions of the genome are found in the active states in each cell type, for both mouse and human. Interestingly, excluding the ‘unmarked’ state, the fraction of each genome that is in the H3K27me3-dominated, transcriptionally repressed state is the most variable, suggesting a profound role of transcriptional repression in shaping the cis-regulatory landscape during mammalian development.

Replication domains
Replication-timing, the temporal order in which megabase-sized genomic regions replicate during S-phase, is linked to the spatial organization of chromatin in the nucleus28–31, serving as a useful proxy for tracking differences in genome architecture between cell types32,33. Since different types of chromatin are assembled at different times during the S phase34, changes in replication timing during differentiation could elicit changes in chromatin structure across large domains. We obtained 36 mouse and 31 human replication-timing profiles covering 11 and 9 distinct stages of development, respectively (Supplementary Table 12). We defined ‘replication boundaries’ as the sites where replication profiles change slope from synchronously replicating segments (discussed later). A total of 64,535 and 50,194 boundaries identified across all mouse and human data sets, respectively, were mapped to 4,322 and 4,675 positions, with 64,535 and 50,194 boundaries identified across all mouse and human stages of development, respectively (Supplementary Table 12). We defined orthologous pairs (Supplementary Tables 13–15). We also inferred orthologous relationships among short non-coding RNA genes using a similar phylogenetic approach. We established one-to-one human–mouse orthologues for 151,257 internal exon pairs (Supplementary Table 16) and 204,887 intron pairs (Supplementary Table 17), and predicted 2,717 (3,446) protein coding and non-coding genes35. The list of protein-coding orthologues for 151,257 internal exon pairs (Supplementary Table 16) and 204,887 intron pairs (Supplementary Table 17), and predicted 2,717 (3,446) protein-coding orthologues for 151,257 internal exon pairs (Supplementary Table 16) and 204,887 intron pairs (Supplementary Table 17), and predicted 2,717 (3,446) protein-coding orthologues for 151,257 internal exon pairs (Supplementary Table 16) and 204,887 intron pairs (Supplementary Table 17), and predicted 2,717 (3,446) proteins (respectively, mouse) exons (Supplementary Table 18). Additionally, we mapped the 17,547 human long non-coding RNA (lncRNA) transcripts annotated in Gencode v10 onto the mouse genome.

We found 2,327 (13.26%) human lncRNA transcripts (corresponding to 1,679, or 15.48%, of the lncRNA genes) homologous to 5,067 putative mouse transcripts (corresponding to 3,887 putative genes) (Supplementary Fig. 3, Supplementary Table 19). Consistent with previous observations, only a small fraction of lncRNAs are constrained at the primary sequence level, with rapid evolutionary turnover36. Other comparisons of human and mouse transcriptomes, covering areas including pre-mRNA splicing, antisense and intergenic RNA transcription, are detailed in an associated paper17.

Divergent and conserved gene expression patterns
Previous studies have revealed remarkable examples of species-specific gene expression patterns that underlie phenotypic changes during evolution39–42. In these cases changes in expression of a single gene between closely related species led to adaptive changes. However, it is not clear how extensive the changes in expression patterns are between more distantly related species, such as mouse and human, with some studies emphasizing similarities in transcriptome patterns of orthologous tissues33–45 and others emphasizing substantial interspecies differences46. Our initial analyses revealed that gene expression patterns tended to cluster more by species rather than by tissue (Fig. 2a). To resolve the sets of genes contributing to different components in the clustering, we employed variance decomposition (see Methods) to estimate, for each orthologous human–mouse gene pair, the proportion of the variance in expression that is contributed by tissue and by species (Fig. 2b). This analysis revealed the sets of genes whose expression varies more across tissues than between species, and those whose expression varies more between species than across tissues. As expected, the clustering of the RNA-seq samples is dominated either by species or tissues, depending on the gene set employed (Extended Data Fig. 1a, b). Furthermore, removal of the ~4,800 genes that drive the species-specific clustering (see ref. 47, Supplementary Fig. 1d)

Figure 2 | Comparative analysis of the gene expression programs in human and mouse samples. a, Principal component analysis (PCA) was performed for RNA-seq data for 10 human and mouse matching tissues. The expression values are normalized across the entire data set. Solid squares denote human tissues. Open squares denote mouse tissues. Each category of tissue is represented by a different colour. b, Gene expression variance decomposition (see Methods) estimates the relative contribution of tissue and species to the observed variance in gene expression for each orthologous human–mouse gene pair. Green dots indicate genes with higher between-tissue contribution and red dots genes with higher between-species contributions. c, Neighbourhood analysis of conserved co-expression (NACC) in human and mouse samples. The distribution of NACC scores for each gene is shown. d, A scatter plot shows the average of NACC score over the set of genes in each functional gene ontology category. Highlighted are those biological processes that tend to be more conserved between human and mouse and those processes that have been less conserved (see Supplementary Table 21 for list of genes).
and primary cell types (see Supplementary Methods), the biological processes detected as conserved and species-specific in the larger panel of mismatched human–mouse samples are largely recapitulated, although some pathways are detected with somewhat less significance, probably owing to the smaller number of data sets used (Supplementary Fig. 8).

In summary, the NACC results support and extend the principal component analysis, showing that while large differences between mouse and human transcriptome profiles can be observed (revealed in PC1), genes involved in distinct cellular pathways or functional groups exhibit different degrees of conservation of expression patterns between human and mouse, with some strongly preserved and others changing markedly.

**Prevalent species–specific regulatory sequences along with a core of conserved regulatory sequences**

To better understand how divergence of cis-regulatory sequences is linked to the range of conservation patterns detected in comparisons of gene expression programs between species, we examined evolutionary patterns in our predicted regulatory sequences. Previous studies have identified a wide range of evolutionary patterns and rates for cis-regulatory regions in mammals7,8, but there are still questions regarding the overall degree of similarity and divergence between the cis-regulatory landscapes in the mouse and human. The variety of assays and breadth of tissue and cell-type coverage in the mouse ENCODE data therefore provide an opportunity to address this problem more comprehensively.

We first determined sequence homology of the predicted cis-elements in the mouse and human genomes. We established one-to-one and one-to-many mapping of human and mouse bases derived from reciprocal chained blastz alignments48 and identified conserved cis-regulatory sequences49. This analysis showed that 79.3% of chromatin-based enhancer predictions, 79.6% of chromatin-based promoter predictions, 67.1% of the DHS, and 66.7% of the transcription factor binding sites in the mouse genome have homologues in the human genome with at least 10% overlapping nucleotides, while by random chance one expects 51.2%, 52.3%, 44.3% and 39.3%, respectively (Fig. 3a, Supplementary Information for details). With a more stringent cutoff that requires 50% alignment of nucleotides, we found that 56.4% of the enhancer predictions, 62.4% of promoter predictions, 61.5% of DHS, and 53.3% of the transcription factor binding sites have homologues, compared with an expected frequency of 34%, 33.8%, 33.6% and 33.7% by random chance (Supplementary Fig. 9).

The candidate mouse regulatory regions with human homologues are listed in Supplementary Tables 22–25. Thus, between half and two-thirds of candidate regulatory regions demonstrate a significant enrichment in sequence conservation between human and mouse. The remaining half to one-third have no identifiable orthologous sequence.

The candidate regulatory regions in mouse with no orthologue in human could arise either because they were generated by lineage-specific events, such as transposition, or because the orthologue in the other species was lost. Species-specific cis-regulatory sequences have been reported before44, but the fraction of regulatory sequences in this category remains debatable and may vary with different roles in regulation. We find that 15% (12,387 out of 82,853) of candidate mouse promoters and 16.6% (48,245 out of 291,200) of candidate enhancers (both predicted by patterns of histone modifications) have no sequence orthologue in humans (Supplementary Tables 26, 28, for details please refer to Supplementary Methods section). However, the question remains as to whether these species-specific elements are truly functional elements or simply correspond to false-positive predictions due to measurement errors or biological noise. Supporting the function of mouse-specific cis elements, 18 out of 20 randomly selected candidate mouse-specific promoters tested positive using reporter assays in mouse embryonic stem cells, where they were initially identified (Fig. 3b, Supplementary Table 27). Further, when these 18 mouse-specific promoters were tested using reporter assays in the human embryonic stem cells, all of them also exhibited significant promoter activities (Extended Data Fig. 2a, Supplementary Table 27), indicating that the majority of candidate mouse-specific promoters are indeed functional sequences, which are either gained in the mouse lineage therein) or normalization methods that reduce the species effects reveal tissue-specific patterns of expression in the same samples (Extended Data Fig. 1c). Categorizing orthologous gene pairs into these groups should enable more informative translation of research results between mouse and human. In particular, for gene pairs whose variance in expression is largest between tissues (and less between species), mouse should be a particularly informative model for human biology. In contrast, interpretation of studies involving genes whose variance in expression is larger between species needs to take into account the species variation. The relative contributions of species-specific and tissue-specific factors to each gene’s expression are further explored in two associated papers37,47.
and enhancers are significantly enriched for repetitive DNA sequences, numbers of primate-specific candidate regulatory regions. This suggests (Fig. 3c), in agreement with the divergent transcription patterns of genes involved in immune function (Supplementary Table 32). The remaining 56–60% of candidate mouse regulatory regions with a human orthologue fall into category (1), which is associated with pleiotropic roles of enhancers, as evidenced by activity in multiple tissues. References 22,49 describe the exaptation of conserved regulatory sequences for other functions.

We surveyed the conservation of function in the subset of mouse candidate cis elements that have sequence counterparts in the human genome. Of the 51,661 chromatin-based promoter predictions that have human orthologues, 44% (22,665) of them are still predicted as promoters in human on the basis of the same analysis of histone modifications (Supplementary Table 31, see Supplementary Methods for details). Of the 164,428 chromatin-based enhancer predictions that have human orthologues, 40% (64,962) of them are predicted as an enhancer in human (Supplementary Table 32). The remaining 56–60% of candidate mouse regulatory regions with a human orthologue fall into category (2) or (3) (see earlier), that is, the orthologous sequence in human either performs a different function or does not maintain a detectable function.

One caveat of the above observation is that the tissues or cell samples used in the survey were not perfectly matched. To better examine the conservation of biochemical activities among these predicted cis-regulatory elements with orthologues between mouse and human, we analysed the chromatin modifications at the promoter or enhancer predictions in a broad set of 23 mouse tissue and cell types with the neighbourhood co-expression association analysis (NACC) method described above. Instead of gene expression levels, we selected the histone modification H3K27ac as an indicator of promoter or enhancer activity as previously reported. As shown in Fig. 4a, the promoter predictions (blue) show a significantly higher correlation in the level of H3K27ac in human and mouse than the random controls (red). Similarly, most chromatin-based enhancer predictions in the mouse genome exhibit conserved chromatin modification patterns in the human, albeit to a lesser degree than the promoters (Fig. 4b). NACC analysis on DNase-seq signal resulted in very similar distributions of conserved chromatin accessibility patterns at promoters (Fig. 4c) and enhancers (Fig. 4d). Thus many sequence-conserved candidate cis-regulatory elements appeared to have conserved patterns of activities in mice and humans.

Taken together, these analyses show that the mammalian cis-regulatory landscapes in the human and mouse genomes are substantially different, driven primarily by gain or loss of sequence elements during evolution. These species-specific candidate regulatory elements are enriched near genes involved in stress response, immunity and certain metabolic processes, and contain elevated levels of repeated DNA elements. On the other hand, a core set of candidate regulatory sequences are conserved and display similar activity profiles in humans and mice.

Chromatin state landscape reflects tissue and cell identities

We examined gene-centred chromatin state maps in the mouse and human cell types (see Supplementary Methods) (Fig. 5a, Supplementary Fig. 10). In all cell types, the low-expressed genes were almost uniformly in chromatin states with the repressive H3K27me3 mark or in the state unmarked by these histone modifications. In contrast, expressed genes showed the canonical pattern of H3K4me3 at the transcription start site surrounded by H3K4me1, followed by H3K36me3-dominated states in the remainder of the transcription unit. A similar pattern was seen for
there were lower than those seen in the active chromatin states (Supplementary Fig. 12).

Previous studies revealed limited changes of the chromatin states in lineage-restricted cells as they undergo large-scale changes in gene expression during maturation\textsuperscript{38–40}. The chromatin state maps recapitulated this result, showing very similar patterns of chromatin modification in a cell line model for proliferating erythroid progenitor cells (G1E) and in maturing erythroblasts (G1E-ER4 cells treated with oestradiol) across genes whose expression level changed significantly during maturation (Fig. 5b, Supplementary Fig. 10b). This limited change raised the possibility that the chromatin landscape, once established during lineage commitment, dictates a permissive (or restrictive) environment for the gene regulatory programs in each cell lineage\textsuperscript{60}, and that the chromatin states may differ between cell lineages. We tested this by examining the chromatin state maps for genes that were differentially expressed between haematopoietic cell lineages (erythroblasts versus megakaryocytes), and we found marked differences between the two cell types (Fig. 5c and Supplementary Fig. 10b). Genes expressed at a higher level in megakaryocytes than in erythroblasts were all in active chromatin states in megakaryocytes, but many were in inactive chromatin states in erythroblasts (Fig. 5c). In the converse situation, genes expressed at a higher level in erythroblasts than in megakaryocytes showed more inactive states in the cells in which they were repressed (Supplementary Fig. 10b). These greater differences in chromatin states correlating with differential expression of genes between, but not within, cell lineages support the model that chromatin states are established during the process of lineage commitment. The clustering of cell types together by lineage based on chromatin state maps (Supplementary Fig. 10c) also supports the model that the landscape of active and repressed chromatin is established no later than lineage commitment, and that this landscape is a defining feature of each cell type. Greater differences in chromatin states correlating with differences in gene expression were also observed when comparing average chromatin profiles in human and mouse\textsuperscript{97}.

Mouse chromatin states inform interpretation of human disease–associated sequence variants

To investigate whether the mouse chromatin states were informative on sequence variants linked to human diseases by genome-wide association studies (GWAS), we combined the chromatin state segmentations of the fifteen mouse samples into a refined segmentation, which we used to train a self-organizing map (SOM)\textsuperscript{61} on four histone modification ChIP-seq data sets (H3K4me3, H3K4me1, H3K36me3 and H3K27me3) for each mouse sample. We mapped 4,265 single nucleotide polymorphisms (SNPs) from the human GWAS studies uniquely onto the mouse genome and scored these SNPs onto the trained SOM to determine whether SNP subsets were enriched in specific areas of the genome.
Figure 6 | Human GWAS hits when mapped onto mouse genome are associated with specific chromatin states. a, A self-organization map of histone modification H3K4me1 shows association between kidney H3K4me1 state and specific GWAS hits associated with urate levels (Methods). b, Liver-specific H3K36me3 unit shows enrichment in GWAS hits related to cholesterol, alcohol dependence and triglyceride levels. c, Brain-specific H3K27me3 high unit shows enrichment in GWAS SNPs associated with neurological disorders. d, Characterization of every unit with statistically significant GWAS enrichments in terms of highest histone modification signal in at least one sample. Units with no signal in top 100 map units for every histone modification are listed as none. RPKM, reads per kilobase per million reads mapped.

As shown in Fig. 6a, the highest enriched H3K4me1 unit in the kidney contains five GWAS hits ($P < 3.95 \times 10^{-14}$) on different chromosomes related to blood characteristics such as platelet counts (Fig. 6a, Extended Data Table 2a). Similarly, the second highest enriched unit in liver H3K36me3 contained six GWAS hits ($P < 7.54 \times 10^{-31}$) related to cholesterol and alcohol dependence out of twelve in that unit (Fig. 6b, Extended Data Table 2b). In contrast, one of the highest units in brain H3K27me3 has five GWAS hits ($P < 4.93 \times 10^{-33}$) on different chromosomes associated with brain disorders/response to addictive substances (Fig. 6c, Extended Data Table 2c). This unit is different from the other examples in that it is enriched for H3K27me3 signal in multiple tissues, with brain being the highest. 801 out of the 1,350 units of the map showed statistical enrichment of SNPs of 0.05 after Holm–Bonferroni correction for multiple hypothesis testing, 55% of which (accounting for 1,750 GWAS hits) had signal for at least one histone mark that ranked within the top 100 units on the map (Fig. 6d). The best histone marks for enriched GWAS units were primarily H3K4me1 (23%), H3K36me3 (18%) and H3K27me3 (12%), with H3K4me3 accounting for less than 2% of the remainder. Together these results suggest that the chromatin state maps can be used to identify potential sites for functional characterization in mouse for human GWAS hits. Indeed, ref. 23 shows that conserved DNA segments bound by orthologous transcription factors in human and mouse are enriched for trait-associated SNPs mapped by GWAS.

Large-scale chromatin domains are developmentally stable and evolutionarily conserved

We mapped the positions of early and late replication timing boundaries in each of 36 mouse and 31 human profiles (Fig. 7a). Significantly clustered boundary positions (above the 95th percentile of re-sampled positions) were identified and peaks in boundary density were aligned between cell types using a common heuristic (Extended Data Fig. 3a, b, Supplementary Fig. 13). After alignment, consensus boundaries were further classified by orientation and amount of replication timing separation, resulting in a more stringent filtering of boundaries (Supplementary Figs 14, 15). Overall, we found that 88% of boundary positions (versus 20% expected for random alignment; Fisher exact test $P < 2 \times 10^{-16}$) aligned position and orientation between two or more cell types in both mouse and human (that is, 12% were cell-type-specific, Fig. 7b, Extended Data Fig. 3). Pair-wise comparisons of boundaries were consistent with developmental similarity between cell types (Supplementary Fig. 16).

The earliest and latest replicating boundaries were most well preserved between cell types, while those of mid-$S$ replicating boundaries were highly variable (Extended Data Fig. 3e, f).

Interestingly, the greatest number of boundaries was detected in embryonic stem cells in both species, with significant reduction in boundary numbers during differentiation (Supplementary Fig. 16), consistent with consolidation of domains and by proxy large-scale chromatin organization into larger ‘constant timing regions’ during differentiation$^{23}$. Given that over half of the mouse and human genomes exhibit significant replication timing changes during development$^{6,53}$, these observations support the model that developmental plasticity in replication timing is derived from differential regulation of replication timing within constant timing regions whose boundaries are preserved during development.

Although conservation of replication timing between mouse and human has been reported$^{29,50}$, the conservation of replicating timing boundaries has not been examined. We converted boundary coordinates $\pm 100$ kb across boundary positions between species, revealing significant overlap (Fig. 7c, d; $P < 2.2 \times 10^{-16}$ by Fisher’s exact test relative to a randomized boundary list). The level of conservation of the positions of boundaries improved from a median of 27% for cell-type-specific boundaries to 70% for boundaries preserved in nine or more cell types (Fig. 7c), demonstrating that boundaries most highly preserved during development were the most conserved across species. This was consistent with results for transcription (Fig. 2), as well as the previous observation that suggests that an increased plasticity of replication timing during development is associated with increased plasticity of replication timing during evolution$^{44}$. Together, these findings identify evolutionarily labile versus constrained domains of the mammalian genome at the megabase scale.

Given the link between replication and chromatin assembly, we compared replication timing and levels of other chromatin properties in...
200-kb windows across the genome (Supplementary Fig. 17). Features associated with active enhancers (H3K4me1, H3K27ac, DNase I sensitivity) were more closely correlated to replication timing than features associated with active transcription (RNA polymerase II, H3K4me3, H3K36me3, H3K79me2). By contrast, the correlation of replication timing to repressive features, such as H3K9me3, was poor and cell-type-specific, consistent with prior results. A more stringent comparison of differences in chromatin to differences in replication timing between cell types (Extended Data Fig. 3c, g, Supplementary Fig. 17) again revealed that marks of enhancers, including p300, H3K4me1 and H3K27ac, and DNase I sensitivity were more strongly correlated to replication timing than marks of active transcription.

Conclusion

By comparing the transcriptional activities, chromatin accessibilities, transcription factor binding, chromatin landscapes and replication timing throughout the mouse genome in a wide spectrum of tissues and cell types, we have made significant progress towards a comprehensive catalogue of potential functional elements in the mouse genome. The catalogue described in the current study should provide a valuable reference to guide researchers to formulate new hypotheses and develop new mouse models, in the same way as the recent human ENCODE studies have impacted the research community.

We provide multiple lines of evidence that gene expression and their underlying regulatory programs have substantially diverged between the human and mouse lineages although a subset of core regulatory programs are largely conserved. The divergence of regulatory programs between mouse and human is manifested not only in the gain or loss of cis-regulatory sequences in the mouse genome, but also in the lack of conservation in regulatory activities across different tissues and cell types. This finding is in line with previous observations of rapidly evolving transcription factor binding in mammals, flies and yeasts, and highlights the dynamic nature of gene regulatory programs in different species and in different mammalian species.

The finding of different rates of divergence associated with regulatory programs of distinct biological pathways suggests complex forces driving the evolution of the cis-regulatory landscape in mammals. We discovered that specific classes of endogenous retroviral elements are enriched at the species-specific putative cis-regulatory elements, implicating transposition of DNA as a potential mechanism leading to divergence of gene regulatory programs during evolution. Previous studies have shown that endogenous retroviral elements can be transcribed in a tissue-specific manner, with a fraction of them derived from enhancers and necessary for transcription of genes involved in pluripotency. Future studies will be necessary to determine whether retroviral elements at or near enhancers are generally involved in driving tissue-specific gene expression programs in different mammalian species.

Despite the divergence of the regulatory landscape between mouse and human, the pattern of chromatin states (defined by histone modifications) and the large-scale chromatin domains are highly similar between the two species. Half of the genome is well conserved in replication timing (and by proxy, chromatin interaction compartment) with the other half highly plastic both between cell types and between species. It will be interesting to investigate the significance of these conserved and divergent classes of DNA elements at different scales, both with regard to the forces driving evolution and for implications of the use of the laboratory mouse as a model for human disease.

Online Content

Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 3 February; accepted 24 October 2014.

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Supplementary Information is available in the online version of the paper.

Acknowledgements This work is funded by grants R01HG002399 (B.R.), U54HG007004 (T.R.), 3RC2HG005602 (M.P.S.), GM083337 and GM085534 (D.M.G.), F31CA165863 (B.D.P.), R01DK005753 and R01DK065806 (R.C.H.) from the National Institutes of Health, and Bio211-26205 from the Spanish Plan Nacional and ERC 294653 (to R.G.). J.V. is supported by a National Science Foundation Graduate Research Fellowship under grant no. DGE-0718245. We thank K. Beyer, J. C. R., and R. S. H. for acknowledgment the Wellcome Trust (grant number 095908), the NHGR (grant number U01HG004695) and the European Molecular Biology Laboratory. We thank C. Beisel for helping with the analysis of high-throughput enhancer validation. L.S. is supported by R01HG034997-09. S.L. was supported by grants F32HL110473 and K99HL119617.

Author Contributions F.Y., Y.C., A.B., J.W., V.W., T.R., M.A.Beer, R.C.H., J.A.S., M.P.S., R.G., T.R., D.G.M., and B.R. led the data analysis effort, R.Sandstrom, Z.M., C.D., B.D.P., Y.S., R.C.H., J.A.S, M.P.S., R.G., T.R., D.G.M., and B.R. led the data production. F.Y., M.A.Beer, L.E., Y.C., P.C., A.B., A.K., S.L., Y.J., J.W., R.Sandstrom, R.E.T., E.H., A.P.R., S.N., R.W., H.C., A.B., M.J.P., B.R., R.C.H., and C.A.K. carried out the gene expression analysis of the Drosophila. PLoS Comput. Biol. 7, 1533–1544 (2011).

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Extended Data Figure 1 | Clustering analysis of human and mouse tissue samples. a, RNA-seq data from Illumina Body Map (adipose, adrenal, brain, colon, heart, kidney, liver, lung, ovary and testis) were analysed together with that from the matched mouse samples using clustering analysis. Genes with high variance across tissues were used, resulting in cell samples clustering by tissues, not by species. b, Clustering employing genes with high variance between species shows clustering by species instead of tissues. c, Principal Component Analysis (PCA) was performed for RNA-seq data for 10 human and mouse matching tissues. The expression values are normalized within each species and we observed the clustering of samples by tissue types.
Extended Data Figure 2 | Comparative analysis of sequence conservation in the cis elements predicted in the human and mouse genome.  

**a.** The predicted mouse-specific promoters and enhancers can function in human embryonic stem cells (hESCs). Percentages of predicted enhancers or promoters that test positive are shown in a bar chart.  

**b.** A bar chart shows the percentage of the predicted mouse-specific promoters containing various subclasses of LTR and SINE elements. As control, the predicted mouse cis elements with homologous sequences in the human genome or random genomic regions are included.
Extended Data Figure 3 | Replication timing boundaries preserved among tissues are conserved during evolution. a, Heat map of TTR overlap with positive (yellow) or negative (blue) slope. Replication timing (RT) boundaries were identified as clustered TTR endpoints (grey) above the 95th percentile (dashed line) of randomly resampled positions (black). b, Examples of constitutive boundaries (blue regions) and regulated boundaries (grey regions) highlighted. c, Spearman correlations between differences in chromatin feature enrichment and differences in RT in non-overlapping 200-kb windows. d, Percentage of boundaries preserved between the indicated number of human cell types. e, f, Distribution of boundary replication timing in mouse (e) and human (f) as a function of preservation level between cell types. g, Comparison of changes in replication timing versus various histone marks across a segment of mouse chromosome 6.
Extended Data Table 1 | A seven-state chromHMM model learned from four histone modifications in 15 mouse cell types or lines and six human cell lines is shown

| State | Feature     | H3K27m3 | H3K4m3 | H3K4m1 | H3K36m3 | Average% | Variation |
|-------|-------------|---------|--------|--------|---------|----------|-----------|
| 1     | K4m3        | 0.07    | 0.92   | 0.05   | 0.03    | 0.75     | 0.07      |
| 2     | K4m1/3      | 0.17    | 0.85   | 0.88   | 0.05    | 0.55     | 0.10      |
| 3     | K4m1        | 0.01    | 0.01   | 0.47   | 0.02    | 3.35     | 0.57      |
| 4     | K4m1+K36m3  | 0.01    | 0.05   | 0.59   | 0.71    | 0.58     | 0.23      |
| 5     | K36m3       | 0.00    | 0.00   | 0.01   | 0.42    | 6.31     | 1.54      |
| 6     | Unmarked    | 0.01    | 0.00   | 0.00   | 0.00    | 85.45    | 9.20      |
| 7     | K27m3       | 0.29    | 0.00   | 0.02   | 0.00    | 3.01     | 3.87      |

The numbers represent the emission probabilities of each histone modification (column) in each chromatin state (row). The enriched histone modifications in each state are summarized in the first column. The fraction of genome assigned in each state was calculated (Supplementary Fig. 2). The average and variation of these fraction values across all included cell types/tissues are listed in the last two columns.
Extended Data Table 2 | Self-organizing map of histone modifications shows enrichment of human GWAS SNPs when mapped onto mouse

| rsID       | Description                                |
|------------|--------------------------------------------|
| rs6900341  | Metabolite                                 |
| rs1668871  | Platelet counts                            |
| rs1063856  | Coagulation factor levels                  |
| rs6798928  | Immunoglobulin A                           |
| rs2079742  | Urate levels                               |
| rs1789891  | Alcohol dependence                         |
| rs3811647  | Hepcidin levels                             |
| rs10199768 | Cardiovascular disease risk factors         |
| rs17155315 | QT interval                                 |
| rs12686004 | HDL cholesterol                             |
| rs3890182  | HDL cholesterol                             |
| rs7758229  | Colorectal cancer                           |
| rs6017342  | Ulcerative colitis                          |
| rs603446   | Triglycerides                               |
| rs2266788  | HDL Cholesterol - Triglycerides             |
| rs6056     | Fibrinogen                                  |
| rs641153   | Age-related macular degeneration (CNV)      |
| rs6952808  | Bipolar disorder and schizophrenia          |
| rs2424635  | Bipolar disorder and schizophrenia          |
| rs2023454  | Functional MRI                              |
| rs1715100  | Parkinson's disease                         |
| rs9312648  | Response to amphetamines                    |

a. Kidney-specific H3K4me1 that shows enrichment of specific GWAS hits associated with urate levels and metabolites. b. Liver-specific H3K36me3 unit shows enrichment in GWAS hits related to cholesterol, alcohol dependence and triglyceride levels. c. Brain-specific H3K27me3 signals show enrichment in GWAS SNPs associated with neurological disorders.