Title
Fine mapping of regulatory loci for mammalian gene expression using radiation hybrids.

Permalink
https://escholarship.org/uc/item/7874z8rd

Journal
Nature genetics, 40(4)

ISSN
1061-4036

Authors
Park, Christopher C
Ahn, Sangtae
Bloom, Joshua S
et al.

Publication Date
2008-04-01

DOI
10.1038/ng.113

License
https://creativecommons.org/licenses/by-nc-sa/4.0/ 4.0

Peer reviewed
Fine mapping of regulatory loci for mammalian gene expression using radiation hybrids

Christopher C Park1, Sangtae Ahn2, Joshua S Bloom1,7, Andy Lin1, Richard T Wang1, Tongtong Wu3,7, Aswin Sekar1, Arshad H Khan1, Christine J Farr4, Aldons J Lusis5, Richard M Leahy2, Kenneth Lange6, and Desmond J Smith1

1Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, California 90095, USA

2Department of Electrical Engineering, Signal and Image Processing Institute, Viterbi School of Engineering, University of Southern California, Los Angeles, California 90089, USA

3University of California Los Angeles School of Public Health, Department of Biostatistics, Los Angeles, California 90095, USA

4Department of Genetics, University of Cambridge, Downing Street, Cambridge, CB2 3EH, UK

5Department of Microbiology, Immunology and Molecular Genetics, Department of Medicine, and Department of Human Genetics, University of California, Los Angeles, California 90095, USA

6Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, California 90095, USA

Abstract

We mapped regulatory loci for nearly all protein-coding genes in mammals using comparative genomic hybridization and expression array measurements from a panel of mouse–hamster radiation hybrid cell lines. The large number of breaks in the mouse chromosomes and the dense genotyping of the panel allowed extremely sharp mapping of loci. As the regulatory loci result from extra gene dosage, we call them copy number expression quantitative trait loci, or ceQTLs. The −2log10P support interval for the ceQTLs was <150 kb, containing an average of <2–3 genes. We identified 29,769 trans ceQTLs with −log10P > 4, including 13 hotspots each regulating >100 genes in trans. Further, this work identifies 2,761 trans ceQTLs harboring no known genes, and provides evidence for a mode of gene expression autoregulation specific to the X chromosome.

© 2008 Nature Publishing Group
Correspondence should be addressed to D.J.S. (DSmith@mednet.ucla.edu).
Present addresses: Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544, USA (J.S.B.) and Department of Epidemiology and Biostatistics, College of Health and Human Performance, University of Maryland, College Park, Maryland 20742, USA (T.W.)

Accession codes. NCBI Gene Expression Omnibus database: microarray and CGH data have been deposited under accession number GSE9052.
URLs. SymAtlas data, http://symatlas.gnf.org/SymAtlas/.
Note: Supplementary information is available on the Nature Genetics website

AUTHOR CONTRIBUTIONS
C.C.P., A.S., A.H.K. and C.J.F. carried out the experiments; S.A., J.S.B., A.L., R.T.W. and T.W. did the statistical and computational analysis; C.C.P., A.J.L., R.M.L., K.L. and D.J.S. wrote the paper; D.J.S. devised the study.

Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions
The use of microarrays to identify loci that regulate mRNA expression is a potent addition to the genetics toolkit\(^1\). To date, expression quantitative trait loci, or eQTLs, have been mapped in populations segregating polymorphisms\(^2\)–\(^12\), but power in such studies is limited by the frequency of \(\sim 1\) crossover per chromosome per meiosis.

Radiation hybrid panels have been used to construct high-resolution maps of various genomes, including that of the mouse, for over 30 years (Fig. 1)\(^13\)–\(^15\). When X-rays are used to randomly cleave DNA, neighboring markers are rarely separated, allowing linkage to be evaluated. High radiation doses yield large numbers of DNA breaks, providing resolution that is hundreds of times greater than that obtained by meiotic mapping.

For the construction of a hybrid panel, normal diploid donor cells containing a selectable marker are lethally irradiated by X-rays. The endogenous thymidine kinase (\(Tk^1\)) gene residing on mouse chromosome 11 is often employed as the marker, although other genes can also be used. Donor cells are then fused with recipient \(Tk^1^-\) hamster cells using polyethylene glycol (PEG), and the fusion products are cloned in HAT medium, which selects \(Tk^1^+\) cells. The irradiated donor cells die, as do the recipient hamster cells. The only survivors are mouse–hamster hybrid clones that have received the \(Tk^1\) gene.

In addition to the selectable marker, each hybrid receives an essentially random sample of DNA fragments from the donor genome. Markers in close proximity to each other show similar patterns of retention in the clones, whereas distant markers are independently retained. Thus, genotyping a panel of such hybrids can order markers from the donor genome with odds >1,000:1. As \(\sim 90\%\) of primers designed on the basis of mouse genome sequence do not amplify hamster DNA, PCR was historically used as a simple and rapid genotyping tool.

One widely employed mouse–hamster radiation hybrid panel, T31, originally consisted of 100 cell lines, with an average mouse marker retention frequency of 30.0\% and an average donor fragment size of 10 Mb\(^16\)–\(^18\). The donor cells were male primary embryonic fibroblasts from the inbred mouse strain 129, and the recipient cells were from the A23 male Chinese hamster lung fibroblast–derived cell line (an offshoot of the DON cell line). The selectable marker was \(Tk^1\).

The \(\sim 2 \times 10^4\) breakpoints in the T31 panel allowed ordering of 25,000 PCR markers in the mouse genome at a resolution of 150 kb per marker. By comparison, even a modern set of mouse recombinant inbred strains might carry \(\sim 2 \times 10^3\) recombinations\(^19\). Significant linkage could be detected in the T31 panel between two markers separated by \(< 10\) Mb, consistent with the average donor fragment size. Genetic variation arises in the panel because radiation hybrid clones with a retained gene have one mouse plus two hamster copies compared to only two hamster copies otherwise. Thus, clones retaining an autosomal donor gene usually show a 1.5-fold copy number increase. As the recipient cells are male, the copy number increase for the X chromosome is twofold (that is, two copies versus only one).

The entire mouse genome is uniformly covered in the T31 panel, apart from two small regions that together constitute \(< 0.1\%\) of the genome. As expected for a selectable marker, the retention reaches 100\% in the vicinity of the \(Tk^1\) gene on chromosome 11. By contrast, the retention frequency is 0\% near the p53 (\(Trp53\)) gene\(^20\), also on chromosome 11. An extra copy of the mouse \(Trp53\) gene may cause the hybrids to undergo apoptosis. In addition, there is a very slight retention increase near the centromeres and telomeres, reflecting a minor selective advantage of these regions. Centromeres promote segregation, and telomeres stabilize naked chromosome ends.
Here, we identify regulatory loci using array data from the T31 panel. This mapping relies on changes in gene dosage, whereas classical eQTL mapping relies on naturally occurring intraspecific polymorphisms. We therefore call loci identified using the radiation hybrid method copy number eQTLs, or ceQTLs.

In classical eQTL mapping, cis eQTLs are caused by polymorphisms affecting promoter activity or mRNA stability, whereas trans eQTLs represent polymorphisms in regulatory genes. We also observed cis and trans ceQTLs with the radiation hybrid data, although in this case, they are caused by the distinct mechanism of copy number change. In the radiation hybrid panel, most genes should have cis ceQTLs, as >99% of genes show copy number variation.

RESULTS

Comparative genomic hybridization genotyping

We grew the T31 radiation hybrid (RH) cell lines on selective media. A total of 99 hybrids from the original panel were available. We re-genotyped the hybrids using mouse comparative genomic hybridization (CGH) arrays (Agilent), interrogating 232,626 copy number markers (Supplementary Methods online). DNA from the A23 recipient hamster cell line served as the control channel. The CGH data was analyzed as $\log_{10}(RH/A23)$ copy number ratio averaged over a sliding window of ten markers.

We compared DNA from the recipient A23 cells with hamster kidney DNA using the CGH arrays. There was little evidence of copy number variation in the A23 cells, with only $\sim 10^{-5}$ of markers reaching a value corresponding to a $\log_{10}$ copy number ratio of 1.5 (Supplementary Fig. 1a online). For the radiation hybrid clones, the $\log_{10}(RH/A23)$ copy number ratio was bimodal, with the first mode representing the two-copy baseline and the second mode representing extra copies (Supplementary Fig. 1b). The data were normalized using a Gaussian mixture model (Supplementary Methods).

We found good agreement between the historical PCR genotypes and the CGH results (Fig. 2a and Supplementary Methods). However, there was loss of 31.3 ± 1.9% of the original PCR markers compared to the CGH data, presumably as a result of instability during culture (Fig. 2b). The loss was uniform throughout the genome, so that the re-genotyped hybrids mirrored the historical retention profile. There was also a gain of 1.8 ± 0.17% of the CGH markers, perhaps because of errors in the previous PCR genotyping. The low rate of marker gain was consistent with the apparent copy number stability of the A23 cells.

The average mouse marker retention frequency was 23.9 ± 0.02%, and the average donor fragment size was 7.17 ± 0.115 Mb. We obtained similar results using a mixture model (Supplementary Methods). The retention frequency was stable across the genome (Fig. 2c), with the expected minor increases at the centromeres and telomeres of some chromosomes (Supplementary Fig. 1c). We found the two anticipated extremes of retention frequency on chromosome 11: a ~150-kb radius around Tk1 showing >95% retention and a ~150-kb radius around Trp53 showing <5% retention (Fig. 2d). Apart from these regions, there were no other extremes; therefore, the T31 radiation hybrid panel uniformly covers >99% of the genome.

The CGH data in clustergram format is shown in Figure 2e. The clustergram shows the bimodal nature of the CGH data and the essentially uniform coverage of the mouse genome in the radiation hybrid cell lines. The Tk1 gene is present in all clones, whereas A23 cells have virtually no regions of copy number alteration.
Transcript analysis

Two RNA samples, separated by a freeze-thaw cycle and representing biological replicates, were extracted from each radiation hybrid clone. We labeled the samples and applied them to two mouse microarrays (Agilent) differing by a dye-swap (Supplementary Methods). Labeled RNA from A23 cells served as the control channel. RNA from both donor mouse and endogenous hamster genes were detected with comparable efficiency (Supplementary Methods and Supplementary Figs. 2 and 3 online), allowing regulation of either by extra donor gene copies to be evaluated. A total of 20,145 genes were assayed by the arrays.

Models

We tested each of the 20,145 gene expression levels queried by the expression arrays for dependence on each of the 232,626 CGH markers typed in the radiation hybrid cells. The dependent variable for each gene consisted of the quantity \( \log_{10}(\text{RH expression}/\text{A23 control channel}) \) averaged across the two replicate microarrays, and the independent variable was the \( \log_{10}(\text{RH}/\text{A23}) \) marker copy number ratio. We used two regression models to analyze the data (Fig. 3a and Supplementary Methods). Significance was indicated by \( -\log_{10}P \).

Model 1 regressed the expression of each gene on each marker, identifying trans ceQTLs for distant markers (>10 Mb from a gene) and cis ceQTLs for local markers (<10 Mb)\textsuperscript{26}. The effect size for model 1 was conveyed by the parameter \( \alpha \). Model 2 evaluated the interaction between local and distant markers on the expression of a gene. This model hence explored whether a distant marker affected the expression of two hamster copies of a gene differently than it affected two hamster copies plus an extra mouse copy. The effect size for model 2 was conveyed by the parameter \( \gamma \).

We estimated the null distribution for both models by permutation (Supplementary Methods)\textsuperscript{27–29} and controlled for false discovery rates (FDRs)\textsuperscript{30,31}. In model 1, the FDR procedure was carried out separately for the cis and trans ceQTLs, as cis ceQTLs do not require genome-wide significance thresholds\textsuperscript{32}. For comparability, we used an FDR of <0.4 throughout. We also obtained similar results to model 1 using a weighted regression model (Supplementary Methods and Supplementary Fig. 4 online).

Identifying cis and trans ceQTLs

For model 1 at an FDR of <0.4, we detected 18,810 cis ceQTLs corresponding to a \( -\log_{10}P > 1.1 \) and 29,769 trans ceQTLs corresponding to a \( -\log_{10}P > 4.0 \) regulating 9,538 genes. Examples of regression lines relating gene expression to peak marker CGH data for cis and trans ceQTLs are shown in Figure 3b–d. cis ceQTLs usually had higher \( -\log_{10}P \) values than trans ceQTLs (Fig. 3e).

Figure 4 plots ceQTLs with FDR < 0.4 on the genome, with cis ceQTLs along the diagonal and trans ceQTLs on the off-diagonal. The ceQTL peaks were extremely sharp. The \( -2\log_{10}P \) support interval typically extended only 150 kb, containing an average of <2–3 genes. Linkage was detected within 7 Mb of a ceQTL peak, consistent with the average donor fragment size. Figure 5a–d shows examples of cis and trans ceQTL peaks. Because of the high resolution, it was possible to resolve multiple ceQTLs on a single chromosome, frequently an obstacle in meiotic mapping. For example, in the case of the Ccnb2 (cyclin B2) gene at 70 Mb on chromosome 9, we resolved a closely linked trans ceQTL at 77 Mb, within 10 Mb of the cis ceQTL peak (Fig. 5a). Similarly, for the Ftl1-rs7 (ferritin light chain 1, related sequence 7) gene on chromosome 13, we resolved an additional trans ceQTL at 31 Mb on chromosome 7, within 10 Mb of the principal trans ceQTL at 40 Mb (Fig. 5d). High reproducibility of the biological replicates indicated that the data was of good quality (Fig. 5e,f, Supplementary Methods and Supplementary Table 1 online).
**Cis ceQTLs**

If expression is directly proportional to gene copy number, nearly all genes should show significant *cis* ceQTLs. Indeed, virtually every autosomal gene had a DNA copy number increase of 1.5-fold in 23.9% of the radiation hybrid clones. In model 1, a direct proportion between copy number and expression would imply a *cis* ceQTL effect size $\alpha = 1$. A total of 94% of genes had significant *cis* ceQTLs. Figure 6a shows the distribution of the effect size $\alpha$. The mean was 0.39, indicating a blunting of a simple, direct relationship between copy number and expression. *Cis* ceQTLs were not driven by sequence differences between mouse and hamster (Supplementary Methods and Supplementary Fig. 5 online). Notably, a substantial number of *cis* ceQTLs (6,193 of 18,810 or 32.9%) had a negative $\alpha$, indicating that an extra copy decreases rather than increases gene expression. Analysis of the two replicate datasets indicated that these negative $\alpha$ *cis* ceQTLs may be driven partly by noise, but that at least some are replicable and not due to outliers (Supplementary Methods and Supplementary Fig. 6a – c online). Our observations are consistent with results from segmental aneuploidy syndromes\(^\text{33}\), suggesting that, in a multigene setting, the relationship between gene dosage and expression is much more complex than direct proportionality.

**Trans ceQTLs and hotspots**

The distribution of $\alpha$ for *trans* ceQTLs with FDR < 0.4 is shown in Figure 6b. A total of 27,077 *trans* ceQTLs had a positive $\alpha$ (mean 1.12), indicating induction of the regulated gene, and a total of 2,692 had a negative $\alpha$ (mean $-0.68$), indicating repression. These observations suggest a strong tendency for *trans* ceQTLs to activate rather than repress gene expression. A gene was somewhat more likely to constitute a *trans* ceQTL if it was also a *cis* ceQTL ($\chi^2 = 5.88$, degree of freedom (d.f.) = 1, $P < 0.015$). This relatively weak relationship indicates that a gene that is not a *cis* ceQTL can nevertheless form a *trans* ceQTL, implying that small increases in expression can be magnified in regulated genes.

To identify regulation hotspots, we plotted the number of genes regulated by each marker (Fig. 4, horizontal marginal graph). The high resolution of the radiation hybrid mapping allowed accurate alignment of multiple ceQTLs and confident localization of hotspots. Chromosomes 4 and 5 harbored an unusually large number of hotspots, with five hotspots regulating > 100 genes on each chromosome (Figs. 4, 6c and 6d). At an FDR < 0.4, the top hotspot was on chromosome 5, regulating 614 genes (Fig. 6d). We used transfection to confirm that the *Pcdh7* (protocadherin 7) gene was responsible for this hotspot (below). The second highest hotspot, on chromosome 4, regulated 354 genes (Fig. 6c). For the top five hotspots, which each regulate >200 genes, the $\alpha$ values were nearly all positive (> 99%), indicating induction of the regulated genes.

**Genes regulated by large numbers of *trans* ceQTLs**

In addition to markers that regulate many genes, there were also genes regulated by large numbers (up to 42) of *trans* ceQTLs (Fig. 4, vertical marginal graph; Supplementary Methods). The five genes regulated by the most *trans* ceQTLs with FDR < 0.4 were *Dnpep* (aspartyl aminopeptidase, regulated by 42 *trans* ceQTLs), *Vgll3* (vestigial like 3, 39 *trans* ceQTLs), *Marcksl1* (myristoylated alanine rich protein kinase C substrate-like 1, 38 *trans* ceQTLs), *Prr13* (proline rich 13, 37 *trans* ceQTLs) and *Shfdg1* (split hand/foot malformation (ectrodactyly) type 1, 34 *trans* ceQTLs) (Supplementary Methods).

**Conservation with mouse tissues**

As the radiation hybrid panel is an artificial system, it is reasonable to ask to what extent regulatory relationships in the radiation hybrid panel reflect regulatory relationships in live...
animals. We used the expression levels from the 99 radiation hybrid clones to construct a matrix of correlation coefficients between all gene pairs (Supplementary Methods). We also constructed an analogous matrix using publicly available SymAtlas data. This microarray database gives expression data for all genes across 61 mouse tissues. We evaluated the similarity of the two matrices for the 15,220 genes in common by using the Frobenius norm of the difference between them and comparing this value to a null distribution obtained by permutation. The two datasets showed significantly greater similarity than expected by chance ($P < 10^{-4}$), suggesting that insights on regulation of gene expression obtained from the radiation hybrid data will be applicable to normal mouse tissues. Consistent with this, we also found that the genes regulated by the top hotspot were significantly co-regulated in the SymAtlas dataset ($P = 1.6 \times 10^{-3}$, permutation $t$-test; Supplementary Methods).

**Positional identification of a gene for a trans ceQTL**

As proof of principle that transfection can be used to identify genes responsible for trans ceQTLs, we examined the hotspot on chromosome 5 regulating the most genes (Fig. 6d). The only gene lying under this hotspot was *Pcdh7*, a cadherin superfamily member involved in homophilic cell–cell adhesion.\(^34\)

We lipofected human epithelial kidney 293 (HEK 293) cells and the A23 hamster recipient cells used for the radiation hybrid panel with an expression construct in which a *Pcdh7* isoform (*Pcdh7a*) cDNA was driven by the cytomegalovirus (CMV) promoter (Supplementary Methods). Two biological replicates were obtained for each cell line and evaluated using expression microarrays (Agilent). Cells transfected with empty vector were used for the array control channel.

For both cell lines, the genes predicted to be regulated by *Pcdh7* from the radiation hybrid data were significantly increased in expression compared to the null distribution (HEK 293, $P = 1.4 \times 10^{-4}$; A23, $P = 3.2 \times 10^{-4}$; permutation $t$-test) (Fig. 6e). As expected, these genes were also significantly overexpressed in radiation hybrid clones containing *Pcdh7* ($P < 2.0 \times 10^{-5}$; permutation $t$-test). Despite the large differences in *Pcdh7a* overexpression in the radiation hybrid and transfected cells (~500-fold and 1.44-fold, respectively), the resulting expression changes were similar in both.

**Cis ceQTLs on the X chromosome**

We examined the mean effect size $\alpha$ for the cis ceQTLs on each of the chromosomes. Compared to the autosomes, the X chromosome showed significantly decreased values of $\alpha$ (Welch’s $t = 16.7$, d.f. = 2,671, $P < 2.2 \times 10^{-16}$) (Fig. 6f). The X chromosome also showed a decreased frequency of cis ceQTLs compared to the autosomes at stringent FDR values (at $-\log_{10} P > 4$, FDR < 0.003, mean of 0.14 cis ceQTLs per gene on the autosomes versus 0.07 on the X chromosome, $\chi^2 = 21.9$, d.f. = 1, $P = 2.94 \times 10^{-6}$).

The decreased effect size for X-chromosome cis ceQTLs may represent an autoregulatory property of genes on this chromosome that renders their expression less sensitive to dosage. Male recipient cells were used to construct the radiation hybrid panel, so some clones had both a hamster and a mouse X-chromosome inactivation center.\(^35\) However, the autoregulation represented by decreased cis ceQTL effect size cannot be a result of X-chromosome inactivation. Inactivation of the hamster X chromosome would result in gross functional aneuploidy, and transcription of the donor mouse *Xist* gene would not cause consistent inactivation of the same genes in all radiation hybrid clones, as the mouse *Xist* gene would be ligated to random genome fragments. Further, any inactivation would propagate inefficiently to contiguous genes, as they are usually autosomal.
Thus, the decreased expression resulting from extra copy number on the X chromosome must stem from an additional regulatory effect. Such a mechanism may prevent overexpression of X-chromosome genes in females should inactivation be less than completely effective.

**Mouse regulation of hamster genes**

Model 2 evaluated whether a distant marker regulated a gene differently, depending on whether or not there was an extra mouse copy (Fig. 3a). There were no significant genome-wide interactions in this model, with FDR < 0.58. Thus, mouse and hamster genes behave equivalently in terms of regulation by *trans* eQTLs.

**Functional categorization of *trans* eQTLs**

Recently, a yeast intercross study showed a notable absence of over-represented gene categories near *trans* eQTLs, suggesting no hierarchy of functional classes. We investigated whether the same conclusion was true for our radiation hybrid data. The closest markers to each gene were identified and taken as neighbors, provided the distance between the gene and marker was <1 Mb. The mean number of neighboring markers to a gene was 11.5 ± 0.11, and mean distance between a gene and its nearest marker was 2.1 ± 0.14 kb.

We classified all genes into two categories: those with at least one *trans* eQTL with FDR < 0.4 in their neighboring markers, and those without. We also classified the genes on the basis of whether they belonged to the Gene Ontology (GO) category being tested or not. We then evaluated whether a Gene Ontology category was enriched in *trans* eQTLs using a one-sided Fisher’s exact test on a 2 × 2 contingency table. FDR values were calculated for all tests. We tested a total of 200 Gene Ontology categories with > 70 gene members, with microRNAs considered a separate Gene Ontology category. The results for the 25 categories with \( P < 10^{-3} \) and FDR < 10\(^{-2} \) are shown in Table 1.

Categories pertinent to transcription were prominent, with 44% (11 of 25) relevant (Table 1). Other categories were also significant, including those related to ion transport, cytoskeleton and protein binding. Although their connection to gene expression is less obvious than that of the transcriptional categories, they deserve the same credibility, given the fairly stringent *P* and FDR thresholds.

MicroRNAs have drawn considerable interest as potential *trans* regulators of sometimes as many as hundreds of transcripts. Of note, microRNAs were not significantly over-represented (Table 1). Further, we did not find any examples of microRNAs within 100 kb of the top ten hotspots regulating large numbers of genes (Fig. 3, horizontal marginal distribution). This may reflect the modest degree of regulation characteristic of microRNAs.

The absence of Gene Ontology category enrichment near *trans* eQTLs in the yeast intercross study may represent lack of naturally occurring polymorphisms for genes at network hubs. This relative deficiency might arise because of selective pressure. In contrast, overexpression in radiation hybrid panels may be a more robust strategy to perturb gene expression.

**Trans eQTLs lacking known genes**

The high-resolution mapping using the radiation hybrid panel provided an opportunity to identify *trans* eQTLs with no known genes, including microRNAs (Supplementary Methods). To be conservative, we defined *trans* eQTLs having no genes as those that lacked genes within a 300-kb radius of the peak \( -\log_{10} P \) marker. There were 2,761 such
trans ceQTLs with FDR < 0.4 regulating a total of 5,725 genes (Fig. 7a and Supplementary Methods). In this tally, a locus regulating one or more genes was counted as one trans ceQTL. The number of trans ceQTLs lacking known genes showed a similar decrease in relation to increasing radius from the peak as randomly chosen markers (Kolmogorov-Smirnov test, \( D = 0.167, P = 1 \); Fig. 7a). This observation suggested that trans ceQTLs lacking genes are not found in unusual regions of the genome, such as gene deserts.

An example of a trans ceQTL lacking a known gene is shown in Figure 7b, and an example of a trans ceQTL hotspot with no known genes is shown in Figure 7c. Trans ceQTLs lacking genes had slightly smaller mean \( \alpha \) values than those with genes (Fig. 7d) and also regulated fewer mean numbers of genes (hotspots) (Fig. 7e,f).

**DISCUSSION**

We achieved high-precision mapping of ceQTLs using the T31 radiation hybrid panel, with a \(-2\log_{10}P\) support interval of \(-150\) kb. As yeast two hybrid and co-affinity purification technologies suffer from high false positive and negative rates\(^41\), an orthogonal method for mapping genetic interactions in the mammalian setting is needed. The resolution of the radiation hybrid data is sufficiently high to construct gene networks for mammalian cells, although regulation of a gene by a ceQTL can represent an indirect phenomenon.

We found cis ceQTLs with both positive and negative \( \alpha \) values, suggesting a complex relationship between gene copy number and expression. The high-resolution mapping allowed confident alignment of trans ceQTLs, facilitating hotspot identification. We also identified highly regulated genes. Trans ceQTLs caused induction of target genes much more frequently than repression. Corresponding to their close evolutionary relatedness, mouse and hamster genes were regulated similarly in the radiation hybrid panel.

Expression patterns in the radiation hybrid panel and mouse tissues were markedly similar, suggesting that the insights from the radiation hybrid data will be relevant to normal gene regulation. The high resolution of the radiation hybrid approach combined with the ease of genetic manipulation of mammalian cells means that genes underlying ceQTLs can be identified in a relatively quick and scalable fashion using transfection. As proof of principle, we demonstrated that the Pcdh7 gene was responsible for a gene-regulation hotspot.

We found that, in contrast to naturally occurring alleles, trans ceQTLs were more likely to reside close to transcription factors than expected by chance, suggesting that these factors have a privileged position in genetic networks. It also indicates that overexpression using radiation hybrid panels may be a more reliable route to perturbing gene expression than polymorphisms, which are subject to selection pressure.

We also found trans ceQTLs lacking known genes, indicating that the radiation hybrid data will be a useful discovery source for previously unannotated genes. In addition, genes on the X chromosome showed smaller expression increases than genes on autosomal chromosomes as a result of extra copy number, suggesting a new form of autoregulation supplementing X-chromosome inactivation. This effect may be an evolutionary remnant of a dosage compensation mechanism preceding X inactivation.

Radiation hybrid panels for other species are available, and it will be interesting to see whether regulatory interactions occurring in the mouse–hamster panel are conserved. Pooling the data for multiple panels will further increase power and sensitivity, and synteny breakpoints might provide additional map refinements. In chemical genomics, radiation hybrid panels could identify mammalian drug targets, either through direct assays of cell viability or by microarray analysis of drug-treated clones and recipient cells\(^42\).
METHODS

Microarray and data analysis

Agilent G4415A CGH and G4121A expression microarrays were hybridized according to manufacturer’s instructions. Agilent G4122A expression microarrays were used for the transfection experiments. We analyzed the data using linear models. Full details are in the Supplementary Methods.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Jansen RC, Nap JP. Genetical genomics: the added value from segregation. Trends Genet 2001;17:388–391. [PubMed: 11418218]
2. Brem RB, Yvert G, Clinton R, Kruglyak L. Genetic dissection of transcriptional regulation in budding yeast. Science 2002;296:752–755. [PubMed: 11923494]
3. Wayne ML, McIntyre LM. Combining mapping and arraying: an approach to candidate gene identification. Proc. Natl. Acad. Sci. USA 2002;99:14903–14906. [PubMed: 12415114]
4. Schadt EE, et al. Genetics of gene expression surveyed in maize, mouse and man. Nature 2003;422:297–302. [PubMed: 12646919]
5. Kirst M, et al. Coordinated genetic regulation of growth and lignin revealed by quantitative trait locus analysis of cDNA microarray data in an interspecific backcross of eucalyptus. Plant Physiol 2004;135:2368–2378. [PubMed: 15299141]
6. Morley M, et al. Genetic analysis of genome-wide variation in human gene expression. Nature 2004;430:743–747. [PubMed: 15269782]
7. Dixon AL, et al. A genome-wide association study of global gene expression. Nat. Genet 2007;39:1202–1207. [PubMed: 17873877]
8. Goring HH, et al. Discovery of expression QTLs using large-scale transcriptional profiling in human lymphocytes. Nat. Genet 2007;39:1208–1216. [PubMed: 17873875]
9. Stranger BE, et al. Population genomics of human gene expression. Nat. Genet 2007;39:1217–1224. [PubMed: 17873874]
10. Bystrykh L, et al. Uncovering regulatory pathways that affect hematopoietic stem cell function using ‘genetical genomics’. Nat. Genet 2005;37:225–232. [PubMed: 15711547]
11. Hubner N, et al. Integrated transcriptional profiling and linkage analysis for identification of genes underlying disease. Nat. Genet 2005;37:243–253. [PubMed: 15711544]
12. Chesler EJ, et al. Complex trait analysis of gene expression uncovers polygenic and pleiotropic networks that modulate nervous system function. Nat. Genet 2005;37:233–242. [PubMed: 15711545]
13. Goss SJ, Harris H. New method for mapping genes in human chromosomes. Nature 1975;255:680–684. [PubMed: 1169690]
14. McCarthy LC. Whole genome radiation hybrid mapping. Trends Genet 1996;12:491–493. [PubMed: 9257524]
15. Olivier M, et al. A high-resolution radiation hybrid map of the human genome draft sequence. Science 2001;291:1298–1302. [PubMed: 11181994]
16. McCarthy LC, et al. A first-generation whole genome-radiation hybrid map spanning the mouse genome. Genome Res 1997;7:1153–1161. [PubMed: 9414320]
17. Avner P, et al. A radiation hybrid transcript map of the mouse genome. Nat. Genet 2001;29:194–200. [PubMed: 11586301]
18. Hudson TJ, et al. A radiation hybrid map of mouse genes. Nat. Genet 2001;29:201–205. [PubMed: 11586302]
19. Peirce JL, Lu L, Gu J, Silver LM, Williams RW. A new set of BXD recombinant inbred lines from advanced intercross populations in mice. BMC Genet 2004;5:7. [PubMed: 15117419]

20. Behboudi A, et al. The functional significance of absence: the chromosomal segment harboring Tp53 is absent from the T55 rat radiation hybrid mapping panel. Genomics 2002;79:844–848. [PubMed: 12036299]

21. Jansen RC. Maximum likelihood in a generalized linear finite mixture model by using the EM algorithm. Biometrics 1993;49:227–231.

22. Redner RA, Walker HF. Mixture densities, maximum likelihood and the EM algorithm. SIAM Rev 1984;26:195–239.

23. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA 1998;95:14863–14868. [PubMed: 9843981]

24. Saldanha AJ. Java Treeview–extensible visualization of microarray data. Bioinformatics 2004;20:3246–3248. [PubMed: 15180930]

25. Hughes TR, et al. Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer. Nat. Biotechnol 2001;19:342–347. [PubMed: 11283592]

26. Rockman MV, Kruglyak L. Genetics of global gene expression. Nat. Rev. Genet 2006;7:862–872. [PubMed: 17047685]

27. Churchill GA, Doerge RW. Empirical threshold values for quantitative trait mapping. Genetics 1994;138:963–971. [PubMed: 7851788]

28. Brem RB, Storey JD, Whittle J, Kruglyak L. Genetic interactions between polymorphisms that affect gene expression in yeast. Nature 2005;436:701–703. [PubMed: 16079846]

29. Storey JD, Akey JM, Kruglyak L. Multiple locus linkage analysis of genomewide expression in yeast. PLoS Biol 2005;3:e267. [PubMed: 16035920]

30. Benjamini Y, Hochberg Y. Controlling the false discovery rate—a practical and powerful approach to multiple testing. J. Roy. Statist. Soc. Ser. B. Methodological 1995;57:289–300.

31. Benjamini Y, Yekutieli D. The control of the false-discovery rate in multiple testing under dependency. Ann. Stat 2001;29:1165–1188.

32. Carlborg O, et al. Methodological aspects of the genetic dissection of gene expression. Bioinformatics 2005;21:2383–2393. [PubMed: 15613385]

33. Prandini P, et al. Natural gene-expression variation in Down syndrome modulates the outcome of gene-dosage imbalance. Am. J. Hum. Genet 2007;81:252–263. [PubMed: 17668376]

34. Yoshida K, Yoshitomo-Nakagawa K, Seki N, Sasaki M, Sugano S. Cloning, expression analysis, and chromosomal localization of BH-protocadherin (PCDH7), a novel member of the cadherin superfamily. Genomics 1998;49:458–461. [PubMed: 9615233]

35. Heard E, Distèche CM. Dosage compensation in mammals: fine-tuning the expression of the X chromosome. Genes Dev 2006;20:1848–1867. [PubMed: 16847345]

36. Yvert G, et al. Trans-acting regulatory variation in Saccharomyces cerevisiae and the role of transcription factors. Nat. Genet 2003;35:57–64. [PubMed: 12897782]

37. Lehmann, EL.; Romano, JP. Testing Statistical Hypotheses. New York: Springer; 2005.

38. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism and function. Cell 2004;116:281–297. [PubMed: 14744438]

39. Lim LP, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature 2005;433:769–773. [PubMed: 15685193]

40. Pillai RS. MicroRNA function: multiple mechanisms for a tiny RNA? RNA 2005;11:1753–1761. [PubMed: 16314451]

41. Cusick ME, Klitgord N, Vidal M, Hill DE. Interactome: gateway into systems biology. Hum. Mol. Genet 2005;14((Spec No. 2)):R171–R181. [PubMed: 16162640]

42. Perlstein EO, Ruderfer DM, Roberts DC, Schreiber SL, Kruglyak L. Genetic basis of individual differences in the response to small-molecule drugs in yeast. Nat. Genet 2007;39:496–502. [PubMed: 17334364]
Figure 1.
Radiation hybrid and ceQTL mapping (adapted from ref. 14).
Figure 2.
CGH results. (a) Data for radiation hybrid clone 26 show only small regions of loss compared to historical PCR genotyping data. (b) Data for clone 15 show more extensive loss. (c) Retention frequency from CGH data for chromosome 17. The retention is relatively constant along the length of the chromosome at ~20%. (d) Retention frequency for chromosome 11, showing 0% retention at $Trp53$ and 100% retention at $Tk1$. (e) Clustergram showing an overview of the CGH $\log_{10}(RH/A23)$ copy number ratios for the radiation hybrid clones and the A23 recipient cells (left column). The $\log_{10}$ copy number ratio was downsampled 10 to 1 using a jumping window of ten markers averaged to give a single value. The rows represent the resulting $\log_{10}$ copy number ratios for the downsampled markers, in the same order as they appear in the genome. The columns represent the radiation hybrid clones and A23 cells ordered by similarity.
Figure 3.
Models. (a) Models 1 and 2. (b) Regression between gene expression and CGH copy number ratio at the peak marker for a trans ceQTL at 78 Mb on chromosome 15 with positive $\alpha$, regulating the Cded (cell differentiation and embryonic development) gene on chromosome 2. $\alpha = 3.075$, $-\log_{10} P = 10.370$. (c) Trans ceQTL at 21 Mb on the X chromosome with negative $\alpha$, regulating the dishevelled homolog 1 (Dvl1) gene on chromosome 4. $\alpha = -0.446$, $-\log_{10} P = 6.237$. (d) Cis ceQTL for the Copa (coatamer protein complex subunit $\alpha$) gene on chromosome 1 at 172 Mb has positive $\alpha$. $\alpha = 4.854$, $-\log_{10} P = 10.370$. (e) FDRs and $-\log_{10} P$ values for cis and trans ceQTLs.
Figure 4. Cis and trans ceQTLs. Marker positions are shown on the horizontal axis, and gene positions are shown on the vertical. Chromosome boundaries are shown on both axes. FDRs < 0.4 are shown. The horizontal marginal distribution represents the sum of the gene numbers regulated in trans at each point and indicates markers regulating many genes (hotspots). The probability that a marker would regulate >6 genes, assuming uniform distribution of trans ceQTLs, was 0.24 (FDR < 0.4), whereas the probability that a marker would regulate >12 genes was 0.002 (FDR < 0.01) (Supplementary Methods). The vertical marginal distribution shows the number of trans ceQTLs regulating each gene and indicates highly regulated genes. The probability that a gene would have >3 trans ceQTLs, assuming the trans ceQTLs were equally distributed across genes, was 0.063 (FDR < 0.4), and the probability it would have >6 trans ceQTLs was $9 \times 10^{-4}$ (FDR < 0.01) (Supplementary Methods).
Figure 5.
Individual cis and trans ceQTLs. (a) Cis ceQTL for the Ccnb2 gene on chromosome 9 at 71 Mb. Gene position is indicated by red vertical line. Marker position is shown on the horizontal axis in Mb. Each point represents a single marker on the CGH array. (b) Cis ceQTL for the Prkar1a (regulatory subunit IIα of cAMP dependent protein kinase) gene on chromosome 11 at 110 Mb. (c) Trans ceQTL at 180 Mb on chromosome 2 regulating the Chst4 (carbohydrate sulfotransferase 4) gene on chromosome 8. Major trans ceQTL peak is indicated by red triangle. (d) Trans ceQTL at 40 Mb on chromosome 7 regulating the ferritin Ftl1-rs7 gene on chromosome 13. (e) Replicability for a trans ceQTL. Trans ceQTL at 160 Mb on chromosome 1 regulating the Prdx6-rs1 (peroxiredoxin 6, related sequence 1) gene on chromosome 2. Data before the freeze-thaw cycle. (f) Data after the freeze-thaw cycle.
Figure 6.
Effect sizes and regulatory hotspots. (a) Counts of *cis* ceQTLs with FDR < 0.4 versus $\alpha$. Positive $\alpha$ values represent genes whose expression is increased by an extra copy of the donor mouse gene, whereas negative values represent genes whose expression is decreased. (b) Counts of *trans* ceQTLs with FDR < 0.4 versus $\alpha$. (c) Regulatory hotspots on chromosome 4 versus $\alpha$. (d) Section of chromosome 5 showing hotspot regulating largest number of genes (614) at 56 Mb. The position of *Pcdh7* is indicated by a green triangle. (e) Mean expression increase for genes regulated by the chromosome 5 hotspot in the radiation hybrid panel and cells transfected with *Pcdh7a* compared to cells transfected with empty vector. (f) Mean *cis* ceQTL $\alpha$ values on the autosomes and X chromosome. Error bars, s.e.m.
Figure 7.
Trans ceQTLs lacking known genes. (a) Relationship between number of trans ceQTLs with no genes and radius from peak marker (solid line). Trans ceQTLs regulating >1 gene are counted once only. The same relationship for randomly chosen markers is also shown (broken line). (b) Example of trans ceQTL on chromosome 15 at 15.3 Mb with no known genes underneath regulating the Maoa (monoamine oxidase A) gene on the X chromosome. Known genes are shown as red bars, (c) Example of a trans ceQTL hotspot with no known genes underneath. This trans ceQTL hotspot, located at 74.75 Mb on chromosome 6, regulates 130 genes. (d) Counts of trans loci with no genes and FDR < 0.4 versus α. Trans ceQTLs lacking genes had smaller mean α values than those with genes (0.832 ± 0.01 and 0.986 ± 0.006, respectively, permutation t-test, P < 2 × 10^{-5}; see also Figure 6b). (e) Counts of trans ceQTLs with FDR < 0.4 versus numbers of regulated genes. (f) Counts of trans ceQTLs with no genes and FDR < 0.4 versus numbers of regulated genes. Fewer mean numbers of genes were regulated by trans ceQTLs lacking genes than by trans ceQTLs with genes (11.35 ± 0.48 and 13.49 ± 0.21 genes, respectively, permutation t-test, P < 2 × 10^{-5}).
## Table 1

Gene Ontology analysis of genes near *trans* ceQTL peaks

| Biological process                                                   | GO ID  | Total | Observed | Expected | P value     | FDR        |
|---------------------------------------------------------------------|--------|-------|----------|----------|-------------|------------|
| Multicellular organismal development                                | 7275   | 696   | 331      | 256      | $2.59 \times 10^{-9}$ | $5.18 \times 10^{-7}$ |
| Regulation of transcription                                         | 45449  | 404   | 199      | 149      | $1.79 \times 10^{-7}$ | $7.16 \times 10^{-6}$ |
| Regulation of transcription, DNA dependent                         | 6355   | 1,593 | 679      | 587      | $4.23 \times 10^{-7}$ | $1.41 \times 10^{-5}$ |
| Cell adhesion                                                       | 7155   | 384   | 185      | 142      | $3.00 \times 10^{-6}$ | $7.51 \times 10^{-5}$ |
| Transcription                                                      | 6350   | 1,264 | 540      | 466      | $5.13 \times 10^{-6}$ | $1.14 \times 10^{-4}$ |
| Nervous system development                                          | 7399   | 152   | 81       | 56       | $2.50 \times 10^{-5}$ | $4.51 \times 10^{-4}$ |
| Ion transport                                                       | 6811   | 447   | 203      | 165      | $1.06 \times 10^{-4}$ | $1.41 \times 10^{-3}$ |
| Regulation of transcription from RNA polymerase II promoter         | 6357   | 87    | 49       | 32       | $1.66 \times 10^{-4}$ | $2.08 \times 10^{-3}$ |
| Potassium ion transport                                             | 6813   | 136   | 70       | 50       | $3.39 \times 10^{-4}$ | $3.76 \times 10^{-3}$ |
| Negative regulation of transcription from RNA polymerase II promoter| 122    | 122   | 63       | 45       | $5.84 \times 10^{-4}$ | $5.56 \times 10^{-3}$ |
| Cell differentiation                                                | 30154  | 374   | 168      | 138      | $7.42 \times 10^{-4}$ | $6.15 \times 10^{-3}$ |
| Positive regulation of transcription from RNA polymerase II promoter| 45944  | 167   | 82       | 62       | $7.69 \times 10^{-4}$ | $6.15 \times 10^{-3}$ |
| Cellular component                                                  |        |       |          |          |             |            |
| Actin cytoskeleton                                                  | 15629  | 70    | 43       | 26       | $2.50 \times 10^{-5}$ | $4.55 \times 10^{-4}$ |
| Transcription factor complex                                        | 5667   | 330   | 151      | 122      | $5.08 \times 10^{-4}$ | $5.22 \times 10^{-3}$ |
| Postsynaptic membrane                                              | 45211  | 77    | 43       | 28       | $5.22 \times 10^{-4}$ | $5.22 \times 10^{-3}$ |
| Cytoskeleton                                                        | 5856   | 349   | 158      | 129      | $6.90 \times 10^{-4}$ | $6.00 \times 10^{-3}$ |
| Molecular function                                                  |        |       |          |          |             |            |
| Protein binding                                                     | 5515   | 3,247 | 1,330    | 1,197    | $6.13 \times 10^{-8}$ | $6.13 \times 10^{-6}$ |
| DNA binding                                                         | 3677   | 1,637 | 701      | 603      | $1.17 \times 10^{-7}$ | $6.45 \times 10^{-6}$ |
| Transcription factor activity                                       | 3700   | 766   | 351      | 282      | $1.29 \times 10^{-7}$ | $6.45 \times 10^{-6}$ |
| Sequence-specific DNA binding                                       | 43365  | 395   | 190      | 146      | $2.53 \times 10^{-6}$ | $7.22 \times 10^{-5}$ |
| Transcriptional repressor activity                                  | 16564  | 92    | 53       | 34       | $3.99 \times 10^{-5}$ | $6.66 \times 10^{-4}$ |
| Calcium ion binding                                                | 5509   | 759   | 331      | 280      | $5.58 \times 10^{-5}$ | $8.58 \times 10^{-4}$ |
| Ion channel activity                                                | 5216   | 280   | 134      | 103      | $9.59 \times 10^{-5}$ | $1.37 \times 10^{-4}$ |
| Guanyl-nucleotide exchange factor activity                          | 5085   | 102   | 55       | 38       | $3.18 \times 10^{-4}$ | $3.74 \times 10^{-3}$ |
| GO ID        | Total | Observed | Expected | P value    | FDR       |
|-------------|-------|----------|----------|------------|-----------|
| Voltage-gated ion channel activity | 5244  | 118      | 61       | 6.83 × 10⁻⁴ | 6.00 × 10⁻³ |
| MicroRNAs   | –     | 312      | 100      | 1          | 1         |