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These mutants allowed direct determination in living cells that acylated proteins associate in a manner predicted by models for clustering of proteins into the liquid-ordered phase (5). They cluster with each other in lipid rafts and with full-length caveolin-1, a marker for caveolae, suggesting similar lipid compositions and environments in the two structures. Raft disruption with MJCD not only disaggregates acylated fluorescent proteins but also alters numerous signaling events (15, 16, 21), demonstrating the importance of these domains for cellular function.

References and Notes
13. CFP and YFP were enhanced versions eCFP and eYFP from Clontech. The signal for geranylgeranylation (GerGer) was a COOH-terminal C11L from rho. PalmPalm, an NH2-terminal LLCMFRKIQ from Gap43; MyrPalm, an NH2-terminal MGC58RKLNNIDDE from Lyn kinase. Full-length cDNA of bovine caveolin-1 was fused to the NH2-terminus of GFP and YFP. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
15. Supplementary methods and references are available on Science Online at www.sciencemag.org/cgi/content/full/296/5569/913/DC1.
25. We thank L. Burns, H. Parkey, and M. Patrassi for help with ultracentrifugation and S. Adams, C. Walkup, R. Wachtler, and J. Henley for discussion and suggestions. Supported by NIH grants NS27177 (R.T.), DK54441 (A.N.), ZF32 GM07752 (J.V.), and HHMI.

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Large-Scale Transcriptional Activity in Chromosomes 21 and 22
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The sequences of the human chromosomes 21 and 22 indicate that there are approximately 770 well-characterized and predicted genes. In this study, empirically derived maps identifying active areas of RNA transcription on these chromosomes have been constructed with the use of cytologic polyadenylated RNA obtained from 11 human cell lines. Oligonucleotide arrays containing probes spaced on average every 35 base pairs along these chromosomes were used. When compared with the sequence annotations available for these chromosomes, it was noted that as much as an order of magnitude more of the genomic sequence is transcribed than accounted for by the predicted and characterized exons.

Transcriptionally active regions of the human genome have been mapped by a combination of the alignment of cDNA sequences to genomic sequences and the annotation of genome sequences to predict coding regions (1–4). The goal of this study was to develop an empirical map of the transcriptionally active regions of the human genome at the nucleotide level and to relate this map to the sequence annotations derived from the two general approaches listed above. Previous reports (5) have relied on the annotations of the human genome to guide the authors in choosing which genomic regions to evaluate to determine the transcription profile of a cell type (exon arrays). In contrast, we have
used an empirical approach to create a collection of transcript maps using uniformly spaced oligonucleotide probes (25-nucleotide oligomers) that interrogate either every base or on average every 35 base pairs (bp) of the sequenc-es of human chromosomes 21 and 22 in a systematic fashion. The advantages of this approach include: (i) the identification of new regions of transcription not yet observed by previous experimentation or sequence analysis, (ii) the detection of RNA transcripts that have little or no coding capacity, and (iii) the identification of alternative RNA isoforms of previously annotated genes.

Labeled double-stranded cDNAs made from cytoplasmic polyadenylated [poly (A)] RNA from 11 different tumor and fetal cell lines [S1 (6)] were hybridized to high-density oligonucleotide arrays of two types. The first array design interrogated 362,901 contiguous oligonucleotide arrays of two types. The first array design interrogated 362,901 contiguous nucleotides of DiGeorge syndrome minimal critical region (DGCR) (7–9) on chromosome 22 using a perfect complement (PM) and mismatch (MM) complement oligonucleotide probe for each base (DGCR array). The second design interrogated approximately 35 million nonrepetitive base pairs of chromosomes 21 and 22 (Chrom 21_22 arrays) using 1,011,768 probe pairs synthesized on a three-array set [S2 (6)].

Determination of whether or not a probe pair detected RNA target was made using a range of threshold values for the ratio (R) of PM to MM measurements and for the difference (D) of PM–MM values [S3 (6)]. Because of the overlap of interrogating probes used in the design of the DGCR array, it was possible to join positive probes separated at most by a certain distance (maxgap) and to then reject resultant regions whose length was less than a particular threshold (minrun), thus building maps with contiguous runs (contigs) of RNA [S4 (6)]. Contigs for the Chrom 21_22 array data were not constructed because of the distance between the probes used in this design. By fixing the R and D thresholds for each experiment, it was possible to calculate (i) false positive, specificity, and sensitivity rates on the basis of spiked bacterial RNA transcripts containing specific deletions and (ii) human sensitivity (only for the DGCR array) based on exon sequences detected by reverse transcriptase–polymerase chain reaction (RT-PCR) [S1 and table S1 (6)].

Chromosomes 21 and 22 have at least 225 and 545 genes, respectively, that are either well characterized or predicted. Of these, approximately 127 and 247 are “known genes” (10, 11) that contain 1430 and 3134 exons on chromosomes 21 and 22, respectively (12). Figure 1 provides an overview of the previously identified and array-predicted transcription activity on chromosomes 21 and 22. By dividing the genome sequences of chromosomes 21 and 22 into 57-kb increments (average gene length on chromosome 21) (11), a total of 1,220 gene-sized loci can be created across both chromosomes. Given that the average distance between each interrogating probe pair is 35 bp, the positive probe and exon densities (13) for each locus are plotted and can be compared. The correlation between the exon and positive probe densities demonstrated a significant relationship over the majority of the lengths of both chromosome sequences. Of the 1,011,768 probe pairs that interrogate approximately 35,000,000 nonrepetitive bp of both chromosomes, 26,516 (2.6%) probe pairs are located within the 4,564 annotated exons of well-characterized genes. Of these annotation-focused probes, 69.8 and 40.7% detect RNA transcript variation in the exon density. Above the positive probe density maps are the experimentally verified regions (downward arrows). The DGCR region of chromosome 22 is boxed in (B). High-resolution maps of the DGCR are shown in Fig. 2.
in at least 1 or 5 of 11 cell lines, respectively (Table 1). The percentage of the overall positive probes detected was 34.8 and 9.6% in 1 or 5 of 11 cell lines, respectively. This indicates that 94 and 88% of the probes detecting transcripts are located outside annotated exons in 1 or 5 of 11 cell lines, respectively. Approximately 50% of these positive probes are located <300 bp distant from the nearest annotated exon. This is reflected in the close correlation between the exon and positive probe densities (Fig. 1).

A high-resolution map at 1-bp resolution that describes the locations of annotated exonic sequences and the array-based detected transcriptionally active regions has been developed [S3 and S4 (6)], and four examples of the annotated regions from DGCR are depicted in Fig. 2. The formation of contigs for this map allowed us to lower the estimated false positive rate for each of the 11 cell lines [S5 and S6 (6)]. Similar to that observed with the lower resolution maps of chromosomes 21 and 22, most of the detected transcripts (59.4 to 65.9%) are located away from the annotated exonic and expressed sequence tag (EST) sequences (Table 2).

At this resolution, for example, alternative transcripts in the DGCR6 locus were observed (Fig. 2A). Exons 1 and 5 appear to have RNA transcripts longer than previously represented. Additionally, there is evidence for transcriptional activity within intron 3. RT-PCR analysis and recent studies support these data (14).

Similar alterations could be made to the annotations of three other regions of DGCR (Fig. 2, B through D). RT-PCR analysis and subsequent sequencing of the transcripts in intron 5 revealed an extended version of Di-George syndrome gene E (DGS-E), as well as transcripts 5' to this gene. Additional limited RT-PCR analysis provided confirmatory evidence for the presence of these and other transcripts within the DGCR2 locus (Fig. 2B). Similarly, novel transcripts have been observed and confirmed in intron 1 of DGCR5 (Fig. 2D) and in the vicinity of the highly expressed SCL25A1 gene (Fig. 2C). Thus, these maps have not only been useful in estimating the overall fraction of the human genome that is transcribed but they have also served as a guide for directing further biochemical and molecular efforts to isolate novel transcripts. High-resolution maps for the entire sequence of the DGCR and the nonrepeat sequences of chromosomes 21 and 22 are also available (6).

Fig. 2. High-resolution maps of four regions within DGCR of chromosome 22 (22q11.2). For each map, the contigs predicted by the DGCR array for 6 of the 11 cell lines analyzed is presented. Below the array map are cartoons derived from the Sanger hand-curated map of this region (19) or from GenBank. Selected regions suggested by the array map were further analyzed with the use of RT-PCR. The sequenced products from these analyses are mapped below the Sanger and ESTs maps (A) DGCR6 gene region [Golden Path (GP) sequence 15,833,950 – 15,840,390], (B) DGCR2 region [GP sequence 15,959,850 – 16,057,850], (C) SLC25A1 gene and flanking region [GP sequence 16,098,590 – 16,107,090], and (D) DGCR5 exon 1 region [GP sequence 15,898,300 – 15,905,040].
A total of 63 individual, array-predicted transcription loci on chromosomes 21 and 22 distant from annotated exons and ESTs were selected. Our expectations were that these "annotation or transcript poor" loci would represent the predictions with the highest probability of being identified as false positives by the array, and, thus, successful identification of transcribed loci in such regions would serve as strong supportive evidence for the array-guided maps. Furthermore, any transcribed regions identified distant from known exons or ESTs are more likely to represent novel transcripts.

PCR primers were designed at or near positive probes or contigs detected by the arrays. Two approaches were used for the verification: RT-PCR on cytosolic poly(A)-RNA and PCR on cDNA libraries prepared from cytosolic poly(A)-RNA of HepG2, NIH3T3, and PC3 cell lines ([51]). Positive RT-PCR or PCR products were identified in 44 of 63 loci [table S2 (6)]. Northern hybridization experiments were also conducted using poly(A)+ RNA from 7 of the 11 cell lines and cloned RT-PCR products from 16 loci as probes. Seventeen of 16 loci yielded detectable RNA transcripts ranging in size from 0.6 to 10 kb, of which three loci were characterized by multiple examples of distinct or indistinct size [for examples see fig. S3 (6)].

The RT-PCR and sequence analyses of the cytosolic poly(A)+ RNA samples and cDNA libraries indicated that at least 44 of 63 loci predicted by the array experiments to be sites of novel transcripts were transcribed. The filter-based hybridization experiments and large number of cycles required to detect RT-PCR or PCR products strongly suggest that the observed novel RNAs are present at low copy number per cell, providing some explanation as to why these transcripts have not previously been observed.

This survey of cytoplasmic poly(A)+ RNA obtained from 11 developmentally diverse cell lines indicated that there may be as much as an order of magnitude greater sites of transcription of mature RNA transported into the cytoplasm than can be accounted for by the current annotation of the sequence of the human genome. However, it is important to note that the number of transcriptionally active sites does not necessarily correspond to an equal number of genes. The maps obtained in this study do not provide a description of the structural relationships among the detected regions of transcription (i.e., the start and termination of each exon in a transcript). The detection of two adjoining regions of positive probes does not necessarily require that both probe sets are detecting the same RNA molecule. In fact, because of our use of labeled double-stranded cDNAs as targets in these experiments, the data presented here does not distinguish the strandedness of the detected RNAs. In some cases, detected expressed sequences cannot necessarily be guaranteed to originate from the transcription of chromosomes 21 and 22. Expression from paralogs in other genomic loci could lead to the same observed signals. However, the central conclusion to be drawn from these data is that a significantly larger proportion of the genome is transcribed and specifically transcribed as mature cytoplasmic poly(A)+RNA than previously considered.

Why were these sequences not observed previously? The answer to this question may reside in three observations. First, many of these detected transcripts may be structurally connected to other partially characterized RNAs (i.e., ESTs). Second, our estimates of the relative abundance of the majority of detected transcripts is very low, based on the required levels of RT-PCR and PCR amplification required to detect them in the RNA and cDNA libraries and the time of exposure needed to detect those observed by Northern hybridization [fig. S3 (6)]. Lastly, the sequences for many cDNA clones are discarded as possible heteronuclear RNA contaminants because of the low coding potential found.

The answer to the question of what function these transcripts might have requires additional large-scale characterization of the observed novel transcripts. Interestingly, noncoding RNAs are emerging as a rapidly expanding functional class of transcripts important for splicing, nuclear and ribosomal structures, telomeric sequence addition, transport and insertion of proteins into membranes, down-regulation of translation, and chromosomal inactivation (16, 17). Though for many of these non-coding RNA groups hundreds of new members are being identified in mammalian cells (18), the function of these identified transcripts must await additional characterization and perhaps reveal a hidden transcriptome.

Table 1. Proportion of chromosomes 21 and 22 transcribed. Analyzed were 1,011,768 probe pairs, of which 26,516 query exons as annotated in the known mRNA databases such as RefSeq, Sanger hand-curated and GenBank. ESTs not included as part of expressed portion of genome. ([2]) Numbers in parentheses indicate percent positive probe pairs.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Positive probes overall</th>
<th>Positive probes in exons</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 of 11</td>
<td>268,466 (26.5%)</td>
<td>17,924 (67.6%)</td>
</tr>
<tr>
<td>5 of 11</td>
<td>98,231 (9.7%)</td>
<td>10,903 (41.1%)</td>
</tr>
</tbody>
</table>

Table 2. Proportion of DGCGR (22q11.2) transcribed. The values are calculated on the basis of 213,009 probe pairs interrogating nonrepetitive (NR) bases of which 61,842 probe pairs are located within annotated expressed regions of the DGCGR. The target false positive (FP) rate is that of each individual cell line [55 and 56 (6)]. Expr. bases refers to the databases mentioned in Table 1 plus all the GenBank mRNAs and ESTs mapping to this region.

<table>
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<tr>
<th>Cell lines</th>
<th>FP</th>
<th>Positive NR bases</th>
<th>Positive NR expr. bases</th>
<th>Positive NR nonexpr. bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 of 11</td>
<td>3%</td>
<td>50,885 (23.9%)</td>
<td>17,421 (34.2%)</td>
<td>33,464 (65.8%)</td>
</tr>
<tr>
<td>5 of 11</td>
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<td>63,908 (30.0%)</td>
<td>21,788 (34.1%)</td>
<td>42,120 (65.9%)</td>
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<tr>
<td>1 of 11</td>
<td>5%</td>
<td>11,623 (5.5%)</td>
<td>4,724 (40.6%)</td>
<td>6,899 (59.4%)</td>
</tr>
<tr>
<td>5 of 11</td>
<td>5%</td>
<td>20,097 (9.7%)</td>
<td>7,477 (37.2%)</td>
<td>12,620 (62.8%)</td>
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</tbody>
</table>