In the new study, Mahoney *et al.*[1] take a different approach to identifying synthetic lethal mutations that could be revealed during OV infection. Their strategy involved treating partially OV-sensitive tumor cell lines with RNA interference (RNAi) directed against expressed cellular genes. Using an arrayed library of approximately 18,000 genes, they then used the oncolytic Maraba virus[21] to probe for genes that sensitized tumor cells to viral oncolysis. Remarkably, they uncovered RNAi-targetable genes that could specifically sensitize tumor cells over 10,000-fold to Maraba infection. The RNAi screen identified a number of gene products involved in the unfolded protein response (UPR), including dedicated transcription factors (ATF6α, ATF6B), the endoribonuclease IRE1α, and its downstream product XBP-1. They also identified proteins associated with the endoplasmic reticulum (ER)-associated degradation (ERAD) pathway that removes misfolded polypeptides from the ER and targets them for proteolytic degradation. The striking enrichment of proteins involved in the UPR and ERAD pathways suggested that Mahoney and colleagues had identified a key pathway that could complement cell killing by Maraba and perhaps other rhabdoviruses. Importantly, combination of Maraba infection with knockdown of UPR/ERAD genes did not sensitize normal skin or lung fibroblasts or normal human astrocytes. To close the loop, the workers then chemically synthesized known inhibitors of IREα and demonstrated that these could block UPR and synergize with Maraba in tumor cell killing.

The authors' experiments also revealed that tumor cells had "rewired" their UPR/ERAD pathways—in the sense that they have come to a new equilibrium with respect to ER stress—leading to a tumor-specific activation of an apoptotic pathway triggered by OV infection that is caspase 2–dependent. This led to a prediction that an "ER preload" by RNAi inactivation of IRE1α could lead to enhanced tumor cell killing by other chemical compounds that work through caspase 2 activation. Indeed, doxorubicin treatment following IRE1α knockdown specifically increased tumor cell killing.

Once again, exploration of virus–host interactions has led to a new understanding of the myriad pathways that control the life and death of mammalian cells. Many questions remain. Is tumor cell killing by all OVs enhanced by ER preload or is Maraba virus uniquely sensitive? Can other synthetic lethal mutations be identified by screening with different OVs? How frequently do tumor cells rewire their UPR/ERAD pathways? Although it remains unknown whether rhabdoviruses such as Maraba will become viable cancer therapeutics, the synthetic lethal screening approach described by Mahoney *et al.*[1] illustrates the value of studying how oncolytic viruses replicate within and kill cancer cells. Although many of us believe that OVs will eventually become viable anticancer therapeutics, the results from this group suggest that, at a minimum, studying the biology of OV–host interactions will reveal previously unappreciated cancer-specific pathways that could potentially identify combination drug approaches that might be less toxic, and yet more effective, in cancer patients.

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lncRNAs: Finding the Forest Among the Trees?

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doi:10.1038/mt.2011.251

One of the greatest surprises revealed when the first draft of the human genome was completed in 2003 was the total number of genes, which at ~20,500 fell well below even the most conservative of estimates.[1] The numerous eukaryal genomes sequenced since then have continued to confound the common-sense notion that gene number and organismal complexity should be positively correlated, with examples such as the sponge and paramecium, both of whose gene numbers exceed that of humans.

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A corollary of the low human gene number is that the proportion of the genome that encodes protein, at just 2%, was also lower than expected, with the remainder largely discounted as nonfunctional, or "junk DNA." In 2005, new light was shed on these noncoding regions of the genome when both large-scale complementary DNA sequencing and genome tiling arrays revealed that the majority of these areas were transcribed into RNA.[2,3] These observations raised two fundamental questions: is the transcription of noncoding regions of the genome biologically meaningful, and could noncoding RNAs reconcile the apparent disparity between gene number and complexity? In a study reported recently in *Nature*, Guttman and colleagues took an important step toward answering these questions by demonstrating that knockdown of the vast majority of long noncoding RNAs (lncRNAs) expressed in embryonic stem (ES) cells affects gene expression patterns in a manner similar to that of knockdown of well-known ES cell regulators.[4]

Although functionality for noncoding RNAs (ncRNAs) has long been established by their roles in the translational and spliceosomal machinery, as well as for dosage compensation by imprinting of the X chromosome by the ncRNA *XIST*, their widespread role has remained contentious. The presence of thousands of lncRNAs was most profoundly brought to light by the large-scale complementary DNA sequencing of the mouse genome as part of RIKEN's functional annotation of the latter, which identified more than 30,000 lncRNAs.[2] Subsequent transcriptomic analyses in humans have identified a comparable number of lncRNAs, with even conservative estimates rivaling the number of annotated protein-coding genes. In support of the case for widespread biological roles for lncRNAs, microarrays targeting thousands of lncRNAs revealed dynamic expression profiles of distinct subsets of lncRNAs in various developmental systems, including ES cell differentiation.[5] Similarly, *in situ* hybridization of hundreds of lncRNAs in the adult mouse brain revealed a remarkable degree of specificity at the tissue, cell-type, and subcellular levels.[6] Combined with conservation of primary sequence and splice sites,[7] as well as the growing number of functionally characterized lncRNAs in the literature, it seemed increasingly likely that lncRNAs were biologically important.[8] Nevertheless, counterarguments maintained that low expression levels were inconsistent with function and that experimental artifact or spurious transcription in regions of open chromatin could reconcile the occurrence of ncRNAs in transcriptomic studies.[9,10]

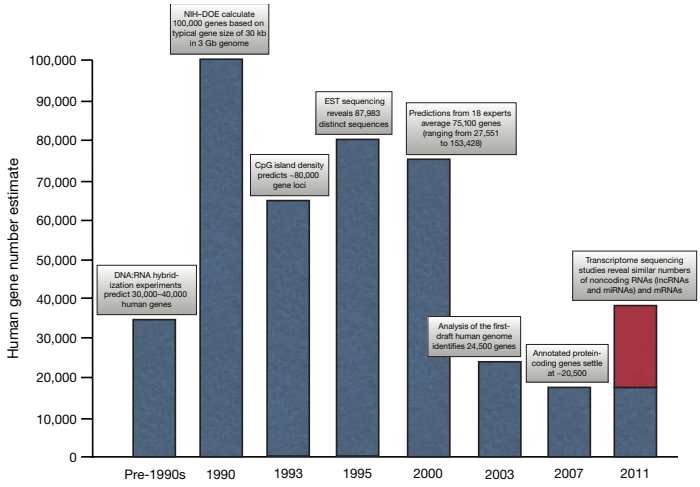

Figure 1 The rise and fall (and rise) of human gene counts. Estimates of human gene numbers have varied dramatically over the past few decades.[16] The considerable variation in gene counts can be accounted for largely by differences in gene definition. Earlier estimates involving hybridization and expressed sequence tag (EST) sequences did not discriminate between coding and noncoding RNAs, whereas the stricter definition of gene counts introduced in 2007 considered only protein-coding genes. Further apparent disagreement in gene numbers arises as a result of genes being defined as independent loci versus distinct sequences. When we consider the introduction of noncoding RNAs as genes, the number of distinct loci returns to early estimates of ~30,000–40,000. lncRNA, long noncoding RNA; mRNA, messenger RNA; miRNA, microRNA; NIH–DOE, National Institutes of Health–Department of Energy.

Guttman *et al.* tackled head-on the question regarding the extent of lncRNA functionality. Targeting 226 lncRNAs that had previously been shown to be expressed in ES cells, the team successfully knocked down the expression of 147 lncRNAs. Microarrays were then used to assess the relative impact on global gene expression profiles 4 days after knockdown. As a result, the authors found that a staggering 93% (137 of 147) of lncRNA knockdowns have a significant effect on gene expression. In further characterizing the roles of the lncRNA knockdowns in ES differentiation, they found that 26 led to increased exit from the pluripotent state and 30 produced expression patterns similar to those of specific differentiation lineages, suggesting that these lncRNAs act as repressive regulators for such differentiation.

The molecular roles of lncRNAs described to date have been highly diverse, including roles in forming nuclear structures, regulation of alternative splicing, and directing imprinting, and are therefore unlikely to share any unifying mechanism.[11,12] However, increasing evidence suggests that a significant proportion are involved in chromatin remodeling and are speculated to recruit generic chromatin-modifying complexes to specific regions in the genome.[13] With this concept in mind, the authors screened antibodies against 28 chromatin complexes and found 74 lncRNAs associated with 11 different complexes. These results provide further support that many lncRNAs exert their regulatory function *in trans* through interaction with epigenetic modifying machinery. Nevertheless, this forms just one aspect of the diverse functional repertoire of lncRNAs. Another significant emerging theme in lncRNA function is a *cis*-acting role in facilitating enhancer activity. Several studies have demonstrated that enhancers are transcribed by RNA polymerase II and that this expression activates gene expression.[14,15] Given the diverse biochemical characteristics of RNA, in terms of both its structural and catalytic properties, as well as the large range of lncRNAs sizes, which can range from hundreds to tens of thousands of nucleotides, it is likely that we have only begun to uncover the possible mechanisms through which lncRNAs can act.

Given the remarkable proportion of lncRNAs that impart measurable phenotypes and increasing numbers with demonstrated regulatory roles in controlling

gene expression, it is opportune to reflect upon the tens of thousands of lncRNAs that have been identified to date in the mammalian transcriptome. In consideration of the high functional validation rate in ES cell–expressed lncRNAs, it is reasonable to expect that lncRNAs expressed in other biological systems will reveal similar degrees of functionality. This realization has a profound impact on the manner by which the genome imparts information to the cell and how we interpret and design genome-wide studies. In light of genome-wide association studies revealing that the majority of disease- or other phenotype-associated regions fall within noncoding areas of the genome, it is particularly pertinent to consider whether these regions encode lncRNAs or other classes of ncRNAs. Furthermore, the scarce annotation of lncRNAs in public databases means that lncRNAs are poorly represented on exome arrays. Consequently, the wide-scale deployment and application of exome arrays is likely to be premature, as their coverage of the functional components of the genome is not as comprehensive as is widely perceived.

The traditional definition of a gene is a sequence of DNA that occupies a specific location on a chromosome and determines a particular characteristic of an organism. In light of the rapidly expanding proportion of lncRNAs that are functional, it is clear that the gene number of ~20,500 announced in 2007, when it was defined as including only protein-coding genes, no longer serves as a meaningful count of functional genetic loci. Indeed, the predominantly protein-centric view of genes may soon be overturned as the number of functional noncoding genes can by most reasonable measures be anticipated to outnumber protein-coding genes in the near future. Because lncRNAs often show remarkably specific expression profiles, both temporally and spatially, it is unlikely that transcriptomic sequencing efforts to date will have mapped the true breadth of noncoding expression in the genome. Therefore, earlier estimates of gene numbers may ultimately prove to have been on the mark after all (**Figure 1**).

From a therapeutic perspective, the expansion of gene numbers provides a wealth of new opportunities. A preponderance of new technologies are becoming available that provide generic approaches to targeting specific RNAs. Indeed, given the specificity of ncRNA expression, their targeting may also be harnessed to yield more specific outcomes. Although the task ahead of dissecting the function of thousands of new genes is daunting, having a substantially more complete picture of the regulatory architecture underpinning our normal function and development will herald a new era in understanding the molecular basis of disease and offer the potential of a world of new therapeutic possibilities.

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Gene Transfer Using HACs: A Key Step Closer to *Ex Vivo* Gene Therapy Using Autologous Gene–Corrected Cells to Treat Muscular Dystrophy

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doi:10.1038/mt.2011.254

Duchenne muscular dystrophy (DMD) is a progressive muscle-wasting disease caused by mutations in an X-linked gene encoding for the muscle-cell structural protein dystrophin.[1,2] Gene therapy strategies to treat DMD face the daunting challenge of how to deliver the very large dystrophin gene to the entire musculature of patients. Cell replacement therapy, also being investigated as a treatment for DMD, aims to deliver the patient's own *ex vivo* gene-corrected cells to replace the diseased muscle and/or stimulate its growth and repair. Of course, such a strategy faces the same challenge of how to deliver such a large gene to the cells and then how to distribute the corrected cells throughout the muscles of the patient. However, a recent study by Tedesco *et al.*[3] takes us one step closer to a possible solution to these problems by making use

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