

## Chipping Away at HIV Pathogenesis

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### THE NEW AND IMPROVED GENETIC CODE

Completion of the Human Genome Project (1,2) has spawned a new set of questions aimed at discovering its meaning. With 2.91 billion basepairs (bp), characterizing this is no easy task. Traditionally, investigators have been confined to mining the human genome a few genes at a time. Fortunately, recent technology has allowed investigators to study it in a comprehensive manner, with the aim of translating genetic sequence information into physiologic relevance. Methodologies such as microarray (cDNA and oligonucleotide) and high-density oligonucleotide array (GeneChips from Affymetrix) analysis allow the interrogation of thousands of genes simultaneously, revealing their expression patterns and the subsequent cellular pathways associated with a particular perturbation. This is particularly useful in studying the effects of microbes on their hosts, which allows both genomes to be investigated simultaneously.

### GENECHIP TECHNOLOGY IN GENOMICS RESEARCH

Differential expression data can provide a clearer understanding of cellular pathways triggered by specific stimuli. GeneChip expression probe arrays assist in genomics research by quantitatively and simultaneously monitoring the expression of thousands of genes (3,4). For example, the U95Av2 Chip has a platform of over 12000 gene-specific oligonucleotides anchored to a glass substrate. GeneChip probe arrays identify mRNA expression level changes of greater than twofold between experiments, and are able to detect mRNA transcripts from the level of only a few copies per cell to more than several hundred thousand copies. In contrast to spotting methods, in which a single clone is used to analyze each mRNA, GeneChip expression arrays use approximately 16 pairs of specific oligonucleotide probes to interrogate each transcript with specificity. GeneChip expression arrays are capable of specifically detecting individual gene transcripts and splice variants and can even differentiate among closely related members of gene families. Each GeneChip expression array contains probes that correspond to a number of reference and control genes. Data from different experiments can be normalized and scaled to compare

multiple experiments quantitatively. These results are typically validated by quantitative real-time RT-PCR.

GeneChip technology has broad applications. Since 1998, the number of publications involving expression monitoring using high-density oligonucleotide probe arrays has increased by approximately sevenfold (see <http://www.affymetrix.com/resources/papers.shtml> for a complete list). The fields of cancer (5–23), toxicology (24–26), cardiology (27–28), nutrition and aging (29–34), bacteriology (35–38) and mycology (39–45) have all benefited from this technology. Surprisingly, there are only a few publications describing Human Immunodeficiency Virus (HIV)-induced changes in host-gene-expression patterns (46–48) and in identifying HIV-associated mutations using the HIV PRT probe array (49–52).

## OVERVIEW OF HIV PATHOGENESIS

Since the presentation of HIV in the early 1980s, billions of dollars have been devoted to its eradication. As of December 2000, 21.8 million people have died globally from acquired immunodeficiency syndrome (AIDS). In addition, 5.3 million people were newly infected in the year 2000 (53). These statistics clearly demonstrate the need for more resources to further the investigation into HIV pathogenesis. Although great progress has been made, the effect on host target cells has not been fully characterized. The inability to directly examine HIV as an intracellular pathogen on a comprehensive level has been a limiting factor. Several aspects of HIV pathogenesis are targets for study by GeneChip probe arrays. At each stage of the infection process, probe arrays could identify changes in gene-expression target and bystander immune cells. The following is a brief overview of potential areas of investigation.

### *Host Entry*

HIV invades the human host through mucosa and blood. There, it has access to its target cells, CD4+ T lymphocytes and monocyte/macrophages. Attachment of the virion is mediated by the interaction of viral-envelope glycoproteins (gp120-gp41) with host cellular-receptor CD4. A conformational change in gp120 allows gp41 to bind to coreceptors such as CXCR4 or CCR5, facilitating cell-viral membrane fusion. The initial contact between HIV and its target cell may be enough to alter host-cell gene expression, as HIV carries host-encoded proteins such as CD80 and CD86 on its surface (54–56).

### *Manifestation of Disease*

Clinically, HIV infection can be defined in three stages: (1) primary, (2) asymptomatic, and (3) symptomatic disease progression. During acute infection, within the initial 6 mo, viral replication is intense, as are both humoral and cellular factors that act to control it (57). In this early stage, flu-like symptoms—which appear in some but not all people—probably reflect the humoral and cellular changes that are occurring. The next phase of HIV progression is the asymptomatic period, which can last for several years. This stage is reflected by relatively low levels of HIV antigens and a high turnover of virus. CD4+ cells also turnover rapidly, but their numbers remain relatively steady because of the balance between replenishment and death. Finally, AIDS develops,

CD4+ levels decline, opportunistic infections arise, and death ensues. Each of these stages involves different systems of viral replication and immune response; GeneChip analysis may be an ideal method to evaluate these differences.

### ***Target-Cell Depletion and Apoptosis***

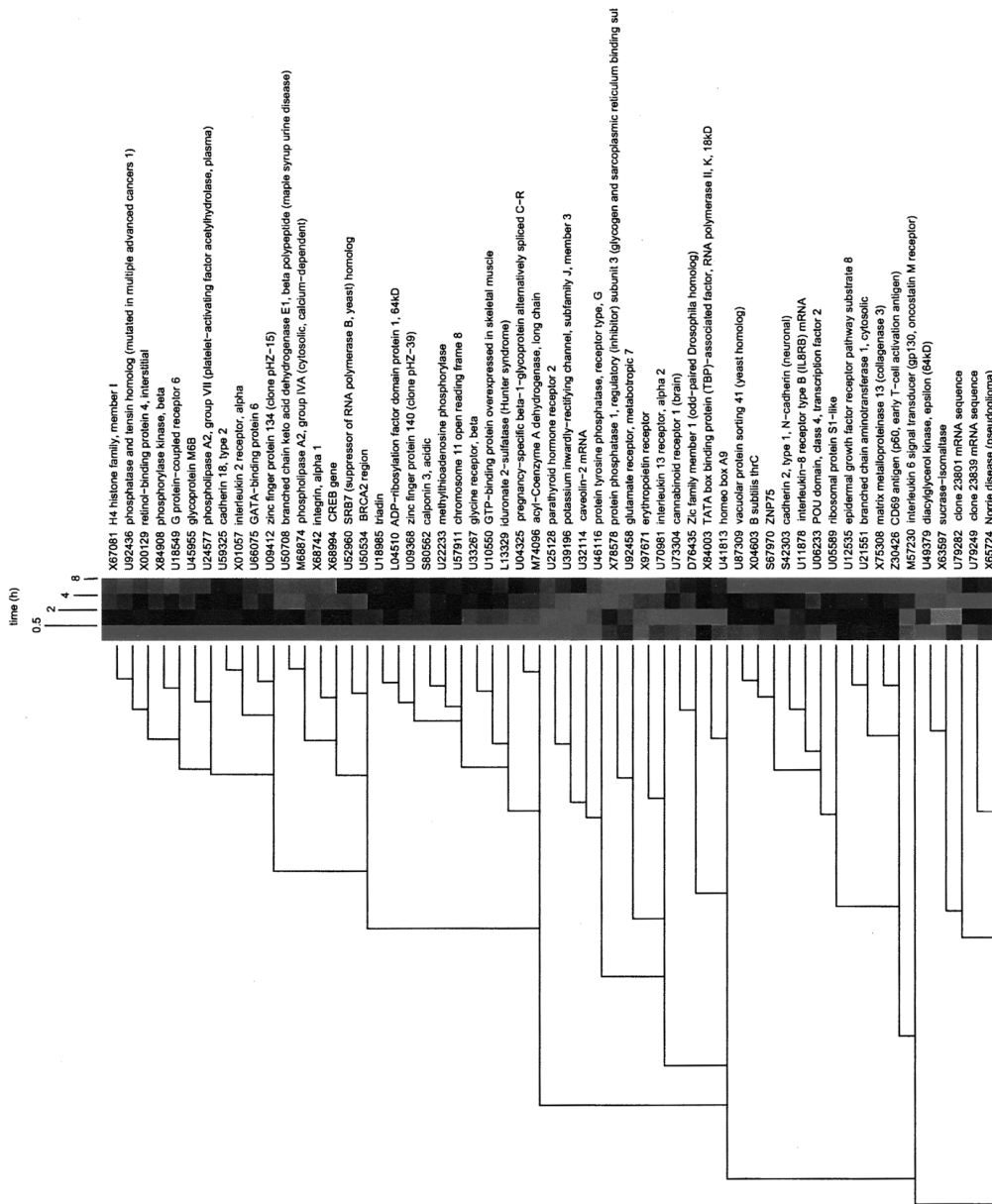
One of the hallmarks of HIV progression is CD4+ T-cell depletion. The decline in cell number results from a complex mixture of infected and bystander-cell apoptosis and direct killing by HIV. As CD4 cell numbers decline, viral load increases, directly contributing to pathogenesis. It has been reported the HIV gp120 crosslinking to CD4 receptor enhances susceptibility to apoptosis (58,59). Other HIV proteins implicated in target-cell apoptosis include nef, tat, protease, and vpr (60–63). It is clear that this battle between the immune system and the virus could be explored more finitely using probe-array technology. This would aid in the discovery of apoptosis inhibitors, which could boost the immune response to infection.

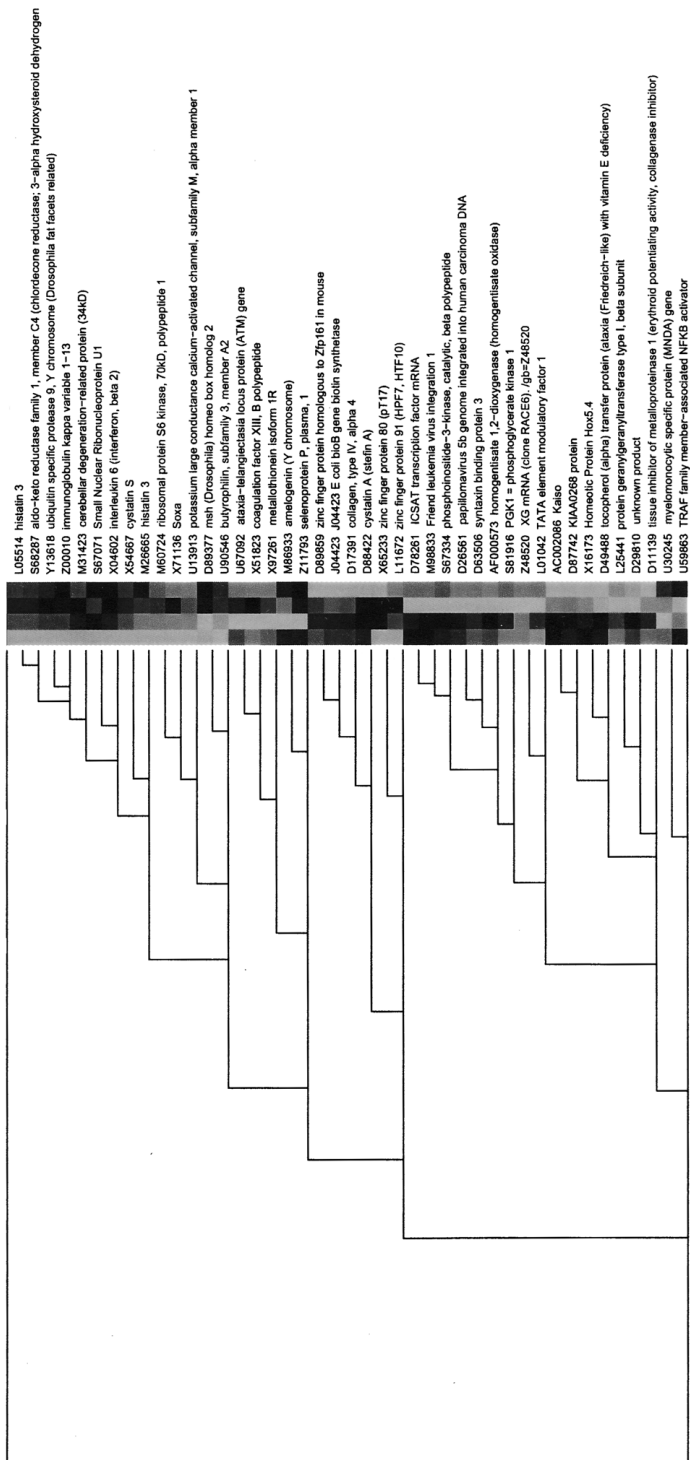
## **BIOINFORMATIC ANALYSIS OF HIV EFFECTS**

The utility of gene-array experimental data has been limited by the paradoxical occurrence of too much and too little data. The number of mRNA copies detected for thousands of gene loci represents too much data to be comprehended by unaided human cognition. This scientific problem has been likened to “trying to drink from a fire hose.” At the same time, the paucity of semantic information—the *meaning* of gene activation patterns—derives from the fact that GeneChip and gene-array analyzers output only signal-strength information about individual loci, and is accompanied by minimal identifying information such as a GenBank accession number and a short description associated with that accession number. Bioinformatics, the science of developing methodologies for interpretation of biological research, has become an essential tool for understanding genomics. This field of computer science was precipitated by the Human Genome Project as a means of managing the vast amount of information now at our disposal. Organizing gene-expression data into clusters (for example, see Fig. 1) points to co-regulated genes and indicates potential pathways as a consequence of the experimental conditions. The bottleneck of interpreting results from new technologies such as GeneChip is aided by bioinformatic analysis.

Recently, we published data (46) on the HIV-1/host-cell relationship over a 72-h time period. Using HuFL GeneChips, we monitored 6800 human genes in a CEM-GFP lymphoblastoid cell line infected by HIV<sub>LAI</sub> (0.5 MOI). In our paper, we reported these responses to infection: (1) after 72 h, one-third of the total transcripts present in the host cells represented HIV transcripts; (2) mitochondrial and DNA-repair gene expression was decreased, (3) *p53* and the *p53*-induced product Bax were upregulated, and (4) caspases 2, 3, and 9 were activated. HIV-1 transcription resulted in the repression of a large portion of cellular RNA expression and was associated with the induction of apoptosis only in infected cells but not bystander cells. The CEM-GFP host gene responses imply that the subversion of the cell transcriptional machinery for the purpose of HIV-1 replication is similar to that observed in genotoxic stress. This process may play a role in HIV-induced apoptosis, ultimately leading to CD4+ T-cell loss and immunosuppression.

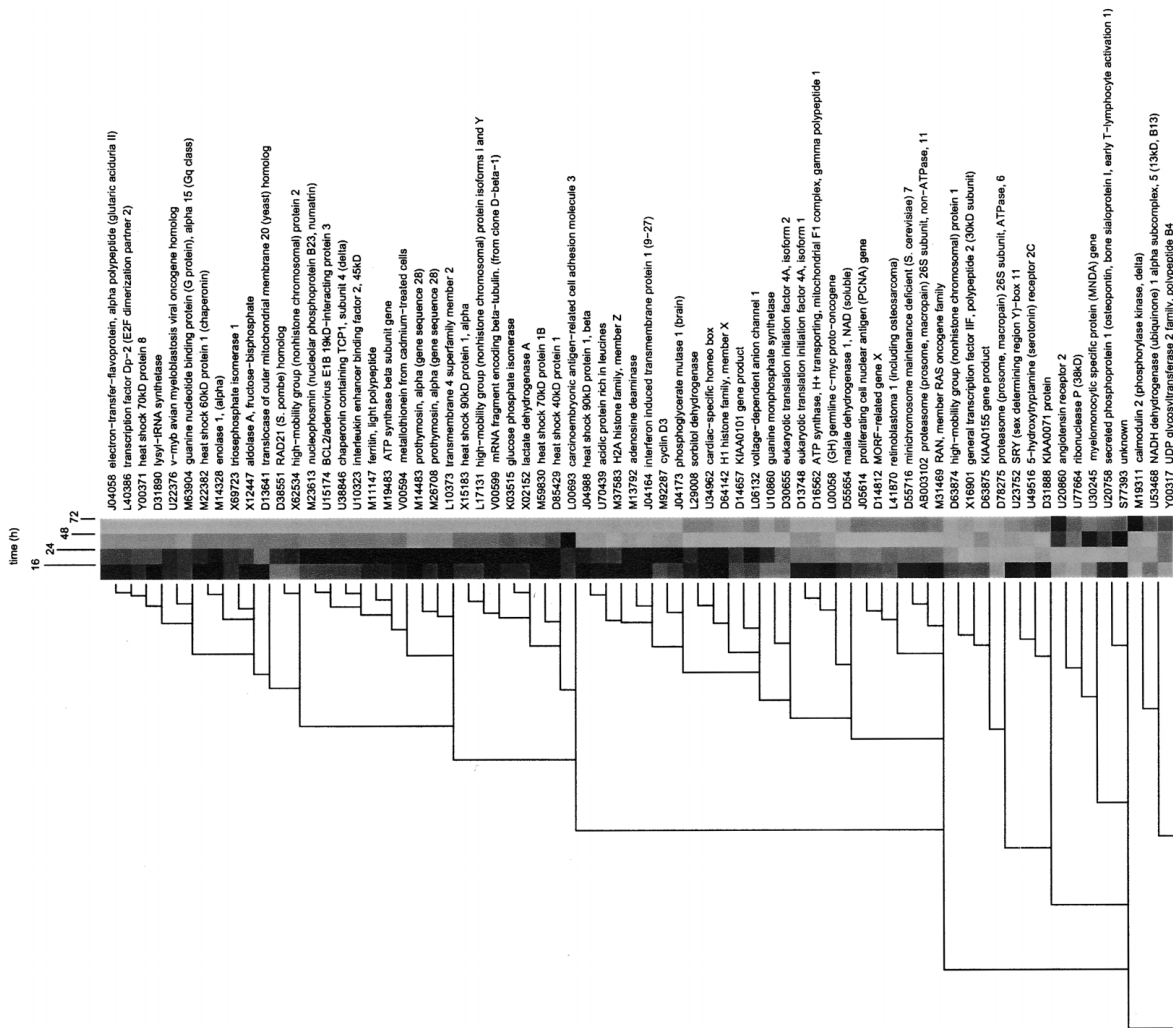
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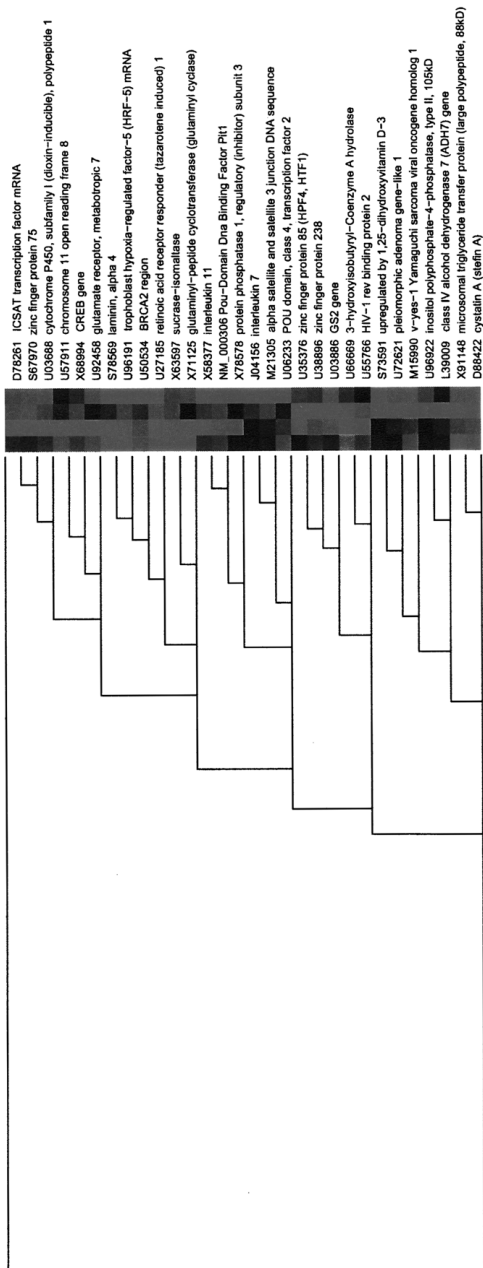




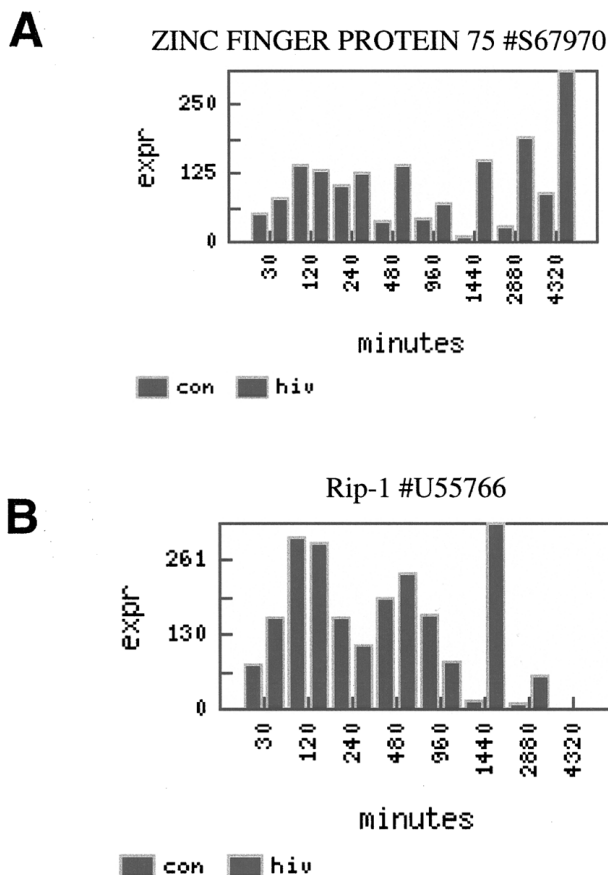
**Fig. 1.** Cluster analysis of CEM-GFP host-cell genes modulated by HIV within 8 h post-infection (A). (Continued)

B





**Fig. 1. (Continued).** and 16-72 hrs (B) post-infection. Green = downregulated in HIV, red = upregulated in HIV.



**Fig. 2.** Expression of zinc-finger protein 75 (**A**) and rev-binding protein (**B**) in uninfected (con) and HIV<sub>LAI</sub> infected CEM-GFP cells over a 72-h time-course.

Further examination of the GeneChip data revealed some interesting results. Specifically, a number of zinc finger (ZNF) proteins all belonging to the Kruppel group were significantly modulated during HIV infection, such as ZNF 75 (Fig. 2A). The human genome is estimated to contain 300–700 ZNF genes. ZNFs, which bind nucleic acids, perform many key functions; the most important is regulating transcription. The C2H2 ZNF family is characterized by repeated ZNF motifs of approximately 28 amino acids, which chelate a zinc ion using 2 cysteine and 2 histidine residues. The vast majority of these ZNFs are classified as Kruppel-like because they contain conserved 6-amino acid H/C links—regions that connect successive finger repeats. About one-third of Kruppel-like ZNFs contain a conserved motif of approximately 75 amino acids, called the Kruppel-associated box (KRAB), in their N-terminal nonfinger region (64).

Another gene previously associated with HIV was highly upregulated in our experiments. This gene, HIV-1 rev-binding protein 2, (also known as rev-interacting protein (Rip-1) accession number U55766) was highly expressed at 24 h and 48 h after infection (see Fig. 2B). The functional relevance of such an upregulation is under investigation.



## FUTURE OF GENOMICS

Discovery-driven approaches in genomics using high-density gene-expression microarrays have led to the establishment of new avenues of research. They have also enabled the identification of pathways and signal-transduction events pertinent to the process of HIV-1 infection. High-density microarray interrogation of gene expression represents a powerful tool to explore the relationships between an infectious agent and its host. The magnitude of knowledge gained by the global survey of effects of pathogens such as HIV on its host will provide a solid foundation for future studies. The study of proteins in a global context may also play an equally important role in the analysis of differential expression of human genomic sequence information.

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