Preface

Since the advent of microbial genome sequencing and the development of algorithms to compare and annotate genomes, an enormous wealth of information has become available to the scientific community. This information is further extended by technologies such as DNA microarrays that use sequence information to analyze genomic expression patterns, proteomics to analyze the translation of these patterns into protein products, and a variety of methods of functional analysis to determine the ultimate phenotypic manifestation of the genes themselves. The analysis of this treasure trove is far from complete, but initial findings have already revolutionized the field of microbiology. Microbiologists have made strong inroads into utilizing this information for drug discovery, vaccine development, and diagnostics. This information continues to be an integral part of the study of the fundamentals of pathogenesis, how organisms interact with each other and with their host environment, and will undoubtedly point to places where intervention will have a significant positive impact on human health. *Pathogen Genomics: Impact on Human Health* is intended to review recent developments in this unfolding story.

The utility of genomics extends from the smallest viral genomes to larger more complex organisms, including humans. Although significant progress has been made studying diverse collections of microorganisms, including plant pathogens, thermophilic Archaebacteria, and other organisms thriving in extreme environments, the scope of this book has been limited to pathogenic organisms that interact with a human host. Clearly, the genomics of all sequenced human pathogens could not be addressed in a single volume, but rather organisms were chosen to give a balanced presentation of viruses, bacteria, fungi, and protozoa. The goal is to bridge these disciplines and to explore the impact genomics has had on the discovery and choice of drug targets, selection of antigenic determinants for vaccine development, diagnostics, and our understanding of pathogenesis. Common sets of tools, such as genomic comparisons and microarray analysis, are used to explore many of these organisms. The findings from these analyses offer unique insights into the fundamental nature of each pathogen, as well as common strategies adopted by diverse pathogens to be successful in the human host.

Genomic comparisons and computational data mining have been used to identify the metabolic capabilities of specific pathogens and have revealed how

they have adapted to unusual host environments. They have pointed to pathways that are unique to an organism, and have identified pathways that are shared among all prokaryotes, are particular to fungi or protozoa, or are common to all life. The analysis of these data has had a significant impact on the identification and selection of targets for antibacterial, antifungal, and antiparasitic drug discovery, as well as providing candidates for the development of diagnostic tools and vaccines. Some of these findings are reviewed in Chapters 5, 7, 11–14, and 17. Chapter 9 explores the changing nature of epidemiological analysis, from plasmid fingerprinting through sequence-based typing, where advancements in genomic analysis have driven the technological development of new investigative tools for identifying the nature of nosocomial outbreaks.

In silico comparisons of strain-to-strain variations can be used to generate historical genealogies of infectious diseases. In Chapter 6, Behr and Gordon discuss comparisons of genomes of attenuated and virulent strains of organisms such as *Mycobacterium tuberculosis*. These types of analyses are critical to our understanding of the genetic basis of the evolution of virulence, and provide candidate genes whose inactivation may lead to the development of improved live attenuated vaccine strains or may serve as components of subunit vaccines. The use of genomic comparisons for the identification of fungal virulence determinants and vaccine candidates is reviewed in Chapters 15 and 16. Similarly, the identification of proteins involved in the pathogenicity of *Entamoeba histolytica* and *Borrelia burgdorferi*, or with possible utility as vaccine candidates, is explored in Chapters 10 and 18.

Genome analysis using microbial DNA microarrays began with the first eukaryotic genome sequenced, *Saccharomyces cerevisiae (1)*. The yeast arrays have been used extensively for exploring changes in expression profiles resulting from changes in growth conditions, in addition to other microarray applications such as mapping gene cross-over events *(2)*. Microarrays have aided our fundamental understanding of metabolic pathways that are important for antifungal drug discovery, as well as antifungal drug resistance. Chapter 12 reviews microarray analyses of yeast, with particular emphasis on the studies of the effect of inhibitors on ergosterol synthesis in both *Saccharomyces cerevisiae* and *Candida albicans*.

More recently, bacterial arrays have become readily available in the form of hybridization filters (Sigma-Genosys, The Woodlands, TX) for organisms such as *Escherichia coli*, *Helicobacter pylori*, and *Bacillus subtilis*. DNA microarrays containing open reading frames derived by PCR, or oligonucleotides representing these ORFs, continue to be developed and are especially useful in studying pathogenic organisms. One important application of this technology is the examination of the response to a variety of treatments, including antimicrobial drug addition. Chapter 8 reviews the development and utility of bacterial microarray technologies.

Viral microarray technology has taken a two-pronged approach: examination of viral-encoded genes on DNA chips and examination of the host cell

response to viral infection. The goal of both approaches is to determine the full complement of genes that are critical to viral propagation, virulence, or control of latency. Chapters 1, 2, and 3 focus on Herpesviruses, Human Papilloma Virus, and Human Immunodeficiency Virus, exploring the utility of microarrays for the identification of novel antiviral drug targets and analysis of viral/host interactions. An additional chapter in the viral section covers the rational design of gene therapeutics for HIV/AIDS, based upon the sequence of HIV-1 subtypes and identification of useful RNA sites that can be targeted by ribozymes (Chapter 4).

Analysis of microbial genomes has revealed that a significant portion of each genome is of unknown function. Entire operons can be identified that are common to bacterial pathogens, yet the functions of these genes have yet to be elucidated. Through genomics one can identify them and using deletion analysis one can show that they are essential to bacterial survival. Functional genomics can begin to provide clues as to what their role is, thus providing information on how to set up high throughput screens to identify novel classes of inhibitors. Several technologies can be utilized to explore the function of unknown proteins, including the use of protein comparisons to define motifs and domains similar to known proteins and threading algorithms for finding similarities in the 3D structure. Other methods, such as the yeast two-hybrid system, seek to find binding partners that may provide a clue to function. High throughput phenotypic microarrays have also been used to simultaneously test a large number of cellular phenotypes and allow novel functions to be assigned to genes *(3)*. Although it is often important to ascribe a function to a target prior to high throughput screening, several methods have been developed to find ligands that bind to proteins, with the idea that amongst the molecules that bind will be inhibitors of function. These types of screens provide the raw materials for further drug development. Many of these technologies are discussed in Chapters 5, 7, 8, 11, 12, 13, 14, 15, and 16.

As a result of the explosion of pathogen and host genomic information, a new era is at hand. The fundamental nature of target evaluation and drug discovery has been radically changed. The wealth of information available will add significant insights to our knowledge of protein function and pathogen physiology, and the exploitation of these findings for the discovery of novel agents to combat pathogenic organisms will continue in the exciting years ahead.

Karen Joy Shaw, PhD

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2
Chipping Away at HIV Pathogenesis

Steffney Elise Rought, Roman Sasik, Davey Mitchell Smith, and Jacques Corbeil

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

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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 aymptomatic 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.

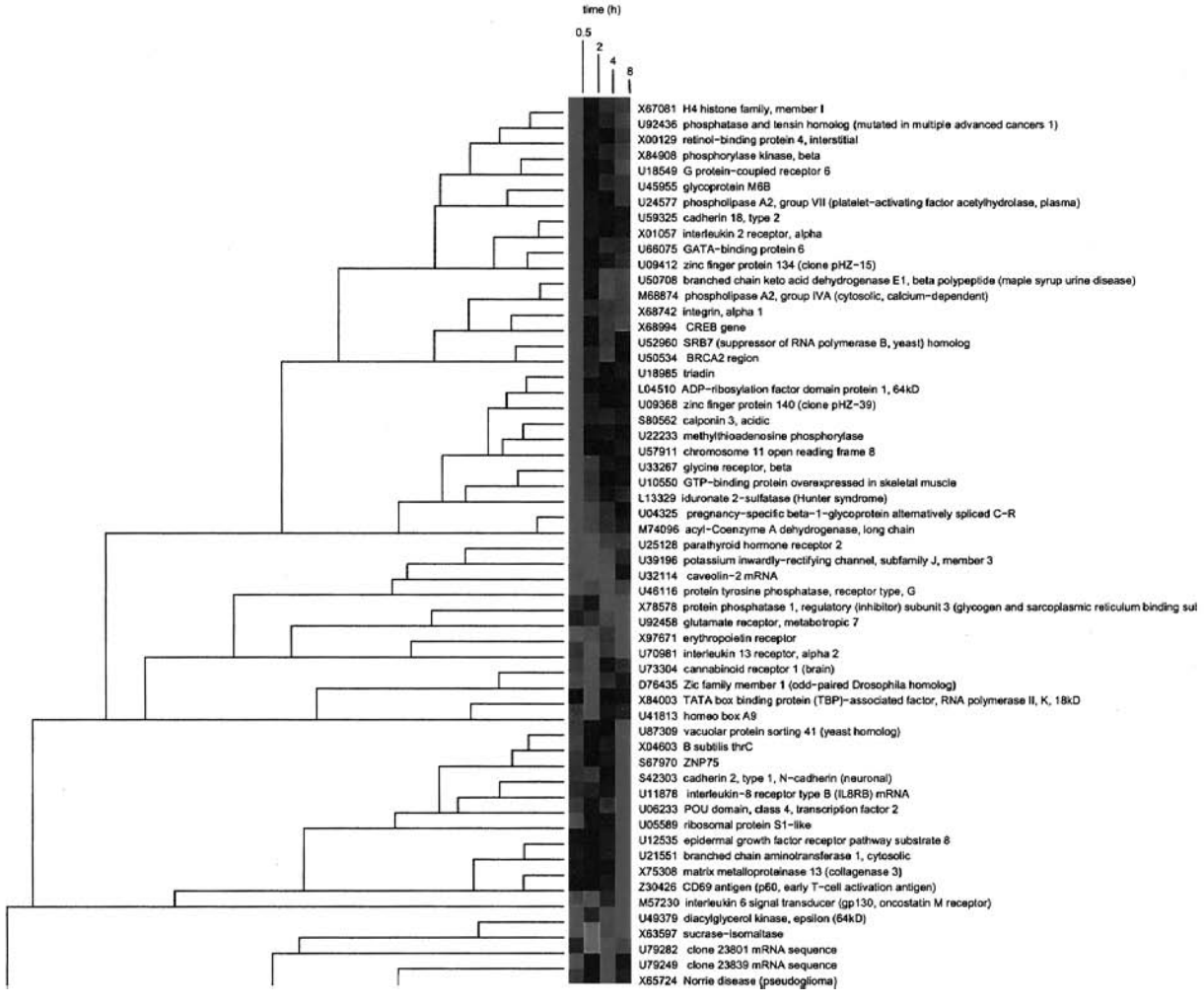
Target-Cell Depletion and Apoptosis

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_{LAI} (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.


A
time (h)
0.5
2
4
8
X67081 H4 histone family, member I
U92436 phosphatase and tensin homolog (mutated in multiple advanced cancers 1)
X00129 retinol-binding protein 4, interstitial
X84908 phosphorylase kinase, beta
U18549 G protein-coupled receptor 6
U45955 glycoprotein M6B
U24577 phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)
U59325 cadherin 18, type 2
X01057 interleukin 2 receptor, alpha
U66075 GATA-binding protein 6
U09412 zinc finger protein 134 (clone pHZ-15)
U50708 branched chain keto acid dehydrogenase E1, beta polypeptide (maple syrup urine disease)
M68874 phospholipase A2, group IVA (cytosolic, calcium-dependent)
X68742 integrin, alpha 1
X68994 CREB gene
U52960 SRB7 (suppressor of RNA polymerase B, yeast) homolog
U50534 BRCA2 region
U18985 triadin
L04510 ADP-ribosylation factor domain protein 1, 64kD
U09368 zinc finger protein 140 (clone pHZ-39)
S80562 calponin 3, acidic
U22233 methylthioadenosine phosphorylase
U57911 chromosome 11 open reading frame 8
U33267 glycine receptor, beta
U10550 GTP-binding protein overexpressed in skeletal muscle
L13329 iduronate 2-sulfatase (Hunter syndrome)
U04325 pregnancy-specific beta-1-glycoprotein alternatively spliced C-R
M74096 acyl-Coenzyme A dehydrogenase, long chain
U25128 parathyroid hormone receptor 2
U39196 potassium inwardly-rectifying channel, subfamily J, member 3
U32114 caveolin-2 mRNA
U46116 protein tyrosine phosphatase, receptor type, G
X78578 protein phosphatase 1, regulatory (inhibitor) subunit 3 (glycogen and sarcoplasmic reticulum binding sul
U92458 glutamate receptor, metabotropic 7
X97671 erythropoietin receptor
U70981 interleukin 13 receptor, alpha 2
U73304 cannabinoid receptor 1 (brain)
D76435 Zic family member 1 (odd-paired Drosophila homolog)
X84003 TATA box binding protein (TBP)-associated factor, RNA polymerase II, K, 18kD
U41813 homeo box A9
U87309 vacuolar protein sorting 41 (yeast homolog)
X04603 B subtilis thrC
S67970 ZNP75
S42303 cadherin 2, type 1, N-cadherin (neuronal)
U11878 interleukin-8 receptor type B (IL8RB) mRNA
U06233 POU domain, class 4, transcription factor 2
U05589 ribosomal protein S1-like
U12535 epidermal growth factor receptor pathway substrate 8
U21551 branched chain aminotransferase 1, cytosolic
X75308 matrix metalloproteinase 13 (collagenase 3)
Z30426 CD69 antigen (p60, early T-cell activation antigen)
M57230 interleukin 6 signal transducer (gp130, oncostatin M receptor)
U49379 diacylglycerol kinase, epsilon (64kD)
X63597 sucrase-isomaltase
U79282 clone 23801 mRNA sequence
U79249 clone 23839 mRNA sequence
X65724 Norrie disease (pseudoglioma)

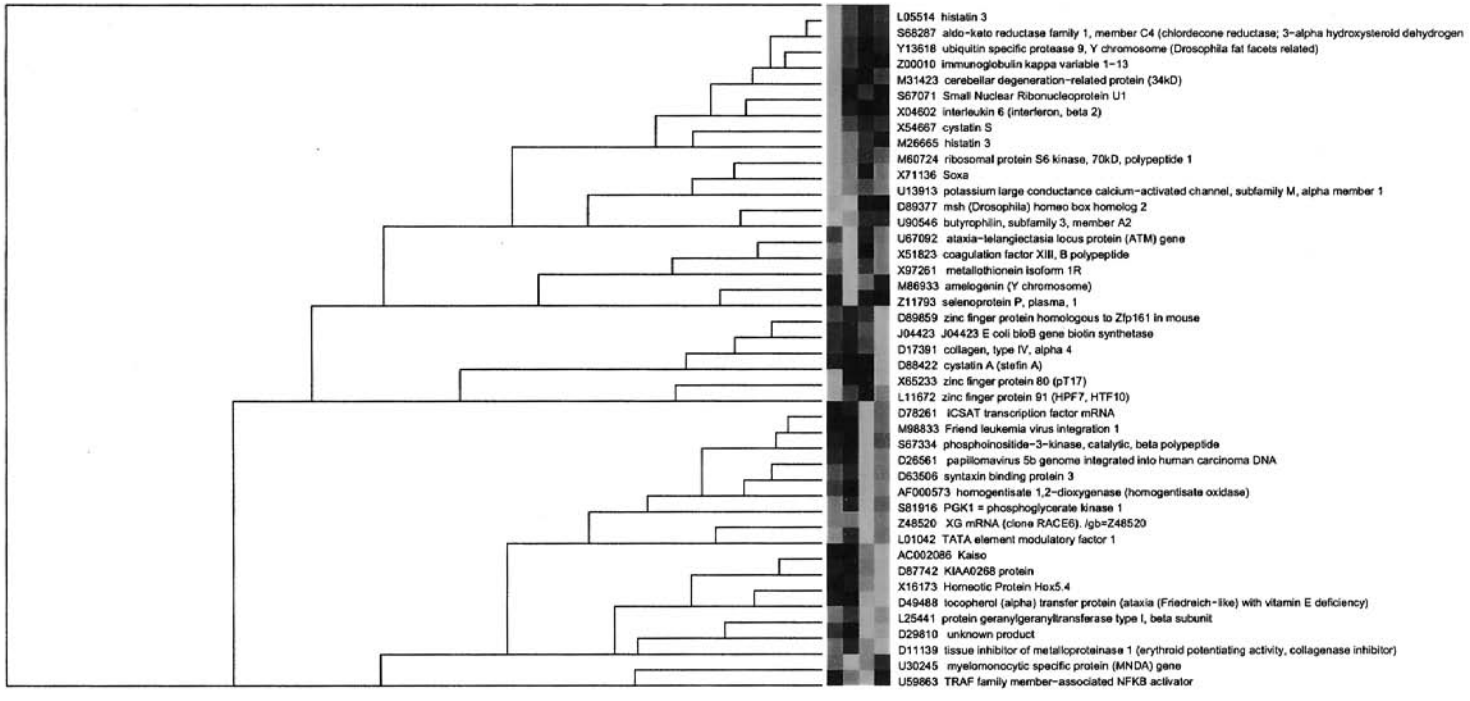


Fig. 1. Cluster analysis of CEM-GFP host-cell genes modulated by HIV within 8 h post-infection **(A).** (*Continued*)

B
time (h)
16
24
48
72
J04058 electron-transfer-flavoprotein, alpha polypeptide (glutaric aciduria II)
L40386 transcription factor Dp-2 (E2F dimerization partner 2)
Y00371 heat shock 70kD protein 8
D31890 lysyl-tRNA synthetase
U22376 v-myb avian myeloblastosis viral oncogene homolog
M63904 guanine nucleotide binding protein (G protein), alpha 15 (Gq class)
M22382 heat shock 60kD protein 1 (chaperonin)
M14328 enolase 1, (alpha)
X69723 triosephosphate isomerase 1
X12447 aldolase A, fructose-bisphosphate
D13641 translocase of outer mitochondrial membrane 20 (yeast) homolog
D38551 RAD21 (S. pombe) homolog
X62534 high-mobility group (nonhistone chromosomal) protein 2
M23613 nucleophosmin (nucleolar phosphoprotein B23, numatrin)
U15174 BCL2/adenovirus E1B 19kD-interacting protein 3
U38846 chaperonin containing TCP1, subunit 4 (delta)
U10323 interleukin enhancer binding factor 2, 45kD
M11147 ferritin, light polypeptide
M19483 ATP synthase beta subunit gene
V00594 metallothionein from cadmium-treated cells
M14483 prothymosin, alpha (gene sequence 28)
M26708 prothymosin, alpha (gene sequence 28)
L10373 transmembrane 4 superfamily member 2
X15183 heat shock 90kD protein 1, alpha
L17131 high-mobility group (nonhistone chromosomal) protein isoforms I and Y
V00599 mRNA fragment encoding beta-tubulin. (from clone D-beta-1)
K03515 glucose phosphate isomerase
X02152 lactate dehydrogenase A
M59830 heat shock 70kD protein 1B
D85429 heat shock 40kD protein 1
L00693 carcinoembryonic antigen-related cell adhesion molecule 3
J04988 heat shock 90kD protein 1, beta
U70439 acidic protein rich in leucines
M37583 H2A histone family, member Z
M13792 adenosine deaminase
J04164 interferon induced transmembrane protein 1 (9-27)
M62267 cyclin D3
J04173 phosphoglycerate mutase 1 (brain)
L29008 sorbitol dehydrogenase
U34962 cardiac-specific homeo box
D64142 H1 histone family, member X
D14657 KIAA0101 gene product
L06132 voltage-dependent anion channel 1
U10860 guanine monphosphate synthetase
D30655 eukaryotic translation initiation factor 4A, isoform 2
D13748 eukaryotic translation initiation factor 4A, isoform 1
D16562 ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1
L00058 (GH) gamma c-myc proto-oncogene
D55654 malate dehydrogenase 1, NAD (soluble)
J05614 proliferating cell nuclear antigen (PCNA) gene
D14812 MORF-related gene X
L41870 retinoblastoma 1 (including osteosarcoma)
D55716 minichromosome maintenance deficient (S. cerevisiae) 7
AB003102 proteasome (prosome, macropain) 26S subunit, non-ATPase, 11
M31469 RAN, member RAS oncogene family
D63674 high-mobility group (nonhistone chromosomal) protein 1
X16901 general transcription factor IIF, polypeptide 2 (30kD subunit)
D63875 KIAA0155 gene product
D78275 proteasome (prosome, macropain) 26S subunit, ATPase, 6
U23752 SRY (sex determining region Y)-box 11
U49516 5-hydroxytryptamine (serotonin) receptor 2C
D31888 KIAA0071 protein
U20660 angiotensin receptor 2
U77664 ribonuclease P (38kD)
U30245 myelomonocytic specific protein (MNDA) gene
U20758 secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)
S77393 unknown
M19311 calmodulin 2 (phosphorylase kinase, delta)
U53468 NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5 (13kD, B13)
Y00317 UDP glycosyltransferase 2 family, polypeptide B4

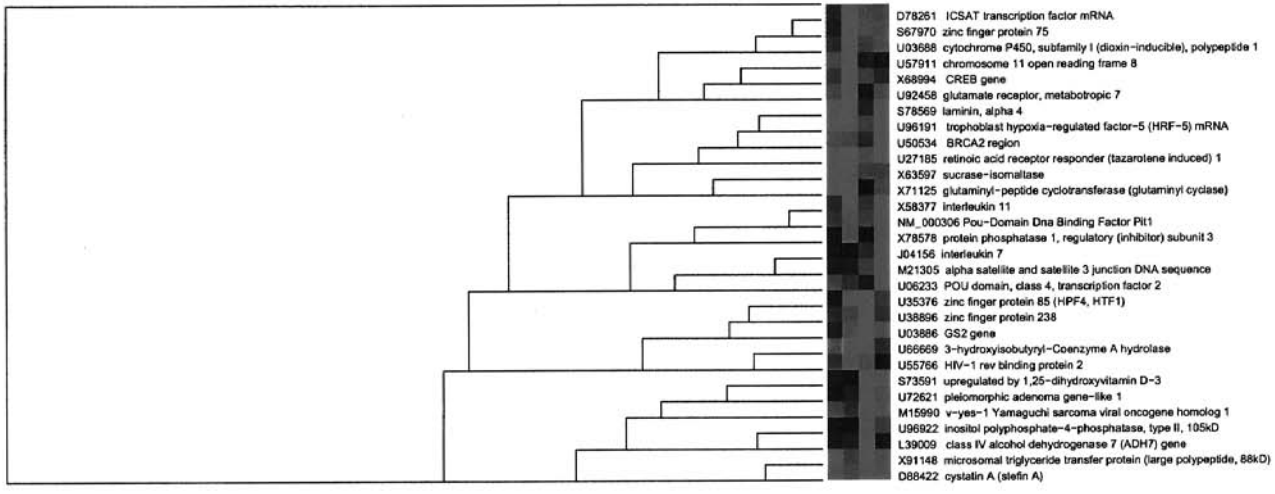


Fig. 1. *(Continued).* and 16-72 hrs **(B)** post-infection. Green = downregulated in HIV, red = upregulated in HIV.

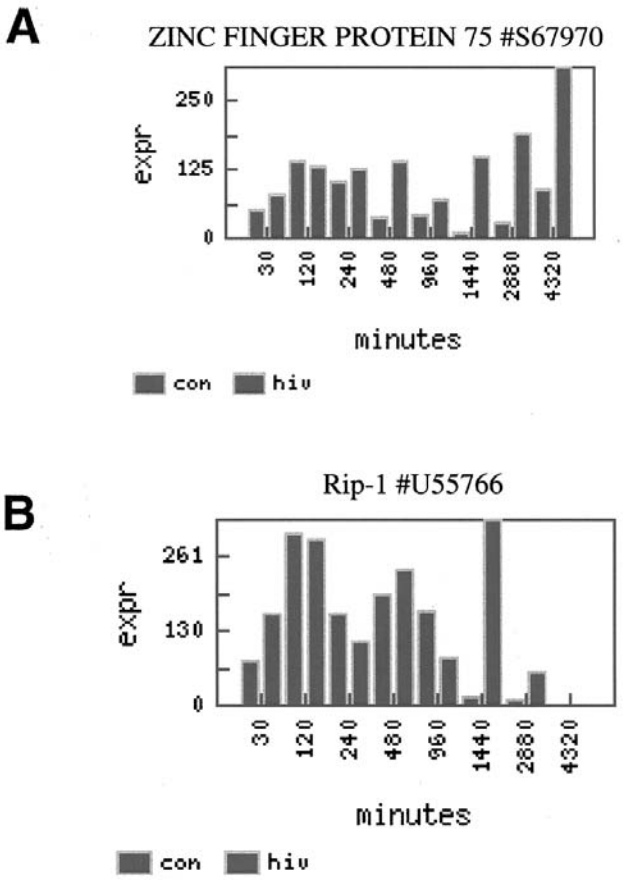


Fig. 2. Expression of zinc-finger protein 75 **(A)** and rev-binding protein **(B)** in uninfected (con) and HIV_{LAI} infected CEM-GFP cells over a 72-h time-course.

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 know 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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