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Virogenomics: the virus–host interaction revisited
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Genomics tools allow us to assess gene expression ‘genome wide’ providing an unprecedented view on the host-side of the virus–host interaction. The success of the application of these tools crucially depends on our ability to reduce the total information load while increasing the information density of the data collected. In addition to the advanced data analysis algorithms, gene annotation-pathway databases, and theoretical models, specifically designed sets of complementary experiments are crucial in translating the collected genomics data into palatable knowledge. A better understanding of the molecular basis of virus–host interactions will support the rational design of improved and novel intervention strategies for viral infections.

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Introduction
Till date the genome sequence of many virus species and their hosts is known and a range of novel tools has become available to study virus–host interactions at the molecular level. The advent of genomics tools provides us with an unprecedented view on the ‘host-side’ of this interaction. Together with advances in high-throughput technology, bioinformatics, and statistics this progress allows us to assess gene expression controlling the host response to viral infections in a genome wide fashion. With the latest generations of microarrays this can be achieved at the mRNA level with one single microarray. Microarray technology also extends to genotyping of the host (SNP analysis), and diagnostics by the identification of imprints in the host transcriptome characteristic for certain clinical conditions. In addition recent progress in the field of proteomics allows for the measurement of the expression levels of hundreds of proteins in a single biological sample, for example by mass spectrometry. Consequently, the ‘genomics revolution’ offers high-throughput tools to study the complex virus–host interaction with changing expression levels of many genes and gene pathways as a direct function of viral and host genome properties. The challenge is to translate and digest this avalanche of information into palatable knowledge.

The principle of microarray assisted mRNA profiling has first been described in 1987 using a collection of cDNA fragments spotted on filter paper [1,2]. Since then the technology has maturated considerably [3] and presently a range of high quality commercial microarray platforms is available either based on long DNA oligonucleotides (single probe per transcript) or short DNA oligonucleotides (multiple probes per transcript). Most of the available expression microarrays cover all genes or even all expressed exons of a certain organism [4]. In 1998, Zhu et al. first applied mRNA expression profiling to characterize the innate response to a viral infection (hCMV) in vitro [5]. Microarray technology has been more widely used in this field since 2001. Over the past years more than 200 papers reporting the results of mRNA expression studies of the host response to virus infection have been published, in which usually a restricted set of experimental design formats was applied. Here we review the recent developments in mRNA profiling of the innate antiviral response by highlighting in vitro studies that represent different experimental formats which allow optimal data analysis in ‘virogenomics’.

Virogenomics: formats of experimental design
Basic design: single virus, single cell type
Most early studies aimed at the characterization of the transcriptional response in a single cell type to a single virus. Examples are the studies on influenza virus, HIV-1, HSV-1, and RSV [6–11]. In general these studies were limited in size and the results generally provided a global description of the (innate) antiviral response mainly expressed in terms of sets of either up-regulated or down-regulated genes that usually supported earlier observations. As it is difficult to interpret these results in isolation, Jenner and Young performed a meta-analysis on the data obtained from 32 studies that involved 77 different pathogen–host interactions [12]. They were able to define a common host-transcriptional response in addition to a set of specific subresponses. Obviously the studies of this nature are ‘conservative’ in design and will at best identify shared (sub) responses that are strong enough to be detected against the intrinsically high level of noise because of the diversity in infection model systems and microarray platforms that were used in the original studies.
Basic design: time course format
Most of the in vitro virogenomics studies, have used a time course design. As the host response to virus infection is dynamic a time course design is required when measuring the expression levels of many genes simultaneously without having pre-existing knowledge about the expression dynamics for most of the genes represented on the microarray. Piqueras et al. [13] elegantly demonstrated the power of the simple time course format. Using purified DCs from healthy donors it was shown that influenza virus triggers a ‘coordinated chemokine production program’ in three successive waves. This program allows for a coordinated mobilization of different immune effectors in response to viral infection: at different time points different sets of chemokine messengers are expressed that are associated with the attraction of neutrophils, CTLs, NK cells, memory T cells, and also naive T and B lymphocytes. The format nicely revealed a gene expression time pattern associated with the role of DCs in orchestrating a mounting immune response.

Now that the costs involved in microarray experiments are decreasing, larger comparative virogenomics studies are performed with experimental formats that provide a better context for data analysis. These formats can be divided in those that are ‘virus-oriented’ and those that are ‘host-oriented’, depending on whether they target the virus or the responding host system in the interaction studied.

Virus-oriented design: multiple viruses, single cell type
An early example of this format is a study performed by Huang et al. in 2001 [6]. The transcriptional response of DCs to different pathogens, Escherichia coli, Candida albicans, and influenza virus, was monitored. Both a shared core response and pathogen-specific programs for each of these pathogens were observed showing that DCs sense diverse pathogens and elicit tailored pathogen-specific immune responses. In a gene expression profiling study of a similar design we observed the induction of tightly regulated responses in lung epithelial cells to a set of respiratory viruses that segregated with the phylogenetic origins of the viruses involved (manuscript in preparation). Two other studies used a similar comparative approach but more closely related pathogens: human coronavirus 229E (HCoV-229E) that is usually associated with common cold and the coronavirus that causes SARS (SARS-CoV). Tang et al. [14] compared the transcriptional response of these two viruses in a human epithelial cell line of liver origin (Huh7 cells) and Cheung et al. did the same with primary macrophages [15]. At two and four hours postinfection, much more perturbation of cellular gene transcription was observed after the infection of liver epithelial cells with SARS-CoV than with HCoV-229E. Predominantly genes associated with apoptosis, inflammation, stress response, and procoagulation were up-regulated. In contrast to HCoV-229E (and influenza A virus, that was also included in this study), SARS-CoV did induce chemokine messengers for, for example CXCL10 (IP10) and CCL2, but not for IFN-β being a key component of innate immunity upon infection of macrophages [15]. This profile could explain certain key features of the pathogenesis of SARS.

Virus-oriented design: manipulated viruses
In several mRNA expression profiling experiments the response induced by virus infection is compared to that induced by exposure to UV inactivated virus preparations, in order to identify replication-dependent and replication-independent changes in gene expression [11,7,16]. In general live virus infections induce more changes in gene expression. First of all this is because of the triggering of Toll-like receptors and similar pathogen-associated motif sensing receptor systems by, for example dsRNA molecules that are synthesized during viral replication. Exposure to nonreplicating antigen only induces relatively mild and short lasting responses. More and more mRNA expression profiling studies use molecularly cloned viruses in which genes are mutated, deleted, or inserted. With this approach the effect of well-defined modifications of the viral genome is evaluated in its natural context. Using this approach Geiss et al. [17] examined the ‘downstream’ effects of NS1 protein expression during infection with either wt influenza A virus or del NS1 mutant influenza viruses in a human lung epithelial cell line (A549). Deletion of the NS1 gene increased the number and magnitude of expression of cellular genes involved in the IFN, NF-kB, and other antiviral pathways. Interestingly, a recombinant influenza virus carrying the 1918 pandemic NS1 gene was more efficient at blocking the expression of IFN-regulated genes than a closely related (wt) influenza virus (A/WSN/33). This demonstrated the contribution of the NS1 gene to viral pathogenesis by enabling the virus to disarm antiviral defense systems.

Virus-oriented design: individually expressed viral genes
This format is reciprocal to the previous format: upon expression of an individual viral gene the transcriptional response is measured in order to identify the function of the viral protein. This format has been applied to genes of, for example lentiviruses, hepatitis viruses, and herpes viruses [18–20]. The results obtained with this approach are highly specific and can only be interpreted in the context of detailed virus-specific information. An inherent disadvantage of this approach is that the effect of viral gene expression is not evaluated in the context of virus replication.

Host-oriented design: single virus, multiple cell types
Relatively few studies perform mRNA profiling in multiple cell types. The following studies clearly demonstrated the added value of this approach. Adamo et al. [21] performed mRNA profiling on rubella virus infected...
primary human fetal fibroblasts and human adult lung fibroblasts. Although the gene expression levels of many functional gene categories were similarly perturbed, a marked difference between the two cell types was observed in genes associated with apoptosis (both for proapoptotic and antiapoptotic genes). Because fetal fibroblasts did not undergo apoptosis when infected with rubella virus it was postulated that this could promote fetal virus persistence. Another study by Sato et al., performed mRNA profiling on a set of genetically modified viruses in genetically modified host animals will ultimately be the best format to study virus–host interactions at the molecular level. However, some quite successful in vivo virogenomics experiments have recently been carried out in various species including nonhuman primates and also agriculturally relevant animals like chickens and cattle [29,30].

Host genomics analysis of the highly pathogenic 1918 influenza A virus infection in nonhuman primates indicated that atypical expression of the innate immune response may be a crucial determinant of the severity and outcome of infection [31]. From these overall gene expression patterns, detailed pathogenic pathways were however difficult to elucidate. Similarly, the analysis of host responses to SARS-CoV infection in the lungs of adolescent cynomolgus macaques revealed the induction of a strong innate immune response characterized by the stimulation of various cytokine and chemokine genes, including a wide range of type I interferons, interleukin (IL)-6, IL-8, and IP-10 (Figure 1A [32**]). Using immunohistochemistry, we revealed that these antiviral-signaling pathways, including the type 1 IFN-induced nuclear translocation of phosphorylated signal transducer and activator of transcription 1, were differentially regulated in infected and noninfected cells (Figure 1B). This suggests that, although SARS-CoV blocks IFN signaling in infected cells, locally produced IFNs are capable of activating noninfected cells and possibly can prevent the infection of these cells. It may be expected that in vivo virogenomics studies using series of genetically modified viruses in genetically modified host animals will ultimately be the best format to study virus–host interactions at the molecular level.

Conclusions and future outlook

With the advent of novel genomics tools, the studies addressing virus–host interaction at the molecular level have entered a new era. Microarray-assisted transcriptional profiling has provided us with a wealth of information about the role and function of host genes and gene-interacting
networks in virus–host interactions. The complex and
dynamic nature of these interactions involving large num-
bers of genes turns genomics studies in this field into a huge
information processing and data management challenge.
There are several key areas that need to be specifically
addressed to benefit optimally from the genomics tech-
nologies that have become available over the past decade;
the most important areas are listed below.

Experimental design
Careful design of complementary sets of experiments
using different formats of virus–host interaction, each
focusing on slightly different aspects should reduce the
total information load while increasing the information
density of the data collected. Figure 2 summarizes the
experimental design formats discussed in this paper.

Technology
The standardization of protocols and the technology plat-
forms should support further integration of data analysis
between individual experiments, and platforms. Rela-
tively low levels of interplatform variation [33] among
the microarrays platforms that are currently used open
new opportunities for meta-analysis of separately gener-
ated data sets.

Data analysis and data management
The field of bioinformatics and statistics in the arena of
transcriptomics and proteomics has developed alongside
with the development of genomics tools. Uniform
approaches to identify differentially expressed genes,
gene-interacting networks, and pathways support inte-
grated data analysis. Adherence of data reporting to the
MIAME standard needs to be more enforced [34].

Gene annotation
Information on gene function especially regarding uniform
definitions of biological pathways and processes is a notor-
ious bottleneck in data analysis. Global analysis of mRNA
expression profiles for example generally starts with a
gene-enrichment type of analysis to test for over-repres-
entation of particular pathways or functions on the basis of
data produced by the Gene Ontology Consortium [35] or
that is available through (collections of) other data bases.
It is encouraging to note that the Gene Ontology Consortium recently launched a program to improve the functional and pathway annotation of especially immune response related genes. The more uniform and detailed information becomes available about the role of individual genes, the more the genomics field will benefit from the available advanced analysis algorithms.

**Modeling gene-interacting networks**

Development and implementation of mathematical and other models based on currently known and newly identified gene-interacting networks is pivotal to improve data interpretation.

In conclusion, the success of the application of genomics tools like microarrays in studies on the complex and highly dynamic virus-host interaction crucially depends on our ability to discard most of the data that have been collected in an intelligent and appropriately selective way. This will lead to a better understanding of the molecular basis of virus-host interactions, which will support the rational design of improved and novel intervention strategies for viral infections.

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**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Kulesh DA, Clive DR, Zarlinga DS, Greene JJ: Identification of interferon-modulated proliferation-related cDNA sequences. *Proc Natl Acad Sci U S A* 1987, 84:8453-8457.

2. Augenlicht LH, Wahrman MZ, Halsey H, Anderson L, Taylor J, Lipkin M: Expression of cloned sequences in biopsies of human colonic tissue and in colonic carcinoma cells induced to differentiate in vitro. *Cancer Res* 1987, 47:6017-6021.

3. Schena M, Shalon D, Davis RW, Brown PO: Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 1995, 270:467-470.

4. Kwan T, Benovoy D, Dias C, Gurd S, Provencher C, Beaulieu P, Hudson TJ, Sladek R, Majewski J: Genome-wide analysis of transcript isoform variation in humans. *Nat Genet* 2008, 40:225-231.

5. Zhu H, Cong JP, Mamotra G, Gingeras T, Shenk T: Cellular gene expression altered by human cytomegalovirus: global monitoring with oligonucleotide arrays. *Proc Natl Acad Sci U S A* 1998, 95:14470-14475.

6. Huang Q, Liu D, Majewski P, Schulte LC, Kom JM, Young RA, Lander ES, Hacohen N: The plasticity of dendritic cell responses to pathogens and their components. *Science* 2001, 294:870-875.

7. Geiss GK, Bumgarner RE, Hammersmark E, Cunningham D, Katze MG: Global impact of influenza virus on cellular pathways is mediated by both replication-dependent and -independent events. *J Virol* 2001, 75:4321-4331.

8. Geiss GK, Bumgarner RE, An MC, Agy MB, van’t Wout AB, Hammersmark E, Carter VS, Upchurch D, Mullins JI, Katze MG: Large-scale monitoring of host cell gene expression during HIV-1 infection using cDNA microarrays. *Virology* 2000, 266:8-16.

9. van’t Wout AB, Lehrman GK, Mikheeva SA, O’Keeffe GC, Katze MG, Bumgarner RE, Geiss GK, Mullins JI: Cellular gene expression upon human immunodeficiency virus type 1 infection of CD4(+) T-cell lines. *J Virol* 2003, 77:1392-1402.

10. Mosiman KL, Macgregor PF, Rozmus JJ, Goryachev AB, Edwards AM, Smiley JR: Herpes simplex virus triggers and then disarms a host antiviral response. *J Virol* 2001, 75:750-758.

11. Zhang Y, Luxon BA, Casola A, Garofalo RP, Jamaluddin M, Brasier AR: Expression of respiratory syncytial virus-induced chemokine gene networks in lower airway epithelial cells revealed by cDNA microarrays. *J Virol* 2001, 75:9044-9058.

12. Jenner RG, Young RA: Insights into host responses against pathogens from transcriptional profiling. *Nat Rev Microbiol* 2005, 3:281-294.

13. Piqueras B, Connolly J, Freitas H, Palucka AK, Banchereau J: Upon viral exposure, myeloid and plasmacytoid dendritic cells...
produce 3 waves of distinct chemokines to recruit immune effectors. Blood 2006, 107:2613-2618.

14. Tang BS, Chan KH, Cheng VC, Woo PC, Lau SK, Lam CC, Chan TL, Wu AK, Hung IF, Leung SY et al.: Comparative host gene transcription by microarray analysis early after infection of the Huh7 cell line by severe acute respiratory syndrome coronavirus and human coronavirus 229E. J Virol 2005, 79:6190-6193.

15. Cheung CY, Poon LL, Ng IH, Luk W, Sia SF, Wu MH, Chan KH, Yuen KY, Gordon S, Guan Y et al.: Cytokine responses in severe acute respiratory syndrome coronavirus-infected macrophages in vitro: possible relevance to pathogenesis. J Virol 2005, 79:7819-7826.

16. Martinez I, Lombardia L, Garcia-Barreno B, Dominguez O, Melero JA: Distinct gene subsets are induced at different time points after human respiratory syncytial virus infection of A549 cells. J Gen Virol 2007, 88:570-581.

17. Geiss GK, Salvatore M, Tumpey TM, Carter VS, Wang X, Basler CF, Taubenberger JK, Bumgarner RE, Palese P, Katze MG et al.: Cellular transcriptional profiling in influenza A virus-infected lung epithelial cells: the role of the nonstructural NS1 protein in the evasion of the host innate defense and its potential contribution to pandemic influenza. Proc Natl Acad Sci U S A 2002, 99:10736-10741.

18. Sundstrom M, Chatterji U, Schaffer L, de RS, Elder JH: Feline immunodeficiency virus OrfA alters gene expression of splicing factors and proteasome-ubiquitination proteins. Virology 2008, 371:394-404.

19. Tang W, Lazzaro CA, Campbell JS, Parks WT, Katze MG, Fausto N: Responses of nontransformed human hepatocytes to conditional expression of full-length hepatitis C virus open reading frame. Am J Pathol 2007, 171:1831-1846.

20. Lucchesi W, Brady G, ttrich-Breilholz O, Krich M, Russ R, Farrell PJ: Differential gene regulation by Epstein–Barr virus Type 1 and Type 2 EBNA2. J Virol 2008, 82:7456-7466.

21. Adamo MP, Zapata M, Frey TK: Analysis of gene expression in fetal and adult cells infected with rubella virus. Virology 2008, 370:1-11.

22. Sato H, Honma R, Yoneda M, Miura R, Tsukiyama-Kohara K, Ikeda F, Seki T, Watanabe S, Kai C: Measles virus induces cell-type specific changes in gene expression. Virology 2008, 375:321-330.

Transcriptional profiling study with wt MV and a MV deletion mutant and protein manipulations in transcriptional profiling studies of the virus-host interaction.

23. Tian B, Zhang Y, Luxon BA, Garofalo RP, Casola A, Sinha M, Brasier AR: Identification of NF-kappaB-dependent gene networks in respiratory syncytial virus-infected cells. J Virol 2002, 76:6800-6814. This paper nicely demonstrates the power of specific host gene expression manipulations in transcriptional profiling studies of the virus-host interaction.

24. O'Donnell SM, Holm GH, Pierce JM, Tian B, Watson MJ, Chari RS, Ballard DW, Brasier AR, Dermody TS: Identification of an NF-kappaB-dependent gene network in cells infected by mammalian reovirus. J Virol 2006, 80:1077-1086.

25. Elco CP, Guenther JM, Williams BR, Sen GC: Analysis of genes induced by Sendai virus infection of mutant cell lines reveals essential roles of interferon regulatory factor 3, NF-kappaB, and interferon but not toll-like receptor 3. J Virol 2005, 79:3920-3929.

26. Frederiksen BL, Keller BC, Frenek J, Katze MG, Gale M Jr: Establishment and maintenance of the innate antiviral response to West Nile Virus involves both RIG-I and MDA5 signaling through IPS-1. J Virol 2008, 82:609-616. A study elegantly demonstrating the power of functional genomics in dissecting the innate host response.

27. Chan G, Bivins-Smith ER, Smith MS, Yurochko AD: Transcriptome analysis of NF-kappaB- and phosphatidylinositol 3-kinase-regulated genes in human cytomegalovirus-infected monocytes. J Virol 2008, 82:1040-1046.

28. Peng T, Zhu J, Hwangbo Y, Corey L, Bumgarner RE: Independent and cooperative antiviral actions of beta interferon and gamma interferon against herpes simplex virus replication in primary human fibroblasts. J Virol 2008, 82:1934-1945.

29. Cogburn LA, Porter TE, Duclos MJ, Simon J, Burgess SC, Zhu JJ, Cheng HH, Dodson JB, Burnsie J: Functional genomics of the chicken — a model organism. Poult Sci 2007, 86:2059-2094.

30. Aich P, Wilson HL, Kaushik RS, Potter AA, Babiuk LA, Griebel P: Comparative analysis of innate immune responses following infection of newborn calves with bovine rotavirus and bovine coronavirus. J Gen Virol 2007, 88:2749-2761.

31. Kobasa D, Jones SM, Shiaya K, Kash JC, Copps J, Ebihara H, Hatta Y, Kim JH, Halfmann P, Hatta M et al.: Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. Nature 2007, 449:319-323.

32. Lang de A, Baas T, Teitel T, Leijten LM, Rain B, Osterhaus AD, Haagmans BL, Katze MG: Functional genomics highlights differential induction of antiviral pathways in the lungs of SARS-CoV-infected macaques. PLoS Pathog 2007, 3:e112. A study demonstrating the power of combining genomics tools with traditional in situ staining techniques.

33. Shi L, Reid LH, Jones WD, Shippy R, Warrington JA, Baker SC, Davis AP, Dolinski K, Dwight SS, Eppig JT et al.: The MicroArray Gene Expression (MIAME)-toward standards for microarray data. Nat Genet 2001, 29:365-371.

34. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT et al.: Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 2000, 25:25-29.

35. Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, Aach J, Ansorge W, Ball CA, Causton HC et al.: Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat Genet 2001, 29:365-371.

36. Diehl AD, Lee JA, Scheuermann RH, Blake JA: Ontology development for biological systems: immunology. Bioinformatics 2007, 23:913-915.