

## MINIREVIEW

Viruses: Hostages to the Cell<sup>1</sup>Peter Ghazal,<sup>2</sup> Juan Carlos González Armas, José J. García-Ramírez, Sabine Kurz, and Ana Angulo*Department of Immunology and Department of Molecular Biology, Division of Virology, The Scripps Research Institute, La Jolla, California 92037**Received July 3, 2000; accepted July 27, 2000**"All viruses come from cells."*

–Variation from Rudolf Virchow (1855)

The quotation reminds us that viruses are obligate parasites and as such their intimate relationship with their host plays a key and vital role to their survival. In this review we emphasize the role of the host, at the cellular level, in governing both acute and latent infections.

*Infecton and Viral Homeostasis.* Figure 1 illustrates the complex interplay that takes place between a pathogen and host. In the immune-competent individual, primary control of an acute infection is dispensed by both innate and adaptive (specific) immune responses. To the pathogen this presents a formidable barrier. However, the immune system is not completely sterilizing and in many cases pathogens have adapted by using general and specific strategies to additionally weaken the immune barrier (Ploegh, 1998). For these reasons primary infection with a number of clinically significant human viruses invariably results in the establishment of viral persistence or latency. Examples of these include for instance human immunodeficiency virus, human cytomegalovirus, Epstein–Barr virus, Varicella zoster, herpes simplex, human papillomavirus, hepatitis C virus, and hepatitis B virus. Under these conditions a relatively stable but highly dynamic state of equilibrium (homeostasis) between the host and virus is maintained (reviewed in Ghazal *et al.*, 2000). Thus, for a number of human virus adaptations there is usually a tendency toward a dynamic balance that ensures survival of both pathogen and host. In addition to the immune pathways that contribute to viral homeostasis, a secondary level of control

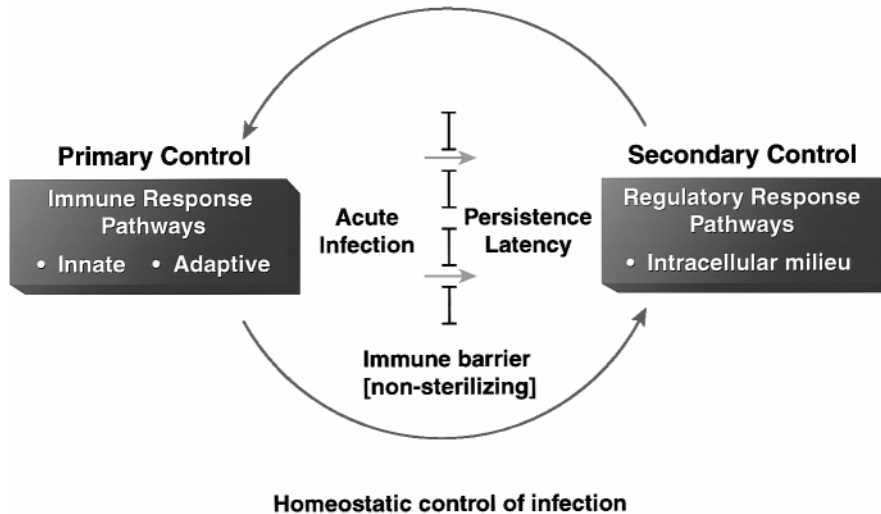
is exerted by nonimmune pathways that interrelate with immunity and are connected to the obligate dependency of the virus on its host. This secondary tier of control plays an important role in modulating infections at the cellular level.

There are three categories of regulatory response pathways that collectively provide the secondary level of control in the infected cell (Ghazal *et al.*, 2000). These are essentially the cell cycle, apoptosis, and (intra- and extracellular) signaling pathways, all of which reflect changes in the intracellular milieu of the cell (Fig. 1 and for a more detailed discussion of these topics, see Op De Beek *et al.*, 1997; Pease and Murphy, 1998; Roulston *et al.*, 1999; Fortunato *et al.*, 2000). Here we address the basis of both immune and nonimmune pathways as checkpoints in modulating the program of viral gene expression. In addition, we propose a new model, the cellular clockwork model, of an infection that underscores the significance of alterations in cellular factors and the intracellular milieu, together with viral factors in the control of virus replication. For the purpose of this review, we illustrate these issues for nuclear DNA viruses; however, many of the principles that we discuss can be extended to RNA and cytoplasmic DNA viruses.

*Genome Complexities and Fatal Restrictions.* The genetic complexity exhibited by viral genomes ranges from  $10^3$  to  $10^5$ . By contrast, our genome has a complexity that approximates  $2 \times 10^9$ . The economy of genetic space afforded by a pathogen has two important consequences. The first is the loss of autonomy due to its reliance on host-encoded gene functions. The second is that it limits the potential for redundancy and thus under certain conditions may lessen flexibility of the virus to use alternate strategies (pathways). In other words, the price to pay for being an obligate parasite is total dependence on the host's environment for its structural and synthetic machinery. For this reason we argue that the dependence of the virus on cellular gene products provides an important part of the secondary level of hierarchical control described above. There are multiple mech-

<sup>1</sup>This is Publication No. 13408-IMM from The Scripps Research Institute.

<sup>2</sup>To whom correspondence and reprint requests should be addressed at Department of Immunology IMM-1, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037. Fax: 858 784-9272. E-mail: [ghazal@scripps.edu](mailto:ghazal@scripps.edu) or [peter.ghazal@ed.ac.uk](mailto:peter.ghazal@ed.ac.uk).



**FIG. 1.** Homeostatic control of infection. Primary control is exerted by immune responses and dependency on host factors provides a secondary level of hierarchical control at the cellular level. See text for details and discussion on the principles of viral homeostasis.

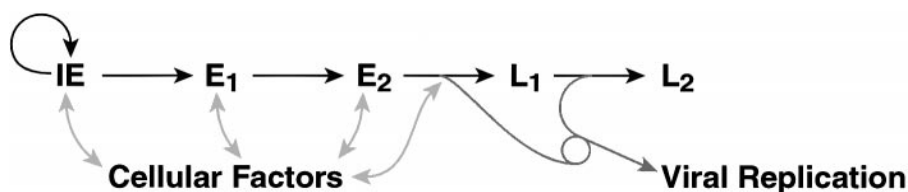
anisms by which the dependence of the virus on host cell processes can affect the viral expression cycle. These include but are not limited to posttranscriptional/translation control of viral or cellular gene products, RNA and protein stability, regulation of protein and RNA transport, as well as vesicular transport and of course control via specific transcription factors. All of these processes can impact on the transcription and replication cycle of the virus genome. This line of reasoning leads to an interesting corollary: that host pathways may exist that are essential for the viability of the pathogen but that may not be necessary for the survival of the host cell. What we mean here is that the cell may have multiple pathways (for instance, pathways A, B, and C) that converge to a defined biological output (pathway D) and to which the virus has adapted to use one alone (pathway A), such that if pathway A is inhibited, then productive infection is blocked but cell survival is ensured by shunting to the alternate pathways (B or C). This possibility, while still hypothetical, provides a new dimension in thinking about infections and is open to experimentation.

An exaggerated view of the points raised above is that once a virus enters a cell it becomes a potential prisoner of its host. This, we argue, provides an important basis of the secondary level of control (Fig. 1). In this connection, the program of virus gene expression (viral transcription

cycle) represents one of the principle levels at which control is exerted. We discuss below the viral transcription cycle with the view that the cell can hold a virus hostage.

*Viral Transcription Cycle: "The Text Book View."* The transcription and replication of virus genomes are divided into temporal phases. The division of viral gene expression into temporal phases (prior to and after viral DNA replication) serves as one of the key checkpoints to productive infection. Figure 2 illustrates the temporal cascade of a typical ("traditional or text book view") viral transcription–replication cycle for a nuclear DNA virus. For viruses with small genomes (e.g., parvoviruses), this involves only a few gene products for each kinetic class while more complex viral genomes (e.g., members of the herpes virus family) exhibit more complex patterns of expression.

One of the first molecular events occurring after deposition and uncoating of a virus in the interior of the cell is transcription of a set of genes that are generally termed immediate-early (IE) genes. These genes do not require viral gene products for their activation but can be influenced by virion proteins as is exemplified by herpes simplex and the activation of its IE promoters by VP16/alphaTIF (see Wagner *et al.*, 1995). The IE promoters use and are strictly dependent on both specific and general



**FIG. 2.** Viral transcription–replication cycle: "the text book view." See text for details.

cellular transcription factors. In many cases the IE proteins establish autoregulatory feedback loops to control their own expression. The next genes activated are the early ( $E_1$ ,  $E_2$ , etc.) class of genes. These genes also use the cellular transcription apparatus but are now dependent on viral IE regulatory proteins for their activation. Early genes encode additional regulatory factors that contribute to coordinate gene control and essential replication factors for viral DNA synthesis. After the initiation of viral replication, late ( $L_1$ ,  $L_2$ , etc.) genes are next activated and mainly encode proteins necessary for virus morphogenesis. Similar to the early kinetic class, late genes are also interdependent on host- and virus-encoded regulatory factors. Viruses that do not directly or indirectly require the host transcription machinery are often dependent on trans-acting cellular host factors for their synthesis (Moyer *et al.*, 1986). Emphasis is most often placed on the parasitic nature of this interdependence, between host and virus factors, in which viruses convert (usurp) the cell for the purpose of controlling their self-replication. This is achieved and orchestrated by viral factors. In this view of virus infections the necessary use of host cell factors is especially conducive to pathological abuse. While this may and does occur on occasions, the importance of the cell in also orchestrating these events is overlooked.

**Viral Transcription Cycle: "The Cellular Clockwork Model."** As commented above, emphasis of the viral transcription cycle is most often placed on virus regulatory factors that drive the temporal phases of expression by opportunistically usurping the cellular transcription machinery. Here we propose a model for the virus transcription cycle that gives equal significance to cellular factors in effecting coordinate viral gene expression. We have termed this the cellular clockwork model to embrace the concept that the temporal phases of the viral transcription–replication cycle are coordinate and interdependent on the levels and kinetic changes in cellular factors (expression, stability, modifications, etc.).

Figure 3 illustrates the "cellular clockwork view" for regulating the temporal program of viral gene expression. In this model the face of the clock represents cellular factors. The temporal cascade of viral gene expression sets time intervals of the clock. The arrows illustrated in the cellular clock show the interaction flow that is necessary for each temporal class. Finally, the hand of the clock signifies the interdependence between viral and cellular factors in progressing through the viral expression cycle. An important prediction that follows from this model is that the cellular clockwork can be internally and externally altered (set) to either promote or inhibit the ability of the virus to complete its cycle. This model is consistent with studies that have shown that cell heterogeneity can have a critical role in the establishment of infections and the ability of cellular trans-acting factors to inhibit viral infection (e.g., Hernández *et*

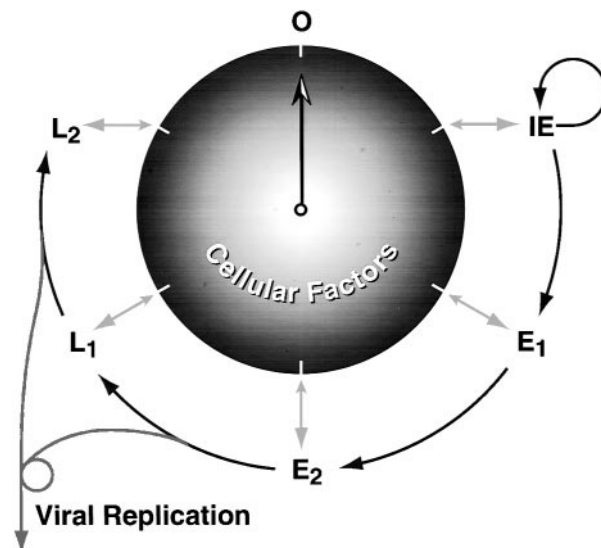


FIG. 3. Viral transcription–replication cycle: "the cellular clockwork model." See text for details.

*et al.*, 1994; De la Torre *et al.*, 1989). Hence, this model extends the textbook view of the transcription–replication cycle by suggesting that once a license to initiate the viral cycle is granted, the virus can be kept in check by molecular brakes of the cell. Indeed, this can occur at the level of the initial infection such that if the hand of the clock were set to 0, then the virus would enter but remain in a latent state. Conversely, hormonal or stress-related signaling events that alter the state of the cellular clockwork within a latently infected cell can also represent triggers for reactivation by moving the hand to the IE time interval. This latter point is still the least understood area of the host–pathogen relationship. In an acute infection, a virus might encode proteins that attempt to shift the hand of the cellular clock to the IE step as mentioned above for the IE promoters of HSV using VP16/alpha TIF.

The model gives equal and interdependent weight to both viral and cellular factors such that one cannot efficiently progress to the next stage of temporal expression until appropriate changes have coordinately taken place. Thus, the cellular clockwork model provides insight into how an infected cell's fate is set and invokes novel antiviral strategies for interruption of an infection. In this case, the model predicts that drugs that target the cellular clockwork machinery, instead of specific viral targets, may well provide nontraditional and novel approaches for controlling infections.

**Viral Cycle: "Cellular Clockwork and Immunity."** Figure 4 illustrates the interrelatedness of immune (e.g., antiviral cytokines) and nonimmune (e.g., vitamin and hormonal signaling) pathways in modulating the viral program of gene expression. While nonimmune pathways can influence the transcription–replication cycle of a virus (e.g., Angulo *et al.*, 1998; Guidotti *et al.*, 1999), immune-mediated antiviral regulatory responses likely

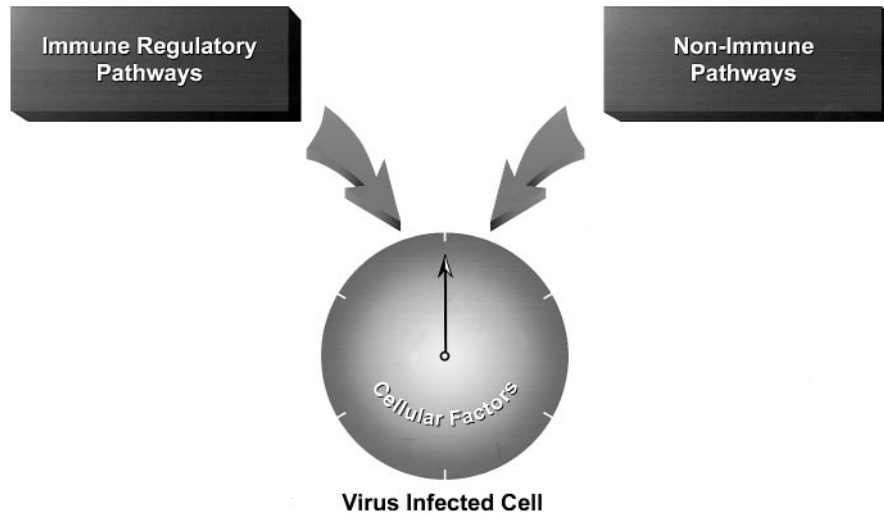


FIG. 4. The relationship between immune and nonimmune pathways with the cellular clockwork model for infection.

play a critical role in the control of virus multiplication. A well-established link between extracellular signaling regulatory pathways and the immune response would be antiviral cytokines, such as the interferon and lymphotoxin pathways (Stark *et al.*, 1998). Such mediators can act locally at the site of infection to limit virus replication directly or indirectly by conferring an antiviral refractory state on neighboring uninfected cells. These cytokines induce cellular genes that subsequently limit the ability of viruses (RNA and DNA) to complete their replication cycle (Sen and Ransohoff, 1993). Historically many of these responses were recognized as an immediate innate response to infection and that viral infections were resolved by the adaptive response involving antigen-specific destruction of infected cells by cytotoxic lymphocytes. However, there is now increasing evidence to indicate that many viral infections are also controlled by noncytopathic, cytokine-dependent mechanisms developed by cytotoxic T lymphocytes (Guidotti and Chisari, 1999). While the precise mechanism is not known, the sensitivity of viruses to antiviral cytokines appears to be dependent on the ability of the infected cell to produce, in part, specific antiviral factors. Alternatively, it is possible that noncytolytic immunity may operate by also perturbing the positive (proviral) factors of the cellular clockwork. The implication of the latter possibility is that it would become extremely difficult to generate escape mutants to the cytokine-mediated alteration of the infected cell. In support of this, for example, the antiviral state induced by IFN- $\alpha$  has been shown to impose constraints on vesicular stomatitis virus infection, which fail to be completely overcome by genetic change (Novella *et al.*, 1996). In other words, essential cellular pathways for virus multiplication may be blocked by antiviral cytokines but are for the cell nonessential for survival.

The virus under these conditions becomes a hostage of the cell.

*Concluding Remarks.* Viruses are obligate parasites and as such have had to pay the price of losing their autonomy. We have emphasized the program of virus gene expression as one of the principal levels by which control of an infection is exerted. So who is in control? At one end of the spectrum it is assumed that the virus decides its own fate while at the other it may be argued that the host cell is the primary decision-maker for licensing virus replication. The answer depends on the pathogen and host, but the process of adaptation leads toward a little of both. This is a dynamic process, which we have previously referred to as viral homeostasis. We continue this line of thought and develop a new model, the cellular clockwork model, which accounts for the pivotal role played by host cell factors in orchestrating, in partnership with virus factors, the viral expression cycle. The key feature of this model is that it predicts that changes in cellular factors (these can be alterations in RNA expression levels, protein degradation, or modification, etc.) upon infection form an absolutely essential component for driving or preventing viral expression. Thus, at the cellular level a dynamic interaction takes place between virus and host factors that can lead to proviral or antiviral states. These intracellular states are susceptible to both immune and nonimmune regulatory pathways. Ultimately, the strategy of a virus in primary infections and perhaps more importantly in latency is to exploit the dynamic equilibrium between host and pathogen at both the cellular and whole organism level.

## ACKNOWLEDGMENTS

This work was supported in part by the NIH. We thank Juan Carlos de la Torre, Ed Wagner, and Jean Spence for helpful comments.

## REFERENCES

- Angulo, A., Chandraratna, R. A. S., LeBlanc, J. F., and Ghazal, P. (1998). Ligand induction of retinoic acid receptors alters an acute infection by murine cytomegalovirus. *J. Virol.* **72**, 4589–4600.
- De La Torre, J. C., de La Luna, S., Diaz, J., and Domingo, E. (1989). Resistance to foot-and-mouth disease virus mediated by trans-acting cellular products. *J. Virol.* **63**, 2385–2387.
- Fortunato, E. A., McElroy, A. K., Sanchez, I., and Spector, D. H. (2000). Exploitation of cellular signaling and regulatory pathways by human cytomegalovirus. *Trends Microbiol.* **8**, 111–119.
- Ghazal, P., García-Ramírez, J., González-Armas, J. C., Kurz, S., and Angulo, A. (2000). Principles of homeostasis in governing virus activation and latency. *Immunol. Res.* **21**, 219–223.
- Guidotti, L. G., and Chisari, F. V. (1999). Cytokine-induced viral purging—Role in viral pathogenesis. *Curr. Opin. Microbiol.* **4**, 388–391.
- Guidotti, L. G., Eggers, C. M., Raney, A. K., Chi, S. Y., Peters, J. M., Gonzalez, F. J., and McLachlan, A. (1999). *In vivo* regulation of hepatitis B virus replication by peroxisome proliferators. *J. Virol.* **73**, 10377–10386.
- Hernandez, A. M., Carrillo, E. C., Sevilla, N., and Domingo, E. (1994). Rapid cell variation can determine the establishment of persistent viral infection. *Proc. Natl. Acad. Sci. USA* **91**, 3705–3709.
- Moyer, S. A., Baker, S. C., and Lessard, J. L. (1986). Tubulin: A factor necessary for the synthesis of both Sendai virus and vesicular stomatitis virus RNAs. *Proc. Natl. Acad. Sci. USA* **15**, 5405–5409.
- Novella, I. S., Cilnis, M., Elena, S. F., Kohn, J., Moya, A., Domingo, E., and Holland, J. J. (1996). Large-population passages of vesicular stomatitis virus interferon-treated cells select variants of only limited resistance. *J. Virol.* **70**, 6414–6417.
- Op De Beeck, A., and Caillet-Fauquet, P. (1997). Viruses and the cell cycle. *Prog. Cell Cycle Res.* **3**, 1–19.
- Pease, J. E., and Murphy, P. M. (1998). Microbial corruption of the chemokine system. *Semin. Immunol.* **10**, 169–178.
- Ploegh, H. L. (1998). Viral strategies of immune evasion. *Science* **280**, 248–253.
- Roulston, A., Marcellus, R. C., and Branton, P. E. (1999). Viruses and apoptosis. *Annu. Rev. Microbiol.* **53**, 577–628.
- Sen, G. C., and Ransohoff, R. M. (1993). Interferon-induced antiviral actions and their regulation. *Adv. Virus Res.* **42**, 57–102.
- Stark, G. R., Kerr, I. M., Williams, B. R., Silverman, R. H., and Schreiber, R. D. (1998). How cells respond to interferons. *Annu. Rev. Biochem.* **67**, 227–264.
- Wagner, E. K., Guzowski, J. F., and Singh, J. (1995). Transcription of the herpes simplex virus genome during productive and latent infection. *Prog. Nucleic Acids Res. Mol. Biol.* **51**, 123–165.

Viruses' host interactions are exemplified by lytic virus multiplication: One or more virions infect a host cell, which is then converted into a factory for the synthesis of new viruses. Virions accumulate in the cell, which eventually disintegrates and scatters its contents. However, this is not the only possible type of virus-host interaction. Many viruses establish a state of lysogeny (see Lysogeny), in which the viral genome remains in a stable condition (a provirus) inside the cell. Lysogenized cells may multiply indefinitely, like other cells, each member of the clone retaining its own copy of DNA. Viruses / immunology. Genome, Viral. Humans. Immunity. Models, Biological. Transcription, Genetic. Virus Replication. Viruses / genetics\*. Viruses are obligate, intracellular parasites. A virus must first recognize and attach to a specific living cell prior to entering it. After penetration, the invading virus must copy its genome and manufacture its own proteins. Finally, the progeny virions must escape the host cell so that they can infect other cells. During the budding process, the cell does not undergo lysis and is not immediately killed. However, the damage to the cells that the virus infects may make it impossible for the cells to function normally, even though the cells remain alive for a period of time. Most productive viral infections follow similar steps in the virus replication cycle: attachment, penetration, uncoating, replication, assembly, and release ((Figure)). Attachment. Virus-first hypothesis: Viruses evolved from complex molecules of nucleic acid and proteins either before or at the same time as the first cells appeared on Earth, billions of years ago. Transmission. A virus exists only to reproduce. When it reproduces, its offspring spread to new cells and new hosts. The makeup of a virus affects its ability to spread. Antiviral drugs have been developed largely in response to the AIDS pandemic. These drugs do not destroy the pathogen, but they inhibit their development and slow down the progress of the disease. Antivirals are also available to treat infection with the herpes simplex virus, hepatitis B, hepatitis C, influenza, shingles, and chicken pox. Vaccines. Vaccinations are generally the cheapest and most effective way to prevent viruses. A virus is a tiny infectious agent that reproduces inside the cells of living hosts. When infected, the host cell is forced to rapidly produce thousands of identical copies of the original virus. Unlike most living things, viruses do not have cells that divide; new viruses assemble in the infected host cell. But unlike simpler infectious agents like prions, they contain genes, which allow them to mutate and evolve. Over 4,800 species of viruses have been described in detail out of the millions in the