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A cross-species view on viruses

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Abstract

We describe the creative ways that virologists are leveraging experimental cross-species infections to study the interactions between viruses and hosts. While viruses are usually well adapted to their hosts, cross-species approaches involve pairing viruses with species that they don't naturally infect. These cross-species infections pit viruses against animals, cell lines, or even single genes from foreign species. We highlight examples where cross-species infections have yielded insights into mechanisms of host innate immunity, viral countermeasures, and the evolutionary interplay between viruses and hosts.

INTRODUCTION

Model organisms have been critically important in biomedical research, and are particularly useful for studying conserved biological processes and pathways that operate by similar rules across diverse species. Indeed, our understanding of viral pathogenesis in humans has greatly benefited from research conducted in model organisms. However, the dynamic interplay between hosts and viruses in nature (Figure 1) is difficult to recapitulate in laboratory-based studies that employ a single viral clone infecting an isogenic host population. First, in the process of virus host-switching, a virus of one species evolves the ability to infect and spread in a second species. In this process, genetic differences between these species, not genetic similarities, are what dictate the evolutionary adaptations required by the virus. Second, viruses and the host genes that encode defenses against them are known to be exceptional for their genetic diversity. Therefore, the results of experiments using clonal hosts and clonal viruses in the laboratory may not always reveal the spectrum of possible host-virus interactions that truly exists in nature. Third, in studies of viruses infecting their natural host species, including cell lines derived from those species, host defense mechanisms can be masked because the viruses have already evolved to evade them. In all of these instances, experiments conducted in non-host species (referred to here as heterologous species) can be highly informative. Here we consider both the strengths and limitations of approaches involving infections of heterologous animals, heterologous cell lines, and even cell lines differing only by the expression of single genes from heterologous species. We also highlight examples where these types of approaches have revealed the evolutionary dynamics driving counter-evolution and adaptation between viruses and hosts.

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Cross-Species Infections Involving Heterologous Genes

In the 1970's and 1980's, scientists developed techniques for introducing and expressing foreign genes in mammalian cell lines. It is now common to express a gene from one species in a cell line derived from another species. The usefulness of this approach in virology has been well demonstrated in studies of retroviral restriction factors. For decades retrovirologists have documented tissue and species tropism of mammalian retroviruses, including HIV. Studies on the genetic underpinnings of these patterns ultimately revealed an impressive landscape of host innate immunity genes, including the restriction factor genes *APOBEC3G*, *TRIM5*, *Tetherin*, and *SAMHD1* [1–4]. In some cases, comparisons of cell lines derived from susceptible and resistant human tissues were used to isolate these genes. Notably, the identification of the TRIM5 α restriction factor instead resulted from comparing cell lines derived from susceptible and resistant primate species (Figure 2A). In this case, researchers took advantage of the fact that some rhesus macaque cells are highly resistant to HIV-1 infection, isolating a cDNA from these cells that conveyed HIV-1 resistance when expressed in human cells [5]. After its discovery, sequence comparisons of different non-human primate TRIM5 α orthologs were exploited to quickly map the genetic determinants of virus recognition (Figure 2B) [6–8]. Analyses of the molecular evolution of the *TRIM5* gene aided in these studies [6], as signatures of recurrent positive selection often accumulate in the exact regions of host genes that modulate host-virus interactions [9,10]. Several non-human primate species were found to encode unique variants of TRIM5 α resulting from gene fusion events, constituting novel restriction factors with different viral specificities when expressed in human cells [11–13]. A human-monkey chimera of *TRIM5*, as well as natural primate orthologs with strong activity against HIV, are now being developed for human gene therapy [14,15]. It is important to recognize that this potent anti-retroviral gene is constitutively expressed in many human cell types, yet lies silent because HIV has evolved to escape its detection. The powerful antiviral activity of TRIM5 α was only revealed when HIV was paired with cells of a heterologous host species to which the virus is not yet well adapted. Removing HIV from the context of the human genetic landscape uncovered how exquisitely vulnerable HIV is to naturally existing host factors that may ultimately prove vital to eradicating this deadly human pathogen.

After many similar studies, we now appreciate that it is common for the interactions between host innate immunity proteins and viruses to be highly species-specific [6,16–24]. For instance, the NS1B protein of influenza B binds and antagonizes the interferon-induced ISG15 protein of humans and other primates, but not of mice or dogs [25]. This potentially contributes to the narrow host range of influenza B viruses, which have only been found to infect humans. Heterologous gene studies performed in tissue culture have also motivated live animal studies in some cases. For instance, the evolution of viral escape was observed in monkeys encoding restrictive alleles at the *APOBEC3G* and *TRIM5* restriction factor loci [23,26]. An intriguing new twist on this heterologous gene approach is to resurrect extinct forms of host genes, including both ancient cell surface receptors and ancient restriction factors, and to test their activity when paired against viruses in the context of modern cells [24,27,28]. In these studies, functional differences between extinct and modern genes reveal the evolutionary pathways by which hosts adapted to their viral challenges over time. In summary, the approach of studying single genes from heterologous or extinct species provides unique insight into host innate immunity, viral escape, and the evolutionary events that are driven by these interactions.

Cross-Species Infections in Heterologous Cell Lines

Virologists often hunt for particular cell lines that are highly permissive for the replication of a certain virus in order to recover high viral titers. These cell lines may be derived from species other than the one from which the virus was isolated or from tissues other than the

ones in which the virus normally replicates. For instance, human influenza virus is commonly grown in MDCK (dog kidney) and MDBK (bovine kidney) cells because these cell lines are more permissive for flu replication than other lines (Figure 3). It is important to consider that, by ignoring other less permissive cell lines, we may be casting aside opportunities to discover new restriction factors and other cellular factors of importance to particular viral life cycles.

It is also common to use heterologous cell lines to attenuate viruses, sometimes for the purpose of creating vaccine strains [29]. During the process of attenuation, viruses undergo selection for increased fitness in cells of the new host species, at the expense of viral fitness in the original host species. Critical genetic differences between the two species, as well as the context of the infection (i.e. cell lines from a particular tissue vs. whole organisms) drive viral adaptation. For example, vaccinia virus, which is presumed to have arisen from cows and/or horses, was passaged nearly 600 times in chicken cells to create an attenuated strain used to vaccinate humans. The genome of this strain contains several large deletions and hundreds of mutations [30]. All major deletions accumulated in the distal regions of the vaccinia genome, which is enriched for genes involved in counteracting specific host defenses, including adaptive immunity. Losses of these regions imply a considerable fitness cost to maintaining anti-host proteins that are expendable for efficient replication in chicken cells. The acquired point mutations may also reflect adaptations specific to the innate immune defenses of chickens, but this has not been demonstrated. The growing field of experimental evolution has the potential to help us understand the adaptive processes that viruses undergo when they acclimate to novel hosts. These approaches involve serial passage of a virus through a novel host, and are especially effective when viral aliquots are collected and catalogued throughout the course of the adaptation process [31–40].

In some cases, more specific mechanistic insights have been gained from infections of heterologous cell lines. For example, the interferon-induced gene product Protein Kinase R (PKR) limits viral replication by blocking global protein translation in the cell. To circumvent this immune strategy, many viruses encode antagonists of PKR, including herpes simplex viruses, influenza viruses, poxviruses, and cytomegaloviruses [41]. Infections in diverse primate cell lines were used to demonstrate that two of these viral antagonists, TRS1 of cytomegalovirus and K3L of vaccinia virus, each have substantially different specificities for inhibiting the PKR proteins of different species [18,42]. The authors of these studies proposed that constant evolutionary struggle between viral antagonists and host defenses uniquely shaped these interactions in each primate species. In this case, selection has tailored more than just interaction affinities between viral antagonists and PKR, as the variants of TRS1 from human and Old World monkey cytomegaloviruses inhibit PKR by different mechanisms. Therefore, a cross-species viewpoint can help reveal key functional differences in how viruses adapt to defeat host immunity.

Cross-Species Infections in Animals

In nature, new diseases arise when existing viruses adapt to infect new species. In most known cases this involves the viral genome acquiring a series of mutations that ultimately makes it more fit in the new host (Figure 4). Cross-species infections of live animals in laboratory settings can be useful for understanding virus evolution in new hosts. A compelling example is the recent adaptation of H5N1 avian influenza to ferrets, the most common animal model system for studying influenza transmission in humans. H5N1 is an avian flu virus that is highly lethal to chicken flocks, and has also been infecting humans through zoonotic transmission since 1997. The well-publicized anxiety around H5N1 avian flu stems from its extremely high case fatality rate in humans [43]. H5N1 does not yet transmit efficiently from human to human via aerosolization and respiratory inhalation, which is taken to mean that the virus requires additional mutational changes before epidemic

spread in humans can result. In line with this hypothesis, three recent studies identified small combinations of viral mutations that result in respiratory transmission of this virus between ferrets in neighboring cages [40,44,45]. Some uncertainties about these studies persist. For instance, past transmission studies in ferrets have not always been accurate in predicting transmission or pathogenesis in humans [46]. However, this work illustrates how cross-species infections of animals may reveal exactly how viruses adapt to new hosts, including humans.

The implications of intra-species genetic diversity can also be studied with experimental approaches. For example, the MHC loci are some of the most genetically diverse loci in human and other mammalian genomes [47]. A recent study reported experimental evolution of Friend virus by serial passage through three different mouse strains that are genetically identical except at their major histocompatibility (MHC) loci [48]. The study demonstrated an important role for specific host MHC genotypes in the evolutionary trajectory of the virus by showing that viral fitness was improved in mice with MHC genotypes “familiar” to the virus. This experiment nicely illustrates a central tenet of the Red Queen hypothesis [49], namely that unique host genotypes exert unique selective pressure on viruses. The same concept has also been demonstrated in primates, where retroviruses have been shown to take specific and reproducible evolutionary trajectories depending on the particular restriction factor alleles of their host [23,26]. Thus, viruses evolve in response to local host genotypes but, by doing so, exert selective pressure on host populations to continually diversify allelic repertoires at loci involved in host defense. In this way, the evolution of host and virus populations are intimately linked. Further studies exploiting genetic differences between closely related strains and species holds great promise for revealing the fundamental rules that govern co-evolution in host and virus populations.

Virus evolution during host switching can also be monitored in the wild. An interesting and often-cited case involves the repeated release of the myxoma poxvirus in Europe and Australia as a form of biological control over invasive European rabbit populations [50]. Historically, this virus was known to cause mild disease in South American rabbits, but was found to be lethal to European rabbits. However, after release into European rabbit populations, the emergence of less virulent viral forms quickly followed. Attenuation of viral pathogenicity resulted in increased rabbit survival times, which may have optimized viral transmission to new rabbits through insect vectors. Rabbit populations also adapted to widespread exposure to myxoma virus, but not to the level observed in rabbit species from South America. While this is as an illustrious example of host-virus co-evolution, the mutations underlying adaptation have not yet been identified. It will be challenging to determine which specific mutational changes conveyed the observed fitness improvements, as a majority of the mutations that accumulated in host and virus genomes are predicted to have been evolutionarily neutral or even slightly deleterious. This limitation pertains to most experiments that involve sampling from infection dynamics unfolding in nature, and can be especially vexing in highly heterogeneous virus populations and for protocols with sparse sampling. On the other hand, the genetic basis of adaptation in both host and virus genomes has been successfully described in another major model for host-switching of viruses in nature: the emergence of carnivore parvoviruses in dogs in the 1970's [27,51,52]. Future studies may reveal the molecular details of the cross-species transmission of myxoma virus and the host-virus genetic conflict that unfolded in the context of this ecologically complex system.

Future Directions

By pairing viruses with heterologous host species at the level of organisms, cell lines, and single genes, species-specific differences are leveraged to provide insight into the struggle for survival that exists between viruses and hosts [53,54]. In turn, mechanistic studies of

host-virus interactions benefit from this evolution-based perspective, as observations of positive selection and species-specific mutational patterns can be used to guide the functional dissection of host-virus interactions [9]. These studies have led to the identification of novel aspects of the innate immune system and have revealed corresponding viral escape pathways. In order to enhance the power of this approach, it will be important to curate panels of cell lines from species that constitute viral reservoirs in nature, as well as from species that may serve as new or intermediate hosts for emerging disease. By extending the model organisms paradigm with a cross-species view of virology, which incorporates the vast genetic diversity driving the dynamics of host-virus interactions, we may be poised to gain the upper-hand in these continuing struggles for survival.

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HIGHLIGHTS

- Dynamic aspects of virology often aren't well suited to traditional model system approaches
- Many viruses are well adapted to their natural hosts, masking pathways of immunity
- Cross-species infections yield unique insight into innate immunity
- Viral adaptation can be effectively studied in novel host cells and species

Figure 1. A cross-species view on viruses and hosts

Different types of virus-host dynamics are illustrated. The hypothetical phylogenetic trees depict class-specific genetic divergence of viruses (left) and species-specific genetic divergence of hosts (right). Not shown are additional genetic differences that exist within host and viral populations. All of these genetic differences have the potential to contribute to viral host range, which may be broad or narrow (colored triangles), and may make some viruses more likely to evolve to expand their host range (dotted line). This dynamic interplay between hosts and viruses is difficult to recapitulate in laboratory-based studies that employ a single viral clone infecting an isogenic host population.

Figure 2. A heterologous gene approach for identifying and characterizing viral immunity proteins

A) Observations of susceptible and resistant cell lines are common in virological research, and often these cells represent different species. In cases where resistance is conveyed by a dominant genetic factor, as would be the case with a cellular restriction factor or other immunity protein, the genetic basis for resistance can be uncovered by performing the illustrated screen. A cDNA library prepared from the resistant cell line is introduced into the susceptible cell line. The resulting cells are screened for a cDNA clone that conveys resistance. This scheme was used to identify the HIV restriction factor, TRIM5 α [5]. **B)** Once cellular immunity proteins have been identified, heterologous gene studies can also be used to finely map the genetic determinants of viral recognition. In this case, multiple orthologs of the gene of interest (here, “gene X”) from related species will be required. These orthologous genes are introduced into a common cell background, and these cells are then tested for susceptibility or resistance to the virus of interest. Phenotypic differences can be compared to the genotypes of each ortholog as shown on the top right, where black tick marks indicate mutational differences compared to the top blue ortholog of species 1. By comparing unique mutations in the resistant (red) versus susceptible (blue and green) orthologs, the genetic determinants of viral detection can be identified (orange arrows). Of these, the best candidate protein regions or residues (dark orange arrows) will be those with signatures of positive selection over the evolution of these species (bottom right, positions under positive selection indicated with asterisks). This scheme was used to identify the region of TRIM5 α that conveys HIV/SIV recognition [6].

Type of cross-species infection:	Component tested:	Common uses in research:	Examples discussed in text:
A. Heterologous species	Whole organism	<ul style="list-style-type: none"> * Viral attenuation * Pathogenesis studies of human viruses in animal models	<p>South American Rabbit → myxoma virus → European Rabbit</p>
B. Heterologous cell line	All cellular proteins	<ul style="list-style-type: none"> * Viral attenuation * Convenience in virology assays	<p>human influenza → dog cells</p>
C. Heterologous gene	One cellular protein	<ul style="list-style-type: none"> * Studying host genetics * Studying viral escape	<p>HIV → human cells expressing one macaque gene</p>

Figure 3. Cross-species infections at the level of organisms, cells, and genes

A) Cross-species infections in nature form the basis for the emergence of new diseases. Experimental cross-species infections are commonly used in the laboratory for viral attenuation and evolution studies, and sometimes out of necessity. For instance, most research on the human hepatitis C virus was historically performed in chimpanzees, because this virus was not easily studied in tissue culture. **B)** Sometimes cell lines from heterologous species are used in tissue culture-based virology experiments. In cases where incompatibility is observed between a virus and a cell line of a heterologous species, this presents an opportunity to identify cellular barriers to infection in a genetically tractable system (see Figure 2). **C)** It is becoming more common to express single genes from one species in cell lines derived from a second species. This allows one to study the significance of genetic divergence at a single host locus with regards to viral replication. Also, in such systems viral evolution experiments elucidate how a virus can escape specific cellular blocks.

Figure 4. The process of virus host-switching

New diseases arise when existing viruses acquire novel hosts. This diagram illustrates the steps by which a virus is transmitted from its original host to a new host species. While all organisms are continuously exposed to the viruses of other species, infection resulting in virus replication and potentially illness (step 1) is thought to be a relatively rare event. Rarer still will be infections that are successful enough to transmit between individuals in the new host species (step 2). Of these, only some will progress to the point of epidemic or pandemic spread through the new host species (step 3). In theory, each of these steps may or may not require the acquisition of novel mutations in the viral genome, although existing evidence suggests that additional mutations usually do accumulate in viral genomes as viruses become more and more adapted to a particular host. Virus mutations can be acquired through point mutation, insertion, deletion, recombination, or reassortment. The acquisition of combinations of mutations may be required for viruses to advance through this process. Figure adapted in part from [55].