POXVIRUS TROPISM

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Abstract | Despite the success of the WHO-led smallpox eradication programme a quarter of a century ago, there remains considerable fear that variola virus, or other related pathogenic poxviruses such as monkeypox, could re-emerge and spread disease in the human population. Even today, we are still mostly ignorant about why most poxvirus infections of vertebrate hosts show strict species specificity, or how zoonotic poxvirus infections occur when poxviruses occasionally leap into novel host species. Poxvirus tropism at the cellular level seems to be regulated by intracellular events downstream of virus binding and entry, rather than at the level of specific host receptors as is the case for many other viruses. This review summarizes our current understanding of poxvirus tropism and host range, and discusses the prospects of exploiting host-restricted poxvirus vectors for vaccines, gene therapy or tissue-targeted oncolytic viral therapies for the treatment of human cancers.

ZOONOSIS
The infection of a novel host species, usually humans, by an animal virus that normally does not use man as a reservoir host.

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Despite remarkable advances in the control and treatment of infectious diseases, the problem of emerging and re-emerging pathogens is likely to be one of the main issues of medical and public health in the twenty-first century[1]. Viral diseases are of particular concern because advances in the field of antiviral drugs have lagged behind those of bacteriocidal drugs and antibiotics. Instead, the use of vaccines and good medical practices remain the traditional strategies to control viral infections. Also, particularly in the case of emerging viral pathogens, the development of antiviral therapies and vaccines can lag behind the time of viral emergence by years, or even decades. As the experience with severe acute respiratory syndrome (SARS) taught us, new members from neglected virus families can cross into humans from unsuspected reservoirs, necessitating rapid advances in our understanding of novel virus–host dynamics before the development of effective vaccines and drugs can even be contemplated[2]. Indeed, if there is one certainty in this new century, it is that viral pathogens will continue to emerge in the human population. It is therefore worthwhile to consider lessons that have been learned from the one viral pathogen — variola virus — that has killed more members of the human population over the span of recorded history than all other infectious diseases combined.

When, in 1980, the World Health Organization (WHO) certified that the world was finally free of smallpox as an extant human disease, all known stocks of variola virus were rounded up and ceremoniously relegated to 'death row'[3]. The two remaining WHO-approved variola virus stocks were stored in 'frozen limbo'; however, fears have increased that these official stocks are not the only ones remaining[4,5]. The terrorist attacks in the United States on 11 September 2001, which were closely followed by anthrax release, only increased fears that variola virus stocks could be acquired and used as deliberate agents of mass mortality. Needless to say, the subsequent increase in funding to research programmes that aim to counter this threat has resulted in the resurgence of research into select pathogens that exhibit human tropism.

Today, the focus of research on variola virus is directed towards the development of novel antiviral drugs and safer vaccines[6,7], but it is also an appropriate juncture to ask a more fundamental question: why is variola virus a human-specific pathogen? One of the reasons that determined the success of the WHO smallpox eradication programme was the fact that no animal reservoirs of variola virus have ever been found. Many poxviruses are capable of zoonotically infecting man[8–10], and it is likely that variola virus is derived from an ancient ZOONOSIS that originated from an animal host species that

Table 1 | **Examples of poxvirus host ranges**

Poxvirus	Genus	Reservoir host	Zoonotic host	Replication range in cultured cells
Variola	Orthopoxvirus	Human	None	Most mammalian cells
Molluscum contagiosum	Molluscipoxvirus	Human	None	Differentiated human keratinocytes
Monkeypox	Orthopoxvirus	Rodents, squirrels	Monkeys, humans	Most mammalian cells, not PEK cells
Cowpox	Orthopoxvirus	Rodents	Humans, cows, cats, foxes, zoo animals	Most mammalian cells, including CHO cells
Vaccinia*	Orthopoxvirus	Unknown	Wide range, including humans	Most mammalian cells, not CHO cells
Ectromelia	Orthopoxvirus	Rodents	Laboratory mice	Most mammalian cells, not CHO cells
Orf‡	Parapoxvirus	Ungulates	Humans, cats	Primary ovine and bovine fibroblasts
Tanapox	Yatapoxvirus	Rodents? Insects?	Humans, monkeys	Selected primate cells
Myxoma	Leporipoxvirus	Rabbit (brush)	Rabbit (European)	Rabbit, selected primate and human tumour cells

*Several orthopoxviruses that can infect humans are thought to be derived from vaccinia — for example, rabbitpox, buffalopox, Cantagalo and Araçatuba viruses. ‡Several parapoxviruses can infect man — for example, paravaccinia, bovine papular stomatitis, deerpox and sealpox viruses. CHO; Chinese hamster ovary; PEK, pig embryo kidney.

is now extinct[4]. In general, poxviruses show species specificities that range from narrow to broad, but we still know little about the fundamental mechanisms that mediate the host tropism of individual poxviruses. Even if variola virus never again infects humans, there are other poxviruses that can cause serious human disease. In 2003, an outbreak of human monkeypox occurred in the mid-western United States due to the inadvertent importation of monkeypox virus in a shipment of rodents from west Africa[11,12]. Fortunately, the strain that caused this outbreak was more benign in humans than the more pathogenic variant that is found in central Africa, which results in mortality rates of 10–15% (REFS 13,14). The animal reservoir for monkeypox in Africa remains unknown, although several indigenous members of the squirrel species are likely candidates, but the features that predispose this virus to zoonotically infect man and other primates are unknown[15]. If monkeypox were to establish a reservoir status in a susceptible north American rodent species, such as prairie dogs[16], the public health consequences would be considerable.

This review considers what is currently known about the fundamental mechanisms that mediate the species specificities and host tropisms of poxviruses, and discusses the prospects for exploiting host-restricted poxvirus vectors for vaccines, gene therapy and tissue-targeted oncolytic viral therapies.

Three levels of viral tropism

Part of the challenge in identifying specific poxvirus/host tropism determinants is the fact that at least three levels of tropism can be defined, each of which involves different aspects of virus–host interactions. The first level of tropism — cellular tropism — refers to the observation that virus replication can be permissive, semi-permissive or abortive in cultured cells of different lineages or species. The second level refers to the frequently observed increased levels of virus replication in specific host organs or tissues, which can be influenced by factors that mediate cellular tropism as well as by tissue-specific antiviral responses. The third level, which manifests with overt pathogenesis and symptoms of disease in the infected organism, is influenced by the first two levels of tropism as well as by the overall host immune and inflammatory responses. Each of the three tropism levels have important roles in determining whether a virus will exhibit tropism for a given host species. In general, in a reservoir host, the virus causes relatively low pathogenicity and is harboured and transmitted while resulting only in subclinical infection. Zoonotic infections, however, are generally discovered only after species transfers that lead to increased virus pathogenicity or novel disease.

Although many poxviruses show strict species specificities in terms of their reservoir or zoonotic hosts, in tissue-culture cells, these specificities can vary markedly such that cells derived from vertebrate species that are not considered PERMISSIVE HOSTS can sometimes be productively infected *in vitro*. For example, myxoma virus is a rabbit-specific poxvirus that has been used to eradicate feral rabbits in Australia[17] but, *in vitro*, myxoma virus replicates robustly in selected transformed cells that are derived from humans and other primates[18]. In fact, *in vitro*, individual poxviruses exhibit a unique host-cell specificity that can be distinct from its *in vivo* host range (TABLE 1).

For many other viruses, tropism specificity in cultured cells is mainly determined by specific receptors that need to be engaged for virus binding and entry[19,20] but, for poxviruses, no specific host-cell receptors have been identified. Although there are correlations between the expression levels of cell surface receptors and permissiveness to certain poxviruses[21,22], subsequent work has shown that poxviruses bind and enter both permissive and RESTRICTIVE CELLS, but downstream intracellular events are aborted specifically in restrictive cells[23]. Therefore, poxviruses can probably bind to and enter a wide range of mammalian cells, but the ability of a given poxvirus to fully complete the replication cycle varies markedly between cells of different lineages or species origins.

The second and third levels of poxvirus tropism — tissue and organism tropisms — determine the distribution and dissemination of the virus in an infected host, and both tropisms affect the ability of the virus to spread between hosts. Therefore, virus spread and pathogenesis are intimately influenced by the innate

PERMISSIVE HOST
A host species that manifests overt disease when exposed to a specific virus.

RESTRICTIVE CELLS
Cells that do not allow completion of the virus life cycle when exposed to a specific virus.

Figure 1 | **Examples of host-restricted poxviruses.** Some poxviruses, like variola major (smallpox) of humans (**a**), ectromelia virus (mousepox) of mice (**b**) or camelpox virus of camels (**c**) remain largely restricted to one host species and rarely, if ever, cause zoonotic infections outside of that species. Other poxviruses (TABLE 1) can infect multiple zoonotic host species. Part **a** is reproduced with permission from the WHO web site (see the Online links box); part **b** is reproduced with permission from REF. 199 © (1982) Academic Press; part **c** was kindly provided by U. Wernery (United Arab Emirates) and H. Meyer (Germany).

and acquired immune responses of the infected host, which are themselves manipulated by the numerous immunomodulatory proteins that are elaborated by poxviruses[24]. Although the three levels of tropism are highly interdependent, each has unique features that interact to regulate the specificity of poxvirus–host interactions. Ultimately, it is the summation of these interactions that determines which infections will be permissive in a specific host species, and which of these will manifest as overt disease.

Cellular tropism: poxvirus replication

Poxviruses that infect vertebrates are of the subfamily *Chordopoxvirinae* and share several biological features. All are large, brick-shaped DNA viruses, with genomes that range from 130–300 kb, and all replicate exclusively in the cytoplasm of infected cells[25]. The *Chordopoxvirinae* are subdivided into eight genera, and members of at least half of these (the orthopoxviruses, parapoxviruses, molluscipoxvirus and yatapoxviruses) can infect man, either exclusively — for example, variola virus and molluscum contagiosum virus — or zoonotically[8,9]. The consequences of these infections range from severe disease associated with high mortality (FIG. 1) to benign infections that resolve over time[10]. Poxvirus particles from members of the various genera are morphologically similar (FIG. 2) and the main viral proteins that comprise poxvirus virions are thought to be largely conserved in terms of both structure and function.

So far, several dozen poxviruses have been sequenced and an NIH-sponsored web site (see the Poxvirus Bioinformatics Resource Center in the Online links box) is dedicated to maintaining an up-to-date repository of all the publicly available poxvirus genome sequences. These poxvirus genomes share several common features that collectively denote membership of the poxvirus family. All have linear double-stranded DNA genomes that include terminal inverted repeat sequences and hairpin termini and which comprise several hundred closely spaced open reading frames[25]. Of these open reading frames, at least 90 are specifically conserved among the various poxviruses and are required for poxvirus replication and morphogenesis, whereas the remainder are more divergent, owing to differences in adaptive evolution between the various poxvirus members[26–28]. It is the specific repertoire of these so-called non-conserved genes that gives each poxvirus its unique characteristics of host range, immunomodulation and pathogenesis[24]. Generally, poxvirus genes that are non-essential for replication in tissue-culture cells, but that influence the pathological profile of the virus in an infected host, are referred to as virulence genes, and targeted gene-knockout analysis has been used to identify the roles of many such poxvirus genes, particularly in vaccinia and myxoma viruses[29–31]. The deletion of some of these virulence genes can result in the inability of the virus to replicate in a subset of cultured cells that are normally permissive for the wild-type virus[29]. These so-called host-range genes have generated some insights into the nature of poxvirus tropism at the level of the infected cell and will be discussed separately below.

As illustrated in FIG. 3, the poxvirus replication cycle is a complex sequence of cytoplasmic events that begins with binding to the cell surface and subsequent fusion of virus and mammalian cell membranes. The intracellular replication cycle has been most well studied for vaccinia virus, which is the vaccine strain that was used to eradicate smallpox, but the essential features are highly conserved amongst other poxviruses[25]. Two distinct infectious virus particle types — the intracellular mature virus (IMV) and the extracellular enveloped virus (EEV) — can initiate the infectious cycle[32]. The IMV and EEV virions differ in their surface glycoproteins and in the number of wrapping membranes, and they are thought to enter cells by different mechanisms[33–35]. So far, several virion proteins have been shown to be crucial for binding of the virion to the cell surface, but the cell determinants of binding are thought to be ubiquitously expressed GLYCOSAMINOGLYCANS or components of the extracellular matrix[36–40]. After binding, the fusion event between the virion and the host cell membranes is still poorly understood, but at

GLYCOSAMINOGLYCANS
A group of polysaccharides with repeating disaccharide units that are linked to proteoglycans located at the surface of most mammalian cells.

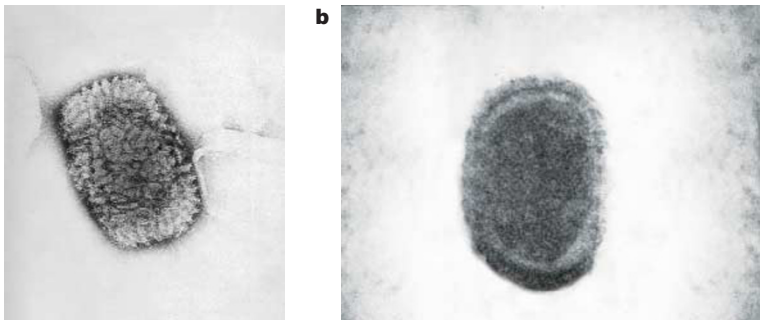

Figure 2 | **All poxviruses are morphologically similar.** Electron microscopic images reveal that poxviruses share common features of size and shape. For example, vaccinia virus (**a**; image courtesy of CDC) can infect a broad range of hosts but is very similar in size, shape and morphology to poxviruses (intracellular mature virus (IMV) forms) that are highly host restricted, such as molluscum contagiosum virus (**b**; image courtesy of CDC/Fred Murphy/Sylvia Whitfield), which has only been shown to infect man and replicates exclusively in human basal keratinocytes.

least one conserved virion protein (VV-A28) has been linked to this fusion/entry event that ultimately releases the virion core structure into the cytoplasm[41]. Although no specific cell receptors are known to be required for virion fusion and entry, there is evidence that virion binding and/or entry is associated with rapid signalling events in several host protein-kinase cascades, and it is likely that these signalling events can influence subsequent replication stages[35,42–44]. The study of signalling that is initiated by virus entry in mammalian cells is a growing field[45], particularly owing to increasing evidence of the crucial role of cell receptors, such as TOLL-LIKE RECEPTORS (TLRs), that act as 'sentries' that activate antiviral pathways[46]. The precise roles of TLRs in the control of poxvirus infections remain to be elucidated, but there is evidence that at least some poxviruses can block TLR signalling[47,48].

Once the virion and host membranes have fused and the virus core has been released into the cytoplasm, the endogenous RNA polymerase and encapsidated transcription factors that comprise the viral transcriptosome begin the first cascade of early viral gene expression, which synthesizes viral mRNA under the control of viral early promoters[25,49]. Then, by a poorly understood process known as core (or second stage) uncoating, as-yet-unidentified host and viral factors induce the dissolution of the core structure. This uncoating step releases the viral DNA into the cytoplasm, where it can function as a template for DNA replication and the subsequent waves of intermediate and late transcription. Unlike early transcription, which is believed to be exclusively under the control of viral transcriptosome factors that are encapsidated within the core, the subsequent intermediate and late transcription stages require cooperation with host-derived transcription factors that contribute to the efficiency of these latter two waves of viral gene expression[50–56].

Concomitant with the accumulation of late viral gene products is the progressive morphogenesis and assembly of infectious virus particles, initially as IMV virions, which assemble and migrate via microtubule-mediated trafficking and wrapping with Golgi-derived membranes to form intracellular enveloped virus (IEV). The IEV form loses one of its outer membrane wrappings as it fuses with the cell membrane to form the cell-associated enveloped virus (CEV), which is either propelled towards neighbouring cells by ACTIN-TAIL POLYMERIZATION under the virion, or is released directly as free EEV particles. It is thought that the CEV and EEV forms are particularly important for rapid cell–cell spread *in vivo*, whereas the IMV form probably contributes to virus dissemination only after late stage cell death and membrane rupture[57,58]. The actin-based extrusion of IEV and CEV is under the control of several host proteins, including N-WASP (neuronal Wiskott–Aldrich syndrome protein), Nck (novel cytoplasmic kinase), WIP (WASP-interacting protein) and kinases of the Src/Abl families[59–61].

In addition to the host *trans*-acting factors mentioned above, poxviruses express an array of modulatory proteins that modify both the intracellular and extracellular environments of the infected cell. These virus-encoded proteins collectively modulate a wide range of antiviral defence responses that are triggered by the virus infection and which include important host pathways such as apoptosis, interferon induction of the antiviral state, stress-induced signalling cascades, MHC-restricted antigen presentation and pro-inflammatory pathways[24]. The particular host-response factors that are encoded by individual poxviruses are responsible for the ability of each poxvirus to respond to the various antiviral mechanisms that are encountered in the infected host, as well as during the progressive migration of the virus through diverse cell types and tissues.

Restriction events in poxvirus-infected cells

Our knowledge of which regulatory factors control the main intracellular steps that determine whether a given poxvirus infection will be permissive or restrictive is still relatively limited, but a few general observations can be made (TABLE 2). At the level of virion binding and entry, all of the currently known cellular determinants that are required for a poxvirus virion to bind and initiate virus–host membrane fusion are ubiquitous surface elements, such as glycosaminoglycans, or extracellular matrix components[37–41]. It is now believed that the binding and entry of poxviruses into mammalian cells is an efficient process, and any restriction events that limit poxvirus replication specifically in non-permissive cells occur after the virus has entered the cell and initiated the replication cycle. Even highly restricted poxviruses such as molluscum contagiosum virus, which cannot be propagated *in vitro* in any known cell line and which replicates productively only in human basal keratinocytes, can bind and enter non-permissive mammalian cells in culture[62]. Similarly, chordopoxviruses such as vaccinia virus can bind, enter and initiate the viral replication cycle even in non-permissive insect cells[63]. However, once the virus core enters the cytoplasm and initiates the first steps of early gene expression, the 'tug of war' between the infecting virus and the target cell begins in earnest. At least four categories of intracellular events have been identified as potential restriction points

TOLL-LIKE RECEPTORS (TLRs). Surface receptors that are pattern-recognition sentinels for recognizing pathogen infection and inducing innate antimicrobial responses.

ACTIN-TAIL POLYMERIZATION A motility mechanism that assists the extrusion of certain pathogens, such as poxviruses, to facilitate infection of neighbouring cells.

MITOGENIC STIMULATION The process by which many poxviruses express growth factor homologues that can trigger neighbouring cells from quiescence into an inappropriate S-phase that increases virus replication levels.

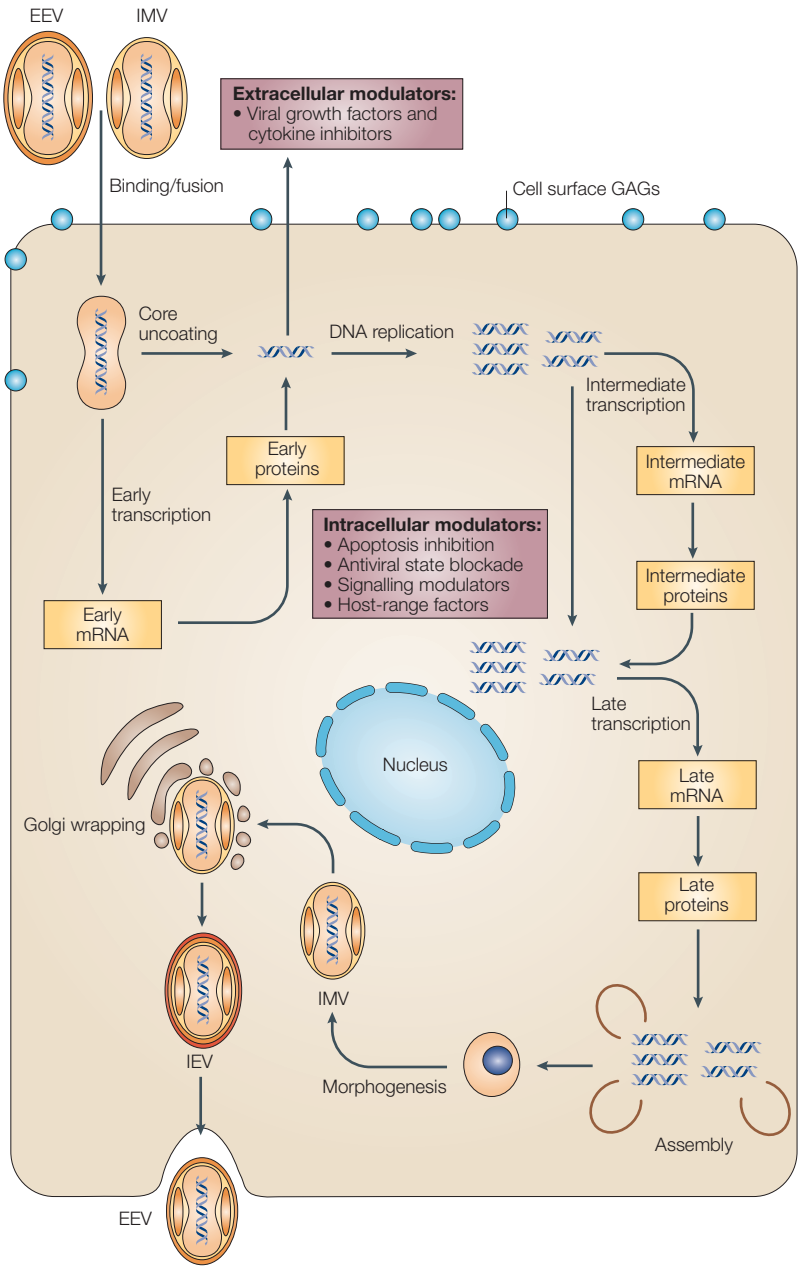

Figure 3 | **Poxvirus replication cycle.** All poxviruses replicate in the cytoplasm of infected cells by a complex, but largely conserved, morphogenic pathway. Two distinct infectious virus particles — the intracellular mature virus (IMV) and the extracellular enveloped virus (EEV) — can initiate infection[32]. The IMV and EEV virions differ in their surface glycoproteins and in the number of wrapping membranes[33–35]. The binding of the virion is determined by several virion proteins and by glycosaminoglycans (GAGs) on the surface of the target cell or by components of the extracellular matrix. Fully permissive viral replication is characterized by three waves of viral mRNA and protein synthesis (known as early, intermediate and late), which are followed by morphogenesis of infectious particles. The initial intracellular mature virus (IMV) is transported via microtubules (not shown in the figure) and is wrapped with Golgi-derived membrane, after which it is referred to as an intracellular enveloped virus (IEV). The IEV fuses to the cell surface membrane to form cell-associated enveloped virus (CEV; not shown), which is either extruded away from the cell by actin-tail polymerization (not shown) or is released to form free EEV. EEV might also form by direct budding of IMV, therefore bypassing the IEV form. Poxviruses also express a range of extracellular and intracellular modulators, some of which are defined as host-range factors that are required to complete the viral replication cycle. Poxviruses can be markedly diverse in their portfolio of specific modulators and host-range factors, which determine tropism and host range. Non-permissive poxvirus infections generally abort at a point downstream of the binding/fusion step.

for regulating whether a given poxvirus infection will proceed to completion, and it is likely that more such control checkpoints remain to be identified (TABLE 2).

Cell-cycle control. The first of these potentially regulatable events is the cell-cycle status of the infected cell. Poxviruses have been thought to be less S-phase-dependent than many other viruses, but there is evidence that the ability of poxviruses to MITOGENICALLY STIMULATE quiescent cells markedly increases viral replication levels. Many poxviruses encode growth factors, as homologues of either epidermal growth factor (EGF) or vascular endothelial growth factor (VEGF), that act in a paracrine manner to stimulate the onset of mitosis in neighbouring cells, and targeted deletions of the growth factor genes of vaccinia and myxoma virus have been shown to result in severe attenuation in infected animals[24,29–31]. There is also some evidence that poxviruses can directly perturb the activity of specific cell-cycle components in the infected cell, but whether there is a direct link with cell tropism is currently unknown[64–66]. Microarray data of HeLa cells that are infected with vaccinia virus strain WR indicate that, although the expression of most cellular genes is repressed, the expression of a small percentage (~3%) is robustly upregulated[67]. It would be of interest to compare and contrast these induced genes with the patterns of gene expression that are seen in comparable infections with host-restricted virus variants but, so far, the only available data is for the attenuated modified vaccinia virus Ankara (MVA), which induces the expression of many more cellular genes than the WR strain[68].

Cell lineage and differentiation state. The second intracellular event that regulates the efficiency of poxvirus replication is the lineage and differentiation state of the infected cell. For example, some poxviruses are dependent on the precise differentiation state of the host cell, such as the restriction of productive replication of molluscum contagiosum virus to keratinocytes that arise from the basal epidermal layer of the skin, mentioned above. In this case, the cellular factors that are required for the virus to complete its replication cycle beyond the stage of early gene expression are unknown, but it is noteworthy that the molluscum contagiosum virus encodes fewer immunomodulatory proteins than any other poxvirus that can infect humans[69,70]. Another example of the dependence of poxvirus infection on the specific cell lineage is shown by studies using differentiated DENDRITIC CELLS. Owing to the importance of dendritic cells for the immunogenic responses to poxvirus-based vaccine vectors, many studies have been conducted to examine the ability of poxvirus-infected dendritic cells to present foreign antigens[71–75]. Interestingly, whereas vaccinia virus is permissive for most cell types, infection of either mature or immature dendritic cells results in abortive infection after early gene expression, indicating that these cells have some specific defect that renders them refractory to productive vaccinia virus infection[76–80]. Currently, the basis of the

Table 2 | **Host–virus interactions that might regulate poxvirus/cell tropism**

Level of host–virus interactions	Viral factors that require host-cell components	Interacting host-cell factors and pathways
Virus binding and entry		
EEV binding	Unknown	Unknown
IMV binding	VV-A27, D8, H3	Glycosaminoglycans, laminins
Fusion/endocytosis	VV-A28, others?	Host membranes, raft-dependent?
Intracellular events		
Cell-cycle control	Viral growth factors (VGF, vVEGF)	S-phase regulators, p53
Differentiation state	Unknown	Cell lineage factors
Complementing factors		
Core uncoating	Core structural protein(s) (?)	Unknown
Transcription	Viral RNA polymerase complex	Intermediate/late transcription factors
Protein folding	Core protein 4a	Hsp90
Virion trafficking	VV-A36 of IEV	N-WASP, Nck, WIP, Src/Abl-kinases
Signal transduction		
Antiviral state	VV-E3L/K3L, tyrosine phosphatase	Interferon signalling, PKR, STAT
Kinases	Unknown targets	PAK1, ERK1/2
Signalling	VV-KIL, N1L, A52R; MC159L; M150R	NF-κB
Apoptosis	M-T5, M-T2, M-T4, M11L, VV-F1L, SPI-1, SPI-2, EV-p28	Cell death machinery

CEV, cell-associated enveloped virus; EEV: extracellular enveloped virus; ERK1/2, extracellular regulated kinases 1, 2; EV, ectromelia virus; Hsp90, heat shock protein 90; IMV: intracellular mature virus; MC, molluscum contagiosum; M, myxoma; NF-κB, nuclear factor-κB; Nck: Novel cytoplasmic kinase; N-WASP, neuronal Wiskott–Aldrich syndrome protein; PAK1, p21-activated kinase 1; PKR, protein kinase R; SPI, serine proteinase inhibitor; STAT, signal transducer and activator of transcription; VGF, vaccinia growth factor; vVEGF, viral vascular endothelial growth factor; VV, vaccinia virus; WIP, WASP-interacting protein.

deficiency of vaccinia replication in dendritic cells is unknown, but the identification of any complementing factor that is missing in dendritic cells, or a lineage-specific antiviral pathway, would be of considerable interest.

Complementing host factors. The third category of intracellular event that is required for poxvirus replication involves the many *trans*-acting factors that must be hijacked by the virus to complete its replication cycle. Some of these, such as the yet-to-be-identified host-core-uncoating factor(s), are thought to exist because cell-to-cell differences are noted in the uncoating stage. Other essential host features that are required by poxviruses, such as the translational machinery in the cytoplasm, are ubiquitous in growing mammalian cells, and are not thought to directly influence tropism. However, the availability of *trans*-acting transcription factors from the host cell that are required as components for intermediate and late viral transcription, such as VITF-2, might be rate-limiting in certain cells[50–52,56]. Similarly, any deficits in important cell regulatory elements of the microtubule-based or actin-based motility machinery would be expected to compromise the morphogenesis or egress of infectious virus. One example of a *trans*-acting factor that has been shown to directly modulate poxvirus propagation is Hsp90 — a MOLECULAR CHAPERONE that associates with the viral factories and regulates the efficiency of vaccinia virus replication by interacting with the viral core protein 4a, which is crucial for virion assembly[81].

Signal transduction. The fourth category of intracellular events that regulate poxvirus replication is the diverse signal-transduction pathways that coordinate the intrinsic cell responses to the virus infection. Perhaps the best studied of these is the interferon-mediated antiviral state, for which almost all viruses have evolved defence mechanisms[82–84]. In the case of poxviruses, the anti-interferon strategies include inhibitors of interferon induction, receptor mimics that scavenge interferon ligands, phosphatases that block the STAT-mediated signal-transduction pathway and inhibitors of the interferon-induced protein mediators of the antiviral state, such as protein kinase R (PKR)[24,82–84]. Indeed, there is increasing evidence that the induced interferon responses are crucial for maintaining the species barrier for some poxvirus infections. For example, myxoma virus is a rabbit-specific poxvirus that is non-permissive in primary murine fibroblasts (FIG. 4), but when interferon responses are ablated with neutralizing antibodies or drugs that prevent interferon induction, or alternatively by using cells that are derived from knockout mice deficient in components of the interferon pathway, the fibroblasts become fully permissive[85]. In fact, STAT1-deficient mice can be lethally infected by myxoma virus, whereas wild-type mice are completely resistant[85]. Furthermore, the interferon pathway is an important restriction determinant of myxoma virus replication in primary human fibroblasts[86]. In contrast to the well-studied interferon system, the mechanisms by which some of the other signalling pathways can manipulate poxvirus replication are less well understood. For example, the activation of host cell p21-activated kinase 1 (PAK1) is required for optimal replication of myxoma virus[23] and extracellular signal-regulated kinases 1 and 2 (ERK1,2) activation is necessary for optimal vaccinia infection[44]. More recently, it has been observed that many poxviruses prevent the activation of pro-inflammatory signalling cascades,

DENDRITIC CELLS
Cells of the immune system with characteristic tree-like projections. They participate in the recognition of pathogens and initiate the early phases of the host antiviral responses.

MOLECULAR CHAPERONE
Host proteins that assist in the folding or trafficking of host (and possibly viral) proteins.

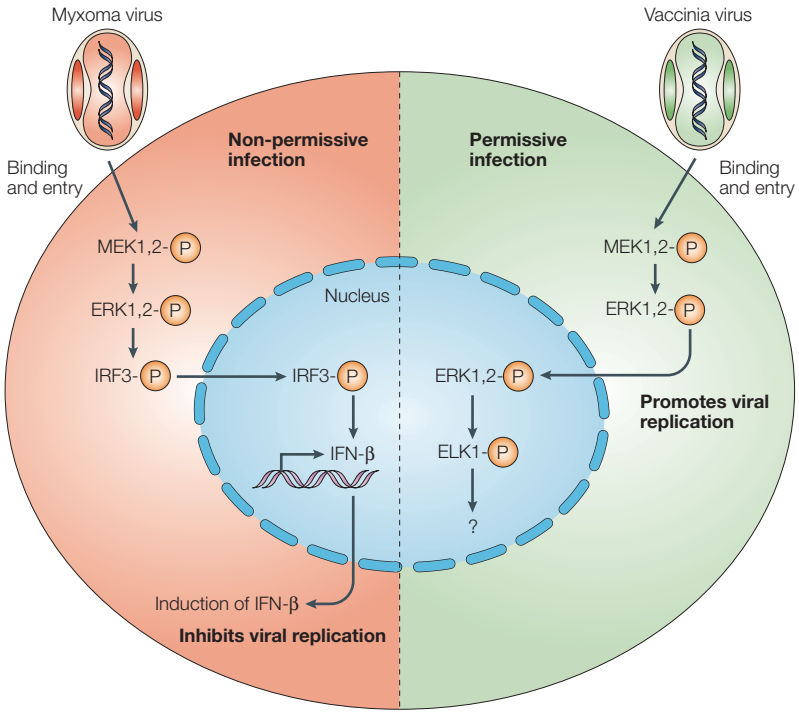

Figure 4 | **Intracellular signalling events modulate poxvirus tropism.** A comparison of the infection of primary murine embryo fibroblasts (pMEFs) by two poxviruses, one of which (myxoma virus) is non-permissive because it is prematurely aborted by an induced type-I interferon response, whereas the other (vaccinia virus) is fully permissive. Both infections are characterized by the induced activation of MEK1,2, which then phosphorylates extracellular signal-regulated kinase (ERK)1,2. However, in the non-permissive infection by myxoma virus, phosphorylated ERK1,2 remains in the cytoplasm where it induces the activation of interferon regulatory factor 3 (IRF3), which then migrates to the nucleus where it initiates the transcriptional upregulation of β-interferon (IFN-β). In the case of the permissive infection by vaccinia virus, the activated ERK1,2 migrates to the nucleus where it activates ELK1 but does not activate IRF3 or the interferon genes. Inhibitors of ERK1,2 activation, such as U0126, render pMEFs permissive for replication of myxoma virus but, in contrast, inhibit the replication of vaccinia[44,85,200].

such as those transduced through NF-κB, by the concerted actions of multiple signalling inhibitors[48,87–91]. Finally, all poxviruses encode a wide range of inhibitors of apoptosis, a process that is frequently triggered during poxvirus infection[92–94]. Although this subject is too extensive to be covered in this review, one recent notable development is the appreciation that poxviruses must control the mitochondrial checkpoint of apoptotic signalling to productively infect mammalian cells[95–97].

Poxvirus host-range genes

The study of viral host-range genes and the interactions of their products with host cells have provided insights into the nature of poxvirus tropism. In fact, the first host-range mutants that were described for animal viruses came from seminal work in the 1960s with rabbitpox virus mutants that failed to replicate in pig kidney cells[98–104]. Later, the locus that mediates this host-range phenotype was mapped to a specific virus gene that encodes the SERPIN SPI-1 (REFS 105,106). Although the exact host-cell targets of SPI-1 that mediate host range for any of the orthopoxviruses remain to be deduced[107,108], the SPI-1 protein of rabbitpox virus can bind and inhibit CATHEPSIN G, which is similar in terms of substrate specificity to a classic serpin[109]. Interestingly, although there is evidence that SPI-1 is not a crucial determinant for poxvirus virulence in infected animals[110,111], vaccinia constructs that are deleted for SPI-1 are reported to be attenuated in mice but still remain potently immunogenic when used as vaccines[112].

In addition to SPI-1, several other poxvirus host-range genes have been identified (TABLE 3), generally by indirect methods. For example, deletion of some non-essential poxvirus genes by targeted recombination has resulted in conditional replication defects in specific cells, and these have been termed host-range genes to denote this phenotypic defect. In some cases, the classification of a host-range gene has been made only after the screening of virus gene knockout clones against panels of normally susceptible cells[29,113].

As the identification of poxvirus host-range genes has been largely fortuitous, our understanding of the range of host pathways with which the protein products of these genes interact is incomplete. Nevertheless, one theme that has emerged is that the known protein products of poxvirus host-range genes are localized within infected cells, which is consistent with the need to circumvent intracellular barriers to complete the virus replication cycle (TABLE 3). The other noteworthy point is that the known host-range proteins are biochemically diverse, and no single poxvirus encodes versions of all the members. Rather, individual poxviruses have evolved their own unique subsets of host-range genes.

The first poxvirus host-range genes to be identified at the molecular level were from the vaccinia virus. Early work had indicated that certain isolates of vaccinia virus that had spontaneous gene deletions were compromised for growth in human cells[114–116]. Later, the K1L and C7L genes were implicated as being required for completion of the replication cycle of vaccinia virus in human cells[113,117,118]. Vaccinia is also unable to complete its replication cycle in Chinese hamster ovary (CHO) cells owing to an intracellular abort that occurs shortly after virus binding and entry, at the stage of intermediate gene expression[119]. The K1L/C7L defect could be rescued by another host-range gene from cowpox virus, designated CHOhr or CP77, which had been shown to be necessary for cowpox replication in CHO cells[113,120,121]. Insertion of the CHOhr gene into vaccinia virus or ectromelia virus allows these viruses to grow in CHO cells for which they are normally restricted[122–125]. The CHOhr gene can also rescue the host-range defects that are imparted by the loss of the K1L gene from vaccinia virus[126,127]. Also, whereas growth of a modified vaccinia virus that is deleted for K1L is usually restricted at the stage of early protein synthesis in rabbit kidney (RK13) cells, the expression of K1L in cells transfected with the K1L gene complements the loss of the K1L gene and allows growth of the K1L-minus vaccinia virus in RK13 cells[128]. K1L and CHOhr are both members of the ankyrin-repeat superfamily of proteins, which are known to be important for protein–protein interactions[129]. There is some evidence that CHOhr affects the translation efficiency of viral intermediate proteins at the level of eukaryotic-translation initiation

SERPIN
Serine proteinase inhibitor, designed to bind and inhibit specific target proteinases. Poxviruses are the only viruses to express active members of this superfamily.

CATHEPSIN G
A host serine proteinase that can form inhibitory complexes with the poxviral SPI-1 serpin.

Table 3 | **Poxvirus host-range genes**

Gene	Protein type	Cultured cells with defects in virus tropism*
Myxoma virus		
M-T5	Ankyrin repeats	Rabbit T cells, human tumour cells
M-T2	TNF receptor	Rabbit T cells
M-T4	ER-localized	Rabbit T cells
M11L	Mitochondrial	Rabbit T cells
Vaccinia virus		
E3L	PKR inhibition	HeLa cells, CEF (MVA-E3L$^-$)
K3L	dsRNA-BP	BHK cells
B22R/SPI-1	Serpin	Human keratinocytes, A549
C7L‡	Cytoplasmic	Hamster Dede cells
K1L‡	Ankyrin-repeats	RK13 cells
Rabbitpox virus		
SPI-1	Serpin	PK15 cells, A549
Ectromelia virus		
p28	E3-ubiquitin ligase	Mouse macrophages
Cowpox virus		
C9L/CP77/CHOhr	Ankyrin repeats	VV-C9L$^+$ grows on CHO cells; VV-K1L/C9L$^+$ grows on RK13 cells

*Host-range defect is specifically exhibited by viral gene knockout constructs or viral recombinants engineered to express heterologous host ranges genes. ‡Double gene knockout (C7L/K1L) of vaccinia virus (VV) unable to replicate in PK1 cells or most human cells. BHK, baby hamster kidney; CEF, chicken embryo fibroblasts; CHO, Chinese hamster ovary; dsRNA-BP, double-stranded-RNA-binding protein; ER, endoplasmic reticulum; MVA, modified vaccinia Ankara; PK15, pig kidney 15; PKR, protein kinase R; RK13, rabbit kidney 13; SPI-1, serine proteinase inhibitor 1; TNF, tumour-necrosis factor.

factor 2α (eIF2α) phosphorylation[125]. Recently, K1L was shown to inhibit the activation of NF-κB in RK13 cells, apparently by inhibiting the degradation of the inhibitor protein Iκβ, which also possesses ankyrin repeats[89]. Although the basis for the molecular properties of K1L, C7L and CHOhr remains to be elucidated, it is presumed that the loss of such genes has important roles in the human-cell restriction of some of the attenuated strains of vaccinia virus like NYVAC and MVA[130–135].

The only other known poxvirus ankyrin-repeat host-range protein is M-T5 of myxoma virus, which is required for the replication of this virus in rabbit T lymphocytes[136]. Recently, this viral protein was also shown to be required for the replication of myxoma virus in several transformed human cells[18]. Again, the role of M-T5 remains to be deduced, but it is clearly not a rabbit-specific modulator, presumably because its unidentified host-cell targets are broadly recognized across species barriers.

So far, the poxviral host-range genes for which the host target is best understood are the E3L and K3L genes of vaccinia virus, which have been extensively characterized for their ability to counteract host interferon responses[24]. The E3L gene products comprise two related dsRNA-binding proteins that oppose the activation of important mediators of the antiviral state, particularly PKR and OAS (2′,5′-oligoadenylate synthetase), whereas K3L mimics the host factor eIF2α and functions as a pseudo-substrate for PKR[137]. Furthermore, E3L can interfere with the induction of type I interferon by blocking the activation of interferon regulatory factor 3 (IRF3) and IRF7 (REF. 138). Vaccinia virus constructs that lack E3L are restricted for replication in many cells[139,140], whereas K3L-minus vaccinia infection is abortive specifically in baby hamster kidney (BHK) cells[137,140]. There is some evidence that the precise levels of dsRNA and PKR that are induced in infected cells determine the hierarchy of importance of E3L and K3L in host-cell tropism[137]. This ability of poxviruses to counteract the inhibitory properties of interferon is linked with the inhibition of apoptosis, and can directly affect the replication and antigen-presenting potential of non-replicating poxvirus vaccines[141,142].

The most recent example of a poxvirus host-range protein for which a specific biochemical function has been ascribed is the p28-RING zinc-finger protein of ectromelia virus (EV-p28). EV-p28 is essential for virulence in mice and deletion of the EV-p28 gene renders ectromelia virus unable to productively replicate in mouse macrophages[143,144]. The EV-p28 protein functions as an E3-ubiquitin ligase[145,146], so presumably the inability of the EV-p28-minus virus to direct substrate proteins for ubiquitination and degradation contributes to the non-permissive phenotype in infected macrophages, but the relevant host targets remain to be identified.

Tropism for tissue and organism

When poxviruses from a long-term evolutionary host cross into a novel species, marked differences in pathogenesis can sometimes occur. For example, the rabbit-specific myxoma virus is relatively non-pathogenic in its evolutionary host, the *Sylvilagus* (brush) rabbit, but is almost 100% fatal in the *Oryctolagus* (European) rabbit[147]. On the other hand, some poxviruses, such as variola virus, are thought to have never spontaneously crossed into another host species, but can be experimentally manipulated to cause disease if injected intravenously at high dosages into particular primate hosts[148,149]. The study of poxvirus pathogenesis, and particularly the host determinants that influence virus replication and dissemination in diverse tissues, is in its infancy[10]. In fact, knowledge about the genetic loci that control intrinsic immunity to most viral infections is still limited, but there have been recent advances in the identification of specific host-restriction factors, particularly for infection by retroviruses[150]. At present, the ectromelia virus, which causes mousepox, is the only poxvirus for which there is information on the host genetic loci that influence susceptibility to infection[151].

The study of mouse strains that exhibit variable resistance or susceptibility to infection by ectromelia virus has revealed important clues about how host genetics influences poxvirus pathogenesis and host range. Ectromelia virus is highly infectious in all strains of laboratory mice, but induces lethal disease only in strains with particular genetic backgrounds (for example, CBA, A/J, BALB/c or DBA/2) and is readily cleared in other strains (for example, C57/BL or AKR), which are considered to be resistant but can be silent carriers of mousepox[151]. Resistant mouse strains are characterized

by a more integrated immune response to ectromelia virus, which includes robust early innate immune responses (particularly interferon induction, macrophage activation and natural killer (NK) responses), as well as efficient adaptive responses (mediated by $CD8^+$ cytotoxic T cells, $CD4^+$ T-helper (T_H) cells and antibodies)[152–154]. Breeding experiments indicate that resistance is dominant over susceptibility, and four genes that confer resistance to mousepox (designated *Rmp1–4*) have been mapped[155–160]. Furthermore, other genetic loci that affect immune responses and susceptibility to ectromelia continue to be discovered in mice[161–163]. In general, the polarization of T_H cells of the host immune response is believed to be crucial in determining whether a given poxvirus infection will be subclinical and resolve, or will progress to systemic disease[164–166]. Specifically, the ability of an infected immunologically naive host to mount a T_H1-POLARIZED IMMUNE RESPONSE is regarded as crucial for the control of poxvirus infection and for recovery[164,167]. This point was dramatically emphasized when it was observed that a recombinant ectromelia virus that expresses interleukin 4, a potent T_H2 cytokine, was lethal in normally non-suceptible mice[168].

Host-restricted poxvirus vectors

In addition to the need to better understand the factors that control disease pathogenesis, host range and zoonoses, there are practical issues relating to the potential manipulation of poxvirus tropism. The most important of these is the development of host-restricted poxvirus vectors as safe platforms for vaccines or gene delivery[169–172]. The vaccinia virus strains that were used to eradicate smallpox were effective and highly immunogenic, but caused high rates of post-vaccination medical complications that are now considered excessive by modern safety standards[173,174]. At present, there are more people with some form of immune compromise than during the smallpox eradication era up to the mid-1970s, and so efforts have been directed towards the development of poxvirus-based vectors that are restricted for replication in humans.

Non-replicating poxvirus vaccines. Generally, these efforts involve two related strategies — the isolation of vaccinia variants (for example, MVA, LC16m8 or NYVAC) that show reduced virulence, or the development of poxvirus platforms such as canarypox (ALVAC) and fowlpox (TROVAC) that are naturally non-permissive for human cells. There is increasing evidence that such non-replicating vaccines are safer than the original vaccinia strains and yet are still comparably immunogenic[130,175–180].

The subject of non-replicating poxvirus vector development is too extensive for this review, but a few of the defining features can be summarized using MVA as the prototypical example (FIG. 5). MVA was derived from a Turkish smallpox vaccine strain (Ankara) that, after more than 500 passages in chicken cells, became defective for replication in human cells and avirulent in test animals[181]. From 1968–1980, MVA was inoculated into more than 100,000 individuals in Germany with no reported secondary complications and it is now considered to be a suitable platform for the next generation of safer smallpox vaccines and recombinant poxvirus vectors[182]. Genomic mapping and sequencing studies have revealed that MVA lost nearly 30 kb of genomic information during its extended passage in chicken cells and has multiple deletions and mutations compared with the parental strain[183]. Many of these genetic alterations were in host-response genes, and it is assumed that these deletions render MVA unable to complete its replication cycle in human cells[184,185]. Importantly, MVA was shown to retain a copy of the E3L host-range gene, and a targeted E3L deletion rendered the virus unable to replicate even in chicken embryo fibroblasts[142]. To facilitate the generation of MVA-based recombinant vectors, another host-range gene that is missing from MVA, K1L, was exploited in targeted insertion vectors as a selection marker to allow the replication of MVA–K1L-expressing virus in normally non-permissive rabbit RK13 cells[186–188]. Another technical advantage of MVA over other vaccinia strains is that the loss of immunomodulatory genes has caused the virus to induce excessive activation of infected human dendritic cells, which possibly explains its enhanced immunogenicity[75]. Future advances in the exploitation of MVA and other human-restricted poxvirus vaccine vectors will probably focus on modifying the immune responses of the infected host to specifically optimize presentation of key immunogenic epitopes by the non-replicating vector.

Poxviruses as oncolytic vectors. In the future, host-restricted poxviruses might be exploited as therapeutic oncolytic viruses. In addition to the use of poxvirus vectors to deliver cancer immunotherapeutics, or to provide vaccine vehicles for tumour-specific cellular epitopes[169,171,189], a wide range of viruses that exhibit increased replication or pronounced cytopathology in transformed cells have been explored as potential therapeutic agents to target and kill cancer cells[190–192]. For example, attempts have been made to harness vaccinia virus as an oncolytic vector to specifically target cancer cells[193,194]. Although wild-type vaccinia virus shows no specific predilection to bind and infect transformed cells, several studies have shown increased viral replication levels in tumours[195]. Furthermore, a vaccinia virus with deletions of the genes that encode thymidine kinase and the vaccinia growth factor showed preferential replication in rapidly growing tumour cells while becoming attenuated for overall virulence[196]. An important technical advantage of poxvirus-based vectors is the ability to insert multiple genes to increase the therapeutic potential of the virus or to assist in its visualization[195,197]. Although attempts have been made to target vaccinia binding to specific cell types by engineering virion surface proteins that mediate host cell binding[198], such attempts have never circumvented the ability of the virus to bind to and enter mammalian cells promiscuously. It is likely that future use of oncolytic poxviruses will involve

T_H1 IMMUNE RESPONSE
A host response to a pathogen that is skewed to the preferential activation of cell-mediated pathways, especially cytotoxic T-cells. By contrast, T_H2 immune responses are skewed towards the activation of humoral patyhways, especially antibodies.

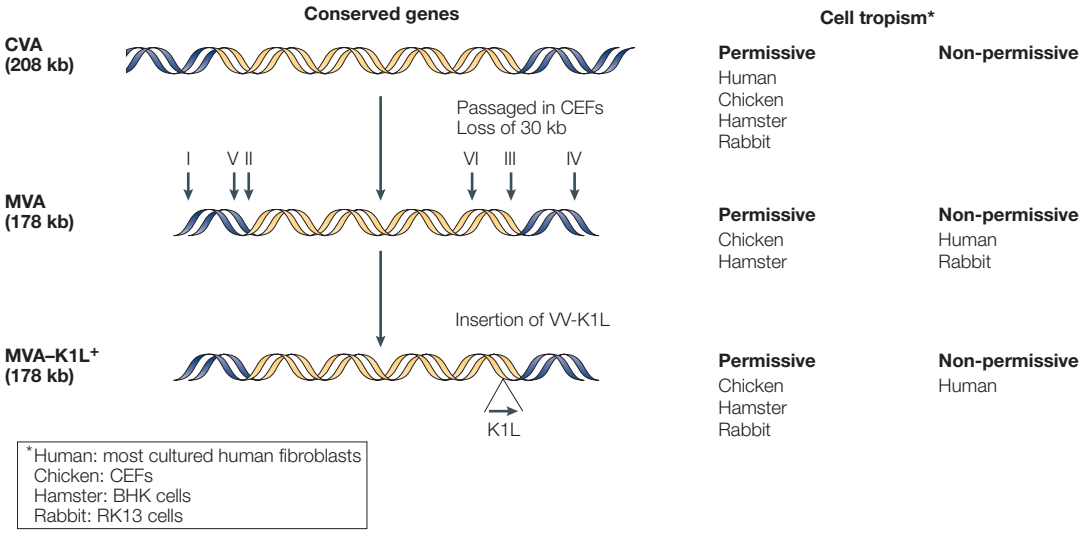

Figure 5 | **Origin of modified vaccinia Ankara strain.** Modified vaccinia Ankara (MVA) was derived from chorioallantois vaccinia Ankara (CVA), which is a smallpox vaccine strain that was used originally in Turkey and that was adapted for growth in chicken cells. After more than 500 passages in chicken embryo fibroblasts (CEFs), the CVA strain lost more than 30 kb of viral sequences that mapped to six main sites (denoted I–VI) and lost the ability to replicate in almost all mammalian cells, including human and rabbit kidney (RK13) cells, but was permissive for chicken embryo fibroblasts and baby hamster kidney (BHK) cells. MVA that is engineered to express the vaccinia K1L host-range gene regained the ability to replicate in RK13 cells, but not in human cells.

exploiting the signalling differences between normal and transformed cells so that the oncolytic virus will spread efficiently in tumour cells, as well as deliver therapeutic transgenes to assist in tumour killing and immunotherapy. In this regard, it should be noted that some poxviruses, such as myxoma virus, that are normally restricted to non-human cells, can nevertheless replicate robustly in human tumour cells[18], and provide additional platforms with which to explore poxvirus-based oncolytic therapies.

Concluding remarks

It has been a quarter of a century since smallpox was eradicated by the WHO vaccination programme. Although the potential re-emergence of smallpox as a consequence of deliberate bioterrorism has been the subject of intense speculation, the appearance of any pathogenic poxvirus that spreads efficiently from human-to-human would be considered an immediate public health crisis. The 2003 human monkeypox outbreak in the United States illustrates how vulnerable the human population is to the emergence and re-emergence of viral pathogens from unsuspected sources. In the case of poxviruses, we know little about the features that govern the species tropism of poxvirus–host relationships, or the hurdles that need to be overcome to initiate zoonotic poxvirus infections in non-evolutionary hosts. The best available evidence indicates that poxviruses bind to and enter mammalian cells promiscuously, but their ability to complete the complex cytoplasmic replication cycle that is needed to generate progeny virus, and then to spread successfully to a new host, can vary markedly between cells of different lineages and host species.

On the basis of our current knowledge, all the main determinants of poxvirus tropism at the cellular level are intracellular events that take place downstream of virus binding and entry. Although signals from specific sentinel host-cell receptors can probably regulate subsequent poxvirus replication, it seems that poxviruses do not require specific host-cell receptors for virus adsorption and fusion events, for the efficient internalization of the virus core structure or for the initiation of early transcription. Rather, the main features that functionally regulate subsequent events in the infected cell are the requirement for various *trans*-acting factors from the host cell and the ability to inhibit diverse cellular antiviral responses such as apoptosis and the interferon pathway. Also, the ultimate outcome of a given infection is potently influenced by the unique portfolio of immunomodulatory and host-range genes that give each poxvirus unique properties of host range, pathogenesis and the potential for host-to-host spread.

As more information is gathered about the molecular basis for tropism determinants of poxviruses, it is likely that new strategies will be uncovered to experimentally manipulate the natural species barriers that regulate zoonotic infections. This knowledge will facilitate the engineering of poxviruses as safer vectors for vaccines and gene therapy, and as tissue-targeted oncolytic viruses to treat human neoplasms. However, the sobering conclusion remains that even if the final stocks of variola virus are destroyed, the potential for the emergence of other poxvirus-derived human pathogens remains. Hopefully, as the general principles that govern poxvirus tropism and host range are better understood, we will also be better prepared to respond to other zoonotic virus infections.

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Competing interests statement

The author declares competing financial interests: see Web version for details.

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