Why are rabbits uniquely sensitive to myxoma virus? *Cherchez l'interferon!*

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Rabbits are the only animals susceptible to myxoma virus. The induction of interferon α/β by myxoma virus in nonpermissive mouse cells seems to be crucial to maintaining this species restriction.

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Myxoma virus, a member of the poxvirus family, causes a generalized, lethal disease in European rabbits (*Oryctolagus* species) and a localized benign cutaneous fibroma in wild rabbits in the Americas (*Sylvilagus* species). No other animal species is known to be susceptible to myxoma virus infection. The species barrier is so strict that in the 1950s the virus was deliberately released into the Australian countryside in an attempt to control the spread of European rabbits, a pest species in Australia. The mechanism responsible for this strict species barrier has not been explained, but it is known that, with some exceptions, the species barrier also exists at the level of isolated cells in culture. In this issue, Wang and colleagues[1] report that inoculation of nonpermissive mouse embryo fibroblast (MEF) cultures with myxoma virus leads to the activation of the mitogen-activated protein kinase (MAPK) Erk1/2, followed by the activation of interferon (IFN) regulatory factor 3 (IRF3) and ensuing induction of IFN-α and IFN-β synthesis. Newly synthesized IFN-α and IFN-β then trigger activation of the transcription factor STAT1, resulting in the establishment of cellular resistance to virus infection. The authors' provocative conclusion is that the interferon response triggered by myxoma virus accounts for the species barrier of poxvirus infection. This claim is based on the demonstration that MEF cultures become permissive for myxoma virus infection if myxoma virus–induced interferon induction or the effects of interferon are blocked. Furthermore, mice deficient in STAT1, which is known to be required for most interferon actions, succumb to myxoma virus infection upon intracranial inoculation, providing the first report of a lethal myxoma virus infection outside rabbits.

Key to the findings reported by Wang *et al.*[1] is the observation that inoculation of MEFs with myxoma virus leads to the phosphorylation of Erk1/2. Many other viruses, including influenza, human immunodeficiency virus and various herpesviruses, have previously been shown to activate Erk1/2 during productive viral infection. In permissive cells infected with another poxvirus, vaccinia virus, Erk activation was required for virus multiplication[2]. In contrast, Wang *et al.*[1] show that myxoma virus induces Erk1/2 activation in abortively infected cells (**Fig. 1a**). The signal responsible for Erk1/2 activation has not been identified, but because cycloheximide inhibited the activation process, it is likely that virus uptake, virus uncoating (which in poxviruses requires protein synthesis[3]) and perhaps some other biosynthetic events are required. Although activated Erk1/2 normally translocates to the nucleus, Erk1/2 activated by myxoma virus remains localized predominantly in the cytoplasm. Most importantly, Erk1/2 activation by myxoma virus seems to be responsible for the failure of myxoma virus to replicate in MEFs, because U0126, a selective inhibitor of MEK, the immediate upstream activator of Erk1/2, caused a marked increase in myxoma virus replication. Depletion of Erk1/2 by treatment with a specific antisense oligonucleotide also rendered the resistant MEFs permissive for myxoma virus infection. An apparent consequence of Erk1/2 activation by myxoma virus infection is the rapid phosphorylation and

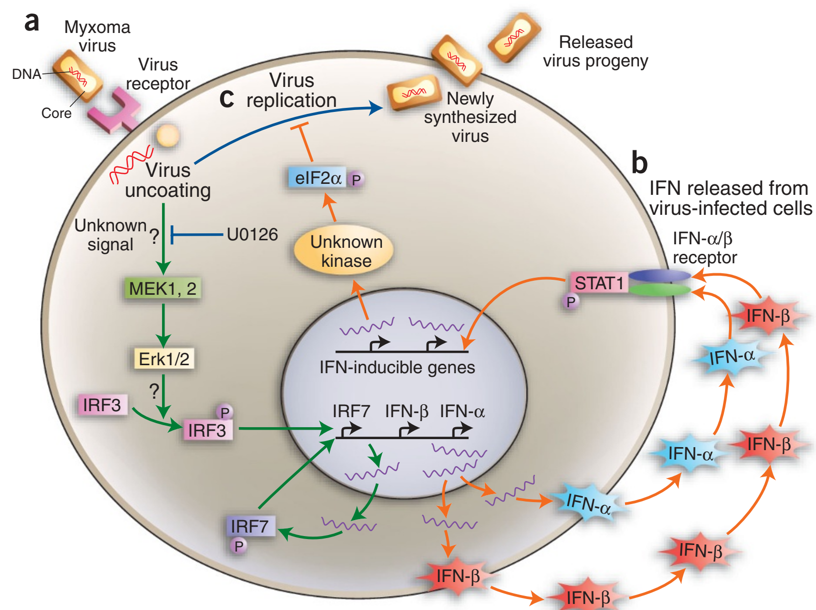

Figure 1 Resistance of nonpermissive MEFs to myxoma virus infection is mediated by Erk1/2 MAPK–triggered stimulation of IFN-α and IFN-β synthesis. (**a**) Inoculation of MEF cultures with myxoma virus does not result in efficient virus multiplication, as judged by the absence of late virus products. However, inoculation with myxoma virus evokes Erk1/2 signaling, activation of IRF3 by phosphorylation and *de novo* synthesis of IRF7. Activated IRF3 and IRF7 translocate to the nucleus and trigger transcription of IFN-β and IFN-α genes, followed by synthesis and secretion of IFN-β and IFN-α. (**b**) IFN-α and IFN-β released from the cells that had encountered myxoma virus bind to IFN-α/β receptors on other cells in culture, which triggers STAT1 activation, resulting in synthesis of proteins that render cells resistant to myxoma virus infection. Cellular blockade of myxoma virus replication correlates with phosphorylation of translation factor eIF2α, which leads to inhibition of protein synthesis. The kinase responsible for eIF2α phosphorylation is apparently not the double-stranded RNA–dependent kinase, PKR, usually implicated in interferon-mediated antiviral actions, but another unknown serine-threonine kinase. (**c**) If synthesis of IFN-α and IFN-β is disrupted in the myxoma virus–inoculated cells by the MEK inhibitor U0126 or by other means, cells become permissive for myxoma virus infection, resulting in full virus replication.

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nuclear translocation of IRF3, a key mediator of the transcriptional activation of the genes encoding IFN-α and IFN-β[4]. A role for Erk1/2 in IRF3 activation and interferon induction has not been previously reported, but whether Erk1/2 phosphorylates IRF3 directly or through activation of another kinase must still be resolved. Myxoma virus infection also led to *de novo* synthesis, activation and nuclear translocation of IRF7, which is required to induce expression of the full repertoire of IFN-α genes[5,6] (**Fig. 1b**). The Erk1/2-dependent induction of IFN-α and IFN-β upon myxoma virus infection of MEFs is responsible for the failure of myxoma virus to replicate, because neutralization of interferon with antibodies, or the use of IFN-α/β receptor–deficient or STAT1-deficient cells, resulted in fully permissive myxoma virus replication[1].

The transcription factor STAT1 is required for the majority of interferon-mediated actions[7]. Wang *et al.*[1] show that the interferon-induced, STAT1-dependent suppression of myxoma virus replication is independent of three well-known antiviral mechanisms[8], namely the interferon-inducible double-stranded RNA-dependent protein kinase (PKR), RNase L (the target of the interferon-inducible enzyme 2-5A synthetase) and Mx (a GTPase that interacts with viral nucleocapsids). Resistance to myxoma virus correlated with serine phosphorylation of the translation factor EIF2α, which leads to its inactivation. EIF2α phosphorylation in MEFs seemed to be independent of PKR, the kinase most often implicated in interferon-induced phosphorylation of EIF2α, and also of three other known EIF2α kinases, leading the authors to postulate the existence of a previously unknown interferon-dependent EIF2α kinase (**Fig. 1c**).

Wang *et al.*[1] are the first to link Erk1/2 activation to interferon induction. Their data support the conclusion that myxoma virus–induced Erk1/2 activation is required for myxoma virus–induced expression of IFN-α/β in MEFs. However, the exact role of Erk1/2 is not known. The authors have not ruled out the possibility that Erk1/2 might be inhibiting a pathway, either viral or cellular, that suppresses interferon production in this system. Whether Erk1/2 activation is sufficient for induction of IFN-α/β has also not been addressed. Other necessary events may be triggered by the interaction of a viral pathogen-associated molecular pattern, such as myxoma virus DNA or newly synthesized viral RNA, with a pattern recognition receptor, most likely Toll-like receptor 3 (TLR3), TLR-7 or TLR-9, such as has been demonstrated for interferon induction by other viruses[9].

Many other poxviruses are known to infect only one animal species (for example, human smallpox virus and raccoon, goat, sheep and camel poxviruses)[10]. In other virus families the species barrier is often determined by the presence or absence of specific virus receptors on the cell surface, but there is no evidence that receptors control the species barrier for poxvirus infections[3]. Has the work of Wang and coworkers[1] solved the 100-year-old riddle of the strict species barrier characteristic for many poxviruses? The proverbial jury is still out. A case in point is the final, crowning experiment in the paper by Wang *et al.*[1], which shows STAT1-deficient mice succumb to myxoma virus infection. To produce a lethal infection, the authors inoculated a high dose of virus intracranially, which does not resemble the common route of transmission in nature. However, it is known that STAT1-independent interferon-induced antiviral mechanisms exist[7], and thus some residual interferon-mediated antiviral actions may be working in STAT1-null mice. It is possible that mice deficient in IFN-α/β receptors could be more highly sensitive to myxoma virus than STAT1-deficient mice. Better yet, why not examine mice deficient in both IFN-α/β and IFN-γ receptors, which are exquisitely sensitive to many virus infections[7]?

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Are dendritic cells afraid of commitment?

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Dendritic cell subsets are thought to become committed to the dendritic cell lineage once they have differentiated from lymphoid or myeloid lineage precursors. However, this tenet has been challenged by data showing plasticity among the different subsets.

Two main subsets of conventional dendritic cells (DCs) have been characterized in mice, originally called the 'myeloid' DCs ($CD11c^+CD11b^+CD8\alpha^-$) and the 'lymphoid' DCs ($CD11c^+CD11b^-CD8\alpha^+$). In addition, in both mice and humans, precursor DCs of the plasmacytoid lineage (plasmacytoid precursor DCs) are cells present in various organs and specialized to efficiently produce large amounts of type I interferon in response to most viruses. Hematopoietic precursor cells committed to the generation of lymphoid (common lymphoid progenitor) or myeloid (common myeloid progenitor) lineages give rise to a mixture of $CD11c^+CD11b^+$ and $CD11c^+CD8\alpha^+$ DCs as well as plasmacytoid precursor DCs, indicating that the original definition of myeloid and lymphoid DCs is not reflected by their precursors[1] (**Fig. 1**). This plasticity may reflect the importance of maintaining the ability to promote the differentiation of the DC subset most suited to provide an effective response against an invading pathogen. Once differentiated, however, DC subsets have been assumed to become committed. However, in this issue of *Nature Immunology*, Zuniga *et al.*[2] show

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