A24R and A35R help Vaccinia play the genetic accordion

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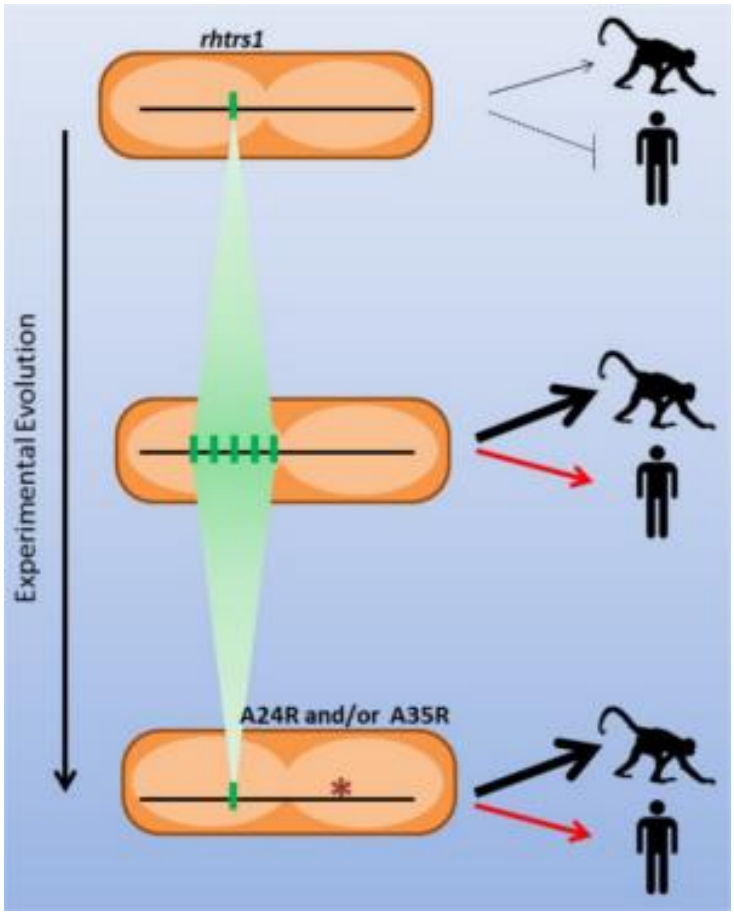

A vaccinia virus expressing one copy of the PKR antagonist rhtrs1 replicates poorly in African green monkey cells and not at all in human or rhesus cells (top). Amplification of rhtrs1 improves replication of the virus in several primate cells (middle). Adaptive mutations in the viral genes A24R or A35R allows for a collapse of the amplification while maintaining the replication potential (bottom).

Image provided by Dr. Greg Brennan.

Many human diseases caused by viruses, such as HIV and Ebola, first emerged from animal sources. Identifying which potential pathogens will cross the species barrier and lead to an epidemic is a challenging endeavor. It is therefore of paramount importance to identify new genetic and molecular signatures of viruses that are poised to adapt to new hosts. One of the reasons why viruses cannot freely cross the species barrier is a group of antiviral proteins known as restriction factors. One well-described restriction factor is protein kinase R (PKR), a host protein that inhibits protein synthesis in response to double-stranded RNA, a hallmark of viral replication. Because inhibition of protein synthesis represents a broad antiviral activity, many viruses have evolved PKR antagonists. For example, vaccinia virus (VACV) harbors two PKR antagonists, E3L and K3L. Intriguingly, PKR is evolving rapidly in primates, presumably as a strategy to evade PKR

antagonists. How viruses like VACV adapt to inhibit PKR from a new host species is not well understood.

A previous Fred Hutch study from Dr. Adam Geballe's Laboratory (Human Biology Division) reported that genetic amplification of a rhesus macaque cytomegalovirus gene, *rhtrs1*, a weak antagonist of PKR alleles from African green monkey (AGM) cells, was able to rescue the replication of a recombinant VACV that lacked its native PKR antagonists (E3L and K3L; Brennan et al., 2014). Gene amplification is a well-known adaptation mechanism, because of increases in both gene dosage and gene frequency. Previous studies suggested that adaptive mutations arise primarily in the amplified locus, with subsequent collapse of the amplification. A new study by the Geballe lab published in the *Journal of Virology*, led by former post-doctoral fellow Dr. Greg Brennan, currently at Western University of Health Sciences, identified mutations in VACV A24R and A35 genes, which arose after experimental evolution of a VACV isolate that contained an amplification of rhtrs1.

The authors first serially passaged VACV with amplified *rhtrs1* and identified 6 viral isolates that replicated better than the original virus. A combination of Southern blotting and deep sequencing identified fixed mutations in two genes, A24R and A35R, from isolates that harbored a single copy of *rhtrs1*. The investigators then examined activation of the PKR pathway by both immunoblotting and metabolic labeling, which revealed that either A24R or A35R mutated viruses indeed inhibited the PKR pathway downstream of PKR phosphorylation. A24R encodes the catalytic subunit of the viral polymerase whereas A35R is a conserved gene involved in modulation of host adaptive immunity through unknown mechanism(s).

Previous studies showed that other A24R mutations are resistant to the antiviral drug isatin β-thiosemicarbazone (IBT). To address whether the A24R mutations in this study behaved similarly, the authors infected AGM cells with either the A24R or the A35R mutant and found that the former but not the latter was partially resistant to IBT. Finally, the researchers tested whether A24R or A35R-mutated viruses could provide a replication benefit in cells other than the AGM cells used in this study. To this end, the authors infected human primary fibroblasts (HFF) or rhesus macaque fibroblasts (RF) with A24R and A35R mutated viruses and found that each replicated better than the original virus with amplified *rhtrs1*. In summary, this study is the first to show that adaptive mutation in the context of an amplified PKR antagonist can occur outside of the amplified locus, allowing the amplification to collapse to a single copy.

"I think the most important part of this study is identifying two new ways for vaccinia virus to evade PKR activation, particularly since neither of these genes have been implicated in PKR evasion before and both adaptive mutations happened outside of the genetic accordion of a known PKR

antagonist, RhTRS1. More broadly though, what I'm most excited about is the possibility of genetic accordions acting as "molecular footholds". The idea is that gene amplification provides a broad replication benefit and allows the virus to infect new host species. Once in that host though, it's possible that the adaptive mutations in one new host may be very different than those in a second new host. If this is true, then gene amplification may be an early biomarker for viruses at higher risk to cross species barriers, and developing technologies to screen for gene amplification in the field may help biosurveillance efforts," said Dr. Brennan.

Brennan G, Kitzman JO, Shendure J, Geballe AP. (2015). Experimental evolution identifies VACV mutations in A24R and A35R that antagonize the PKR pathway and accompany collapse of an extragenic gene amplification. J Virol, 89:9986-9997.

See also:

Brennan G, Kitzman JO, Rothenburg S, Shendure J, Geballe AP. 2014. Adaptive gene amplification as an intermediate step in the expansion of virus host range. PLoS Pathogens. Doi: 10.1371/journal.ppat.1004002.

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