TRS1 lives solely to antagonize PKR

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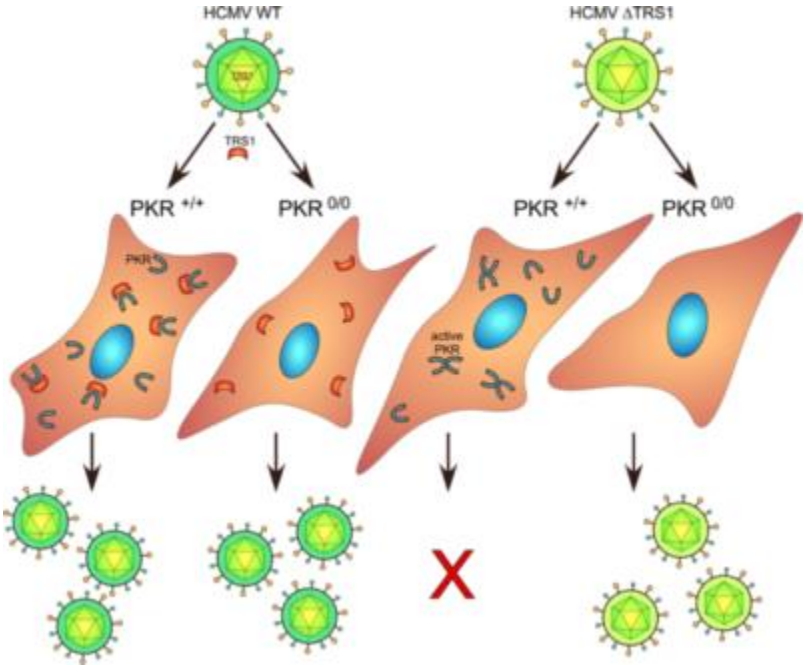

Schematic of the study. Wild-type (WT) Human cytomegalovirus (HCMV) can replicate efficiently in both wild-type (PKR+/+) and PKR-deficient cells (PKR 0/0). in contrast HCMV mutants that either lacks the protein kinase R (PKR) antagonist TRS1 or expresses a TRS1 mutant that cannot bind to PKR, replicate in PKR 0/0 but not PKR +/+ host cells.

Image provided by Dr. Stephanie Child.

The recent Zika virus outbreak illustrates the importance and complexity of virus-host interactions. To thwart viruses, host cells have evolved a wide variety of defense mechanisms. One well-characterized innate defense pathway involves Protein Kinase R (PKR), which responds to double-stranded RNA (dsRNA), a byproduct of viral replication. Activation of PKR results in phosphorylation of the eukaryotic initiation factor 2 (eIF2α), which in turn inhibits protein synthesis and prevents viral replication. Many viruses that infect human cells encode one or more PKR antagonists in their compact genomes. For example, human cytomegalovirus (HCMV) encodes two closely related proteins, TRS1 and IRS1, which can each can inhibit the PKR pathway. Previous studies from the Geballe Laboratory (Clinical Research and Human Biology Divisions) had demonstrated that a mutant HCMV lacking both TRS1 and IRS1 (HCMV [*Δ*I/*Δ*T]) was unable to replicate in cell culture. However, because both TRS1 and IRS1 participate in many other protein-protein interactions and may have other biochemical activities, it remains unclear which, if any, of these are important for HCMV replication.

A new Fred Hutch follow-up study from Dr. Adam Geballe's Lab, led by former graduate student Dr. Jacquelyn Braggin and published in *Virology*, addressed this question by first identifying TRS1 mutants that can no longer bind to PKR and then by generating PKR-deficient cells with CRISPR gene editing technology. To generate TRS1 mutants, the authors used a C-terminal charged-cluster-to-alanine (CCTA) method, wherein charged clusters of amino acids, which are likely to be surface-exposed, are mutated to alanine. After testing seven such mutants for PKR binding by utilizing a pulldown assay, the investigators successfully identified one CCTA mutant that could not bind to PKR, termed TRS-Mut1. Importantly, a recombinant HCMV expressing TRS1-Mut1 (HCMV [TRS1-Mut1]) could not replicate in human fibroblasts, similar to HCMV [*ΔI/ΔT*] and in contrast to HCMV expressing wild-type TRS1 (HCMV [TRS1]). Next, the authors evaluated PKR activation by monitoring levels of both total and phosphorylated PKR and eIF2α by immunoblotting, which revealed that indeed HCMV [TRS1-Mut1] was unable to prevent PKR activation. Finally, they tested whether PKR inactivation could rescue the replication prowess of either HCMV [TRS1-Mut1] or HCMV [*ΔI/ΔT*]. To this end, the scientists knocked down and knocked out PKR by shRNA and CRISPR/Cas9 gene editing, respectively. Strikingly, both HCMV [TRS1-Mut1] and HCMV [*ΔI/ΔT*] were able to replicate in PKR-deficient cells. In summary, while TRS1 and IRS1 can inhibit the autophagy, RNAseL and PKR pathways and bind to PKR and dsRNA, only the PKR inactivation activity appears to be required to viral replication. Said Dr. Geballe: "Among the surprising results of this study was our finding the a mutant virus that has the TRS1 gene but not the IRS1 gene does not block the PKR pathway completely. We are conducting experiments now to try to distinguish whether a simple gene dosage effect (having two redundant PKR antagonists) accounts for these results or if TRS1 and IRS1 play complementary but non-redundant roles in blocking the PKR pathway. Our results also raise questions about the importance of other functions of TRS1 and IRS1 during the CMV replication cycle. For example, TRS1 and IRS each block autophagy, a complicated "self-eating" cellular process that is proviral in some viral systems and antiviral in others. By eliminating the need for TRS1 or IRS to block the PKR pathway (by deleting PKR from the host cell) we can now explore whether the impact of TRS1 and IRS1 on autophagy is beneficial or harmful to the virus. Finally, knowing that antagonizing PKR is the only essential function of TRS1 and IRS1 will help push forward translational studies in which, in collaboration with investigator at University of Minnesota and Virginia Commonwealth University, we are attempting to develop a safe vaccine for use in preventing congenital transmission of CMV"

Braggin JE, Child SJ, Geballe AP. 2015. Essential role of protein kinase R antagonism by TRS1 in human cytomegalovirus replication. *Virology*. 489:75-85.

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