

# Cytomegalovirus TRS1 Must Bind Dsrna to Evade Detection by PKR

June 17, 2013

G Brennan

Cytomegalovirus (CMV) is a  $\beta$ -herpesvirus which causes severe disease in the immunocompromised (e.g. transplant patients), and it is a major infectious cause of birth defects. For a productive infection, viruses must evade or inactivate several host restriction factors that would otherwise halt virus replication. Protein kinase R (PKR) is one such restriction factor, inducing translational shutoff in response to double stranded RNA (dsRNA), a byproduct of most viral infections. CMV has evolved two PKR antagonists, TRS1 and IRS1; however, their exact mechanism of action is unclear. In a recent study published in *Virology*, Drs. Craig Bierle (Program in Molecular and Cellular Biology) and Adam Geballe (Human Biology and Clinical Research Divisions), along with Katie Semmens (University of Washington), demonstrate direct interaction between TRS1 and dsRNA, proposing a model in which TRS1 must bind to both dsRNA and to PKR in order to inhibit translational shutoff.

Previous truncation analyses demonstrated that dsRNA binding is necessary for TRS1-mediated inhibition of PKR. However, TRS1 encodes a noncanonical dsRNA binding domain, so it is unclear whether TRS1 directly binds dsRNA. To address this question, Bierle, *et al.* synthesized and purified TRS1 using a baculovirus system, and they confirmed that TRS1 directly binds dsRNA in the absence of other proteins. Using this purified TRS1, the authors determined that the TRS1 dissociation constant ( $K_d$ s) for binding to dsRNA was as low as 230 nM. However, in the same experiment they found that PKR bound dsRNA with a higher affinity ( $K_d$ s ~ 90 nM). At face value, these data would suggest that TRS1 cannot directly compete with PKR for free dsRNA. However, the authors also showed that TRS1 is expressed at significantly higher concentrations than PKR at relevant time points during infection, leaving open the possibility that TRS1 may sequester some dsRNA during infection.

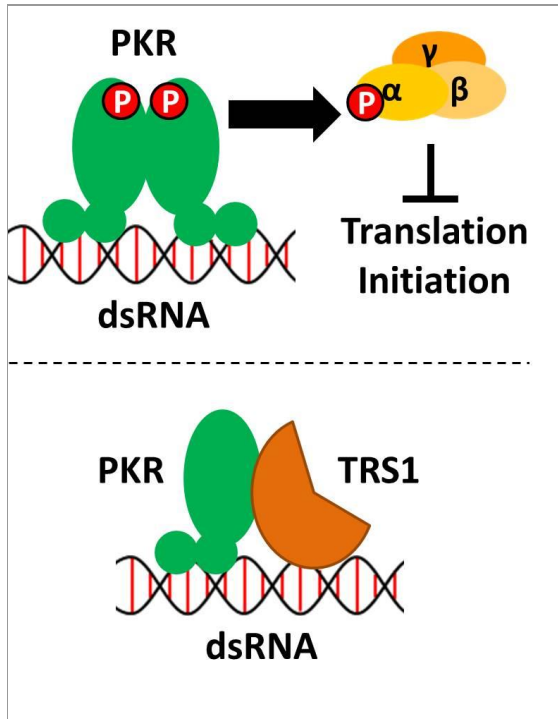
The dsRNA binding domain (dsRBD) of TRS1 is encoded by a motif present only in  $\beta$ -herpesviruses. Reasoning that charged amino acids are frequently involved in protein-nucleic acid interactions, the authors chemically modified arginine residues either before or after exposure to poly I:C, a dsRNA mimetic. Chemical modification before, but not after, the addition of poly I:C abolished TRS1 dsRNA

binding, confirming that arginine residues are critical for this function of TRS1. Combining these data and previous studies mapping the TRS1 dsRBD, the authors targeted eight conserved arginine or lysine residues for alanine mutagenesis to define residues critical for dsRNA binding. All of the single mutations reduced dsRNA binding modestly, and one significantly reduced dsRNA binding. However, Bierle et al. found that mutating either a group of three tightly clustered arginines in the center of the previously mapped domain, or a cluster of four amino acids at the 3' end of the domain, reduced dsRNA binding even more potently. All of these mutations retained the ability to bind PKR, with the exception of the four amino acid mutant, which did not express well, demonstrating that binding dsRNA and binding PKR are genetically separable phenotypes. These dsRNA binding-deficient mutants were unable to rescue replication of a virus deleted for its PKR antagonist, confirming that dsRNA binding is necessary for PKR inhibition by TRS1.

This study confirms that basic residues in the N-terminal half of TRS1 are critical for the direct binding of dsRNA, and that this interaction is necessary to inhibit PKR. Similarly, previous studies from the Geballe lab suggest that TRS1-PKR binding mediated by the C-terminus of TRS1 is also necessary for PKR inhibition. Taken together, these studies provide support for a trimolecular model of PKR inhibition, in which TRS1 binds to both dsRNA and PKR through separate domains to prevent PKR activation. "Given how divergent TRS1 is from other dsRNA binding proteins, having purified protein has opened the door to biochemical and structural studies of TRS1 function," said Dr. Bierle.

[Bierle CJ, Semmens KM, Geballe AP](#). 2013. Double-stranded RNA binding by the human cytomegalovirus PKR antagonist TRS1. *Virology*. Epub ahead of print, doi: 10.1016/j.virol.2013.03.024.

Also see: [Hakki M and Geballe AP](#). 2005. Double-stranded RNA binding by human cytomegalovirus pTRS1. *J Virol*. 79(12):7311-8.



*Image modified from an image provided by Dr. Craig Bierle.*

Schematic of the PKR activation pathway (top). PKR dimerizes and autophosphorylates upon binding dsRNA. Activated PKR phosphorylates eIF2 $\alpha$ , inhibiting translation initiation. Trimolecular model of PPKR inhibition (bottom). Cytomegalovirus TRS1 is proposed to bind both PKR and dsRNA, preventing the activation of PKR.