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The importance of transmembrane domain interactions in the viral control of apoptosis

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ABSTRACT

Viral control of apoptosis occurs through the expression of viral encoded anti-apoptotic B-cell lymphoma 2 (BCL2) analogs. These proteins are thought to restrain apoptosis by interacting with cellular BCL2 family members. We identified that protein-protein interactions between cellular and viral BCL2 transmembrane domains are crucial for the viral protein's function.

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In multi-cellular organisms, tissue development and homeostasis relies on tight control of programmed cell death. Furthermore, miss-regulation of apoptosis, or any other cellular mechanism that participates in the control of cell fate, has a strong impact on developed organisms as it usually leads to cancer, auto-immunity, or neurodegeneration among other disorders. Accordingly, programmed cell death is heavily regulated. This control relies primarily on the B-cell lymphoma 2 (BCL2) protein family. The BCL2 family, consisting of approximately 20 proteins, includes pro-survival, pro-apoptotic, and apoptosis activators. Pro- and anti-apoptotic BCL2 family members share four sequence homology domains (BCL2 Homology domain 1-4, BH1-4). On the other hand, activators of the BH3-only subgroup solely have the BH3 domain. Besides, most BCL2 proteins have a transmembrane domain (TMD) in the carboxyl-terminal (Ct) end that facilitates insertion into the target membrane.

Interactions among BCL2 proteins are crucial for the regulation of apoptosis. Anti-apoptotic BCL2 proteins inhibit the activation of pro-apoptotic members of this family through direct interaction or sequestering BH3-only activators. After an apoptotic stimulus, pro-apoptotic proteins and/or BH3-only activators will be released and, in turn, induce cell death through mitochondrial membrane permeabilization. These interactions among BCL2 family members were thought to occur through soluble domains, particularly through the BH3 domain. However, a seminal work proposed that interactions between pro- and anti-apoptotic TMDs can occur.¹

Apoptosis and autophagy also play a major role in the control and clearance of infectious diseases. It is generally accepted that apoptosis-induction is, in most infections, beneficial to the host. Eliminating infected cells mitigates the propagation of the infection and stimulates an appropriate immune response.² To elude the cell death mediated antiviral response viruses have developed multiple strategies to control apoptosis. These include masking of internal cellular sensors,

caspase regulation, signaling cascade modulation, and mimicking of BCL2 regulators with viral homologs known as viral BCL2s (vBCL2s; singular, vBCL2).^{3,4}

There is a structural homology between vBCL2s and their cellular counterparts. As most cellular BCL2 (cBCL2) many vBCL2s present a hydrophobic amino acid stretch on their Ct end. Using an *in vitro* assay based on the *E. coli* leader peptidase, an assay that allows for quantitative description of the membrane insertion capability of short sequences,⁵ we found that the Ct hydrophobic regions of herpesviral (HHV4, HHV8, and BOHV4) and poxviral (VACV, MYXV, and ORFV) vBCL2s can insert into endoplasmic reticulum (ER)-derived membranes as TMD despite a theoretical low hydrophobicity score for some of these regions.⁶ To avoid confusion, here we use the viral acronym to refer to the vBCL2 protein.

Next, we assessed whether the TMD of these vBCL2s exhibits any sort of self-association properties. For this purpose we used two approaches, a bimolecular fluorescent complementation (BiFC) assay adapted for the study of intramembrane interactions⁷ and BLaTM, a genetic tool designed to study TMD-TMD interactions in the bacterial membrane.⁸ Our results indicated that all vBCL2 TMDs can form homo-oligomers in mitochondrial membranes. Additionally, we investigated the potential TMD-TMD interactions between vBCL2 and pro-, anti-apoptotic, and BH3-only cBCL2s.

Our BiFC-based screening revealed that most viral TMDs can interact with multiple cellular TMDs. However, the particularities of these intramembrane protein-protein interaction networks varied from virus to virus, revealing distinctive mechanisms of action. Of note, we observed similar connection circuits among closely related viruses. These similarities could not have been inferred by the analysis of the TMDs sequences, which suggests a structural pattern underlying the sequence that governs intramembrane interactions.

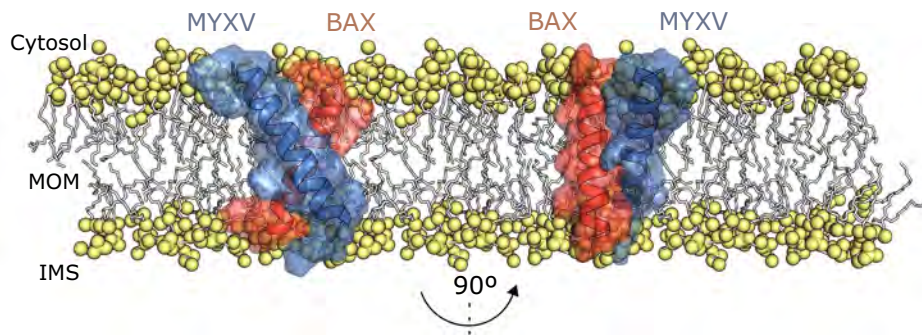


Figure 1. Interaction between BAX and myxovirus vBCL2 transmembrane domains. Model of a putative dimer between MYXV transmembrane domain (TMD, blue) and Bax TMD (red) obtained with the PredDIMER algorithm.⁹ The position of the mitochondrial outer membrane (MOM) was adjusted manually. The cytosolic and the inner membrane side (IMS) of the MOM are indicated. TMDs are shown with a cartoon (for the peptide backbones) and a surface (for side chains) representations.

An in-depth analysis of the TMD-TMD interactions between MYXV and the pro-apoptotic protein Bax on one hand, and HHV8 and BCL2 on the other, revealed that these intramembrane interactions are governed by ridge-groove arrangements created by an adequate disposition of large and small residues, where glycine residues play a key role by maximizing intimate contacts (Figure 1).

Next, to analyze whether the observed TMD-TMD interactions are required to control cellular apoptosis, we transfected HeLa cells with vBCL2 either with or without the TMD. We completed our study by including chimeras in which the TMD of each vBCL2 protein was replaced by the TMD of TOMM20, a mitochondrial protein that cannot establish TMD-TMD interactions with any cBCL2. Additionally, cells were either treated with doxorubicin or infected with VACV to induce apoptosis. Our results demonstrated that, once the TMD was removed the vBCL2 proteins could not promote survival or stop apoptosis (measured by Trypan blue staining and flow cytometry using propidium iodide staining and phosphatidylserine labeling (FITC-Annexin V), or by Caspase 3/7 levels). Similarly, the chimeras carrying the TMD of TOMM20 could not control apoptosis. These results suggest that TMD-TMD hetero-oligomerizations are crucial for modulating cell death regardless of the nature of the apoptotic stimulus.

Our work expands our knowledge about how viruses interact with their host and point to the membrane hydrophobic core as a new playground for host-viral interactions. Furthermore, these results increase our understanding of how viruses control cellular apoptosis and how apoptosis is regulated in the cell. The necessity of TMD-TMD interactions for successful apoptosis inhibition opens a new avenue for the development of therapeutic drugs against viral pathogens characterized by short- and long-term deregulation of programmed cell death.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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