Role of Chinook salmon PKR and PERK in protein translation inhibition during Golden Shiner Virus infection

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Understanding the role of Chinook salmon PKR in the antiviral response during Viral Haemorrhagic Septicaemia Virus (VHSV) infection

Research team

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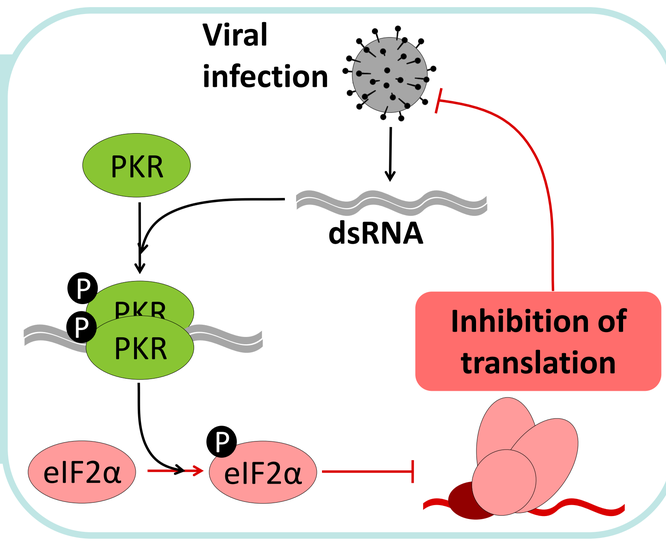
Background

The interferon-induced antiviral response is mediated through a wide range of interferon-stimulated genes (ISGs). The **double-stranded RNA-activated protein kinase R** (PKR) is one of the most studied proteins encoded by an ISG and is recognized as a multifunctional key factor of innate immunity. In mammals and in fish, it is known to be involved in cellular processes in response to stress signals, including **inhibition of protein translation initiation** *via* phosphorylation of the eukaryotic initiation factor 2α (eIF2α).

Aims

- **Is PKR from Chinook salmon (*Oncorhynchus tshawytscha*) induced *in vitro* during VHSV infection?**
- **Does Chinook salmon PKR inhibit host protein translation like its mammalian counterparts?**
- **To what extent is Chinook salmon PKR involved in the inhibition of VHSV replication?**



Three PKR isoforms are expressed during VHSV infection in CHSE-derived cell line

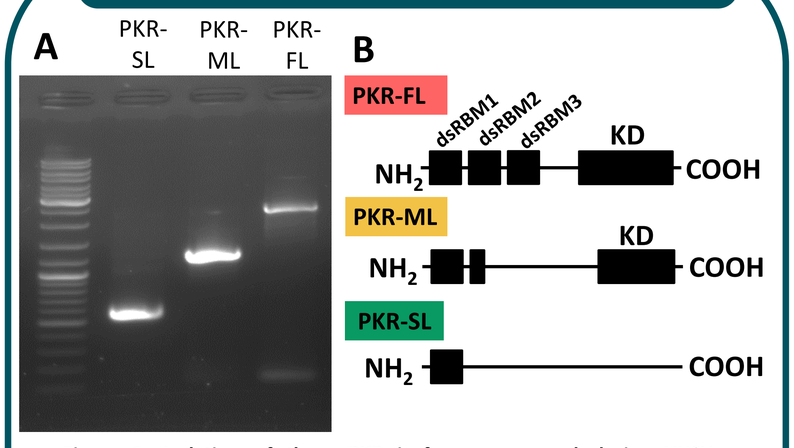


Figure 1: Isolation of three PKR isoforms expressed during $VHSV_{tomato}$ infection in EC cells. (**A**) Electrophoresis on 1% agar gel of PKR isoforms (FL = full length, ML = medium length, SL = short length) amplified from cDNA of CHSE-derived cells (EC) infected with $VHSV_{tomato}$ (Biacchesi *et al.*, 2010). (**B**) Schematic representation of the 3 isolated PKR isoforms. dsRBM = dsRNA binding motif, KD = kinase domain.

Development of a CHSE-derived cell line knocked-out for *pkr* by CRISPR-Cas9 genome editing

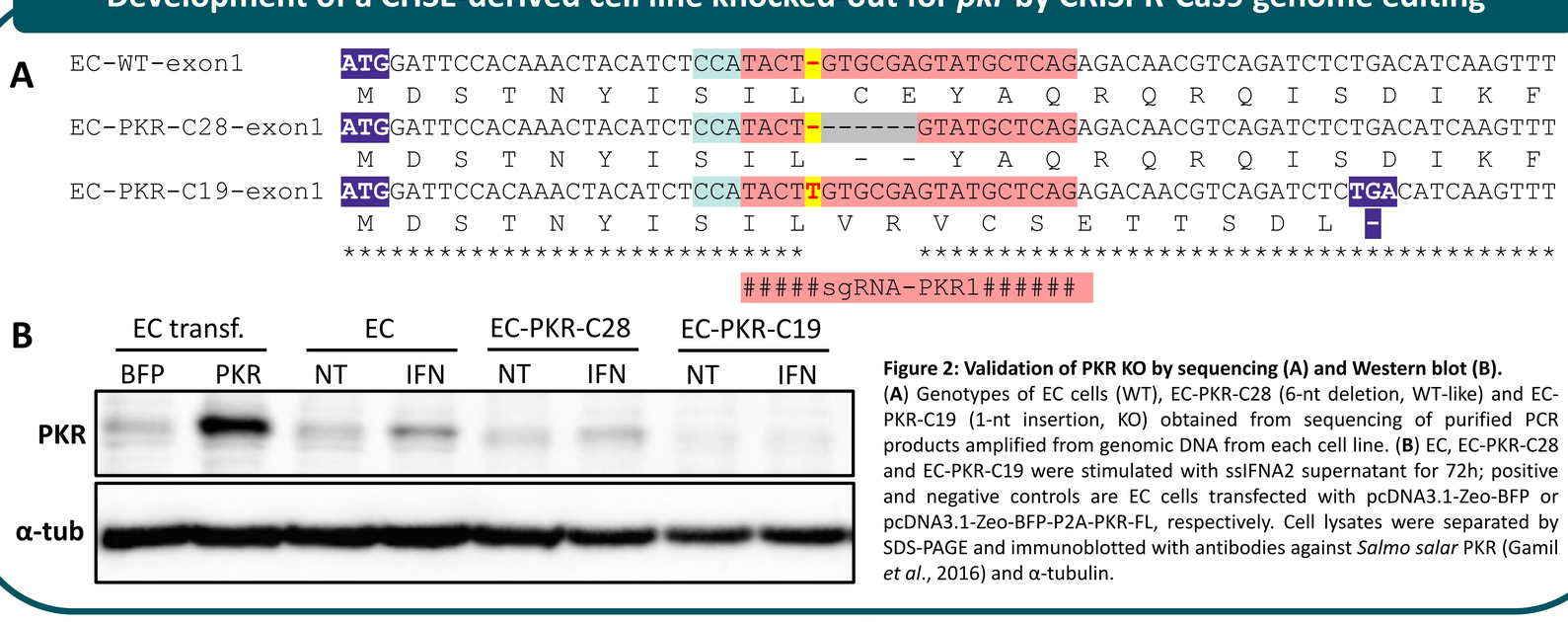
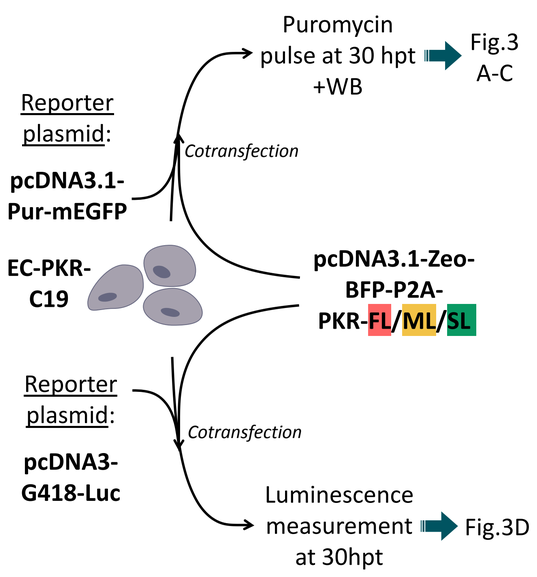


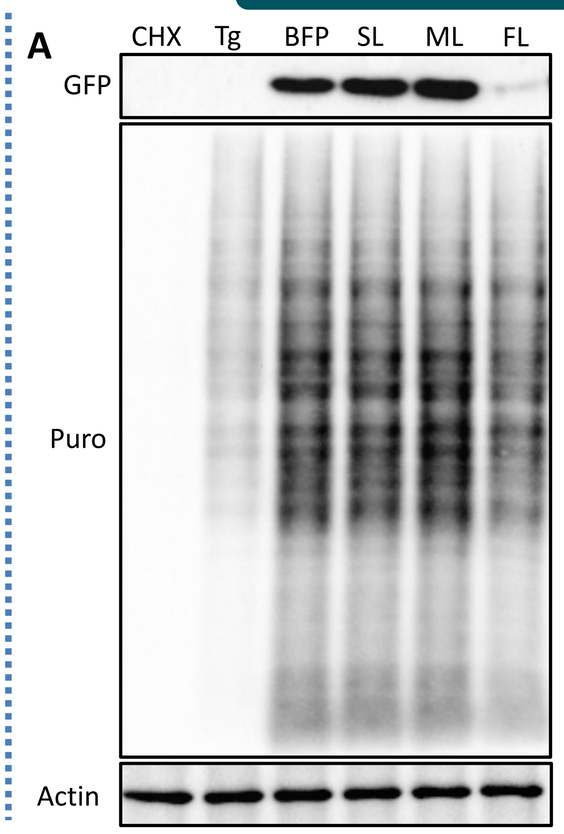
Figure 2: Validation of PKR KO by sequencing (A) and Western blot (B). (**A**) Genotypes of EC cells (WT), EC-PKR-C28 (6-nt deletion, WT-like) and EC-PKR-C19 (1-nt insertion, KO) obtained from sequencing of purified PCR products amplified from genomic DNA from each cell line. (**B**) EC, EC-PKR-C28 and EC-PKR-C19 were stimulated with ssIFNA2 supernatant for 72h; positive and negative controls are EC cells transfected with pcDNA3.1-Zeo-BFP or pcDNA3.1-Zeo-BFP-P2A-PKR-FL, respectively. Cell lysates were separated by SDS-PAGE and immunoblotted with antibodies against *Salmo salar* PKR (Gamil *et al.*, 2016) and α-tubulin.

Only one PKR isoform inhibits host protein translation

Overexpression study

Materials and methods





B C D

Figure 3: PKR inhibits host protein translation. (**A**) EC-PKR-C19 cells were cotransfected with pcDNA3.1-Pur-BFP and plasmids encoding the different PKR isoforms. At 8 hpt, mock transfected cells were treated with cycloheximide (CHX, 50 µg/mL, 24h) or thapsigargin (Tg, 2µM, 45 min). At 30 hpt, cells were pulsed with puromycin (5 µg/mL) for 15 min and cell lysates were separated by SDS-PAGE and immunoblotted with antibodies against GFP, puromycin and actin. (**B;C**) Quantification of (A). GFP (**B**) and puromycin (**C**) signal intensity normalised to actin signal intensity and graphed as fold change relative to BFP transfected cells. Bars show means ± SD from two pooled independent experiments. (**D**) EC-PKR-C19 cells were cotransfected with pcDNA3-G418-Luc and plasmids encoding the different PKR isoforms. Luminescence signal intensity normalised to fluorescence signal intensity and graphed as fold change relative to BFP transfected cells. Data shown are means ± SD and are representative of 2 independent experiments. *, $p < 0.05$; ****, $p < 0.0001$, one-way ANOVA with Tukey's post-hoc multiple comparison tests.

Overexpression of PKR-FL inhibits VHSV replication while PKR KO favours its replication

Overexpression study

Figure 4: PKR inhibits $VHSV_{tomato}$ replication. EC-PKR-C19 cells were transfected with pcDNA3.1-Zeo-BFP or plasmids encoding PKR isoforms. At 24 hpt, cells were infected with $VHSV_{tomato}$ (MOI 1) and fluorescence was measured at different time points post-infection. Fluoresence signal intensity graphed as fold change relative to BFP transfected cells; data shown are means ± SD from 6 independent experiments, *, $p < 0.05$, two-way ANOVA with Tukey's post-hoc multiple comparison tests.

KO study

Figure 5: Invalidation of PKR favours $VHSV_{tomato}$ replication. EC, EC-PKR-C28 and EC-PKR-C19 cells were infected with $VHSV_{tomato}$ (MOI 1) and fluorescence was measured at different time points post-infection. Data shown are means ± SD from 8 independent experiments. Two-way ANOVA with Tukey's post-hoc multiple comparison tests were performed on log-transformed data: *, $p < 0.05$ starting from 72 hpi.

Perspectives

- Study the **expression profile of PKR isoforms** over the course of VHSV infection
- Explore if endogenous PKR inhibits **host protein translation during VHSV infection** (KO study)
- Identify if the PKR-dependent modulation of VHSV replication impacts the **extracellular viral titre**
- Investigate the role of PKR in regulating the antiviral response *via* **transcriptomic study**

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Biacchesi S. *et al.*, J Virol., 2010, doi: 10.1128/JVI.00343-10; Gamil AA. *et al.*, Viruses, 2016, doi: 10.3390/v8060173

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