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Innate immune responses to SAV3 infection in vitro

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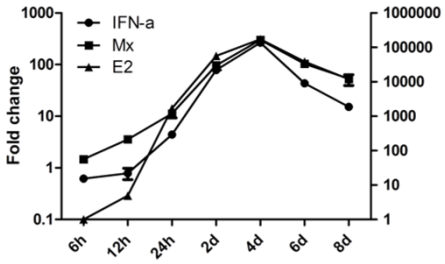
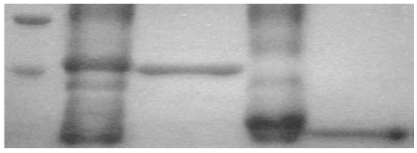
Two motivations

Protection against infection with alphaviruses is reliant on neutralizing antibodies and an intact innate immune response (IFN-induced)



Cellular responses to SAV-3 infection in TO cells (salmon macrophage cell line) correlated with E2 transcript levels

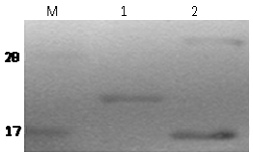
Cloning and expression of IFNα



rIFNα

trIFNα

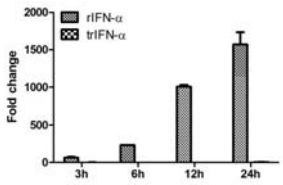
Cloned and expressed IFNα as full length and truncated form



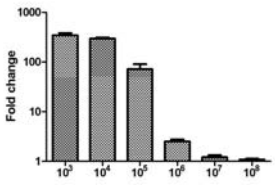
Western blot analysis of rIFN-α (lane 1) and trIFN-α (lane 2) in bacterial lysate. The predicted molecular weight of full length and truncated IFN-α are approximately 19.6 and 16.0 kDa respectively (M – marker lane).

Cheng et al., J Virology, 84(17):8903-12, 2010

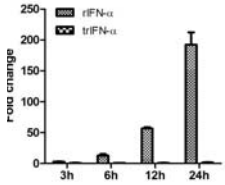
Functional activity of rIFNα



Mx – time course



Mx – dose-response



ISG15 – time course

Induction of Mx and ISG15 expression in TO cells after treatment with full length recombinant (rIFNα) and truncated (trIFNα)

Documenting the functional activity of rIFNα

Cheng, et al. J Virology, 84(17):8903-12, 2010

Effect of rIFNα pretreatment – protection against CPE

TO cells were pretreated with rIFNα for 24h prior to SAV-3 infection – serial dilution of rIFNα

Induce an antiviral state

Monitor to what extent IFNα treated cells are protected against virus infection

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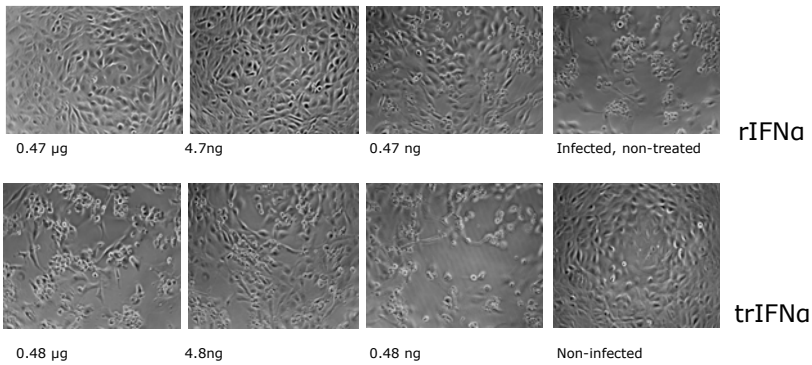
Protection against CPE



CPE occurs at IFNα concentration of 0.47ng/ml while for trIFNα there is no protection against CPE

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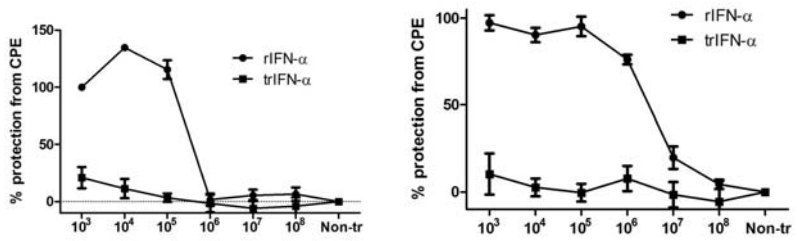
Protection against CPE (quantification)

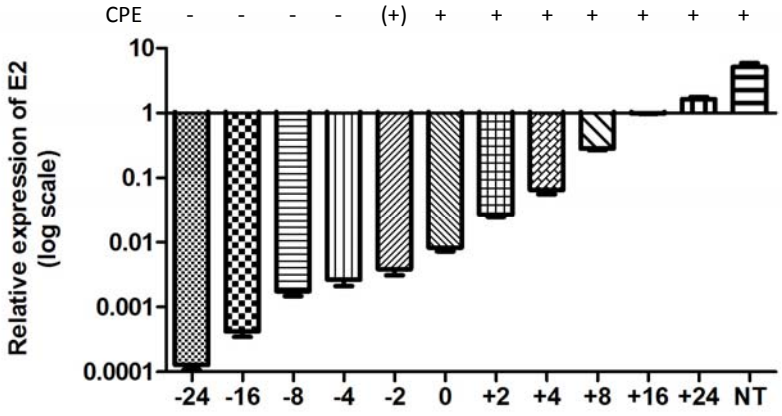


Cell viability of cell cultures subjected to the different treatments was assayed for viability (quantified using the CellTiter 96® AQueous One solution cell proliferation assay kit (Promega) at day 10 post infection when strong CPE developed in untreated cells)

Pretreatment of TO cells and CHSE cells gave a full protection against virus-induced CPE and 100% viability at 4.7ng/ml.
Findings are concordant with morphology assessment.

Cheng, et al. J Virology, 84(17):8903-12, 2010

Timing of IFN-α treatment



Treatment 4 to 24h prior to infection results in marked reduction in virus replication (2000 to 40000-fold reduction) and protection against CPE

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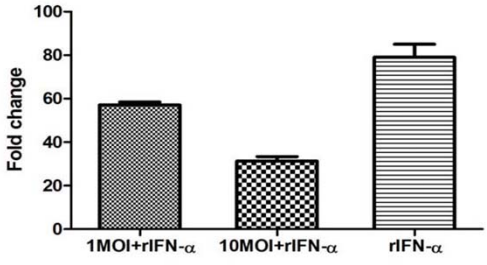
How is SAV3 manipulating the responses in infected cells?

Inhibition of ISG mRNA expression in response to IFNα

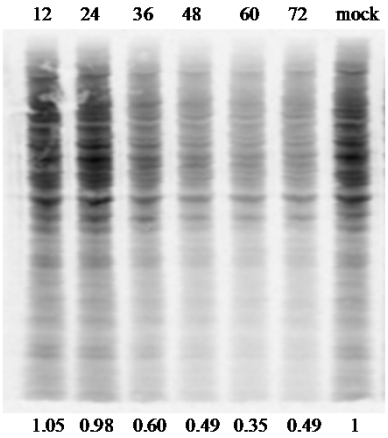


Inhibition of Mx mRNA transcription by SAV-3

TO cells were infected with 1 or 10 MOI SAV-3 or left non-infected . 12 h post infection cells were treated with IFN-for 12 h and analysed for Mx expression by real-time PCR

Protein shut-down post infection

- Confluent TO cells grown in 6-well plate and infected with SAV-3. Cells were grown for 36h and then washed and pulsed with ^{35}S-Met. At different times post pulsing the cells were harvested and electrophoresed and transferred to a membrane.
- The membrane was exposed in a phosphor-imager cassette and then scanned using a Typhoon.
- The protein amount was quantified with ImageJ software and the value was expressed relative to mock infected control and corrected for protein amount loaded in each lane.
- The results show 40% reduction by 36h and 65% by 60h post infection
- Mock infected to the far right.



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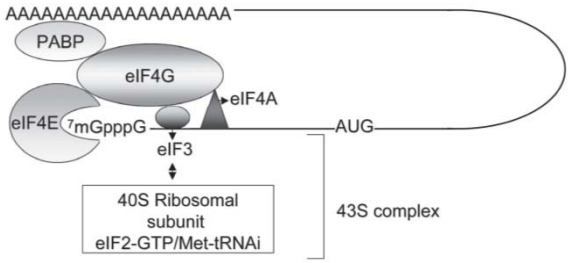
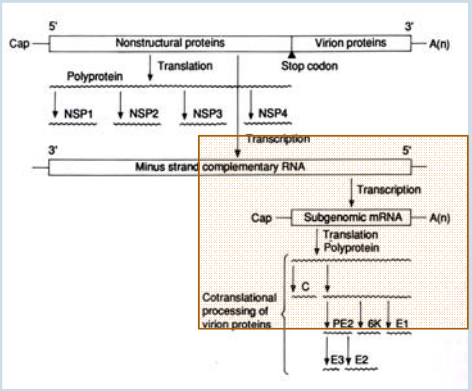
Translation initiation

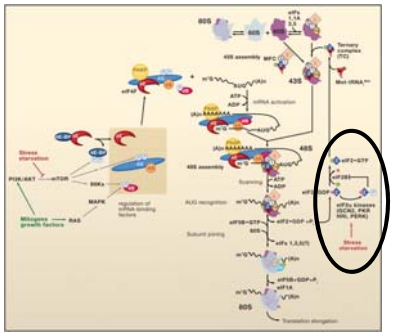


Mechanism of cap-dependent translation initiation. Schematic representation of the closed-loop model of translation initiation. In this model, the eIF4F complex interacts with both the 5'end of the mRNA (via eIF4E) and the poly(A) tail (via PABP) and recruits the 40S ribosomal subunit via its interaction with eIF3

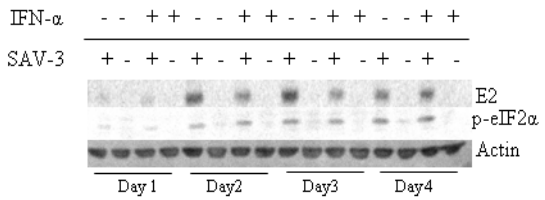
Cap-dependent initiation at subgenomic level (26S)



Subgenomic mRNA translation is cap-dependent



Translation of 26s subgenomic RNA is probably resistant to eIF2α phosphorylation



- TO cells were infected with SAV-3 (MOI 1) and at day 1, 2, 3, 4 postinfection, uninfected (-) and infected (+) cells were treated with rIFN-α for 16 h. Expression of E2, p-eIF2α and actin protein was detected by western blot.
- SAV-3 infection results in PKR induction and phosphorylation of eIF2α
- Envelope protein E2 are synthesized in the presence of eIF2αphosphorylation which points towards 26S sub-genomic mRNA translation being resistant to eIF2α phosphorylation

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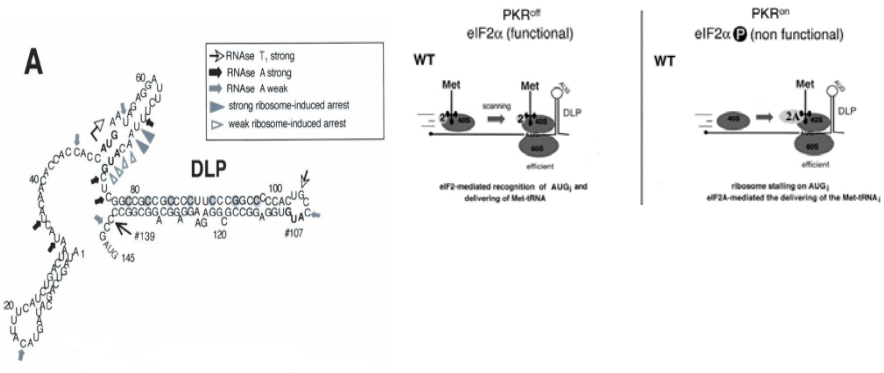
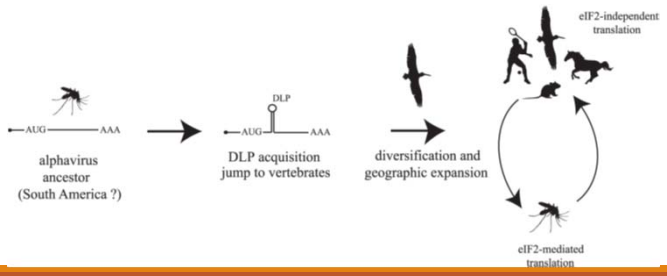
Translational resistance of Sindbis virus 26s mRNA to eIF2α phosphorylation



Ventoso et al., 2009

Conclusions

- SAV3 replicates under high concentrations of IFNα
- Pretreatment (>4h prior to infection) of cell cultures results in protection against CPE and lowering of virus production
- Initiation of translation is cap-dependent, both for primary translation from in-coming +strand and for subgenomic mRNA translation
- Formation of structural proteins from subgenomic mRNA occurs at high rate despite eIF2a phosphorylation and protein shutdown likely because of compensatory guiding of initiation complex by secondary structure formation of the subgenomic mRNA



Ventoso, J. Virol. 2012, 86(17):9484.