# Do cellular defences help Herpes simplex virus 1 regulate viral gene expression?

## Introduction

- For a virus to successfully create new progeny and conduct viral gene expression it must first take over the cellular machinery in the host cell allowing the virus to synthesize viral proteins.
- The cells do not go down without a fight as restriction-factor-mediated intracellular intrinsic, and innate immune responses protect the host cells by preventing replication from taking place within them (Lanfranca et al., 2014).
- Through years of coevolution, Herpesviruses have developed ways to surmount host defenses to enable their own gene expression (Full and Ensser, 2019).
- Prior student research showed production of a late viral protein mRNA was potentially dysregulated in cells lacking an intact defense suggesting that host defenses may not be exclusively detrimental to the virus.

## Aims

- To investigate if there is dysregulation of viral mRNA production in cells lacking an intact immune response versus those capable of responding to HSV-1 infection.

## Methodology

- Cells with and without an intact antiviral response were split, counted, and grown in multiwell dishes overnight.
- The following day cells were infected with 5pfu per cell of HSV-1 and left for 4 hours for preliminary tests or 2, 3, 4 and 5 hours for the final data set.
- RNA prep was done using the Monarch Total RNA mini-prep kit
- One Step RT-qPCR using primers for VP5 and GAPDH was performed on purified total RNA from cells with and without an intact antiviral response and on no template (NTC) and minus reverse transcriptase (–RT) controls.

## Optimization and Results

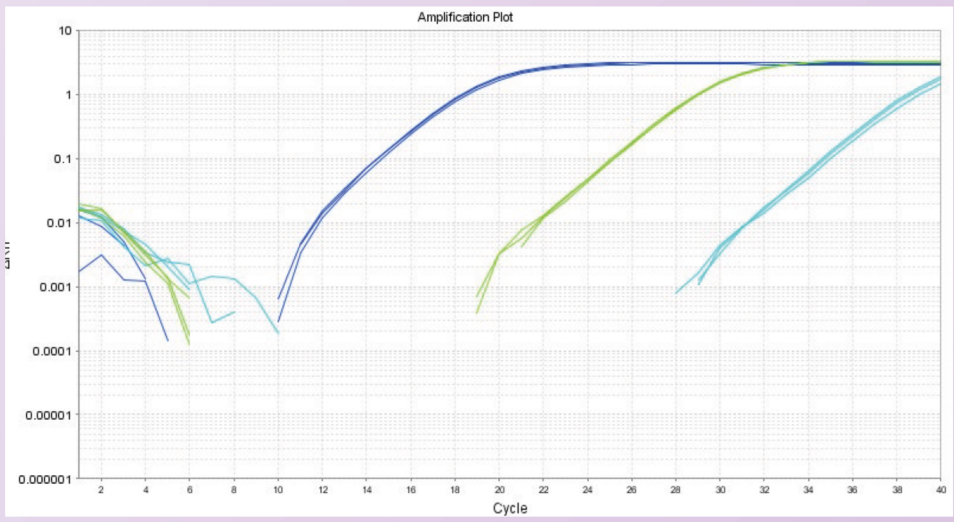


***Figure 1: Initial one-step qRT-PCR*** shows amplification of viral DNA in –RT controls
Cells lacking antiviral response (4hpi) -RT control of infected cells
Uninfected cells

- Our initial results showed an unexpected amplification of product in the –RT reactions suggesting carried over viral DNA was being amplified.
- Following this a different supplier of RNA prep kit was used with no change in outcome

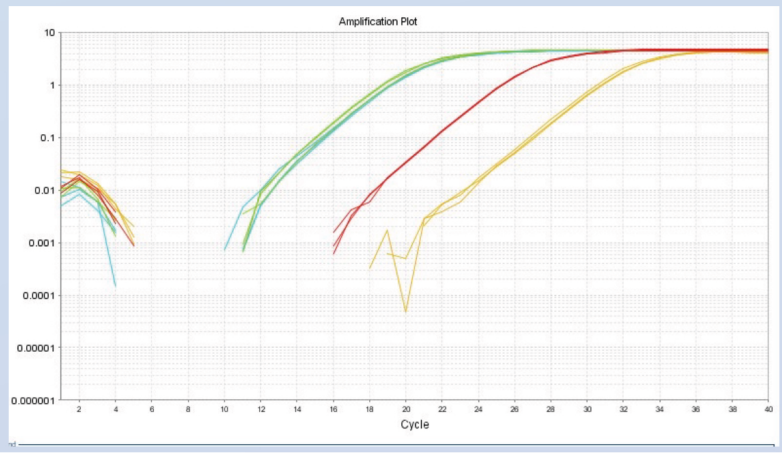


***Figure 2:*** Double DNase treatment removes sufficient vDNA to allow accurate quantitation of mRNA
DNase x1 -RT control DNase x1 DNase x2 -RT control DNase x2

- Next, we used a double DNase treatment with the original RNA purification kit.
- This was to see if treating twice would eliminate background vDNA amplification.
- This worked well shifting the –RT amplification curve to later cycles comparative to the +RT reactions.

- Finally, we tested a dilution series of RNA (1:10, 1;100, 1;1000), with 1:100 being optimal to reduce –RT amplification to a point where it was insignificant and would not interfere with mRNA quantitation.

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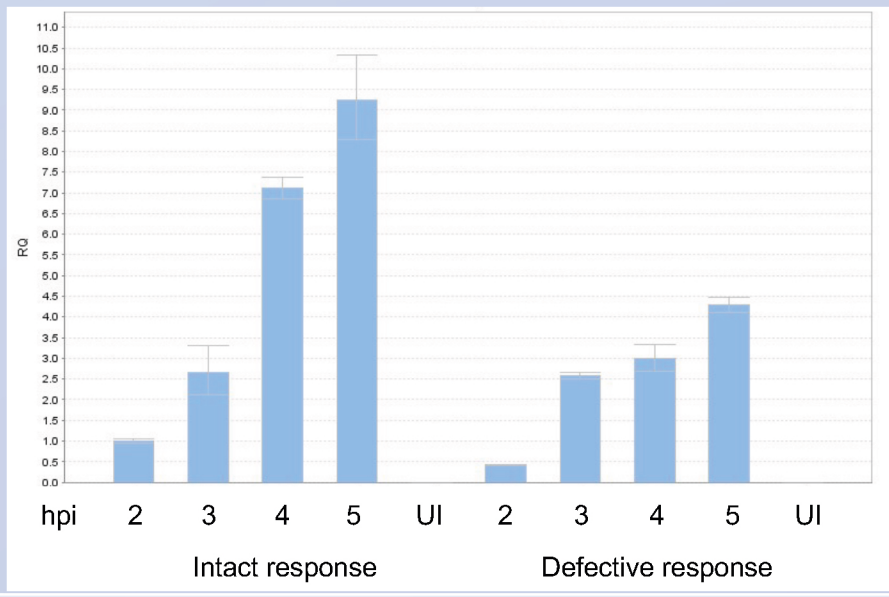


**Figure 3:** Expression of VP5 at 5 hpi in cells with or without an antiviral response shows a similar pattern. Data relative to intact 2hpi mRNA levels.

Very similar amplification values for both cells types were seen at 2 and 3hpi, but at 4hpi and 5hpi mRNA levels were ~2 times higher in intact cells than those lacking an antiviral response. Though in both cases the trend to increasing expression over time was the same.

## Discussion

- From the results, we can see that the trend we were expecting is not present. Both cell types show increasing RNA levels, as opposed to what was suggested may be the case by prior research.
- Initial data shows the importance of –RT controls in one step RT-qPCR, as without these we would have misinterpreted the results which would have also measured contaminating vDNA.
- Finally, it presents the fact that RNA prep kits are not completely effective as even when optimizing the conditions multiple times, background DNA amplification can occur (although insignificant).

**References:** Full, F. and Ensser, A. (2019) Early Nuclear Events after Herpesviral Infection. Journal of Clinical Medicine, 8(9). Lanfranca, M.P., Mostafa, H.H. and Davido, D.J. (2014) HSV-1 ICP0: An E3 Ubiquitin Ligase That Counteracts Host Intrinsic and Innate Immunity. Cells, 3(2), 438-454.

**Student:** Evie Muñoz-Pujol

**Supervisor:** Dr Ashley Roberts

University of Lincoln
Lincoln Academy of Learning and Teaching

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