Understanding RNA Polymerase II Transcriptional Regulation Upon HSV-1 Infection

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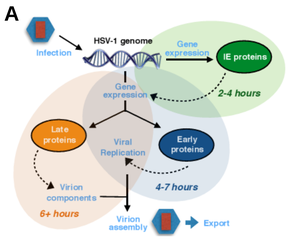
Background

Herpes Simplex Virus 1 (HSV-1) is a large double stranded DNA virus that encodes ~80 genes temporally regulated in three classes: immediate early (IE), early (E), and late (L) genes (**A**).

None of these genes code for general transcription factors or an RNA polymerase, therefore HSV-1 hijacks host cell transcription machinery to transcribe its genome.

A



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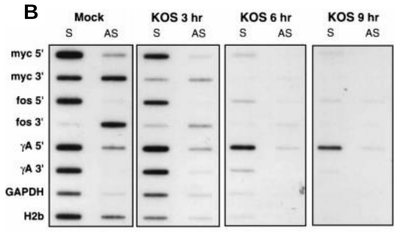
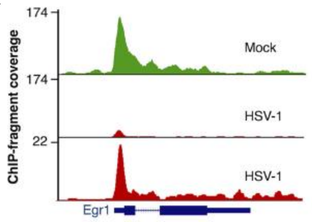


HSV-1 potently represses host cell transcription by 6h post-infection[1] (**B**) through the near-complete removal of RNA Polymerase II (Pol II) from the host genome as seen by Pol II ChIP-seq experiments in mouse NIH-3T3 cells[2] (**C**). This effect is seen globally, with 98% of genes undergoing a >2-fold repression as compared to Mock infected cells (**D**).

C



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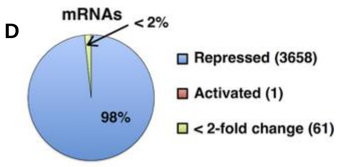
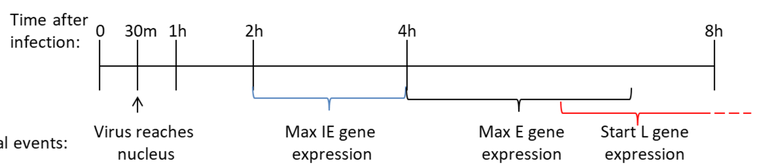


Key Questions

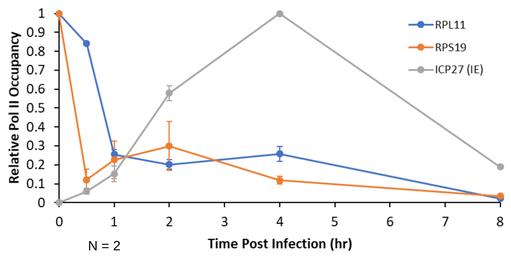
1. Are the losses of Pol II occupancy on host genes and the presence of Pol II occupancy on viral genes temporally correlated?
2. Which viral proteins are involved in repressing host transcription?

Pol II occupancy is lost as early as 30m post-infection

I monitored Pol II occupancy on the host and viral genomes along a timecourse of infection with wildtype (WT) HSV-1 by Pol II ChIP-qPCR in HEK 293 cells. These time points encapsulate major events in the viral lifecycle.



Pol II occupancy is lost from some genes as early as 30 minutes post-infection. Furthermore, loss of Pol II occupancy on the host genome coincides with an increase of Pol II occupancy on viral genes.

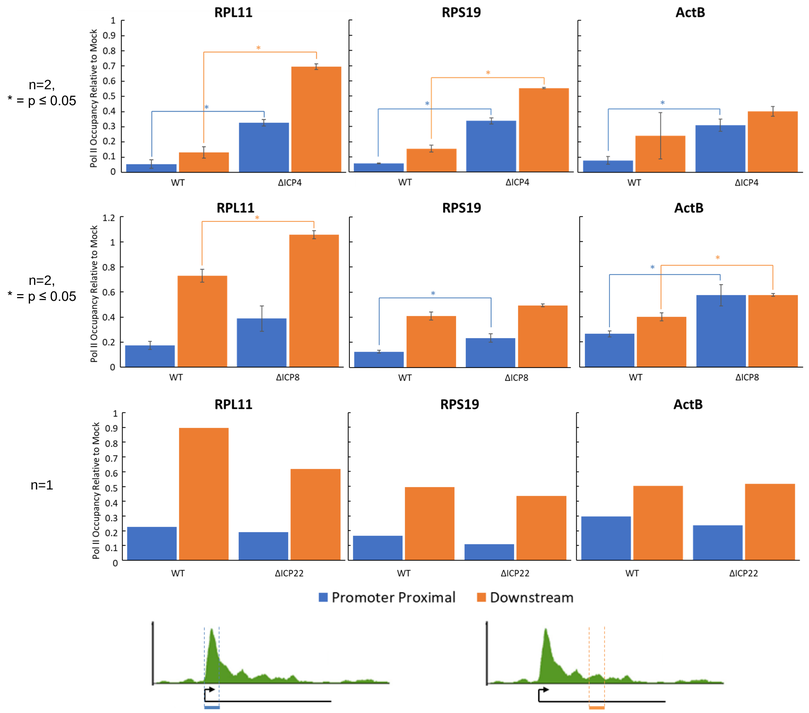


Identifying viral proteins that mediate host transcription

Given the kinetics of repression observed with the WT HSV-1 virus, I decided to focus on the HSV-1 IE proteins and ICP8, since these are the viral proteins expressed early during infection and could play a role in altering host transcription.

Protein	Class	Function with transcription machinery
ICP0	IE	**Alters histone dynamics** on host cell chromatin
ICP4	IE	Interacts with **TFIIB, TFIID, Mediator**
ICP22	IE	**Blocks TFIIE association**, interacts with P-TEFb, induces Pol IIi CTD pattern
ICP27	IE	**Binds to Pol II CTD**, alters splicing, conserved in all mammalian herpesviruses
ICP47	IE	Promotes immune evasion
ICP8	E	**Co-purifies with Pol II**, viral replication compartments

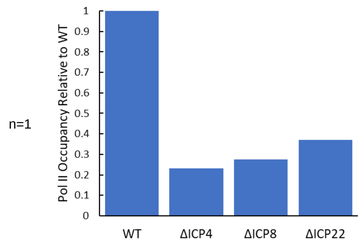
To see if a viral protein mediates Pol II occupancy on host genes, I infected HEK 293 cells with mutant HSV-1 viruses that lack an individual viral protein (ΔICP_) for 4h and performed Pol II ChIP-qPCR for various host genes. If Pol II occupancy at a gene increases upon the deletion of a viral protein, then that viral protein mediates Pol II occupancy in some manner.



Deletion of either immediate early protein ICP4 or early protein ICP8, but not ICP22, partially recovers Pol II occupancy on select host genes.

Pol II occupancy changes on viral genome

To see how Pol II occupancy changes on viral genes upon infection with mutant viruses, Pol II ChIP-qPCR was performed looking at the viral early gene Thymidine Kinase.

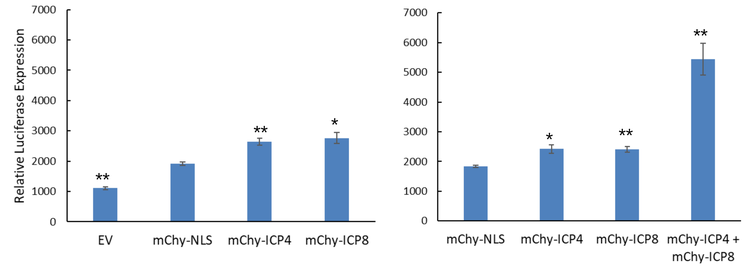


Infections with WT and mutant viruses were done at the same multiplicity of infection for 4h. Input chromatin from these samples gave approximately equal Ct values by qPCR, suggesting that infectivity is not reduced in mutant viruses.

Regardless of which viral protein is deleted, Pol II occupancy on viral genomes is reduced.

Exogenous ICP4 and ICP8 activate luciferase expression

To see what effect these viral proteins have on host transcription in the absence of infection, HEK 293 cells were co-transfected with a luciferase reporter plasmid driven by 3xAP-1 sites and either: empty vector (EV), mCherry-NLS, or a plasmid expressing an mCherry-tagged viral protein.

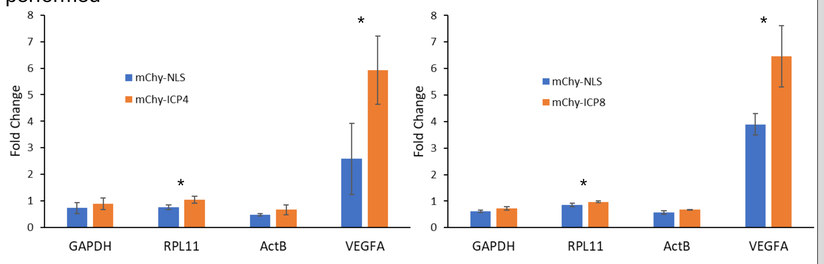


Left: 1ug of each plasmid was transfected. Right: 500ng of each plasmid was transfected supplemented with 500ng of EV unless otherwise noted. Statistical significance determined by comparing samples to mChy-NLS. N=3; * = p < 0.0025; ** = p < 0.0010.

The effects of ICP4 and ICP8 on luciferase expression are additive.

Exogenous ICP4 and ICP8 do not repress transcription

Untransfected HEK 293 cells were sorted alongside those transfected with either mChy-NLS, mChy-ICP4, or mChy-ICP8 to see what effect these viral proteins have on endogenous, chromatinized genes. Total RNA was harvested and qRT-PCR was performed



N=3; * = p < 0.05

N=2; * = p < 0.05

Future Directions

- What effect do the other IE viral proteins have on host gene transcription?
- How do ICP4 and ICP8 alter the ability of Pol II to bind to host genome?
- What is the mechanism by which Pol II is lost from the host genome?

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References

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