Understanding RNA Polymerase II Transcriptional Regulation Upon HSV-1 Infection

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Introduction

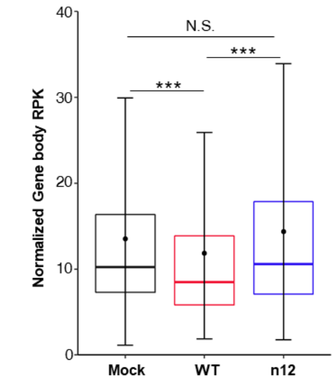
Herpes Simplex Virus 1 (HSV-1) has long been known to globally repress host transcription upon infection. The mechanism of repression is due to loss of host Pol II on the host genome immediately upon infection and not due to rapid degradation of host mRNAs[1]. A recent study has identified the immediate early viral protein ICP4 as a key regulator of Pol II occupancy on the host genome[2]. Additionally, DNA binding proteins, including host transcription machinery, are sequestered into viral replication compartments upon the onset of viral DNA replication[3]. The current belief is that ICP4 helps recruit DNA binding proteins into viral compartments; therefore, transcription machinery is limited for host mRNA transcription.

While it is clear that ICP4 is involved in transcriptional reprogramming upon infection, most data focus on transcriptional regulation in the context of the viral genome. My goal is to characterize the role that ICP4 has on host transcription upon viral infection.

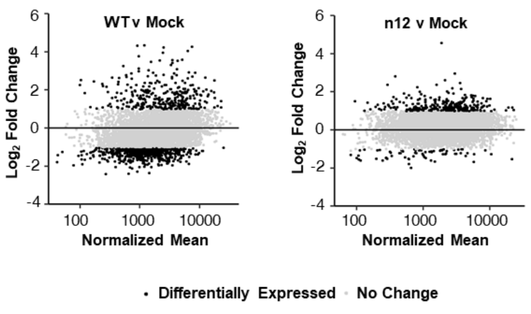
1. Abrisch, R.G., Eidem, T.M., Yakovchuk, P., Kugel, J.F., Goodrich, J.A. (2016) *J. Virol*. **90**:2503-2513.
2. Dremel, S.E., DeLuca, N.A. (2019). *eLife*. **8**:e51109.
3. McSwiggen, D.T., … Tjian, R., Darzacq, X. (2019). *eLife*. **8**:e47098.

Deletion of viral protein ICP4 attenuates host cell transcriptional reprogramming upon HSV-1 infection

To determine the effect of ICP4 in viral infection, I infected human HEK 293 cells with WT HSV-1 and the n12 virus, which is a modified HSV-1 strain that codes for a truncated, nonfunctional ICP4. I infected cells for 4h at a MOI of 10 pfu/cell and performed Pol II ChIP-seq in infected cells as well as uninfected cells in biological duplicates. I also sequenced their respective input chromatin. I used a Pol II antibody that binds to the N-terminal region of Rpb1, the largest subunit of Pol II, that ignores the phosphorylation state of the C-terminal domain.

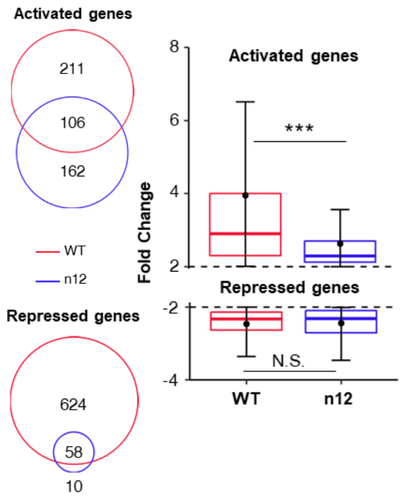


I first compared gene body (+250 to 3'-end) RPK normalized to a constant sequencing depth for all mRNA genes considered actively transcribed across the three samples. As expected, WT infection globally reduced Pol II occupancy on mRNA genes. **n12-infected cells saw a statistically significant recovery of Pol II occupancy over WT-infected cells.**



I performed differential gene expression analysis comparing WT or n12 infection to uninfected cells (mock) using DESeq2. Genes were considered activated or repressed if they have a p-value < 0.05 and a $\log_2$ fold change ≥ 1 or ≤ -1 respectively. **n12 infection has a greatly reduced number of repressed genes compared to WT infection.**

WT and n12 infection activate similar numbers of genes while n12 infection represses fewer genes



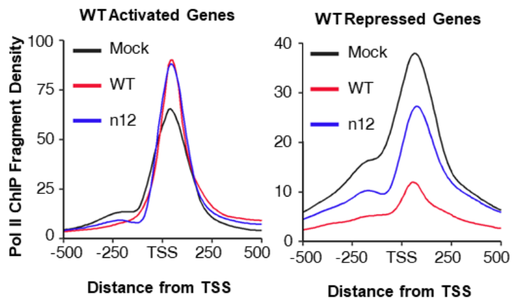
Differential gene expression analysis showed that WT infection activated **317** genes and repressed **682** genes while n12 infection activated **268** genes and repressed **68** genes. The overlaps between activated and repressed genes are represented as Euler diagrams. The incomplete overlap between activated gene sets suggests that ICP4 plays a role in activating a subset of genes during infection.

I plotted the fold changes for activated and repressed genes as a box plot to compare magnitudes of change between infections. **WT activated genes are activated to a higher magnitude than n12 activated genes, while there is no difference in magnitude between repressed genes.**

I performed gene ontology analysis using DAVID to look at which biological processes were enriched in the activated or repressed gene lists for WT or n12 infected cells. **Shown below are the enriched GO-terms for WT and n12 activated genes**. There was no enrichment for repressed genes in either infection.

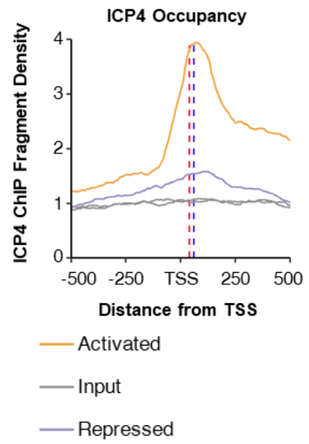
ID	Term	Count	P-value	P-adj value
WT Activated Genes				
GO:0000122	negative regulation of transcription from RNA polymerase II promoter	28	5.52E-05	3.33E-02
GO:0006366	transcription from RNA polymerase II promoter	23	3.98E-05	3.59E-02
GO:0051591	response to cAMP	7	9.87E-05	4.44E-02
n12 Activated Genes				
GO:0007399	nervous system development	16	9.24E-06	1.51E-02
GO:0051412	response to corticosterone	5	8.72E-05	4.66E-02

ICP4 is important for the loss of promoter proximal Pol II and the loss of divergent Pol II



I made histograms surrounding the transcription start site (TSS) for WT activated genes (left) and WT repressed genes (right) using the ChIP-seq data from mock, WT, and n12 infected cells. Pol II occupancy increases at the TSS in both WT and n12 infection at WT activated genes, **therefore the increase in Pol II occupancy does not require ICP4.** Interestingly, divergent transcription peaks (upstream of TSS) are lost upon WT infection and are partially restored upon infection with n12 virus, suggesting that **ICP4 could modulate divergent transcription**. For WT repressed genes, **ICP4 is necessary for the reduction in Pol II occupancy**.

ICP4 occupancy is highest on WT activated genes

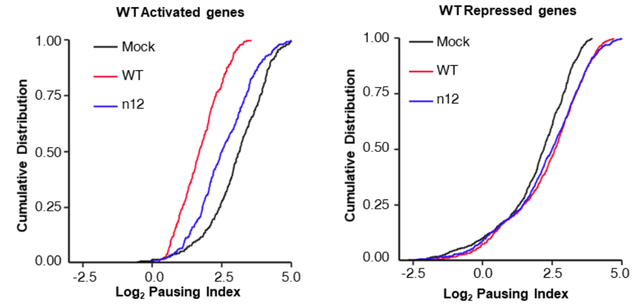


It has been shown that ICP4 binds to the host genome at early time points of infection (2h and 4h) primarily around host gene promoters[2]. I used publicly available ICP4 ChIP-seq data from a 4h WT infection in MRC5 cells at a MOI of 10 and made a TSS histogram over the WT activated and repressed genes along with input chromatin.

ICP4 occupancy correlates with genes with high Pol II occupancy upon infection. The dashed lines represent the position of the promoter proximal peak for WT activated genes (red) and WT repressed genes (blue). **ICP4 predominately binds around the Pol II promoter proximal peak and not around upstream regulatory elements.**

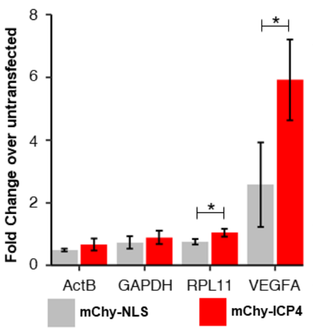
ICP4 regulates Pol II promoter proximal pausing on activated genes but not repressed genes

Because ICP4 bound around promoter proximally paused Pol II, I examined the effect ICP4 had on pausing. I calculated pausing indices for all WT activated genes (left) and WT repressed genes (right) by $\log_2$ transforming promoter proximal RPK (TSS through +250) divided by gene body RPK (+250 to 3'-end) and plotting as a normalized cumulative distribution function.



WT infection greatly reduces the pausing index for activated genes. Pausing is partially restored to mock-infected levels upon n12 infection, suggesting that **ICP4 regulates pausing at activated genes**. P-value: mock v WT: $p \sim 0$; mock v n12: $p < 6.3 \times 10^{-8}$; WT v n12: $p < 2.2 \times 10^{-16}$. By contrast**, there was no difference in pausing between WT and n12 infection at repressed genes**. P-value: mock v WT: $p < 3.9 \times 10^{-9}$; mock v n12: $p < 1.5 \times 10^{-8}$; WT v n12: $p = 0.2403$.

Overexpression of ICP4 in the absence of infection slightly activates host transcription



I wanted to see the effects of ICP4 on host transcription in the absence of infection. I sorted HEK 293 cells transfected with either mChy-NLS (gray) or mChy-ICP4 (red). Total RNA was harvested, and qPCR was performed for a variety of host genes. Fold changes over untransfected cells are plotted after normalizing to cell number. **Overexpression of ICP4 slightly activates select host genes in the absence of infection**. n = 3. I will perform qPCR against repressed genes revealed by ChIP-seq to see if exogenous ICP4 can repress transcription in the absence of infection.