ZIKA VIRUS TRANSIENTLY ALTERING HOST CHROMATIN ACCESSIBILITY DURING INFECTION

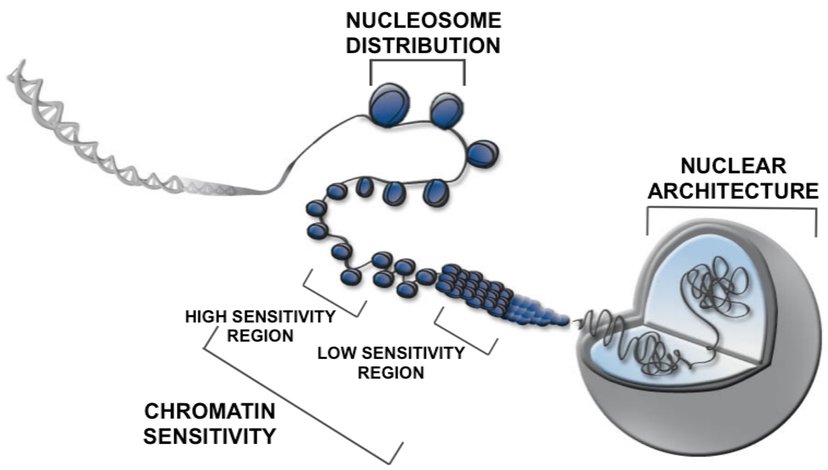
Brandon D. Buck[1], Sarah C. Ogden[1], Lauren A. Cole[1], Athanasios Vouzas[1], Jane Benoit[1], Peiyao Zhao[1], Hengli Tang[1], and Jonathan H. Dennis[1,2]

[1] Department of Biological Science, The Florida State University, Tallahassee, Florida 32306-4295, USA

[2] The Center for Genomics and Personalized Medicine, The Florida State University, Tallahassee, Florida 32306-4295, USA

CHROMATIN ARCHITECTURE & ZIKA INFECTION

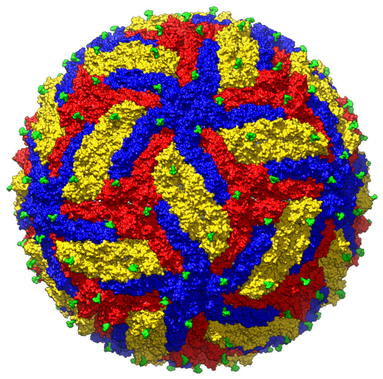
The foundation of chromatin architecture begins with the DNA of a eukaryotic cell wrapped around histone proteins. The complex formed by the 146 base pairs of DNA wrapped around 8 histone proteins is referred to as a nucleosome. Nucleosomes are utilized in the storage and compaction of genomic DNA into the nucleus of the cell, commonly known as chromatin. The positioning of nucleosomes contributes to which areas of DNA are accessible for transcription. Nucleosomal positioning and occupancy can be altered by *cis* and *trans* acting factors. These *cis* and *trans* factors contribute to a temporal transient shift in nucleosomal positioning during a stress on the cell. This shift has been observed surrounding the Transcription Start Site (TSS) throughout the eukaryotic host genome, referred to as a Genomic Transient Intermediate State. Until this study, there has not been a recorded GTIS in relation to nucleosomal sensitivity throughout the host's genome. This study continued the highly resolved temporal analysis of host nucleosomes during viral infection. The team of Sexton *et al*., utilized a Kaposi's-Sarcoma-associated-Herpes Virus (KSHV) with latent viral reactivation via doxycycline in iSLK.219 cells (2014). However, in this study the stress utilized was the PRVABC59 Puerto Rican strain of Zika virus on SNB-19 glial cells. This set up allowed for a temporal analysis of nucleosomes during a new stress and model while also observing the effects of Zika on the host genome.



ZIKA VIRUS: STILL A THREAT

The Zika virus (ZIKV) is a single stranded positive sense RNA virus in the same family as the Dengue virus, *Flaviviridae*. The ZIKV was first isolated in 1947 in Uganda. The initial studies of ZIKV showed viral presence and structural deformities in the infected mice brains. Current human cases and studies support the initial findings. The ZIKV has had multiple outbreaks since its original discovery in 1947. The outbreaks have occurred in 2007: Yap Island, 2013-2014 French Polynesia, and 2015-2016 Brazil and the Americas. During the French Polynesia Zika virus outbreak there was a recorded increase of Guillain-Barré syndrome cases. A recent outbreak of ZIKV in 2015 identified the Zika virus in the amniotic fluid of two pregnant women in Brazil, which led to concern of microcephaly in developing fetuses. The ZIKV has been discovered in fetal brain tissue without the presence of other *flaviviruses*. Further research has supported the connection between ZIKV and microcephaly through an in vitro Zika infection with human embryonic cortical neural precursor cells.

This virus has had numerous outbreaks within the last 13 years and is in a primed condition to result in another outbreak. One of the primary insect vectors that can transmit the virus is the *Aedes aegypti* mosquito, which currently is present in 61 countries and territories. The current track record of the virus strengthens the urgency to further understand how it is biologically resulting in increased Guillain-Barré and microcephaly cases. One of the gaps in knowledge is the relationship of the Zika virus with the host's structural genome. This temporal study shows the relationship between chromatin architecture changes throughout ZIKV infection.



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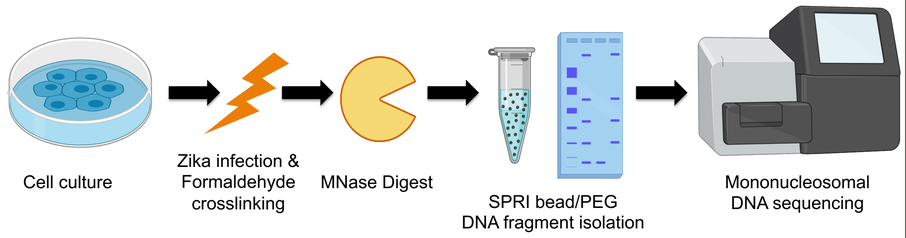
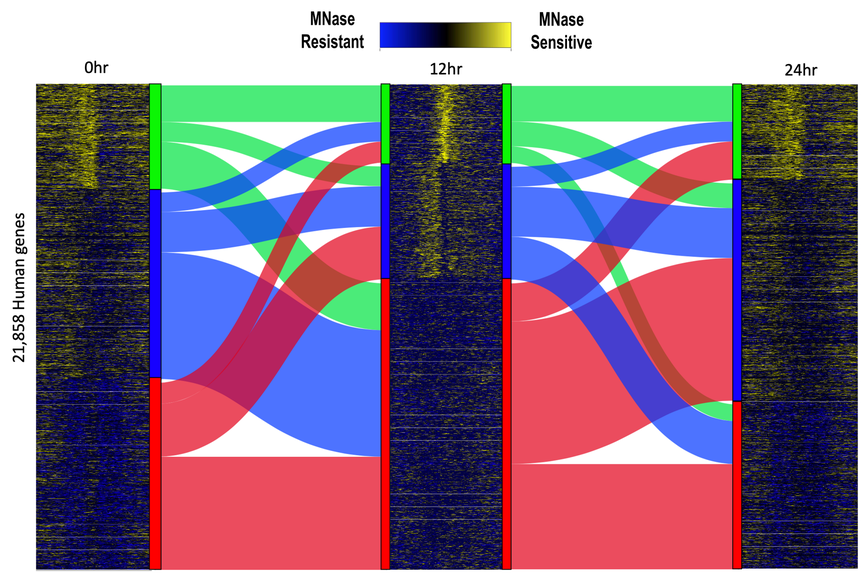
MONONUCLEOSOMAL DNA ISOLATION FROM SNB-19 GLIAL CELL ZIKA INFECTION



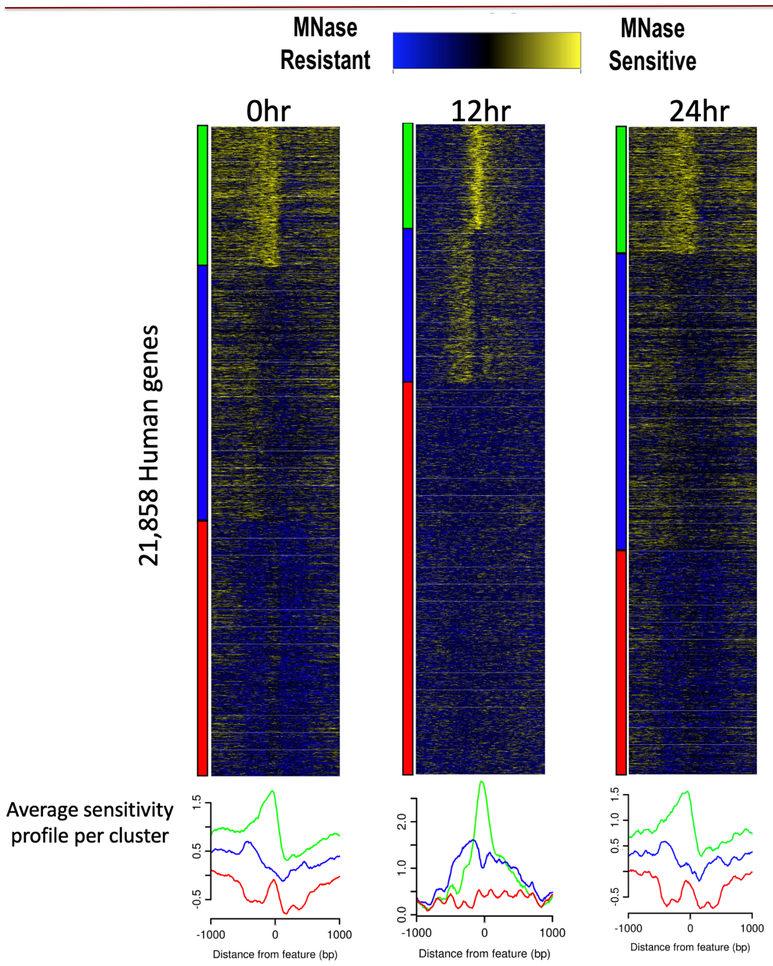
- SNB-19 Glial cells were cultured to 2.5 x 10^7 cells per 0, 12, and 24-hour Zika infection time point
- SNB-19 cells were infected with the Puerto Rican strain of Zika virus (PRVABC59), and formaldehyde crosslinked at each time point.
- Light (20 Unit) and Heavy (200 Unit) Micrococcal Nuclease digests were performed for each sample
- Nucleosomal DNA fragment laddering was confirmed through gel electrophoresis
- Mononucleosomal DNA fragments were isolated with SPRI beads and Polyethylene glycol
- Sequence libraries were prepared and sequenced

TRANSIENT CHROMATIN ARCHITECTURAL CHANGES THROUGHOUT ZIKA INFECTION



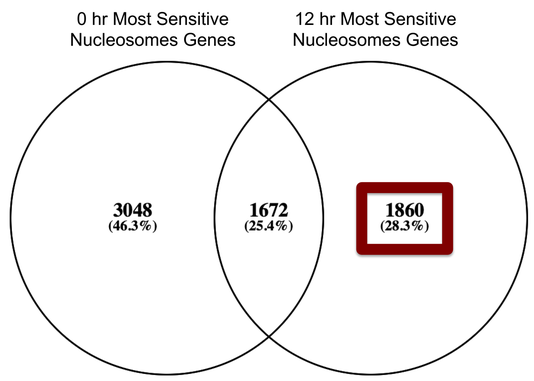
39.12% of all genes in the human genome began in an initial cluster of chromatin architecture at the beginning of Zika infection, experienced a change in their chromatin architecture at the 12-hour time point, and returned to their original cluster at 24 hours. The alluvial plot represents the symmetric transient nature of these genes experiencing chromatin architectural changes. The transient nature of these genes represents an acute response to the Zika virus.

NUCLEOSOME SENSITIVITY INCREASE AT 12 HR ZIKA INFECTION



Genomic nucleosome sensitivity (yellow) and resistance (blue) were observed by comparing the log ratio of light 20 Unit and heavy 200 Unit MNase digests through the use of heatmaps. The heatmaps represent the nucleosome sensitivity of every gene in the human genome surrounding the Transcription Start Site. The heatmap shows genes with nucleosome sensitivity increased at 12 hours post Zika infection. The increase in nucleosome sensitivity at 12 hours was transient, as it returned to basal levels at 24 hours. Unique genes only acquiring increased nucleosomal sensitivity at 12 hours were selected to further analyze. These genes accounted for 8.5% of the host's entire genome. A gene ontology of these genes processes showed connections to: Telencephalon regionalization (p-value= 3.33 x 10^{-4}), and Cerebral cortex cell migration (p-value= 9.77 x 10^{-4}).

INDEPENDENT SENSITIVE GENES AT 12 HR ZIKA INFECTION



The Venn Diagram represents the genes with the most sensitive nucleosomes during the 0 and 12-hour Zika infection time points. The 1860 genes that are marked acquired their nucleosomal sensitivity at 12 hours of Zika infection. The 0 hour and shared sensitivity genes were omitted from the Gene Ontology. This was to focus on determining the processes of the genes with the most sensitive nucleosomes at 12 hours of infection.

ZIKA VIRUS IMPACT ON HOST CHROMATIN ARCHITECTURE AND ACCESSIBILITY

The data supports the Genomic Transient Intermediate State (GTIS) occurring around the transcription start site during the 12th hour of Zika infection. The first finding had nucleosomes gaining sensitivity at 12 hours and then returning to basal states by 24 hours. At this time point some of the genes involved were connected to telencephalon regionalization, and cerebral cortex cell migration. These two processes provide support for Zika's involvement with microcephaly as the sensitive nucleosome genes affected during infection code for cells to migrate to the brain. The Zika virus could exploit this function allowing for passage to the developing fetal brain. Once present, these cells could proceed to carry out further viral replication and assembly, resulting in developmental abnormalities. Supporting studies showed that an infected pregnant woman could infect the developing fetus through the amniotic fluid. In vitro, Zika can also infect human embryonic cortical neural precursor cells, further supporting the narrative that Zika can result in microcephaly. The findings of this study provide a new aspect on the ways in which Zika affects the hosts chromatin on a temporal scale.

The study conducted by Sexton *et al.*, was the first discovery of a Genomic Transient Intermediate State (2014). Their study involved the temporal repositioning of nucleosomes during viral infection. This study proposes the first discovery of a Genomic Transient Intermediate State in association with nucleosomal sensitivity. Nearly 40% of the human genome presented a symmetric transient change in chromatin architecture. 8,551 genes started in their original clusters based on their chromatin architecture, changed clusters at 12 hours ZIKV infection, and then returned to their basal cluster at 24 hours. This massive change is critical when considering the hosts response to the virus. These genes promptly responding to the virus and then returning to their original architecture could be involved with an acute response to ZIKV.

Further studies would involve RNA-seq analysis at the same 0, 12, and 24-hour time points during Zika infection. The gene expression data would be compared to the genes associated with altered and unaltered chromatin architecture. This analysis would provide greater understanding on the relationship between change in gene expression and change with chromatin architecture during Zika infection. Finding associated changes in genomic transcription will provide the details of where the Zika virus is altering the host's genome and impacting gene regulation on a temporal scale during infection. This would lay the groundwork into developing a mechanistic analysis of how the virus is causing a disruption in nucleosomal sensitivity. Due to the change in nucleosome sensitivity on a large genomic scale this provides support to investigate ATP-dependent chromatin remodelers. Another contributor could be markers from histone post-translational modifications allowing for specified remodeling to occur. These findings could lead to future studies and ways to combat the Zika virus.