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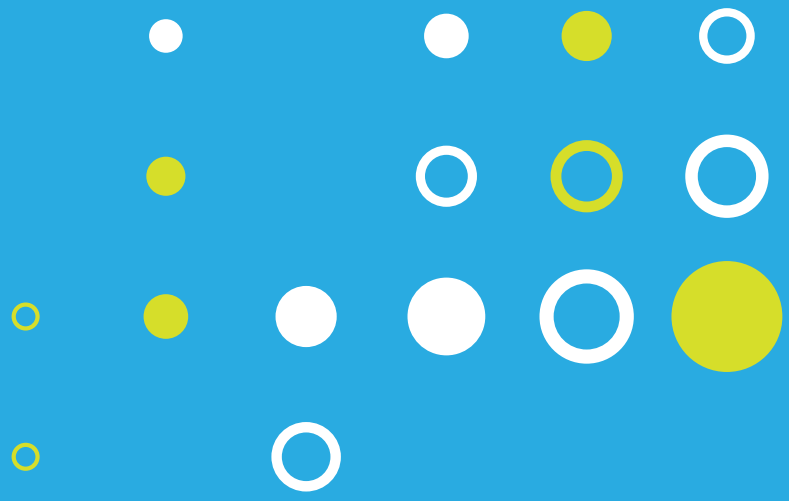

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that synthetic peptides derived from these regions are active at micromolar concentrations against conidia from the phytopathogenic fungus Fusarium graminearum and we have characterized their action. Here, we continue the study of SmAPα1-21 mode of action (sequence: KLCEKPSKTWFGNCGNPRHCG; Minimum Inhibitory Concentration, MIC: 32 μM) and explore the correlation between the biological activity and primary structure of the α-core of DefSm2-D flower defensin focusing on the relevance of histidine 19. New peptides were designed by modifying the parent peptide (SmAPH19R and SmAPH19A, where His19 was replaced by Arg or Ala, respectively) and synthesized by the Fmoc solid phase method. Antifungal activity was determined against F. graminearum. Conidia membrane permeability was assessed by visualizing the influx of the membrane impermeant fluorescent red dye propidium iodide by confocal laser scanning microscopy (CLSM) after challenging conidia with each peptide. Reactive oxygen species (ROS) production was monitored on conidia with H2DCF-DA probe by fluorescence spectroscopy and CLSM. The peptides were derivatized with fluorescein and rhodamine B and subcellular localization in conidia was studied by CLSM by colocalization with the cell wall marker Trypan Blue. Transmission electron microscopy (TEM) was used to study the ultrastructural effects of SmAPα1-21 in conidial cells. SmAPα1-21 induced morphological changes in the cell wall and peroxisome biogenesis in F. graminearum conidia. SmAP2H19A and SmAP2H19R were found to be active against F. graminearum (MIC SmAPH19R: 40 μM and SmAPH19: 100 μM). The replacement of His19 by Ala produced a decrease in the net charge of one unit at pH 5.5 with a significant increase in MIC, thus evidencing the importance of the positive charge in position 19 of the antifungal peptide. All three peptides produced permeabilization of the conidia membrane and induced oxidative stress through ROS production. However, the replacement by Ala turned all the processes slower. SmAPH19R and SmAPH19A were localized in the conidia cell wall whereas SmAPα1-21 was internalized, first entering through the basal and apical cells of the macroconidia. As the incubation times were prolonged, SmAPα1-21 localized in all the cells of the spores with a non homogeneous distribution in the cell cytoplasm. SmAPα1-21 has a multi-step mechanism of action against F. graminearum conidia that involves at least alteration of the fungal cell wall, membrane permeabilization, peroxisome biogenesis, and induction of oxidative stress. The extracellular localization of peptides SmAPH19R and SmAPH19A highlights the role of the His 19 residue in the internalization.

MI-02

CONTRASTING STUDY AMONG TRYPANOSOMATIDS REVEALS CONSERVED CHROMATIN ORGANIZATION AROUND TRANS SPLICING-ACCEPTOR SITE

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Trypanosoma cruzi, *Trypanosoma brucei* and *Leishmania major*, usually known as TriTryps, are the causal agents of animal and human sickness. TriTryps are characterized by having complex life cycles, alternating between a mammal host and an insect vector. One peculiarity of these organisms is that their genes are organized in long transcriptional units that give rise to polycistronic transcripts, which maturate into mRNA by a process known as trans-splicing. Even though gene expression regulation occurs mainly post-transcriptionally, it has been recently shown that chromatin plays a role in modulation. In this work, we made a comparative analysis of genome-wide chromatin organization and its potential impact on gene expression for the parasite stage present in the insect vector for the TriTryps using MNase-seq and RNAse-seq data, publicly available or generated by our laboratory. To compare average nucleosome positioning and mRNA patterns, we predicted the most likely trans-splicing acceptor sites and used them as reference points to plot average nucleosome or RNA-seq signals. By representing MNase-seq data, we corroborated the presence of a mild nucleosome depleted region (NDR) around trans-splicing acceptor sites (TASs) in *T. cruzi* and *L. major*; but not in *T. brucei*, as previously reported. However, when analyzing H3 ChIP-seq data, we uphold that TAS protection in *T. brucei* is due to a non-histone complex instead of a well-position nucleosome, as previously claimed. Moreover, we showed that this nucleosome organization around TASs is not just an average, since the same layout is conserved in most of the genome.

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Furthermore, the strand-specific analysis revealed that the NDR is not exactly at the TAS but a few base pairs upstream of that point. We corroborated that this trough, is coincident with a footprint of DNA-RNA duplex, as previously observed.
Additionally, it was previously shown that average nucleosome density around TAS correlates with average RNA-seq signals. To test how strong is that correlation, we performed gene clustering using k-means with either nucleosome occupancy or mRNA signals relative to TAS as predictor variables. From the MNase-seq clustering, we observed a homogenous distribution of average nucleosome density in the three organisms except for a subset of genes with unusually high nucleosome density in *T. cruzi*. As opposed, from RNA-seq analysis, we obtained well-defined gene clusters for the three organisms supported by high silhouette values. Particularly, we observed that there is a subset of genes with markedly high mRNA levels compared to the rest, but the correspondence between nucleosome density and mRNA signal is only partial. To have a better understanding of the role and conservation of those subsets of genes with unusual characteristics among TriTryps, we are currently performing GO and Metabolic pathway analysisanalyses.

MI-03

EVASION OF THE CELLULAR IMMUNE RESPONSE BY THE NS5 PROTEIN OF THE DENGUE VIRUS. IMPACT ON THE PATHOGENESIS OF THE FOUR SEROTYPES FOR A RATIONAL DESIGN OF TETRAVALENT VACCINES

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Dengue virus (DENV) is the most important mosquito-borne virus, with an estimated infection rate of 390 million people per year. Despite its global burden, there are no antiviral agents or universal vaccines available to cope with this infection. Vaccine development has been a great challenge due to the presence of four serotypes (DENV1-4). A previous exposure to any of the serotypes can lead to a more severe clinical manifestation upon infection with a heterologous one. This feature, along with the rapid expansion of co-circulation of DENV serotypes and the increasing infection incidence, highlights the importance to understand differences among serotypes and their interaction with the host cell.
DENV is a single-stranded RNA virus that relays on ten viral proteins to carry out its replication cycle and counteract the immune response. NS5 is the largest and most conserved protein across serotypes. It comprises two domains, the N-terminal methyltransferase (MTase) domain, which is essential for viral RNA capping, and the C-terminal RNA-dependent RNA-polymerase in charge of RNA synthesis. NS5 is also a potent antagonist of type I interferon signaling pathway through STAT2 degradation. In this study we demonstrate that NS5 further interferes with nuclear factor-κB (NF-κB) activation cascade by degrading the host protein ERC1 in the course of infection.
ERC1 is a cellular protein with multiple functions. It is a regulatory subunit of the IKK complex involved in NF-κB activation pathway, it participates in the docking and/or fusion of Rab6-positive vesicles at the cell cortex and it forms a complex that drives cell motility. We found that during DENV2 infection, ERC1 is degraded in a proteasome-dependent manner, mechanism that requires UBR4 as E3 ubiquitin ligase. We determined that the MTase domain is the viral counterpart essential and sufficient for ERC1 degradation and that not all NS5 from the four serotypes are capable of ERC1 degradation. Based on this difference between the MTase domain of DENV1, 2, 3 and DENV4, we mapped the amino acid residues responsible for ERC1 degradation. We generated a recombinant DENV2 that exchanges serotype properties with DENV4 by a single amino acid substitution. Infection with this virus led to higher levels of cytokine expression and secretion, resembling that reported for DENV4, and increased cell motility. NF-κB regulates the expression of genes related to immune responses. Viruses have thus developed a variety of strategies to modulate this pathway. In this regard, although DENV infection triggers the production of large amounts of pro-inflammatory cytokines, infected cells block further NF-κB activation, albeit the underlying mechanism has not been fully described. In the