cells infected with high MOI. Strikingly, large relative quantities of mRNA for SH and M22 proteins were consistently found. The analyses of viral RNAs engaged in polysomes showed that the translation profile was similar to that found for the overall detection of viral RNAs. Western blot and immunofluorescence analysis for viral proteins for which antibodies are available were in agreement with this, revealing that viral protein F was present in larger quantities than M over time post infection. These results are novel and may help to understand the relative importance of different HRSV gene products in the replication of tis agent in diferent tissues and cell types.

BV215 OROPOUCHE VIRUS ASSEMBLY IN MAMMALIAN CELLS REQUIRES THE ACTIVITY OF HOST ESCRT PROTEINS

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Oropouche virus (OROV) is a Bunyavirus that can cause Oropouche fever in humans, a febrile illness that can lead to meningitis. Because little is known about OROV replicative cycle this study aimed to describe the intracellular pathway and host factors involved in OROV assembly in HeLa cells. Toward this goal, cells were inoculated with OROV (MOI = 1) and the dynamics of viral onestep replication cycle was monitored at different time points postinfection (p.i.). Aquantification by TCID50 assay showed that during the first hours intracellular viral titers were continuously reduced and barely detected at 6 h p.i., indicating virus eclipse. This was followed by a rapid increase in viral titers in cell lysates and culture supernatants, reaching peak levels at 24h p.i. Accordingly, viral proteins were detected by immunoblot in cell lysates at 9 h p.i and in culture supernatants at 24 h p.i. After 9h p.i. large vesiclelike structures enriched in OROV proteins were detected by immunofluorescence at a pericuclear region, indicating viral factories. These factories contained early endosome protein and the endoplasmic reticulum (ER) resident membrane protein. A transGolgi marker showed a dispersed pattern throughout the cytoplasm and also colocalized with the viral factories. In contrast, a cis-Golgi marker did not colocalize at any time point p.i. with these factories suggesting that Golgi apparatus may not be the main site of viral assembly. ImmunoEM analysis of infected cells revealed large multivesicular bodies structures (MVBs) that contained virus particles and were often associated with the ER. This data prompted us to verify a possible role for the ESCRT (Endosomal Sorting Complexes Required for Transport) machinery in viral replication. Knockdown of Tsg101/ ESCRTI and Alix led to a strong reduction in OROV production (40% ±13.3% and 35%±14.8%, respectively) and compromised the formation of prominent viral factories, as intracellular OROV staining remained restricted to small puncta dispersed throughout the cytoplasm. The superexpression of a dominant negative form of the AAATPase Vps4A, which disrupts the MVB pathway, led to an enlargement in the area of the viral factories (146%±63.2%), where the Vps4A mutant accumulated. Together our data presents new insights into cell compartments and host factors involved in OROV biogenesis indicating that OROV requires the host MVB pathway with the recruitment of the ESCRT machinery for a proper virus formation.

PIV102 MOLECULAR CHARACTERIZATION OF GRAPEVINE ENAMOLIKE VIRUS, A NOVEL PUTATIVE MEMBER OF THE GENUS ENAMOVIRUS

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The genus Enamovirus, family Luteoviridae, consists of one recognized viral species, Pea enation mosaic virus 1 (PEMV1) and two putative members, Alfalfa enamovirus1 (AEV1) and Citrus vein enation virus (CVEV). We encountered a novel Enamovirus, tentatively named Grapevine enamolike virus (GELV), in a Vitis vinifera 'Cabernet Sauvignon' vine in an experimental field in the municipality of Bento Gonçalves, Brazil. The symptoms in this host were those of severe grapevine leafroll disease and reddish leaves. To characterize the viromes of this sample, dsRNA was extracted from 30g of bark scrapings. Sequencing data was generated from a cDNA library that was constructed by Macrogen. The Illumina HiSeq2000 platform was used to generate about 20 million reads. CLC Genomics Workbench software was used for quality trimming and de novo contig assembly from the reads. All contigs were analyzed using NCBI's Blastx program against the viral RefSeq database. Bioinformatic analysis indicated that the longest contig (6206 bp, GenBank accession code KX645875) shares only 49% identity

with PEMV1 (Query coverage 25%, E value: 9e130) thus indicating that GELV is a distinct member of the genus Enamovirus based on established criteria. To confirm the high throughput sequencing (HTS) results, dsRNA and total RNA were extracted from fresh plant material and screened by RTPCR using the specific primers (SetF: 5'TTCCCTTGGGAGACTCGGTTCTAT3' and SetR: 5'AAACATGACCACCCGTCTCATAGC3'). The resulting amplicon (735 bp) was cloned, sequenced and determined to be 99% identical with corresponding sequences generated by HTS. Grafttransmissibility of GELV was confirmed by grafting source vines onto P1103 cultivar rootstock (13+/16) and confirming the infection by RTPCR. This is the first report of a virus in the family Luteoviridae infecting grapevines. Further investigation using HTS lead to the discovery of this novel virus in three different samples of grapevines (cvs. CG 90450, Semillon, and Cabernet Franc) and using RT-PCR in another sample (cv. Malvasia Longa). To gain insight into the virus organization and evolution, the 6206 bp contig was subjected to further bioinformatics analysis. Five ORFs were predicted and analyzed for conserved elements. Field surveys and biological studies are currently underway to determine the prevalence of GELV in Brazil, evaluate its potential natural spread, and assess its effect on vine performances and wine quality.

PIV117 IDENTIFICATION AND FUNCTIONAL ANALYSES OF THE COTTON BLUE DISEASE RESISTANCE LOCUS

Fausto, A.K.S.; Moura, M.O.; da Franca, T.S.. Romanel, E.; Vaslin, M.F.S.

Cotton blue disease (CBD) is a major cotton disease in Brazil. It is transmitted by Aphis gossypii and its causal agent is the Cotton leaf roll dwarf virus (CLRDV). CBD resistance is controlled by one single dominant locus, however nothing is known about it. Previously, we mapped the Cbd resistance locus, identifying two ORFs, named Cbd1 and Cbd2, respectively. The promoter region of these two genes is bidirectional and presented many cis elements related to: salicylic acid, auxin responses and biotic and abiotic stress. CBD1 is a low density lipoprotein receptor (LDL) and CBD2, an arginyl tRNA transferase (ATE) implicated in the Nend rule leading target specific proteins to degradation by 26S proteasome. The expression of GhCBD1 and GhCBD2 in organs of five cotton cvs (two susceptible, two resistant and one showing middle resistance to CLRDV) showed no differences that could justify their responses in infection. Studying GhCBD1 relative expression during viral infection we observed that its expression is 9to-189x induced in all points (24 hpi, 5, 15 and 25dpi) in susceptible cv. However, its expression suffer just a slight variation in the resistant cv. along the infection. By the other side, GhCBD2/ATE was suppressed 24hpi (7x reduced) and induced at 5dpi (12x more expressed) in susceptible cvs. An opposite pattern was seen in the resistant cv (4x more expressed at 24 dpi and 5x reduced at 5 dpi). Investigation of similar effects in Arabidopsis thaliana Col. under CLRDV infection showed a significant increase (8x) of AtATE1 after CLRDV infection. Arabidopsis pATE1:GUS revealed an increase GUS activity in shoot and root apical meristems as well in young leaves compared with noninfected plants. 35S:ATE1 plants blocked viral infectivity. Thus, the data suggest that to prevent CLRDV spreading, the expression of CBD2/ATE must be induced in 24hpi. So, CBD2/ATE could act by inhibition of the replication and/or viral spread. In this sense, we saw that the CLRDV's movement protein (ORF4) has the necessary amino acids to be an ATE target. We suggests, CBD2/ATE can be a candidate protein to generate resistance, possibly, leading viral movement protein to 26S proteasome for degradation via the Nend rule.

PIV118 DICER-LIKE PROFILE EXPRESSION DURING VIRAL INFECTION IN SUSCEPTIBLE AND RESISTANT COTTON

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RNA silencing is an important antiviral defense mechanism in plants. RNA silencing ou RNAi pathway is triggered when viral doublestrand intermediary RNAs (dsRNA) are generated during viral replication in the first steps of virus infection. Plant Dicerlike (DCL) ribonucleases, specially DCL2 and 4, recognize these dsRNA strands and dicers then, producing 21–24 nucleotide short interfering RNAs (siRNAs). The viral siRNAs are incorporated into RISC complexs, which recognizes and destroys siRNA complementary target RNAs. Arabidopsis thaliana presents four DCLs (DCL1–4), however, the cotton DCLs have not yet been