	Título de la Publicación	Nombre de la Revista / Libro	Vínculo o DOI	Indexador	Autoría
2016	Molluscum contagiosum virus transcriptome in abortively infected cultured cells and a human skin lesion	JVI	https://doi.org/10.1128/JVI.02911-15	SCOPUS	PA
Importancia: El Molusco contagio es un poxvirus frecuentemente encontrados en niños. Su replicación in vitro no es posible por lo cual se estudia su cascada de expresión con diversos métodos que incluye el RNA-Seq. Entender la cascada de expresión génica permite conocer los mecanismo del virus para su ciclo viral.					

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

Molluscum Contagiosum Virus Transcriptome in Abortively Infected Cultured Cells and a Human Skin Lesion

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ABSTRACT

Molluscum contagiosum virus (MOCV), the only circulating human-specific poxvirus, has a worldwide distribution and causes benign skin lesions that may persist for months in young children and severe infections in immunosuppressed adults. Studies of MOCV are restricted by the lack of an efficient animal model or a cell culture replication system. We used next-generation sequencing to analyze and compare polyadenylated RNAs from abortive MOCV infections of several cell lines and a human skin lesion. Viral RNAs were detected for 14 days after MOCV infection of cultured cells; however, there was little change in the RNA species during this time and a similar pattern occurred in the presence of an inhibitor of protein synthesis, indicating a block preventing postreplicative gene expression. Moreover, a considerable number of MOCV RNAs mapped to homologs of orthopoxvirus early genes, but few did so to homologs of intermediate or late genes. The RNAs made during *in vitro* infections represent a subset of RNAs detected in human skin lesions which mapped to homologs of numerous postreplicative as well as early orthopoxvirus genes. Transfection experiments using fluorescent protein and luciferase reporters demonstrated that vaccinia virus recognized MOCV intermediate and late promoters, indicating similar gene regulation. The specific recognition of the intermediate promoter in MOCV-infected cells provided evidence for the synthesis of intermediate transcription factors, which are products of early genes, but not for late transcription factors. Transcriptome sequencing (RNA-seq) and reporter gene assays may be useful for testing engineered cell lines and conditions that ultimately could provide an *in vitro* replication system.

IMPORTANCE

The inability to propagate molluscum contagiosum virus, which causes benign skin lesions in young children and more extensive infections in immunosuppressed adults, has constrained our understanding of the biology of this human-specific virus. In the present study, we characterized the RNAs synthesized in abortively infected cultured cells and a human skin lesion by next-generation sequencing. These studies provided an initial transcription map of the MOCV genome, suggested temporal regulation of gene expression, and indicated that the *in vitro* replication block occurs prior to intermediate and late gene expression. RNA-seq and reporter assays, as described here, may help to further evaluate MOCV gene expression and define conditions that could enable MOCV replication *in vitro*.

Molluscum contagiosum virus (MOCV) is the sole member of the *Molluscipoxvirus* genus of the *Chordopoxvirinae* subfamily of the *Poxviridae* (1). Although many poxviruses cause zoonoses, variola virus (the causative agent of smallpox) and MOCV are the only known human-specific poxviruses (2, 3). MOCV has a worldwide distribution and commonly infects young healthy children, where it causes papular skin lesions that may persist for many months before spontaneous resolution (4). However, widespread disfiguring skin lesions may occur in individuals with immunodeficiencies. For the latter, the most successful therapy is treatment of the underlying immunodeficiency. Although several MOCV variants have been recognized by restriction endonuclease analysis and limited DNA sequencing, they produce indistinguishable lesions (4).

Knowledge of MOCV is limited because of the lack of either a cell culture system or useful animal model. The inoculation of primate cells with MOCV produces an abortive infection with cell rounding and related cytopathic effects (CPE) (5). However, the cells regain a more normal appearance after 48 h (6, 7). Evidence that MOCV gene expression is necessary for CPE was supported by the ability of inhibitors of RNA and protein synthesis to prevent this phenomenon (7, 8). Following infection, electron microscopy revealed MOCV cores within the cytoplasm, consistent with early gene expression, but the disassembly of the cores or assembly of new virus particles was not observed (7). More direct evidence for early gene expression in human fibroblasts was obtained by RNA-DNA hybridization and reverse transcription-PCR (RT-PCR) (7,

Received 17 November 2015 Accepted 12 February 2016
Accepted manuscript posted online 17 February 2016
Citation Mendez-Rios JD, Yang Z, Erlandson KJ, Cohen JI, Martens CA, Bruno DP, Porcella SF, Moss B. 2016. Molluscum contagiosum virus transcriptome in abortively infected cultured cells and a human skin lesion. J Virol 90:4469–4480. doi:10.1128/JVI.02911-15.
Editor: G. McFadden, University of Florida
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Supplemental material for this article may be found at http://dx.doi.org/10.1128/JVI.02911-15.