Marek’s Disease Virus Reactivation from Latency: Changes in Gene Expression at the Origin of Replication

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ABSTRACT Marek’s disease is a contagious lymphoma of chickens caused by Marek’s disease virus (MDV). MDV replicates in chicken lymphocytes and establishes latency within and transforms chicken CD4+ T-cells. Transformed T-cells are seen as skin leukosis or as lymphomas in visceral organs. A major focus of our laboratory is the functional study of genes flanking the origin of replication. This origin (OriLyt) is contained within the repeats flanking the unique long (U1) region of the genome (IRL and TRL). To the left of this Ori are genes associated with MDV latent/transforming infection [1.8-kb RNA family, pp14, Meq], and to the right (U1) are genes associated with early stages of MDV lytic infection [BamHI-H-encoded protein (Hep), pp38/pp24, Mys].

During latency, MDV suppresses lytic gene expression and has evolved mechanisms for blocking the apoptosis of latently-infected CD4+ T-cells. Of the genes expressed during MDV latency and in the transformed cell, the Meq (Marek’s EcoRI-Q-encoded protein) has been shown to block apoptosis and transactivate gene expression. Upon reactivation to lytic infection, we have found that splice variants of Meq predominate and that these forms lack several of the domains important to Meq trans-activation and trans-repression.

We have found that rightward from the origin of replication, a family genes, including phosphoprotein 38 (pp38) are expressed during early stages of reactivation. Three separate open reading frames (Hep, Mys, and pp38) are encoded by distinct transcripts from this region. We are now determining the kinetics of expression of these transcripts and their relative abundance during reactivation.

(Key words: Marek’s disease virus, Meq oncoprotein, origin of replication, phosphoprotein 38)

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INTRODUCTION

Marek’s disease is a prevalent, contagious lymphoma of chickens caused by a herpesvirus called Marek’s disease virus (MDV), for review see (Calnek and Witter, 1997; Venugopal, 2000). MDV is unique in that it is the only known acutely-transforming α-herpesvirus that causes lymphomas in its host. Moreover, MDV-induced lymphoma formation can be prevented by vaccination with related apathogenic herpesviruses, MDV-2 (Schat and Calnek, 1978a; Schat and Calnek, 1978b), herpesvirus of turkeys (HVT) (Witter et al., 1970; Witter et al., 1976) or attenuated MDV-1 strains (Churchill et al., 1969a,b; Rispens et al., 1972). Despite the success of MDV vaccination in controlling losses to the poultry industry, the vaccines are not sterilizing, and strains of increased virulence (vvMDV, vv+MDV) have continued to evolve in the field (Witter, 1983, 1997).

MDV infection proceeds from the inhalation of virus-containing dander (Beasley et al., 1970) and can first be readily detected in the bursa of Fabricius, thymus, and spleen as a productive/restrictive replication in B- and T-cells. The mechanism of early MDV spread from the lungs to lymphocytes is unknown, but phagocytic cell types have been implicated (macrophage, dendritic cells, ellipsoid cells of the spleen) (Jeurissen et al., 1989).

MDV infection of B- and T-cells appears to be fundamentally different in that B-cells support lytic infection (productive/restrictive) and T-cells can either support lytic (CD8+, some CD4+) or latent (primarily CD4+) infection (Shek et al., 1983; Calnek et al., 1984). Following an initial stage of lytic infection that can last from days to weeks, a period of cell-associated latency follows. By about 2 wk postinfection, MDVreactivates from latently-

Abbreviation Key: b-ZIP = basic, leucine-zipper protein; CEF = chicken embryo fibroblasts; FFE = feather follicle epithelium; Hep = BamHI-H-encoded protein; HVT = herpesvirus of turkeys; Meq = Marek’s EcoRI-Q-encoded protein; Mys = Mys-encoded protein; ORF = open reading frame; Ori = origin of lytic replication; PEST = proline, glutamic acid, serine/threonine-rich motif; pp14 = phosphoprotein of 14-kDa apparent molecular weight, pp24/pp38 = phosphoprotein family of 24- and 38-kDa apparent molecular weights; Rb = retinoblastoma protein (cell-cycle regulator), 3’-RACE = 3’ rapid amplification of cDNA ends; TGF-β = transforming growth factor-β, vIL-8 = MDV-encoded interleukin 8 homolog.

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infected T-cells at peripheral sites, including the feather follicle epithelium (FFE). The FFE then serves as the site of egress from the host as infectious dander is shed into the environment (Calnek et al., 1970).

Progression from MDV lytic to latent infection appears to be regulated by the initial host response to MDV infection (Buscaglia et al., 1988). This initial latency establishment may be complex, involving both host and virus factors regulating the level to which the MDV genome is repressed (Volpini et al., 1995). In the MDV-transformed cell line model for MDV latency, this repression mechanism does not appear to be DNA sequence specific, nor is it likely to involve methylation of the MDV genome (Parcells et al., 1999). In another model for MDV latency employing a reticuloendotheliosis virus-(REV) transformed T-cell line, RECC-CU91, we have likewise found the MDV genome to be repressed through a global mechanism not dependent upon genome methylation (Parcells, unpublished observations).

Reactivation of MDV from latency is likely to involve specific interactions with permissive cell types or may be regulated in the host by a drop in cytokine or lymphokine levels in peripheral tissue. The MDV genome can be induced to undergo some aspects of latency reactivation from MDV-transformed cell lines by a number of different stimuli including: co-cultivation with permissive cells (chicken embryo of fibroblasts; CEF) (Calnek et al., 1981), temperature shift (Calnek et al., 1981), or treatment with 5′-iododeoxyuridine or bromodeoxyuridine (Nazerian, 1975; Dunn and Nazerian, 1977), sodium butyrate (Parcells et al., 2001), or O-12-tetradecanoyl phorbol acetate (Parcells, unpublished observations). Despite the ability to mimic some aspects of reactivation using these stimuli, the in vivo mechanism(s) mediating changes in virus gene expression and reactivation are unknown.

Our focus on MDV latent infection stems from the apparent importance of MDV latency to both the spread of virus (via infection of FFE) and the transformation of T-cells. The regulation of the pattern of gene expression between latent and lytic infection appears to be regulated by or is associated with changes in gene expression within the repeated sequence flanking the unique long region of the genome (IRL). This region encodes a number of MDV-specific genes, two apparent cellular homologs, Marek’s EcoRI-Q-encoded protein (Meq) and MDV-encoded interleukin-8 homology (vIL-8) (Liu et al., 1999a; Parcells et al., 2001), and an origin of lytic replication (Bradley et al., 1989a).

The MDV Origin of Replication

The MDV origin of lytic replication (Ori) was initially identified according to its AT-rich sequence and flanking putative UL9-binding sites (Bradley et al., 1989a). This element was recently demonstrated to function as an Ori (Katsumata et al., 1998), and specific sequences (132 bp repeats, pp38 promoter/enhancer) were found to be essential for this function. Expression at the Ori is complex, with transcripts expressed toward the UL region encoding the phosphoprotein family of 38 and 24 kDa apparent molecular weight (pp38/pp24) family of gene products (see Figure 1). The EcoRI-Q-encoded protein (Meq) and MDV-encoded interleukin-8 homology (vIL-8) (Liu et al., 1999a; Parcells et al., 2001).

The region surrounding the Ori has been implicated in oncogenicity, as duplications within this region that disrupt or alter expression have been associated with attenuation (Bradley et al., 1989b; Ross et al., 1993). Moreover, the Ori contains two DNA-binding sites (MERE-II) of the Meq oncoprotein, which has both transactivator and transrepressor functions (Qian et al., 1995; Qian et al., 1996). Although the Ori is apparently constitutively active in uninfected and MDV-infected CEF in both directions, the regulation of this region has not been examined in T-cells, the site of MDV latency (Shigekane et al., 1999).

An attractive model for the regulation of MDV lytic versus latent infection, therefore, involves the binding of Meq-Meq homodimers to the Ori and repressing the expression of the pp38 gene family, while transactivating the 1.8 kb, pp14, and Meq promoters. To understand the regulation of these regions, our laboratory has been involved in defining the transcripts expressed from both sides of the MDV Ori during latency, reactivation, and lytic infections.

The pp38/pp24 Family of Gene Products

Originally described as a transformation-associated antigen (Silva and Lee, 1984; Ikuta et al., 1985), phosphoproteins 38 and 24 (pp38/pp24) are highly-expressed, cytoplasmic proteins with early lytic expression kinetics (Cui et al., 1990, 1991; Chen et al., 1992). Moreover, the induction of reactivation correlates with a marked increase in pp38 expression (Parcells et al., 1999), and this expression has been used to discern lytically-infected from latently-infected lymphocytes (Baigent et al., 1998). Conversely, Xie et al. (1996) have suggested that pp38 expression is essential for the maintenance of latency in lymphoblastoid cell lines, but the nature and mechanism of action of pp38 are currently unknown. Recently, Reddy et al. (2002), demonstrated that the pp38 open reading frame is nonessential for MDV replication in culture, in vivo, and transformation, although no cell lines were established from the pp38-knockout virus.

To identify the function(s) of the pp38 gene, we performed reverse transcriptase-PCR and 3′ rapid amplification of cDNA ends (3′-RACE) to precisely mapirst products expressed in this region (see Figure 2). We found that not only is the previously described 1.8 kb transcript expressed from this region, but that two other gene products are expressed as well. These transcripts potentially encode two open reading frames, BamHI-H-encoded protein (Hep) and mystery protein (Mys). One open reading frame (ORF), termed Hep, encodes a 124 a.a. basic protein with a calculated pI of 12 and net charge of +18 at
pH 7. The second, Mys, encodes an acidic protein of 126 a.a., a calculated pI of 3.7, and a net charge of \(-19\) at pH 7. Among the ORF in the pp38 gene family, Mys is most conserved among the three serotypes of MDV (MDV-2, HVT). The Hep and pp38 ORF are more divergent, with the pp38 protein being the most varied in size (MDV-1 = 290 a.a., MDV-2 = 260 a.a., HVT-78 a.a.). The functions of these ORF are unknown, but due to the timing of their expression, their lack of homology to known enzymes, or virion structural components, it is likely that they affect the permissivity of the host cell during replication.

Hep is a highly-basic protein with well-defined nuclear localization signals (PRRGRRR, and bipartite RRRGRAWENRSGMRRGR). Hep has homology to the L19 ribosomal protein, suggesting a possible role in translational regulation or in RNA-binding.

The pp38 protein is of unknown function, is localized to the cytoplasm of infected cells, and contains a Zinc-binding motif (MSFFAGMLVG) and a retention signal for the endoplasmic reticulum (KSES). Deletion of pp38 results in a decreased lytic infection in vivo (Reddy et al., 2002), yet the role of pp38 in MDV infection remains enigmatic. To date, no cellular interaction partners have been identified.

The Mys protein contains a well-defined, amino-terminal proline, glutamic acid, serine/threonine-rich motif (PEST) sequence (HESLDLDADVSPETISPIEEVEPVLS).
The Meq and vIL-8 Family of Gene Products

Expressed from the origin of replication in the opposite direction of the pp38 family of genes (Figure 2), only the pp38 protein has been identified in infected cells. Although transcripts for the Hep and Mys ORF proteins are expressed during lytic infection, their proteins have not been directly detected, and their relative abundance and interaction with pp38 are currently unknown (Parcells, unpublished observations).

The Meq and vIL-8 Family of Gene Products

Expressed from the origin of replication in the opposite direction of the pp38 family of genes are Meq and vIL-8 (Jones et al., 1992; Liu et al., 1999a; Parcells et al., 2001). Meq is a basic leucine-zipper protein (b-ZIP) having homology to cellular proto-oncoproteins, Jun and Fos (Jones et al., 1992). Meq forms homo- and heterodimers with several cellular b-ZIP proteins (Jun, Fos, CREB, ATF-1, etc.) and can bind to DNA sequences, termed Meq-response elements (MERE) (Qian et al., 1996; Liu and Kung, 2000). Meq also interacts with cyclin-dependent kinase 2 (CDK2) and has the capacity to transactivate and transpress gene expression depending on its dimerization partner and phosphorylation state (Liu et al., 1999a,b; Liu and Kung, 2000). Meq is localized to the nucleus and nucleolus and can bind to a number of cell-cycle regulatory proteins including p53 and Rb (Liu and Kung, 2000). During lytic infection, Meq is expressed as an immediate early gene, although the full-length protein is not routinely detected during lytic infection (Parcells et al., 2001).

Downstream of the Meq gene is a spliced homolog to chicken interleukin 8 (vIL-8) (9E3/CEF4 or cCAF, K60) named viral interleukin 8 (vIL-8), a true late gene with an apparent function in MDV early dissemination in vivo (Parcells et al., 2001). The expression of Meq and vIL-8 are related through a splice variant connecting the amino terminus (basic and b-ZIP domains) of Meq to the second two exons of vIL-8 (Peng et al., 1995; Peng and Shirazi, 1996a). This Meq-vIL-8 fusion protein (Meq-sp), originally described by Peng et al. (1995) is expressed during both lytic and latent infections, but is the primary form of Meq expressed during lytic infection (Peng and Shirazi, 1996a; and Parcells, unpublished observations). Conversely, in latently-infected MDV-transformed cell lines, the full-length Meq form predominates. Thus, Meq-sp may function as a negative regulator of latency/transformation functions of Meq and/or may be involved in the reactivation from latency.

In addition, Meq expression appears to also be regulated at the sense/anti-sense level (Peng and Shirazi, 1996b). A model for MDV regulation of latency, therefore, is based on the observation that Meq/Meq homodimers can bind to CACA-rich sequences at the lytic origin of replication (Qian et al., 1996; Liu and Kung, 2000; and H.-J. Kung, personal communication). Thus, these homodimers may inhibit expression of the pp38 family of gene products. Alteration of the cellular environment, through specific signaling or nonspecific stress signaling could then result in the upregulation of other b-ZIP proteins, displacing Meq/Meq homodimers with Meq/Jun, Meq/ ATF-1, etc., heterodimers. This change in Meq partnering could change Meq from a repressor to an activator of lytic phase genes (MERE-I sequences are present throughout the MDV genome). Moreover, as reactivation commences, the Meq-sp form and Meq anti-sense transcripts would also begin to inhibit Meq full-length expression. Due to the nuclear localization of the Meq-sp protein, this fusion could provide some cellular signaling as well.

Our current goals are to determine the changes in expression at the Ori and their relationship to definable phases of MDV infection and to identify the functions of these different gene products as they relate to latency and reactivation. As we move into the era of microarrays, functional genomics, and proteomics, we move closer to understanding fundamental mechanisms of MDV pathogenicity and oncogenicity.

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