TITLE:  
Temporal profiling of the coding and non-coding murine cytomegalovirus transcriptomes  

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The global transcriptional programme of murine cytomegalovirus (MCMV) involving coding, non-coding and anti-sense transcription remains to date unknown. Here we report an oligonucleotide custom microarray platform capable of measuring both coding and non-coding transcription on a genome-wide scale. By profiling MCMV wild-type and immediate-early mutant strains in fibroblasts we find rapid activation of the transcriptome by 6.5 hours post infection with absolute dependency on ie3, but not ie1 or ie2 for genomic programming of viral gene expression. Evidence is also presented, showing for the first time, genome-wide non-coding and bi-directional transcription at late stages of MCMV infection.
INTRODUCTION:

Murine cytomegalovirus (MCMV) is a ubiquitous beta-herpesvirus with a 235 kilo-base pair genome transcribed in a classical cascade fashion (55). The genome sequence of MCMV has been available for some time (89), yet a systematic study of temporal gene expression during MCMV infection has been lacking. Double stranded PCR based cDNA microarrays were used once previously (105) to validate the expression of a subset of predicted MCMV (ORFs)(15) at a single time point (24 hpi). More advanced microarray technology based on oligonucleotide probes affording increased specificity to distinguish RNA polarity has not been reported. For human cytomegalovirus (HCMV) abundant antisense transcription has been observed (120) raising the possibility that aspects of the CMV life cycle are influenced or regulated by non-coding transcripts. For MCMV, small virus-encoded miRNAs (19, 20, 33) and larger double-stranded RNAs (21, 110) have been reported to be transcribed from multiple loci, however the frequency and abundance of non-coding transcripts throughout the MCMV genome has not yet been measured systematically on a genome-wide scale and at multiple stages of infection.

Here we have investigated the global transcriptional programme of MCMV by constructing a microarray capable of measuring sense and antisense transcripts. Microarrays were designed using 55-mer oligonucleotide probes in sense and antisense orientation to each of the 170 viral open reading frames (ORFS) predicted in the MCMV genome(89). 192 positive control probes were designed against stably expressed mouse genes for normalization purposes and 97 negative control probes were designed against yeast sequences with no homology to mouse or MCMV genomes (probe sequences see Supplementary Table S1). The 55mers were diluted to a concentration of 60uM and inkjet printed (Arrayjet, UK) onto amino-silane coated glass slides with each microarray consisting of six identical sub-arrays. Probes were printed in triplicate per array and have the capacity for developing a total of 18 measurements per probe.
per sample to ensure high technical replication. Target RNA was extracted from infected fibroblasts using PureLink RNA Mini Kits with on-column DNase treatment (Invitrogen, CA, USA). 700ng of purified RNA for each sample was labelled for microarray analysis using the Agilent low input fluorescent linear amplification protocol (Agilent, CA, USA) with 3ug of Cy5 labelled target cRNA hybridised per sample. Hybridized microarrays were washed and subsequently scanned using the Agilent (CA, USA) scanner G2505B.

To perform a systematic analysis of genome-wide transcription in MCMV we infected NIH 3T3 fibroblasts with the parental MCMV strain at an MOI of 1 and performed DNA microarray analysis on total RNA harvested from duplicate cultures at 0.5, 6.5, 24 and 48 hpi. Individual probe signals were background subtracted, median-summarized and log base 2 transformed to form raw data points (Supplementary Table S2). Raw data was quality controlled and normalization between samples was performed based on a subset of 44 positive control probes highly correlated across the data set (Pearson r<0.90). Normalized expression data (Supplementary Table S3) was subjected to a statistically rigorous threshold detection methodology for providing on/off calls for each probe based on receiver operating characteristic (ROC)(12). From these ROC analyses, we evaluated specificity levels corresponding to a given sensitivity of 70%, 80% and 90%. At a moderate sensitivity of 70% we are able to obtain an average specificity of 93%, and this was chosen as affording an optimal balance between identifying true positives and excluding true negatives with stringency (for ROC plots see Supplementary Figures S1 and S2). Accordingly we detect 297 total probes having “on” calls and 163 probes for coding MCMV ORFs making 87.6% of the MCMV genome detectable at 48hpi (for list of genes detected see Supplementary Table S4).

To account for experimental variation, statistical testing (empirical Bayes moderated t-test) was applied between mock and infected groups to identify differential expression of only the
most highly significant MCMV ORFs. Using this more stringent approach, 119 ORFs were found to be significantly activated to a confidence level of \( p < 0.05 \) above mock levels at any time point (Table 1a). This included the DNA polymerase sub-unit M54 (59), known inhibitors of MHC class I surface expression m04 (gp34)(51), m06 (gp48)(90), and the Fc receptor m138 (108). After a single round of replication at 24hpi, a total of 111 MCMV ORFs were detected at the high confidence level. To further validate these findings a sub-set of MCMV ORFs were subjected to qRT-PCR analysis (primer sequences see Supplementary File S1), and in agreement with the microarray results, each test-case showed ORF expression was also detectable by qRT-PCR (Figure 1a).

As previously noted for HCMV microarray analysis, there is no overt positional bias towards expression of coding ORFs based on genomic location could be linked to the patterns of gene expression observed during the infection. MCMV ORFs were annotated based on Rawlinson et al (89) and updated with details from additional publications wherever possible. MCMV ORFs from recent predictions (15, 105) were aligned against 55mer probe sequences identifying five probes overlapping with newly predicted ORFs (m107-m107.2, m16-m16.2, m22-m22.1, M34-m34.2, and M58-m58.1) which were re-annotated accordingly.

As a result of the statistical cut-off (\( p < 0.05 \)), MCMV probes for ORFs M44, M70, M75, m135, m143, m144m, m153 and m157 failed to be included, although these genes have been reported to be expressed in previous MCMV studies or are homologues of HCMV genes reported to be expressed (21, 29, 34, 79, 80, 89, 92, 110, 118). It is most likely that the specific probes for these genes exhibit false negative results. Nevertheless, in this study, we aim to purposefully avoid false positives at the sacrifice of capturing a modest level of false negatives. For this reason, we also do not detect \( ie1 \) or \( ie3 \) expression until 24hpi based on the statistical cut-off.
however, these genes are detectable as early as 0.5hpi and 2.5hpi using a more sensitive qRT-PCR approach (Supplementary Figure S3).

To gain further insight into the transcriptional programming of MCMV, we next sought to profile gene expression from three well-characterized MCMV mutants (5, 23, 36, 72) alongside the parental MCMV strain (111) (for schematic of strains see Figure 1b). To characterize the mutant strains before microarray analysis, we sought to: a) determine equivalent infectious doses at the genomic level by measuring genome / PFU ratio for each stock, b) ensure generating MCMVdie3 in a complementing cell line did not drastically alter infectious particle ratio, c) ensure viral growth phenotypes were consistent with those previously published (5, 36, 72), and d) ensure no viral transcription was occurring from deleted loci. On the basis of qPCR we detect equivalent numbers of MCMV genome copies per PFU for each viral strain (Figure 1c) using as a calibrator reference plasmid containing the m115 (gL) gene (nts 166387-167208 GenBank NC_004065) (for detailed account of this approach see (98)). Equivalent numbers of MCMV genomes were also found inside infected cells at 2hpi from different MCMV strains (data not shown). Multi-step growth curves confirmed viral growth phenotypes (Supplementary Figure S4) and qRT-PCR (Qiagen 1-step, Germany) confirmed transcription was not detectable from the respective deleted loci for ie1, ie2 and ie3 (data not shown). These data show the four MCMV strains are experimentally comparable for downstream gene expression analysis.

Based on previous virologic characterization of these MCMV IE mutants, we expect to observe different gene expression profiles for each MCMV strain. For example, given that removal of the ie2 gene causes no reported phenotype (45), we anticipate few gene expression changes in the MCMVdie2 strain relative to the parental MCMV strain. Alternatively, given ie3 has an indispensable regulatory function and is essential for viral growth (5), we expect little or no
viral gene expression to be detectable from the MCMVdie3 strain. However, for the \textit{ie1} deletion mutant (MCMVdie1) it is less clear given that it has wild-type growth characteristics yet the IE1 protein is well known have transcriptional regulatory activity (24, 39, 76), and is further known to interact with cellular host factors (81, 104). In order to profile the gene expression of each mutant strain, we infected NIH 3T3 fibroblasts in parallel at MOI of 1 and harvested total RNA for microarray analysis at 0.5, 6.5 and 48 hpi, along with mock-infected cells.

Figure 2 shows the comparative activation of viral transcriptomes between the four MCMV strains at 48hpi and indicates the transcriptomes of MCMVdie1 and MCMVdie2 are activated with very similar profiles of viral gene expression to the parental MCMV strain over the 48-hour period (Figure 2a). A similar number (within 10%) of MCMV probes were detectable from MCMVdie1 (103 ORFs), MCMVdie2 (114 ORFs) and the parental strain (113 ORFs) at 48hpi. The degree of similarity in expression profiles between the MCMVdie1, MCMVdie2 and parental MCMV strains suggests that \textit{ie1} and \textit{ie2} have a redundant or negligible transcriptional regulatory role in controlling downstream MCMV gene expression during fibroblast infection. Hierarchical clustering (Figure 2b) further indicates few, if any, differences in the global gene expression profiles of MCMVdie1 and MCMVdie2 compared to the parental MCMV strain. These results could potentially point to a redundant role for the \textit{ie1} and \textit{ie2} genes in controlling downstream viral gene expression, or alternatively a lack of sensitivity in controlling the viral genomic programme in fully permissive fibroblast cells.

In marked contrast to the \textit{ie1} and \textit{ie2} mutant strains, MCMVdie3 exhibited an undetectable level of viral gene expression, suggesting \textit{ie3} acts as a global trans-activator of downstream MCMV gene expression as indicated by previous studies(5) To further examine the transcriptional status of MCMVdie3 using a more sensitive approach, immediate early (IE) and
downstream MCMV genes were measured using qRT-PCR in MCMVdie3-infected cells both in the presence and absence of 50µg/ml cyclohexamide (Sigma UK, C7698) at 2.5hpi. These experiments confirmed IE kinetic class genes were expressed in MCMVdie3, but genes beyond the IE region were not (Supplementary Figure S5).

The design of the MCMV microarray platform enables selective detection of transcripts originating from both strands of the viral genome by having probes designed in sense (S) and antisense (AS) orientation to each MCMV ORF. At 24hpi we detect antisense transcripts from 23 AS loci, five of which (m104as, M113as, m147as, m163as, m168as) have overlapping ORFs on the opposite strand of the genome indicating known or predicted regions of bidirectional transcription based on prior annotation(105). Three other loci were found to have neighbouring but non-overlapping ORFs in their vicinity (M57as, m74as and M88as). An additional 15 MCMV AS probes detected at 24hpi were found to have no overlapping or nearby ORFs located on the opposite strand, indicating previously unknown non-coding transcripts derived from regions outside of MCMV ORFs (m04as, m05as, m06as, m07as, m09as, m13as, m41as, M47as, M50as, M87as, M102as, M115as, m119.2as, m124as, m145as). At 48 hpi an additional eight antisense probes were detectable but all have overlapping or nearby ORFs on the opposite strand of the genome (M48as, m69.1as, M89as, M94as, m108as, m119.3as, m132as, and m144as). In total, evidence of antisense transcription was detected from 35 loci over the four time points as measured by microarray analysis (Table 1 and Figure 3a). 26 of these loci were also found to have significant signal from their corresponding sense (S) probe indicating a potential site of bi-directional transcription. A trend towards antisense transcription occurring more frequently at the terminal ends of the MCMV genome is also noted (Figure 3a).
In order to independently validate AS transcripts identified by microarray analysis, we generated cDNA libraries from MCMV infected fibroblasts pooled from either 4, 8 and 12 hpi (IE library), 16, 24 and 32 hpi (E library) or 40, 60, 80 and 100 hpi (L library). cDNA libraries were generated as described previously for HCMV (120). cDNA clones overlapping AS microarray regions were found at m04as, m74as, M94as (none of which have overlapping ORFs on the opposite strand of the genome) and m148as (which has the m147 ORF on the opposite strand of the genome) (for validated AS transcripts see Table 1b). We also find one large cDNA clone that overlapped three MCMV ORFs (m119.2, m119.3 and m119.4), two of which had potential AS regions identified by microarray probes (m119.2 and m119.3). Four additional AS cDNA clones were found to overlap AS regions not identified by microarray analysis (m19as, M72as, m149as and m151as). These experiments thus reveal for the first time that antisense transcription occurs frequently throughout the MCMV genome, an observation that will likely seed further studies.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

Figure 1 - MCMV ORF detection and characterization of MCMV IE deletion mutant

strains 1a i Microarray signals for 10 MCMV ORFs in mock and MCMV-infected cells. 1a ii qRT-PCR validation of the 10 MCMV ORFs at 24hpi. Y axis represents crossing point (Cp) values from qPCR amplification curves with low Cp values indicating high transcript abundance. 1b Schematic representation of recombinant MCMV IE deletion mutants. The map of the parental MCMV genome is shown at the top with structure of the ie1, ie2 and ie3 transcripts below. Coding exons are shown in black with arrows indicating the direction of transcription and white triangles represent deleted loci. The grey box marks the MCMV enhancer ie1/ie3 promoter. The MCMVdie3 revertant strain (1b iii) was renamed in this study to MCMVdiie2 as the HCMV MIEP is inserted between two HpaI sites spanning the transcription start site of the ie2 gene, disrupting ie2 expression (marked with a cross) 1c Viral titres and genome particle / PFU equivalence of four MCMV strains as determined by qPCR.

Figure 2 - Gene expression program of MCMV, MCMVdie1, MCMVdiie2 and MCMVdie3 2a Volcano plots compare microarray signals from mock and infected samples at 48hpi using p-value (y-axis) and fold change (x-axis) comparisons derived from empirical Bayes testing with two biological replicates per group. 2b Hierarchical clustering of high confidence MCMV probes with each row representing a single probe normalized to its mean value across the data set to show relative expression. Yellow indicates increased expression and blue indicates decreased expression relative to the mean. Genes clustered based on the similarity of their expression profile across the dataset, with similar genes connected at the hierarchical tree on the left. Data represents mean values from two biological replicates.
Figure 3 - MCMV genome activation measured by microarray analysis and qRT-PCR

Transcript abundance of ORFs expressed from the parental MCMV measured using oligonucleotide microarrays at 0.5 hpi (i), 6.5 hpi (ii), 24 hpi (iii) and 48 hpi (iv) in NIH 3T3 cells at MOI1. Histograms represent mean values from two replicate samples after background (mock) subtraction. Transcripts are arranged in order from left to right according to ORF names ranging from m01 up to m170 with sense probes shown in the left boxes and anti-sense probes on the right. All raw data are available in Supplementary Material.
Table 1. High confidence MCMV microarray probes

Table 1a - Microarray probes for coding MCMV ORFs found to be significantly upregulated in MCMV infected cells versus mock to a high confidence level (p<0.05 empirical Bayes). Probe ID, ORF name, MCMV and HCMV gene name, time detected (hpi), protein type, annotation and virion association (based on (49)) are shown in columns. Table 1b - Microarray probes for antisense transcripts found to be significantly upregulated in MCMV infected cells versus mock to a high confidence level (p<0.05 empirical Bayes). AS probes with significant signal also found from the corresponding sense probes are marked with an “S” in the rightmost column. Details of cDNA cloning validation is also noted in the annotation column where applicable.
**Figure 1 - MCMV ORF detection and characterization of MCMV IE deletion mutant strains**

1a i Microarray signals for 10 MCMV ORFs in mock and MCMV-infected cells. 1a ii qRT-PCR validation of the 10 MCMV ORFs at 24hpi. Y axis represents crossing point (Cp) values from qPCR amplification curves with low Cp values indicating high transcript abundance. 1b Schematic representation of recombinant MCMV IE deletion mutants. The map of the parental MCMV genome is shown at the top with structure of the ie1, ie2 and ie3 transcripts below. Coding exons are shown in black with arrows indicating the direction of transcription and white triangles represent deleted loci. The grey box marks the MCMV enhancer ie1/ie3 promoter. The MCMVdie3 revertant strain (1b iii) was renamed in this study to MCMVdiie2 as the HCMV MIEP is inserted between two HpaI sites spanning the transcription start site of the ie2 gene, disrupting ie2 expression (marked with a cross).

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<table>
<thead>
<tr>
<th>Unique Probe ID</th>
<th>QAF</th>
<th>Strand</th>
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<th>ORF (Unique)</th>
<th>Time on</th>
<th>Protein type</th>
<th>Annotation</th>
<th>Virus source</th>
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<td>44032</td>
<td>v123</td>
<td>C</td>
<td>US22 (G12)</td>
<td>i10</td>
<td>6.5hpi</td>
<td>Immediate early</td>
<td>The spliced v123 (i0) gene has sequence similarity to members of the US22 family of HCMV (77)</td>
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<td>44119</td>
<td>v123</td>
<td>C</td>
<td>US22 (G12)</td>
<td>a1</td>
<td>6.5hpi</td>
<td>Immediate early</td>
<td>Set apart in early viral kinetics (a1) and total length of E1 transcript plus E4p is (6.6 kbp), total RNAs 577-658 (93)</td>
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<td>Glycoprotein m02</td>
<td>Sequence variation and early transcriptional kinetics found in wild-derived MCMV isolates, with encoded protein could not be found on cell surface (28)</td>
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<td>v04</td>
<td>GC</td>
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<td>The viral gene product, gp04, forms a complex with M133 (u3) which reaches the cell surface (166) and is required for gp04 recognition of infected cells (129), redirects also antigenic fine epitope of viral glycoprotein (128)</td>
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<td>Virion glycoprotein (vMIA), inhibits the levels of MHC class I molecules and renders the cell refractory to T cell recognition of infected cells (95), redirects also antigenic fine epitope of viral glycoprotein (128)</td>
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<td>Tryptophan family viral</td>
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<td>US15.2 family viral, viral surface antigen (vM15.2) probe overlaps with newly predicted ORF v15.2 (105)</td>
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<td>Maintenase glycoprotein</td>
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<td>Early antigenic early gene (m03)</td>
<td>Anti-apoptotic function (79)</td>
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<td>US22 family homolog</td>
<td>Gag-mX strain showed no obvious growth phenotype (79)</td>
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<td>6.5hpi</td>
<td>DNA polymerase delta subtype</td>
<td>DNA binding protein (3, 4), DNA polymerase delta subtype (59)</td>
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<td>6.5hpi</td>
<td>DNA polymerase delta subtype</td>
<td>Major ssDNA binding protein (3, 4), DNA polymerase delta subtype (59)</td>
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<td>C</td>
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<td>i10</td>
<td>6.5hpi</td>
<td>DNA polymerase delta subtype</td>
<td>Part of the helicase-primase complex of three proteins (M70, M102, and M105) (70, 140)</td>
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<td>M07</td>
<td>C</td>
<td>UL37</td>
<td>Ex2</td>
<td>6.5hpi</td>
<td>Assembly protein and protease</td>
<td>Anti-apoptotic (79), immunoregulatory gene that modulates T helper cell response (97). Found to be a functional oncogene (131) and in vivo and in vitro independent of other replication-essential genes (109, 110)</td>
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<td>UL37</td>
<td>M118</td>
<td>6.5hpi</td>
<td>Tripartite motif transcriptional regulator (58)</td>
<td>Tripartite motif transcriptional regulator (58), regulation of viral transcription by inhibiting caspase-8 activation in a manner similar to members of the anti-apoptotic Bcl family (107). May also be a transcriptional activator (89)</td>
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<td>Tripartite motif transcriptional regulator (58), regulation of viral transcription by inhibiting caspase-8 activation in a manner similar to members of the anti-apoptotic Bcl family (107). May also be a transcriptional activator (89)</td>
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<td>i10</td>
<td>6.5hpi</td>
<td>Receptor transduction homolog</td>
<td>Anti-apoptotic (79), homolog of the large subunit of ribonucleotide reductase (85). Blocks NFkB activation as a result of its inhibitory effect on receptor-interacting protein 1 (RIP1) signaling (83, 139)</td>
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<td>i10</td>
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<td>Capsid</td>
<td>Smallest capsid protein (14)</td>
<td>Smallest capsid protein (14), critical for viral budding (83), required for packaging (84) and is required for Ly49P recognition of infected cells (57). m06/gp48 also antagonizes the effect of Fas/CD95 or Trail.</td>
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<tr>
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<td>DNA polymerase</td>
<td>DNA polymerase alpha type (50)</td>
<td>DNA polymerase alpha type (50), essential for viral replication (50), required for packaging (84) and is required for Ly49P recognition of infected cells (57). m06/gp48 also antagonizes the effect of Fas/CD95 or Trail.</td>
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<td>DNA polymerase</td>
<td>DNA polymerase alpha type (50)</td>
<td>DNA polymerase alpha type (50), essential for viral replication (50), required for packaging (84) and is required for Ly49P recognition of infected cells (57). m06/gp48 also antagonizes the effect of Fas/CD95 or Trail.</td>
</tr>
<tr>
<td>44079</td>
<td>M60</td>
<td>C</td>
<td>UL53 (AP)</td>
<td>i10</td>
<td>6.5hpi</td>
<td>Assembly protein, protease</td>
<td>Assembly protein and protease (11, 66) which cornerstone the domain structure and cleavage site preserved in HCMV UL49</td>
<td>VAP</td>
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<tr>
<td>44080</td>
<td>M02</td>
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<td>UL54 (G12)</td>
<td>i10</td>
<td>6.5hpi</td>
<td>Upper matrix protein</td>
<td>Encodes a structural protein unique to the beta-herpesvirus group, aka gp11 - same family as M04 (91)</td>
<td>VAP</td>
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<td>44082</td>
<td>M04</td>
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<td>UL54</td>
<td>i10</td>
<td>6.5hpi</td>
<td>Upper matrix protein</td>
<td>Encodes a structural protein unique to the beta-herpesvirus group, aka gp11 - same family as M04 (91)</td>
<td>VAP</td>
</tr>
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<td>44089</td>
<td>M09</td>
<td>C</td>
<td>UL59 (G12)</td>
<td>i10</td>
<td>6.5hpi</td>
<td>Phosphoprotein</td>
<td>Encodes a small structural phosphoprotein unique to the beta-herpesvirus group shown to be a modulator of both host and viral genes (136)</td>
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</tr>
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<td>44100</td>
<td>M100</td>
<td>C</td>
<td>UL59 (G12)</td>
<td>i10</td>
<td>6.5hpi</td>
<td>Helicase protease</td>
<td>Part of the helicase-protease complex of three proteins (M09, M100, and M105) (74, 75)</td>
<td>VAP</td>
</tr>
<tr>
<td>44103</td>
<td>M105</td>
<td>C</td>
<td>UL59 (G12)</td>
<td>i10</td>
<td>6.5hpi</td>
<td>Helicase protease</td>
<td>Part of the helicase-protease complex of three proteins (M09, M100, and M105) (74, 75)</td>
<td>VAP</td>
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<tr>
<td>44114</td>
<td>M118</td>
<td>C</td>
<td>UL118</td>
<td>i10</td>
<td>6.5hpi</td>
<td>Possible alternate splice to v110 as for HCMV UV 118 (62, 65)</td>
<td>VAP</td>
<td></td>
</tr>
<tr>
<td>44117</td>
<td>v110</td>
<td>C</td>
<td>UL118</td>
<td>i10</td>
<td>6.5hpi</td>
<td>Possible alternate splice to v110 as for HCMV UV 118 (62, 65)</td>
<td>VAP</td>
<td></td>
</tr>
</tbody>
</table>
Putative glycoprotein

Encodes the 88 kDa Fc receptor glycoprotein (107). Fc receptor specific (m138) deletion mutants show unexpected alterations in virulence and are attenuated in normal and immunosuppressed adult mice (31). In vitro deletion mutates the MCMV gene MUL17, UL161, and UL162 (m138).

Down-modulates the NKG2D ligands MULT-1, H60 (65) and RAE-1 epsilon (8).

Down-regulates the NKG2D receptor (65).

US26 family homolog

Required to block PKR-mediated shutdown of cellular protein synthesis and associated antiviral response (21, 110).

Putative membrane glycoprotein

Spliced gene m142 selectively targets CD86 expression on APCs (68).

US22 family homolog

Located on MCMV virions and involved in virion assembly (110).

VAP

MCMV viral carrier protein gp36.5 (64), the m164-derived peptide 257-AGPPRYSRI-265 is presented by the MHC-I molecule D(d) (45).

Required for MCMV virulence in killing SCID mice and for optimal viral growth in vivo (121).

Glycoprotein

The m152 gene product (gp40) is a member of MGP family that downmodulates MHC class I proteins on infected cells (50), disrupts export of MHC class I complexes from pre-Golgi compartments to the Golgi (122), modulates NK cell proliferation, engages the NKG2D receptor, and can inhibit NK cell-mediated lysis (8).

Threonine-serine-rich glycoprotein of MGP family m145, some homology to EHV1 g(X) (106).

Putative membrane glycoprotein

Spliced gene m142 selectively targets CD86 expression on APCs (68).

Putative membrane glycoprotein

Spliced gene m142 selectively targets CD86 expression on APCs (68).

MCMV virus carrier protein gp36.5 (64), the m164-derived peptide 257-AGPPRYSRI-265 is presented by the MHC-I molecule D(d) (45).

Required for MCMV virulence in killing SCID mice and for optimal viral growth in vivo (121).
<table>
<thead>
<tr>
<th>Protein</th>
<th>Identifier</th>
<th>Function</th>
<th>Comments</th>
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</thead>
<tbody>
<tr>
<td>M50</td>
<td>UL50</td>
<td>Nuclear export</td>
<td>Conserved herpesvirus proteins that form a complex essential for egress of nucleocapsids from the nucleus (94)</td>
</tr>
<tr>
<td>M50</td>
<td>UL50</td>
<td>Nuclear export</td>
<td>M50/p35 inserts into the inner nuclear membrane and is aggregated by M53/p38 to form the capsid docking site (82)</td>
</tr>
<tr>
<td>M52</td>
<td>UL52</td>
<td>Nuclear export</td>
<td>Conserved herpesvirus proteins that form a complex essential for egress of nucleocapsids from the nucleus (94)</td>
</tr>
<tr>
<td>M53</td>
<td>UL53</td>
<td>Nuclear export</td>
<td>M50/p35 recruits the cellular protein kinase C for phosphorylation and dissolution of the nuclear lamina (82)</td>
</tr>
<tr>
<td>M55</td>
<td>UL55 (gB)</td>
<td>Glycoprotein</td>
<td>Glycoprotein B (29, 87)</td>
</tr>
<tr>
<td>M56</td>
<td>UL56 (NM)</td>
<td>Tegument protein and homologue of HCMV terminase subunit and HSV ICp0 (13, 38)</td>
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</tr>
<tr>
<td>M69</td>
<td>UL69</td>
<td>Tegument</td>
<td>Tegument protein similar to HCMV transactivator UL69 (111) which is in HCMV induces a G1 block (118)</td>
</tr>
<tr>
<td>M72</td>
<td>UL72 (dUTPase)</td>
<td>Pulmonary dUTPase enzyme required for nucleotide metabolism, replication, and/or repair (86)</td>
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<tr>
<td>M73</td>
<td>UL73</td>
<td>Tegument</td>
<td>Tegument</td>
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<tr>
<td>M78</td>
<td>UL78</td>
<td>G protein-coupled receptor</td>
<td>G protein-coupled receptor (GCR) homologue, same family as M33 (38). Has subcellular trafficking properties. Mutations in UL78 exhibit reduced replication in cultured cells as well as severe attenuation in the infected host (83)</td>
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<tr>
<td>M79</td>
<td>UL79</td>
<td>Tegument</td>
<td>Tegument</td>
</tr>
<tr>
<td>M83</td>
<td>UL83 (PopMatrix)</td>
<td>Virion associated</td>
<td>Homologue of HCMV - a viral-associated factor with inhibition of factor function (46), 17, 74. M83 mutant has aberrant viral growth and antigenic in E20 mice (118)</td>
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<tr>
<td>M86</td>
<td>UL86 (hCOP)</td>
<td>Capsid</td>
<td>Homologue of HCMV viral capsid protein (9)</td>
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<tr>
<td>M88</td>
<td>UL88</td>
<td>Tegument</td>
<td>Capsid</td>
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<td>M93</td>
<td>UL93</td>
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<td>M89</td>
<td>UL93</td>
<td>Virion-associated</td>
<td>Homologue of HCMV virion protein (9)</td>
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<tr>
<td>M95</td>
<td>UL95</td>
<td>G protein</td>
<td>Glycoprotein M with seven hydrophobic stretches that are potential membrane-spanning regions (66, 93)</td>
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<tr>
<td>M112</td>
<td>Ex1 e1</td>
<td>Early</td>
<td>Early protein activator 1 (EA1)</td>
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<tr>
<td>M114</td>
<td>UL114 (UNG)</td>
<td>Glycosylase</td>
<td>Uracil DNA glycosylase enzyme homologue (114) found in herpesviruses required for nucleotide metabolism, replication, and/or repair</td>
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<td>M115</td>
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<td>Glycoprotein M with seven hydrophobic stretches that are potential membrane-spanning regions (66, 93)</td>
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<td>M117</td>
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<td>M119</td>
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<td>M121</td>
<td>UL121</td>
<td>Potential glycoprotein</td>
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<tr>
<td>M123</td>
<td>UL123</td>
<td>Glycoprotein</td>
<td>Glycoprotein M with seven hydrophobic stretches that are potential membrane-spanning regions (66, 93)</td>
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</tbody>
</table>
immediate early
ie3 exon 5, with mRNA terminating at base 177817(76); total length of Ex2 plus Ex3 plus Ex5 is
611 aa, MW is 68.1 kDa

Putative membrane glycoprotein

Potential ORF located within the origin of replication (89)

Functional homolog of HCMV gO and has key role in determining the entry pathway of MCMV (95)

Table 1b – High confidence antisense probes

<table>
<thead>
<tr>
<th>Unique Probe</th>
<th>ORF</th>
<th>Strand</th>
<th>MCMV / HCMV name</th>
<th>Time on</th>
<th>Protein type</th>
<th>Annotation</th>
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<td>m05as</td>
<td>-</td>
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<td>Antisense transcript</td>
<td>Validated with AS cDNA clone M151 position 155486-155181 (5' to 3') overlapping m04 (AS) and clone length 384nt</td>
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<td>Validated with AS cDNA clone M150 position 155500-155200 (5' to 3') overlapping m05 (AS) and clone length 384nt</td>
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