Identification and characterization of a novel spliced form of the meq transcript in lymphoblastoid cell lines derived from Marek’s disease tumours

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In tumour cell lines established from Marek’s disease (MD) lymphomas L-meq is consistently expressed. It contains a 180 bp insertion encoding additional copies of the proline-rich repeat in the meq open reading frame and its product may contribute to the maintenance of MD virus (MDV) latency. In this study, we identified a novel spliced form of the meq transcript in MD-derived lymphoblastoid cell lines and in MDV-infected cells. This transcript, termed Δmeq, encodes an N-terminal 98 aa of the Meq protein and lacks part of the basic leucine zipper (bZIP) and transactivation domains. In MD cell lines, transcription of L-meq was significantly downregulated, while that of the Δmeq transcript was upregulated during apoptosis. These observations were also confirmed at the protein expression level. Reporter assays using meq- and interleukin-2 (IL-2)-promoter-driven luciferase vectors revealed that ΔMeq suppressed transactivation by L-Meq or Meq in a dose-dependent manner. Immunoprecipitation confirmed that ΔMeq was associated with L-Meq or Meq physically. These results suggest that ΔMeq could be involved in apoptosis in MD cell lines as it works as a negative regulator of L-Meq and Meq by direct interaction.

INTRODUCTION

Marek’s disease virus (MDV) is a cell-associated avian herpesvirus that causes malignant T-cell lymphomas in its natural host, chicken (Calnek, 1992). MDV strains are classified into three serotypes and only the strains of serotype 1 (MDV1) causes lymphomas (Schat, 1987). Marek’s disease (MD) has been controlled by vaccination with attenuated or avirulent MDV1 and/or other serotype strains since the 1960s, and successful vaccination has been of economic benefit to the poultry industry. However, the virulence of MDV strains isolated from the field has increased and the efficacy of vaccines has decreased in the past decade due to the emergence of very virulent (vv) and very virulent plus MDV strains (Witter et al., 2005). Thus, studies for understanding the mechanisms of lymphoma formation by MDV and developing a novel vaccine strategy are required. Since MD represents the first malignant tumour controlled by vaccination, elucidating the mechanisms of tumorigenicity and the principles underlying successful vaccination may provide insights into the development of vaccines against other tumorigenic viruses such as Epstein–Barr virus (Epstein, 2001). It has been suggested that several candidate oncogenes are present in internal/terminal repeat long (IRL/TRL) regions of the MDV genome (Kung et al., 1995). Among these genes, meq, which encodes a 339 aa Meq protein with a basic leucine zipper (bZIP) motif that resembles the Fos/Jun family of oncoproteins, is consistently detected in MDV1-infected cells and tumour cell lines (Jones et al., 1992). Meq contains a proline-rich repeat (PRR) region in its C terminus and may function as both a transcriptional transactivator and a transrepressor with its resemblance to the tumour suppressor factor, WT-1 (Qian et al., 1995; Wang et al., 1993). Meq can dimerize with various factors such as c-Fos, c-Jun, ICP4 and p53 as well as Meq itself (Brunovskis et al., 1996). It was also suggested that Meq/Jun heterodimers bind to the AP-1-like sequence and activate the expression of Meq in vitro (Qian et al., 1995, 1996). The meq gene is only present in MDV1, and Meq was shown to be involved in the maintenance of the transformation status of MD tumour cell lines (Jones et al., 1992; Peng et al., 1995; Tillotson et al., 1988). Over-expression of Meq in the rodent cell line induces morphological transformation of the cells and protects the cells from apoptosis induced by tumour necrosis factor
alpha (TNF-α), C2-ceramide, UV-irradiation or serum deprivation (Liu et al., 1998).

Previously, it was reported that a 180 bp sequence is inserted into the region encoding the C-terminal transactivation domain in the meq open reading frame (ORF) of an attenuated vaccine strain, CVI988 (Lee et al., 2000). The gene, termed L-meq, was also detected in chickens infected with vvMDV1 strains, Md5 and RB1B, during latency (Chang et al., 2002a). The inserted 180 bp sequence encodes three additional copies of PRR and, as a result, L-Meq suppressed transactivation by Meq and may partially contribute to the protection of MD by vaccination (Chang et al., 2002b). The meq gene has been known to be polymorphic and several variants have been reported, including L-meq (Chang et al., 2002c). The presence of multiple spliced transcripts of meq has also been suggested in several studies (Jones et al., 1992; Peng & Shirazi, 1996; Le Rouzic et al., 2002). Nevertheless, the functions of Meq and its variants in the establishment of latency or transformation have not been elucidated clearly.

In this study, we identified a novel spliced form of the meq transcript, termed Δmeq, as well as the L-meq transcript in MD cell lines. We speculate that ΔMeq could work as a negative regulator of Meq and L-Meq, and that ΔMeq could be involved in the loss of the transformed phenotype of MD cell lines because the putative ΔMeq protein lacks a part of bZIP and transactivation domains. Thus, we analysed the expression of the meq transcripts and the Meq proteins in MD cell lines during apoptosis, and investigated the involvement of these meq variants in maintenance of the transformed phenotype. As shown herein, the expression of the ΔMeq protein was upregulated during apoptosis, and ΔMeq would in part repress transactivation by L-Meq or Meq by physical association, suggesting that ΔMeq could play a role in apoptosis in MD cell lines.

**METHODS**

**Cells.** MD-derived lymphoblastoid cell lines, MSB1-O (Akiyama & Kato, 1974), MSB1-cl18, a transplantable subclone derived from MSB1-O (Higashihara et al., 1984), MTB1-cl21 (Ikuta et al., 1987) and HP1 (Powell et al., 1974), were maintained at 41°C, 5% CO2 in RPMI 1640 (Sigma) supplemented with 10% fetal calf serum (FCS; Gibco). Chick embryo fibroblasts (CEF) were prepared from 11-day-old fertile eggs (Shiroyama Farm of Breeding Hens Inc.) and maintained at 37°C in Eagle’s MEM (Nissui) supplemented with 10% tryptose phosphate broth (Difco Laboratories) and 10% FCS. The immortalized CEF cell line, DF-1 (Himly et al., 1998), was maintained at 39°C in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) supplemented with 10% FCS.

**Virus.** A strain of vvMDV1, Md5 (Witter, 1983), was obtained from chicken kidney cell culture of experimentally infected chickens in our laboratory. This virus was propagated in the CEF monolayer. The virus was inoculated onto CEF at 100 pfu per well in a six-well plate format and infected cells were harvested 24 h after virus inoculation.

**Cellular DNA extraction.** MD cell lines, CEF (about 5 × 10⁶ cells) infected with Md5 (CEF/Md5) and uninfected CEF were harvested 24 h after virus inoculation. Lysis buffer (20 mM Tris pH 8.0, 25 mM EDTA pH 8.0, 100 mM NaCl and 0.5% SDS) containing 50 mg protease K ml⁻¹, and incubated at room temperature with gentle rotation for an overnight period. After treatment with RNase, total cellular DNA was extracted with phenol/chloroform-isooamylalcohol (25:24:1) and precipitated with ethanol. The resultant DNA was dissolved in TE buffer (10 mM Tris pH 8.0, 1 mM EDTA pH 8.0).

**Induction of apoptosis in MD cell lines.** Approximately 3 × 10⁶ cells per 5 ml medium of MD cell lines were treated with either actinomycin D (2 mg ml⁻¹), etoposide (50 μM), cycloheximide (100 μg ml⁻¹) or dexamethasone (100 μM). Cells were then harvested after 3, 6 and 9 h (actinomycin D, etoposide) or 6, 12 and 24 h (cycloheximide, dexamethasone). MD cell lines were also cultured under serum-starved conditions (1 or 0.5% FCS) and harvested after 48 h. Cells were washed twice with PBS (pH 7.2) and used for the extraction of total cellular RNA, or lysed in 2× SDS sample buffer for Western blot analysis as described below. Apoptosis was confirmed by the detection of DNA fragmentation as described previously (Takagi et al., 2006).

**Extraction of total cellular RNA and cDNA synthesis.** MD cell lines untreated or treated with either cycloheximide or dexamethasone were resuspended in 1 ml of the TRIzol reagent (Invitrogen), and total cellular RNA was extracted as indicated by the manufacturer. Total cellular RNA of CEF/Md5 and uninfected CEF was extracted as well. Each RNA sample was treated with 10 U DNase I (Promega) and was incubated at 70°C for 15 min for denaturation. Reverse transcription was carried out in a final volume of 30 μl of reaction buffer containing 50 mM Tris/HCl (pH 8.3), 40 mM KCl, 1 mM DTT, 3 mM MgCl₂, 0.5 mM each of dNTPs, 40 U RNase inhibitor (Promega), 200 pmol oligo(dT)₁₅ and 20 U of the RAV-2 reverse transcriptase (Takara). The mixture was incubated at 42°C for 60 min for reverse transcription, then at 70°C for 10 min. Synthesized first strand cDNA (30 μl) was diluted with distilled water up to 100 μl.

**Semi-quantitative RT-PCR.** Synthesized cDNAs were used to amplify the meq-specific sequences. To standardize the quantity of each cDNA, the expression of the β-actin transcript was measured as an internal control. The primers used for meq-specific PCR were M-S (5′-ATGTTCTAGAGCGCCAGGGCGTGC-3′) and M-AS (5′-GGGGGATAGACGATGTGCTGAG-3′) as described previously (Lee et al., 2000). We also employed primers MR-S (5′-ATGTTGGTGTCGTCAGACGAG-3′) and MR-AS (5′-TTGTCGATCTCCTGTAAGA-3′), which amplify the C terminus coding region of meq (Chang et al., 2002a) for specific quantification of the L-meq transcript. For the β-actin amplification, sense (5′-AGTTTCGACGATGTTGAG-3′) and antisense (5′-TTGTCGCAATGCCAGGTTAC-3′) primers were used. PCR was performed in a final volume of 20 μl containing 1.5 mM MgCl₂, 0.25 mM of each of primers, 0.1–0.5 μg of cellular DNA or cDNA samples, 0.2 mM of dNTPs and 0.25 U Taq polymerase (Takara). PCR reaction was carried out as follows: 1 cycle of a denaturation step at 95°C for 5 min, 20 cycles of amplification at 95°C for 30 s, annealing at 53°C for 30 s and extension at 72°C for 30 s (for β-actin) or 30 cycles of denaturation at 95°C for 40 s, annealing at 56°C for 40 s and extension at 72°C for 1 min 20 s (for meq) followed by the final extension step at 72°C for 7 min. PCR products (10 μl) were separated on a 1.5% (β-actin) or 1.2% (meq) agarose gels and visualized by ethidium bromide staining.

**Sequencing of the L-meq and 0.4 kb PCR product.** The PCR products corresponding to the L-meq and 0.4 kb fragment (termed Δmeq) were extracted from the gel and purified using the GeneClean II kit (Bio 101) and cloned into the pGEM-T easy vector (Promega). The constructed plasmids (designated pGEM-Te-L-meq and pGEM-Te-Δmeq) were purified by a standard mini-prep method.
followed by polyethylene glycol precipitation and sequencing by using the Thermo Sequenase Core Sequencing kit (Amersham Life Sciences) and DNA sequencer SQ-5500 (Hitachi).

**Bacterial expression of Meq fusion proteins and generation of rat antisera.** The *meq* gene of the MD5 strain was amplified by PCR using specific primers, gel-purified and cloned into the pGEM-T easy vector. The resultant plasmids designated pGEM-Te-Md5meq and pGEM-Te-Δmeq were digested with EcoRI, and subfragments containing Md5meq or Δmeq were cloned into the EcoRI site of the pET TRX Fusion System 32b (Novagen). Fusion proteins were expressed in a bacterial host, AD494 (DE3) pLysS, by induction with 1 mM isopropyl β-D-thiogalactopyranoside for 9 h. Proteins were purified using the His-Bind Resin and Buffer kit (Novagen) as instructed by the manufacturer. Female Wistar rats (12 weeks old) were injected intramuscularly with 100 μg of the entire fusion proteins with complete Freund’s adjuvant, and boosted at days 14 and 21 after the first immunization followed by intravenous immunization on day 28. Antisera were collected 14 days after the final boost.

**Western blot analysis.** Western blot analysis was performed as follows: cell lysates (approx. 1 × 10^6 cells per 10 μl) were subjected to SDS-PAGE (13 % polyacrylamide) and transferred onto the Immobilon Transfer membranes (Millipore). The blots were incubated with primary antibody (rat antisera at a 1: 1000 dilution) for 1 h, washed three times with PBS containing 0.05 % Tween 20 (PBS-T). Then, the blots were incubated with peroxidase-conjugated goat anti-rabbit IgG (Cosmo Bio) for 30 min and washed three times with PBS-T. Finally, the blot was incubated with 0.5 mg 3,3-diaminobenzidine tetrahydrochloride ml^-1 in PBS solution supplemented with 1/1000 volume of 30 % hydrogen peroxide for detection. The blot was also probed with mouse anti-actin monoclonal antibody (Chemicon International) followed by peroxidase-conjugated goat anti-mouse IgG (H + L) (Jackson ImmunoResearch) to verify the amount of proteins loaded and transferred.

**Construction of the expression plasmids.** The L-meq, meq and Δmeq transcripts were amplified by PCR using primers M-S-Xhol, which contains the Xhol site at the 5′ end, and M-AS. Chicken c-Jun transcript was also amplified by PCR using primers cl-S-Xhol (5′-CTCGAGAAGATTGAGGCCAATTTACGGCA-3′) and cl-AS (5′-GTTTGGTTATACCACAACATCACAG-3′). The amplified fragments were gel-purified and cloned into the pGEM-T easy vector, and digested with restriction enzymes Xhol and NotI. The Xhol–NotI fragments were cloned into the Xhol and NotI sites of the eukaryotic expression vector pCI-neo (Promega), and expression plasmids designated pCI-L-Meq, pCI-Meq, pCI-ΔMeq and pCI-c-Jun were constructed. The *meq* transcripts were also amplified using primers M-S-BglII and M-AS-Xhol, gel purified and digested with BglII and Xhol. These fragments were cloned into the BglII and Xhol sites of the pCMV-Tag1 vector (Stratagene) to construct the expression plasmids of N-terminal FLAG-tagged Meq proteins. These plasmids were designated pCMV-L-Meq, pCMV-Meq and pCMV-ΔMeq, respectively.

**Dual luciferase reporter assay.** The promoter region of the *meq* gene was amplified as described by Levy et al. (2003), and that of the IL-2 gene (−578/+34) was also amplified by PCR. These fragments were cloned upstream of the firefly luciferase gene in the pGL3-Basic vector (Promega). The resultant plasmids, designated pGL3-MP and pGL3-IL2P, were used as reporter plasmids. DF-1 cells were seeded in 24-well plates at 3 × 10^5 cells in 0.5 ml DMEM containing 10 % FCS and incubated overnight at 39 °C. Transfection was carried out using the Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer’s instruction. For each well, a total amount of 0.8 μg of the vectors including pGL3-MP or pGL3-IL2P expression vectors and pRL-tk, which served as an internal control reporter, were transfected. After 24 h incubation, cells were lysed with 1 × Passive Lysis buffer, and a dual luciferase assay was performed according to the manufacturer’s instructions using Luminescencer-INR AB-2100 (Atto). The luminescence intensity of firefly luciferase was normalized to that of the Renilla luciferase.

**Co-immunoprecipitation.** Co-immunoprecipitation of L-Meq/Meq with ΔMeq was analysed as follows. Approximately 80 % confluent DF-1 cells in 12-well plates were transfected with 0.8 μg of each pCMV-Tag1 or pCMV-ΔMeq in combination with pCI-L-Meq or pCI-Meq using the Lipofectamine 2000 reagent. After 24 h incubation, cells were lysed with high salt RIPA buffer (50 mM Tris pH 8.0, 500 mM NaCl, 1 % NP-40, 0.25 % sodium deoxycholate and 1 mM EDTA pH 8.0) supplemented with Complete Protease Inhibitor Cocktail (Roche). Insoluble debris was removed by centrifugation, and the supernatants were pre-cleared using Protein A/G Plus Agarose (Santa Cruz) combined with mouse naive serum by gentle rotation for 1 h at 4 °C. After centrifugation, supernatants were transferred into new tubes. FLAG-tagged ΔMeq and interacting proteins were co-puriﬁed using anti-FLAG M2 monoclonal antibody (Sigma-Aldrich) conjugated with Protein A/G Plus Agarose. Immunoprecipitates were washed six times with high salt RIPA buffer and eluted in 30 μl of 2 × SDS sample buffer. After boiling, eluates were subjected to SDS-PAGE (12.5 % polyacrylamide), and Western blot analysis was performed using anti-ΔMeq serum as described above.

Co-immunoprecipitation of c-Jun with Meq or its variants was analysed by the same method, using pCMV expression vectors and pCI-c-Jun. The blot was probed with rabbit anti-c-Jun polyclonal IgG (Santa Cruz) followed by peroxidase-conjugated goat anti-rabbit IgG (Cappel).

### RESULTS

**Detection of the meq variants in MD cell lines**

Total cellular DNA samples of MD cell lines were prepared to confirm the presence of the *meq* gene by PCR. The L-meq gene was mainly detected in MD cell lines, whereas the *meq* gene was amplified from total cellular DNA extracted from CEF/Md5 (Fig. 1a). The *meq-*specific PCR was also performed using cDNA samples synthesized from total cellular RNA of MD cell lines and CEF/Md5 to detect the *meq* transcripts. L-meq transcript and an approximately 0.4 kb fragment were detected in MD cell lines, while meq transcript and a 0.4 kb fragment were detected in CEF/Md5 (Fig. 1b).

**Comparison of the nucleotide sequences and deduced amino acid sequences**

To further characterize the L-meq gene from MD cell lines and a 0.4 kb fragment obtained from MSB1-O, their nucleotide sequences were determined. L-meq from MSB1-O had a 177 bp insertion and an ORF of 1197 bp that encodes for a putative L-Meq protein with 398 aa. A 0.4 kb band, termed Δmeq, was a newly identified form of the *meq* transcript that encodes part of the Meq protein. The Δmeq encodes an N-terminal region of 98 aa of Meq and a frame-shifted different C terminus of 30 aa. Putative ΔMeq protein lacks part of the bZIP and transactivation domains (Figs 2 and 3). The Δmeq transcript detected in CEF/Md5 has almost the same sequence as that of MSB1-O (data not shown), suggesting that this short form is an alternatively spliced product of both L-meq and meq.
Expression of the meq transcripts in MD cell lines during apoptosis

To investigate the functions of meq and its variants in the maintenance of the transformation by MDV, we treated MD cell lines, MSB1-O and HP1, with cycloheximide, actinomycin D, etoposide and dexamethasone to break the transformation status. We also cultured MD cell lines under serum-starved condition. DNA fragmentation assay confirmed that apoptosis was induced effectively with cycloheximide and actinomycin D, and partially with etoposide and serum starvation. Dexamethasone did not induce apoptosis in MD cell lines (Fig. 4a). Cells were harvested and the expression of the meq transcripts during the treatments with cycloheximide and dexamethasone were evaluated by semi-quantitative RT-PCR. The L-meq and Δmeq transcripts were consistently present in untreated

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**Fig. 3.** Structures of the meq variants. The meq ORF of oncogenic MDV1 encodes a 339 aa protein. The L-meq gene found in MSB1-O contains a 177 bp (encoding 59 aa) insertion into the transactivation domain. The Δmeq transcript identified in this study encodes an N-terminal 98 aa and frame-shifted additional C-terminal 30 aa. TRL, Terminal repeat long region; IRL, internal repeat long region; UL, unique long region; IRS, internal repeat short region; US, unique short region; TRS, terminal repeat short region.
MD cell lines (Fig. 4b). In both of the cell lines treated with cycloheximide, the expression of the L-meq transcript was downregulated and finally under the detectable level during apoptosis. This result was also confirmed by specific amplification of 0.8 kbp fragment of L-meq using MR primers described in Methods. In contrast, the Δmeq expression was remarkably upregulated during apoptosis (Fig. 4b, indicated as 'CHX'). Apoptosis was not induced with dexamethasone, and the expression of the L-meq and Δmeq transcripts was not altered after drug treatment in MD cell lines (Fig. 4b, 'Dex').

Detection of the Meq proteins during apoptosis

Meq protein expression during apoptosis was analysed using a rat antiserum against recombinant Md5meq (rMeq) and ΔMeq (rΔMeq) proteins. The putative 70 kDa L-Meq protein was successfully detected in MD cell lines with both of the antisera, while no specific band was detected with preimmune serum (Fig. 5a, upper panels). In addition, an approximately 18 kDa band corresponding to the ΔMeq protein was detected with anti-rΔMeq antiserum (Fig. 5a, lower panels). Therefore, in the following experiment, we used anti-rMeq antiserum for the detection of the L-Meq protein and anti-rΔMeq antiserum for the detection of the ΔMeq protein.

L-Meq expression was consistently observed in untreated cells (Fig. 5b, indicated as 'Cont.'). In MSB1-O, a decrease in the L-Meq expression and an increase in the ΔMeq expression were observed after the induction of apoptosis with either actinomycin D, etoposide or cycloheximide (Fig. 5b). Similarly, the L-Meq expression was decreased and ΔMeq expression was increased in HP1 during apoptosis induced with either actinomycin D or cycloheximide. In this experiment, as mentioned above, etoposide did not induce apoptosis in MD cell lines effectively, but a slight increase of the ΔMeq expression was observed in etoposide-treated cells (Fig. 5b). Dexamethasone did not induce apoptosis in MD cell lines, and no significant change was observed in the expression of the Meq proteins (Fig. 5b). No notable change was observed in the L-Meq expression, but an increase in the ΔMeq expression was observed in cell lines cultured under serum-starved conditions (Fig. 5b, 1 and 0.5% FCS).

The effect of ΔMeq on transactivation by Meq and L-Meq

In our previous study, transactivation by L-Meq and Meq on the meq promoter has been tested by using a luciferase assay (Chang et al., 2002b). L-Meq itself functioned as a transactivator, but its transactivation was lower than that of Meq, suggesting a suppressive effect of L-Meq on Meq transactivation. It has been reported that Meq, as a heterodimer with c-Jun, could recruit the chicken IL-2 promoter, which has an AP-1 site (Levy et al., 2003). In this work, the effect of ΔMeq on transactivation by L-Meq or Meq was examined by using meq or IL-2-promoter-driven luciferase reporter vectors. As shown in Fig. 6(a), both L-Meq and Meq enhanced transactivation on both of the promoters, and transactivation by L-Meq was lower than that by Meq, comparable to our previous study. In contrast, ΔMeq did not transactivate these promoters, as expected from its structure. Co-transfection experiments showed that ΔMeq negatively regulated the transactivation by L-Meq and Meq in a dose-dependent manner (Fig. 6b).

Binding ability of ΔMeq to L-Meq/Meq and c-Jun

To analyse the inhibitory mechanisms shown by ΔMeq, we examined whether ΔMeq can interact with L-Meq, Meq
and the cellular bZIP protein c-Jun by co-immunoprecipitation. The FLAG-tagged ΔMeq plasmid (pCMV-ΔMeq) or control plasmid (expressing only the FLAG tag peptide) was transfected in combination with native L-Meq or Meq plasmid (pCI-L-Meq or pCI-Meq) into DF-1 cells. L-Meq, Meq and FLAG–ΔMeq were successfully expressed in transfected cells, as confirmed by Western blot analysis (Fig. 7a, upper panels). The result of co-immunoprecipitation revealed that L-Meq as well as Meq were co-precipitated along with FLAG–ΔMeq. By contrast, neither L-Meq nor Meq was precipitated in the absence of FLAG–ΔMeq (Fig. 7a, lower panels). The result of co-immunoprecipitation suggested that Meq and ΔMeq were expressed in the transfected cells, as confirmed by Western blot analysis (Fig. 7a, upper panels). The result of co-immunoprecipitation revealed that L-Meq as well as Meq were co-precipitated along with FLAG–ΔMeq. By contrast, neither L-Meq nor Meq was precipitated in the absence of FLAG–ΔMeq (Fig. 7a, lower panels). Next, the FLAG-tagged Meq and its variants were expressed in combination with c-Jun. We overexpressed c-Jun by co-transfection of the pCI-c-Jun plasmid because endogenous expression of c-Jun was moderate in DF-1 cells (Fig. 7b 'Cont.'). As shown in Fig. 7(b), c-Jun was co-precipitated along with Meq, comparable to the results described by Qian et al. (1995). L-Meq was also able to interact with c-Jun, as expected from its structure containing the entire bZIP domain. On the other hand, c-Jun was not co-precipitated along with ΔMeq, showing that ΔMeq did not interact with c-Jun.

**DISCUSSION**

The meq gene has an important role in oncogenesis and maintenance of the transformation status by MDV1 (Liu et al., 1998). Its gene product, Meq, is a transcription factor with an N-terminal bZIP domain homologous to Jun/Fos oncoproteins (Jones et al., 1992). Meq can interact with itself and cellular bZIP proteins such as c-Jun and c-Fos as well as p53 and C-terminal-binding protein, and can contribute to cellular immortalization (Brunovskis et al., 1996; Brown et al., 2006). A recent study also demonstrated that Meq is a transforming protein that activates transformation-related genes such as JTAP-1, JAC and HB-EGF as well as cell survival pathways, by upregulating proto-oncogenes such as bcl-2, c-jun and c-ski (Levy et al., 2005).
It has been noted that meq is a polymorphic gene. Our previous study reported the presence of the L-meq gene in an attenuated vaccine strain, CVI988 (Lee et al., 2000). The L-meq gene has also been identified in some other mild/virulent MDV1 strains (Shamblin et al., 2004). Interestingly, the L-meq gene was also detected in chickens infected with very virulent MDV1 at 3–5 weeks post-infection (Chang et al., 2002a). We have also shown the presence of other forms of the meq gene, S-meq and VS-meq with sequences encoding different copy numbers of the PRR region (Chang et al., 2002c). The biological significance of the coexistence of meq, L-meq and other forms of meq in MDV-infected chickens has not been elucidated clearly, but L-Meq could exert an inhibitory effect on the transactivation by Meq and may suppress the replication of the oncogenic MDV1 (Chang et al., 2002b).

Several studies have shown the presence of multiple forms of the meq transcript (Jones et al., 1992; Le Rouzic et al., 2002). One of these transcripts, termed Meq-sp, was initially identified in the MD cell line, MKT-1 (Peng et al., 1995). Meq-sp is encoded from an alternatively spliced transcript, and its C terminus is replaced by peptides encoded by the second and the third exons of vIL-8 (Liu et al., 1999). A recent study reported two other spliced variants of meq. One of them was termed Meq/RLORF5a, which encodes the N terminus of Meq and a part of RLORF5a, and the other had an uncharacterized sequence.
...in this study, we have identified another spliced transcript, \( \Delta meq \) downstream of the \( meq \) gene (Jarosinski & Schat, 2007). In this study, we have identified another spliced transcript, \( \Delta meq \), and examined the expression of the \( meq \) transcripts and Meq proteins during apoptosis to investigate the function of L-Meq and \( \Delta meq \) in MDV-transformed cell lines.

The \( \Delta meq \) transcript consists of nt 1–294 and 915–1020 of \( meq \) ORF, and encodes an N-terminal 98 aa of Meq and a frame-shifted C-terminal 30 aa (Figs 2, 3 and refer to GenBank accession no. AB091108). \( \Delta meq \) could be an alternatively spliced transcript because the assumed intron (nt 295–914) has possible CT–AC donor and acceptor sites, which are non-canonical but have been reported in the HHV-6 U83 gene (French et al., 1999). However, the \( \Delta meq \) transcript was not detected in DF-1 cells transfected with pCI-Meq or pCI-L-Meq (data not shown), indicating that some other viral factor(s) could be involved in the splicing of the C-terminal coding region of \( meq \) or \( \Delta meq \). In MD cell lines, L-Meq and \( \Delta meq \) transcripts were detected. Western blot analysis confirmed that the \( \Delta meq \) transcript was expressed as a putative L-Meq protein, and that its expression was decreased during drug-induced apoptosis. The \( \Delta meq \) transcript could also be translated to generate an 18 kDa \( \Delta meq \) protein as predicted from its sequence. The \( \Delta meq \) expression was remarkably increased during drug-induced apoptosis as well as serum-starvation, despite the defective induction of apoptosis (Fig. 5).

It has been reported that Meq homodimer binds to the ACACA motif of the MDV origin of replication (MDV-Ori), and represses the flanking bi-directional promoters overlapping MDV-Ori and regulates the replication of MDV (Levy et al., 2003; Parcells et al., 2003). It was also suggested that Meq-sp was dominantly expressed during the lytic replication phase of MDV, as a putative negative regulator of the functions of Meq (Peng & Shirazi, 1996; Parcells et al., 2003). A recent study described the different functions of Meq and Meq-sp (Anobile et al., 2006). The \( \Delta meq \) also lacks the transactivation domain and predominates during apoptosis in MD cell lines. Therefore, we have speculated that \( \Delta meq \) may exert a negative effect on L-Meq or Meq functions such as transactivation. As expected, \( \Delta meq \) inhibited the transactivation by L-Meq or Meq through the \( meq \) and IL-2 promoters in a dose-dependent manner, while its inhibitory effect may be marginal. The third leucine in the bZIP domain of \( \Delta meq \) is replaced by an arginine, a highly hydrophilic residue (Fig. 2). Hence, \( \Delta meq \) would be able to form only one loop of helical conformation that seems insufficient to interact with L-Meq or Meq. Nevertheless, \( \Delta meq \) could exert its inhibitory effect by direct interaction with L-Meq and Meq. Further studies such as chromatin-immunoprecipitation or electrophoretic mobility shift assay are required to investigate detailed mechanisms of interaction and precise roles of \( \Delta meq \). L-Meq may be involved in the MDV latency as suggested previously (Chang et al., 2002a). In addition, L-Meq may also play a role in cellular transformation, as Meq does, because it exhibited transactivation of both of the promoters used in this study, although its activity was lower than that of Meq. Thus, the downregulation of L-Meq as well as the upregulation of \( \Delta meq \) could be involved in cellular apoptosis. \( \Delta meq \) may also play a role in the lytic infection, part of the reason for this speculation is that it predominates in vvMDV-infected CEFs when the cytopathic effect was confluent (data not shown).

![Fig. 7. (a) Co-immunoprecipitation of either L-Meq or Meq with FLAG-tagged \( \Delta meq \). DF-1 cells were transfected with pCI-L-Meq or pCI-Meq (indicated as 'L' or 'M') in combination with pCMV-Tag1 or pCMV-\( \Delta meq \). Whole-cell lysates (WCL) were immunoprecipitated (IP) with anti-FLAG monoclonal antibody, and the precipitates were subjected to SDS-PAGE (12.5 % polyacrylamide) followed by Western blot (WB) analysis using anti-\( \Delta meq \) antiserum. Asterisk indicates an unspecifically detected protein. Vec., Transfected only with pCMV-Tag1. (b) Co-immunoprecipitation of c-Jun with FLAG-tagged Meq or Meq variants. Cells were transfected with pCI-c-Jun in combination with either pCMV-Tag1 (Vec.), pCMV-L-Meq (L), pCMV-Meq (M) or pCMV-\( \Delta meq \) (\( \Delta \)). Immunoprecipitation was performed with anti-FLAG antibody and precipitates were analysed by Western blot by using anti-c-Jun antibody.](image-url)
In summary, we have reported a novel spliced transcript termed Δmeq. Our results demonstrated that ΔMeq would be a negative regulator of the functions of L-Meq and Meq, and may be involved in apoptosis in MD cell lines. Further study for details of expression manners, interaction partners and functions of Meq variants both in vitro and in vivo will contribute to understanding the mechanisms of MDV oncogenicity and transformation.

REFERENCES


