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Transactivation Activity of Meq, a Marek's Disease Herpesvirus bZIP Protein Persistently Expressed in Latently Infected Transformed T Cells

ZHENG QIAN,[1] PETER BRUNOVSKIS,[1] FRANK RAUSCHER III,[2] LUCY LEE,[3] AND HSING-JIEN KUNG[1]*

Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, Ohio 44106[1]; The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104[2]; and Avian Disease and Oncology Laboratory, USDA Agricultural Research Service, East Lansing, Michigan 48823[3]

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Marek's disease virus (MDV) is an avian herpesvirus that induces a variety of diseases, including T-cell lymphomas, in chickens. In latently infected, transformed lymphoid cells, very few viral transcripts or proteins are detected. We previously described a gene, *meq* (MDV *Eco*Q), which is persistently expressed in MDV-transformed tumor samples and cell lines. *meq* codes for a 339-amino-acid protein with a basic-leucine zipper domain near its N terminus and a proline-rich domain near its C terminus. The basic-leucine zipper domain shows homology with Jun/Fos family proteins, whereas the proline-rich domain resembles that of the WT-1 tumor suppressor protein. These structural features raise the possibility that Meq functions as a transcription factor in regulating viral latency or oncogenesis. In this report, we show that the proline-rich domain is a potent transcription activator when fused to the yeast (*Saccharomyces cerevisiae*) Gal4(1–147) DNA-binding domain. The transactivation activity maps to the C-terminal 130 amino acids, with the last 33 amino acids essential. In the absence of these 33 amino acids, a two-and-one-half proline-rich repeat structure was found to exhibit repression activity. We further show that Meq is able to dimerize not only with itself but also with c-Jun. Meq/c-Jun heterodimers bind to an AP1-like sequence in the *meq* promoter region with an affinity much greater than that of Meq/Meq or c-Jun/c-Jun homodimers. Cotransfection chloramphenicol acetyltransferase assays suggest that the Meq/c-Jun heterodimers can up-regulate Meq expression in both chicken embryo fibroblasts and F9 cells. Our data provide the first biochemical evidence that Meq is a transcriptional factor and identify c-Jun as one of Meq's interacting partners.

Marek's disease virus (MDV) is an avian herpesvirus that causes Marek's disease, a progressive, highly contagious and malignant T-cell lymphoma in chickens, several weeks after infection (9, 29). The lytic MDV infection occurs in many cell types, including B cells, but only the latent infection in T cells results in cell transformation (9, 40).

The MDV genome is about 180 kbp in length and is most related genetically and structurally to the genomes of alphaherpesviruses, such as herpes simplex virus and varicella-zoster virus (7, 10). Cross-hybridization and the accumulating MDV nucleotide sequence data show that MDV and other alphaherpesviruses are colinear in the unique long and short regions but differ substantially in the adjacent repeats (7, 34). There are three serotypes of MDV. Serotype I strains are oncogenic, while serotype II (SB-1) and III (herpesvirus of turkeys) strains are not (39, 51). Serotypes II and III, as well as attenuated serotype I MDV strains, have been successfully used as vaccines against Marek's disease (30, 52).

Apart from its economic significance, MDV provides a good model to study oncogenesis by a herpesvirus. The rapid onset of polyclonal tumors suggests the presence of an MDV-encoded oncogene(s). Currently, little is known about either MDV oncogenes or mechanisms of oncogenesis. Recent studies have focused on regions of the genome (*Bam*D, -H, -I_2, -L, and -Q2 [5, 38, 46, 48]) which are persistently expressed in MDV-induced tumors and transformed cell lines. In vitro passages of MDV result in attenuation of its oncogenicity. This change was shown to be associated with a 132-bp repeat amplification from 1 to 2 copies to 3 to 150 copies within the *Bam*H fragment (14, 43). It was suggested that this amplification disrupts the expression of genes involved in oncogenesis (6, 19). Two short open reading frames are encoded within these transcripts (32). However, it is not clear whether these open reading frames are solely responsible for transforming ability, because variants with multiple copies of the 132-bp repeat are still oncogenic (35). This raises the likelihood that there are additional MDV genes crucial to the oncogenesis process.

Recently we identified a basic-leucine zipper (bZIP) gene, *meq*, located in the *Bam*I_2-*Bam*Q_2/*Eco*Q fragments within the terminal and inverted repeat long regions (TR_L and IR_L) of the MDV genome (17). As a potential oncogene candidate, *meq* is expressed in all MDV-induced tumors and MDV-transformed cell lines surveyed thus far. Its bZIP structure in the N-terminal portion closely resembles that of the *jun*/*fos* oncogene family (17). In the basic region, Meq shares 16 of 22 amino acids (aa) with chicken c-Jun. Following the bZIP is a C-terminal domain containing two and one-half copies of direct proline-rich repeat (PRR) sequence. Prolines make up 36.8% (39 of 106) of the residues in the PRR regions, while they make up 21.5% in the C-terminal region. In this regard, this long C-terminal domain is similar to that of the WT-1 tumor suppressor protein, which also has high proline content (8, 15). Apart from the distinct structural motifs identified above, virtually nothing is known about Meq protein function.

* Corresponding author. Mailing address: Department of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, OH 44106. Phone: (216) 368-6655. Fax: (216) 368-3055.

As a first step, we sought to demonstrate that Meq is a transcriptional transactivator.

We show here that the C-terminal domain of Meq (aa 209 to 339) is able to activate transcription when fused to the Gal4 DNA-binding domain. This transactivation activity requires the last 33 aa which carry an RNA-binding motif. The highly PRR structure in its isolated form displays transrepression properties. We further show that Meq effectively dimerizes with c-Jun and binds as a heterodimer to an AP1-like site in a *meq* promoter. Coexpression of Meq and c-Jun significantly augments transcription from the *meq* promoter. These data provide strong evidence that Meq is a transcriptional regulator with the potential to regulate viral and cellular gene expression.

MATERIALS AND METHODS

Plasmids. The plasmids used to test the transactivation of Gal4 fusion proteins, pG5BCAT, Galvp16, wild-type Gal4 (25), and pSG424 (36) were kindly provided by A. Tacaks with the permission of M. Ptashne. pSG424 is the plasmid that contains the Gal4 DNA-binding domain (amino acids 1 to 147) followed by a multiple cloning region and is referred to as Gal4(1–147) in Fig. 2A and 3A. Our starting material for the *meq* fusion plasmids was a Bluescript-based plasmid containing the entire *meq* coding sequence with an *Nco*I site engineered before the translation start codon and an *Eco*RI site immediately after the stop codon, pBS-MEQNR. The *Kpn*I-*Eco*RI fragment of pBS-MEQNR (coding for aa 129 to 339) was blunt ended with T4 DNA polymerase, ligated to an *Eco*RI linker (8-mer), and cloned into the *Eco*RI site of pSG424, resulting in Galmeq (Fig. 2A). The frameshift mutant, Galmeq-FS, was similarly constructed, except a different *Eco*RI linker (10-mer) was used. GalmeqΔC33 was made by cloning a *Kpn*I-*Bam*HI fragment of pBS-MEQNR into pSG424 (coding for aa 129 to 307). The other deletion mutants, GalmeqΔC171, GalmeqΔC130, and GalmeqΔC87, were generated by incorporating various PCR fragments into the *Eco*RI site of pSG424. These PCR fragments employed Galmeq as a template in conjunction with the following sets of primers. The common 5′ primer corresponds to the Gal4 sequence, 5′-GACATCATCATCGGAAGA-3′. The 3′ primers are as follows: (i) 5′-ATGAATTCGGAACCGGAGCAATAGTG-3′ (for GalmeqΔC 171); (ii), 5′-ATGAATTCGAGGGGGGAAGGCCCC-3′ (for GalmeqΔC130); and (iii) 5′-ATGAATTCCTCCGGAGATGGAGGCT-3′ (for GalmeqΔC87). PCRs generating N-terminal deletions employed pBS-MEQNR as a template, along with the T3 promoter primer (U.S. Biochemical Corp.) and the following 5′ primers: (i) 5′-ATGAATTCCAACCTCCTATCTGTACC-3′ (for GalmeqΔ PRR1 and GalmeqΔPRR12) and (ii) 5′-ATGAATTCGGAATCTTCCCTGC ATTGT-3′ (for GalmeqΔPRR123). To generate GalmeqC33, the *Bam*HI-*Eco*RI fragment of pBS-MEQNR was filled in with the Klenow fragment, ligated to an *Eco*RI linker, and cloned into the *Eco*RI site of pSG424. pMEQ1TKCAT was constructed by ligating a blunt-ended 58-bp *Eco*RI-*Eco*RV subfragment of the MDV *Eco*Q fragment into the blunt-ended *Sal*I site in pBLCAT2 (24). pm1TKCAT and pm2TKCAT were constructed by cloning the 58-bp double-stranded oligonucleotides with the corresponding mutations into the same site of pBLCAT2. The blunted 272-bp *Eco*RI-*Xmn*I subfragment from the MDV *Eco*Q fragment was cloned into the blunt-ended *Hin*dIII-*Xba*I site of pCAT-Basic (Promega, Madison, Wis.), resulting in pMEQ2CAT. The expression plasmid of *meq* was constructed by subcloning the *Nco*I-*Eco*RI fragment of pBS-MEQNR into the *Hin*dIII-*Xba*I site of pRc/CMV (Invitrogen). All constructs were verified by DNA sequencing with the Sequenase kit from U.S. Biochemical Corp. (Cleveland, Ohio).

Cells, transfections, and CAT assays. Chicken embryo fibroblasts (CEFs) were maintained in 1:1 M199-Dulbecco's modified Eagle's medium (BRL-GIBCO) supplemented with 2% chicken serum and 3% fetal bovine serum. Transfections were carried out by the calcium-phosphate method (11), with 2 to 5 μg of chloramphenicol acetyltransferase (CAT) reporter plasmids and 5 to 10 μg of expression plasmids per 100-mm-diameter plate. One microgram of RSV-β-gal plasmid per plate was included as an internal control. All transfections were repeated at least twice with two to three replica plates per combination. Forty-eight to 56 h after transfection, the cells were lysed by a freeze-thaw method in 0.1 M Tris-HCl (pH 7.5). For transfection in F9 cells, the same procedure was followed, except that the cells were lysed 24 h after transfection. CAT assays were performed with a previously described phase extraction method (41). All reactions were repeated to make sure that the CAT conversion was in the linear range (<20%). CAT activities were normalized by assessing the β-galactosidase activity present in each cell extract, assayed as described before (37).

Bacterial expression of MeqbZIP and c-JunbZIP. The N-terminal portion of *meq* (from aa 1 to 129) containing the complete bZIP region was expressed in *Escherichia coli*. To develop the appropriate expression clone, an *Nco*I site was introduced before the first codon of *meq* by PCR. The entire *meq* sequence was then cloned into the *Sma*I site of pBluescript KS$^+$, resulting in MeqNco/BS. The *Bam*HI-*Kpn*I subfragment (containing Meq aa 1 to 129) was then filled in with the Klenow fragment and cloned into a Klenow fragment-blunted *Eco*RI site of pET21b (Novagen, Madison, Wis.). This resulted in a product (MeqbZIP) with a T7 tag at the N terminus and a His-6 tag at the C terminus. The c-junbZIP construct [1] is provided by R. Hanson with the permission of T. Curran. Protein expression was induced with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 6 h. The protein was purified to about 80% homogeneity by nickel chelation chromatography as recommended by the manufacturer (Qiagen). After elution, the protein was dialyzed extensively in a buffer containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [pH 7.9]), 100 mM KCl, 12.5 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, and 20% glycerol.

Gel retardation assays. Complementary oligonucleotides were annealed and labeled with polynucleotide kinase (U.S. Biochemical Corp.) according to the manufacturer's instructions. The labeled double-stranded DNA probes were incubated with a total of 0.1 μg of protein in a reaction buffer containing 25 mM HEPES (pH 7.9), 100 mM KCl, 0.5 mM $MgCl_2$, 1 mg of bovine serum albumin per ml, 10% glycerol, 5 mM dithiothreitol, and 0.1 μg of poly(dI-dC) per ml at 37°C for 30 min. In cases in which both Meq and c-Jun were present, equal moles of proteins were used. The DNA-protein complexes were resolved on 5% nondenaturing polyacrylamide-Tris-glycine gels.

In vitro translation. A *Bam*HI-*Kpn*I fragment of MeqNco/BS (containing aa 1 to 129) was cloned into pCITE-2b (Promega). A *Bam*HI-*Eco*RI fragment of p6CJ-1 (4), which contains the entire coding sequence of the chicken c-*jun* cDNA, was subcloned into pBS-KS+ (Stratagene). The resulting two plasmids were then used as templates for coupled T7-directed in vitro transcription-translation reactions with the TNT system (Promega) according to the manufacturer's instructions.

Immunoprecipitation. Equal volumes (5 μl) of in vitro-translated products were coincubated at 30°C for 30 min prior to the addition of 500 μl of ELB buffer (250 mM NaCl, 50 mM HEPES [pH 7.5], 0.1% Nonidet P-40) and further incubation at 4°C for 1 to 2 h. After addition of specific antibodies and protein A-Sepharose beads, immune complexes were precipitated and extensively washed with ELB buffer prior to resuspension in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) loading buffer. Immunoprecipitated proteins were separated on an SDS-PAGE (12.5% polyacrylamide) gel.

RESULTS

The proline-rich C-terminal domain of Meq has transactivation potential. The Meq protein can be divided into two major domains: the N-terminal bZIP domain (aa 56 to 129) and the C-terminal proline-rich domain (aa 129 to 339) (Fig. 1A). We first set out to test the transactivation potential of the Meq proline-rich domain. To this end, the Meq C-terminal domain, encompassing aa 129 to 339, was fused to the Gal4 DNA-binding domain (residues 1 to 147) (25). This resulted in the fusion construct Galmeq (Fig. 2A). A reporter plasmid, pG5BCAT, which bears five Gal4 binding sites, was cotransfected with Galmeq to CEFs. As an internal control, the RSV-β-gal plasmid was included in the transfection mixture and was used to normalize all CAT values in different extracts. The CAT activity induced by Gal(1–147) (pSG424) was set at a basal level of 1.0. As shown in Fig. 2A, Galmeq activates the reporter gene greater than 50-fold. In contrast, Galmeq-FS, a frameshift fusion construct containing an out-of-frame linker inserted at residue 129, gave only basal activity. This provided the first indication that the proline-rich domain of Meq has transactivation activity: its activity level is nearly 50% of that of wild-type Gal4 and close to 8% of that of Galvp16. Figure 2B shows the dose-response curve of the transactivation by Galmeq. In the range shown, the fold induction of CAT activity is linear with the amount of effector plasmid transfected. In the same range, the cotransfected Galmeq has little effect on the parental plasmid lacking the Gal4-binding sites, pE1bCAT. This ability to activate the parental CAT was not due to differences in Galmeq fusion protein expression, as evidenced by immunoprecipitation of labeled cell lysates with an antibody against the Gal4 DNA-binding domain (data not shown). Taken together, the results presented above demonstrated that the proline-rich C-terminal domain of Meq is capable of regulating transcription.

The transactivation activity of Meq requires the C-terminal 33 aa. To better delineate the transcriptional regulation domain, a series of C-terminal deletion mutants were constructed

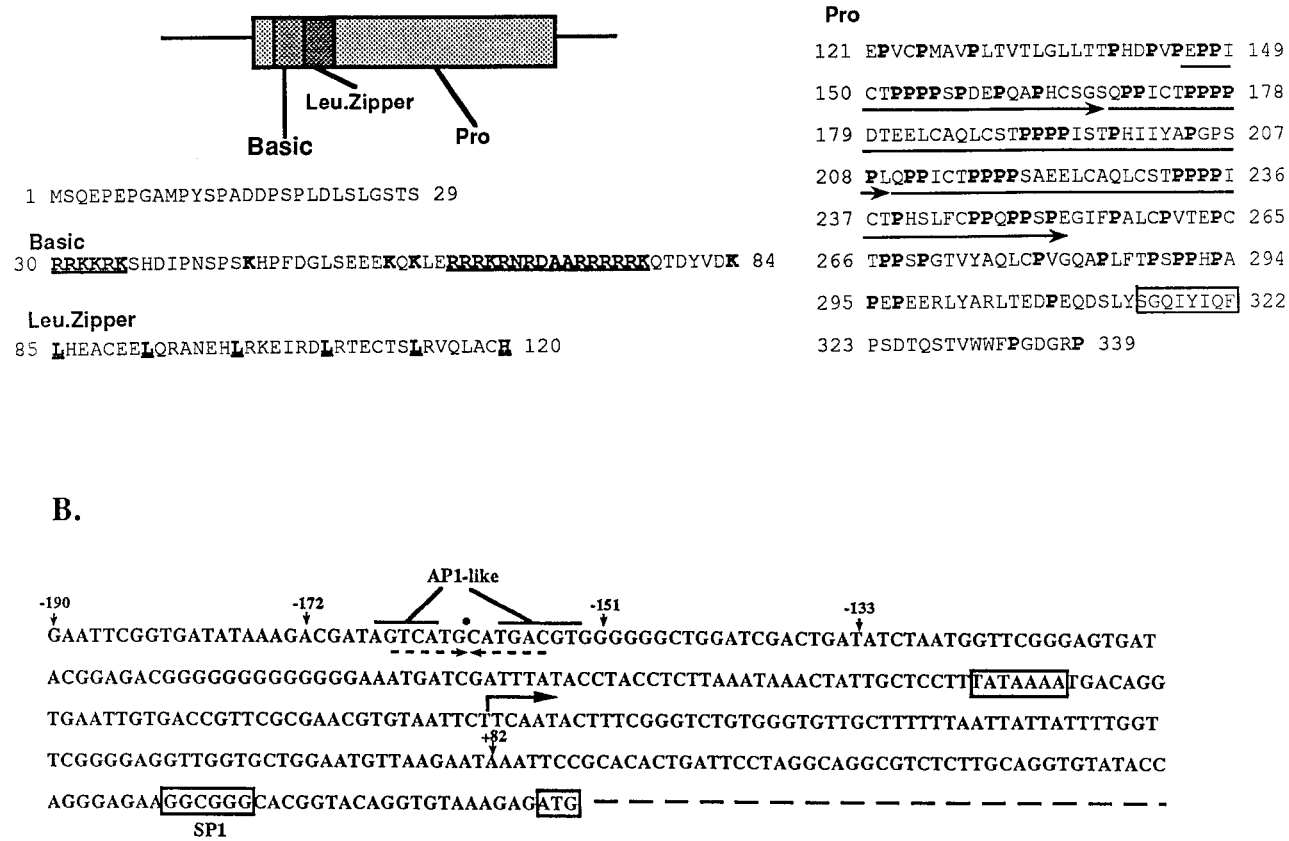

FIG. 1. Meq protein structure (A) and nucleotide sequence of the *meq* promoter region (B). (A) The basic and leucine (histidine) residues are in boldface and underlined. Within the C-terminal region, the proline residues are in boldface and the PRRs are underlined. An octamer consensus RNA-binding motif is boxed. (B) The AP1-like sequences are overlined, and the overlapping palindromic sequence is indicated by both a dot and underlined dashed arrows. The TATA box, an SP1 site, and the translation start codon are boxed. The transcription start site is marked by a solid arrow.

and compared with Galmeq (Fig. 2A). Whereas the expression of Galmeq led to a 55-fold increase in CAT activity, deletion of C-terminal 33 aa (GalmeqΔC33) reduced the activating potential by nearly 30-fold. Further deletion of 87, 130, or 171 aa (ΔC87, ΔC130, and ΔC171) reduced the CAT activity to background levels. This result suggests that the C-terminal 33 aa contain a major activation domain. We then tested whether these 33 aa were by themselves responsible for the transactivation potential of Galmeq. This was tested with a construct containing the C-terminal 33 aa fused to the Gal4 DNA-binding domain (GalmeqC33). Because this construct failed to transactivate the reporter CAT gene, our data indicate that the C-terminal 33 aa are necessary, but not sufficient, to activate transcription.

In the course of this analysis, we also made internal deletions of the PRRs (indicated by arrows in Fig. 2A) in an effort to define their roles in transcriptional regulation. Deletion of the first or first one and one-half PRRs (GalmeqΔPRR1 and Gal meqΔPRR12) slightly increased the transactivation potential, compared with wild-type Galmeq (Fig. 2A). However, when all repeats were removed (GalmeqΔPRR123), the transactivation potential was significantly reduced. This suggests that either the third repeat or at least one of the PRRs is required in concert with the C-terminal region to exert full transactivation potential. The construct GalmeqΔPRR12 delimits the region for full transactivation activity to aa 209 to 339.

The direct repeats within the Meq C terminus have repression activity. In light of the strong repression activity attributed to the proline-rich domain of WT-1 (26, 27), we asked whether the proline-rich segments present in the direct repeats exhibit such an activity. In the cotransfection experiments described above, the E1b TATA sequence was used to drive CAT expression. This reporter construct has a low level of basal activity and is useful in detecting enhancer-dependent activation. It is, however, not very sensitive in detecting repression activity. The herpes simplex virus thymidine kinase (TK) promoter, with a relatively high level of basal activity, is often used for such purposes (24). Using a TK-CAT construct containing five Gal4 DNA-binding sites (pG5TKCAT), we confirmed our previous results which showed that the C-terminal 33 aa are required for maximal transactivation activity (compare the CAT activities of Galmeq and GalmeqΔC33 in Fig. 3A). As before, mutants which carry only the PRRs (GalmeqΔC87, GalmeqΔC130, and GalmeqΔC171) do not have activating function;

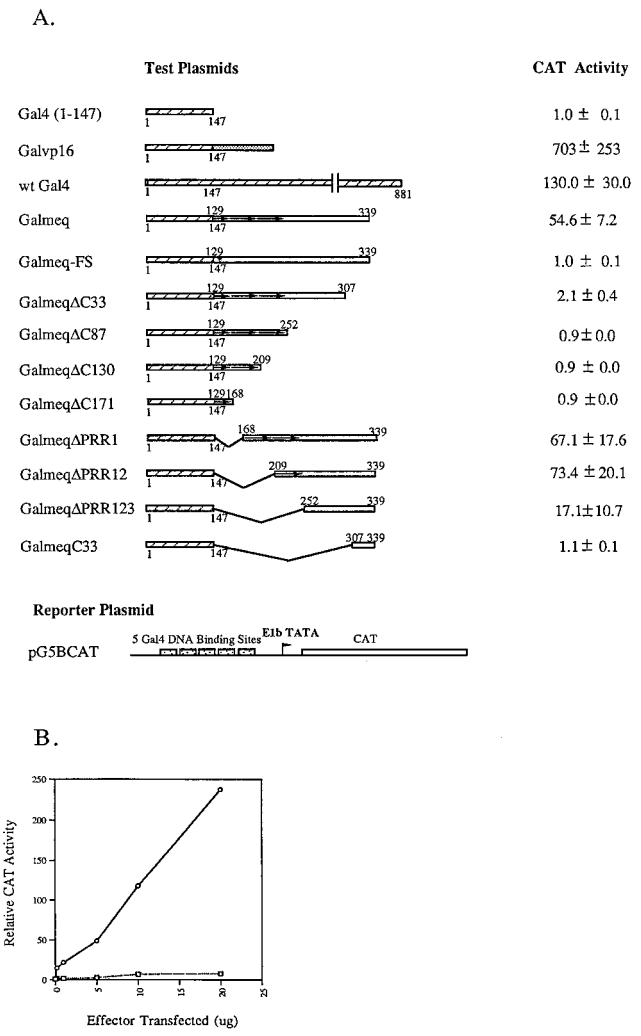

FIG. 2. Transactivation of pG5BCAT by Gal4-Meq fusion proteins. (A) CAT assay results from cotransfection of pG5BCAT with the various Gal4 fusion plasmids. Arrows in the constructs indicate the PRRs. Results were normalized by comparison with a β-galactosidase internal control (see Materials and Methods). wt, wild type. (B) Dose-response curve of Galmeq on the reporter plasmid pG5BCAT (○) and its parental plasmid pE1bCAT (□).

however, with this more sensitive assay, a repression activity of the PRRs could be discerned. Gal4 fusion proteins carrying one-half, one-and-one-half, or two-and-one-half PRRs (Gal meqΔC87, GalmeqΔC130, and GalmeqΔC171) all exhibit a 12- to 15-fold repression activity. This repression activity is about 1/10th that of WT-1 (27). Thus, the repeat structure, while serving an important role in the overall transactivation potential of Meq, has suppression potential in its isolated (presumably exposed) form.

Meq and c-Jun interact cooperatively as heterodimers with the *meq* promoter in vitro. Having attributed the transactivation activity to the C-terminal region of Meq, we then asked whether Meq in its native form can also transactivate target promoters. We would prefer to study physiologically relevant target promoters; however, we presently lack an understanding about optimal Meq binding sites, let alone the promoters regulated by Meq. In light of Meq's homology to the Jun/Fos subfamily of transcriptional regulators in the bZIP region (17), we initially looked for AP1 or AP1-like motifs in MDV promoters, because most herpesvirus promoters are regulated by their own regulatory proteins. Among the handful of MDV promoter sequences available in the literature, interestingly, the promoter of the *meq* gene itself has an AP1-like motif (Fig. 1B). This motif overlaps a perfect palindromic sequence which contains two AP1 half-sites (ATAGTCATGCATGACGT).

To see whether Meq could bind to this site, we used a truncated bacterially expressed *meq* product (MeqbZIP) that carries the N-terminal bZIP domain (aa 1 to 129). In preliminary studies, with a series of deletion mutants, we determined that the DNA-binding capacity of Meq resides solely within the bZIP domain and that MeqbZIP could bind to this site with a moderate affinity (6a). To see whether binding could be enhanced via cooperative interactions with other bZIP products, we examined whether Meq could bind to the site described

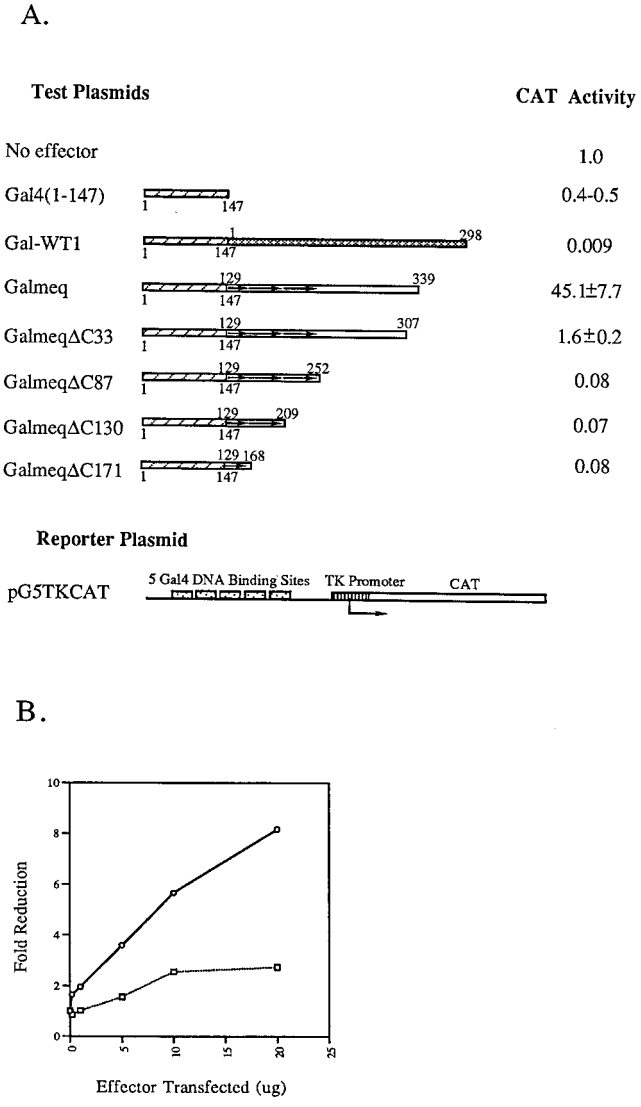

FIG. 3. Repression of pG5TKCAT activity by Gal4-PRR fusion proteins. The TK promoter present in this reporter has some auxiliary sequences besides the minimal TATA box; therefore, it gives higher basal CAT activity in CEF. (A) CAT assay results from cotransfection of pG5TKCAT with the Gal4-PRR fusion plasmids. Arrows in the constructs indicate the PRRs. CAT activities were normalized as described in the legend to Fig. 2. (B) Dose-response curve of GalmeqΔC171 on the reporter plasmid pG5TKCAT (○) and its parental control plasmid pBLCAT2 (□).

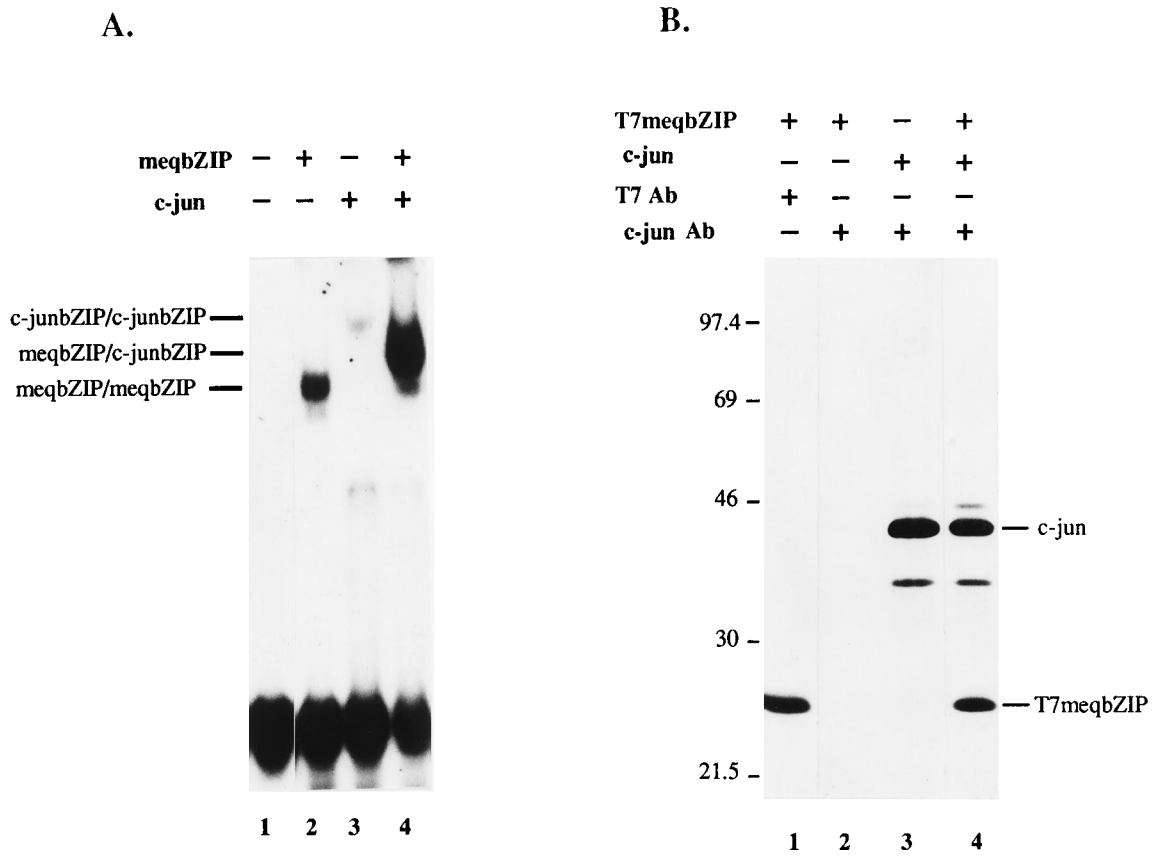

FIG. 4. Interaction between Meq and c-Jun. (A) Gel retardation assay. The double-stranded oligonucleotide probe contains an AP1-like sequence present in the *meq* promoter region (5′-ACGATAGTCATGCATGACGTGG-3′). The positions of three complexes are indicated on the left. (B) Coimmunoprecipitation of in vitro-translated T7meqbZIP and c-Jun. The positions of the two proteins are indicated at right. The extra two bands in lanes 3 and 4 are possibly partially translated or degraded products of c-Jun.

above as a Meq/c-Jun heterodimer. The small size of MeqbZIP allows it to be easily distinguished from bacterially expressed c-JunbZIP (Fig. 4A) or in vitro-translated full-length c-Jun protein (Fig 4B). The differences in size also allow one to easily distinguish homodimer-DNA complexes from the heterodimer complexes, which migrate with an intermediate mobility compared with the other two homodimer complexes. Using the bacterially expressed protein products, together with a labeled oligonucleotide corresponding to the AP1-like motif present in the *meq* promoter, a gel retardation assay was performed. As shown in Fig. 4A, the MeqbZIP homodimer was found to bind to this site with moderate affinity, whereas the binding of the c-JunbZIP homodimer is much weaker. However, when Meqb ZIP and c-JunbZIP were incubated together, a MeqbZIP/c-JunbZIP heterodimer band was observed. This band is intermediate in mobility compared with MeqbZIP/MeqbZIP and c-JunbZIP/c-JunbZIP (Fig. 4A). Taken together, these results suggest that although Meq has a moderate affinity toward the *meq* promoter by itself, its affinity is significantly enhanced when Meq/c-Jun heterodimerization occurs. To further demonstrate Meq's ability to associate with c-Jun, coimmunoprecipitation was performed with in vitro-translated products (Fig 4B). In this experiment, a T7 gene 10-epitope tag (Novagen) was fused to the N terminus of MeqbZIP. This fusion protein can be precipitated by the T7 antibody (lane 1), but not by c-Jun antibody (lane 2), although the latter readily precipitates the in vitro-translated c-Jun (lane 3). Only in the presence of c-Jun, could MeqbZIP be precipitated by c-Jun antibody (lane 4). This further demonstrates that Meq and c-Jun can associate with one another in vitro.

Meq and c-Jun together transactivate the *meq* promoter. To study whether the interaction between Meq and c-Jun has any significance in the transcriptional regulation, a reporter plasmid (pMEQ1TKCAT) containing the AP1-like motif present in the *meq* promoter was constructed. This construct contains a fragment from positions −190 to −133 relative to the transcriptional start site (17) upstream of the *meq* coding region. We chose CEFs as recipient cells, because they represent the natural host for MDV replication and are free of endogenous Meq protein (as would be the case for MDV-transformed T cells). As shown in Fig. 5A, Meq alone has a moderate, but consistent, transactivating effect on this promoter, about three- to fourfold. c-Jun (44) alone has at best a twofold effect. But when c-Jun and Meq are both present, the activation is more than 10-fold, consistent with the gel shift data described earlier. We have also made a reporter construct in which the CAT gene is driven completely by the *meq* promoter. A fragment carrying part of the *meq* upstream region and a portion of the 5′ untranslated region (−190/+82) was cloned into a promoterless CAT construct, pCAT-Basic (Promega). The resulting reporter plasmid, pMEQ2CAT, contains both the AP1-like site and the TATA sequence of the *meq* gene. Once again, a significant activation could be induced when both Meq and c-Jun were present, whereas Meq alone had only a moderate effect (Fig. 5A).

Because there is a low level of c-Jun in CEFs, which could

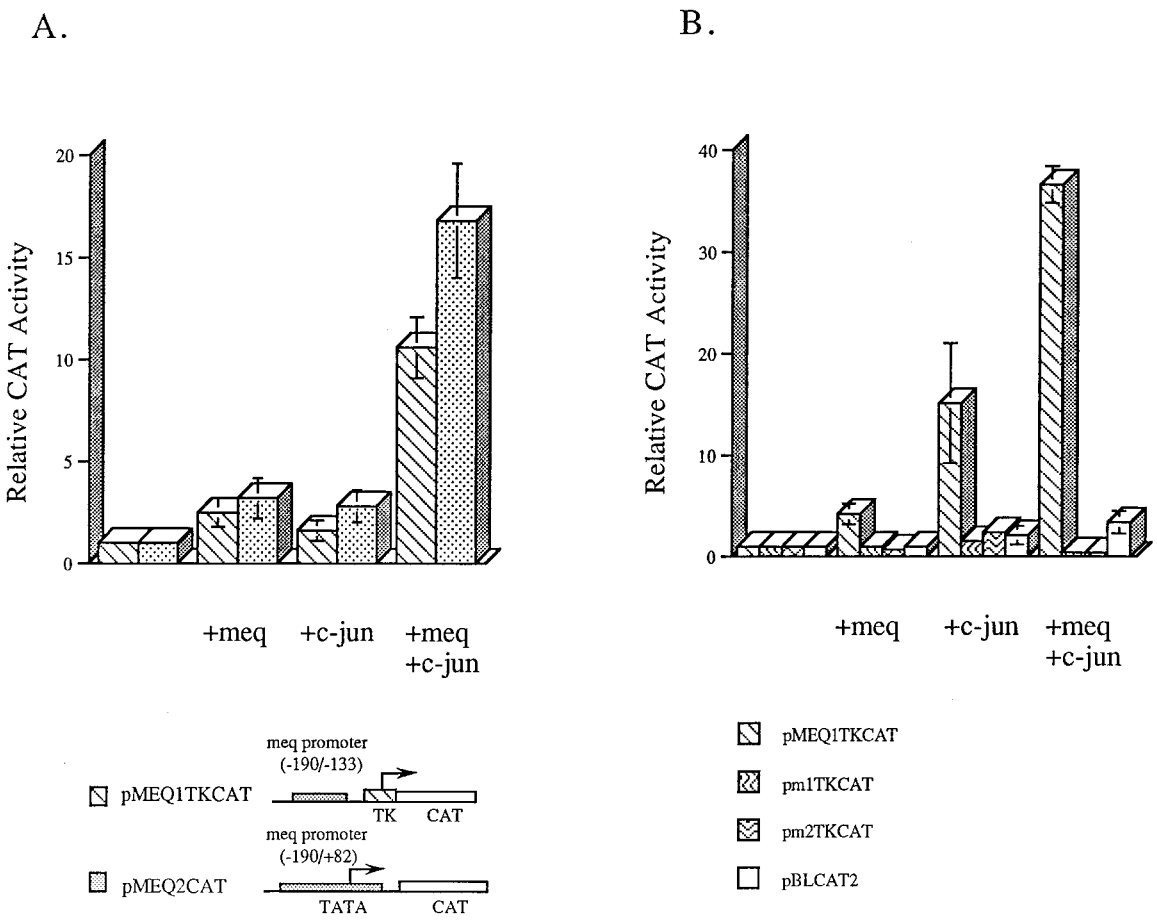

FIG. 5. Transactivation of the AP1-like motif in the *meq* promoter after cotransfection of *meq* and c-*jun* expression plasmids. (A) CAT activities after cotransfection of CEFs. (B) CAT activities after cotransfection of F9 cells. In both cell types, the CAT activities were normalized as described above.

obscure the c-Jun effect, we also tested transactivation in F9 cells, in which the endogenous c-*jun* (and c-*fos*) expression is negligible (12, 22). In these cells, activation by the c-Jun/c-Jun homodimer is more pronounced (~15 fold) (Fig. 5B). Although activation by Meq/Meq remains low (~2-fold), activation by Meq/c-Jun reaches a level that is 30-fold above background. These data, taken together with those from the CEF experiments, clearly demonstrate the synergistic transactivation observed in the presence of both c-Jun and Meq.

Transactivation of the *meq* promoter is via the AP1-like motif. To establish that the AP1-like motif in pMEQ1TKCAT is where the Meq/c-Jun complex exerts its effect, two mutant oligonucleotides were synthesized. The first one, m1, has the palindromic sequence overlapping the AP1-like motif GTC ATGCATGAC replaced by an irrelevant palindromic sequence, CCATCTAGATGG. The second oligonucleotide, m2, has the palindromic sequence replaced by the randomized nucleotides TGCTACGCACTA. In gel retardation assays, both oligonucleotides failed to bind Meq/Meq, c-Jun/c-Jun, or Meq/c-Jun (data not shown). When the corresponding mutations were structured into the reporter plasmid pMEQ1TK CAT, the resulting plasmids, pm1CAT and pm2CAT, no longer responded to the transactivation by Meq/Meq, c-Jun/c-Jun, or Meq/c-Jun in F9 cells (Fig. 5B). The data presented above suggest that Meq and c-Jun, alone or in combination, transactivate the *meq* promoter via the AP1-like palindromic motif.

DISCUSSION

MDV is among the most oncogenic herpesviruses and is the only tumor virus against which successful vaccines have been developed (18). The oncogenic strain (serotype I) and the vaccine strains (serotypes II and III) share a similar genetic organization, particularly with respect to their unique long and unique short regions (31, 53). However, they differ appreciably from one another in the repeat long regions, where by analogy to herpes simplex virus, many of the regulatory proteins are encoded. *meq* is one gene from this region that is unique to oncogenic MDV strains and that is ubiquitously expressed in both tumors and MDV-transformed T-cell lines (17, 38, 46, 48). Meq carries a bZIP domain near its N terminus and a proline-rich domain near the C terminus. Its overall structure thus resembles that of a transcriptional factor. The transcriptional properties of Meq, however, have not been elucidated. In this report, we provide the first biochemical evidence that Meq functions as a transcription factor.

One remarkable feature of Meq is its 210-aa proline-rich domain. This unusually long proline-rich domain is reminiscent of the repression and activation domains of the WT-1 tumor suppressor (8, 15, 26). Recent studies suggest that proline-rich domains may interact with transcriptional factors in regulating transcription (20, 28). The transcriptional factors targeted by proline-rich domains appear to be different from those targeted by acidic or glutamine-rich domains (42, 47).

Proline-rich domains can function either in transactivation or in transrepression, depending on their structures and the context of their binding sites (50). Using Gal4 fusion proteins to target Gal4 DNA-binding sites, we tested the activation and repression potential of Meq. We found that the C-terminal domain has strong activating potential. Full activity requires residues 209 to 339, the last 33 aa of which are absolutely essential. There is no remarkable structure in this 33-aa stretch except for an octamer, SGQIYIQF (boxed in Fig. 1A), matching a consensus RNA-binding motif (33). This motif is present in many RNA-binding proteins, including the *Drosophila melanogaster bcd* (*bicoid*) gene product, which is a transactivator (45). Deletion of a region encompassing this motif in *bcd* severely diminishes its transactivation activity. At present, we do not know whether Meq binds RNA or whether such a binding in any way contributes to transactivation. It is clear, however, on the basis of our deletion analysis, that this region is necessary but not sufficient for Meq's transactivation activity. Full transactivation potential requires additional proline-rich sequences as well. Interestingly, only one PRR is necessary for Meq transactivation activity. In isolated form, these repeats actually exhibit repression activity. These PRRs bear resemblance to the repression domain of WT-1. Moreover, like WT-1, Meq appears to contain both activation and repression domains (50). The overall activity of these domains is likely to be influenced by their particular conformation in cells, which could in turn be regulated by phosphorylation status, by protein-protein interactions, by the context of binding sites in various promoter targets, and by expression of alternatively spliced messages. With regard to the latter, it is noteworthy that MDV-transformed cell lines such as MSB-1 express multiple Meq transcripts (17). Experiments are in progress to determine whether these different messages arise from alternative splicing.

Because *meq* is one of the few genes expressed in latently infected tumor cells, it is likely to play a role in regulating viral latency, cell transformation, or both. *meq* may also play a role in viral replication because it is expressed early in the infection cycle. In the case of Epstein-Barr virus, the viral gene product EBNA2 is a transcriptional factor (13) regulating both viral (e.g., LMP and TP-1 [2, 49, 54]) and cellular (e.g., c-*fgr* [21]) genes involved in latency and transformation. It is also expressed early after infection (3). The transactivation potential of EBNA-2 depends on its interaction with CBF-1 (16, 23). We are therefore interested in testing whether Meq interacts with other cellular factors that promote transactivation potential. We first chose c-Jun as a potential interacting partner, because of its role in oncogenesis and because of the striking resemblance between the bZIP domains of Meq and c-Jun. They share 55% homology in their basic regions, and both contain five leucines and one histidine, equally spaced to facilitate dimerization. The last histidine in the zipper domain is characteristic of the Jun/Fos family. We were able to show that Meq dimerizes readily with c-Jun and that these heterodimers bind to both AP1 (data not shown) and AP1-like motifs, such that heterodimer binding is favored over homodimer binding. We were interested in the AP1-like motif because of its presence in the *meq* promoter. Coexpression of Meq and c-Jun significantly enhanced transcription from the *meq* promoter. This was shown in both CEFs, the natural host for MDV infection, and in rodent F9 embryonic carcinoma cells. That this transcriptional activation is dependent on the AP1-like motif was demonstrated by comparison to mutant promoters with this motif replaced by other palindromic or random sequences. Neither Meq nor c-Jun alone binds the AP1-like motif particularly well, yet the Meq/c-Jun heterodimer displays a high level of affinity toward this site. This suggests that Meq may direct c-Jun into new target sites. It would be of interest to determine which genes (viral or cellular) are regulated by Meq/c-Jun heterodimers.

In our studies, we also noticed that Meq/Meq homodimers are able to modestly increase transcription of the *meq* promoter. This does not appear to be due to dimerization with endogenous c-Jun, as evidenced by the transactivation experiment in F9 cells, which lack detectable c-Jun (12, 22). We do not yet know the natural binding sites of Meq, because we have only studied its transactivation with an AP1-like site in the *meq* promoter. The possibility exists that Meq/Meq homodimers are more potent in regulating other unique target sites.

In summary, this report presents biochemical evidence that Meq can function as a transactivator and that it can interact with c-Jun. c-Jun is the prototype of bZIP family transcriptional regulators. Given the strong implication of c-Jun's role in cell growth and tumorigenesis and the wealth of information regarding its biochemical properties, our findings provide a framework for understanding how Meq may regulate both viral and cellular gene expression in causing transformation.

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