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Chicken Homolog of the *mos* Proto-Oncogene

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We compared the sequence and properties of the chicken *mos* homolog with the previously characterized mouse and human c-*mos* genes. Sequence analysis revealed one major open reading frame of 1,047 base pairs encoding a protein of 349 amino acids. Both the nucleotide sequence and the deduced amino acid sequence showed 62% overall homology to mouse and human c-*mos*, but regions of higher conservation (~70%) occurred in the putative ATP-binding and kinase domains. We detected *mos* transcripts by Northern (RNA) analyses in RNA prepared from chicken and quail ovaries and testes. Evidence for low levels of *mos* RNA expression in adult chicken heart, kidney, and spleen and in the entire embryo was obtained by S1 nuclease protection experiments. In contrast to the low transforming efficiency of human c-*mos* when linked to a mouse retroviral long terminal repeat element, chicken c-*mos* transformed NIH 3T3 cells as efficiently as mouse c-*mos* did. We also show that chicken primary embryo fibroblasts were morphologically altered when infected with an avian retroviral vector containing the chicken c-*mos* coding region.

The *mos* oncogene was originally identified as the transforming gene (v-*mos*) of the acute transforming retrovirus Moloney murine sarcoma virus (Mo-MSV) (13, 37). This virus causes fibrosarcomas in mice and transforms fibroblasts in culture (1, 22). The v-*mos* region contains an open reading frame that encodes a 37,000-dalton *env-mos* fusion protein which has been detected in the cytoplasm of cells acutely infected or transformed by Mo-MSV (27).

The single-copy cellular homolog of v-*mos* was cloned from mouse genomic DNA and was found to be colinear with the viral gene (26). Comparison of the deduced amino acid sequence of the viral and cellular homologs revealed that with the exception of additional N-terminal amino acids, the v-*mos* of the HT-1 strain of Mo-MSV was identical in coding sequence to the proto-oncogene (32). This identity was also reflected in similar transforming efficiencies in DNA transfection assays of NIH 3T3 cells when the *mos* oncogene and proto-oncogene were activated by a Mo-MSV long terminal repeat (LTR) (5, 7). In contrast, the human c-*mos* gene (39), which is 77% homologous to mouse c-*mos*, transforms NIH 3T3 cells 100-fold less efficiently than v-*mos* or mouse c-*mos* genes do when linked to a Mo-MSV LTR (6). Comparisons of the transforming efficiencies of different mouse-human *mos* hybrids indicate that certain domains, notably the C-terminal domain of the *mos* coding region, markedly influence transforming efficiency (6). An overlapping open reading frame in the 5′ portion of human c-*mos* has also been implicated in reducing its transforming efficiency in NIH 3T3 cells (6). In the mouse, an upstream sequence, termed UMS, inhibits transforming activity of mouse c-*mos* in NIH 3T3 cells by acting as a transcriptional terminator (21, 43).

To determine whether the transforming efficiency and regulatory regions of c-*mos* vary in a species-specific fashion, we have begun to examine the properties of the c-*mos* locus from a nonmammalian species, the chicken. The acute transforming retroviruses isolated from this species have provided an abundant source of oncogenes, but no avian virus has been found to contain a *mos* oncogene. We report here the cloning and characterization of the chicken *mos* proto-oncogene and show that the nucleotide sequence and putative amino acid sequence of this c-*mos* gene is 62% homologous to either mouse or human c-*mos*. Like mouse (14, 24, 28, 29) and primate c-*mos* (R. S. Paules, F. Propst, K. J. Dunn, D. G. Blair, K. Kaul, A. E. Palmer, and G. F. Vande Woude, manuscript in preparation), chicken c-*mos* RNA is expressed at high levels in ovaries but at very low levels in other tissues. We also show that the chicken c-*mos* could be activated with proviral LTR sequences to transform NIH 3T3 cells efficiently. Moreover, when inserted into an avian retrovirus vector, chicken c-*mos* morphologically altered chicken primary embryo fibroblasts in culture.

MATERIALS AND METHODS

Cloning of chicken c-*mos* sequences. A total of 10^6 phage from a chicken genomic DNA library prepared in phage vector Charon 4A (12) were screened with a nick-translated ^{32}P-labeled *Ava*I-*Hin*dIII fragment of mouse c-*mos* (30). Hybridizations were performed in 35% formamide at 42°C overnight. Filters were washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and 0.1% sodium dodecyl sulfate at 50°C. Hybridizing phages were isolated, and phage DNA was characterized by restriction enzyme mapping and Southern transfer analyses (33). Subcloning was performed by standard procedures with pBR322, pUC12, M13 mp18, and M13 mp19 as vectors (9, 11, 20, 44).

DNA sequence analysis. All sequencing was performed with the Sanger dideoxy chain termination method with M13 single-stranded DNA (31). The sequencing strategy was based on exonuclease III deletions as described previously (25). A total of 23 different overlapping deletion clones were used for sequencing 1,968 base pairs (bp). Computer analyses of the nucleotide sequence were performed by using programs of Wilbur and Lipman (42) or Stephens (34).

Analysis of RNA. Total RNA was isolated from tissue by the guanidine isothiocyanate-CsCl procedure (10), and poly(A)$^+$ RNA was prepared by selection on oligo(dT)-cellulose (20). Northern (RNA) analysis was performed as described earlier (28, 29) with a nick-translated ^{32}P-labeled

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*Pst*I-*Pvu*II fragment from the c-*mos* coding region. A sensitive S1 nuclease protection assay was used as previously described (3, 28, 29). The probe was prepared by 3′ end labeling a *Sau*3AI fragment obtained from a pUC18 clone containing the downstream 300-bp chicken c-*mos Sac*I coding region fragment with all four ^{32}P-labeled deoxynucleotides (3,000 Ci/mmol) by using the Klenow fragment of *Escherichia coli* DNA polymerase I (28, 29). After digestion with *Apa*LI (in pUC18), a 895-bp fragment was gel purified and annealed (20,000 cpm) to 50 to 100 μg of RNA overnight at 60°C. Under these conditions, there was an excess of probe. S1 digestion, gel separation, and autoradiography were performed as described previously (28, 29). *mos*-containing transcripts will protect an ~225-bp fragment of this probe against digestion by S1 nuclease.

Transformation of NIH 3T3 cells. The following plasmids were used. pM1sp containing one Mo-MSV LTR was described earlier (5). pCMB contains the *Bgl*II fragment possessing the chicken c-*mos* coding region cloned into pBR322 (Fig. 1). pM1CM36 was obtained by filling in the ends of the *Bgl*II fragment with the Klenow fragment of *E. coli* DNA polymerase I and ligating it into pM1sp cut with *Sma*I. pM1CM32 was obtained by digestion of pM1CM36 with *Xba*I and religation of the larger fragment. pM1CM33 was derived by blunt-end ligation of the filled in *Xba*I-*Bgl*II fragment from pCMB containing the *mos* coding region into pM1sp cut with *Sma*I. pTS1 contains the Mo-MSV LTR and mouse c-*mos* (7), and pLh04 contains the Mo-MSV LTR and human c-*mos* (6). Plasmid DNA was purified twice on CsCl gradients and linearized with *Bam*HI or *Eco*RI. Transfection of NIH 3T3 cells was performed as described previously with dexamethasone (0.125 μM) in the culture medium (6, 7).

Transformation of chicken embryo fibroblasts. The genomic clone of chicken c-*mos* was inserted into the plasmid Cla12Nco (15, 16) in three steps. Cla12Nco contains the polylinker from pUC12N (38) flanked by *Cla*I sites. The region upstream from the *Nco*I site (between *Nco*I and *Cla*I in Cla12Nco) derives from the region upstream from the *src* initiator ATG in the SRA strain of Rous sarcoma virus (15). The segment of chicken c-*mos* between *Nco*I and *Sst*I (bp 760 to 805; Fig. 2) was synthesized in vitro. Two different c-*mos* DNA segments were made. One precisely matched the original c-*mos* sequence encoding a proline (CCA) at the second codon. This clone was called c-*mos*-P. In the second construction, the second codon was changed to GCA (glycine) to more closely match the optimal sequence surrounding initiator codons (19). This clone was called c-*mos*-G. After insertion of the synthetic segment, the central region of c-*mos* was inserted as an *Sst*I segment. The resulting plasmid was then recut with *Bst*XI and *Sma*I, and the downstream portion of c-*mos* was inserted as a *Bst*XI-to-*Bgl*I segment. To permit ligation of *Bgl*I to *Sma*I, the protruding *Bgl*I end was digested away with the Klenow fragment of *E. coli* DNA polymerase I before cutting with *Bst*XI. After assembly, the plasmid was mapped with several restriction enzymes, including *Nco*I, *Bst*XI, and *Sst*I to confirm its structure.

The *Cla*I fragment containing the reassembled chicken c-*mos* gene was then excised from the plasmid and inserted into the proviral form of the avian retroviral vector RCAS (16) at the *Cla*I site to yield plasmid RCAS-*mos*. The plasmid DNA was purified from *E. coli* by banding it twice in CsCl-ethidium bromide gradients. A total of 10 μg of this DNA was transfected onto primary chicken embryo fibroblasts as a calcium phosphate precipitate (41).

RESULTS

Cloning and nucleotide sequencing of chicken c-*mos*. Southern transfer analyses of chicken genomic DNA with mouse c-*mos* as the probe revealed only one hybridizing fragment, indicating that *mos* homologous sequences were present as a

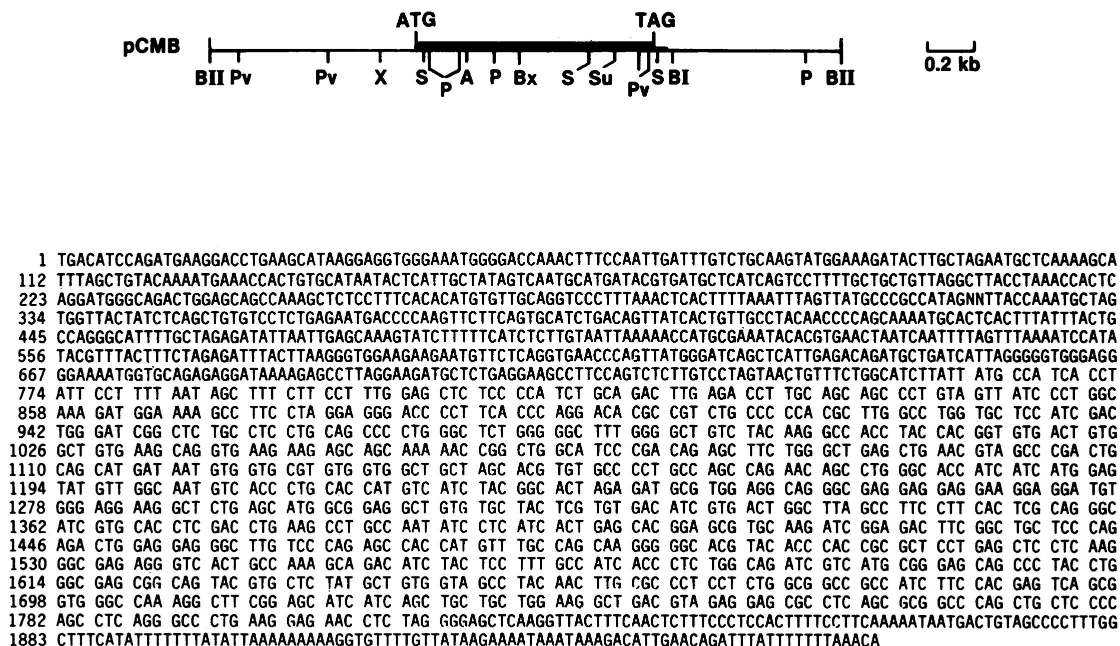

FIG. 1. Restriction map and nucleotide sequence of the chicken c-*mos* locus. The initiation codon (ATG), stop codon (TAG), and open reading frame (black bar) are indicated. A, *Acc*I; BI, *Bgl*I; BII, *Bgl*II; Bx, *Bst*XI; RI, *Eco*RI; P, *Pst*I; Pv, *Pvu*II; S, *Sac*I and *Sst*I; Su, *Sau*3AI; X, *Xba*I. The open reading frame is shown in triplet codons, and two overlapping poly(A) addition consensus signals are underlined.

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human     1      LALRPY RS F   V A P  S SEL ---A LL
mouse     1      LSLCRY PR L   V S S  I LVA RKAG LF
chicken   1  MPSPIPFNSFLPLELSPSADLRPCSSPVVIPGKDGKAFLG

human    38  -ATL  APR  R    C    EQV  LQR  A    S
mouse    41  -TTP  APG  R    F    EQV  MHR  S    S
chicken  41  GTPSPRTRRLPPRLAWCSIDWDRLCLLQPLGSGGFGAVYK

human    77     R  P  I   N CT NRL   R       V   R
mouse    80     H  P  I   N CT DLR   R       I   R
chicken  81  ATYHGVTVAVKQVKKSSKNRLASRQSFWAELNVARLQHDN

human   117  I        RT AGS         FG      Q    AAG
mouse   120  I        RT EDS         FG      Q    ATR
chicken 121  VVRVVAASTCAPASQNSLGTIIMEYVGNVTLHHVIYG-TR

human   157  H-PEGDAGEPHCRTGGQ  LGKCLK  L V N  L
mouse   160  S-PE-----PLSC-REQ  LGKCLK  L V N  L
chicken 160  DAWRQGEEEEGGCGRKALSMAEAVCYSCDIVTGLAFLHSQ

human   196  S V           S QDV   S     EK EDLLCFQTP
mouse   193  S L           S QDV   S     QK QVLRCRQAS
chicken 200  GIVHLDLKPANILITEHGACKIGDFGCSQRLEEGLSQSHH

human   236  SYPL      R   L    GV P       A     MTTK
mouse   233  PHHI      Q   I    IA P       G     MTTR
chicken 240  VCQQGGTYTHRAPELLKGERVTAKADIYSFAITLWQIVMR

human   276  QA  S  R HIL      D   S SA V ED LP QR GD
mouse   273  EV  S  P YVQ      N   S AG V TA LT KT QN
chicken 280  EQPYLGERQYVLYAVVAYNLRPPLAAAIFHESAVGQRLRS

human   316  V QR  RPSAAQ PS RL LVD TSLKAE G    346
mouse   313  I QS  EARALQ PG EL QRD KAFRGA G    343
chicken 320  IISCCWKADVEERLSAAQLLPSLRALKENL     349
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FIG. 2. Comparison of the deduced amino acid sequences of the human (38), mouse (35), and chicken c-*mos* open reading frames. Amino acids homologous to all three species are underlined and shown only for chicken c-*mos*. Gaps are indicated by a dash.

single copy (data not shown). We cloned the *mos* homologous sequences from a chicken genomic DNA library prepared by Dodgson et al. (12), and three isolates were obtained from 10^6 phages. Southern transfer analysis showed that all three isolates were identical and that the hybridizing sequences were localized within a 2.8-kilobase (kb) *Bgl*II restriction fragment (Fig. 1). This fragment was cloned into pUC12, and subclones derived from it by exonuclease III digestion were used for nucleotide sequencing by the Sanger dideoxy chain termination procedure (31). We derived 1,968 bp of sequence information and localized *mos* homologous sequences between nucleotides 765 and 1,700 (Fig. 1). This region of homology contains an open reading frame of 1,047 bp corresponding to a coding region of 349 amino acids which is homologous to the deduced human and mouse c-*mos* amino acid sequences.

Comparisons of the chicken, mouse, and human c-*mos* loci. Comparisons of the nucleotide sequence of the chicken c-*mos* with the mouse and human c-*mos* loci showed a 62% homology within the conserved *mos* open reading frame (data not shown). No significant regions of homology upstream or downstream from this region were observed. Thus, no sequences were found that were homologous to the UMS region in the mouse c-*mos* locus or the MUH region present in both the mouse and human c-*mos* loci (8, 21, 43). Two overlapping poly(A) addition consensus signals, AA TAAA (40), were found 120 bp downstream of the open reading frame (Fig. 1). Poly(A) addition signals were also present at similar distances from c-*mos* stop codons in the mouse (36), human, and African green monkey genes and are apparently used in normal tissues of those species (28, 29; Paules et al., in preparation).

Comparison of the deduced amino acid sequences of the three c-*mos* genes showed that, whereas the human and mouse sequences are 77% homologous to each other, the deduced chicken c-*mos* protein sequence is only 62% homologous to either the mouse or human proteins (Fig. 2). However, there are regions of higher homology that divide the coding region into three conserved domains (residues 45 to 157, 185 to 230, and 244 to 301 of chicken c-*mos*; Fig. 2) which are ~70% conserved among the three species. Amino acid sequence homology of v-*mos* protein to bovine cyclic AMP-dependent protein kinase (2) and to *src* kinase oncogenes (2, 17, 18, 35, 36) has been previously reported. The homology is greatest in the ATP-binding and kinase domains, and amino acid residues in these regions are highly conserved among the *mos* proteins of the three species (i.e., residues 70 to 92 and 204 to 270 in chicken *mos*; Fig. 2). The putative ATP-binding domain is separated from the putative kinase domain by a region of total nonhomology (residues 157 to 169 in chicken c-*mos*; Fig. 2) which corresponds to a region in other kinase oncogenes where large insertions are found (4, 17). The putative kinase domain is further divided by a stretch of 13 amino acid residues of low homology. The N terminus (residues 1 to 44 in chicken c-*mos*) and C terminus (residues 309 to 349 in chicken c-*mos*; Fig. 2) are also less well conserved and show only 41 and 27% homology, respectively, among the three genes.

Expression of chicken c-*mos* RNA. c-*mos* RNA expression has been detected in several mammalian species (24, 28, 29; Paules et al., in preparation), and in each species, the highest levels have been detected in gonadal tissue. In chickens, two *mos* transcripts are detected in ovaries (1.4 and 3.3 kb), whereas in testes, one major RNA transcript (1.4 kb) is observed (Fig. 3). The same size ovary- and testis-specific *mos* RNA transcripts are detected in quail (Fig. 3). Whereas both *mos* transcripts present in chicken ovaries are detected with a probe containing sequences of the *mos* open reading frame (Fig. 3), a probe obtained from the *Pst*I-*Bgl*II fragment at the 3′ end of the chicken c-*mos* clone pCMB (Fig. 1) only detects the larger 3.3-kb transcript (not shown). This result suggests that the two chicken ovarian transcripts differ in their 3′ untranslated region. We were unable to detect RNA transcripts of discrete sizes by Northern analysis in other

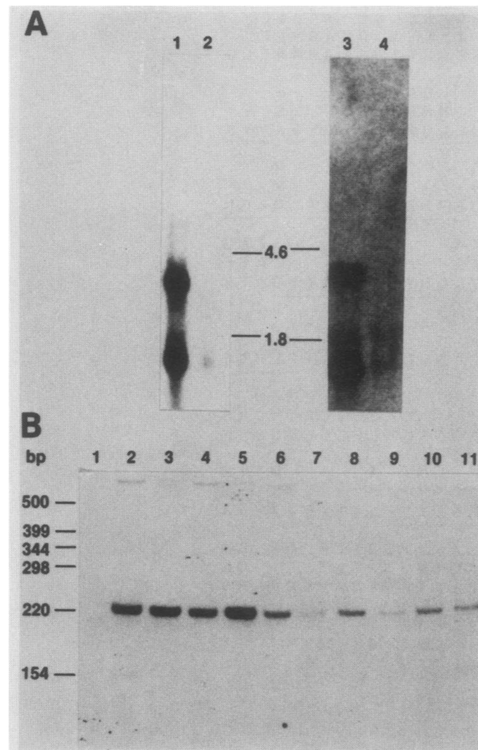

FIG. 3. Detection of chicken c-*mos* transcripts. (A) Northern analysis of RNA from ovaries and testes of chicken and quail. Total RNA (25 μg) was analyzed in each lane. The probe was a ^{32}P-labeled, nick-translated *Pst*I-*Pvu*II fragment from the c-*mos* coding region. rRNA sizes are indicated (in kilobases). Lanes: 1, chicken ovary; 2, chicken testes; 3, quail ovary; 4, quail testes. The detection of a 1.8-kb band in quail RNA is due to background hybridization to 18S ribosomal RNA and was only observed in this particular experiment. X-ray film was exposed for 2 days. (B) S1 nuclease protection analysis. The probe was a 3'-end-labeled *Sau*3AI fragment containing 225 bp from the downstream end of the c-*mos* coding region. Lanes: 1, 50 μg of yeast tRNA; 2, 100 μg of total testes RNA; 3, 50 μg of poly(A)$^+$ testes RNA; 4, 100 μg of whole 4-day-old embryo RNA; 5, 50 μg of poly(A)$^+$ 4-day-old embryo RNA; 6, 100 μg of 10-day-old whole embryo RNA; 7, 100 μg of 17-day-old whole embryo RNA; 8, 50 μg of poly(A)$^+$ 17-day-old whole embryo RNA; 9, 100 μg of whole heart RNA; 10, 100 μg of whole kidney RNA; 11, 100 μg of whole spleen RNA. The X-ray film was exposed for 17 days.

adult chicken tissues or in RNA extracted from whole embryos. However, we were able to demonstrate the presence of *mos* transcripts in embryonic and adult chicken tissue by S1 nuclease protection analysis. With an end-labeled 895-bp DNA fragment probe containing the downstream portion of the c-*mos* coding region, we detected the expected 225-bp (*Sau*3A-*Sac*I; Fig. 1) fragment with RNA from 4-, 10-, and 17-day-old whole embryos and from adult testes, heart, kidney, and spleen (Fig. 3). RNA from adult chicken muscle and liver were negative under these assay conditions (results not shown).

Transforming activity of chicken c-*mos* in NIH 3T3 cells. Mouse c-*mos* can be activated by an LTR upstream or downstream of the coding region and, when activated, can morphologically transform NIH 3T3 cells in culture (7). Typical transformation efficiencies for upstream LTR constructs vary from 1,700 to 7,500 foci per pmol (6, 7). We have shown that human c-*mos* can be activated in the same manner but at a 10- to 100-fold lower efficiency (6). To test the transforming activity of chicken c-*mos*, several plasmids were constructed having Mo-MSV LTR sequences at varying distances upstream from the c-*mos* coding region. pM1CM32 and pM1CM33, with the LTR placed ~200 bp upstream from the chicken c-*mos* coding region transformed NIH 3T3 cells very efficiently (700 to 4,700 foci per pmol), whereas pM1CM36, with the LTR enhancer and promoter regions placed 900 bp upstream, was 100-fold lower in transforming activity (Fig. 4). In these assays, the mouse (pTS1) and human (pLh04) LTR constructs transformed with the same efficiencies as previously described (6, 7) (Fig. 4). The transforming efficiencies of the chicken c-*mos* plasmids (pM1CM32 and pM1CM33) are comparable to that of mouse c-*mos* constructs and are also 10- to 100-fold higher than that of the activated human c-*mos* constructs (Fig. 4). The foci produced by the chicken c-*mos* plasmids were easily distinguishable and strongly resembled mouse *mos* foci (6, 7). Cell lines derived from several foci contained the expected chicken c-*mos* DNA sequences and expressed *mos* RNA transcripts (data not shown). We conclude from these analyses that chicken c-*mos* can transform mouse cells as efficiently as mouse c-*mos*.

Transforming activity of chicken c-*mos* in chicken embryo fibroblasts. To test whether chicken c-*mos* is an active transforming gene in the homologous species, the two c-*mos* proto-oncogene constructions c-*mos*-P and c-*mos*-G (see Materials and Methods) were inserted into the avian retroviral vector RCAS (16). This vector is replication competent and, after transfection, the viruses spread throughout the culture. A total of 10 μg of each plasmid, RCAS *mos*-P and RCAS *mos*-G, were transfected onto chicken embryo fibroblasts by standard procedures (15, 41). After approximately

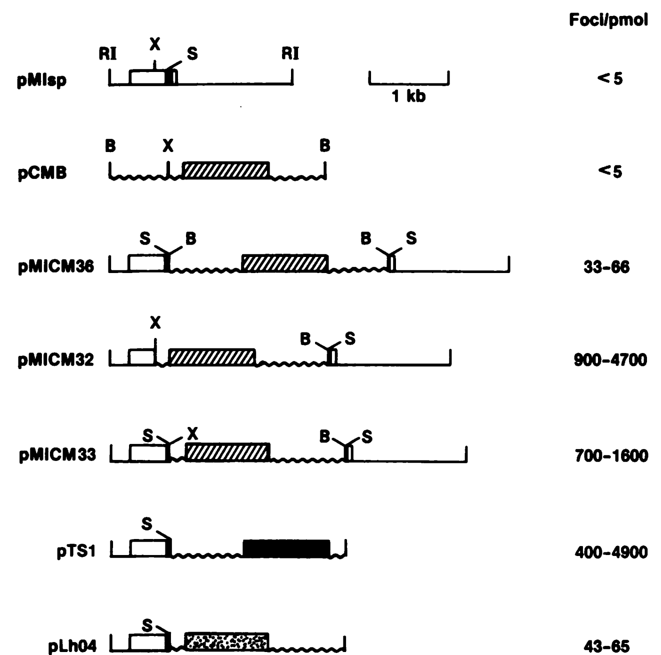

FIG. 4. Transforming activities of LTR c-*mos* plasmids in NIH 3T3 cells. Results represent ranges of foci obtained from the following number of separate assays: pM1sp, 2; pCMB, 2; pM1CM36, 2; pM1CM33, 4; pM1CM32, 5; pTS1, 2; and pLh04, 2. Symbols: □, Mo-MSV LTR; ▨, chicken c-*mos*; ■, mouse c-*mos*; ▩, human c-*mos*; ——, mink genomic DNA; ∿∿∿, genomic DNA. RI, *Eco*RI; B, *Bgl*II; S, *Sma*I; X, *Xba*I.

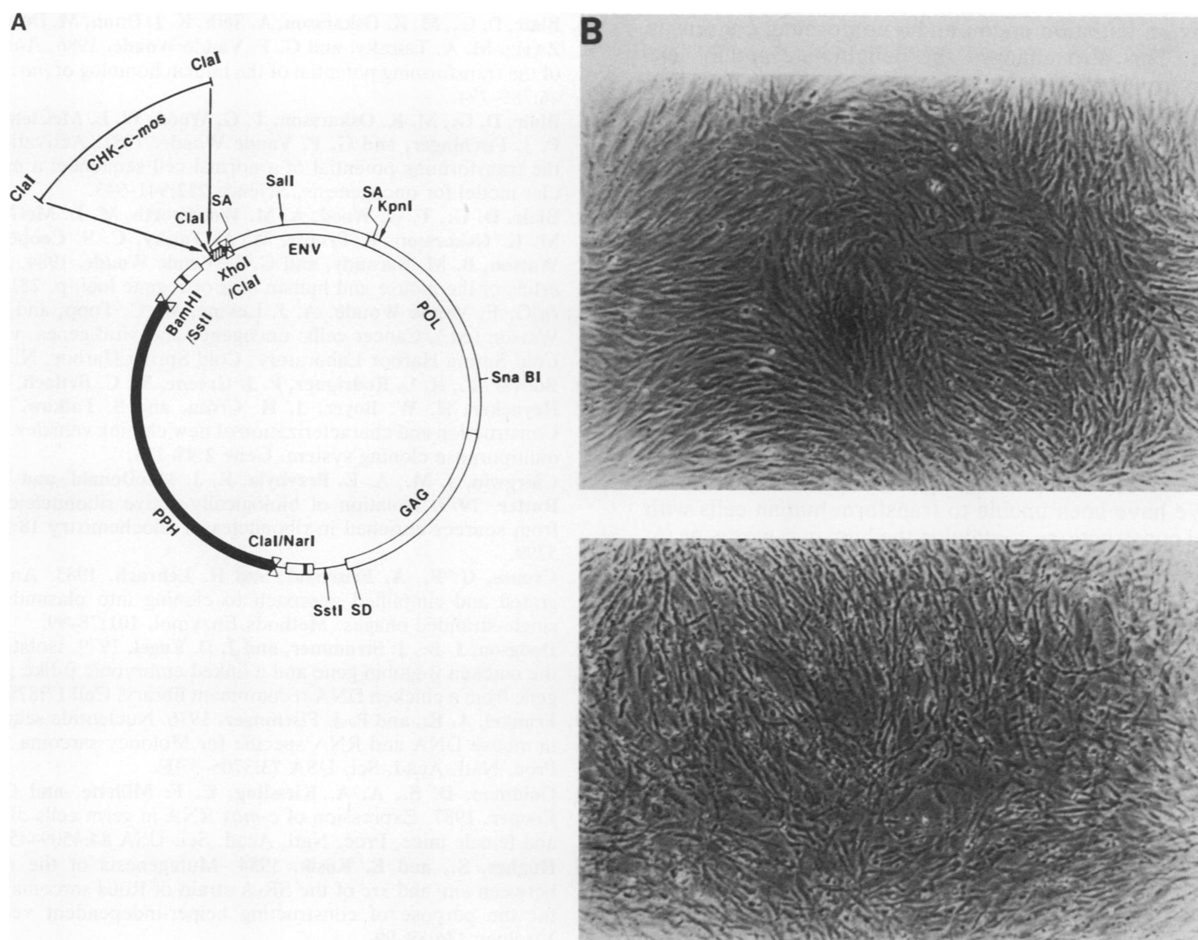

FIG. 5. Restriction map of chicken c-*mos* retrovirus vectors RCAS-*mos*-P and RCAS-*mos*-G and the transformation of chicken embryo fibroblasts by RCAS-*mos*-G. (A) RCAS-*mos* vectors contain the chicken c-*mos* coding region inserted into the replication-competent vector RCAS (16) as described in Materials and Methods. (B) Upper panel: uninfected chicken embryo fibroblasts; lower panel: chicken embryo fibroblasts transformed with RCAS-*mos*-G.

2 weeks in culture, the culture transfected with RCAS *mos*-G showed significant morphological alterations (Fig. 5), whereas the culture infected with RCAS *mos*-P was indistinguishable from control cultures (not shown).

DISCUSSION

The coding region of the *mos* proto-oncogene is less well conserved between species than most other proto-oncogenes. However, greater conservation is found in those coding regions previously shown to be homologous to other members of the kinase oncogene family (2, 17, 18, 35, 36). In the N-terminal portion, residues in the putative ATP-binding domain (2, 17, 18, 35, 36) are conserved, whereas in the C-terminal portion, residues homologous to the kinase domain are conserved.

RNA transcripts have been identified in mouse and primate tissues, with the highest levels found in gonads (14, 24, 28, 29; Paules et al., in preparation). In the mouse, c-*mos* expression in gonads has been shown to be developmentally regulated and expression is primarily confined to germ cells (14, 24, 28). As in mice, in adult birds, we find the highest levels of c-*mos* transcripts in ovaries. However, we also find c-*mos* expression in early (4-day-old) embryos which decreases in older embryos. In heart, kidney, and spleen, we detected *mos* in RNA by S1 nuclease protection experiments, but no discrete-size-class RNA transcripts were observed in Northern analysis from the same tissues. In the mouse, similar observations were made with RNA from immature testes and some adult tissues (28). We suspect that either the level of the transcripts is too low for detection by Northern analysis or the transcripts are initiated from multiple sites, or both.

Sequences which inhibit transforming activity of c-*mos* have also been identified in the human and mouse c-*mos* loci (6, 21, 43). The pM1CM36 LTR construct containing 900 bp of sequences upstream from chicken c-*mos* has diminished transforming efficiency. No overlapping reading frame is present in the chicken c-*mos* locus, as is found in primates (6; Paules et al., in preparation). However, as in the mouse locus (28), the chicken c-*mos* locus is preceded by numerous ATG codons which are followed immediately by termination codons. Such ATGs influence the translational efficiency of the yeast GCN-4 regulatory gene (23), and if the upstream chicken c-*mos* sequences are present in the ovarian and testis transcripts, it is possible that they could also influence translation. Likewise, in all species thus far analyzed, the nucleotide sequences surrounding the conserved c-*mos* ATG do not conform with good consensus initiator codons and morphological alterations were only observed in chicken embryo fibroblasts with the RCAS-*mos* retroviral vector

containing an initiation codon and a conforming consensus sequence. This also changed the penultimate amino acid from Pro to Gly, and we cannot exclude the possible influence of this change.

The *mos* proto-oncogene from all species tested can be activated by an LTR to transform NIH 3T3 cells in culture or to produce tumors in nude mice (6, 7). The transforming activity of human c-*mos* was shown to be 100-fold lower than that of mouse c-*mos* (6). One possibility for this difference could be the large divergence between mouse and human sequences, making mouse cells inappropriate targets for testing the oncogenic potential of human c-*mos*. However, we show in this study that the more distantly related chicken c-*mos* transforms mouse cells as efficiently as mouse c-*mos*. Moreover, the chicken c-*mos*-G can morphologically alter cells from the homologous species, although the alterations are not as profound as those seen when the RCAS vectors express v-*src* or v-*ras* (16; S. Hughes, unpublished observations). We have been unable to transform human cells with retroviral constructions containing the human c-*mos* gene (6; D. G. Blair, unpublished observations). It should be noted, however, that the form of chicken c-*mos* shown to produce this effect differs from the normal c-*mos* at the second amino acid (Gly was substituted for Pro). This modification was made in an attempt to provide a better initiation codon for c-*mos*. Whether the different biological effects seen with the two forms of c-*mos*, c-*mos*-G and c-*mos*-P, are the result of the more efficient translation of c-*mos*-G or whether it is a more potent form of the protein or some combination of these effects is unclear, and we are currently investigating these questions. Genetic distance per se, therefore, cannot account for the low level of transforming activity of human c-*mos*. It is possible that the human gene (and that of old-world monkeys) (Paules et al., in preparation) has a reduced transforming potential. Obviously, this reduction could provide a selective advantage to the species. Moreover, if *mos* transforming function is a measure of proto-oncogene function, this finding raises the possibility that in old-world primates, its normal cellular function may be altered.

ACKNOWLEDGMENTS

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