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Isolation and characterization of *fra-2*, an additional member of the *fos* gene family

(*fos*-related gene/cellular transformation/transcription factor AP-1/immediate early gene/cell cycle)

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ABSTRACT **Antiserum raised against a Fos peptide (amino acids Lys-127 to Arg-140 of chicken c-Fos) recognizes a 46-kDa Fos-related protein in cell lysates of growth-stimulated chicken embryo fibroblasts. Induction of the 46-kDa protein is transient but is slightly prolonged relative to c-Fos following growth stimulation. Using a mixed oligonucleotide probe encoding the peptide antigen, we have cloned the chicken genomic locus that encodes this protein and have determined its gene structure. This locus consists of four exons, each of which has some homology with the corresponding exons of the chicken c-*fos* gene, and it expresses a 6-kilobase mRNA after growth stimulation. The deduced amino acid sequence of the gene (323 amino acids) contains a "leucine zipper" and includes five distinct regions that exhibit strong sequence homology to the recently identified *fos*-related antigens Fra-1 (rat) and FosB (mouse) as well as c-Fos. Since the other regions of the gene have little homology to any of these three proteins, this gene was named "*fra-2*." When the *fra-2* gene was overexpressed by an avian retrovirus vector, it caused transformation of chicken embryo fibroblasts. The *fra-2* gene product formed a complex in cells with the c-Jun protein, indicating that c-*fos* and *fra-2* share biological and biochemical functions.**

The v-*fos* oncogene was detected in two murine osteosarcoma viruses, FBJ-MuSV (1) and FBR-MuSV (2), as well as chicken nephroblastoma virus, NK24 (3). The cellular counterpart, the c-*fos* gene, encodes a 62-kDa nuclear phosphoprotein (c-Fos) that is believed to play a pivotal role in the transduction of extracellular signals into changes in gene expression (4). In many cell types, c-Fos is induced rapidly and transiently by a variety of agents and has been reported to function as a transcriptional regulator for several target genes (4–7). c-Fos has been shown to function in association with Fos-associated proteins (8, 9), such as the c-*jun* gene product (c-Jun) (10, 11) and the *jun*-related gene products (JunB, JunD) (12).

We noticed that one of our anti-Fos antisera recognized a 46-kDa protein, the synthesis of which was induced by growth stimulation of chicken embryo fibroblasts (CEF). As we were interested in the biological function of this protein, we cloned the chicken genomic DNA and analyzed the gene structure.§ This gene is another *fos*-related gene, *fra-2*, distinct from the two recently identified *fos*-related genes, *fra-1* (13) and *fos-B* (14). We will further report the transforming activity of the *fra-2* gene.

MATERIALS AND METHODS

Growth Conditions and Transfection Procedure. Primary and secondary CEF preparation, cell culture conditions, and virus assays were essentially as described previously (15–17). For the DNA transfection, plasmids pF2C1 (carrying chicken genomic *fra-2*), pFM4 (carrying mouse genomic c-*fos*), and pDS3 (carrying no oncogene) (16) were completely digested with *Sal* I and ligated with *Sal* I-digested pREP to produce an intact proviral DNA structure as described previously (15–17). The ligated DNAs (total of 4–6 μg of DNA) were introduced into CEF (60-mm dishes) by the calcium phosphate transfection method, and replication-competent viruses were recovered from the culture fluids (15–17). Confluent cells were made quiescent by incubation for 18 hr in medium containing 0.2% calf serum. Cultures were stimulated by adding calf serum to a final concentration of 10% with or without cycloheximide at 10 μg/ml.

Cloning of Genomic DNA. Mixed oligonucleotides 5′-YCTNCGYTTYTCYTCYTCYTCNGGNGANARYTGYT-CNACYTT-3′ (#467 probe; at degenerate positions Y = T or C, N = A, G, T, or C, and R = A or G) were synthesized on the basis of the amino acid sequence of Fos peptide 1 (amino acids Lys-127 to Arg-140 of chicken c-Fos). Under low-stringency conditions, the #467 probe detected a 2.5-kilobase (kb) fragment in chicken genomic DNA digested with *Bgl* II. *Bgl* II digestion fragments of chicken DNA of the size range 2–3 kb were cloned in the *Bam*HI site of λ Charon 27 and were screened by #467 probe to yield λBB2.5. To isolate the entire gene locus, we made a chicken genomic library by partially digesting with *Sau*3AI and cloning in the *Bam*HI sites of λ Charon 40A. From this library, four positive clones, λ001–λ004, were isolated, using the 2.5-kb λBB2.5 *Bgl* II fragment as a probe under stringent hybridization conditions.

Plasmid Construction and DNA Analysis. DNA fragments that hybridized to the serum-inducible 6-kb mRNA were subcloned in pUC118 plasmid in both orientations. From each subcloned plasmid, a series of deletion derivatives was generated (18) and DNA sequence was determined by the dideoxy chain termination method.

The DNA fragment that includes the entire coding sequence of the *fra-2* gene [46 nucleotides upstream from the initiation codon (the *Sac* II site) to 53 nucleotides downstream from the termination codon (the *Nco* I site)] was isolated from λ002. The fragment was blunt-ended by treatment with the Klenow fragment of DNA polymerase and inserted into the *Bgl* II site that was blunt-ended. The resulting plasmid was named pF2C1.

Protein Analysis. A rabbit polyclonal antibody raised against Fos peptide 1 (Lys-Val-Glu-Gln-Leu-Ser-Pro-Glu-Glu-Glu-Glu-Lys-Arg-Arg) has been described (17). Polyclonal antisera to two species of Fra-2 peptide were prepared: Fra-2 peptide 1 (Val-Lys-Gln-Glu-Pro-Val-Glu-Glu-Glu-Ile-Pro-Ser-Ser-Ser-Leu) and Fra-2 peptide 2 (Leu-Asp-Gln-Glu-Ser-Pro-Leu-

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Abbreviation: CEF, chicken embryo fibroblasts.
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§The sequences reported in this paper have been deposited in the GenBank data base [accession nos. D90104 (*fra-2* exon 1), D90105 (*fra-2* exon 2), D90106 (*fra-2* exon 3), and D90107 (*fra-2* exon 4)].

Ser-Pro-Ser-Glu-Ser-Cys-Ser-Lys) were synthesized, conjugated with keyhole lympet hemocyanin, and used to immunize rabbits as described previously (17). Preparation of a rabbit polyclonal antiserum raised against total Jun protein expressed in *Escherichia coli* will be described elsewhere.

For ^{35}S labeling, each cell culture (60-mm dish) was incubated in L-methionine-free minimal Eagle's medium for 3 hr and further incubated in the presence of 600 μCi (1 Ci = 37 GBq) of Trans^{35}S (ICN) for the time indicated. The nuclear fraction was prepared from cells as described previously (19). Preparation of total cell lysates in denaturing conditions (0.5% SDS) and the procedure for immunoprecipitations were described previously (17). Cell lysates were also prepared in nondenaturing conditions as follows. Cells were washed three times with Tris/glucose and lysed with RIPA buffer (17). The lysates were collected in a microcentrifuge tube and cellular nuclei were disrupted by one cycle of freezing and thawing. The lysates were clarified by centrifugation at 2°C for 1 hr at 12,000 × *g*.

RESULTS

Antiserum Against Fos Peptide 1 Recognizes a Serum-Inducible 46-kDa Fos-Related Protein. We have previously raised a polyclonal antiserum against a synthetic peptide corresponding to a sequence in the c-Fos protein (Fos peptide 1) (17). This peptide is located N-terminal to the basic region of the DNA-binding domain of c-Fos and is completely conserved among chicken (20), mouse (21), and human (22) c-Fos. Using anti-Fos peptide 1, we first analyzed kinetics of c-Fos induction after growth stimulation of quiescent CEF. As shown in Fig. 1*A*, c-Fos was detected in the nuclear fraction. It was induced rapidly and transiently after serum addition as reported for mouse (23, 24) or rat (9) cells, and the synthetic rate reached maximum at 20–40 min after the stimulation. Two other serum-inducible protein bands with apparent molecular masses of 46 and 40 kDa in SDS gels were also immunoprecipitated by this antiserum (Fig. 1*A*). When cell lysates were prepared under denaturing conditions, the 40-kDa protein band was not precipitated by the anti-Fos peptide 1 (Fig. 1*B*), indicating that it is a Fos-associated protein. Immunoprecipitation analysis using anti-Jun antiserum has further shown that this protein is the endogenous c-*jun* gene product (data not shown).

The 46-kDa protein, on the other hand, was immunoprecipitated by anti-Fos peptide, even when the lysates were denatured in 0.5% SDS (Fig. 1*B*), and its induction was slightly prolonged compared with that of c-Fos (Fig. 1*A*). From the cell lysates labeled with $H_3{}^{32}PO_4$, the 46-kDa band as well as c-Fos (25) was immunoprecipitated by anti-Fos peptide 1 (data not shown). We have concluded that this nuclear phosphoprotein is a Fos-related antigen. We occasionally observed another serum-inducible band of 44 kDa which, as discussed below, may represent a differently modified form.

Cloning of the *fos*-Related Gene from the Chicken Genomic DNA and Analysis of the *fra-2* Gene Locus. On the basis of the amino acid sequence of Fos peptide 1, mixed oligonucleotides were synthesized and used as a probe (#467 probe) for the Southern blot analysis of the chicken DNA under low-stringency conditions. Among the *Bgl* II fragments, #467 probe detected a 2.5-kb fragment as well as the 3.8-kb fragment that is derived from the chicken c-*fos* gene (20). The 2.5-kb fragment was cloned as described in *Materials and Methods* (λBB2.5). Under stringent conditions, the λBB2.5 insert detected a 6-kb mRNA in serum-induced CEF but not in the quiescent CEF (Fig. 1*D*). This 6-kb mRNA was further shown to be superinduced in the presence of the protein

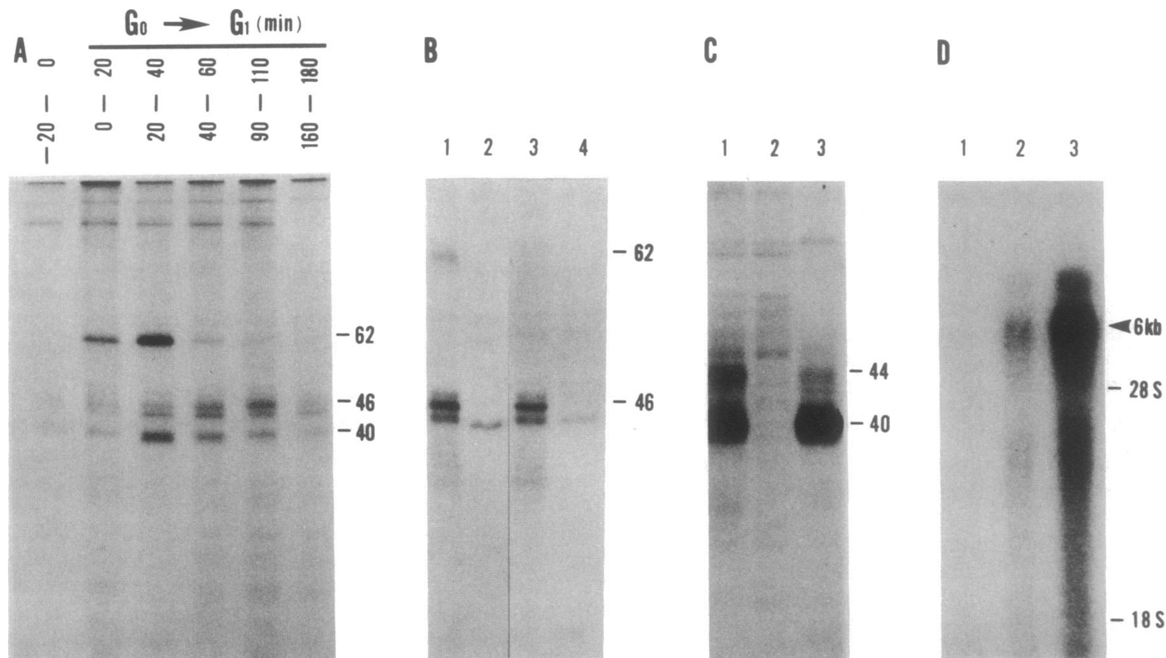

FIG. 1. Expression of the *fra-2* gene product and the *fra-2* mRNA. (*A*) Serum-starved CEF were stimulated by the addition of calf serum and pulse-labeled for the time indicated (in min). The nuclear fractions of the ^{35}S-labeled CEF were prepared, immunoprecipitated by anti-Fos peptide 1, and subjected to electrophoresis. Molecular masses are indicated in kDa. (*B*) Cell lysates prepared from the serum-stimulated CEF (pulse-labeled 80–100 min after the stimulation) in denaturing conditions were immunoprecipitated with anti-Fos peptide 1 (lane 1), anti-Fos peptide 1 preabsorbed by the peptide antigen (lane 2), anti-Fra-2 peptide 2 (lane 3), and anti-Fra-2 peptide 2 preabsorbed by the peptide antigen (lane 4). (*C*) Cell lysates were prepared from logarithmically growing F2C1-infected CEF (labeled for 1 hr) in nondenaturing conditions and immunoprecipitated with anti-Fos peptide 1 (lane 1), anti-Fos peptide 1 preabsorbed by the antigen peptide (lane 2), and anti-Jun (lane 3). (*D*) Poly(A)$^+$ RNA was isolated from CEF that were stimulated by calf serum for 0 min (lane 1), 30 min (lane 2) without cycloheximide, or 60 min with cycloheximide (lane 3). Samples were analyzed by electrophoresis in 1% agarose/formaldehyde gels (1 μg of RNA per lane), transferred to nitrocellulose filters, and hybridized with the BB2.5 insert labeled from random primers. Positions of 18S and 28S rRNAs are indicated.

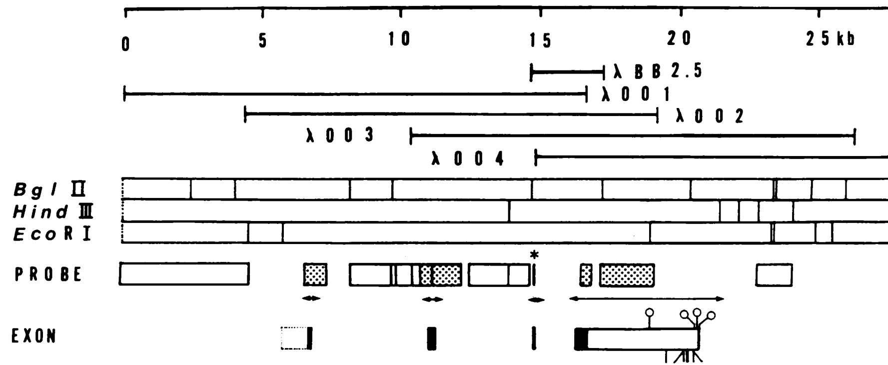

FIG. 2. Restriction endonuclease map of the *fra-2* gene locus and the DNA probes used for the Northern blot analysis. The filled-in boxes and the asterisk (#467 probe) show the DNA fragments that hybridize to the serum-inducible 6-kb mRNA, and the arrows indicate the sequenced regions. The box and line at the bottom represent the exon–intron structure and the coding regions are shown in the black boxes. The putative poly(A) addition signal AATAAA (♀) and the nucleotide sequence ATTTA (|) are also shown.

synthesis inhibitor cycloheximide, as has also been reported for c-*fos* mRNA (26). This indicates that the λBB2.5 insert is likely to represent a part of an immediate early gene that shares some sequence similarity with c-*fos*.

Using the λBB2.5 insert as a probe, we next screened an entire chicken genomic library, and four λ clones were isolated from a single locus (Fig. 2). Several restriction fragments were prepared from this locus and used as probes for a Northern blot analysis similar to that shown in Fig. 1*D* to detect the expressed regions. DNA fragments from four separate regions were shown to detect the serum-inducible and cycloheximide-superinducible 6-kb mRNA. The DNA sequences around these regions were determined and compared with the c-*fos* coding sequences. Each of the four regions contained a nucleotide sequence that exhibits homology to c-Fos in exons 1, 2, 3, and 4. Each nucleotide sequence was flanked by consensus sequences observed in intron–exon junctions (27) (Fig. 3). From these results, the amino acid sequence encoded by this locus was deduced; the locus encodes a protein composed of 323 amino acid residues (molecular weight of 35,241). A DNA-binding domain consisting of a "leucine zipper" structure and a basic region (28, 29) is located in the middle of the coding sequence. Adjacent to the basic region there is a sequence of 12 amino acids that is also included in the 14 amino acid Fos peptide 1, suggesting that this region was recognized by the anti-Fos peptide 1 antiserum.

When the amino acid sequence was compared with the sequences of c-Fos (chicken), Fra-1 (rat), and FosB (mouse) (Fig. 4), five distinct regions of homology were observed (regions 0–4); four of them (regions 1–4) were previously pointed out as conserved regions between c-Fos and Fra-1 (13). The segments between the conserved regions (open boxes in Fig. 4) vary considerably in length and sequence. From these results, we concluded that the protein encoded by this locus is different from c-Fos, Fra-1, and FosB, and we have named this gene "*fra-2*" to denote that it is a *fos*-related gene.

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    TGTTTTTTTGGTTGTTTTTTTTTTTTGTCGGCTTTCCGCTTTTTCTTTTTTTCTTTTTTTTCCCTTTTTCTATTTTTCCCCCCCTTCTTCTTCTCCCGCTGCGGACTCTCCCCCGGCTGC

                                                                                                  SacII
    GGGAGGCGCGAGGCAGAGCCCGAGAGGTCGGCACGGAGCAGGGGGCGGGGAGACGGCGAGGGAGCGGCGGCCGCGGCGCGGGAAGGCGGGGACGCGGCTCCCCCGGGCCGGCCTCGGACC

  1 MetTyrGlnAspTyrProGlySerPheAspThrSerSerArgGlySerSerGlySerProGlyHisProGluProTyrSerAlaGlyAlaAlaGlnGln
    ATGTACCAGGACTATCCCGGGAGCTTCGACACCTCCTCCAGAGGCAGCAGCGGCTCCCCGGGACACCCCGAGCCCTACTCCGCCGGCGCAGCCCAGCAGGTAGGGCCGCCTCCGCCCCGT

 34                             LysPheArgValAspMetProGlySerGlySerAlaPheIleProThrIleAsnAlaIleThrThrSerGlnAspLeuGlnTrpMetValGlnPro
    ---CTCCCCCACCTTTCCTCCTAGAAATTCCGAGTAGATATGCCAGGATCAGGCAGTGCTTTTATTCCCACGATCAACGCCATCACAACCAGCCAAGACCTGCAGTGGATGGTGCAGCCC

 66 ThrValIleThrSerMetSerSerProTyrSerArgSerHisProTyrSerHisProLeuProProLeuSerSerValAlaGlyHisThrAlaLeuGlnArgProGlyValIleLysThr
    ACCGTCATCACCTCCATGTCCAGCCCGTACTCTCGCTCGCACCCCTACAGCCACCCACTGCCGCCGCTGTCCTCGGTGGCTGGACACACGGCCCTTCAGCGACCGGGCGTGATCAAAACC

106 IleGlyThrThrValGlyArgArgArgArgAspGluGln                                                     LeuSerProGluGluGluGluLysArgArgIleArg
    ATCGGCACCACAGTGGGACGGAGACGAAGGGATGAGCAGGTAACTGTGTGAGCAGGAGGA---TTTCTTGGCACTTGCCCATAGCTGTCGCCTGAGGAAGAAGAGAAGCGAAGGATCCGG

131 ArgGluArgAsnLysLeuAlaAlaAlaLysCysArgAsnArgArgArgGluLeuThrGluLysLeuGlnAla                                                Glu
    AGAGAGAGGAACAAGCTGGCAGCTGCTAAATGTCGTAACAGGCGCCGAGAGCTAACAGAGAAACTCCAGGCGGTACGTGCTCTGCATGCATT----TTATTCCCTTTTTGTCTGCAGGAA

156 ThrGluValLeuGluGluGluLysSerValLeuGlnLysGluIleAlaGluLeuGlnLysGluLysGluLysLeuGluPheMetLeuValAlaHisSerProValCysLysIleSerPro
    ACTGAGGTGCTGGAGGAGGAAAAGTCAGTGCTTCAAAAAGAGATTGCTGAGCTCCAGAAGGAGAAGGAGAAACTAGAGTTCATGCTGGTTGCTCACAGCCCTGTGTGTAAAATCAGCCCT

196 GluGluArgArgSerProProThrSerSerLeuGlnSerValArgThrGlyAlaSerGlyAlaValValValLysGlnGluProValGluGluGluIleProSerSerSerLeuValLeu
    GAGGAACGTCGGAGCCCACCAACCAGCAGCCTCCAGAGCGTTCGGACTGGAGCGAGCGGAGCAGTGGTGGTGAAGCAGGAGCCTGTGGAGGAAGAGATCCCATCTTCCTCTTTGGTCCTT

236 AspLysAlaGlnArgSerValIleLysProIleSerIleAlaGlyGlyTyrTyrGlyGluGluAlaLeuAsnThrProIleValValThrSerThrProAlaIleThrProGlySerSer
    GACAAAGCTCAGAGGTCTGTCATTAAGCCCATCAGCATTGCTGGAGGTTATTATGGGGAGGAGGCACTCAACACTCCCATCGTGGTGACCTCGACACCAGCCATCACTCCTGGTTCCTCC

276 AsnLeuValPheThrTyrProAsnValLeuAspGlnGluSerProLeuSerProSerGluSerCysSerLysAlaHisArgArgSerSerSerSerGlyAspGlnSerSerAspSerLeu
    AACTTGGTGTTCACCTACCCCAATGTCTTGGATCAGGAGTCTCCTCTCTCCCCGTCCGAGTCCTGCTCCAAAGCTCACCGGAGGAGCAGCAGCAGCGGCGACCAGTCCTCGGATTCCTTG

316 AsnSerProThrLeuLeuAlaLeuTER                                                    NcoI
    AACTCTCCCACCTTGCTGGCATTGTAATCCCCTGAGGCCCCCCCATTGCCAGTGTGTTACATCCCCCGCCCGGCTCCATGGGGAGACCCCTCCATGGGATTAGAGACAGGCACAGGATCG

    TTCAAGCACAAGGGCAGCAAGAACAAGAATGGGGAAATGCTGCAGCTCCAGGAAAGAGAGTGAGGACCAATGCCAGCTCCCTGGAGGCAGGAAATGGCAAGGGTGGGACTGATGCACCAG
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FIG. 3. Nucleotide sequence and the deduced amino acid sequence of the *fra-2* gene. Numbers at the left refer to the first amino acid on each line. Only parts of introns are indicated. The *Sac* II site and the *Nco* I site that were used for the construction of pF2C1 are shown.

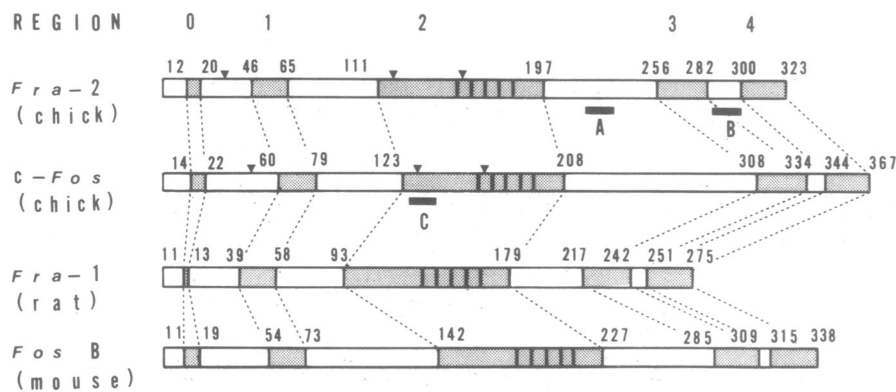

FIG. 4. Comparison of the *fra-2*, c-*fos*, *fra-1*, and *fosB* gene products. The five homologous regions are numbered from 0 to 4. Numbers above each product are amino acid residues. Five bold vertical lines indicate the leucine residues that form the leucine zipper structure. The arrowheads indicate the position of introns present in the nucleotide sequence of the *fra-2* and c-*fos* genomic DNA. Three peptides that were used for the antigen are indicated by bars: A, Fra-2 peptide 1; B, Fra-2 peptide 2; and C, Fos peptide 1.

Approximately 2.2, 3.8, 3.9, and 4.0 kb downstream from the termination codon of the *fra-2* gene, there are four putative poly(A) addition signals (Fig. 2). Several ATTTA sequences are present in the 3′ untranslated region; this sequence is reported to contribute to the instability of mRNA (30) and is also detected in 3′ noncoding regions of c-*fos* (20–22), *fra-1* (13), and *fosB* (14). The 5′ untranslated leader of the mRNA is probably longer than that shown in Fig. 3, because the size of the mRNA was estimated to be 6 kb by Northern blot analysis (Fig. 1*D*).

Detection of the 46-kDa Protein by Two Anti-Fra-2 Antisera. To confirm the genomic structure by protein analysis, we synthesized two peptides (Fra-2 peptide 1 and peptide 2) in the deduced amino acid sequence of Fra-2 that lack homology to any part of c-Fos, Fra-1, or FosB (Fig. 4 and *Materials and Methods*). Both of the antisera specifically immunoprecipitated a protein with an apparent molecular mass of 46 kDa from serum-stimulated cells (Fig. 1*B*) and not from the quiescent cells. This protein band was not detected when the antisera were preabsorbed by the corresponding antigen, and it comigrated with the 46-kDa protein immunoprecipitated by anti-fos peptide 1 (Fig. 1*B* and unpublished data). Neither anti-Fra-2 peptide serum immunoprecipitated the c-Fos protein, which is consistent with the lack of sequence homology between c-Fos and the Fra-2-specific peptides used to raise the antisera. This result indicates that the *fra-2* gene cloned in this study encodes the serum-inducible 46-kDa protein. The apparent molecular weight of this protein in SDS gels (46,000), therefore, is higher than the calculated molecular weight (35,241), as has also been observed for c-Fos (25), Fra-1 (31), and FosB (14).

Transforming Activity of the *fra-2* Gene. High expression of c-*fos* causes cellular transformation of fibroblasts (16, 32). To examine whether the *fra-2* gene has transforming potential like c-*fos*, we constructed a Rous sarcoma virus variant (F2C1) that carries the chicken *fra-2* genomic DNA instead of the v-*src* sequence. It has been reported that the c-*src* and c-*fos* genomic DNA that were inserted into the same plasmid system were recovered as the properly processed form in the virus genome (15, 16).

After DNA transfection, the virus stock was recovered and was used to infect fresh CEF. The titer of the transforming virus in the F2C1 virus stock was 5.0×10^5 focus-forming units (FFU)/ml, while that of the c-*fos*-containing virus, FM4, was 5.0×10^6 FFU/ml. The focus formation by F2C1 took a longer time (usually more than 7 days after infection) and the foci were smaller than those formed by FM4. F2C1-infected CEF formed small colonies (about 0.4 mm in diameter) in soft agar by 3 weeks with an efficiency equivalent to the formation of larger colonies (about 0.7 mm in diameter) by FM4-infected CEF. From these results, we conclude that the *fra-2* gene has transforming activity.

Cell lysates of F2C1-infected CEF (in the logarithmic phase of growth) were prepared in nondenatured condition. From these lysates, a 44-kDa protein and a 40-kDa protein were immunoprecipitated with the two species of anti-Fra-2 antisera (data not shown) and anti-Fos peptide 1 (Fig. 1*C*). This overexpressed 44-kDa protein was coimmunoprecipitated with c-Jun (40 kDa) (16) by the anti-Jun antiserum (Fig. 1*C*). These results indicate the *fra-2* gene product forms a complex with the c-*jun* gene product in cells.

Only a 44-kDa protein, and not the 46-kDa protein, was detected in F2C1-infected CEF that were growing (Fig. 1*C*) or resting. The 44-kDa band, however, was shifted to the 46-kDa band immediately after the resting F2C1-infected CEF were stimulated to grow (unpublished results), suggesting that the 46-kDa protein is a different form (possibly a highly phosphorylated form) of the 44-kDa protein, and that the modifying activity is rapidly induced after the serum stimulation as observed for c-Fos (refs. 33 and 34 and unpublished data). The serum-inducible endogenous *fra-2* gene product, the 46-kDa protein, was also shown to form a complex with c-Jun (data not shown).

DISCUSSION

We have cloned and characterized *fra-2*, an additional member of the *fos* gene family. After growth stimulation, *fra-2* mRNA (6 kb) was promptly transcribed, and it was superinduced in the presence of cycloheximide, indicating that this gene is one of the immediate early genes (35). The sequence alignment shows that amino acid sequences conserved among Fra-2, c-Fos, Fra-1, and FosB are contained in five regions. Region 2, the longest and most highly conserved region, contains the leucine zipper structure and the basic region, suggesting that, like c-Fos (8–11), Fra-1 (31), and FosB (14), Fra-2 also may form heterodimers with c-Jun that recognize a specific DNA sequence such as the binding site for transcription factor AP-1. This possibility was supported by the observation that the *fra-2* gene product forms a complex with c-Jun in growth-stimulated cells and cells overexpressing the *fra-2* gene product.

It is noteworthy that the structures of c-*fos* (21, 22) and *fra-2* are very similar. In both genes, the coding sequences are divided into four exons and each exon encodes at least one segment of the five highly homologous regions. Two introns of each gene are inserted at the same positions of region 2. This conservation of intron–exon structure suggests that c-*fos* and *fra-2* (and probably *fra-1* and *fosB*) belong to

a gene family (the *fos* gene family) that originated from a single ancestral gene.

To analyze the transforming activity of the *fra-2* gene, we constructed a *fra-2*-containing retrovirus (F2C1) and demonstrated that it can transform CEF. This result, as well as the observations that c-Fos and Fra-2 form a complex with c-Jun in the cells, indicates that the two genes are similar not only in structure but also in function. However, the difference in the induction kinetics of these two genes after growth stimulation suggests they have distinct roles in transcriptional regulation in response to extracellular signals.

In the process of preparing this paper, we noticed that one of the *fos*-related genes isolated from a human cDNA library by Matsui *et al.* (36) is likely to be the human counterpart of the *fra-2* gene.

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