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An Avian Transforming Retrovirus Isolated from a Nephroblastoma That Carries the *fos* Gene as the Oncogene

MAKOTO NISHIZAWA,[1] NAOAKI GOTO,[2] AND SADAAKI KAWAI[1]*

Department of Tumor Virus Research, The Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108,[1] and Department of Veterinary Pathology, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753,[2] Japan

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A new avian transforming retrovirus, NK24, was isolated from a chicken with a nephroblastoma. This transforming virus induced fibrosarcomas with osteogenic cell proliferation and nephroblastomas in vivo and transformed fibroblast cells in vitro. From extracts of NK24-transformed cells, anti-*gag* serum immunoprecipitated a 100-kilodalton nonglycosylated protein with no detectable protein kinase activity. An NK24 provirus present in infected quail cells was molecularly cloned and subjected to nucleotide sequence analysis. The genome of NK24 was 5.3 kilobases long and had a 1,126-base-pair sequence of cellular origin in place of a viral sequence of avian leukosis virus containing the 3′ half of the *gag* gene and the 5′ half of the *pol* gene. Although the entire *env* gene was retained, it appeared to be inactive, possibly owing to the loss of function of its splice acceptor site as a result of a second deletion of 1,598 bases in the 3′ half of the *pol* gene that extended to the acceptor site. Nucleotide sequence analysis revealed that the NK24 virus contained the *fos* gene, previously identified as the oncogene of FBJ and FBR murine osteosarcoma viruses. Unlike the v-*fos* gene products of FBJ and FBR, which suffer a structural alteration at their carboxyl termini, the NK24 v-*fos* gene product seemed to have the same carboxyl-terminal structure as the chicken c-*fos* gene product. A comparison of the structures of the products of the NK24 v-*fos* and mouse c-*fos* genes suggested that the *fos* gene product consists of highly conserved regions and relatively divergent regions.

The viral oncogenes of acute transforming retroviruses are responsible for the neoplastic transforming abilities of the retroviruses. These viral oncogenes are known to be acquired by transduction of normal cellular counterparts. To date, more than 20 oncogenes have been identified in retroviruses of birds and mammals. Some viral oncogenes have been found in multiple virus isolates of the same species. For instance, the most well-characterized oncogene, *src*, was originally identified in Rous sarcoma virus (RSV) (35) and was reidentified in two recently isolated strains of avian viruses, S1 and S2 (12). Some other oncogenes have been isolated from viruses from different species. The *fps* oncogene of avian Fujinami sarcoma virus and the *fes* oncogene of feline Snyder-Theilen sarcoma virus are known to have originated from the same cellular counterparts of chickens and cats (33).

Some retroviruses are known to induce a wide spectrum of neoplasms, including sarcomas, leukemias, and carcinomas. However, most known transforming retroviruses were originally isolated from sarcomas or leukemias, and only a few were isolated from carcinomas. We are interested in the mechanism of induction of the chicken nephroblastoma, which is histologically similar to Wilms' tumor of humans (11), and have tried to isolate transforming viruses from affected tissues.

In this work, we characterized a new avian retrovirus, isolated from a nephroblastoma, that induces fibrosarcomas, osteosarcomas, and nephroblastomas in chickens and transforms chicken embryo fibroblast (CEF) cells in vitro. The oncogene of the virus was found to be the *fos* gene, previously identified in murine osteosarcoma viruses. Structural analysis of the NK24 *fos* gene showed that the chicken v-*fos* gene was highly divergent from that of murine viruses but had some highly conserved regions.

* Corresponding author.

MATERIALS AND METHODS

Cells and viruses. Fertilized chicken and quail eggs were supplied by the Nippon Institute of Biological Science, Tokyo, Japan. CEF and quail embryo fibroblast (QEF) cultures were prepared as described previously (17). The preparation of Rous-associated virus-7 (RAV-7), isolation of a helper virus by the endpoint dilution method, focus assay, and soft agar colony formation were described previously (16, 21, 39).

Immunoprecipitation and gel electrophoresis. Virus-producing or non-virus-producing transformed cells were starved in serum-free medium for 60 min, labeled with [^{35}S]methionine (500 Ci/mmol; Amersham Corp., North Chicago, Ill.) at 100 μCi/ml for 6 h, and then lysed in RIPA buffer (150 mM NaCl, 10 mM Tris hydrochloride [pH 7.2], 1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulfate, 2 mM phenylmethylsulfonyl fluoride). The cell lysates were immunoprecipitated with antiserum. Rat antiserum from a rat inoculated with rat 3Y1 cells transformed by the Bryan high-titer strain of RSV was described elsewhere (20). The procedures for immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis were as described previously (19).

DNA probes. pPvuIIDG, a subclone of the RSV long terminal repeat (LTR) *gag*-specific 1.6-kilobase (kb) *Pvu*II fragment (5), and p*fos*-1, a plasmid that contains a mouse v-*fos*-specific 1.3-kb fragment (4), were kindly supplied by J. M. Bishop (University of California) and I. M. Verma (Salk Institute), respectively. Hybridization experiments were carried out as described previously (36).

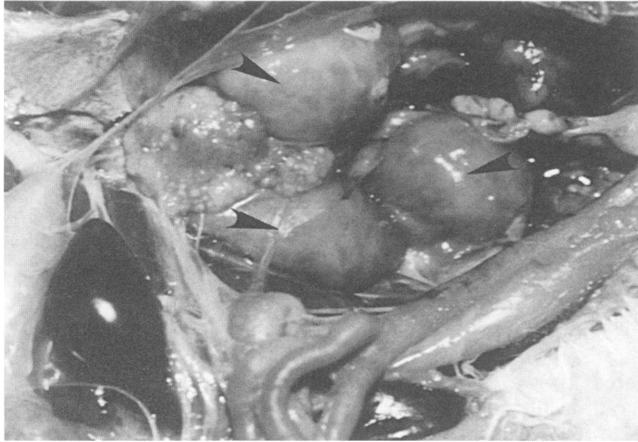

FIG. 1. Original lateral nephroblastomas (arrowheads) from which the NK24 virus was isolated.

Molecular cloning. High-molecular-weight DNA prepared from NK24-infected QEF cells was partially digested with *Eco*RI endonuclease and size fractionated by sucrose density gradient centrifugation. The fractionated DNA was ligated with the Charon 4A *Eco*RI arm and packaged into phage particles in vitro. The recombinant phage were screened with a ^{32}P-labeled RSV LTR *gag*-specific probe. The DNAs of the purified phage clones were subjected to restriction mapping and hybridization experiments to distinguish clones that contained the NK24 provirus genome from RAV-7 clones. Two *Eco*RI fragments of 4.0 and 1.3 kb which were derived from one clone, 2401, and seemed to contain the NK24 genome were subcloned into plasmid vector pUC-19 and used for further analyses.

Sequence determination. Nucleotide sequences were determined by the dideoxy sequencing method. The restriction fragments to be sequenced were subcloned into plasmid pUC-19 and deleted unidirectionally by the method of Henikoff (15). A series of deleted plasmid DNAs were used as templates in the chain termination sequencing method of Hattori and Sakaki (13). Some guanine-plus-cytosine-rich sequences were determined with deoxy-7-deazaguanosine triphosphate (27), which was kindly supplied by S. Nishimura (National Cancer Center Research Institute, Tokyo, Japan), instead of dGTP in the dideoxy sequencing reaction.

RESULTS

Isolation of a transforming virus, NK24, from a chicken nephroblastoma. Old chickens were obtained from a farm in the northwest Yamaguchi prefecture, Japan, and examined for naturally occurring malignant tumors. At necropsy, bilateral renal tumors were found in one of these chickens (Fig. 1). The tumors consisted of both epithelial and mesenchymal elements, some tubulelike structures, and a network of capillaries, that is, they showed the features of nephroblastomas. Electron microscopic examination revealed many C-type virus particles associated with the tumor cells (data not shown). Parts of the dissected renal tumors were stored at -70°C for use in an attempt to isolate the transforming virus. The frozen tissue (0.5 g) was cut into small pieces with scissors and smashed up with a pestle in a cooled mortar. The tissue was extracted with 5 ml of cold medium, and the mixture was centrifuged at $1,800 \times g$ for 15 min. A sample (0.1 ml) of the supernatant was tested on CEF cells for the presence of focus-forming virus. After incubation for 10 days at 37°C, many small isolated foci of transformed cells were noticed in the CEF test culture. These foci consisted mainly of piled-up cells of an elongated fusiform type (Fig. 2). The culture was repeatedly transferred and overlaid with soft agar-containing medium until it was fully transformed. The virus stock prepared from culture fluids of the fully transformed culture had a titer of 3×10^6 focus forming units per ml.

Biological properties of the NK24 virus. NK24 virus induced tiny, compact colonies of transformed CEF cells in soft agar suspension cultures. When the colonies were picked and grown in fluid cultures after the cells had been dispersed, most of the colonies formed consisted of elongated but rather flat cells. A nontransforming virus of subgroup B, NAV, was isolated from the NK24 virus stock by the endpoint dilution method (39). Non-virus-producing transformed colonies were easily isolated from soft agar suspension cultures infected with a low titer of the virus. In one experiment, 9 of 27 colonies isolated were non-virus producers. Infectious transforming viruses were rescued from these non-virus-producing cell cultures by superinfection with nontransforming avian leukosis viruses. This finding, together with molecular analyses of the viral genome described below, indicated that the NK24 virus is defective in replication.

When about 10^5 focus-forming units of the NK24 virus was inoculated into the wing webs of newborn chickens, palpable tumors began to appear after a latent period of 3 weeks. These tumors grew slowly, and in two of eight virus-infected chickens no sign of tumor formation was seen, even after an observation period of 3 months. The tumors were paler and much harder than those induced by RSV. Upon histological examination, the tumor tissues were found to consist of fibroblasts and osteoblasts and to show calcification. Interestingly, a nephroblastoma was found in one of eight chickens inoculated in the wing webs with the NK24 virus, suggesting that this virus has the potential to induce nephroblastomas. In this experiment, we used a cloned NK24 virus which was rescued from a non-virus-producing transformed colony by superinfecting the colony with Rous-associated virus-1 (RAV-1). An uncloned NK24 virus also induced the same type of tumors at inoculation sites on the wing webs after similar latent periods.

Details of a study of the in vivo pathogenicity of NK24

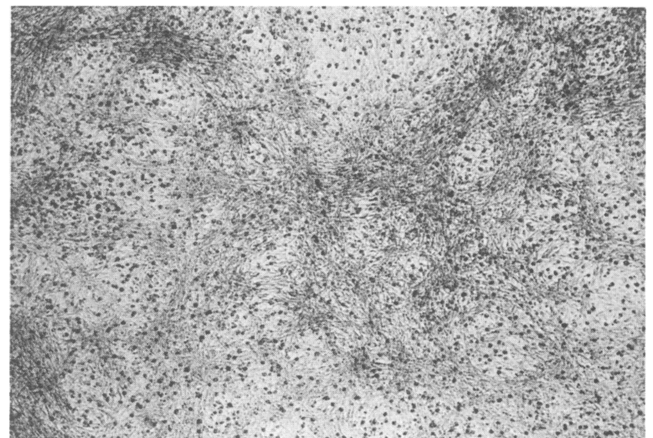

FIG. 2. A focus of transformed cells induced by NK24. CEF cells were infected with NK24, overlaid with 0.7% agar medium, and incubated at 41°C for 1 week.

virus and the histological features of the induced tumors will be described elsewhere.

Analysis of the viral gene product. NK24(NAV)-transformed and NK24-transformed non-virus-producing cells were labeled with [^{35}S]methionine, and cell lysates were immunoprecipitated with rat anti-*gag* serum obtained from a rat inoculated with rat cells transformed by the Bryan high-titer strain of RSV. This rat anti-*gag* serum has been shown not to react with either the *src* gene product (pp60^{src}) or the *env* gene product (gp95) (20). The immunoprecipitates were electrophoresed on an 8.5% polyacrylamide gel. A protein of about 100 kilodaltons (p100) was detected in extracts of both types of transformed cells, and no other proteins were immunoprecipitated from NK24-transformed non-virus-producing cells, while p180 (*gag-pol* fused protein), pr76 (*gag* protein), and other cleaved *gag* products were detected in extracts of NK24(NAV)-transformed cells (Fig. 3). Immunoprecipitation of p100 was abolished by preincubation of the anti-*gag* serum with disrupted Rous-associated virus-2 (RAV-2) virion proteins. These findings indicate that the NK24-specific protein is a *gag*-fused protein of about 100 kilodaltons.

Antisera against the *myc*, *erbB*, and *src* gene products could not immunoprecipitate the p100 protein (data not shown). The gene products labeled with [^{35}S]methionine in the presence and absence of tunicamycin did not differ in mobility in gel electrophoresis, suggesting that the gene product was not glycosylated (data not shown). Moreover, the gene product in the immunoprecipitate did not show any protein kinase activity when tested in an in vitro kinase reaction (data not shown). From these findings, together with the facts that the virus did not induce acute leukemia in vivo and could not transform hematopoietic cells in vitro (data not shown), it seems very likely that the virus does not carry any transforming gene that has been found in avian acute transforming viruses. Therefore, we next tried to identify the *onc* gene contained in the NK24 virus by molecular cloning of a provirus present in NK24-transformed cells and by analysis of its nucleotide sequence.

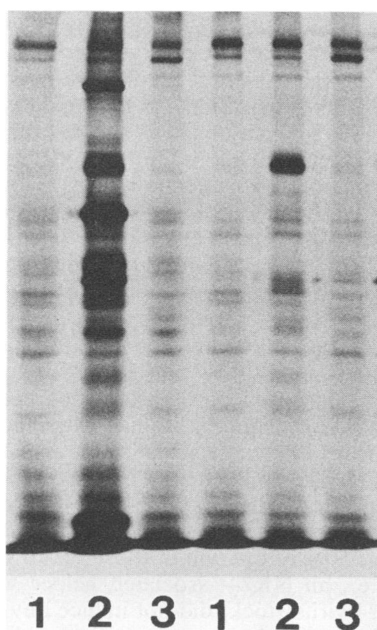

FIG. 3. Immunoprecipitation of NK24 transforming protein. Virus-producing [NK24(NAV)-infected] (leftmost three lanes) and non-virus-producing (NK24-infected) (rightmost three lanes) transformants were starved for 1 h in 2 ml of methionine-free medium supplemented with 5% dialyzed calf serum and then labeled with [^{35}S]methionine (50 μCi/ml) for 6 h in the same medium. Extracts of labeled cells were immunoprecipitated with normal rat serum (lanes 1), rat anti-*gag* serum (lanes 2), and rat anti-*gag* serum preincubated with disrupted RAV-2 virion proteins (lanes 3). The resulting immunoprecipitates were analyzed by 8.5% (wt/vol) polyacrylamide gel electrophoresis.

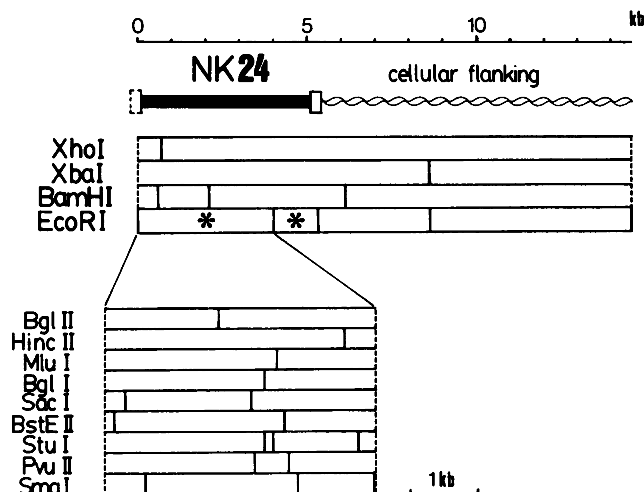

FIG. 4. Restriction endonuclease map of the NK24 provirus genome clone 2401. The position of the provirus genome is indicated by a black bar and white boxes (LTR sequences). This clone contained the cellular 3'-flanking sequence (wavy line) and the whole provirus genome of the NK24 virus except for a part of the 5' LTR sequence. Two *Eco*RI fragments, indicated by asterisks, were subcloned into the plasmid vector and used for further analysis.

Genome structure of NK24. To determine the genome structure of the NK24 virus, we infected QEF cells rather than CEF cells with the NK24 virus, because chicken, but not quail, cell DNA contains endogenous viral sequences that would complicate the identification of a DNA fragment(s) containing NK24 provirus by Southern blot analysis of cellular DNA with a viral sequence as a probe. NK24 virus was recovered from NK24-induced, non-virus-producing transformants by superinfection with RAV-7 and inoculated onto QEF cells. The infected cultures were overlaid with soft agar-containing medium, incubated at 41°C for 1 week to allow the development of transformed cell foci, and then transferred and overlaid repeatedly with fresh soft agar-containing medium until the cultures were fully transformed. High-molecular-weight DNA was then extracted from the NK24(RAV-7)-transformed QEF cells and partially digested with *Eco*RI endonuclease. The fractionated DNA was ligated with the Charon 4A *Eco*RI arm and packaged into phage particles in vitro. Several recombinant phage clones were detected with the LTR *gag* probe. Restriction mapping and hybridization experiments suggested that one clone, 2401, contained the NK24 genome sequence. The restriction map of this clone is shown in Fig. 4. Two *Eco*RI fragments of 4.0 and 1.3 kb (marked by asterisks in Fig. 4) were deduced to contain the viral genome. For confirmation of the biological activity of the cloned DNA, the two fragments were ligated and transfected in the presence of a helper virus DNA clone, pYAV-e (28), onto CEF cells (18). The transforming virus was recovered from the transfected culture, indicating that the two *Eco*RI fragments

together provide the entire genome of the NK24 virus. Then, the two *Eco*RI fragments were subcloned into the pUC vector plasmid and used for further analyses. Restriction mapping of the subcloned *Eco*RI 4.0-kb fragment suggested that its 3′ half contained the oncogene sequence. We found no similarity between the restriction map of this portion and that of any avian retrovirus oncogene sequence reported previously, suggesting that the viral oncogene of NK24 is different from those of other avian transforming viruses, as suggested above. Thus, to determine the structures of the viral genome and the oncogene more precisely, we subjected this portion to sequence analysis.

The nucleotide sequence of the sequenced region is shown in Fig. 5. The recombination junctions were predicted by comparing the sequence with that of the Prague C strain of RSV (32). The 3′ half of the *gag* gene and the consecutive 5′ half of the *pol* gene were found to be replaced by a 1,126-base-pair (bp) sequence that was unrelated to the viral genome. The nonviral NK24-specific sequence was combined at its 3′ end with the viral sequence in the middle of the *pol* gene. However, sequence analysis indicated that the remaining 3′ half of the *pol* gene contained a large deletion of 1,598 bp. The deletion started 55 bp downstream from the recombination site and ended just before the *env* splice acceptor site.

Although the *env* gene remained intact in NK24, the gp85 protein was not detectable in extracts from NK24-transformed cells (data not shown). Thus, it is likely (and possibly the reason for the absence of expression of the *env* gene in NK24) that the genomic RNA of the NK24 virus is not spliced, possibly owing to the loss of a functional *env* splice acceptor site. In support for this notion, blot hybridization analysis of RNA prepared from NK24-infected non-virus-producing cells detected no subgenomic mRNA (data not shown). If the *env* gene were expressed in NK24-transformed non-virus-producing cells, superinfection of the cells with NAV, which has the same *env* gene as NK24, would have been blocked, and the NK24 virus could not have been rescued efficiently as infectious virus. Therefore, the second deletion possibly resulting in inactivation of the *env* gene may not have been fortuitous.

Structure of the viral oncogene. Consistent with the results of viral gene product analysis, we found that the *gag* coding frame was interrupted near the end of p27 and was followed by a 967-bp open reading frame encoding a *gag* fused oncogene product with a molecular weight of 95,000. It should be noted that the guanine-plus-cytosine content of the transduced sequence was high (72%). On comparing the sequence with those of previously reported viral and cellular oncogenes, we found significant homology between the oncogene of NK24 and the *fos* oncogene of FBJ murine osteosarcoma virus (38). Figure 6 shows the alignment of the predicted amino acid sequence of the NK24 oncogene with that of the mouse c-*fos* gene, the cellular counterpart of the v-*fos* gene. The amino-terminal sequence of 45 amino acid residues in the mouse c-*fos* gene product was replaced by a viral *gag* polypeptide sequence in the NK24 oncogene product. The overall homology of the two *fos*-derived polypeptide sequences was 75.4%. Frequent amino acid substitutions and gaps were clustered in two regions of the sequence. We arbitrarily divided the polypeptide sequence into five regions, three homologous portions and two divergent portions, depending on their extents of homology with the sequence of mouse c-*fos*. The homologous portions showed very high homology: in region 3, only 2 of 85 amino acid residues were substituted, and in other highly homologous regions, the homology was as high as 90%. On the other hand, the two divergent regions (regions 2 and 4) exhibited about 50% homology or less.

Derivation of the oncogene of NK24 from chicken c-*fos*, not from another *fos*-related gene. The existence of regions of relatively low homology with the mouse c-*fos* gene raised the question of whether the NK24 oncogene was derived from the chicken c-*fos* gene or another *fos*-related, but distinct, gene. To distinguish between these possibilities, we carried out Southern blot analysis of human peripheral blood cell DNA with two viral oncogene probes derived from NK24 and FBJ murine osteosarcoma virus. These two probes both detected the same restriction fragment under stringent hybridization conditions (Fig. 7). This result suggested that the oncogene of the NK24 virus originated from the chicken c-*fos* gene, not from another *fos*-related gene.

DISCUSSION

Retroviruses cause a variety of tumors. The mechanisms of induction of these tumors involve the expression of activated oncogenes. Some retroviruses contain oncogenes of cellular origin as viral genes, and these viral oncogenes are highly expressed in infected cells and induce the malignant growth of infected cells. In the case of retroviruses without viral oncogenes, cellular oncogenes are thought to be activated by the integration of proviral sequences of the retroviruses into the neighborhood of these cellular oncogenes. For example, lymphatic leukemia and erythroblastosis of chickens have been reported to be induced by the activation of the cellular *myc* gene and *erbB* gene, respectively, by avian lymphatic leukemia viruses (14, 25, 30, 31). Interestingly, avian erythroblastosis viruses, which induce acute erythroblastosis in chickens, are leukosis viruses that have transduced the activated cellular *erbB* gene as its own viral gene during replication in leukemia virus-induced erythroblast cells (43). In chickens, kidney tumors that are histologically similar to Wilms' tumors of humans (11) are known to be induced by leukemia viruses (1–3, 29, 34, 40). Furthermore, we frequently found nephroblastomas as naturally occurring tumors in chickens more than 2 years old; 12 of 96 tumors found in these chickens were nephroblastomas. The mechanism of induction of kidney tumors is unknown, but Westaway et al. (42) found that the c-H-*ras* gene was activated in one case of chicken nephroblastoma. To analyze the mechanism of nephroblastoma induction, we attempted to isolate from chicken nephroblastomas viruses that could reproducibly induce nephroblastomas when inoculated into chickens. Tissues of five of seven nephroblastomas so far examined by electron microscopy have been found to contain many retrovirus particles. We have isolated two infectious viruses, a transforming virus, NK24, and a nontransforming virus, 219V, from two of those tumor tissues. 219V and NAV (i.e., an NK24-associated helper virus isolated from an NK24 virus stock) did not induce any macroscopically detectable visceral tumors, including nephroblastomas, at least in one experiment, when injected into newly hatched chickens. However, both nontransforming viruses induced a high incidence of focal growth of blastic cells in the kidneys which were apparent on histological examination of the kidneys 6 months after inoculation, while RAV-1, a standard lymphatic leukemia virus, did not induce the focal growth of blastic cells (unpublished data). These focal lesions of blastic cells may represent the preneoplastic stage of nephroblastomas. If so, these replication-competent nontransforming viruses may have the potential to activate a certain

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-128  BglII
     TCAGATCTCTCACCTACCGCGCGGGCTCCGGTGATCATTGACTGCTTTAGGCAGAAGTCACAGCCAGATATTCAGCAGCTTATACGGGCA  -39
     SerAspLeuSerProThrAlaArgAlaProValIleIleAspCysPheArgGlnLysSerGlnProAspIleGlnGlnLeuIleArgAla

                                                    gag | NK24 specific
     GCACCCTCCACACTGACCACCCCAGGAGAAATAATCAACTCGCAGGATTTCTGCACCGACCTGGCCGTGTCCAGCGCCAACTTCGTGCCC  52
     AlaProSerThrLeuThrThrProGlyGluIleIleThrSerGlnAspPheCysThrAspLeuAlaValSerSerAlaAsnPheValPro (17)
                                                        (1)
                                              PstI
     ACCGTGACGGCCATCTCCACCAGTCCCGACCTGCAGTGGCTGGTGCAGCCCACCCTCATCTCCTCGGTCGCCCCCTCCCAGAACCGCGGG  142
     ThrValThrAlaIleSerThrSerProAspLeuGlnTrpLeuValGlnProThrLeuIleSerSerValAlaProSerGlnAsnArgGly (47)

     CACCCCTACGGCGTTCCGGCGCCCGCCCCTCCCGCCGCCTATTCCCGCCCCGCCGTGTTGAAGGCGCCGGGCGGCCGCGGGCAGAGCATC  232
     HisProTyrGlyValProAlaProAlaProProAlaAlaTyrSerArgProAlaValLeuLysAlaProGlyGlyArgGlyGlnSerIle (77)

                               PvuII                                BamHI
     GGACGAAGGGGCAAAGTCGAGCAGCTGTCCCCGGAGGAGGAGGAAAAGAGGAGGATCCGCCGGGAGAGGAACAAGATGGCAGCGGCCAAG  322
     GlyArgArgGlyLysValGluGlnLeuSerProGluGluGluGluLysArgArgIleArgArgGluArgAsnLysMetAlaAlaAlaLys (107)

                         SacI            PstI                 PvuII                    PstI
     TGCCGCAACCGGCGGCGGGAGCTCACCGACACTCTGCAGGCGGAGACGGACCAGCTGGAGGAGGAGAAGTCCGCTCTGCAGGCGGAGATA  412
     CysArgAsnArgArgArgGluLeuThrAspThrLeuGlnAlaGluThrAspGlnLeuGluGluGluLysSerAlaLeuGlnAlaGluIle (137)

     GCCAACCTGCTGAAGGAGAAGGAGAAGCTGGAGTTCATCCTGGCGGCGCACCGGCCCGCCTGCAAGATGCCCGAGGAGCTGCGCTTCTCC  502
     AlaAsnLeuLeuLysGluLysGluLysLeuGluPheIleLeuAlaAlaHisArgProAlaCysLysMetProGluGluLeuArgPheSer (167)

                                                                      BglI StuI
     GAGGAGCTGGCGGCCGCCACCGCACTGGACCTGGGCGCACCCAGCCCCGCCGCGGCCGAGGAGGCCTTCGCCCTGCCGCTAATGACCGAG  592
     GluGluLeuAlaAlaAlaThrAlaLeuAspLeuGlyAlaProSerProAlaAlaAlaGluGluAlaPheAlaLeuProLeuMetThrGlu (197)

     GCGCCGCCGGCCGTGCCGCCCAAGGAGCCGAGCGGCAGCGGGCTGGAGCTGAAGGCCGAGCCCTTCGACGAGCTGCTTTTCTCCGCGGGG  682
     AlaProProAlaValProProLysGluProSerGlySerGlyLeuGluLeuLysAlaGluProPheAspGluLeuLeuPheSerAlaGly (227)

         StuI                                                         MluI
     CCGCGGGAGGCCTCCCGCTCGGTGCCTGACATGGACCTGCCCGGAGCCTCCTCCTTCTACGCGTCGGACTGGGAGCCGCTGGGCGCCGGG  772
     ProArgGluAlaSerArgSerValProAspMetAspLeuProGlyAlaSerSerPheTyrAlaSerAspTrpGluProLeuGlyAlaGly (257)

                                         BstEII
     AGCGGCGGGGAGCTGGAGCCCCTCTGCACCCCCGTGGTGACCTGCACCCCGTGCCCTAGCACCTACACCTCCACCTTCGTCTTCACCTAC  862
     SerGlyGlyGluLeuGluProLeuCysThrProValValThrCysThrProCysProSerThrTyrThrSerThrPheValPheThrTyr (287)

                          PvuII
     CCCGAGGCGGACGCCTTCCCCAGCTGCGCCGCTGCGCACCGGAAGGGCAGCAGCAGCAACGAGCCCTCGTCCGACTCCCTCAGCTCCCCC  952
     ProGluAlaAspAlaPheProSerCysAlaAlaAlaHisArgLysGlySerSerSerAsnGluProSerSerAspSerLeuSerSerPro (317)

                                                     ApaI
     ACCCTGCTGGCCTTGTGAGGGGCTCGCCCCGCGCTGACTGACCTGCCGGGCCCCCTCCCCTGCCCACGCGCCCCCACGGACTCGCCACGC 1042
     ThrLeuLeuAlaLeuTER
                (322)                                                                      env splice
       SmaI          NK24 specific | Δpol
     CCCCCGGGACTCCCGGACCTGGGGAGGGCGCTGCTGCCTTGGAACGATGGGACCCTGCCCTGCCTCTGGAGGGAGCGGTCGCTAGGCATT 1132
                                                                                          ^
                                                                                   1598 bases
                                                                                    deleted

     TCTGACTGGGCACCCTGGAAAGGTGAGCAAGAAGGACTCCAAGAAGAAGCCGCCAGCAATAAGCAAGAAAGACCCGGAGAAGACACCCTT 1222

                   Δpol                                                          gp85
     GCTGCCAACGAGAGTTAATTATATTCTCATTATTGGTGTCCTGGTCTTGTGTGAGGTTACGGGGGTAAGAGCTGATGTTCACTTGCTTGA 1312
                                                                                 AspValHisLeuLeuAr

     GCAGCCAGGGAACCTTTGGATTACATGGGCCAACCGTACAGGCCAAACGGATTTCTGCCTCTCCACACAGTCAGCCACCTCCCCTTTTCA 1402
     gGlnProGlyAsnLeuTrpIleThrTrpAlaAsnArgThrGlyGlnThrAspPheCysLeuSerThrGlnSerAlaThrSerProPheGl

     AACATGTTTGATAGGTATCCCGTCCCCTATTTCCGAAGGTGATTTTAAGGGATATGTCTCTGATACAAACTGCTCCACTGTGGGAACTCA 1492
     nThrCysLeuIleGlyIleProSerProIleSerGluGlyAspPheLysGlyTyrValSerAspThrAsnCysSerThrValGlyThrHi

     CCGGTTAGTCTCGTCAGGCATTCCCGGCGGTCCTAACAACACTGCCACCCTCACTTATCGAAAGGTTTCATGCTTGCTGCTAAAGCTGAA 1582
     sArgLeuValSerSerGlyIleProGlyGlyProAsnAsnThrAlaThrLeuThrTyrArgLysValSerCysLeuLeuLeuLysLeuAs

     TGTCTCTATGTGGGATGAGCCACCGGAACTACAGTTGCTAGGTTCCCAGTCTCTCCCTAATATTACTAACGTTACTCAGATCCCTGGTGT 1672
     nValSerMetTrpAspGluProProGluLeuGlnLeuLeuGlySerGlnSerLeuProAsnIleThrAsnValThrGlnIleProGlyVa

                                                                             HincII
     GGCAGGAGGATGCATAGGCTTCACCCCATACGGCAGTCCGGCTGGTGTTTACGGGTGGGGCCGGGGAGAGTTGACACACATCCTCTTAAC 1762
     lAlaGlyGlyCysIleGlyPheThrProTyrGlySerProAlaGlyValTyrGlyTrpGlyArgGlyGluLeuThrHisIleLeuLeuTh

     TAACCCCCCTGATAATCCTTTCTTTAACCGTGCTTCTAATTCTACGGAACCGTTTACGGTGGTGACAGCGGATAGGCACAATCTTTTTAT 1852
     rAsnProProAspAsnProPhePheAsnArgAlaSerAsnSerThrGluProPheThrValValThrAlaAspArgHisAsnLeuPhe
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FIG. 5. Nucleotide sequence of the v-*fos* gene of the NK24 virus. A comparison of the sequence with that of RSV revealed that a sequence containing parts of the *gag* and *pol* genes was replaced by an NK24-specific 1,072-bp sequence. In addition, another deletion of 1,598 bp was found from 55 bp downstream of the transduced sequence in the remaining *pol* gene sequence to the splice acceptor site for *env* mRNA. The nucleotide and amino acid sequences are numbered from the putative 5′ recombination site.

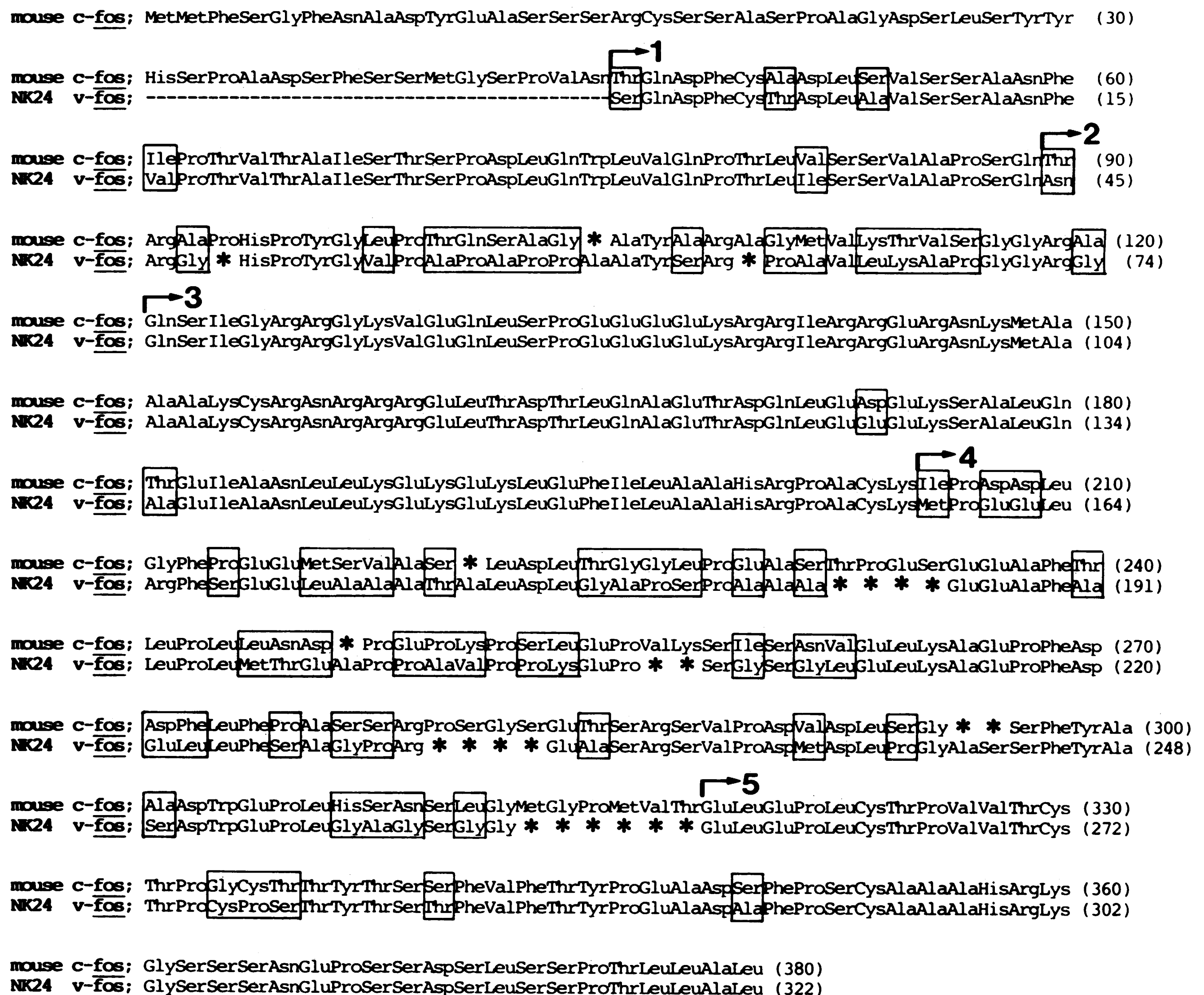

FIG. 6. Comparison of the mouse c-*fos* and NK24 v-*fos* gene products. The amino-terminal portion of the v-*fos* gene product replaced by the amino acid sequence of the *gag* gene is not shown. Asterisks indicate gaps, which were introduced to optimize alignment. Amino acids that differ from each other are boxed. Note that substitutions and gaps are clustered in two separate regions. To distinguish these regions of low homology from other, highly homologous regions, we arbitrarily divided the c-*fos* gene product into five regions. Numbers in boldface type above the sequence indicate the start of each region. Numbers in parentheses on the right indicate the position from the start of the *fos*-specific protein of the amino acid shown at the end of each line.

cellular oncogene that is involved in the induction of nephroblastomas. In this sense, it seems suggestive that the NK24 virus contains the v-*fos* gene as an oncogene. The *fos* gene may be responsible for the induction of at least some nephroblastomas of chickens, just as the *erbB* gene is responsible for erythroblastosis induction. Thus, it may be interesting to see whether and to what extent the expression of the c-*fos* gene is activated in blastic cells showing focal growth in the kidneys of 219V- and NAV-infected chickens.

When NK24 was inoculated into the wing webs of newborn chickens, fibrosarcomas with osteogenic cell proliferation were induced near the inoculation sites after relatively long latent periods at a high frequency, although not at 100% frequency. The FBJ and FBR strains of murine sarcoma virus, which were isolated from tissues of osteosarcomas and contain the v-*fos* gene (6–8, 37, 41), were reported to induce osteosarcomas in vivo and transform fibroblast cells in vitro (22, 23), although these murine viruses have not been reported to induce nephroblastomas.

These results indicate that avian and murine viruses with the v-*fos* gene have the same target cell specificity for fibroblasts and osteoblasts, although they were isolated from different types of tumors. The fact that a certain strain of avian leukemia virus induced nephroblastomas in one flock of chickens but osteopetrosis in another flock (avian osteopetrosis can be classified as osteogenic osteoblastoma [10]) suggests some common mechanism for the induction of nephroblastomas and osteogenic proliferation in chickens.

A comparison of the deduced amino acid sequence of the NK24 v-*fos* gene with that of the mouse c-*fos* gene revealed that the NK24 v-*fos* gene consists of highly homologous and relatively divergent regions. We used molecular cloning and sequence determinations of the chicken c-*fos* gene to determine whether the divergent regions are intrinsic to the

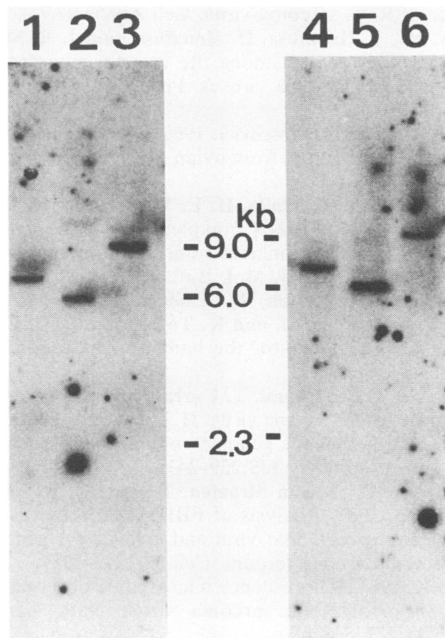

FIG. 7. Southern blot analysis of human DNA with mouse and chicken v-*fos* probes. High-molecular-weight DNA prepared from human peripheral blood was digested with *Hin*dIII, *Bam*HI, or *Eco*RI restriction endonuclease. Samples of 10 μg of the digests were fractionated by electrophoresis on a 0.9% agarose gel and then subjected to Southern blot analysis with the ^{32}P-labeled NK24 v-*fos* probe (lanes 1 to 3) or the ^{32}P-labeled mouse v-*fos* probe (lanes 4 to 6). A 1,047-bp fragment (spanning from nucleotide 1 to nucleotide 1,047 in Fig. 5) covering the entire coding region and part of the 3′ noncoding region of the v-*fos* sequence was used as the NK24 v-*fos* probe. Lanes 1 and 4, *Hin*dIII digest; lanes 2 and 5, *Bam*HI digest; lanes 3 and 6, *Eco*RI digest. ^{32}P-end-labeled *Hin*dIII fragments of lambda DNA were used as molecular weight markers.

chicken c-*fos* gene or are also divergent from the corresponding regions of the chicken c-*fos* gene. As described in the accompanying paper (9), a comparison of the coding regions of the c-*fos* and v-*fos* genes showed no amino acid substitution except for most of the first exon, which was replaced by the viral *gag* sequence in the NK24 v-*fos* gene. Therefore, the predicted NK24 *fos* gene product shows no structural alteration of the *fos*-specific region, unlike the murine FBJ and FBR v-*fos* gene products, which have carboxyl termini different from those of the c-*fos* gene products and contain several mutations. It is unknown whether the substitution of the amino-terminal portion derived from the first exon of the c-*fos* gene by the *gag*-derived polypeptide has any effect on the transforming ability of the chicken c-*fos* gene product. However, it is not surprising that no structural change was found in the c-*fos* gene-derived portion, because the mouse c-*fos* gene product is known to be able to induce the transformation of cultured rat fibroblasts (26). In the mouse c-*fos* gene, a controlling element of 67 nucleotides which would regulate the expression of the c-*fos* gene posttranscriptionally (24, 26) was found 123 to 189 nucleotides upstream from the polyadenylation signal in the 3′-noncoding region. When the c-*fos* gene linked to the viral LTR was introduced into cultured rat fibroblasts, the removal of this controlling element was found to confer the c-*fos* gene with transforming ability (24). In the chicken c-*fos* gene, an almost identical 67-nucleotide stretch was found in the corresponding location (only one nucleotide was substituted). It is noteworthy that this controlling element was not present in the v-*fos* sequence transduced in the NK24 virus.

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