JOURNAL OF VIROLOGY, Nov. 1982, p. 674–682
0022-538X/82/110674-09$02.00/0

Vol. 44, No. 2

FBJ Murine Osteosarcoma Virus: Identification and Molecular Cloning of Biologically Active Proviral DNA

TOM CURRAN,[1] GORDON PETERS,[2] CHARLES VAN BEVEREN,[1] NATALIE M. TEICH,[2] AND INDER M. VERMA[1]*

Tumor Virology Laboratory, The Salk Institute, San Diego, California 92138[1] and Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX, England[2]

Received 14 June 1982/Accepted 27 July 1982

A 12.0-kilobase *Eco*RI restriction fragment containing FBJ murine osteosarcoma virus (FBJ-MSV) proviral DNA was identified in FBJ-MSV-transformed nonproducer rat cells and molecularly cloned in bacteriophage Charon 30 (λFBJ-1). A 5.8-kb *Hin*dIII fragment containing the entire FBJ-MSV proviral DNA was isolated from λFBJ-1 and subsequently subcloned in plasmid pBR322 (pFBJ-2). The DNA from recombinant plasmid pFBJ-2 was able to induce morphological transformation of rat fibroblasts in tissue culture. Transfected cells contained the p55 and p39 antigens specific for cells transformed by FBJ-MSV (T. Curran and N. M. Teich, J. Virol. **42**:114–122, 1982). The organization of the FBJ-MSV provirus was analyzed by restriction endonuclease mapping, and a region of nonhomology with the helper virus was delineated. Sequences specific for this region (presumably the viral *fos* gene) were subcloned and used as a probe to identify related sequences present in the normal genomes of cells from a variety of mammalian species (cellular *fos*). A single-size (3.4 kilobases long) class of RNA hybridizing to the viral *fos* probe was identified in FBJ-MSV-transformed cells.

The acutely oncogenic retroviruses encode sequences responsible for cellular transformation in vitro and induction of neoplasia in vivo (2, 3). It is now clear that these cellular sequences, referred to as *onc* genes, are related to and derived from counterparts in the normal cellular genome (3). Thus, viral oncogenesis is a consequence of the expression of acquired cellular sequences. In the majority of cases, the acquisition of normal cellular sequences occurs at the expense of all or part of the viral structural genes. Therefore, such viruses are defective for replication and must be propagated in the presence of a helper virus. Two modes of viral oncogene expression have been discerned in cells infected with acutely oncogenic retroviruses. The acquired cellular sequences may be expressed as an independent viral gene product (6) or, alternatively, as a fusion protein containing peptides derived from the viral structural proteins (44).

At present, over a dozen viral *onc* (v-*onc*) genes have been distinguished, each differing in nucleotide sequence and each with a distinct corresponding cellular homolog (c-*onc*) (1, 12, 13, 15, 18, 19, 21, 26–28, 31, 34, 35). A major aim of current retroviral research is the identification and isolation of novel v-*onc* genes, since they present an unique opportunity for the experimental manipulation of specific cellular genes the perturbation of which can result in neoplastic growth.

In 1966, the FBJ murine osteosarcoma virus complex was isolated by Finkel, Biskis, and Jinkins (hence the name FBJ) from a spontaneous tumor in a 260-day-old CFI mouse (17). Serial passage of cell-free tumor extracts in newborn mice resulted in virus which induces osteosarcomas with a latency as short as 3 weeks (17). The tumors can arise anywhere along the bones, although they appear occasionally in the abdominal peritoneum and the diaphragm, and they appear first as a cortical thickening or as small areas of increased density in the soft tissue adjacent to the bone (17, 43). Proliferation appears to begin at the periosteum, and growth proceeds peripherally, with late involvement of the deep cortex. The FBJ virus complex consists of a replication-competent helper murine leukemia virus (FBJ-MLV) and a replication-defective transforming murine sarcoma virus (FBJ-MSV) (23, 24). The two components of the FBJ virus complex have been isolated separately in tissue culture: FBJ-MLV by endpoint dilution and FBJ-MSV by the establishment of nonproducer transformed rat cells (10). A 55,000-dalton phosphoprotein (p55), which is a potential product of the FBJ-MSV oncogene (v-*fos*), was identified by employing sera from rats bearing tumors induced by inocu-

lation of nonproducer cells (11). In addition to p55, tumor-bearing rat sera (TBRS) also precipitated a 39,000-dalton protein (p39) presumably of host origin (10, 11). These proteins could not be precipitated from cells transformed by a variety of other acutely oncogenic retroviruses, which suggests that v-*fos* is a novel viral oncogene. To obtain more direct evidence for the existence of the v-*fos* gene, we isolated a molecular clone of the FBJ-MSV provirus by recombinant DNA techniques. This clone, which proved to be biologically active, was used to identify the v-*fos* gene cellular homolog (c-*fos*) in a number of different species. In addition, a single species of FBJ-MSV-specific RNA of 3.4 kilobases (kb) was detected in transformed cells.

MATERIALS AND METHODS

Cells and viruses. All cells were grown in Dulbecco modified Eagle medium supplemented with 10% heat-inactivated calf serum and antibiotics. The following cell lines were used: 208F (29), a thioguanine-resistant variant of Rat-1 cells (25); NRK cells (14); RS2 (10), FBJ-MSV-transformed nonproducer 208F cells; RKS3 (10), FBJ-MSV-transformed nonproducer NRK cells; RLS1 (10), 208F cells infected with FBJ-MLV and FBJ-MSV; and RL1 (10), 208F cells infected with FBJ-MLV clone L1. Large-scale preparation of virus and purification of 70S viral RNA was carried out as previously described (11). FBJ-MLV was quantitated by the XC plaque assay (32), and FBJ-MSV was quantitated by a modification (11) of the focus assay of Hartley and Rowe (20). DNA transfection by calcium phosphate precipitation with NIH/3T3 DNA as the carrier was essentially as described previously (9), except that 208F cells, grown in the presence of 10^{-6} M dexamethasone, were used as recipients.

Preparation and analysis of cellular DNA and RNA. High-molecular-weight cellular DNA was prepared in the following manner. Confluent dishes of cells were washed twice in buffer containing 10 mM Tris (pH 7.5), 1 mM EDTA, and 0.1 M NaCl and lysed in the same buffer plus 1% sodium dodecyl sulfate (SDS). The lysates were removed by scraping the dishes with a rubber policeman, and the bulk of cellular proteins was digested with 500 μg of pronase per ml at 37°C for 3 to 4 days. We extracted nucleic acids twice with phenol and twice with chloroform and recovered DNA by spooling it onto a glass rod after ethanol precipitation. It was then suspended in buffer containing 10 mM Tris (pH 7.5) and 1 mM EDTA and dialyzed for 2 to 3 days against a total of 2 liters of the same buffer.

Detection of specific DNA fragments was carried out by agarose gel electrophoresis followed by the transfer-hybridization procedure described by Southern (37). Radioactive probes were prepared by nick translation of cloned DNAs (30) or by calf thymus DNA-primed reverse transcription of viral RNA (38). Total cellular RNA was purified by guanidine thiocyanate lysis followed by centrifugation through CsCl as described previously (8). Samples of RNA (2 to 10 μg) were lyophilized, dissolved in buffer containing 20 mM MOPS (morpholinopropanesulfonic acid; pH 7.0), 1 mM EDTA, 5 mM sodium acetate, 2.2 M formaldehyde, and 50% formamide, and denatured at 60°C for 5 min. After addition of sucrose to 5% and bromophenol blue to 0.025%, the samples were applied to flatbed agarose gels containing 0.8% agarose, 20 mM MOPS (pH 7.0), 1 mM EDTA, 5 mM sodium acetate, and 2.2 M formaldehyde. Electrophoresis was carried out in the same buffer without formaldehyde. After the gels were run, they were stained for 30 min in electrophoresis buffer containing 5 μg of ethidium bromide per ml and then destained for 30 min in the same buffer without ethidium bromide. The gels were viewed under UV light, and the positions of the 28S and 18S rRNAs were noted. The RNA in the gels was then transferred to nitrocellulose as described previously (39), and hybridization was carried out as described for the Southern transfer procedure.

Recombinant DNA procedures. A 300-μg portion of RKS3 cellular DNA was digested to completion with *Eco*RI, and the resulting fragments were separated by agarose gel electrophoresis. The region of the gel containing fragments of approximately 12 kb each was excised, and the DNA was purified by electroelution and DEAE-cellulose chromatography (45). Charon 30 vector DNA was digested with *Eco*RI, and the left (21.5-kb) and right (12-kb) arms were separated by agarose gel electrophoresis. The separated arms were eluted from the gel with glass powder as described previously (42). Approximately 2 μg of the left arm, 1 μg of the right arm, and 0.6 μg of target DNA were ligated with T4 DNA ligase (21, 41). The ligated chimeric DNA was packaged in vitro with an efficiency of approximately 10^5 to 10^6 viable phage particles per μg of DNA (4). The 12-kb *Eco*RI fragment and the 5.8-kb *Hin*dIII fragment from one recombinant phage (λFBJ-3) were isolated by gel electrophoresis and subcloned into the unique *Eco*RI and *Hin*dIII sites of plasmid pBR322 (41).

Immunoprecipitation. The preparation of TBRS2, which recognizes p55, has been described elsewhere (10). [^{35}S]methionine labeling of cell cultures and immunoprecipitation of cell lysates were performed as described previously (10, 11). Immune complexes were electrophoresed in the presence of SDS on slab gels containing 10% polyacrylamide (22), and the gels were processed for fluorography (5). The ^{14}C-labeled protein molecular weight markers (Amersham International) used were myosin (molecular weight, 200,000), phosphorylase *a* (molecular weight, 92,500), bovine serum albumin (molecular weight, 69,000), ovalbumin (molecular weight, 45,000), carbonic anhydrase (molecular weight, 30,000) and lysozyme (molecular weight, 14,300).

RESULTS

Identification and molecular cloning of FBJ-MSV proviral DNA. All of the available virus-producing cell lines release more FBJ-MLV than FBJ-MSV (10, 23, 24), which makes it difficult to prepare a radioactive probe specific for FBJ-MSV sequences. However, it was assumed that the FBJ-MSV genome, like those of other MSVs, would have helper virus sequences at its termini. Therefore, a probe was prepared by reverse transcription of a mixture of FBJ-MLV RNA and FBJ-MSV RNA (in which there

was a 50-fold excess of FBJ-MLV). This probe was hybridized to restriction enzyme digests of high-molecular-weight DNA isolated from FBJ-MSV-transformed nonproducer NRK cells (RKS3) and control uninfected NRK cells. A single DNA fragment (5.8 kb with *Hin*dIII, 12.0 kb with *Eco*RI) was identified in RKS3 DNA (Fig. 1A, lanes 1 and 2). Other fragments, which hybridized to a lesser extent, were also detected in uninfected NRK cellular DNA and may represent FBJ-MLV-related endogenous rat virus sequences (lanes 3 and 4). If it is assumed that the probe hybridized to MLV sequences present at the termini of the FBJ-MSV genome, then the data suggest that *Eco*RI and *Hin*dIII did not cleave within the FBJ-MSV provirus and that an entire copy of the FBJ-MSV genome was contained within each restriction fragment.

A 300-μg portion of RKS3 DNA was digested with *Eco*RI, and the resulting fragments were separated by agarose gel electrophoresis. The approximate region of the gel containing the 12.0-kb restriction fragments was cut out, and the DNA was purified by electroelution and DEAE-cellulose chromatography. The DNA was then ligated to *Eco*RI-cleaved Charon 30 vector DNA and packaged in vitro to generate viable phage particles. Over 20,000 phage plaques were screened with a cDNA probe synthesized by reverse transcription of FBJ complex viral RNA. Six plaques which hybridized to the probe were identified, and DNAs from two recombinant phage clones were purified for further analysis. The *Eco*RI insert from one of the recombinant phages, λFBJ-3, was subcloned into the unique *Eco*RI site of plasmid pBR322 (pFBJ-1) (Fig. 1A, lanes 5 and 7) by procedures previously described (40). Digestion of pFBJ-1 DNA with *Hin*dIII generated a number of fragments, including a 5.8-kb fragment similar in size to the fragment obtained from proviral DNA (lanes 6 and 8). The *Hin*dIII fragment containing the entire FBJ-MSV proviral DNA was also subcloned in the unique *Hin*dIII site of plasmid pBR322 (pFBJ-2).

Figure 1B displays a detailed restriction endonuclease map of plasmid pFBJ-2. This restriction map is in close agreement with a partial restriction map of the FBJ-MSV provirus generated by restriction endonuclease analysis of RKS3 cellular DNA (T. Curran, Ph.D. thesis, University of London, London, Great Britain, 1982). Recently, the complete nucleotide sequence of pFBJ-2 DNA has been determined (C. Van Beveren, unpublished data), and the physical map shown here was generated by a combination of conventional methods and nucleotide sequence analysis.

Biological activity of pFBJ-2. To assess whether cloned FBJ-MSV proviral DNA retained the capability to induce transformation of rat fibroblasts in tissue culture, we introduced pFBJ-2 DNA into 208F cells by calcium phosphate precipitation (9). The efficiency of morphological transformation was approximately 150 focus-forming units per pmol (Table 1). The morphology of transformed 208F cells in a focus induced by FBJ-MSV (Fig. 2A, panel i) closely resembled that of 208F cells transformed by pFBJ-2 DNA (Fig. 2A, panel ii). To determine whether the viral genome in pFBJ-2-transformed cells could be rescued, we isolated an individual focus of pFBJ-2-transformed 208F cells (TX.F1)

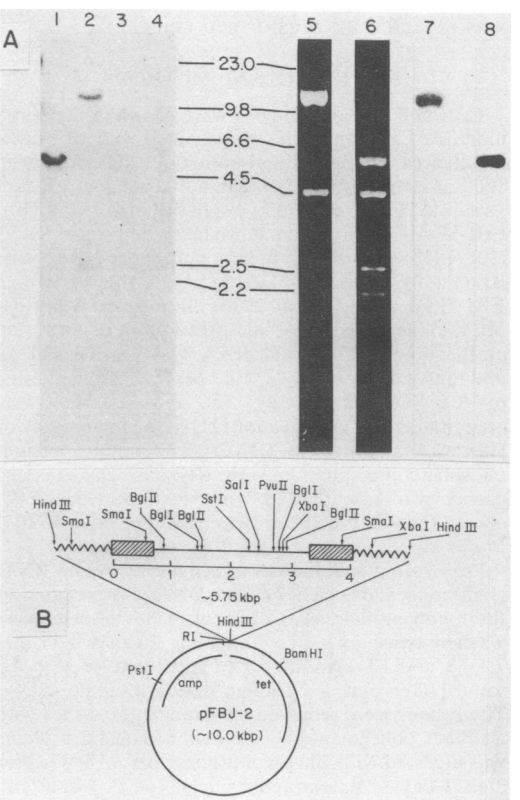

FIG. 1. Identification of FBJ-MSV provirus in high-molecular-weight cellular DNA and in recombinant plasmid DNA. (A) DNAs from RKS3 cells (lanes 1 and 2), uninfected NRK cells (lanes 3 and 4), and recombinant plasmids pFBJ-1 (lanes 5 and 7) and pFBJ-2 (lanes 6 and 8) were digested with *Hin*dIII (lanes 1 and 3), *Eco*RI (lanes 2, 4, and 5), and *Hin*dIII plus *Eco*RI (lane 6). The resulting fragments were then analyzed by agarose gel electrophoresis, Southern transfer, and hybridization with a probe made by reverse transcription of FBJ viral RNA. Lanes 5 and 6, Same digests as in lanes 7 and 8 but visualized by ethidium bromide staining. Bacteriophage λ DNA digested with *Hin*dIII was used for size standards (numbers in middle indicate lengths of the standard in kb). (B) Physical map of recombinant plasmid pFBJ-2.

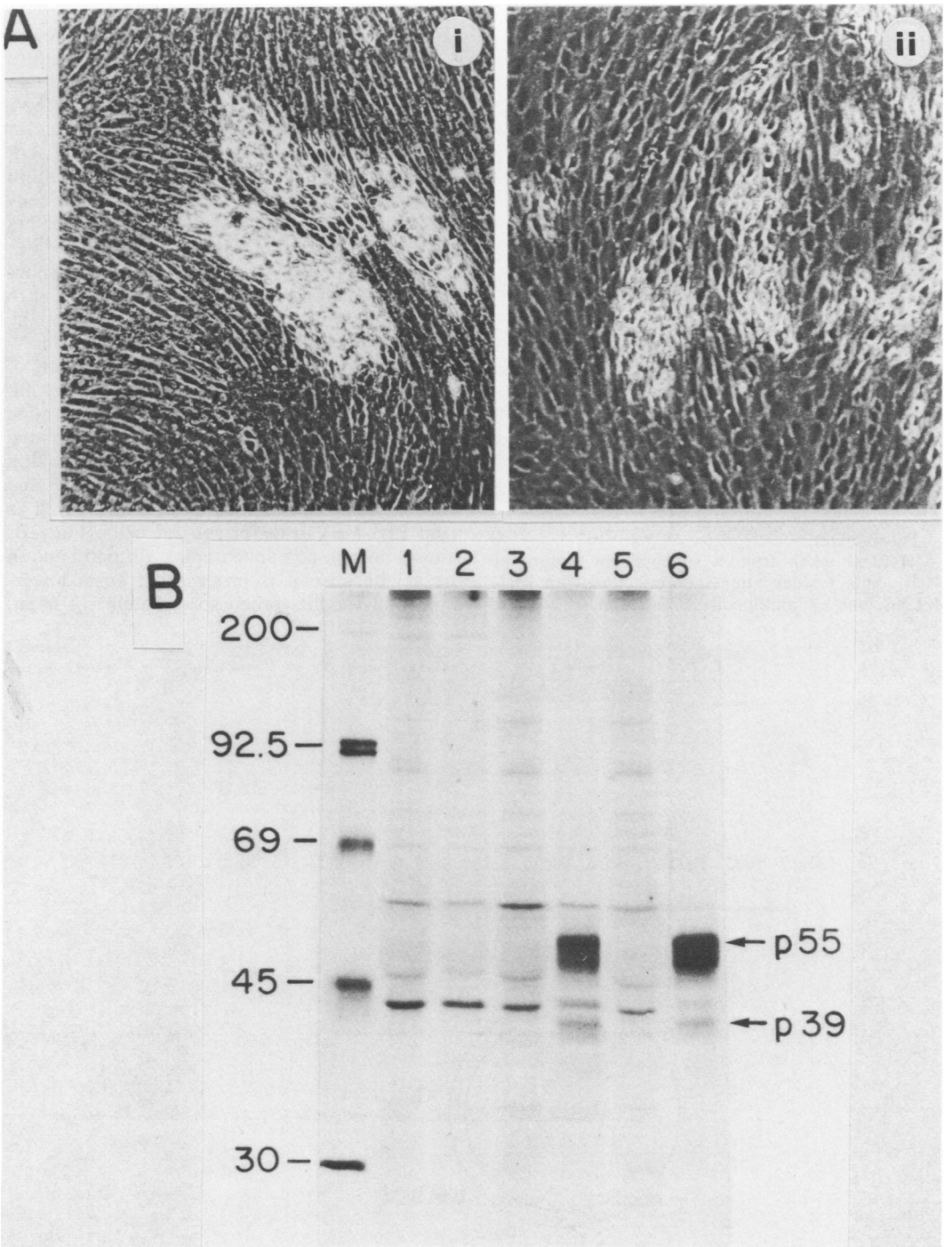

FIG. 2. Morphology of pFBJ-2-transformed cells and immunoprecipitation with TBRS2. (A) Cells were photographed under phase-contrast microscopy (×400). (i) Focus induced by FBJ-MSV on 208F cells at 2 weeks postinfection. (ii) pFBJ-2-transformed 208F cells passaged once after transfection. (B) [^{35}S]methionine-labeled extracts of 208F cells (lanes 1 and 2), 208F cells transformed by pFBJ-2 (TX.F1) (lanes 3 and 4), and FBJ-MSV-transformed nonproducer cells (RS2) (lanes 5 and 6) were immunoprecipitated with normal rat serum (lanes 1, 3, and 5) and TBRS2 (lanes 2, 4, and 6) and analyzed by electrophoresis on a 10% SDS-polyacrylamide gel. Lane M, Molecular weight markers; the numbers on the left indicate molecular weights (×10^3).

TABLE 1. Biological activity of cloned FBJ-MSV provirus

Procedure	Efficiency of transformation	
	FFU[a]	PFU/ml[b]
Transfection with:		
Carrier DNA	<0.2[c]	
pFBJ-2 DNA[d]	150.0[c]	
pH1 DNA[e]	20.0[c]	
Infection with:		
TX.F1 supernatant[f]	<10[g]	<10
TX.F1 clone L1 supernatant[h]	1.4×10^5[g]	3.7×10^6

[a] Focus-forming units (FFU) on 208F cells.
[b] XC plaque-forming units on NIH/3T3 mouse cells.
[c] Per picomole.
[d] The entire recombinant plasmid DNA shown in Fig. 1B was used for transfection.
[e] This clone contains Harvey MSV circular DNA permutated by cleavage at the *Eco*RI site (7).
[f] Harvest taken from a subconfluent culture of pFBJ-2-transformed cells (TX.F1).
[g] Per milliliter.
[h] Harvest taken from a subconfluent culture of TX.F1 cells 1 week after superinfection with FBJ-MuLV (clone L1; multiplicity of infection, 0.1 to 0.5).

by micromanipulation and superinfected it with FBJ-MLV. Transforming virus could be readily detected in virus harvests from this culture (Table 1). The rescue efficiency was comparable to that obtained by superinfection of FBJ-MSV-transformed nonproducer cells (10).

It has been previously demonstrated that sera from rats bearing tumors induced by inoculation of FBJ-MSV-transformed nonproducer rat cells precipitate two proteins with molecular weights of 55,000 (p55) and 39,000 (p39) from FBJ-MSV-transformed cells (11). p55, which is a phosphoprotein, can also be detected among the in vitro translation products of the virion RNA of the FBJ complex and is a strong candidate for the v-*fos* product. [^{35}S]methionine-labeled extracts of pFBJ-2-transformed cells were analyzed by immunoprecipitation, using TBRS to determine whether these two antigens were also associated with molecularly cloned FBJ-MSV proviral DNA. Both p55 and p39 could be precipitated from rat cells transformed by pFBJ-2 as well as from FBJ-MSV-transformed rat cells (Fig. 2B, lanes 4 and 6). p55 appeared as a broad band in the gel because it is processed from a lower-molecular-weight precursor protein (Curran,

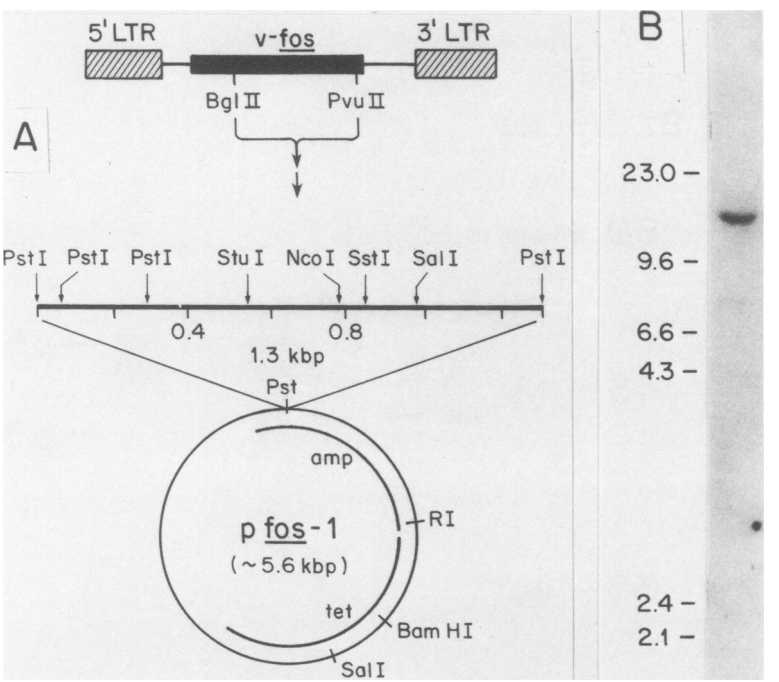

FIG. 3. Restriction endonuclease analysis and subcloning of *fos*-specific sequences. (A) Physical map of p*fos*-1 showing the v-*fos*-specific region. pFBJ-2 was cleaved with *Bgl*II and *Pvu*II and subcloned into the *Pst*I site of pBR322 by deoxycytidine-deoxyguanosine tailing. (B) Southern transfer and hybridization analysis of BALB/c mouse liver cell DNA with nick-translated *fos*-specific probe. High-molecular-weight DNA was digested with *Eco*RI, and the resulting fragments were separated by electrophoresis on a 0.7% agarose gel before transfer and hybridization. Bacteriophage λ DNA digested with *Hin*dIII was used for size standards (numbers on left indicate lengths of the standards in kb).

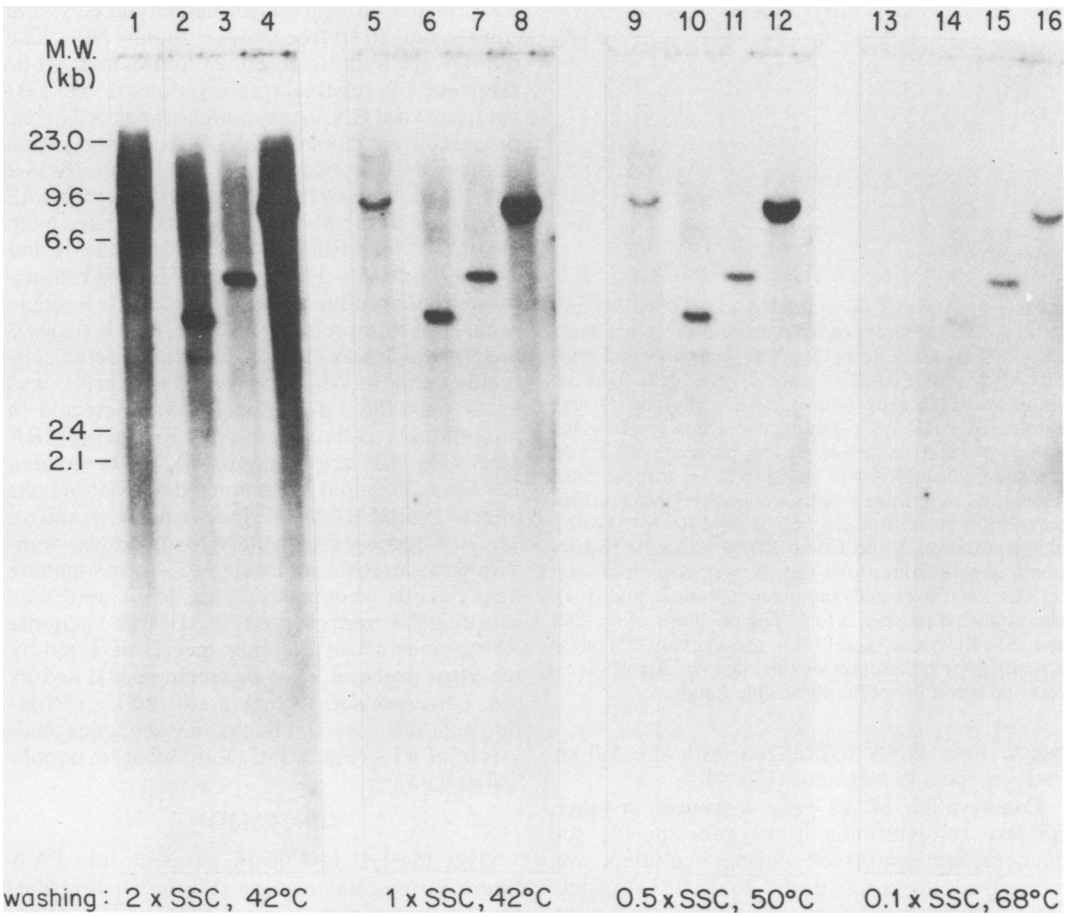

FIG. 4. Identification of cellular sequences homologous to v-*fos* in vertebrate species. High-molecular-weight DNAs were isolated from chicken (lanes 1, 5, 9, and 13), mink (lanes 2, 6, 10, and 14), mouse (lanes 3, 7, 11, and 15), and human cells (lanes 4, 8, 12, and 16) and digested with *Eco*RI, and the resulting fragments were analyzed by agarose gel electrophoresis and Southern transfer. A nitrocellulose filter containing one set of samples was hybridized with the nick-translated, 1.0-kb, *Pst*I *fos*-specific fragment and then washed under different stringency conditions as indicated. Autoradiograms were exposed for 72 h. However, when washing was carried out at 68°C in 0.1 × SSC, exposure was for only 24 h. Bacteriophage λ DNA digested with *Hin*dIII was used for size standards (numbers on left indicate lengths of molecular weight [M.W.] standards in kb).

Ph.D. thesis). Thus, it appeared that recombinant plasmid pFBJ-2 was capable of inducing both the morphological transformation of rat cells typical for FBJ-MSV and the expression of the p55 and p39 antigens found only in FBJ-MSV-transformed cells.

Cloning of *fos*-specific probe. The transforming gene of most retroviruses has a counterpart in the normal cellular genome. To test whether the oncogenic sequences encoded by FBJ-MSV were also present in uninfected mouse or rat cells, we needed a probe specific for the *fos* gene sequences. The FBJ-MSV proviral DNA clone, pFBJ-2, also contained helper viral sequences and therefore could not be used as a specific probe. However, by a combination of restriction endonuclease analyses and nucleotide sequencing data, we found that a majority of the v-*fos*-specific information resided in the region between *Bgl*II and *Pvu*II (Fig. 1B). The *Bgl*II-*Pvu*II fragment was isolated from pFBJ-2 DNA, extended with oligodeoxycytidine residues by using terminal transferase, and annealed to deoxyguanosine-tailed *Pst*I-cleaved pBR322 DNA (a kind gift from R. Müller, The Salk Institute, San Diego, Calif.), as described previously (21). Figure 3A shows the structure of this *fos* gene-specific probe. Cleavage of plasmid p*fos*-1 with *Pst*I generates three *fos*-specific fragments, one of 1.0 kb and two small fragments of 60 and 260 nucleotides. A single major band of 15.0 kb was identified in *Bam*HI-cleaved

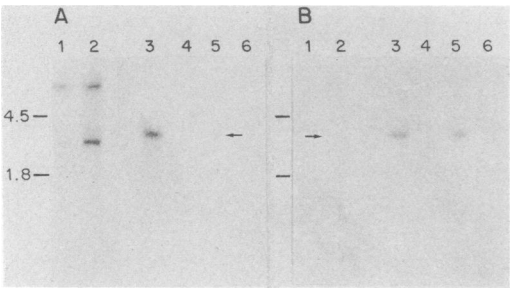

FIG. 5. Analysis of viral RNA in FBJ-MSV-transformed cells. Samples of RNA from 208F cells infected with FBJ-MLV (lane 1), 208F cells infected with FBJ-MLV plus FBJ-MSV (lane 2), RS2 cells (lane 3), uninfected 208F cells (lane 4), RKS3 cells (lane 5), and uninfected NRK cells (lane 6) were analyzed by agarose gel electrophoresis in the presence of 2.2 M formaldehyde. RNA was transferred to nitrocellulose filters and hybridized with radioactive probes prepared by reverse transcription of viral RNA (A) or by nick translation of the 1.0-kb *Pst*I *fos*-specific restriction fragment from p*fos*-1 (B). A 2-μg portion of total cellular RNA was analyzed in lanes 1 and 2, and 10 μg was analyzed in lanes 3 to 6. The positions of the 28S and 18S rRNAs, 4.5 and 1.8 kb, respectively (33), were visualized by ethidium bromide staining. Arrows indicate the positions of faintly visible bands.

mouse liver DNA hybridized with the 1.0-kb *Pst*I *fos*-specific fragment (Fig. 3B).

Conservation of *fos* gene sequences in other species. To determine if *fos* gene-specific sequences are conserved during evolution, we hybridized the v-*fos* probe to *Eco*RI-digested DNAs from a variety of species under different hybridization conditions. A single *fos*-specific DNA fragment was detected, which, in the case of human, mouse, and mink DNAs, remained relatively unaffected by increased washing stringencies, suggesting extensive sequence homology (Fig. 4). However, the c-*fos* gene in the chicken genome appears to be less conserved, since little or no hybridization could be observed after washing with 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 68°C (Fig. 4, lane 13). No hybridization was observed with DNAs isolated from amphibians and lower species, including species of *Torpedo*, *Drosophila*, and *Dictyostelium* (data not shown). Thus, like many other oncogenes, the *fos* gene sequences are relatively conserved during evolution.

Analysis of viral RNA in cells infected with FBJ-MLV and FBJ-MSV. The mode of v-*fos* transcription in FBJ-MSV-transformed cells was analyzed by the Northern transfer procedure (39). Total cellular RNAs were purified from uninfected cells, cells infected with FBJ-MSV alone, cells infected with FBJ-MLV, and cells infected with FBJ-MLV plus FBJ-MSV. Samples of RNA were denatured with formamide, fractionated on agarose-formaldehyde gels, and transferred to nitrocellulose filters (39). The filters were hybridized either to a cDNA probe prepared by reverse transcription of the FBJ complex viral RNA or to a nick-translated probe prepared from cloned v-*fos* DNA. Hybridization to the cDNA probe revealed two major size (8.2 and 3.2 kb) classes of RNA in cells infected with FBJ-MLV alone and also in cells infected with FBJ-MLV plus FBJ-MSV (Fig. 5A, lanes 1 and 2). In contrast, RNA from FBJ-MSV-transformed nonproducer cells contained a single species of virus-related RNA of 3.4 kb (lanes 3 and 5), which was not present in uninfected cells (lanes 4 and 6). When the *fos*-specific probe was used, only the 3.4-kb species was detected in nonproducer cells and in cells producing FBJ-MSV (Fig. 5B, lanes 2, 3, and 5). No *fos*-related RNA was detected in uninfected cells or in cells infected with FBJ-MLV alone (lanes 1, 4, and 6). Thus, it appeared that the v-*fos* gene was transcribed as an RNA molecule of 3.4 kb containing helper virus sequences. This RNA molecule presumably represented FBJ-MSV genome RNA, since it was the only species detected by the v-*fos* probe in virus-producing cells, and its size corresponded to that predicted by restriction endonuclease and nucleotide sequence analysis of pFBJ-2 (Fig. 1B; C. Van Beveren, unpublished data).

DISCUSSION

Over the last few years, research into RNA tumor viruses has made a substantial contribution to our understanding of neoplastic transformation. In particular, characterization of the acutely oncogenic viruses has allowed the investigation of some of the basic mechanisms of virus-induced oncogenesis at the molecular level. FBJ-MSV is an acutely oncogenic virus which encodes a gene (v-*fos*) responsible for cellular transformation in vitro and osteosarcoma induction in vivo. The exclusive association of FBJ-MSV with osteosarcomas (10, 16, 17, 43) suggests that v-*fos* is unrelated to other known MSV oncogenes. In addition, two proteins, p55 and p39, can be precipitated from FBJ-MSV-transformed cells but not from cells transformed by other MSVs (11). To study the genome organization of FBJ-MSV and to obtain a molecular probe specific for the *fos* gene, we have molecularly cloned FBJ-MSV proviral DNA from transformed nonproducer rat cells. The FBJ-MSV provirus is approximately 4.0 kb in length. It is bounded by two long terminal repeats of approximately 600 bases and has a substituted region of approximately 1.5 kb. Although no *env*-related proteins can be detected in FBJ-MSV-transformed nonproducer cells (10), the genome does contain residual *env* se-

quences (Van Beveren, unpublished data). Since FBJ-MSV was isolated from a spontaneous tumor, the parental virus from which these sequences were derived is not known. However, it is hoped that nucleotide sequence analysis of the most abundant helper virus in the FBJ complex, which has recently been molecularly cloned (T. Curran, unpublished data), will enable an accurate delineation of these regions. Nucleotide sequence analysis of helper virus regions in the FBJ-MSV genome (Van Beveren, unpublished data) suggests that the parental virus may be related to AKR-MLV (40).

Evidence that the recombinant plasmid pFBJ-2 contained an entire biologically active copy of the FBJ-MSV provirus was obtained from DNA transfection experiments. pFBJ-2 DNA transformed rat fibroblast cells with an efficiency of 150 focus-forming units per pmol. The morphology of the cells transformed by pFBJ-2 DNA was identical to that of rat cells transformed by FBJ-MSV. In addition, transforming virus could be efficiently rescued from the transfected cells by superinfection with FBJ-MuLV (Table 1). We are currently examining the potential of this rescued virus to induce osteosarcomas after inoculation into newborn mice.

All of the v-*onc* genes isolated to date are related to sequences present in the normal genetic complement of vertebrate species (3). By using a probe specific for the v-*fos* gene, a cellular counterpart (c-*fos*) has been identified in mouse, human, mink, and chicken chromosomal DNAs. The reduced stability of the hybrid formed between the *fos* probe and chicken DNA suggested a lower degree of conservation in this species (Fig. 4). However, unlike a probe specific for v-*mos* (I. Verma, unpublished data), the c-*fos* probe formed a hybrid of high stability with human DNA, suggesting a greater degree of conservation. A single major DNA fragment was detected in mouse DNA cut with *Eco*RI and *Bam*HI, suggesting that there is only a single copy of the c-*fos* sequences in the mouse genome. Recently, nucleotide sequence analysis of the mouse c-*fos* gene indicated that it contained at least four intervening sequences (F. van Straaten, unpublished data).

In FBJ-MSV-transformed cells, the v-*fos* gene was apparently transcribed as a single RNA molecule of 3.4 kb which contained both *fos* and helper virus sequences (Fig. 5). Two RNA species of 8.2 and 3.2 kb were identified in cells infected with FBJ-MLV and were thought to represent genome RNA and envelope mRNA, respectively.

The *fos* gene of the FBJ-MSV is a novel oncogene isolate. It does not cross-hybridize with probes specific for *mos*, *abl*, ras^{Ha}, *fes*, *src*, *myc*, *myb*, and *erb* oncogenes (R. Müller and I. M. Verma, unpublished data). In addition, the size of the mouse or human genomic DNA fragment which was cleaved with *Bam*HI (Fig. 3B) or *Eco*RI (Fig. 4) and which hybridizes with the *fos* probe is different from that of the fragments reported to hybridize with *fms*- or *sis*-specific sequences (13, 15). Although expression of some of these v-*onc* genes occasionally results in osteosarcoma development (36), v-*fos* is exclusively associated with this specific and somewhat rare disease (10, 16, 17, 43). Thus, when expressed under the control of a viral promoter, *fos* sequences stimulate the growth and differentiation of bone tissue, albeit neoplastic. Therefore, it is tempting to postulate that the c-*fos* gene has a primary role in promoting the growth and differentiation of normal bone tissues. This hypothesis is supported by the recent observation (R. Müller, D. J. Slamon, J. M. Tremblay, M. J. Cline, and I. M. Verma, Nature [London], in press) that c-*fos* is expressed at relatively high levels in the bones of newborn mice.

ACKNOWLEDGMENTS

We thank F. Blattner and colleagues for the Charon 30 vector, E. Scolnick and colleagues for cloned Harvey MSV DNA, and R. A. Bosselman for in vitro-packaging reagents. We acknowledge with gratitude the competent technical assistance of John Hunts and thank Clive Dickson, Rolf Muller, and Flip van Straaten for fruitful discussions.

T. C. is supported by a Damon Runyon-Walter Winchell Cancer Fund fellowship (DRG 551). This research was supported by a Public Health Service grant from the National Cancer Institute and a grant from the American Cancer Society.

ADDENDUM IN PROOF

We have now shown that virus rescued from TX.FI cells induces osteosarcomas, with a latency and pathology equal to those of tumors induced by FBJ-MSV, after inoculation into newborn mice.

LITERATURE CITED

1. **Anderson, P. R., S. G. Devare, S. R. Tronick, R. W. Ellis, S. A. Aaronson, and E. M. Scolnick.** 1981. Generation of BALB-MuSV and Ha-MuSV by type C virus transduction of homologous transforming genes from different species. Cell **26**:129–134.
2. **Bishop, J. M.** 1978. Retroviruses. Annu. Rev. Biochem. **47**:35–88.
3. **Bishop, J. M.** 1981. Enemies within: the genesis of retrovirus oncogenes. Cell **23**:5–6.
4. **Blattner, F. R., B. G. Williams, A. Blechl, K. Denniston-Thompson, H. E. Faber, L. Furlong, D. J. Grunwald, D. O. Kiefer, D. D. Moore, J. W. Schumm, E. L. Sheldon, and O. Smithies.** 1977. Charon phages: safer derivatives of bacteriophage lambda for DNA cloning. Science **146**:159–161.
5. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
6. **Brugge, J. S., and R. L. Erikson.** 1977. Identification of a transformation-specific antigen induced by an avian sarcoma virus. Nature (London) **269**:346–348.
7. **Chang, E. H., J. M. Maryak, C.-M. Wei, T. Y. Shih, R. Shober, H. L. Cheung, R. W. Ellis, G. L. Hager, E. M.**

Scolnick, and D. R. Lowy. 1982. Functional organization of the Harvey murine sarcoma virus genome. J. Virol. **35**:76–92.
8. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.
9. **Coraso, C. M., and M. L. Pearson.** 1981. Enhancing the efficiency of DNA-mediated gene transfer in mammalian cells. Somatic Cell Genet. **5**:1031–1044.
10. **Curran, T., and N. M. Teich.** 1982. Identification of a 39,000-dalton protein in cells transformed by the FBJ murine osteosarcoma virus. Virology **116**:221–235.
11. **Curran, T., and N. M. Teich.** 1982. Candidate product of the FBJ murine osteosarcoma virus oncogene: characterization of a 55,000-dalton phosphoprotein. J. Virol. **42**:114–122.
12. **DeFeo, D., M. A. Gonda, H. A. Young, E. H. Chang, D. R. Lowy, E. M. Scolnick, and R. W. Ellis.** 1981. Analysis of two divergent rat genomic clones homologous to transforming gene of Harvey murine sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **78**:3328–3332.
13. **Donner, L., L. A. Fedele, C. F. Garon, S. J. Anderson, and C. J. Sherr.** 1982. McDonough feline sarcoma virus: characterization of the molecularly cloned provirus and its feline oncogene (v-*fms*). J. Virol. **41**:489–500.
14. **Duc-Nguyen, H., E. N. Rosenblum, and R. F. Zeigel.** 1966. Persistent infection of a rat kidney cell line with Rauscher murine leukemia virus. J. Bacteriol. **92**:1133–1140.
15. **Eva, A., K. C. Robbins, P. R. Anderson, A. Srinivasan, S. R. Tronick, E. P. Reddy, N. E. Ellmore, A. T. Galen, J. A. Lautenberger, T. S. Papas, E. H. Westin, F. Wong-Staal, R. C. Gallo, and S. A. Aaronson,** 1982. Cellular genes analogous to retroviral *onc* genes are transcribed in human tumour cells. Nature (London) **295**:116–119.
16. **Finkel, M. P., and B. O. Biskis.** 1968. Experimental induction of osteosarcomas. Prog. Exp. Tumor Res. **10**:72–111.
17. **Finkel, M. P., B. O. Biskis, and P. B. Jinkins.** 1966. Virus induction of osteosarcomas in mice. Science **151**:698–701.
18. **Franchini, G., J. Even, C. J. Sherr, and F. Wong-Staal.** 1981. *Onc* sequences (v-*fes*) of Snyder-Theilen feline sarcoma virus are derived from noncontiguous regions of a cat cellular gene (c-*fes*). Nature (London) **290**:154–157.
19. **Goff, S. P., E. Gilboa, O. N. Witte, and D. Baltimore.** 1980. Structure of the Abelson murine leukemia virus genome and the homologous cellular gene: studies with cloned viral DNA. Cell **22**:777–785.
20. **Hartley, J. W., and W. P. Rowe,** 1966. Production of altered foci in tissue culture by defective Moloney sarcoma virus particles. Proc. Natl. Acad. Sci. U.S.A. **55**:780–786.
21. **Jones, M., R. A. Bosselman, F. A. v.d. Hoorn, A. Berns, H. Fan, and I. M. Verma.** 1980. Identification and molecular cloning of Moloney mouse sarcoma virus-specific sequences from uninfected mouse cells. Proc. Natl. Acad. Sci. U.S.A. **77**:2651–2655.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
23. **Levy, J. A., J. W. Hartley, W. P. Rowe, and R. J. Huebner.** 1973. Studies of FBJ osteosarcoma virus in tissue culture. I. Biological characteristics of the C-type viruses. J. Natl. Cancer Inst. **51**:525–549.
24. **Levy, J. A., P. L. Kazan, C. A. Reilly, Jr., and M. P. Finkel.** 1978. FBJ osteosarcoma virus in tissue culture. III. Isolation and characterization of non-virus-producing FBJ-transformed cells. J. Virol. **26**:11–15.
25. **Mishra, N. K., and W. L. Ryan.** 1973. Effect of 3-methylcholanthrene and dimethylnitrosamine on anchorage dependence of rat fibroblasts chronically infected with Rauscher leukemia virus. Int. J. Cancer **11**:123–130.
26. **Oskarsson, M., W. L. McClements, D. G. Blair, J. V. Maizel, and G. F. Vande Woude.** 1980. Properties of a normal mouse cell DNA sequence (*sarc*) homologous to the *src* sequence of Moloney sarcome virus. Science **207**:1222–1224.
27. **Parker, R. C., H. E. Varmus, and J. M. Bishop.** 1981. Cellular homologue (c-*src*) of the transforming gene of Rous sarcoma virus: isolation, mapping, and transcriptional analysis of the c-*src* and flanking regions. Proc. Natl. Acad. Sci. U.S.A. **78**:5842–5846.
28. **Perbal, B., and M. A. Baluda.** 1982. Avian myeloblastosis virus transforming gene is related to unique chicken DNA regions separated by at least one intervening sequence. J. Virol. **41**:250–257.
29. **Quade, K.** 1979. Transformation of mammalian cells by avian myelocytomatosis virus and avian erythroblastosis virus. Virology **98**:461–465.
30. **Rigby, P. W., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. **113**:237–251.
31. **Robins, T., K. Bister, C. Garon, T. Papas, and P. Duesberg.** 1982. Structural relationship between a normal chicken DNA locus and the transforming gene of the avian acute leukemia virus MC29. J. Virol. **41**:635–642.
32. **Rowe, W. P., W. E. Pugh, and J. W. Hartley.** 1970. Plaque assay techniques for murine leukemia viruses. Virology **42**:1136–1139.
33. **Rubstov, P. M., M. M. Musakhanov, V. M. Zakharyev, A. S. Krayev, K. G. Skryabin, and A. A. Bayev.** 1980. The structure of the yeast ribosomal RNA genes. I. The complete nucleotide sequence of the 18S ribosomal RNA gene from *Saccharomyces cerevisiae*. Nucleic Acids Res. **8**:5779–5794.
34. **Shalloway, D., A. D. Selentz, and G. M. Cooper.** 1981. Molecular cloning the characterization of the chicken gene homologous to the transforming gene of Rous sarcoma virus. Cell **24**:531–541.
35. **Shibuya, M., H. Hanafusa, and P. C. Balduzzi.** 1982. Cellular sequences related to three new *onc* genes of avian sarcoma virus (*fps*, *yes*, and *ros*) and their expression in normal and transformed cells. J. Virol. **42**:143–152.
36. **Soehner, R. L., and L. Dmochowski.** 1969. Induction of bone tumours in rats and hamsters with murine sarcoma virus (Harvey and Moloney) and their cell-free transmission. Nature (London) **224**:191–192.
37. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
38. **Taylor, J. M., R. Illmensee, and J. Summers.** 1976. Efficient transcription of RNA into DNA by avian sarcoma virus polymerase. Biochim. Biophys. Acta **442**:324–330.
39. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
40. **Van Beveren, C., E. Rands, S. K. Chattopadhyay, D. R. Lowy, and I. M. Verma.** 1982. Long terminal repeat of murine retroviral DNAs: sequence analysis, host-proviral junctions, and preintegration site. J. Virol. **41**:542–556.
41. **Verma, I. M., M. H.-T. Lai, R. A. Bosselman, M. A. McKennett, H. Fan, and A. Berns.** 1980. Molecular cloning of unintegrated Moloney mouse sarcoma virus DNA in bacteriophage λ. Proc. Natl. Acad. Sci. U.S.A. **77**:1773–1777.
42. **Vogelstein, B., and D. Gillespie.** 1979. Preparation and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. U.S.A. **76**:615–619.
43. **Ward, J. M., and D. M. Young.** 1976. Histogenesis and morphology of periosteal sarcomas induced by FBJ virus in NIH Swiss mice. Cancer Res. **36**:3985–3992.
44. **Witte, O. N., N. Rosenberg, M. Paskind, A. Shields, and D. Baltimore.** 1978. Identification of an Abelson murine leukemia virus-encoded protein present in transformed fibroblast and lymphoid cells. Proc. Natl. Acad. Sci. U.S.A. **75**:2844–2492.
45. **Wu, R.** 1979. Recombinant DNA. Methods Enzymol. **68**:1–555.