Proc. Natl. Acad. Sci. USA
Vol. 78, No. 6, pp. 3328–3332, June 1981
Biochemistry

Analysis of two divergent rat genomic clones homologous to the transforming gene of Harvey murine sarcoma virus

(*sarc* genes/recombinant DNA/Southern blot analysis/heteroduplex analysis)

DEBORAH DEFEO*, MATTHEW A. GONDA†, HOWARD A. YOUNG†, ESTHER H. CHANG‡, DOUGLAS R. LOWY‡, EDWARD M. SCOLNICK*, AND RONALD W. ELLIS*§

*Laboratory of Tumor Virus Genetics, National Cancer Institute, Bethesda, Maryland 20205; †Biological Carcinogenesis Program, Frederick Cancer Research Center, Frederick, Maryland 21701; and ‡Dermatology Branch, National Cancer Institute, Bethesda, Maryland 20205

Communicated by Philip Leder, January 27, 1981

ABSTRACT **Harvey murine sarcoma virus (Ha-MuSV) is a mouse–rat recombinant retrovirus that encodes a protein designated p21, required for virally induced transformation. Using a radiolabeled DNA fragment from the p21 coding region, we have detected homologous DNA sequences in the normal DNA of rats and of several other vertebrate species. Moreover, many tested cells from these species contain low levels of a p21 protein that is highly related to viral 21. Now we report two independent fragments from normal rat DNA containing sequences (*sarc*) homologous to the Ha-MuSV transforming region that were cloned in the bacteriophage vector Charon 4A. *Sarc* sequences in the one fragment are completely colinear with the viral sequences and share apparently all restriction endonuclease sites. *Sarc* sequences in the second fragment have several sets of intervening sequences and lack some restriction endonuclease sites found in the viral transforming region. Despite the presence of these intervening sequences in the second *sarc* fragment, we have been able to ligate this *sarc* fragment to the long terminal repeat sequence of Ha-MuSV and to induce cellular transformation and high levels of p21 expression upon transfection of this DNA to NIH 3T3 mouse cells. These results suggest that elevated levels of p21, normally expressed at low levels in a variety of cells, can induce cellular transformation.**

Retroviruses can be grouped into two broad classes based upon their biological activities: the nontransforming leukemia or leukosis viruses and the transforming sarcoma or acute leukemia viruses (1). The molecular biology of the transforming viruses, such as Rous sarcoma virus, Moloney murine sarcoma virus (Mo-MuSV), Abelson murine leukemia virus, and Harvey murine sarcoma virus (Ha-MuSV), has been of special interest because viruses of this class enable cellular transformation related to a defined genetic sequence and its gene product(s) to be studied in great detail and under a high degree of experimental control. Accordingly, the genetic origin and transforming proteins of these viruses have come under close scrutiny (2–10).

Our laboratory has been investigating the molecular biology of Ha-MuSV, a replication-defective retrovirus derived after injection of a mouse leukemia virus into a rat (11). Similar to sequences in the above-mentioned viruses, the Ha-MuSV *src* gene sequences are derived from (rat) cellular unique sequences (10). These viral *src* sequences encode the transformation-specific p21 protein (12, 13) and the p21 biochemical activities, which specifically use guanine nucleotides (14, 15). Consistent with the conserved nature of these sequences is the expression of a related, but distinguishable p21 *sarc* protein in normal cells from a wide variety of species (16).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact

In order to understand better any differences in the structure, biology, and regulation of expression between the viral p21 *src* and the related cellular p21 *sarc* protein, we have initiated studies into the structure and function of rat *sarc* genes. In this report, we describe the molecular cloning of two distinct fragments of rat cellular DNA whose sequences are homologous to the Ha-MuSV *src* gene. The length of homology of each *sarc* sequence to the viral *src* sequence is consistent with the size of the Ha-MuSV transforming region, which we have described (10, 17). One *sarc* sequence (in clone Ha-MuSV–*sarc* I) is apparently colinear with the viral *src*, a result analogous to the viral *src* and cellular *sarc* sequences of Mo-MuSV (4). The second *sarc* sequence (in clone Ha-MuSV–*sarc* II), despite being interrupted by three sets of intervening sequences, has the coding information to induce cellular transformation in association with elevated levels of (cellular) p21 expression.

MATERIALS AND METHODS

DNA Biochemistry. These techniques have been described (10, 18, 19).

Cloning Subgenomic Viral Fragments in pBR322. Procedures for the transformation of *E. coli* and the molecular cloning of viral DNA in pBR322 have been described (19). This technology was utilized to clone the following fragments of Ha-MuSV DNA.

BS-9. This 0.45-kilobase pair (kbp) cloned portion of the *src* region has been described (10.)

HB-11. After digestion of the full genome-length clone H-1 DNA (10) with *Bam*HI and *Eco*RI, the 2.3-kbp fragment, of which the entire *src* region is a subset, was purified by preparative gel electrophoresis before cloning at the *Bam*HI and *Eco*RI sites of pBR322.

HLTR. The details of the cloning of HLTR, the Ha-MuSV long terminal repeat (LTR) clones, will be presented elsewhere. The LTR represents an amalgam of 5′- and 3′-terminal sequences juxtaposed during circularization of retroviral DNA after infection of cells and reverse transcription (20–23). These sequences are 0.3–0.9 kbp upstream of clone HB-11 in Ha-MuSV DNA.

Isolation of Endogenous *sarc* Sequences. The Ha-MuSV clone BS-9 probe was employed in two approaches for isolating endogenous *sarc* sequences. The Ha-MuSV–*sarc* II clone was isolated from a Charon 4A library, the gift of T. Sargent and J. Bonner, of adult Sprague–Dawley rat liver DNA partially di-

Abbreviations: Mo-MuSV, Moloney murine sarcoma virus; Ha-MuSV, Harvey murine sarcoma virus; kb, kilobase(s); kbp, kilobase pair(s); LTR, long terminal repeat.

§ To whom reprint requests should be addressed.

gested by *Hae* III. The Ha-MuSV–*sarc* I clone was isolated as follows. An *Eco*RI limit digest of 1 mg of Fisher rat embryo DNA was resolved by centrifugation at 100,000 × *g* for 20 hr at 20°C on a 10–40% linear sucrose density gradient in 1 M NaCl/2 mM EDTA/10 mM Tris, pH 7.5. Fractions containing *sarc* sequences (as determined by agarose gel electrophoresis, transfer to nitrocellulose, and blotting with ^{32}P-labeled Ha-MuSV *src* probe) were pooled, ligated to Charon 4A arms (24), and packaged (25), and the recombinant phage were screened for *sarc* sequences (26).

Heteroduplex Preparation and Electron Microscopy. These techniques were performed as described (17, 27). DNA molecules used in these analyses include (*i*) Ha-MuSV clone HB-11; (*ii*) Ha-MuSV–*sarc* II clone: 13.5-kbp insert and its 5.4-kbp fragment with *Bam*HI and *Eco*RI termini, subcloned as described in *Results and Discussion*; and (*iii*) Ha-MuSV–*sarc* I clone: 13-kbp insert and its 2.8-kbp fragment with *Eco*RI and *Bam*HI termini, subcloned as described in *Results and Discussion*.

RESULTS AND DISCUSSION

Identification of the Rat *sarc* Sequences. Although similar in principle to that of other transforming retroviruses, the genetic organization of Ha-MuSV is more complex insofar as its 5.4-kilobase (kb) RNA genome is composed of three, rather than two, sets of sequences (8–10): (*i*) 0.1 kb at the 5′ end and 0.9 kb at the 3′ end, derived from the helper-independent virus Moloney murine leukemia virus; (*ii*) 3.5 kb sequences—highly homologous to a family of greatly reiterated rat genes, which are retroviral-like sequences expressed in many rat cells—sometimes referred to as 30S sequences on the basis of the sedimentation coefficient of their RNA transcriptional product (28, 29); and (*iii*) 1.0 kb of *src* sequences, completely bracketed by 30S sequences, that are required for transformation, encode the transformation-specific p21 protein, and originate from rat cellular sequences, which are highly conserved evolutionarily (10). As an initial step for investigating rat *sarc* sequences related to Ha-MuSV, we had defined a viral *src* probe from this latter set of sequences (10).

The *src* probe was employed to detect *sarc* sequences in Southern blot analysis of *Eco*RI-digested rat cellular DNA (Fig. 1, lane 1). Two major bands (13 and 20 kbp) were detected. In addition, a doublet at 1.7 kbp can be found. The intensity of hybridization of the *src* probe to this doublet has not been reproducible, ranging from that in Fig. 1 to one virtually unnoticeable (data not shown). Therefore, we are unable to determine thus far the precise number (two or more) of rat *sarc* genes related to Ha-MuSV *src*. We have hybridized the *src* probe to Southern blots of *Eco*RI-digested DNAs from a variety of normal rat cells, including Sprague–Dawley, and consistently have detected the 13-kbp and 20-kbp bands (data not shown).

Cloning of the *sarc* Sequences from Rat DNA. The *src* clone was utilized as a probe for cloning *sarc* sequences from two sources of rat DNA, a partial *Hae* III-digested genomic library and recombinant phage containing *Eco*RI-limit-digested fragments of 13 kbp and 20 kbp. A positive clone that was detected among the phage-containing *Eco*RI-limit-digested fragments contained a 13.0-kbp DNA insert, henceforth referred to as Ha-MuSV–*sarc* I. Restriction digestion of this insert produced a 2.8-kbp fragment, with *Bam*HI and *Eco*RI termini, which hybridized to the *src* probe (data not shown). This 2.8-kbp fragment was subcloned in pBR322 and digested by *Acc* I; a 0.3-kbp fragment (see Fig. 3 *Top*, map position 0.2–0.5 kbp) that did not hybridize to Ha-MuSV *src* was purified by preparative gel electrophoresis. This 0.3-kbp fragment detected only the 13-kbp rat *Eco*RI band (Fig. 1, lane 3), demonstrating that the Ha-MuSV–*sarc* I clone represents the 13-kbp band; this relationship is consistent with the derivation of this clone by *Eco*RI limit digestion of rat cellular DNA.

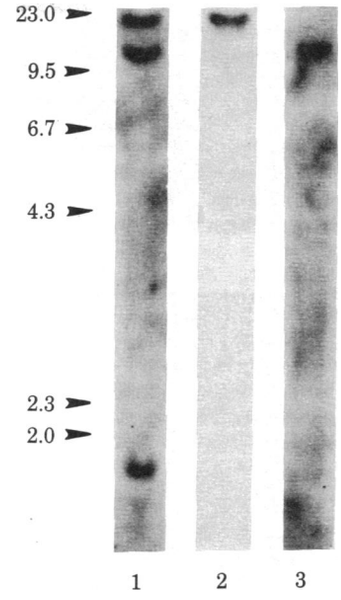

FIG. 1. Southern blot analysis of restriction endonuclease-digested high molecular weight DNA from Fisher rat embryo cells. Cellular DNA (50 μg) was digested with *Eco*RI, electrophoresed, blotted, and hybridized to [^{32}P]DNA probes (1.5 × 10^7 cpm each). Lanes: 1, Ha-MuSV *src*; 2, 1.4-kbp non-*sarc* fragment produced by *Xba* I digestion of the Ha-MuSV–*sarc* II clone; 3, 0.3-kbp non-*sarc* fragment produced by *Acc* I digestion of the Ha-MuSV–*sarc* I clone.

Upon screening the partial *Hae* III-digest library, a clone was isolated, *Eco*RI digestion of which released three inserts of 13.5 kbp, 2.0 kbp, and 1.5 kbp; only the 13.5-kbp insert contained sequences hybridizing to the *src* probe. This 13.5-kbp fragment was found to be immediately adjacent to phage sequences in the Charon 4A vector (data not shown), and we assumed that this fragment corresponds to an *Eco*RI-limit-digestion fragment of more than 13.5 kbp because it was generated by a partial *Hae* III digestion of rat cellular DNA. Restriction digestion of this insert, henceforth referred to as Ha-MuSV–*sarc* II, revealed a 5.4-kbp fragment with *Bam*HI and *Eco*RI termini which hybridized to the *src* probe (data not shown). This fragment was subcloned in pBR322 and digested with *Xba* I; a 1.4-kbp fragment (see Fig. 3 *Bottom*, map position 2.3–3.7 kbp) that did not hybridize to Ha-MuSV *src* (data not shown) was purified by preparative gel electrophoresis. This 1.4-kbp fragment detected only the 20-kbp rat *Eco*RI band (Fig. 1, lane 2), demonstrating that the Ha-MuSV–*sarc* II clone is derived from the 20-kbp band.

Structural Comparison of Cellular *sarc* and Viral *src*. In order to survey the gross structural relationship between the Ha-MuSV *src* and the cellular *sarc* sequences, we performed heteroduplex mapping among these DNA molecules (Fig. 2). A comparison of Ha-MuSV–*sarc* II (13.5-kbp fragment) with viral *src* revealed 0.9 kbp of homology interrupted in *sarc* by three intervening sequences of 0.2, 0.2, and 0.8 kbp (Fig. 2 *a–c*). In contrast, Ha-MuSV–*sarc* I (13.0-kbp fragment) and viral *src* hybridized to an uninterrupted 0.9-kbp homoduplex (Fig. 2 *g–i*). The heteroduplex formed between the two *sarc* clones (5.4- and 2.8-kbp pBR322 subclones) was consistent with these results: 0.9 kbp of homology interrupted in the Ha-MuSV–*sarc* II clone by three intervening sequences (Fig. 2 *d–f*). These results indicate that *sarc* genes may contain several or no introns and may be present in more than one copy per haploid genome.

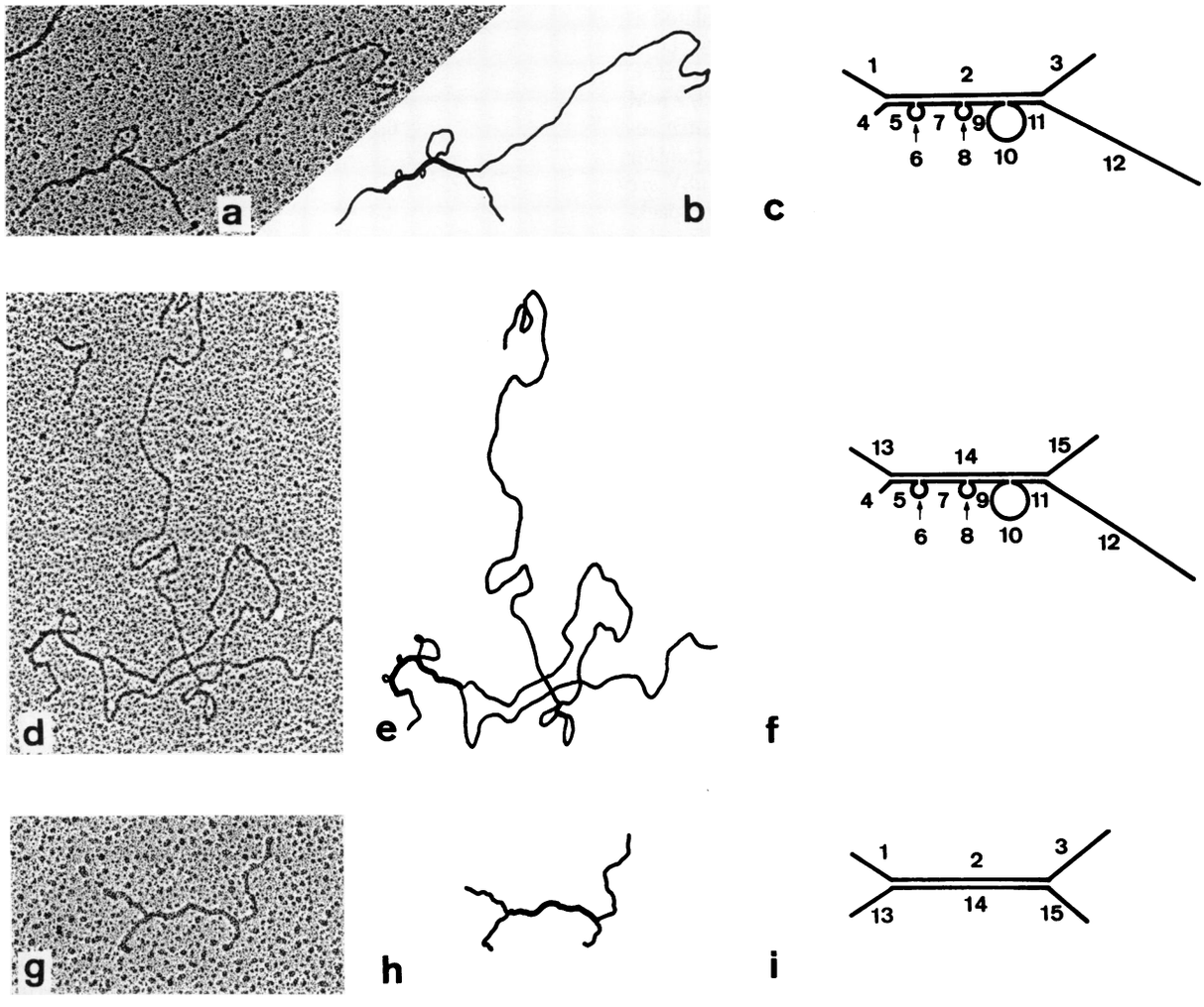

FIG. 2. Heteroduplex analyses of Ha-MuSV *src* and cellular *sarc* sequences. Molecules presented in micrographs (×120,000) and schematics are Ha-MuSV *src* and Ha-MuSV–*sarc* II (*a–c*), Ha-MuSV–*sarc* I and Ha-MuSV–*sarc* II (*d–f*), and Ha-MuSV–*sarc* I and Ha-MuSV *src* (*g–i*). Schematic representations (c, f, i) of the heteroduplexes, illustrated by the 2.3-, 5.4-, and 2.8-kbp subcloned fragments of Ha-MuSV, Ha-MuSV–*sarc* II, and Ha-MuSV–*sarc* I, respectively, are based on measurements of over 15 molecules. Single-stranded ϕX174 (5375 bases) and double-stranded simian virus 40 DNA (5240 bp) were used as size standards. Contour lengths, measured from tracings with a digital length calculator, were 1 μm = 3040 bases = 3080 bp. The length measurements in kb were as follows: 1 = 0.56 ± 0.07, 2 = 1.00 ± 0.07, 3 = 0.68 ± 0.04, 1–3 = Ha-MuSV *src* (clone HB-11); 4 = 0.05 ± 0.01, 5 = 0.24 ± 0.03, 6 = 0.22 ± 0.03, 7 = 0.19 ± 0.03, 8 = 0.21 ± 0.2, 9 = 0.18 ± 0.02, 10 = 0.89 ± 0.04, 11 = 0.35 ± 0.05, 12 = 3.40 ± 0.30, 4–12 = Ha-MuSV–*sarc* II; 13 = 0.70 ± 0.06, 14 = 1.10 ± 0.09, 15 = 1.03 ± 0.10, 13–15 = Ha-MuSV–*sarc* I.

Desiring to achieve a finer comparison of the clones, we constructed restriction endonuclease maps of the pBR322 subclones of both *sarc* sequences (Fig. 3). The colinearity of the Ha-MuSV–*sarc* I/viral *src* heteroduplex is strongly reinforced by the total conservation of a series of sites in the 5′ half of the homologous sequences (Fig. 3 *Top* and *Middle*; *Sst* II, *Bgl* I, *Sma* I, *Sac* I, *Hin*dIII, *Pvu* II). In contrast, Ha-MuSV–*sarc* II (Fig. 3 *Bottom*) has retained in this block of six sites only three sites (*Sac* I, *Hin*dIII, *Pvu* II) that are clustered in the viral *src* (Fig. 3 *Middle*); in addition, Ha-MuSV–*sarc* II contains a second *Pvu* II site. The close similarity between Ha-MuSV–*sarc* I and viral *src* suggests that *src* was derived from Ha-MuSV–*sarc* I. In this regard, the origin of the Ha-MuSV *src* sequences would be analogous to that of Mo-MuSV, whose *src* gene is derived from a colinear cellular *sarc* gene (4, 30). It is noteworthy that the heteroduplexes among these molecules revealed no substantial homology between the 30S sequences immediately flanking viral *src* and sequences bracketing either cellular *sarc*. Since Ha-MuSV was formed from three components, these data would suggest that Ha-MuSV arose after at least two recombinational events.

Biological Activity of *sarc*. In contrast to that of Ha-MuSV–*sarc* I, the structure of Ha-MuSV–*sarc* II is significantly divergent from viral *src*. Therefore, we sought biological evidence for its relationship to the Ha-MuSV transforming sequences. Unlike clone H-1 DNA, both Ha-MuSV–*sarc* II still linked to Charon 4A and the 5.4-kbp *Eco*RI–*Bam*HI clone of Ha-MuSV–*sarc* II failed to induce foci of transformed NIH 3T3 cells in the DNA transfection assay. It has been reported that the viral LTR, which contains a promoter-like sequence of the same 5′–3′ polarity as the viral genome (31), significantly enhances the transformation efficiency of viral *src* genes (23, 32). Indeed, clone HB-11, the Ha-MuSV subclone that lacks an LTR, also induces no transformed cell foci. Accordingly, we ligated clone HLTR, the Ha-MuSV LTR with synthetic *Eco*RI termini, to the *Eco*RI–*Bam*HI subclone of L clone *sarc*. Transfection of this preparation resulted in a low but significant level of focus formation (Table 1).

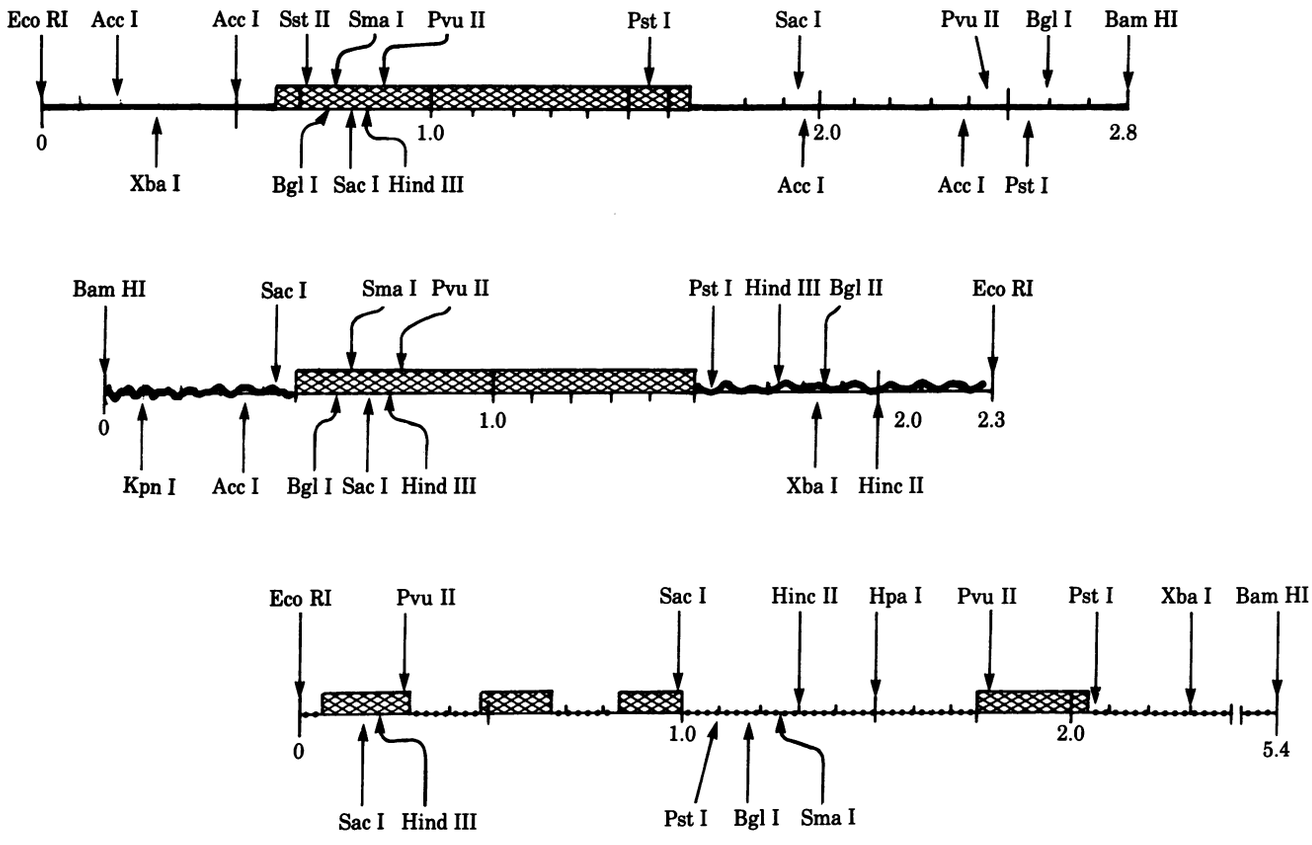

FIG. 3. Restriction endonuclease maps in kbp of viral *src* and cellular *sarc* sequences. Restriction site placements are accurate to ±0.05 kbp. As deduced from both heteroduplex analysis and Southern blot hybridization, the homologous *src* and *sarc* sequences in the three fragments are highlighted by crosshatches. (*Top*) Ha-MuSV–*sarc* I (2.8-kbp fragment). (*Middle*) Ha-MuSV *src* (2.3-kbp fragment = clone HB-11). (*Bottom*) Ha-MuSV–*sarc* II (5.4-kbp fragment). Sequences in each molecule that are nonhomologous to sequences in other molecules are denoted by solid (*Upper*), wavy (*Middle*), and dotted (*Lower*) lines.

To confirm the specificity of the focus induction, we tested cell extracts for the presence of the transformation-specific viral protein p21. Immunoprecipitation of cell extracts with Ha-MuSV-specific antiserum detected high levels of p21 in each of three independent foci (Fig. 4; data for two clones are not shown). Therefore, despite the structural differences between Ha-MuSV–*sarc* II and the viral *src*, the ability of this cellular DNA clone to transform cells and to induce p21 expression unambiguously demonstrates its close relationship to the viral *src*.

Table 1. Infectivity of DNA clones

DNA	Treatment	Foci per μg of viral DNA*
H-1†	Undigested	2600
HLTR‡	Self-ligated	0
Ha-MuSV–*sarc* II/λ§	Undigested	0
Ha-MuSV–*sarc* II¶	Self-ligated	0
HLTR‡ + Ha-MuSV–*sarc* II‡¶	Ligated	86

* With calf thymus DNA as carrier, NIH 3T3 cells were transfected with 0.3–1.0 μg of cloned DNA by means of the calcium precipitation procedure (32) as outlined (33). Numbers of foci were counted from duplicate dishes and were standardized to micrograms of viral DNA. The data represent the average of two experiments.

† H-1 DNA still was residing in pBR322 for the transfection.

‡ HLTR and Ha-MuSV–*sarc* II were purified free of pBR322 by preparative gel electrophoresis prior to ligation and transfection.

§ The 13.5-kbp insert still was residing in Charon 4A for the transfection.

¶ *Eco*RI–*Bam*HI subclone of Ha-MuSV–*sarc* II.

The ability of Ha-MuSV–*sarc* II to induce cellular transformation has several implications. Even though we have not proven formally that Ha-MuSV–*sarc* II encodes p21, the enhanced p21 levels in the transformed cells and the Ha-MuSV–*sarc* II/viral *src* homology make this interpretation a likely one. Therefore, it is clear that a mere change in the level of expression of a normal cellular gene and gene product may be sufficient to induce the transformed phenotype. It had been shown previously that Mo-MuSV cellular *sarc* also could induce cellular transformation after ligation to the viral LTR sequence (34). However, as noted above, Mo-MuSV *sarc* is not expressed in normal cells (2, 3), and no gene product has been identified formally yet. Our results are consistent with those of both Rous sarcoma virus and Abelson murine leukemia virus, wherein the viral *src* proteins are expressed in virally transformed cells at higher levels than are their related cellular *sarc* proteins in normal cells (35, 36). However, in neither of these cases has the cellular *sarc* gene been molecularly cloned as a prelude to inducing high expression levels of the actual cellular *sarc* proteins. The findings obtained with Mo-MuSV and Ha-MuSV *sarc* genes suggest that other *sarc* genes also may be capable of profoundly influencing cellular proliferation. One could speculate that such a change in the expression of *sarc* genes could occur in spontaneously arising tumors if a strong promoter of RNA synthesis were present to the left of the *sarc* gene.

Our ability to transfect these *sarc* sequences and transform cells (37) allows us to obtain yields of Ha-MuSV–*sarc* II-encoded p21 approaching or exceeding those of Ha-MuSV p21 in virally transformed cells (15). Thus, we can begin to study whether the

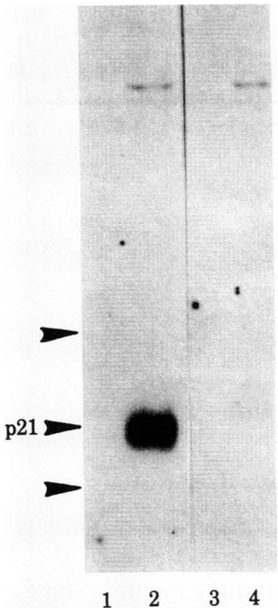

FIG. 4. Immunoprecipitation of p21 from extracts of NIH 3T3 cells transformed by Ha-MuSV–*sarc* II. Cells were labeled for 24 hr at 37°C with [^{35}S]methionine, cell extracts were made, and immunoprecipitations were performed as described (12, 13). Ha-MuSV–*sarc* II-transformed NIH 3T3 cells (lanes 1 and 2), uninfected NIH 3T3 cells (lanes 3 and 4), normal rat serum (lanes 1 and 3), and Ha-MuSV antiserum (lanes 2 and 4) were used. The arrows indicate molecular weight markers: 25,700 (α-chymotrypsinogen) and 18,400 (β-lactoglobulin).

biochemistry of viral p21 differs qualitatively from that of cellular p21 in terms of its ability to mediate different phenotypes of cellular transformation. Furthermore, the functional and evolutionary relationship between Ha-MuSV–*sarc* I and Ha-MuSV–*sarc* II remains to be clarified. Ha-MuSV–*sarc* I may not be expressed in normal cells, as is the case with Mo-MuSV *sarc*, which also lacks introns (4, 30). Accordingly, a critical question for us at this juncture is whether the two cellular *sarc* genes are expressed in different types of normal cells or at varying states of differentiation in the rat. Such studies could enable us to understand better the role, if any, of p21 in normal cells (16).

We have been able to obtain cell transformation and high level p21 expression with Ha-MuSV–*sarc* I, using a construction similar in principle to that used for Ha-MuSV–*sarc* II.

We thank Jean Maryak, Ralph Shober, and Maureen Weeks for fine technical assistance; Peter Gruss, Ray Jones, and Thomas Shih for stimulating discussions; and Lorraine Shaughnessy for careful preparation of the manuscript.

1. Bishop, J. M. (1978) *Annu. Rev. Biochem.* **47**, 37–85.
2. Frankel, A. E., Neubauer, R. L. & Fischinger, P. J. (1976) *J. Virol.* **18**, 481–490.
3. Scolnick, E. M., Howk, R. S., Anisowicz, A., Peebles, P. T., Scher, C. D. & Parks, W. P. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4650–4654.
4. Oskarsson, M., McClements, W. L., Blair, D. L., Vande Woude, G. F. & Maizel, J. V. (1980) *Science* **207**, 1222–1224.
5. Witte, O. N., Rosenberg, N. E. & Baltimore, D. (1979) *Nature (London)* **281**, 396–398.
6. Hughes, S. H., Payvan, F., Spector, D., Schimke, R. T., Robinson, H. L., Payne, G. S., Bishop, J. M. & Varmus, H. E. (1979) *Cell* **18**, 347–360.
7. Stehelin, D., Guntaka, R. V., Varmus, H. E. & Bishop, J. M. (1976) *J. Mol. Biol.* **101**, 349–365.
8. Shih, T. Y., Williams, D. R., Weeks, M. O., Maryak, J. M., Vass, W. C. & Scolnick, E. M. (1978) *J. Virol.* **27**, 45–55.
9. Chien, Y. H., Lai, M., Shih, T. Y., Verma, I. M., Scolnick, E. M., Roy-Burman, P. & Davidson, N. (1979) *J. Virol.* **31**, 752–760.
10. Ellis, R. W., DeFeo, D., Maryak, J. M., Young, H. A., Shih, T. Y., Chang, E. H., Lowy, D. R. & Scolnick, E. M. (1980) *J. Virol.* **36**, 408–420.
11. Harvey, J. J. (1964) *Nature (London)* **204**, 1104–1105.
12. Shih, T. Y., Weeks, M. O., Young, H. A. & Scolnick, E. M. (1979) *Virology* **96**, 64–79.
13. Shih, T. Y., Weeks, M. O., Young, H. A. & Scolnick, E. M. (1979) *J. Virol.* **31**, 546–556.
14. Scolnick, E. M., Papageorge, A. G. & Shih, T. Y. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5355–5359.
15. Shih, T. Y., Papageorge, A. G., Stokes, P. E. Weeks, M. O. & Scolnick, E. M. (1980) *Nature (London)* **287**, 686–691.
16. Langebeheim, H., Shih, T. Y. & Scolnick, E. M. (1980) *Virology* **106**, 292–300.
17. Young, H. A., Gonda, M. A., DeFeo, D., Ellis, R. W., Nagashima, K. & Scolnick, E. M. (1980) *Virology* **107**, 89–99.
18. Hager, G. L., Chang, E. H., Chan, H. W., Garon, C. F., Israel, M. A., Martin, M. A., Scolnick, E. M. & Lowy, D. R. (1979) *J. Virol.* **31**, 795–809.
19. Chang, E. H., Maryak, J. M., Wei, C.-M., Shih, T. Y., Shober, R., Cheung, H. L., Ellis, R. W., Hager, G. L., Scolnick, E. M. & Lowy, D. R. (1980) *J. Virol.* **35**, 76–92.
20. Hsu, T. W., Sabran, J. L., Mark, G. E., Guntaka, R. V. & Taylor, J. M. (1978) *J. Virol.* **28**, 810–818.
21. Shank, P. R., Hughes, H. S., Kung, H.-J., Majors, J. E., Quintrell, N., Guntaka, R. V., Bishop, J. M. & Varmus, H. E. (1978) *Cell* **15**, 1383–1395.
22. Gilboa, E., Goff, S., Shields, A., Yoshimura, F., Mitra, S. & Baltimore, D. (1979) *Cell* **16**, 863–874.
23. Blair, D. G., McClements, W. L., Oskarsson, M. K., Fischinger, P. J. & Vande Woude, G. F. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3504–3508.
24. Sternberg, N., Tiemeier, D. & Enquist, L. (1977) *Gene* **1**, 255–280.
25. Holm, B. & Murray, K. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3259–3263.
26. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–182.
27. Davis, R. W., Simon, M. & Davidson, N. (1971) *Methods Enzymol.* **210**, 413–428.
28. Tsuchida, N., Gilden, R. & Hatanaka, M. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 4503–4507.
29. Scolnick, E. M., Vass, W. C., Howk, R. S. & Duesberg, P. H. (1979) *J. Virol.* **29**, 964–972.
30. Van Beveren, C., Goddard, J. G., Jonas, V., Bems, A. J. M., Doolittle, R. F., Donoghue, D. J. & Verma, I. M. (1981) *Nature (London)* **289**, 258–262.
31. Dhar, R., McClements, W. L., Enquist, L. W. & Vande Woude, G. F. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3937–3941.
32. Chang, E. H., Ellis, R. W., Scolnick, E. M. & Lowy, D. R. (1980) *Science* **210**, 1249–1251.
33. Graham, F. L. & van der Eb, A. J. (1973) *Virology* **52**, 456–461.
34. McClements, W. L., Dhar, R., Blair, D. G., Enquist, L., Oskarsson, M. & Vande Woude, G. F. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45**, 760–768.
35. Collett, M. S., Brugge, J. S. & Erikson, R. L. (1978) *Cell* **15**, 1363–1369.
36. Witte, O. N., Rosenberg, N. E. & Baltimore, D. (1979) *Nature (London)* **281**, 396–398.
37. Lowy, D. R., Rands, E. & Scolnick, E. M. (1978) *J. Virol.* **26**, 291–298.