to reduce the amount of virus in HIV-1–infected individuals.

Serum concentrations of DAB_{486} IL-2 used in the experiments described here (10^{-8} M) have been safely achieved in patients with IL-2R–expressing malignancies (*16*). Binding and internalization of DAB_{486} IL-2 by high-affinity IL-2R–bearing cells occurs within 30 min of exposure, resulting in maximal protein synthesis inhibition within 4 to 6 hours and cytotoxicity within 48 to 72 hours (*24*). Therefore, despite the short serum half-life of DAB_{486} IL-2 (5 to 10 min), continuous administration of DAB_{486} IL-2 to patients should not be necessary.

Antitumor effects have been observed in refractory leukemia-lymphoma patients, and these effects have occurred despite the presence of antibodies to diphtheria toxin in some patients, and the presence of elevated soluble IL-2R levels in all patients. This observation is consistent with data obtained by ourselves and others that suggest that soluble IL-2Rs do not interfere with binding of IL-2 to the high-affinity IL-2R (*25*). Extensive immunological monitoring has been an important feature of early clinical trials, and patient data have demonstrated that DAB_{486} IL-2 has no generalized immunosuppressive properties (*15, 16*). The experimental results reported here indicate that DAB_{486} IL-2 selectively eliminates HIV-1–infected cells from mixed T cell cultures while sparing uninfected cells. In addition, DAB_{486} IL-2 inhibits production of viral proteins and infectious virus in T cells and inhibits viral RT in HIV-infected monocytes. Thus, the emergence of IL-2Rs on monocytes and lymphocytes that are replicating HIV-1 may provide the basis for early therapeutic intervention with targeted toxins in HIV infection.

Table 2. Incubation of T cell cultures with DAB_{486} IL-2–eliminated infectious virus. $CD4^+$ T cells were infected with HIV-1, washed, and then mixed with uninfected cells 1 day later, as described in Table 1. Mixtures (uninfected:infected T cells) are a ratio of 10:1. Cells were cultured in IL-2 (10^{-9} M), with and without DAB_{486} IL-2 (10^{-8} M) added on days 1 and 3. Cultures were washed and fed every 48 hours as described in Table 1. At several time points, T cells (5×10^5) were removed, washed three times, and cocultivated with 5×10^5 uninfected H9 cells. Cultures were incubated for an additional 6 days and screened for production of p24 in the supernatants. These data are representative of two experiments, each with duplicate culture wells.

T cell cultures	DAB_{486} IL-2	Days after infection	p24 (pg)
Uninfected		0	
HIV-1–infected		0	>500
Mixtures		0	>500
Mixtures	10^{-8} M	0	>500
Mixtures		1	>500
Mixtures	10^{-8} M	1	>500
Mixtures		6	>500
Mixtures	10^{-8} M	6	0
Mixtures		9	>500
Mixtures	10^{-8} M	9	0

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17. However, cultures of both DAB_{486} IL-2–treated and control cells achieved similar densities after 2 weeks incubation (*23*).
18. Functional assays that measured responses to IL-2 and phytohemagglutinin also revealed no abnormalities in the DAB_{486} IL-2–treated cell populations (*23*).
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Requirement of Heparan Sulfate for bFGF-Mediated Fibroblast Growth and Myoblast Differentiation

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Basic fibroblast growth factor (bFGF) binds to heparan sulfate proteoglycans at the cell surface and to receptors with tyrosine kinase activity. Prevention of binding between cell surface heparan sulfate and bFGF (i) substantially reduces binding of fibroblast growth factor to its cell-surface receptors, (ii) blocks the ability of bFGF to support the growth of Swiss 3T3 fibroblasts, and (iii) induces terminal differentiation of MM14 skeletal muscle cells, which is normally repressed by fibroblast growth factor. These results indicate that cell surface heparan sulfate is directly involved in bFGF cell signaling.

BASIC FGF IS A PROTOTYPE OF THE heparin-binding growth factor (HBGF) family that includes acidic fibroblast growth factor (aFGF), int-2, *hst*, FGF-5, k-FGF, and KGF, polypeptides that control cell growth and differentiation (*1*). HBGFs bind to heparan sulfate and to high-affinity protein receptors on cell surfaces (*2*). Cross-linking of iodinated bFGF to cell surfaces identifies multiple high-affinity receptors that bind the growth factor with dissociation constants in the 10^{-9} to 10^{-12} M range (*1, 3*). Among these are the *flg* and *bek* gene products, transmembrane proteins that contain cytoplasmic domains with tyrosine kinase activity (*4*). FGF binding triggers intracellular phosphorylation of several proteins, including the receptors themselves, which may lead to FGF action (*5*).

Heparan sulfate binds bFGF with a dissociation constant of 10^{-8} to 10^{-9} M and is present on proteoglycans found in the extracellular matrix or anchored in the plasma membrane (*2, 6, 7*). An example of the latter is syndecan (*8*). Syndecan derived from several cell types, including NMuMG mammary epithelial cells, Swiss 3T3 cells, or MM14 myoblasts, binds to bFGF affinity columns (*9, 10*), and expression of syndecan in lymphoblastoid cells confers FGF binding on these cells (*11*). Indirect roles for heparan sulfate in bFGF activity include extracellular stabilization and storage of FGF (*12, 13*). A direct role in bFGF activity has not been apparent, although deletions of putative heparin or heparan sulfate binding regions

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from the growth factor dramatically reduce its biological activity (*14*). Therefore, we have questioned whether a direct role exists for cell-surface heparan sulfate in the action of bFGF (*10*).

Swiss 3T3 fibroblasts proliferate in response to FGF (*15*). We used two methods to assess the role of heparan sulfate in this response: (i) treatment of the cells with heparitinase (*16*) or (ii) culture in sodium chlorate to block sulfation (*17*). Iodinated bFGF binding sites were distinguished as heparan sulfate (Fig. 1A) or protein receptors (Fig. 1B) by salt washes at neutral or low pH, respectively (*2, 18*). As expected, treatment with either heparitinase or chlorate reduced the specific binding of bFGF to heparan sulfate by more than 80% (Fig. 1A). Maximal reduction in binding occurred at 30 mM; however, simultaneous addition of 10 mM sulfate and 30 mM chlorate, which allows sulfation to occur (*17*), permitted bFGF binding. Diminished iodinated FGF binding closely parallels the reduced sulfation of proteoglycans and is specific for heparan sulfate, as enzymatic removal of chondroitin sulfate is without effect (*9*).

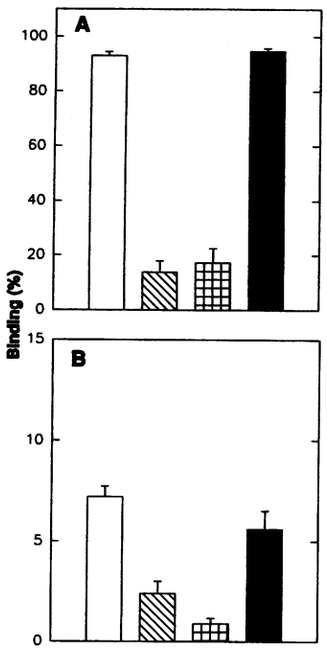

Fig. 1. Binding of iodinated bFGF to heparan sulfate and cell surface receptors of Swiss 3T3 cells. Cells were cultured in 30 mM chlorate and 0.1% FBS (*17*), then were trypsinized and replated in no chlorate (open bars), 30 mM chlorate (crosshatched bars), or 30 mM chlorate + 10 mM sulfate (solid bars). After an additional 24 hours, heparitinase (diagonal lines; 1 mIU/ml for 2 hours) (*16*) was added to the medium of selected wells in no chlorate, and then cells were chilled to 4°C to measure binding of iodinated bFGF to heparan sulfate (**A**) or cell surface receptors (**B**) (*18*). Nonspecific binding in the presence of 1 μM cold ligand was about 15% of the total and has been subtracted. Nonspecific binding was not altered by heparitinase or chlorate treatments. (n = 3, ±SEM)

Surprisingly, specific binding of bFGF to the protein receptors parallels binding to heparan sulfate. Heparitinase or chlorate treatments reduce binding to these sites by 66% or 88%, respectively (Fig. 1B). In other experiments, heparitinase treatment reduced receptor binding by as much as 90%. Cross-linking of iodinated bFGF to cell surfaces identified protein receptors of 140 to 160 kD (Fig. 2). Competition with a 100-fold excess of cold bFGF abolished the binding of labeled ligand (*19*). Both chlorate and heparitinase treatments substantially reduced binding. Furthermore, binding in cells treated with chlorate was restored by low concentrations of exogenous heparin, demonstrating that the receptors are available at the cell surface, but are unable to bind bFGF unless heparan sulfate (or heparin) is present.

To directly question the importance of heparan sulfate–mediated binding to the action of bFGF, we examined the growth of Swiss 3T3 fibroblasts. The mitogenic response to 10 pM bFGF was inhibited by more than 90% if the cells were cultured in 30 mM chlorate, but it was restored by the addition of 10 mM sulfate (Fig. 3A). Chlorate at 10 mM was less effective, consistent with its lesser effect on bFGF binding to heparan sulfate. We examined concentrations of bFGF up to 500 pM and found that chlorate inhibits bFGF-induced proliferation by more than 80%. Chlorate specifically inhibits bFGF action, as growth in 100 to 400 pM epidermal growth factor (EGF) (Fig. 3A) or 100 to 400 pM platelet-derived growth factor (*19*) was not affected. Furthermore, culturing cells simultaneously in 10 pM bFGF and 5 mIU heparitinase reduced ^{3}H-labeled thymidine incorporation by 61% (*19*). Lastly, the chlorate blockage of bFGF-mediated growth could be overcome by exogenous heparin at concentrations as low as 1 ng/ml. This restoration not only demonstrates the ability of the cells to grow normally in chlorate, but also confirms the requirement of heparan sulfate or heparin for the action of bFGF.

The requirement for heparan sulfate also extends to the bFGF-mediated repression of MM14 skeletal muscle cell differentiation. FGF deprivation results in the expression of skeletal muscle genes and fusion into myotubes (*20*). The cells also withdraw from the cell cycle, although this alone is not sufficient to induce differentiation (*20*). Blockage of FGF signaling can therefore be detected by two independent assays: (i) examining bromodeoxyuridine (BrdU) incorporation into DNA to assess exit from the cell cycle and (ii) staining for myosin heavy chain to assess the activation of muscle-specific genes.

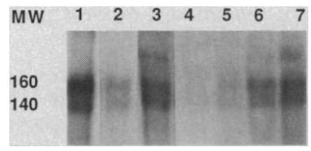

Fig. 2. Cross-linking of iodinated bFGF to cell surface receptors. Swiss 3T3 cells were subjected to DSS cross-linking after incubation with 300 pM iodinated bFGF, as in Fig. 1, then extracted for PAGE (*27*). Lane 1, nontreated; lane 2, heparitinase; lane 3, 30 mM chlorate + 10 mM sulfate; lanes 4 to 7, 30 mM chlorate; lane 5, chlorate + heparin (1 ng/ml); lane 6, chlorate + heparin (10 ng/ml); lane 7, chlorate + heparin (100 ng/ml).

Treatment of MM14 cultures with chlorate reduces [^{3}H]thymidine incorporation by 95% at concentrations of bFGF that promote maximal growth, and supplementing the sulfate concentration to 10 mM nullifies this inhibition (*21*). As occurs with the 3T3 cells, up to 90% of the specific binding of iodinated bFGF to cell surface receptors is abolished by treatment with either the sulfation inhibitor or heparitinase (*21*). These effects on proliferation were visualized directly by fluorescent staining of BrdU incorporation into newly synthesized DNA (Fig. 4A). In addition, staining for myosin heavy chain in companion cultures showed that proliferating myoblasts remained as single, myosin-negative cells (Fig. 4E). However, if the cells were cultured with bFGF in the presence of bFGF neutralizing antibodies (*22*), over 95% of the cells failed to incorporate BrdU, stained for muscle-specific myosin, and fused to form myo-

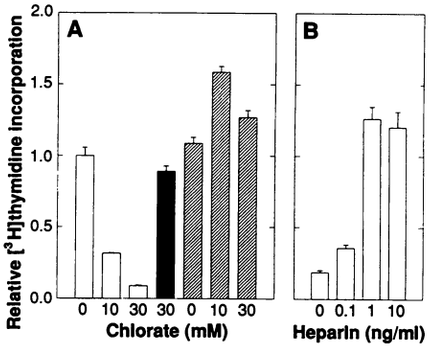

Fig. 3. Growth of Swiss 3T3 fibroblasts in chlorate. (**A**) Chlorate blocks response to bFGF but not EGF. Cultures were chlorate-treated and serum-starved (*17*). We added bFGF (10 pM) (open bars), EGF (400 pM) (diagonal lines), or bFGF + sulfate (solid bar) for 18 hours, then measured [^{3}H]thymidine incorporation into newly synthesized DNA during a 4-hour pulse label (*28*). Relative incorporation is compared to cells cultured in bFGF without chlorate. (**B**) Heparin restores the mitogenic activity of bFGF. Heparin was added with 10 pM bFGF to serum-starved cultures treated with 30 mM chlorate. (n = 4, ±SEM)

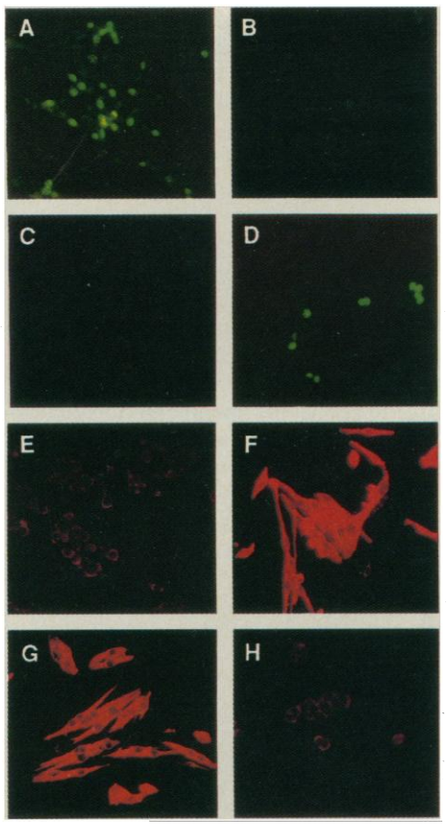

Fig. 4. Differentiation of MM14 myoblasts in chlorate. Myoblasts were plated in medium containing 150 pM bFGF. The cells were nontreated (**A** and **E**), or received a 1:1000 dilution of bFGF-neutralizing antibody (**B** and **F**), or were cultured in 30 mM chlorate (**C** and **G**) or 30 mM chlorate + 10 mM sulfate (**D** and **H**). BrdU (10 μM) was added for 1 hour, 35 hours after plating; then the cells were fixed in methanol and acid-treated for staining with fluorescein isothiocyanate–conjugated antibody to BrdU (A through D) (*29*) or fixed in 4% paraformaldehyde and permeabilized with 0.5% Triton to stain with antibody to myosin heavy chain and Texas red–conjugated second antibody (E through H). Other cultures were deprived of bFGF and contained myosin-producing myotubes at the time of fixation (not shown).

tubes (Fig. 4, B and F). This differentiation program was similarly initiated by culture in 30 mM chlorate, despite the continued presence of bFGF (Fig. 4, C and G). Simultaneous addition of chlorate and 10 mM sulfate restored bFGF signaling and prevented differentiation (Fig. 4, D and H).

These data demonstrate that cell surface heparan sulfate is critical for the action of bFGF on fibroblasts and myoblasts. Similar results have been obtained with aFGF or k-FGF on MM14 myoblasts (*21*). The effect of chlorate appears specific for the proteoglycan-mediated growth mechanism, as the Swiss 3T3 cells retain the ability to grow in serum and other growth factors, despite the chlorate treatment. Indeed, the MM14 myoblasts respond to chlorate not simply by ceasing proliferation but by embarking on an intricate differentiation program. The chlorate concentrations that reduce FGF activity correspond closely to the amounts required to block bFGF binding through effects on proteoglycan sulfation (*9, 10*). In the MM14 cells, radiosulfate incorporation into proteoglycans in 20 mM chlorate is reduced by 89% (*21*).

The primary consequence of reducing the sulfation of heparan sulfate is that it abolishes a binding site required for bFGF activity. Although heparan sulfate stores FGF in the extracellular matrix (*12*), a more direct mechanism appears necessary, namely, its participation in the binding of FGF to its receptor. This binding requirement is supported by a report (*23*) showing that FGF receptors expressed in heparan sulfate–deficient CHO cells bind FGF only if exogenous heparin is added. Our data suggest, therefore, that the intrinsic affinity of the cell surface receptor for bFGF appears to be low, whereas the heparan sulfate–FGF complex is recognized with high affinity. FGF may undergo a change in conformation when bound to heparan sulfate. Alternatively, a direct interaction between the receptor and cell surface proteoglycans may also be required. Although details of the mechanism remain to be worked out, it is clear that heparan sulfate is required for biological activity of the growth factor. The growth and differentiation of cells in vivo may be regulated not only by the expression of FGF and its receptor but also by the temporal and spatial expression of heparan sulfate proteoglycans.

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16. Heparitinase E.C. 4.2.2.8 [heparinase III (*24*)] from ICN Biochemicals recognizes sites common in heparan sulfate but rare in heparin.
17. Chlorate ion is a competitive inhibitor of sulfation because it competes for sulfate recognition by ATP-sulfurylase (*25*). Thus, the effects of the inhibitor can be enhanced by reduction of sulfate concentrations in the medium or negated by raising of sulfate concentrations to 10 mM. Ionic concentration was maintained at 0.15 M by adjustment of sodium chloride concentrations. Because Swiss 3T3 cells can use cysteine as a source of sulfate by means of sulfur oxidation (*26*), cysteine concentration was also diminished to 50 μM. We cultured the cells in chlorate for 48 hours, then trypsinized to remove residual sulfated proteoglycans. No differences were noted in DNA content or gross morphology of cells cultured in either the presence or the absence of chlorate for several days. Quiescent cells were generated by culture for 24 hours in 30 mM chlorate and 10% fetal bovine serum (FBS); then the FBS was reduced to 0.1% in chlorate for an additional 24 hours. The cells were then trypsinized and replated in media with or without chlorate and other treatments. Unlike the Swiss 3T3 fibroblasts, 30 mM chlorate is effective on the MM14 myoblasts in normal sulfate concentrations (0.8 mM sulfate).
18. Human recombinant bFGF was iodinated by the chloramine T method (*3*) to a specific activity of 1 to 2 Ci/μM, determined by comparison with unlabeled bFGF in MM14 myoblast growth assays (*19*). Cells were incubated with 300 pM iodinated bFGF at 4°C in a solution of Dulbecco's minimum essential medium, 0.1% bovine serum albumin, and 10 mM Hepes (pH 7.4), then were washed three times in phosphate-buffered saline. In selected experiments, porcine mucosal heparin was included with the iodinated ligand in concentrations ranging from 0.1 to 100 ng/ml. FGF bound to heparan sulfate was removed by two washes with 2 M NaCl containing 10 mM Hepes (pH 7.4), then two washes with 2 M NaCl containing 10 mM sodium acetate (pH 4.0) to remove receptor-bound ligand (*2*). Alternatively, bound ligand was cross-linked to cell surface receptors by incubation for 30 min at 4°C in 250 μM disuccinimidylsuberate (DSS), and the cells were extracted for polyacrylamide gel electrophoresis (PAGE) analysis (*27*).
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30. We thank S. Gandy and H. Monis for expert assistance in these experiments, C. Hart of Zymogen for mouse IgG monoclonal antibodies to bFGF, and C. Basilico, New York University School of Medicine for k-FGF. Supported by NIH grants HD21881 to A.C.R. and AR39467 to B.B.O. B.B.O. is the recipient of PEW and SHAW scholarships. A.K. is supported by NIH training grant 5T32H007118.

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Identification of p53 as a Sequence-Specific DNA-Binding Protein

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The tumor-suppressor gene p53 is altered by missense mutation in numerous human malignancies. However, the biochemical properties of p53 and the effect of mutation on these properties are unclear. A human DNA sequence was identified that binds specifically to wild-type human p53 protein in vitro. As few as 33 base pairs were sufficient to confer specific binding. Certain guanines within this 33–base pair region were critical, as methylation of these guanines or their substitution with thymine abrogated binding. Human p53 proteins containing either of two missense mutations commonly found in human tumors were unable to bind significantly to this sequence. These data suggest that a function of p53 may be mediated by its ability to bind to specific DNA sequences in the human genome, and that this activity is altered by mutations that occur in human tumors.

THE GENE FOR THE NUCLEAR PHOSphoprotein p53 is the most commonly mutated gene yet identified in human cancers (*1*); missense mutations occur in tumors of the colon, lung, breast, ovary, bladder, and several other organs (*2, 3*). One of the important challenges of current cancer research is the elucidation of the biochemical properties of the p53 gene product and the way in which mutations of the p53 gene affect these properties. Although some biological characteristics of p53 have been defined, such as its ability to suppress the growth of in vitro–transformed murine cells (*4*) or human cancer cells (*5*), the biochemical basis of this suppression remains unknown.

As a step toward understanding such properties, we have attempted to determine whether p53 binds to specific DNA sequences within the human genome. Two previous lines of evidence stimulated these studies. First, p53 can provide a transcriptional activation function when fused to a DNA-binding polypeptide such as GAL4 (*6*). Second, p53 can bind nonspecifically to DNA (*7*), and such nonspecific binding was altered in each of 15 human tumor-derived or murine-transforming forms of mutant p53 tested (*8, 9*). Because many proteins with a specific DNA-binding function also bind nonspecifically to DNA (*10*), we have suggested that a sequence-specific binding ability of p53 might exist and be a functional target of p53 mutations (*9*).

To identify a sequence-specific binding site, cloned DNA sequences were screened by means of an immunoprecipitation technique (*11, 12*). Two classes of clones were tested. The first consisted of 400 clones containing inserts of 300 to 1000 bp obtained randomly from the human genome. The second class consisted of cosmid and plasmid clones chosen because they contained sequences that might be important in normal growth control (*12*). Each clone was digested with an appropriate restriction endonuclease, end-labeled with ^{32}P, and incubated with p53 from a lysate of cells infected with a recombinant vaccinia virus expressing p53 (*11*). Labeled DNA fragments that bound to p53 were then recovered by immunoprecipitation with monoclonal antibodies against p53 (anti-p53). Of the more than 1400 restriction fragments tested, only two, both from the second class of clones, bound reproducibly to p53 under the experimental conditions used: a 259-bp Hinf I fragment (fragment A) of clone 772 C_{BE} (Fig. 1A, panel 2), and a 190-bp Hinf I fragment (fragment B) of clone λ 5R (Fig. 1A, panel 3); these fragments bound to a far greater extent (at least tenfold more) than any of the other labeled fragments of larger or smaller size present in the same assay mixes.

Subsequent efforts were concentrated on fragment A. First, it was demonstrated that detection of the binding of fragment A was dependent on both p53 protein and anti-p53. Lysates from cells infected with wild-type vaccinia virus (devoid of p53) were not able to specifically immunoprecipitate fragment A (Fig. 1B). Similarly, the precipitation of fragment A was dependent on the presence of anti-p53 (Fig. 1B). The binding was evident in lysates prepared from either human HeLa cells or monkey BSC40 cells

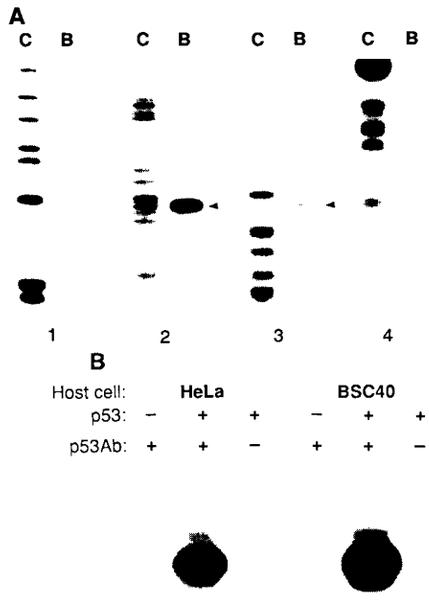

Fig. 1. (**A**) Screening for fragments bound by p53 by an immunoprecipitation assay (*11*). Clones (*12*) were cleaved by a restriction endonuclease, end-labeled, and incubated with lysates of vaccinia virus–infected cells synthesizing wild-type p53. After immunoprecipitation with anti-p53 (*17*), bound DNA fragments were recovered and separated on a nondenaturing polyacrylamide gel. Panel 1 contains the hFosAva2 clone; panel 2, 772 C_{BE}; panel 3, λ 5R; panel 4, a pool of clones with inserts of randomly cloned human genomic sequences. The 772 C_{BE} and λ 5R contain Hinf I fragments (259 and 190 bp, respectively) that bound p53 relatively strongly (arrowheads). C, control lane, containing 2% of the labeled DNA used in the binding reactions. B, bound DNA recovered from the immunoprecipitate. (**B**) Tests for dependence on p53 and specific antibody. Cell lysates were produced by infection with vaccinia virus that did (+) or did not (−) contain an insert of wild-type p53 cDNA. Immunoprecipitation was performed with anti-p53 (+) or normal mouse immunoglobulin G (−).

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