Molecular Cloning and In Vitro Expression of a cDNA Clone for Human Cellular Tumor Antigen p53

ED HARLOW,1* NICOLA M. WILLIAMSON,1 ROBERT RALSTON,2 DAVID M. HELFMAN,1 AND TIMOTHY E. ADAMS1

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724;1 and Hooper Research Foundation and Department of Microbiology and Immunology, School of Medicine, University of California, San Francisco, California 94143

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Three clones for the human tumor antigen p53 were isolated from a cDNA library prepared from A431 cells. One of these clones, pR4-2, contains the entire coding region for human p53. This clone directs the synthesis of a polypeptide with the correct molecular weight and immunological epitopes of an authentic p53 molecule in an in vitro transcription-translation reaction. Although the pR4-2 clone contains the coding region for p53, it is not a full-length copy of the human p53 mRNA. Northern analysis showed that the p53 mRNA is approximately 2,500 nucleotides long, whereas the pR4-2 insert is only 1,760 base pairs in length. Analysis of the DNA sequence of this clone suggests that the human p53 polypeptide has 393 amino acids. We compared the predicted amino acid sequence of the pR4-2 clone with similar clones for the mouse p53 and found long regions of amino acid homology between these two molecules.

A number of studies have shown that primary rat cells can be transformed in vitro by cotransfecting an activated ras gene with any of a series of cellular or viral genes (28, 50). Transformed foci have been observed after the cotransfection of an activated ras gene with the gene for the cellular myc protein, polyoma large-T antigen, adenovirus E1A proteins, or MC29 gag-myc fusion protein. Recent studies by three groups demonstrated that cotransfection of the gene that encodes the mouse cellular tumor antigen p53 with an activated human ras gene also yields transformed foci (12, 21, 42). Results of earlier work had suggested that p53 might play a role in some types of transformation. These suggestions were based not only on comparison of the biochemistry of p53 in normal and transformed cells but also on studies of the immune response of animals to some types of tumors. This work has shown that (i) sera from laboratory animals with tumors or from human patients with some types of neoplasia often contain circulating antibodies specific for p53 (9, 11, 29, 32, 49); (ii) transformed cells often have higher levels of p53 than their normal cell counterparts (3, 10, 11, 20, 48, 53); (iii) in cells transformed by simian virus 40 (SV40) or adenovirus, p53 is found in a stable, high-molecular-weight complex with either the SV40-coded large-T antigen or the adenovirus-coded E1B 57-kilodalton protein (29, 32, 35, 52); (iv) p53 appears to play an important role in the movement of quiescent cells into the S phase after serum stimulation (37, 38); and (v) the synthesis of p53 is temporally regulated after stimulation of cells with mitogens (40, 46). These studies suggest that p53 may play an important role in the regulation of cell division in some cell types, but how p53 may be involved in these processes is not known.

We present here the isolation, characterization, and nucleic acid sequence of a p53 cDNA clone from human A431 cells that has the coding potential for a full-length p53.

MATERIALS AND METHODS

Cells and antibodies. All cells were grown in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum. A431, ME5, and SV40 cells were the kind gifts of D. Gospodorowicz, A. DeLeo, and W. Topp, respectively. The HeLa and 293 cells were grown and provided by B. Ahrens. PAb122 (14) and PAb421 (17) are monoclonal antibodies specific for p53, PAb416(17) is specific for SV40 large-T antigen, and M73 is specific for the adenovirus E1A proteins. All of these antibodies have been described and characterized previously (see reference 8 for the correct nomenclature of the PAb series) except for M73, which is a recent isolate (E. Harlow and C. Schley, submitted for publication).

Metabolic labeling and immunoprecipitations. Semi confluent cultures of cells were prepared for labeling by aspirating the medium and washing the monolayers once with Dulbecco modified Eagle medium without methionine. The cells were then labeled with [35S]methionine (>1,000 Ci/mmol, 0.5 mCi per 100-mm dish; Amersham Corp.) for 3 h in 2.0 ml of Dulbecco modified Eagle medium without methionine. The monolayers were washed once with phosphate-buffered saline (150 mM NaCl, 10 mM sodium phosphate [pH 7.2]), and 1.0 ml of cold Nonidet P-40 lysis buffer (1.0% Nonidet P-40, 50 mM Tris [pH 8.0]) was added. The lysates were incubated for 30 min on ice and then transferred to a 1.5-ml microfuge tube. The nuclei and cellular debris were removed by centrifugation for 2 min, and the supernatant was precleared by treatment with 50 µl of fixed Staphylococcus aureus Cowan I cells (23). S. aureus Cowan I cells were prepared by being washed once in NET-GEL (150 mM NaCl, 5 mM EDTA, 0.05% Nonidet P-40, 0.02% NaN3, 0.25% gelatin, 50 mM Tris [pH 7.5]). The S. aureus Cowan I pellet was suspended in the lysate and then incubated for a further 30 min on ice. The S. aureus Cowan I cells were removed by centrifugation for 10 min at 4°C. The supernatant was carefully removed and divided into portions

* Corresponding author.
for immunoprecipitation. A 50-μl sample of tissue culture supernatant from the appropriate hybridoma culture was added to each tube, and the reaction was kept on ice for 1 h. A 100-μl sample of a 3% solution (dry wt/vol) of protein A-Sepharose (Pharmacia Fine Chemicals) in NET-GEL was added to each tube. The tubes were then rocked at 4°C for 1 h. The beads were collected by centrifugation and washed three times in NET-GEL. After the final wash they were suspended in 50 μl of sample buffer (2% sodium dodecyl sulfate [SDS], 10% glycerol, 100 mM dithiothreitol, 20 mM Tris [pH 6.8]) and then heated to 90°C for 10 min. The polypeptides were then separated on 10% polyacrylamide gels (27). The gels were prepared for fluorography as described by Bonner and Laskey (5).

Isolation of mRNA. Total cellular RNA was isolated by disrupting cells in guanidinium lysis buffer (4 M guanidinium thiocyanate, 2.0% Sarkosyl, 140 mM 2-mercaptoethanol, 10 mM EDTA, 50 mM Tris [pH 7.5]) and then centrifuging the RNA through a CsCl cushion as described by Chirgwin et al. (6). Polyadenylated [poly(A)]+ RNA was then prepared by selection on an oligodeoxynucleotide cellulose column (Collaborative Research, Inc.) column (34).

Construction and screening of A431 cDNA library. Poly(A)+ mRNA from A431 cells was used as a template for the construction of a cDNA library. The library was prepared as described by Helfman et al. (19). Briefly, the first strand was synthesized by reverse transcriptase on an mRNA template primed with oligodeoxynucleotide. The mRNA was removed, and the second strand was synthesized by the Klenow fragment of *Echerichia coli* DNA polymerase with the 3’ OH of the first strand as an initiation site after the formation of a hairpin. The cDNA was blunted, and then EcoRI synthetic linkers were ligated to the 3’ end of the cDNA. The hairpin was removed with S1 nuclease, and Sall linkers were ligated to the cDNA. The cDNAs were then digested with EcoRI and Sall restriction endonucleases and ligated into either EcoRI- and Sall-cut pUC8 or pUC9 plasmids (39). The resultant DNA molecules were introduced into the DH5 strain of *E. coli* by transformation as described by Hanahan (15). Ampicillin-resistant colonies were screened for the presence of p53 sequences by the high-density screening methods of Hanahan and Meselson (16). The colonies were hybridized under the conditions described by Benchimol et al. (2) with nick-translated probe from the mouse p53 cDNA clone 9 (2).

DNA sequencing. The DNA sequence of the pR4-2 insert was determined by the dideoxy sequencing method of Sanger et al. (51) as described by Bankier and Barrell (1). Restriction enzyme fragments of the pR4-2 insert were subcloned by blunt-end ligation to *Smal*-cut and phosphatase-treated M13mp8 RFI DNA (the kind gift of W. Herr). Representative clones were grown, single-stranded DNA was purified, and these DNAs were used as templates for the dideoxy sequencing reactions. The sequencing reactions were labeled with [35S]dATP (New England Nuclear Corp.), and the products were run on either the gradient polyacrylamide gel system of Biggin et al. (4) or standard 6% polyacrylamide sequencing gels. Three oligonucleotide primers were prepared on an Applied Biosystems DNA synthesizer and were used to complete the sequencing of the p53 insert.

Southern and Northern hybridizations. Appropriate DNA or RNA samples were subjected to electrophoresis in agarose gels as described by Maniatis et al. (34). The nucleic acids were transferred to nitrocellulose as suggested by the nitrocellulose manufacturer, Schleicher and Schuell, Inc. Radioactive probe was prepared by nick translation as described previously (34).

**In vitro transcription and translation.** The pR4-2 insert from *SalI* to EcoRI was subcloned into plasmid pSP64 (Promega Biotech). RNA with the same sense as the human p53 mRNA was synthesized by SP6 RNA polymerase (26, 36). This RNA was then added to a rabbit reticulocyte translation reaction (44), and the protein products were analyzed.

**RESULTS**

Characterization of p53 polypeptides in A431 cells. The human A431 cell line (13), derived from an epidermoid carcinoma of the vulva, was chosen as a convenient source for the preparation of a human cDNA library. The A431 cell is a well-characterized line which has been used to study the interaction of epidermal growth factor with its receptor. Figure 1 shows the immunoprecipitation of p53 from A431 cells with monoclonal antibodies PAb122 and PAb421 (14, 17). These antibodies were raised against mouse p53, but previous experiments have shown that they bind p53 from a number of mammalian species, including humans (10, 17, 53). As described by Crawford (7), the PAb421 antibody also immunoprecipitates a series of polypeptides in the 40- to 60-kilodalton range from epithelial cells. These proteins have been identified as members of the keratin family, and their presence suggests that A431 cells were derived from an epithelial parental cell. In general, other monoclonal antibodies specific for p53 do not precipitate the keratins, but it appears that the PAb122 antibody also recognizes one of these polypeptides. This band is not always found in im-

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**FIG. 1.** Immunoprecipitation of p53 proteins from A431 cells. A431 and SV80 cells at similar stages of confluency were metabolically labeled with [35S]methionine, and extracts of these cells were prepared for immunoprecipitation. Monoclonal antibodies specific for the adenovirus EIA proteins (M73), SV40 large-T antigen (PAb416), or p53 (PAb122 and PAb421) were added to samples of the A431 or SV80 extracts. The immune complexes were collected on protein A-Sepharose, washed, and separated on SDS-polyacrylamide gels.
mune precipitations from A431 cells with PAb122, and we assume that the affinity of this monoclonal antibody for this polypeptide is lower than that of PAb421. Similar types of cross-reactions have been reported for a number of monoclonal antibodies, and these reactions have been reviewed by Lane and Koprowski (30).

Previous studies have shown that some human cell lines synthesize p53 species of the two distinct molecular weights (10, 53). Either or both of these bands may be seen in immunoprecipitations from a particular human cell line, and the pattern of the bands is characteristic of an individual cell line. A431 cells synthesize only one of the p53 polypeptides (Fig. 1). An example of the p53 human doublet is also shown in Fig. 1. When the PAb122 and PAb421 antibodies are used to immunoprecipitate p53 from an SV40-transformed fibroblast cell, SV80, the p53 species can be resolved into two distinct forms. The p53 polypeptide from A431 cells comigrates with the higher-molecular-weight band of the human doublet. Immune complexes between the PAb122 and PAb421 antibodies and polypeptides from SV80 cells also contain SV40 large-T antigen. These antibodies do not bind to large-T antigen directly, but they precipitate large-T antigen because it forms a complex with p53 (10, 53).

Isolation and characterization of a cDNA clone for human p53. Approximately 500,000 colonies of a cDNA library prepared from A431 poly(A)⁺ mRNA were screened for the presence of p53 sequences by hybridization at low stringency with a mouse p53 cDNA probe (2). The conditions used for hybridization were those described by Benichou et al. (2). Three colonies which carried p53-related sequences were isolated, and plasmids were purified from these bacteria for further analysis. These cDNA clones were designated pR1-2, pR4-2, and pR9-2 and contained inserts of approximately 800, 1,700, and 1,200 base pairs, respectively (Fig. 2). Figure 2 also demonstrates cross-hybridization of the mouse cDNA probe to plasmid sequences under the conditions of low stringency used here. The pR4-2 insert was chosen for further analysis. The locations of a number of restriction enzyme sites in the pR4-2 sequence were determined (Fig. 3). Figure 3 also shows the strategy used to determine the DNA sequence of pR4-2. The nucleotide sequence of the pR4-2 insert was determined by the dideoxy sequencing method, and the DNA sequence is shown in Fig. 4. This insert has 1,760 base pairs bounded by SalI and EcoRI restriction sites. In the directional cloning scheme that was used to synthesize this cDNA, SalI and EcoRI linkers were used to prepare the cDNA ends for ligation into the vector (see above for a description).

The pR4-2 insert has one large open reading frame which would code for 393 amino acids beginning at the methionine codon at nucleotide 215 and terminating at the stop codon TGA at nucleotide 1394. The predicted amino acid sequence of this open reading frame is shown in Fig. 4. The predicted molecular weight of the polypeptide coded by pR4-2 is approximately 43,500. This is less than the apparent molecular weight of the corresponding human doublet determined by immunoprecipitation.
FIG. 4. DNA sequence and predicted amino acid sequence of the cDNA insert from clone pR4-2. The DNA sequence of the 1.7-kilobase insert of pR4-2 was determined by the dye-terminator sequencing method. The sequence was determined for both strands of the pR4-2 insert from at least two independent clones with the orientations shown in Fig. 3B. The subclones shown in Fig. 3B allowed the double-strand sequencing of all the pR4-2 insert except for three short regions where it was difficult to complete the determination on both strands. These final regions were sequenced by using synthetic oligonucleotide primers. In addition, the predicted amino acid sequence of the open reading frame is shown in the single-letter amino acid code.
lar weight of human p53 deduced from SDS-polyacrylamide gel electrophoresis. Although the reason for these differences is unknown at present, both the mouse and the human p53 species are rich in proline residues. Other workers have speculated that the abundance of proline residues may impart an unusually extended and rigid structure to the p53 polypeptides and that this structure may cause them to migrate slowly during electrophoresis (54).

Although the pR4-2 clone appears to contain the entire coding region for the human p53 (see below), it is not a full-length copy of the p53 mRNA. The cDNA clone does not have a polyadenylate [poly(A)] tract at its 3' terminus, nor does it have a 3' poly(A) addition signal. Hybridization of Northern blots of poly(A)^+ mRNA from A431 and other human cell lines with a probe prepared from the pR4-2 plasmid has shown that the p53 mRNA is approximately 2,500 nucleotides in length (Fig. 5). Taken together, these data suggest that the pR4-2 clone is missing approximately 750 nucleotides of noncoding sequences.

Two observations confirm that the pR4-2 clone is an authentic p53 clone. To ensure that the pR4-2 clone contained the entire coding region of p53, the protein products from an in vitro transcription and translation reaction (26, 36) were checked for the presence of the p53 polypeptide. The pR4-2 insert was subcloned behind the SP6 promoter in plasmid pSP64 (Fig. 6A). The resultant plasmid contains the SP6 promoter upstream of the pR4-2 open reading frame. This plasmid was linearized, and RNA which should contain the coding region of the human p53 was synthesized by SP6 RNA polymerase. This RNA was translated in a rabbit reticulocyte lysate, and the protein products were either run directly on SDS-polyacrylamide gels or immunoprecipitated first with monoclonal antibodies specific for p53. These results are shown in Fig. 6B. The RNA synthesized in vitro codes for a polypeptide with the proper molecular weight and immunological epitopes of an authentic p53 protein. In separate experiments, we showed that the p53 polypeptide synthesized in these reactions can also be immunoprecipitated with PAb421 (data not shown). Interestingly, although the p53 synthesized in the in vitro reactions has p53-specific epitopes, the anti-p53 monoclonal antibodies do not appear to immunoprecipitate all of the p53 polypeptides produced in these lysates. We assume that some of the in vitro products either do not display the correct epitope to allow antibody binding or undergo a posttranslational modification that inhibits the binding. Whether these in vitro observations have any functional significance is not known at present. Figure 6B also shows the immunoprecipitation with PAb122 of a smaller polypeptide with a molecular weight of approximately 46,000. A number of previous studies showed that uncapped mRNA initiates at internal methionine residues when translated in a rabbit reticulocyte lysate (for example, see Paucha et al. [43]). Because this polypeptide migrates with the same mobility as an in vitro transcription-translation product from a plasmid containing the SP6 promoter and p53 sequences which delete the methionine at nucleotide 215 (data not shown), we assume that this polypeptide arises from an internal initiation event at the second in-frame methionine at nucleotide 332. As can be seen from comparisons of the unprecipitated tracks, a number of putative internally initiated products can be found in these reactions. However, only the 53,000- and 46,000-molecular-weight species can be precipitated with PAb122 and PAb421.

We have compared the predicted amino acid sequence of the longest open reading frame in pR4-2 with the amino acid sequence predicted from similar clones of the mouse p53 (22, 45, 54) (Fig. 7). These comparisons show that the mouse and human p53 polypeptides have long regions of homology, particularly in the amino-terminal 10% and the carboxy-terminal 75%. The presence of these stretches of homology further supports the authenticity of the pR4-2 clone.

**DISCUSSION**

Results from a number of laboratories have led investigators to speculate on a possible role for p53 in the regulation of the cell cycle. These speculations have been based on findings in three areas. First, the synthesis of p53 increases after the stimulation of resting cells with mitogens (40, 46). This was shown both in mouse 3T3 cells stimulated with fetal calf serum and in mouse splenocytes stimulated with concanavalin A. Mercer et al. (37, 38) extended these studies to show that in the 3T3 system, stimulated cells can be blocked from entering the S phase by microinjecting monoclonal antibodies specific for mouse p53. Second, a number of studies showed that changes in the synthesis, phosphorylation, and level of p53 are often coincident with the loss of cell cycle regulation characteristic of transformation (10, 11, 20, 41, 47, 48). Third, cotransfection studies showed that p53 can be grouped with a set of oncogenes whose protein products appear to be involved in the loss of cell cycle control found in established cell lines (12, 21, 28, 42, 50). Although these observations provide a framework for speculation on the function of p53, they do not explain the biochemical mechanisms by which p53 may act. One approach to initiating these studies is to understand in more detail the biochemical properties of purified p53 protein. At present, no convenient source has been identified that allows the ready purification of p53. To circumvent this problem we have begun studies designed to overproduce p53 in mam-

![Fig. 5. Northern hybridizations of human mRNA. Poly(A)^+ mRNA was separated on a 1.2% formaldehyde-agarose gel, transferred to nitrocellulose, and hybridized with a human p53 cDNA probe. The Rhôs analysis shown is from the epidermoid carcinoma cell line A431, the adenovirus-transformed embryonic cell line 293, the cervical carcinoma cell line HeLa, and a secondary cell culture of epidermal cells from a patient (M.E.) with hereditary adenomatosis of the colon and rectum.](http://mcb.asm.org/Downloaded-from/mcb.asm.org)
malian recombinant DNA vector systems. We present here the isolation and characterization of a cDNA clone prepared from the human cell line A431, which contains the entire coding region of human p53.

Clone pR4-2 was one of three human cDNA clones isolated containing p53 sequences, and it contains all of the nucleic acid sequence necessary to encode a full-length p53 polypeptide. Analysis of the sequence of this clone has shown one unusual feature. Early in the studies presented here, we attempted to synthesize human p53 under the control of a heterologous promoter by cloning the insert from pR4-2 into a number of mammalian expression vectors. Although the vector constructions appeared to be correct, we were never able to demonstrate the synthesis of the human p53 protein (data not shown). Analysis of the DNA sequence of pR4-2 may explain these observations. Within the 5' untranslated region at nucleotides 62 to 80 lies a stretch of 18 adenine residues. At 24 nucleotides upstream of this oligoadenylate sequence is a consensus poly(A) addition signal. Any transcript initiating upstream of this site would be expected to be processed to remove the p53 coding region. The presence of the adenine residues downstream of the poly(A) addition signal also suggests that the first 80 nucleotides of the pR4-2 insert may be a second cDNA segment that was ligated in a tail-to-head arrangement to the 5' end of the authentic p53 cDNA during the cloning procedure. Alternatively, if the 5' untranslated region is a faithful copy of the p53 mRNA, these sequences may be involved in some type of posttranscriptional regulation. We currently favor the possibility that these sequences represent a cloning artifact, and we are attempting to isolate other clones from this region to confirm this possibility.

We have compared the sequence of the pR4-2 insert with the cDNA sequence of a second human clone isolated by Matlashewski et al. (33; kindly supplied before publication). These workers isolated a partial cDNA clone from an SV40-transformed human fibroblast library. This clone begins at nucleotide 514 in the sequence described here, and the two cDNA sequences are colinear with the exception of the two nucleotides at positions 1032 and 1712. The difference at 1032 (adenine versus guanine) would change a histidine to an arginine in the predicted amino acid sequence. The second difference (adenine versus guanine) is in the 3' untranslated region. We believe that these differences most probably reflect authentic differences in the DNA sequence of the two cell lines from which the cDNA libraries were prepared. Similar differences have been reported for murine p53 cDNA clones. Confirmation that the differences in the human cDNA sequences are authentic must await the isolation and sequencing of independent clones from these regions.

Because the pR4-2 clone contains the entire coding region for the human p53, we were able to compare the predicted amino acid sequence of the human p53 of A431 cells with the predicted sequence of mouse p53. Three full-length clones
FIG. 7. Comparison of the predicted amino acid sequences of the human and mouse p53 genes. The predicted amino acid sequence of the human p53 shown in Fig. 4 was compared with the predicted amino acid sequence of the mouse cDNA reported by Jenkins et al. (22), Pennica et al. (45), Zakut-Houri et al. (54), and Benchimol et al. (2). Only amino acids where all the reported sequences have identical residues are marked as matches.
were reported for mouse p53 (22, 45, 54). Two of these clones were prepared from cell lines derived from BALB/c mice, and one was prepared from cell lines from a 129 mouse. Although Jenkins et al. (22) and Zakut-Houri et al. (54) isolated cDNA clones from BALB/c mice, the DNA sequences of these clones are surprisingly different. The origin and significance of these differences are unknown at present. However, comparisons between the mouse and human sequences show that these proteins are similar throughout much of the primary sequence. The pR4-2 insert has the coding potential for 393 amino acids, beginning from a methionine codon at nucleotide 215. We assume that this methionine residue serves as the initiation site for protein synthesis because there are no methionine codons either in frame or out of frame to the 5′ side of this triplet and there is a stop codon in the same reading frame 105 base pairs upstream. The region around this methionine codon also has many of the hallmarks of the mRNA sequence and structure suggested by Kozak (24, 25) to be important in the initiation of translation. At the amino terminus of the predicted sequence for the mouse p53 there are two methionine residues in the same reading frame, giving the amino acid sequence M-T-A-M-E-E-. The sequence of the human amino terminus is M-E-E-. There are no data to suggest which of the two ATG codons acts as the initiator. As might be expected, there is no detectable nucleic acid homology in the DNA sequences upstream of the M-E-E- coding region. Starting at the second methionine residue of the predicted mouse p53 sequence, there are 387 amino acid residues before the stop codon (386 for the sequence from Jenkins et al. [22]). This is six fewer than the 393 amino acids for the human sequence. In the best alignment, there are 304 identical matches between these two sequences. The proportion of identical matches is lower in the region between amino acids 27 and 84, where it drops to 21 of 57. The longest stretches of identity are from residues 155 to 186 and 236 to 267. In addition, the hydropathy profiles and the secondary structure predictions of human and mouse p53 are similar except for the region between residues 27 and 84.

Several studies have shown that p53 from human cells can be distinguished from mouse p53 by biochemical and immunochemical methods. These differences include the number and relative mobility of p53 polypeptides on SDS-polyacrylamide gels, the binding affinity for SV40 large-T antigen, and the binding species-specific monoclonal antibodies. The isolation of a full-length human cDNA clone should enable us to examine the physical basis for these differences. Although the human p53 has a larger relative molecular weight (approximately 2,000 or 3,000 on SDS-polyacrylamide gels), the predicted difference is only about 200. The only apparent difference in amino acid composition between these two molecules is in the number of proline residues. The human p53 has 45 prolines, whereas the mouse p53 has only 38. If the unusual mobility of p53 on SDS-polyacrylamide gels compared with the predicted molecular weight is based on the number of prolines, then the higher percentage of proline residues in the human p53 may account for the difference in mobility between the human and mouse polypeptides. The human and mouse p53 can also be distinguished by the appearance of the pattern of bands. Human p53 often runs as a doublet on SDS-polyacrylamide gels. Because the p53 from A431 appears only as a single band, none of the information we have been able to gain from the studies presented here has helped us understand the origin of the human p53 doublet. Another distinguishing characteristic between human and mouse p53 is their relative affinity for the SV40 large-T antigen. Whereas the mouse p53 binds very tightly to the SV40 large-T antigen, p53 from primate cells has been shown to have a lower binding affinity (18). We are currently exchanging regions of the mouse and human cDNAs in an attempt to identify the area of p53 that is responsible for the differences in binding in vivo. The human and mouse p53 can also be selectively immunoprecipitated with monoclonal antibodies specific for either the mouse or human p53. Although the human-specific antibodies have not yet been tested (31, 53) for binding to the p53 synthesized by the pR4-2 clone, we have noticed that both of the monoclonal antibodies we used only precipitate a small fraction of the p53 polypeptide synthesized in our in vitro assays (see above).

We also compared the human and mouse p53 sequences with other polypeptides in several of the protein data banks and found regions of weak homology with a number of other proteins. Because the regions of homology are not large and because they often are centered on a stretch of proline and alanine residues, it is difficult to understand the significance of these observations. The region has 10 alanine residues and 9 proline residues within a 21-amino-acid stretch between residues 68 and 89. The proteins which show the greatest homology are the adeno virus E1A proteins, the polyoma large-T antigen, and the human c-myc protein. All three of these proteins are nuclear and all complement an activated ras gene to yield transformed foci when cotransfected into normal rat cells. Since p53 is also a nuclear protein and complements ras these, these homologies may suggest some common structural domain. However, little importance can be placed on these observations until more is known about the functions of these proteins. We hope that the isolation of the clones described here will help in our understanding of the role p53 plays in both normal and transformed cells.

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