INTERNATIONAL REVIEW OF EXPERIMENTAL PATHOLOGY, VOL. 25

The 53,000-Dalton Cellular Protein and Its Role in Transformation

LIONEL CRAWFORD

Molecular Virology Laboratory, Imperial Cancer Research Fund
London, England

I. Introduction 2
A. Nomenclature 3
B. Methodology 4

II. p53 in SV40-Transformed Cells 7
A. p53 in SV40-Transformed Rodent Cells 7
B. p53 in Cells Transformed by SV40-Related Viruses 12

III. p53 in Other Transformed Cells 13
A. Chemical Carcinogen-Induced Tumor Cells 13
B. Embryonal Carcinoma Cells 14
C. Studies with Abelson Murine Leukemia Virus-Transformed Cells 14
D. Adenovirus-Transformed Cells 15

IV. Epstein–Barr Virus-Transformed Cells 16

V. p53 in Infected Cells 16

VI. p53 in Human Transformed and Tumor Cell Lines 18
A. SV40-Transformed Cells 18
B. Tumor Cell Lines 19
C. Anti-p53 Antibodies in Sera 23

VII. Properties and Quantitation of p53 Protein 25

VIII. Cellular Location and Control of p53 33
A. Cellular Location of p53 33
B. Control in SV40-Transformed Cells 35
C. Control in Differentiating Cells 36

IX. The Gene(s) for p53 37
A. Chromosomal Location of the Gene(s) for p53 37
B. Cloning of the Gene(s) for p53 38

X. The Function of p53 39
A. Tumorigenicity 39
B. Embryonic Antigen 41

Copyright © 1983 by Academic Press, Inc.
All rights of reproduction in any form reserved.

C. Protein Kinase 41
D. Transforming Gene 42
E. Cell Cycle-Related Protein 43

XI. Conclusions 44

References 47

I. Introduction

Tumor cells are extremely diverse in their properties and the search for some property which they have in common, and which distinguishes them from normal cells, has been largely to no avail. p53 stands out from the other characteristics of tumor cells, because it seems to be altered in a great variety of tumor cells. The interest in p53 came initially from the unexpected convergence of two major areas of cancer research, tumor viruses on the one hand and tumor specific transplantation antigens (TSTA) on the other.

Work with DNA tumor viruses had defined particular virus-coded proteins as being primarily responsible for transformation. In the case of simian virus 40 (SV40) the transforming protein appeared to be large-T, a polypeptide with an apparent molecular weight of 94,000 (Tegtmeyer *et al.*, 1975; Rundell *et al.*, 1977). This is the major product of the early region of the virus (the region expressed before the onset of DNA replication in the infected cell). The small size of the virus genome made it seem unlikely that any other protein could be coded in this region, but in fact a second protein, small-t, is coded in the same region (Crawford *et al.*, 1978). In the closely related polyoma virus there is even a third early protein, middle-T, which has a molecular weight of 55,000 (Ito *et al.*, 1977a, b). The discovery of polyoma middle-T antigen prompted an intensive search for an equivalent virus-coded protein in the same size range in SV40-infected and -transformed cells. None was found but the search did produce a protein which was host coded, rather than virus coded, and was associated with the virus-coded large-T antigen to form a complex (Lane and Crawford, 1979). It was this association which allowed p53 to be clearly distinguished from the general immunoprecipitation background and from proteolytic degradation products of large T.

The other line of research was quite distinct from the work on DNA tumor viruses and was concerned with understanding the mechanism of transplantation rejection. Cells derived from tumors induced by chemical carcinogens, such as methylcholanthrene, can be used to immunize syngeneic animals. These animals then show increased resistance to a second dose of the same cells. Each tumor cell line is highly individual (Prehn and Main, 1957) and immunizes effectively against itself but against very few other cell lines, even if these others are induced by the same carcinogen or are derived from two different tumors in the same animal. Transplantation rejection is cell mediated, and it has proved difficult to define the relevant target molecules and to obtain a humoral response. There is, however, a humoral response to some methylcholanthrene-induced tumor cell lines, such as Meth A, which is directed toward p53 and not toward the transplantation antigen (DeLeo *et al.*, 1979). The anti-p53 activity in sera of mice bearing Meth A tumors was used to show that p53 was by no means confined to Meth A or even to methylcholanthrene-induced tumor cell lines but was found in cells transformed by a great variety of chemicals, viruses, and physical agents (DeLeo *et al.*, 1979). This result generated a great deal of excitement and stimulated much of the work on p53 that followed.

A. Nomenclature

The nomenclature used in the literature for p53 is rather confusing, partly because of the fact that it was found in a variety of different systems and not until later was it realized that the same protein was involved. There are mobility differences between the p53s of different species, although this cannot entirely account for the wide variation in monomer molecular weight given for p53. Variations in gel systems in different laboratories also cause variations in gel mobility of the p53 band, as do variations in loading of IgG heavy chain since the samples loaded on the gels are usually immunoprecipitates and the serum inputs vary considerably. In addition to names derived from apparent molecular weight, other names have been used by various groups. "SV40 middle-T" is probably the most misleading since it implies that it is virus coded and analogous to polyoma middle-T. "Tau" has also been used both for p53 (Simmons *et al.*, 1980) and for polyoma middle-T. "NVT" (nonviral T antigen) has also been used (Smith *et al.*, 1979a). It has been agreed by several of the groups working in this field to use p53 in preference to other names for the protein as an interim arrangement pending the elucidation of the function of p53 and the consequent displacement by a functional name (Crawford, 1982a). There is clearly an advantage in using the same term for p53 even though the name is somewhat arbitrary. There is still the danger that not all the proteins referred to as p53 are the same (or corresponding) species. A list of properties expected of "authentic" p53 has been suggested (Crawford *et al.*, 1981), although it is not always easy to ensure that the protein of interest satisfies all of them. However, it is clear that possessing one or two properties (such as size and phosphorylation) is not sufficient to guarantee identity with p53, and even reactivity with a p53-specific monoclonal antibody is not by itself

sufficient (see later). In practical terms reaction of a phosphorylated polypeptide with two or more p53-specific monoclonal antibodies to generate a band from the immunoprecipitate that runs in the 53,000-molecular-weight region on a gel is a good indication that the protein is p53. Proteins which appear to be p53 on the basis of currently available data are listed in Table I. A number of other proteins have been found in transformed cells which share some properties with p53 but should not yet be considered to be p53. One protein in particular, the 53K protein found in association with a nuclear antigen in cells transformed by Epstein–Barr virus (Luka *et al.*, 1980), does not now appear to correspond to p53 (Luka *et al.*, 1983; D. Lane, personal communication) (Section V).

B. Methodology

p53 is a minor component, even in transformed cells, and probably contributes between 1 and 100 ppm of the total cellular proteins. Special techniques are therefore necessary to study it. Most procedures make use of the specificity of antibodies, either polyclonal or more recently monoclonal, and little progress would have been made without these immunochemical reagents. All the techniques do, however, have limitations and these can become very important when the techniques are pushed to the limits of their sensitivity.

1. *Labeling, Immunoprecipitation, and Gel Electrophoresis*

The most commonly used method for studying p53 has been labeling of cells by growing them in the presence of radioactive amino acids or $^{32}P_i$.

TABLE I
Proteins Corresponding to p53

48K (but not 55K of the same authors)	Melero *et al.* (1979a)
p50	Rotter *et al.* (1980)
pp53	Crawford *et al.* (1981)
53K	Lane and Crawford (1979); Crawford *et al.* (1979a)
54K	Linzer and Levine (1979)
55K	Kress *et al.* (1979); Mora *et al.* (1980)
55K (but not 60K polyoma middle-T of the same authors)	Hutchinson *et al.* (1978)
56,000 d	Chandrasekaran *et al.* (1981a)
Tau	Chang *et al.* (1979)
NVT	Smith *et al.* (1979a)

This is followed by immunoprecipitation to bring down the p53 selectivity and electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate (SDS) to separate on the basis of size the polypeptides present. Attempts to make quantitative estimates of p53 based on this approach are fraught with difficulties, some of which are discussed in detail later. At best the results with short pulses of radioactivity give an indication of the *rate of synthesis* of p53, rather than the *amount* of p53. Extended labeling, for periods of at least one cell generation time, would give incorporation related to the amount of p53 present, but this is seldom done. The efficiency of the immunoprecipitation step and the specificity of the antisera or antibody used at this stage also affect the results markedly. Since the heavy chain of IgG runs in the same region of SDS gels as p53, and most of the antibodies used are IgGs, this affects the appearance of p53 on the gels. This is most severe where double-antibody precipitation is used, less severe with staphylococcal protein A as immunoadsorbent (Kessler, 1975), and least troublesome when monoclonal antibodies are used, because the IgG here is entirely specific so that only very small amounts are needed.

2. *Immunofluorescence*

Cells, either unfixed for surface staining or fixed for staining internal components, are treated with antiserum or monoclonal antibody, usually followed by a second antibody specific for the first antibody, and conjugated with fluorescent compound. Although this method is only semiquantitative, it generates results that relate to the amount of p53 and are unaffected by the rate of synthesis or any of labeling variables that affect the previous method. It does, however, rely heavily on the specificity of the antiserum or monoclonal antibody used and can be misleading where the antibody recognizes other molecules in addition to p53.

3. *Monoclonal Antibodies*

Polyclonal antisera have been used widely for both immunoprecipitation and immunofluorescence, but they can cause problems, first because they contain a mixture of antibodies of different affinity and specificity, and second because only a small fraction of the immunoglobulin present is active. Monoclonal antibodies (Köhler and Milstein, 1975) avoid both these problems and have been useful in helping to unravel some of the complexities of p53. Some properties of the presently available antibodies are summarized in Table II. Cross-blocking studies using these antibodies (Crawford, 1982b) define four antigenic areas on mouse p53, one of them common to mouse and human p53 and three specific for mouse p53. The area defined by PAb122, PAb410, and PAb421 can be subdivided because PAb421 and PAb122 block each others binding, but their determinants are

TABLE II
MONOCLONAL ANTIBODIES REACTIVE WITH p53

Antibody	Specificity	Other activities	Reference
PAb122	Rodent and primate p53	130,000 MW protein	Gurney *et al.* (1980); Kuypers (1982)
PAb410, PAb421	Rodent and primate p53	80,000 MW protein Mouse and human skin component(s)	Harlow *et al.* (1981b); Kuypers (1982); P. Real and L. Old (personal communication)
RA3-2C2	Mouse p53	B lymphocyte differentiation antigen, p35	Coffman and Weissman (1981)
200.47	Mouse p53	Human nuclear component	Dippold *et al.* (1981)
PAb607	Mouse p53		L. Gooding (unpublished data)
PAb1005	Human p53		Levine (1982)

not identical. This is shown by the fact that PAb421 blocks the binding of another antibody, PAb1005 (anti-human p53), on human p53 but that PAb122 does not (D. Lane, personal communication). The three sites (PAb421, PAb122, and PAb1005) on human p53 are therefore close or overlapping.

Some of these antibodies react with other host proteins in addition to p53 (Table II), although this problem is not peculiar to p53. It was previously found with several of the set of anti-SV40 large-T monoclonal antibodies studied by Harlow *et al.* (1981b; Crawford *et al.*, 1982b) and in an increasing number of other instances reviewed by Lane and Koprowski (1982). So long as reasonable precautions are taken this need not detract from the usefulness of monoclonal antibodies.

4. *Quantitative Assays*

Statements about the amount of p53 have frequently been based on labeling and immunoprecipitation. Because of variation in the stability of p53 in different cells, and under different conditions, this is at best risky and can be misleading. By using monoclonal antibodies, assays have been devised which give a reliable indication of the *amount* of p53. One assay described by Lane *et al.*, (1982) makes use of PAb421 (Table II) and PAb204, an anti-SV40 large-T antibody (3C4 of Lane and Hoeffler, 1980). Competition between free p53 and p53 complexed to SV40 large-T is then used to get an estimate of p53.

Other assays (Benchimol *et al.*, 1982) have made use of the moderate number of anti-p53 monoclonal antibodies now available (Table II) and used them in pairs to set up a "sandwich"-type assay. One monoclonal antibody is bound passively to the wells of polyvinyl chloride (PVC) microtiter plates. Cell extract is then added and the p53 is allowed to react with the antibody. The second antibody radioactively labeled with ^{125}I then acts as a probe for bound p53. The amount of ^{125}I bound provides a measure of p53. Not all the antibodies listed in Table II can be used with each other since they block each others' binding in some combinations. Values obtained with this assay are given in Section VII.

II. p53 in SV40-Transformed Cells

A. p53 IN SV40-TRANSFORMED RODENT CELLS

Immunoprecipitation of extracts of SV40-transformed cells gives a prominent band of p53, whether the cells are labeled with [^{35}S]methionine or $^{32}P_i$ (Fig. 1). In the early work the serum used for this type of study usually came from hamsters bearing tumors of SV40-transformed cells (Tegtmeyer *et al.*, 1977). The precipitation seemed virus specific in that normal hamster sera did not precipitate p53; nor did anti-T sera precipitate a similar band from extracts of the untransformed parental cells from which the SV40-transformed cells had been derived. In this respect it was like the virus-coded large-T antigen, the product of the SV40 *A* cistron (Rundell *et al.*, 1977), and breakdown products of large-T such as the polypeptide with a molecular weight of 60,000 (Lane and Robbins, 1978) which shares determinants with large-T (Crawford *et al.*, 1979b). Since the hamster sera used were polyclonal, the activities against large-T and p53 could have been independent or overlapping.

In contrast to the apparently virus-specific nature of p53 there was strong evidence that p53 was influenced by the source of the transformed cell, different proteins being found in transformed cells from the different mammalian species (Section VII). As discussed later, these differences included the mobility of the proteins on polyacrylamide gel electrophoresis and the patterns of methionine-labeled tryptic peptides. The accumulating evidence that p53 was at the same time related to the virus in some way, but also influenced by the host cell, led to the suggestion that the protein might be part virus coded and part host coded. The alternative that there was an association of p53 with a virus-coded protein was also suggested (Gaudray *et al.*, 1978), but there was no clear way of deciding between the several alternatives. By making use of a specific antiserum directed against the gel-

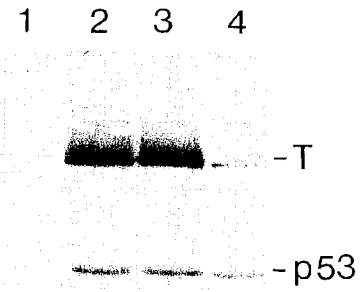


FIG. 1. P53 is a phosphoprotein in transformed cells. Extracts of $^{32}P_i$- or [^{35}S]methionine-labeled cells (SVA31E7 SV40-transformed mouse cells) were immunoprecipitated with hamster serum and the proteins were separated by electrophoresis on a 7 to 20% polyacrylamide gel. The tracks are as follows: (1) $^{32}P_i$ label, normal hamster serum; (2 and 3) $^{32}P_i$ label, hamster anti-T serum; (4) [^{35}S]methionine label, hamster anti-T serum. [Taken from Lane and Crawford (1980) with permission.]

purified polypeptide of SV40 large-T (Lane and Robbins, 1978), Lane and Crawford (1979) were able to produce clear evidence for the existence of a p53–SV40 large-T complex. The anti-T polypeptide serum gave coordinate precipitation of large-T and p53 from extracts of transformed mouse cells, the ratio of the two proteins remaining constant over a range of antiserum concentrations (Fig. 2). The same anti-T polypeptide serum was active against large-T isolated by gel electrophoresis, but had no activity against isolated p53. Most tumor sera also had no activity against isolated p53. Fortunately, sera from mice bearing tumors of SV40-transformed cells did show some anti-p53 activity and proved that the isolated p53 was still sufficiently intact to be recognized by suitable antibodies. The importance of the idea of a complex was that it explained both how p53 could be immunoprecipitated by anti-T sera, either serum from tumor-bearing animals or specific antipolypeptide serum, while at the same time sharing no sequences or antigenic determinants with SV40 large-T. The clearest proof that p53 was host coded came from comparison of p53 from SV40- transformed cells with p53 from embryonal carcinoma cells (Linzer and Levine, 1979) (Section III,B). The protein appeared to be the same whether SV40 was present or not. Similarly, p53 could be immunoprecipitated from mouse cells transformed by polyoma virus rather than by SV40, and the isolated polypeptide reprecipitated with the mouse anti-T serum which was known to have anti-p53 activity (Lane and Crawford, 1979). The immunological case for the existence of the p53–large-T complex was completed by showing that specific anti-p53 polypeptide serum, lacking any activity against large-T, also precipitated SV40 large-T from extracts of SV40- transformed cells (McCormick and Harlow, 1980). Other physical evidence for the complex came from the cosedimentation of p53 and SV40 large-T on sucrose gradients (McCormick *et al.*, 1979). Only a fraction of the large-T present was complexed with p53, but most of the p53 was complexed to large-T. The complex was clearly large, sedimenting at about 23 S, corresponding to a molecular weight of 600,000 to 1,000,000 if a globular shape is assumed (McCormick and Harlow, 1980). An example of this type of analysis is shown in Fig. 3. Immunoprecipitation of alternate fractions with specific antiserum to large-T and with anti-p53 monoclonal antibody makes the sedimentation of p53 with its associated large-T very clear. The mouse p53–large-T complex is stable to nonionic detergent [1% Nonidet P-40 (NP-40)], 5 m*M* ethylenediaminetetraacetic acid (EDTA), 2 *M* NaCl, and 0.1 *M* dithiothreitol, but disrupted by exposure to pH 10 or to 2% SDS (McCormick and Harlow, 1980). Reconstruction of the complex *in vitro* is more difficult but some success has been achieved with purified T antigen from SV80 cells and with D2 protein (McCormick *et al.*, 1981). This SV40 large-T-related protein is produced in cells infected with the adenovirus–SV40 hybrid virus Ad2+D2. Addition of the purified proteins to nuclear extracts from a va-

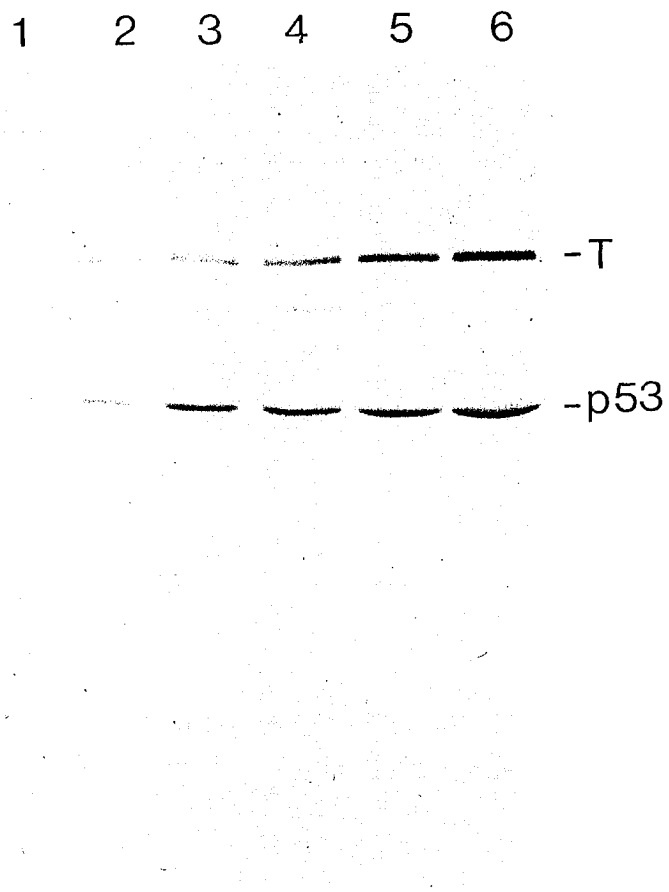


FIG. 2. P53 and SV40 large-T are precipitated coordinately by specific anti-T polypeptide serum. Equal aliquots of an [^{35}S]methionine-labeled extract of SVA31E7 cells were immunoprecipitated with increasing amounts of antiserum. The specific anti-T sera were made against gel-purified large-T (Lane and Robbins, 1978) and had no activity against p53 in the direct binding radioimmunoassay (Lane and Crawford, 1979). The tracks are as follows: (1) normal serum 30 μl; (2) to (5) increasing anti-T serum 1, 3, 10, 30 μl with decreasing normal serum 29, 27, 20 and 0 μl; (6) 100 μl anti-T serum. [Taken from Lane and Crawford (1979) with permission].

riety of mouse cells resulted in formation of a complex which was resistant to 0.5% NP-40, 0.4 *M* LiCl, or 2 *M* urea. The complex formed *in vitro* sedimented more slowly than the natural complex, 12 S as compared to 23 S, but the increase in sedimentation of p53 from 8 S in the absence of large-

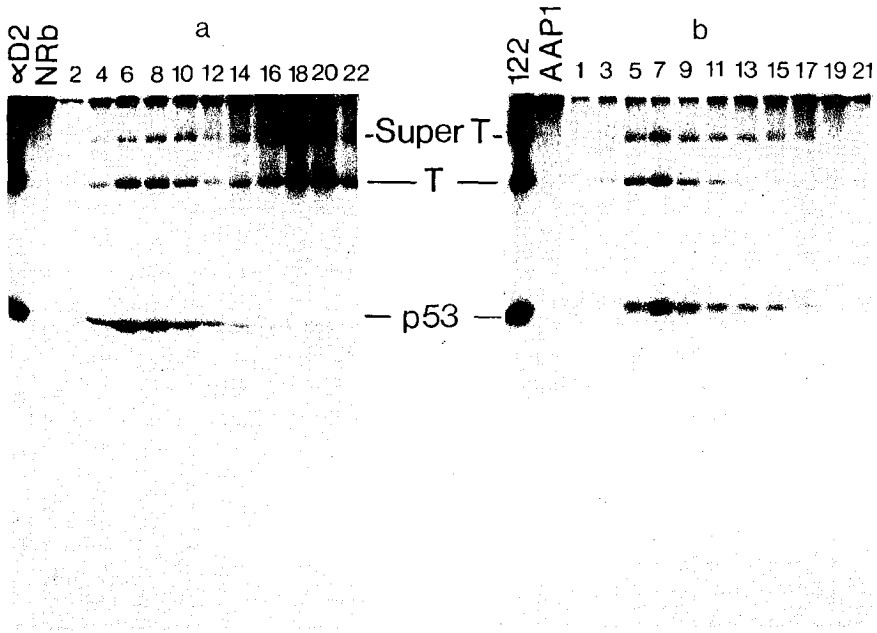


FIG. 3. The complex of p53 with SV40 large-T is large and heterogeneous. An extract of $^{32}P_i$-labeled SV40-transformed mouse cells was layered onto a 5 to 20% sucrose gradient and centrifuged for 15 hours at 25,000 rpm (McCormick and Harlow, 1980). Alternate fractions were immunoprecipitated with (a) anti-SV40 large-T serum (anti-D2 protein serum, kindly provided by D. Lane) or (b) anti-p53 monoclonal antibody (PAb122; Gurney *et al.*, 1980) and the samples were run on a 10% polyacrylamide gel. The two tracks on the left of each set are the unfractionated sample as added to the gradient. Sedimentation was from right to left and the main peak of complex is in fraction 7, corresponding to about 22 S (E. Harlow, unpublished results).

T to 12 S in the complex was significant. The main criterion for complex formation was that p53 became immunoprecipitable by specific antiserum directed against the D2 protein.

The complex between p53 and SV40 large-T seems to be very specific. No other cellular protein complexes in the same way to SV40 large-T, although two proteins different from p53 do complex with SV40 small-t (Yang *et al.*, 1979). There are a few examples of other virus-coded proteins which complex with p53, including viruses closely related to SV40 and adenovirus (Section III,D). The overall specificity encourages the idea that complex formation is important to the virus or to the cell, or to both. It would also seem likely that matching configurations of specific regions of both p53

and large-T would be required for complex formation. However, it has not been possible to localize these sites yet, beyond saying that the extreme ends of the large-T polypeptide are not involved. In the D2 protein already mentioned, the first 100 or so amino acid residues at the N terminus of large-T are replaced by adenovirus-coded sequences, yet this protein complexes with p53. Viable deletion mutants lacking sequences at or near the C terminus of large-T, such as *dl* 1263 and *dl* 1265, also complex with p53 (Denhardt and Crawford, 1980). A variety of truncated polypeptides, related to large-T but lacking parts of the C-terminal half of the polypeptide, have been examined in transformed cells. None of them appear to complex with p53 (Chaudry *et al.*, 1982; Clayton *et al.*, 1982; Colby and Shenk, 1982). It is not clear whether this indicates that the deleted sequences interact with p53 or whether the effect is an indirect one. Most of the truncated, large-T-related polypeptides are unstable and do not form the oligomers characteristic of large-T. Oligomerization may be essential for complexing with p53. Temperature-sensitive (ts) large-T in *tsA*-transformed cells also shows reduced complex formation with p53 at the nonpermissive temperature (Linzer *et al.*, 1979). This effect may also be indirect via dissociation of large-T oligomers or direct through some allosteric change in large-T configuration. One approach to locating the regions of large-T and p53 involved in complex formation would be to isolate monoclonal antibodies which interfered with complex formation. The localization of the binding sites for interfering antibodies should help considerably in defining these regions.

It is not clear what effect the association of p53 with large-T has on their properties. The ATPase activity of SV40 large-T is not affected by the association with p53 (R. Clark, personal communication; E. Harlow, personal communication). The ability of SV40 large-T to bind the origin region of SV40 DNA is also unaffected by p53 (Reich and Levine, 1982). As discussed later, the stability of p53 is increased by association with large-T, but because little is known about the function of p53 we cannot say whether this is altered or not.

B. p53 in Cells Transformed by SV40-Related Viruses

Viruses closely related to SV40, such as JC and BK, show similar large-T–p53 complexes in transformed mouse cells (Simmons *et al.*, 1980). This is consistent with the extensive homology seen in their nucleic acid sequences and T-antigen properties. Polyoma virus is less closely related to SV40 than JC and BK, but there is still extensive nucleic acid homology and amino acid sequence homology between its large-T antigen and the

SV40 large-T antigen (Soeda *et al.*, 1980). Polyoma-transformed cells show a p53 band (Lane and Crawford, 1979) as do polyoma-infected cells (Hutchinson *et al.*, 1978), but there is as yet no clear evidence for association of the p53 with polyoma large-T or with either of the other two virus-coded T antigens, middle-T and small-t, but it is quite possible that there is a loose association which is disrupted by the procedures used so far. Polyoma infection causes an increase in the rate of synthesis and amount of p53, but the increase does not look very large since the cells often subjected to 3T6 polyoma virus infection already contain a moderately high level of p53. The molecular weights of polyoma middle-T and p53 are close but they are not known to have much else in common. The molecular weights of middle-T and p53 were given as 60,000 and 55,000, respectively, by Hutchinson *et al.* (1978), but since tryptic peptide mapping showed no similarity of the 55,000 MW protein to the virus-coded antigens, it is likely that this protein corresponds to p53. The lack of any association of polyoma large-T with p53 means that some other explanation must be given for the presence of p53 in immunoprecipitates of T-antigens from infected and transformed cells with anti-T sera. Either the sera may contain anti-p53 antibodies or the p53 may have been coprecipitated as a result of aggregation or trapping with the immune complexes containing the viral T antigens.

III. p53 in Other Transformed Cells

A. Chemical Carcinogen-Induced Tumor Cells

Tumors induced in mice by polycyclic hydrocarbons have been studied extensively by tumor immunologists, and although they show strong transplantation rejection the antigens concerned are very diverse. The diversity of transplantation antigens clearly complicates study of this type of tumor, particularly since the response is cellular and there is little or no production of a specific humoral immune reaction. The transplantation antigens of these tumors are displayed on the cell surface and it was only when reactions with internal components were studied that a common element was found—p53.

In the study of DeLeo *et al.* (1979), 15 different antisera were raised in mice to 11 different tumor cell lines. Of these, only two against the methylcholanthrene-induced tumor cell lines, Meth A and CMS4, showed anti-p53 activity. Parallel lines such as CMS3 and CMS5 did not generate anti-p53 activity, and this must be taken into account when attempting to explain how p53 elicits an antibody response in spite of being a host protein

occurring in normal as well as transformed cells. Once the antisera had been produced they were used to examine a wide variety of tumor lines from tumors induced by methylcholanthrene, RNA tumor viruses, DNA tumor viruses, X rays, and spontaneously transformed cells. Extracts of [^{35}S] methionine-labeled cells were immunoprecipitated with anti-Meth A antiserum and control sera. In all cases they showed specific precipitation of a band at about 53,000, together with additional bands in some lines. In normal cells such as mouse embryo fibroblasts and spleen cells no band was observed, although we know now that p53 is present at a low level in some normal cells rather than being completely absent. Normal thymocytes did show incorporation of [^{35}S]methionine into p53 although this is not an indication of a high level of p53 in these cells. At this stage it was clear that p53 was host coded but there was no evidence for it being coded by any of the exogenous RNA tumor viruses tested. One RNA tumor virus-coded product was similar in size. This 53,000-dalton protein found in Kirsten murine sarcoma virus (MuSV)-transformed cells could be distinguished from the p53 contained in the same cells: first, it was synthesized by *in vitro* translation of the virus RNA, and second, it had an isoelectric point quite different from that of p53 (DeLeo *et al.,* 1979) (Section VII).

B. Embryonal Carcinoma Cells

Tumors derived from immature pluripotent cells are unusual since they can grow as malignant tumors or, under the right conditions, be assimilated into developing embryos and behave as normal cells. As already mentioned, embryonal carcinoma lines incorporate high levels of [^{35}S]methionine into p53, and this was very important as an early indication that p53 was a host protein (Linzer and Levine, 1979). The high level of methionine incorporation is usually taken as an indication of a high level of p53 in these cells, and although this is not correct (Section VII), embryonal carcinoma cells have been widely used in studies of the control of p53, especially in relation to differentiation (Section VII,C).

C. Studies with Abelson Murine Leukemia Virus-Transformed Cells

The injection of mouse cells transformed by Abelson murine leukemia virus (MuLV) into mice or rats induces antibodies against the proteins of the helper virus, the P120-transformed protein of the Abelson virus, and, in addition, against p53. The protein was intially identified as P50 (Rotter *et al.,* 1980, 1981) before it was shown to be the same p53 as demonstrated previously in other retrovirus-transformed cells. The sera from Abelson

MuLV tumor-bearing mice were used in immunoprecipitation tests to show that the protein was phosphorylated and cellular in origin, rather than virus coded (Rotter *et al.,* 1980). Monoclonal antibodies were also used and initially led to some confusion. The antibody RA3-2C2 (Coffman and Weissman, 1981) was generated by fusion of rat spleen cells (immunized with Abelson virus-transformed cells) with mouse myeloma cells. Although it is an IgM it binds well to staphylococcal protein A and has been widely used as an anti-p53 monoclonal antibody. The original isolation of the hybridoma was as a producer of antibodies against antigens expressed on the surface of mouse B cells and B-cell precursors (Coffman and Weissman, 1981). The molecule concerned in this reaction may be a p35 component which can be surface labeled on the appropriate cells. The activity of RA3-2C2 against both p53 and the B lymphocyte differentiation antigen appears to be another example of the type of chance cross-reaction already mentioned and seen with a variety of monoclonal antibodies. So long as cells lacking the B lymphocyte differentiation antigen are used the reaction of RA3-2C2 with p53 is quite specific and the antibody seems to have a high affinity for p53. Nevertheless, it is still necessary to be cautious in interpreting the results of immunofluorescence with RA3-2C2 and, if possible, to confirm the results with other anti-p53 monoclonal antibodies that recognize determinants which are distinct from the RA3-2C2 determinant (Section VIII).

Using both the polyclonal mouse serum and the monoclonal antibody RA3-C2 it was shown that p53 was prominent in a variety of transformed cell lines (Rotter *et al.,* 1981) and in cultures of primary mouse tumors (Rotter, 1982). The amount of p53 was not measured, but incorporation of [^{35}S]methionine and of $^{32}P_i$ into p53 were both increased in the tumor cells as compared with normal cells. As also shown by Jay *et al.* (1979), thymocytes are unusual among normal cells in that they do show incorporation of [^{35}S]methionine into p53 but not incorporation of $^{32}P_i$, in contrast to the transformed cells (Rotter, 1982).

D. Adenovirus-Transformed Cells

The complex of p53 with an adenovirus-coded protein, E1b 58K, has been reported (Sarnow *et al.,* 1982a). Previous studies on adenovirus-infected cells had given no indication of such a complex and this may have been due to the fact that primate cells were used. As has already been mentioned, the association of p53 with SV40 large-T is weak in primate cells and the same may be true of other complexes involving primate p53. By changing to mouse cells where it was known that the complex of SV40 large-T with p53 was very stable, the chances of detecting a complex may have been considerably increased. Whether or not this was the reason, a complex

of p53 with the adenovirus early protein E1b 58K was detected. The 58K protein is immunoprecipitated with p53 when either one of the monoclonal antibodies directed against p53, RA3-2C2 (Coffman and Weissmann, 1981) and PAb122 (Gurney *et al.*, 1980) is used. It is important in this context that both antibodies were used since each has cross-reactivities which have, in the case of RA3-2C2, caused problems in other work. The converse coimmunoprecipitation of p53 by anti-58K monoclonal antibodies (Sarnow *et al.*, 1982b) completes the evidence for the complex. The importance of this observation is that it provides clear evidence for the importance of the p53–tumor antigen complex in transformed cells. What still has to be explained is why cells transformed by SV40 and its close relatives BK and JC contain the p53–large-T complex, yet polyoma virus-transformed cells do not appear to. Adenovirus is much more distant from SV40 than polyoma virus is, but has a similar complex. The similarity may arise from convergence of the two tumor antigens, large-T and E1b 58K, with respect to this p53 binding function, and certainly encourages speculation on the possible role of the complex (Lane and Harlow, 1982).

IV. Epstein-Barr Virus-Transformed Cells

Cells derived from Burkitt lymphomas or transformed by Epstein–Barr virus (EBV) *in vitro* contain a 53K protein which as been studied intensively for several years. The evidence now is that this 53K protein is not the p53 with which we are concerned in this review (D. Lane, personal communication; G. Klein, personal communication). Burkitt lymphoma cells do contain p53 (Section VI,B) as well as 53K, and this complicates the situation. However, the protein purified by Luka *et al.* (1980) and of which the N-terminal sequence was determined by Jornvall *et al.* (1982) appears to be quite distinct from p53. The complex of 53K with Epstein–Barr virus nuclear antigen (EBNA) in Epstein–Barr virus-transformed cells is therefore not analogous to that found in SV40-transformed cells and in adenovirus-transformed cells (Section III,D), and we shall therefore not discuss it further here.

V. p53 in Infected Cells

The complex of p53 with SV40 large-T in transformed cells has received far more attention than that in infected cells. From the point of view of the virus it seems likely that the ability of large-T to form a complex with p53 is important. If not, then this ability must be a consequence of some other function important to the virus, otherwise it would be unlikely to have

been retained. Complex formation may indeed be a way of accentuating the function of p53 by stabilizing it as discussed later (Section VIII,B). The presence of p53 in SV40-infected monkey cells was reported by Simmons (1980), and the complex of p53 with SV40 large-T in infected cells was studied by Harlow *et al.* (1981a). They found that the complexes in infected and transformed monkey cells were similar but that in both cases they were less stable than the large-T–p53 complex previously studied in mouse cells. Infection of monkey cells caused a fivefold stimulation of the incorporation of [^{35}S]methionine into p53. Incorporation of $^{32}P_i$ was stimulated even more, approximately 30-fold (Harlow *et al.*, 1981a). The complex in extracts of monkey cells transformed by UV-irradiated SV40 sedimented rapidly on sucrose gradients (Fig. 4). Both the anti-p53 monoclonal antibody and the specific anti-T serum precipitate p53 and large-T together in fractions near the bottom of the gradient. Only a small fraction of the SV40 large-T present sedimented with p53; most of the large-T sedimented more slowly and when immunoprecipitated did not coprecipitate any p53.

It is difficult to tell what effect p53 or the complex has on virus replication in monkey cells since there is no system lacking p53 with which com-

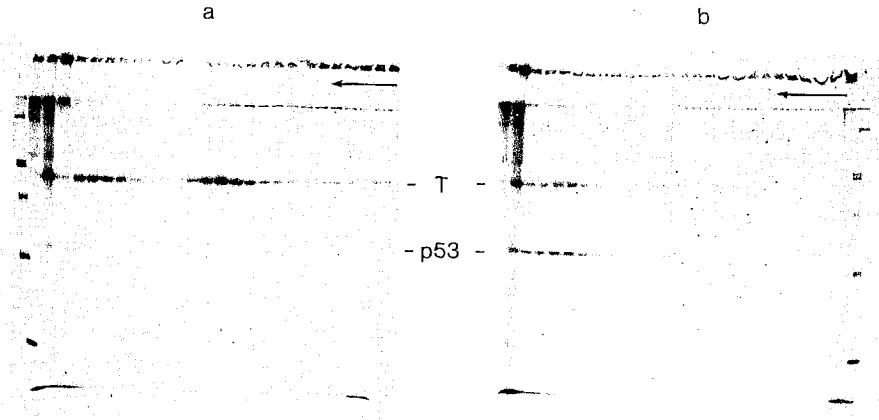


FIG. 4. Transformed monkey cells also contain the p53–SV40 large-T complex. Cultures of UV-irradiated SV40-transformed monkey cells (C2; Gluzman *et al.*, 1977) were labeled with $^{32}P_i$ phosphate and fractionated by sedimentation through a 5 to 20% sucrose gradient. Sedimentation was from right to left, as indicated by the arrows. Each fraction was split: (a) half was precipitated with anti-SV40 large-T (anti-D2) serum and (b) half was with anti-p53 monoclonal antibody (PAb122; Gurney *et al.*, 1980). The outside tracks are marker proteins and the other pair of tracks at the left of each set is the unfractionated sample precipitated with normal serum (left) and antiserum or antibody (right). The complex is at the bottom of the gradient and may be compared with the complex from the transformed cells (Fig. 3). (E. Harlow, unpublished results.)

parison can be made. Some human tumor cell lines such as HeLa seem to lack p53 (Section II,B) and can be compared with cells that do contain p53. E. Harlow (personal communication) has found that the yield of progeny virus from SV40-infected HeLa cells is about 100-fold higher than that obtained from C33I, a line which produces a high level of p53 (Section VI,B). Whether this indicates that the presence of p53 helps to protect C33I cells from SV40 infection or whether the resistance is completely unconnected with the presence of p53 in C33I cells remains to be seen.

Infection of mouse cells with SV40 gives an abortive infection with consequences which are very like transformation in the short term (Linzer *et al.*, 1979; Melero *et al.*, 1979b; Carroll *et al.*, 1980). It has the advantage that the kinetics of the change in p53 can be followed, in conjunction with the synthesis of the SV40 large-T in wild-type and temperature-sensitive mutant infected cells. In pulse-labeling experiments the rate of synthesis of p53 started to rise soon after the onset of large-T synthesis and remained high after large-T synthesis had reached its maximum and started to decline (Linzer *et al.*, 1979; Carroll *et al.*, 1980). In mouse cells infected with the temperature-sensitive mutant *tsA58,* which produces an altered large-T, the induction of the synthesis of both large-T and p53 were reduced at the nonpermissive temperature, indicating a need for a functional large-T for p53 synthesis (Linzer *et al.*, 1979).

VI. p53 in Human Transformed and Tumor Cell Lines

A. SV40-Transformed Cells

Since human cells are permissive or semipermissive for SV40, infection usually leads to cell death and production of progeny virus. Those transformed cells which do grow out are to some extent altered either with respect to cellular metabolism, or more frequently, viral metabolism. In the most commonly used line, SV80 (Todaro *et al.*, 1966), the SV40 genome which has been integrated into the cell DNA is altered. The T antigen produced in these cells is defective for viral replication and contains several mutations as compared to wild-type SV40 (W. Gish and M. Botchan, quoted by Clark *et al.*, 1981). These alterations do not inactivate the transforming function of the T antigen, but should be borne in mind when considering its activity in complexing with p53. A variety of SV40 mutants have been described in which the replication function and the transforming function have been separated to a greater or lesser extent (Pintel *et al.*, 1979; Cosman and Tevethia, 1981; Stringer, 1982). The first transformed permissive cells

were obtained by infecting monkey cells with SV40 which had been UV irradiated to inactivate replication (Gluzman *et al.*, 1977). The stability of the p53–large-T complex in these cells is similar to that formed in infected monkey cells with replication-competent, wild-type SV40 (Harlow *et al.*, 1981a). The relatively low stability of the human p53–large-T found in SV80 cells is probably not related to the alterations which inactivate the replication function of large-T and, on the contrary, seems to be a general property of primate p53. Early studies with monoclonal antibodies (Gurney *et al.*, 1980) detected only a fraction of the complex in SV80 cell extracts because of this instability. However, more recent studies have shown that all of the large-T in SV80 cells is complexed to p53, if gentle techniques of extraction and assay are used (Lane *et al.*, 1982), but there is apparently free p53 in addition to that complexed to large-T in SV80 cells (Lane and Crawford, 1980). The situation is somewhat different from that in mouse cells where there is free large-T but all the p53 is complexed with large-T (Crawford *et al.*, 1979a; McCormick and Harlow, 1980). The p53 from SV80 cells shows heterogeneity both in mobility, appearing as a doublet on one-dimensional gels, and in p*I* (Crawford *et al.*, 1981). The two mobility components are very similar in their partial proteolysis patterns and do not seem to be the result of varying amounts of glycosylation, since their mobilities are unaffected by tunicamycin treatment of the cells before and during labeling (Crawford *et al.*, 1981). The following properties of p53 in SV80 cells have been used as criteria for comparison with p53 proteins in other human cells: (1) size and phosphorylation; (2) reaction with monoclonal antibody PAb122; (3) association with SV40 large-T; (4) isoelectric focusing pattern; and (5) partial proteolysis pattern. Taken together, they ensure that the proteins are closely related to the p53 found in SV80 cells rather than being just cross-reacting species.

This may seem to be rather overdoing it, but the importance of using more than one criterion and more than one monoclonal antibody before concluding that the protein under consideration is p53 has been emphasized by the problems that have arisen with EBV-transformed cells (Section IV) and normal human cells (Section VI,B).

B. Tumor Cell Lines

With the precedent of mouse cells transformed by all sorts of agents and the availability of a monoclonal antibody with activity against human p53 it was natural to look for evidence of p53 in human cell lines derived from malignant tumors (Crawford *et al.*, 1981). Many of the cell lines examined contained p53, although there were exceptions. The evidence for the presence of p53 initially was incorporation of $^{32}P_i$ into p53 immunoprecipitated

by the monoclonal antibody PAb122 (Gurney *et al.*, 1980). Using the same labeling conditions p53 was not detected in the cultures of normal cells, both fibroblastic and epithelial, examined. The detection of p53 in the tumor cell lines and not in normal cells could have been a consequence of different rates of synthesis or phosphorylation rather than simply amount of protein, the problem already discussed with mouse cells. In principle, immunofluorescence should give a result related to amount of protein rather than rate of synthesis, and a study using this approach (Dippold *et al.*, 1981) appeared to show that transformed cells contained more p53 than normal cells and that rapidly dividing cells contained more than stationary cells, both very important results. The implication was that p53 was a cell-cycle protein and that some alteration in control of p53 levels could directly mediate the change in growth control characteristic of transformed cells. The monoclonal antibody used in these studies was 200.47, which reacted with mouse p53 in both immunoprecipitation and immunofluorescence tests, and because the human cells did contain p53, it was natural to assume that the nuclear immunofluorescence produced by 200.47 in human cells was due to p53. Extending these studies with other monoclonal antibodies initially gave confusing results. Cells that contained substantial amounts of p53 complexed to SV40 large-T and reactive with PAb421 (SV80) did not give immunoprecipitation of p53 or SV40 large-T with 200.47 In contrast, cells that were strongly positive for nuclear immunofluorescence with 200.47 (SK-MEL-28 and SK-MEL-64) showed only small amounts of incorporation of $^{32}P_i$ into p53 when precipitated with PAb421 even less when precipitated with 200.47. My explanation of these results is as follows. The antibody 200.47 is specific for mouse p53 (or probably rodent p53 because it reacts very weakly with rat p53) and does not react significantly with human p53. The immunofluorescence seen on either rapidly dividing normal human cells or tumor cells such as SK-MEL-28 or SK-MEL-64 is due to some cross-reacting antigen in these cells. Cross-reactions are not uncommon with monoclonal antibodies, and in particular anti-p53 antibodies, as discussed in Section I,B,3. Because p53 comprises a family of proteins differing in charge, and in some cases mobility on SDS polyacrylamide gels (Section VII), it is always possible that different p53 molecules predominate in different cell types. For example, the p53 in SV40 transformed cells may be different from that in methylcholanthrene transformed cells, although they may share many antigenic determinants. If 200.47 recognized a form of p53 that was predominant in mouse cells but found only rarely in human cells, this could account for the results obtained. Certainly the predominant form of p53 found in SV40 transformed human cells association with SV40 large-T reacts weakly, if at all, with the monoclonal antibody 200.47.

Direct radioimmunoassay of p53 in SK-MEL-28 and SK-MEL-64 by a more recently devised method (Benchimol *et al.*, 1982) showed that they contained less than 1% of the amount of p53 found in SV80 cells. They should therefore have shown only 1% of the immunofluorescence. In fact, SV80 cells are negative for immunofluorescence with 200.47 instead of being 100 times brighter than SK-MEL-64. Reevaluation of these results has several important consequences. We can no longer use the 200.47 data to show that p53 is nuclear in location in human cells not transformed by SV40 (see Section VIII,A). Neither can the data be used as an indication that p53 is found in normal human cells, although we know from other studies that normal cells in culture do contain a low level of p53. The most serious loss is the evidence that the amount of p53 in normal human cells is high in rapidly dividing, untransformed cells and low or undetectable in stationary cells. This is a much quoted result, and although it may be true that p53 is a protein involved in control of cell division (see Section X,E), none of the 200.47 data on human cells can be used in support of this idea. It should be emphasized that all the data obtained on mouse cells with 200.47 are unaffected by this reevaluation.

Another example of an unexpected activity of an anti-p53 monoclonal antibody was noted in these studies when PAb421 was used in parallel with 200.47. Sections of both human and mouse skin showed strong cytoplasmic fluorescence with PAb421 and not with 200.47 (P. Real and L. Old, personal communication). The material responsible for this fluorescence may include a group of polypeptides with molecular weights between 45,000 and 70,000 (Fig. 5). These polypeptides may correspond to cytokeratins and are seen in epidermis but not in dermis of transformed mouse cells. They are peculiar to PAb421, that is, they were not seen with any of the other anti-p53 monoclonal antibodies tested.

The problem of obtaining reliable estimates of the amount of p53 could not be solved by the type of radioimmunoassay used for mouse p53 (see Sections I,B,4 and VII) as we did not have monoclonal antibodies with the right attributes. By making use of the observation that some breast cancer patients had anti-p53 activity in their sera (see Section VI,C), a modified assay was set up (Benchimol *et al.*, 1982). Monoclonal antibody PAb421 was used to bind limiting amounts of p53 to the wells of PVC microtiter trays. The bound p53 was then reacted with polyclonal p53 serum (from a breast cancer patient) followed by an ^{125}I-labeled monoclonal antibody directed against human IgG (8a4) (Lowe *et al.*, 1982). Some of the results obtained using this assay are shown in Table III. Overall the agreement with the results of $^{32}P_i$ incorporation is good, because SV40-transformed cells contain the highest level of p53, followed by a range of tumor cell lines. The highest of these are close to SV80, and there is a range of more than 100-fold down to teratocarcinoma cell lines. Three tumor cell lines con-

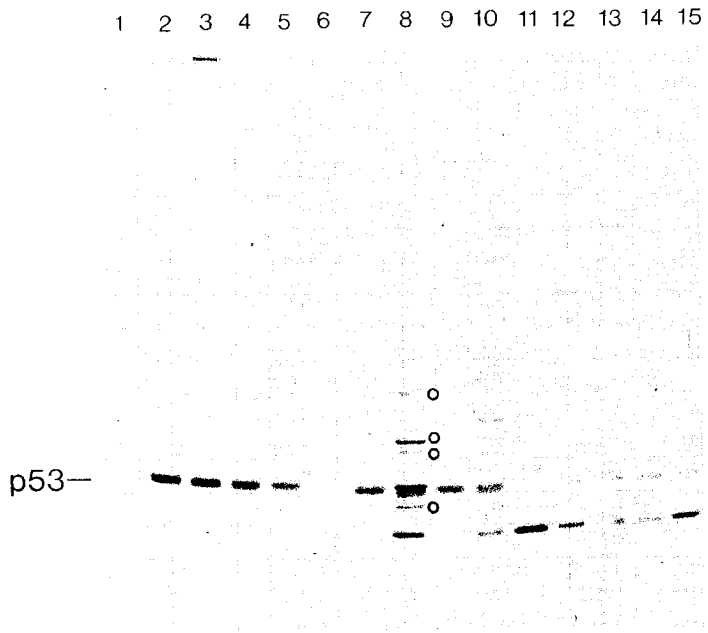


FIG. 5. An anti-p53 monoclonal antibody reacts with additional polypeptides found in epidermis. Cultures of mouse epidermis, and mouse dermis from newborn mice (kindly provided by M. Pera), were labeled with [^{35}S]methionine. In parallel a culture of spontaneously transformed mouse cells (CQ15) (Table IV) was labeled to give a control p53 band. All the extracts were precipitated with monoclonal antibodies as follows: (1–5) CQ15 cells; (6–10) epidermis; (11–15) dermis; (1,6 and 11) PAb419 (anti-T control); (2,7, and 12) PAb122; (3,8, and 13) PAb421; (4,19, and 14) antibody 200.47; and (5,10, and 15) antibody RA3-2C2. PAb122, PAb421, 200.47, and RA3-2C2 are all anti-p53 monoclonal antibodies and bring down a p53 band in all three extracts. Only with PAb421 and epidermis (track 8) are additional bands seen above and below p53 (marked "o").

tained too little p53 to be detected in the assay: HeLa, EJ, and T24. Using a variety of other methods, we still have not obtained evidence that they contain p53; certainly they contain less p53 than normal human cells. Normal human cells contain low levels of p53 by this assay, more in embryo lung than normal fibroblasts. The p53 in normal human cells is readily labeled by [^{35}S]methionine incorporation but not by $^{32}P_i$ incorporation (K. Leppard, unpublished). The p53 they contain is authentic as far as can be

TABLE III
RELATIVE LEVELS OF p53 IN HUMAN CELLS

Cell	Description	Amount of p53[a] (%)
SV80	SV40-transformed fibroblast	100
C33I	Cervical carcinoma cell line	80
Raji	Burkitt lymphoma cell line	40
MB 157	Mammary carcinoma cell line	30
BT 20	Mammary carcinoma cell line	18
T47D	Mammary carcinoma cell line	11
Daudi	Burkitt lymphoma cell line	4
Hs578T	Mammary carcinosarcoma cell line	2.8
HT 1080	Fibrosarcoma	2.4
NALM 1	Chronic myelogenous leukemia	1
NALM 6	Chronic myelogenous leukemia	1
Tera 1	Teratocarcinoma cell line	1.5
Tera 2	Teratocarcinoma cell line	0.5
HEL	Human embryo lung fibroblasts, fourth passage	0.5

[a]The amounts of p53 in detergent extracts of the cell lines listed were measured by the radioimmunoassay described by Benchimol *et al.* (1982) for human p53, using PAb421 as first antibody, serum P407 (anti-p53), and ^{125}I-labeled antibody 8a4 (anti-human IgG) as probe. All of the values are corrected for the total protein content of the extract and are given relative to SV80 as 100%. Other cell lines which gave background values in the assay (< 0.3%) were HeLa, EJ, T24 and normal skin fibroblasts, milk fibroblasts, and milk epithelial cells. These data are from Benchimol *et al.* (1982) and the origins of the cell lines used are given there.

determined from tryptic peptide comparisons but different from tumor cell p53 in its level of phosphorylation.

C. ANTI-p53 ANTIBODIES IN SERA

Although p53 was clearly present in substantial amounts in tumor cell lines in tissue culture, we were concerned initially that this might be a consequence of growth in culture. Many tumors, particularly highly malignant ones, do not give rise to cell lines that grow in culture. The cell lines available may therefore not be representative of the cells in the tumors as they occur in patients (Fogh and Trempe, 1975). To try and answer this type of reservation we set up a survey of anti-p53 activity in sera from breast cancer patients and normal women. The reasoning was that if p53 was altered in amount, type, or presentation in tumors, it might elicit an antibody response. The survey showed anti-p53 activity in about 9% of sera from breast cancer patients and none in the control sera (Crawford *et al.*, 1982a). Several possible explanations can be offered for the low frequency, but clearly

some patients do produce a humoral response to p53, and this encourages us to think that p53 is affected in tumors as well as in tumor cell lines.

We have recently carried out a similar survey of sera from lung cancer patients, lung cancer being chosen because it is a common cancer and a major cause of death in men and increasingly in women also. In conjunction with I. Smith, P. Sappino, and B. Ponder of the Royal Marsden Hospital, we screened sera from 77 patients and found 8 of them positive. The frequency of sera positive for anti-p53 antibodies is again low, comparable to that seen in the sera from breast cancer patients. It does demonstrate that production of antibodies against p53 is not restricted to breast cancer patients but may be more general. The method of screening sera for activity is now very simple, using a plate binding assay on PVC microtiter trays (Benchimol *et al.*, 1982), and this should facilitate large-scale studies. All those which appear positive on the plate binding assay are then checked by immunoprecipitation to make sure that they precipitate the SV40 T antigen-associated p53. In one patient where antibodies to p53 were found before an operation to remove the primary mammary carcinoma, they were still present 6 months after the operation. Two years later the titer of this patient's serum had fallen by more than 100-fold and activity was only just detectable in our assay. In other cases, this time patients with lung cancer, the titer of the anti-p53 antibodies fell by about fourfold over a period of 4 months. We would now like to follow the time course of production of p53 antibodies in a large number of patients over an extended period to see whether or not these are isolated cases.

If indeed the production of anti-p53 antibodies is a transient phenomenon this would go some way toward explaining the rather low frequency with which anti-p53 sera were found. If antibody production occurs only during a particular phase of tumor development, we have only a low probability of obtaining serum samples during that phase. Transient antibody production might be connected with the fact that p53 is a normal cellular protein and that tolerance to it is eventually reestablished after removal of the tumor.

The most direct proof that p53 is altered in human tumors is provided by assay of p53 protein levels in the tumor tissue and comparison with the corresponding normal tissue. We have initiated a study of p53 levels in colon carcinomas using the assay already mentioned (Benchimol *et al.*, 1983). The first indications are that a significant number of tumors contain increased amounts of p53), using adjacent sections of colon from the same patient as control tissue (Crawford and Makin, unpublished). If this is substantiated in a larger series, then it would both provide an explanation for the presence of anti-p53 antibodies in the sera of some patients with cancer of the colon, and it would also be a direct proof for alteration of p53 in spontaneous human tumors.

VII. Properties and Quantitation of p53 Protein

Studies on the properties of p53 have played an important part in several of the topics already discussed, for example the establishment of the nature of p53 as a host-coded protein. As already mentioned, p53 is a phosphoprotein in all of the cell lines examined with the possible exception of thymocytes and some normal cells. These label reasonably well with [^{35}S]methionine but do not label significantly with $^{32}P_i$. This may be a technical failure in that these cells contain very little p53, or it may be connected with the instability of p53 since it has a very short half-life in normal cells, as discussed later. Where phosphate is present it is attached to serine and threonine residues, not to tyrosine, and many different sites are phosphorylated to some extent (Van Roy *et al.*, 1981). Two serine residues are phosphorylated in rat, mouse, monkey, and human p53, and all except mouse p53 have an additional threonine residue phosphorylated. The threonine and one of the serine residues map close to the ends of the polypeptide chain (Van Roy *et al.*, 1981).

p53 proteins from different species have different mobilities on gel electrophoresis. Mouse p53 runs close to 50,000 apparent molecular weight and human runs closer to 55,000 molecular weight (Fig. 6). Even closely related species differ considerably, rat p53 running much more slowly than mouse p53 and having an apparent molecular weight of about 56,000. In general all the cell lines of a given species give p53 proteins with similar mobilities. Human cells give p53 which may run as a single or double band with the additional component being the slower one (Crawford *et al.*, 1981). Some of the heterogeneity seen on two-dimensional gels of p53 (Fig. 7) may be due to varying amounts of phosphorylation, but the same general distribution is seen whether $^{32}P_i$- or [^{35}S]methionine-labeled p53 is examined (Fig. 8). We have in general referred to p53 as a protein but it is more correct to consider it as a family of proteins. It shows heterogeneity in mobility in the SDS dimension, indicating differences in size. There is no evidence for glycosylation of human p53 by the dolichol sugar (tunicamycin-inhibited) pathway (Crawford *et al.*, 1981), nor did treatment with endoglycosidase H affect the mobility of mouse p53 (Rotter *et al.*, 1980). In the isoelectric focusing dimension p53 also shows some heterogeneity and streaking. Some of the streaking on nonequilibrium pH gradient electrophoresis (O'Farrell *et al.*, 1977) may be due to aggregation. The isoelectric point of p53 from Meth A mouse cells was estimated to be at pH 6.3 (DeLeo *et al.*, 1979) and from human cells to be at pH 6.7 with a minor species at pH 7.2 (Crawford *et al.*, 1981). The behavior of p53 on two-dimensional gels is quite characteristic and has been used to distinguish p53 from a similar-sized Kirsten

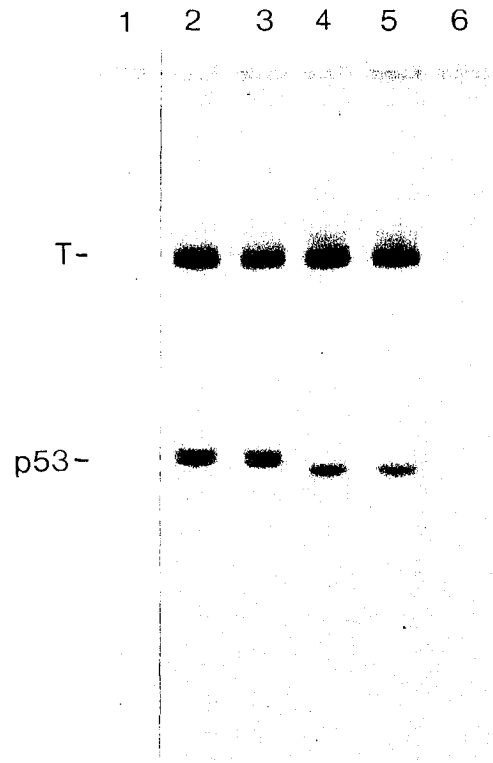


FIG. 6. P53 proteins from different species have different gel mobilities. Extracts of $^{32}P_i$-labeled cells, either SV80 SV40- transformed human cells (1–3) or SVA31E7 SV40-transformed mouse cells were immunoprecipitated with varying amounts of the anti-p53 monoclonal antibody PAb421 (Harlow *et al.*, 1981a). The volumes of hybridoma tissue culture supernatant were 0 (1 and 6), 50 $\mu\lambda$ (2 and 5), or 200 $\mu\lambda$ (3 and 4). Note that the human p53 doublet migrates more slowly than the single band of mouse p53.

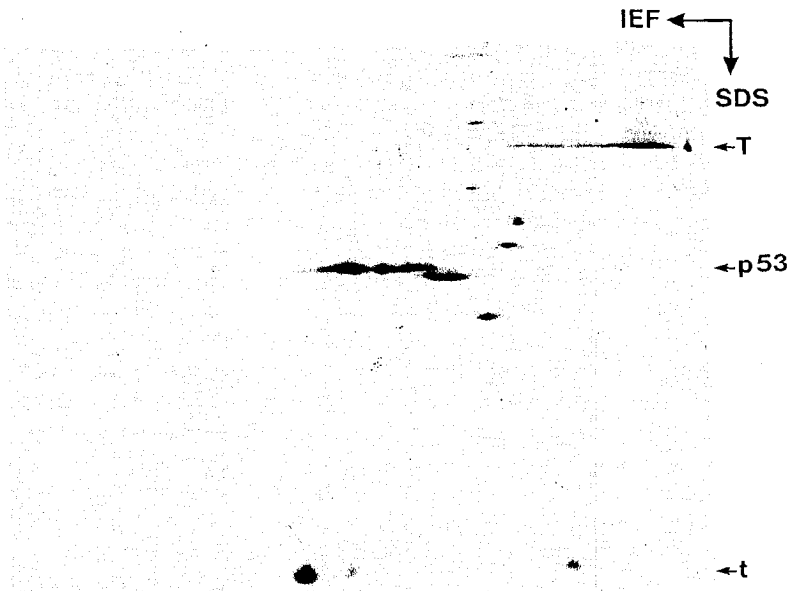
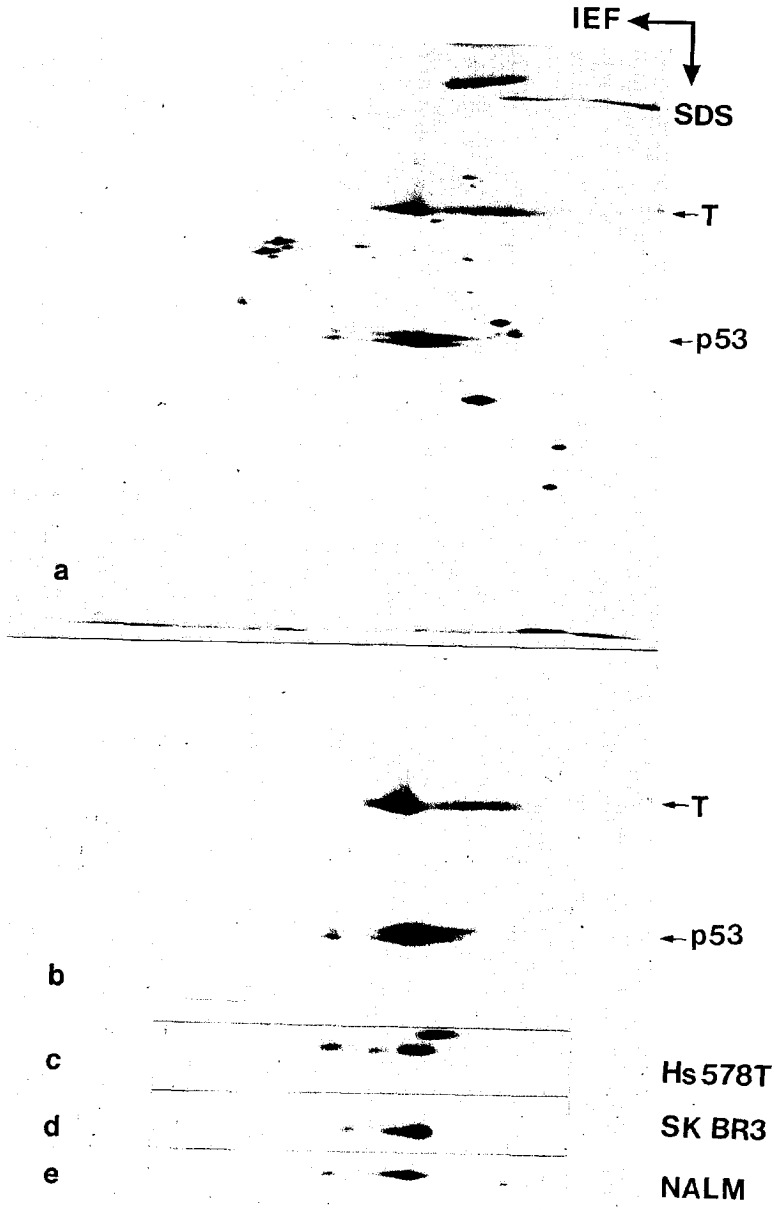


FIG. 7. Mouse p53 is a family of proteins. An extract of [^{35}S]methionine-labeled SVA31E7 cells was immunoprecipitated with anti-T serum and the protein was resolved on a two-dimensional gel (O'Farrell *et al.*, 1977). The nonequilibrium isoelectric focusing (IEF) dimension is horizontal and the SDS dimension is vertical. Mouse p53 is resolved into two mobility components and several species with different charge. [Taken from Crawford *et al.* (1979a). *Cold Spring Harbor Symposia or Quantitative Biology* **44**, 179–187. Copyright © 1980 Cold Spring Harbor Laboratory.]

MuSV-coded protein (DeLeo *et al.*, 1979) and to show that the p53 proteins from human tumor cell lines and from SV80 cells were similar.

On small amounts of material, confirmation of similarity between proteins can be obtained by comparing partial proteolysis patterns (Cleveland *et al.*, 1977). Some regions of the protein are susceptible to cleavage by protease, and limited digestion gives rise to a characteristic pattern of large polypeptides. This has been used on many occasions with p53, for example to show that p53s from embryonal carcinoma cells and SV40-transformed cells were similar (Linzer and Levine, 1979) and that p53s from various human tumor cell lines were similar (Crawford *et al.*, 1981). This type of



comparison has the advantage that it does not require large amounts of protein or radioactivity, and low levels of other proteins can be tolerated as impurities in the p53 preparation. More complete proteolysis, usually with trypsin, gives rise to small peptides. These can then be separated by electrophoresis and chromatography to give rise to characteristic patterns. In the early studies tryptic peptide patterns of [^{35}S]methionine-labeled p53 were used to show that p53 was very different in sequence from SV40 large-T and to show that p53s from different species were similar to each other (May *et al.*, 1979; Smith *et al.*, 1979a; Simmons *et al.*, 1980; Stitt *et al.*, 1981). Rat and mouse p53 were closely similar and shared most of their [^{35}S]methionine-labeled tryptic peptides, whereas mouse and human p53 shared only about half their tryptic peptides. All of this was consistent with p53 being entirely host coded. An example of a tryptic peptide comparison is given in Fig. 9 where both [^{35}S]cysteine and [^{35}S]methionine were used as labels so as to cover a larger fraction of the total amino acid sequence (Crawford *et al.*, 1979a). More recently, tryptic peptide patterns have been used to show that p53 synthesized *in vitro* is similar to that isolated from SV40-transformed or normal cells and synthesized *in vivo* (Maltzman *et al.*, 1981). A few (three to five) additional peptides were seen in the *in vivo*-labeled p53 from the SV40-transformed cells, possibly resulting from post-translational modifications of the protein in these cells (see Section VIII,B). If this is the correct explanation, phosphorylation does not seem to be involved (Maltzman *et al.*, 1981). In a similar study on rat p53, May *et al.* (1982) found that the *in vivo* p53 gave rise to one peptide in addition to those found in the digest of *in vitro*–synthesized p53.

The more basic protein chemical data for p53 such as amino acid composition and N-terminal sequence are not yet available, largely because of the difficulty in getting sufficient pure protein for analysis. The most promising source of protein for protein chemistry appeared to be EBV- transformed cells, but as already mentioned the protein characterized by Jornvall *et al.* (1982) does not appear to be p53. Some data of this sort should be available for p53 both directly from protein chemistry and from sequencing of the gene for p53 (Section IX,B).

Before considering the question of how p53 correlates with transformation it is necessary to have reliable estimates of the amount of p53 in a variety of cells and under a variety of conditions. As already mentioned

FIG. 8. Human p53 phosphoproteins are resolved into several species on two-dimensional gels. The upper panel shows [^{35}S]methionine-labeled SV80 (SV40-transformed human fibroblasts) and the lower panel shows $^{32}P_i$-labeled SV80 cells. The insets show the corresponding regions of gels from human tumor cell lines (Table III) and demonstrate the similarity of p53s from SV40- and nonSV40- transformed human cells [Taken from Crawford *et al.* (1981) with permission.]

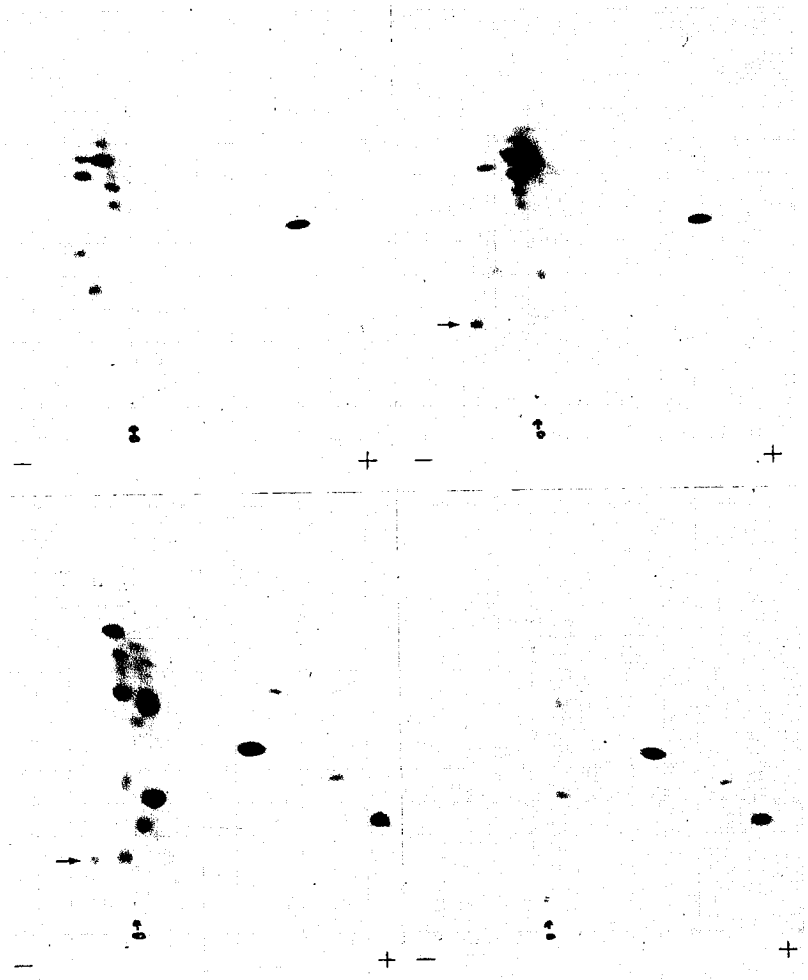


FIG. 9. Comparison of p53 from two different transformed mouse cells. p53 from two derivatives of 3T3 cells, PyA31C2 polyoma-transformed and SVA31E7 SV40-transformed cells, were digested with trypsin and resolved by electrophoresis at pH 4.5 (horizontal dimension) and chromatography (vertical dimension). The upper panel shows [^{35}S]methionine- labeled peptides, and the lower panel shows [^{35}S]cysteine-labeled peptides. In each case the polyoma-transformed cell p53 is on the right and the SV40-transformed cell p53 is on the left. Note that there are only minor differences between the tryptic peptide patterns from the two cell lines (arrows). [Taken from Crawford *et al.* (1979b). *Cold Spring Harbor Symposia on Quantitative Biology* **44**, 179–187. Copyright © 1980 Cold Spring Harbor Laboratory.]

(Section IB), the basic problem is one of amount, and in general estimates have been based on incorporation of [^{35}S]methionine into p53. Two conditions of labeling give easily interpretable results. Short-pulse labeling gives incorporation which is a reflection of the *rate of synthesis* of p53 as compared with other proteins in the cell, so long as the specific activity of the precursor pool is always the same and remains constant during the pulse. The other alternative is very long labeling, i.e., for at least one cell generation time so that all proteins in the cell approach equal specific activity. The amount of incorporation into p53 relative to the total incorporation into the cell then becomes a reflection of the *amount* of p53 in the cell. For practical reasons, labeling periods are usually neither long enough nor short enough to satisfy either of these conditions. Even so it is frequently stated that the amount of p53 is high or low, based on the amount of incorporation. This is only true if the precursor pools are similar in the cells being compared and, more importantly, if the rate of turnover of p53 is the same. The latter condition is often not met, and the conclusion reached can be very misleading. The early estimate of the amounts of p53 in SV40-transformed or abortively infected cells as compared with the same cells mock infected gave values of $<2\%$ for the uninfected mouse cells (transformed cells = 100%). This was using a 4-hour pulse of [^{35}S] methionine (Linzer *et al.*, 1979), and it established clearly that mouse 3T3 cells in culture did contain p53, although in low amounts. At the same time it was shown that two teratocarcinoma cell lines, F9 and PCC4, incorporated high levels of [^{35}S]methionine, 11 to 55% of the SV40-transformed cell line. This seemed to indicate that high levels of p53 could be found in non-SV40-transformed cells, but although this is true, the results with embryonal carcinoma cells are misleading if they are taken to indicate high levels of p53.

Similar labeling studies on p53 in cultures from embryos gave results which encourged the idea that p53 was a fetal antigen. This would fit in well with the results obtained with embryonal carcinoma cells. The method of assaying p53 was to make primary cultures from embryos at successive stages of gestation and label for 3 hours with [^{35}S]methionine (Mora *et al.*, 1980; Chandrasekaran *et al.*, 1981b). Early embryos gave high levels of incorporation into p53, but after 12 days the level began to fall and was very low by 16 days gestation. The high level of incorporation obtained with cultures from early embryos was only seen with primary cultures; secondary cultures gave no detectable p53. The relative amounts of incorporation into transformed cells, teratocarcinoma cells, and early embryo

cultures were 100, 48, and 45 to 52, respectively (Chandrasekaran *et al.*, 1981b). Embryonal carcinoma cells and early embryo cultures are therefore very similar in this respect.

The problem of measuring the amount of p53 protein directly has now been solved by the use of monoclonal antibodies in radioimmunoassays. These assays are extremely sensitive and can be highly specific so that reliable figures for the relative amounts of p53, if not absolute amounts, can be determined routinely. One assay described by Lane *et al.* (1982) makes use of antibodies PAb204 (3C4; Lane and Hoeffler, 1980) and PAb421 and competition between free p53 and p53 complexed to SV40 large-T. In another assay (Benchimol *et al.*, 1982) advantage has been taken of the moderate number of anti-p53 monoclonal antibodies now available by using them in pairs to set up a "sandwich"-type assay. One monoclonal antibody is bound passively to the wells of PVC microtiter plates. Cell extract is then

TABLE IV
RELATIVE LEVELS OF p53 IN MOUSE CELLS

Cell	Description	Amount of p53[a] (%)
SVA31E7	SV40-transformed BALB/c 3T3	100
MCA	Methylcholanthrene-induced C57BL/6 fibrosarcoma	31
CQ15	Spontaneously transformed C3H.Q embryo fibroblast	19
3T6	Mouse embryo fibroblast Swiss	16
Py A31C2	Polyoma-transformed BALB/c 3T3	16
PYS	Parietal yolk sac carcinoma	3
PCC4	Embryonal carcinoma	2
F9	Embryonal carcinoma	2
F9 AC Cl9	Differentiated carcinoma	2
WME 10	Whole mouse embryo, 10 days gestation	≤1
WME 12	Whole mouse embryo, 12 days gestation	≤1
WME 14	Whole mouse embryo, 14 days gestation	≤1
WME 15	Whole mouse embryo, 15 days gestation	≤1
BMK	Baby mouse kidney, strain TO	≤1
3T12	Mouse embryo fibroblast BALB/c	2
3T3.A31	Mouse embryo fibroblast BALB/c	≤1
3T3K	Mouse embryo fibroblast Swiss	≤1
L929	Methylcholanthrene-induced tumor	≤1
NS1	Mouse myeloma	≤1
Thymocytes	Baby mouse thymus, strain TO	≤1

[a] The amounts of p53 in detergent extracts of the listed cells were assayed by one of the radioimmunoassays described by Benchimol *et al.* (1982) for mouse p53 using PAb421 as first antibody and ^{125}I-labeled RA3-2C2 as probe. All the values corrected for the total protein content of the extract are given relative to SVA31E7 as 100%. The values in the table are taken from Benchimol *et al.* (1982) and S. Benchimol (personal communication). The origins and characteristics of the cell lines used are given in Benchimol *et al.* (1982).

added and the p53 is allowed to react with the antibody. The second antibody, radioactively labeled with ^{125}I, then acts as a probe for the bound p53. The amount of ^{125}I bound provides a measure of p53. Not all the antibodies listed in Table II can be used with each other since they block each others' binding in some combinations (Crawford, 1982b). However, this antibody set defines at least four independent determinants on p53 and this is more than sufficient for the purposes of the assay. By checking that consistent results were obtained with several antibody pairs the possibility was ruled out that some undefined peculiarity of one antibody distorted the estimates. The range of values obtained for p53 content of different cells, normalized for protein content, was more than 100-fold (Table IV). By this assay, teratocarcinoma cells contain 1 to 3% of the amount of p53 contained in SV40-transformed cells (SVA31E7) (Benchimol *et al.*, 1982). The same is true for 15-day mouse embryos where we found high levels of incorporation into p53 and low levels of p53 protein (<1% of SVA31E7). Earlier embryos gave higher levels of incorporation but also contained low levels of p53. It is possible to argue that the radioimmunoassay is affected by variables such as the state of aggregation of p53; however, if we accept the results of the assay as being of the right order, then we must conclude that the data on levels of p53 derived from labeling experiments are often suspect. High levels of incorporation can mean high levels of p53 or high rate of synthesis of p53, or some combination of the two, and additional information is needed to decide between these alternatives.

VIII. Cellular Location and Control of p53

A. Cellular Location of p53

In cells transformed or infected with SV40, p53 is associated with large-T antigen and, as a consequence, its location is nuclear (McCormick and Harlow, 1980). The prime function of SV40 large-T for the virus appears to be the initiation of viral DNA replication. It binds specifically to the region of the genome that acts as the origin of bidirectional DNA replication (Jessel *et al.*, 1975; Reed *et al.*, 1975). The association of p53 with large-T does not appear to influence this binding (Reich and Levine, 1982). Apparently the regions on the large-T molecule involved in the binding to p53 and to the origin of replication are quite separate from each other. The association of p53 with large-T is quite stable at acid or neutral pH and nuclei extracted from SV40-transformed cells retain most of their p53. Extraction with detergent at high pH (8 or 8.5) elutes both large-T and p53 without disrupting the nuclei (Tegtmeyer and Andersen, 1981). In cells not

transformed by SV40 it is much more difficult to locate p53 unequivocally, except where substantial amounts of the protein are present. Using the anti-p53 monoclonal antibody 200.47, Dippold *et al.* (1981) were able to show immunofluorescence which was strong on SV40-transformed cells, intermediate on methylcholanthrene-transformed cells, and weak with sarcomas and leukemias. All these were transformed mouse cells and none of the normal mouse cells tested showed immunofluorescence.

In general it appears that cells with substantial amounts of p53, whether SV40 transformed or not, show nuclear fluorescence, both with anti-p53 monoclonal antibodies and with polyclonal anti-p53 serum (McCormick and Harlow, 1980). This is in agreement with cell fractionation studies which gave the first indications that p53 was a nuclear protein in SV40-transformed cells (Anderson *et al.*, 1977; Chang *et al.*, 1977). The situation in normal cells, or in transformed cells where the amount of p53 is small, is less clear. The fluorescence observed is correspondingly weak and it is difficult to be sure that it is p53 specific. Antisera may contain low levels of activity against proteins other than p53, and monoclonal antibodies may also react with such molecules, for example RA3-2C2 with a B lymphocyte-specific differentiation antigen and 200.47 with some nuclear component in human cells. However, both immunofluorescence and cell fractionation studies have indicated that p53 is present in the cytoplasm of normal mouse cells and may be concentrated around the nucleus (Rotter *et al.*, 1981). It is quite possible that the transformed cells have similar amounts of cytoplasmic staining which tends to be swamped by the bright nuclear fluorescence. In Abelson MuLV-transformed lymphoid cells p53 appears to be predominantly cytoplasmic (Rotter *et al.*, 1983). It is not clear whether p53 may move from nucleus to cytoplasm during the cell cycle in normal cells and in cells not transformed by SV40.

There is an extensive literature on the presence of large-T-related molecules on the surface of SV40-transformed cells, and in some cases p53 has also been detected by surface labeling, membrane fractionation, and immunoprecipitation with monoclonal antibodies (Soule and Butel, 1979; Soule *et al.*, 1980; Chandrasekaran *et al.*, 1981a; Santos and Butel, 1982). It is always difficult to be sure that surface-located molecules have not been released by lysis of dead cells and readsorbed on to other cells, but the weight of evidence now supports the idea that some fraction of both large-T and p53 may be surface located. One important distinguishing feature of plasma membrane-associated large-T found recently is that it carries covalently bound palmitate (Klockmann and Deppert, 1983).

Other studies have indicated that molecules like p53 are located on the cell surface and associated there with SV40 large-T in SV40-transformed cells. In SV40-transformed hamster cells, Schmidt-Ullrich *et al.* (1980) showed that plasma membrane preparations contained a protein of about

60,000 molecular weight which was coprecipitated with large-T. By these criteria it appeared to be p53 like, but other evidence, i.e., that it was a glycoprotein and had an isoelectric point at pH 4.5, would not be consistent with what is known of p53 in other species. In SV40-transformed mouse cells p53 had isoelectric points in the region of 6.5 to 7.0 (Crawford *et al.*, 1979a). In the Meth A, methylcholanthrene-transformed mouse cells examined by DeLeo *et al.* (1979), p53 had a p*I* of 6.3. In SV40-transformed human cells p53 had a p*I* of 6.7 to 7.2 and there was no evidence for glycosylation of the protein (Crawford *et al.*, 1981). It is quite possible that the form of p53 present on the cell surface is different from that in the nucleus, having additional carbohydrate which would increase its molecular weight and give a more acidic p*I*. The presence of p53 on the cell surface would help to explain why a host protein like p53 might become immunogenic. Several suggestions have been made to account for the fact that anti-p53 antibodies appear in the sera of tumor-bearing animals. In SV40-transformed cells the association of p53 with large-T could render it antigenic either simply by virtue of being associated with a foreign protein or by undergoing some allosteric change which then renders it immunogenic. The level of activity against p53 is usually much lower than against large-T (Lane and Crawford, 1979). This type of explanation is less easily tenable for the methylcholanthrene-transformed cells and the situation here is even more puzzling. Only certain lines of methylcholanthrene-induced tumor cells elicit anti-p53 antibodies (DeLeo *et al.*, 1979; Jay *et al.*, 1979). The two lines which are effective, Meth A and CMS4, could be contrasted with nine other lines of tumor cells, such as CMS3 and CMS5 which contain p53, but do not induce anti-p53 antibodies. The various lines CMS3, CMS4, CMS5, and Meth A are all distinct from the point of view of transplantation rejection and it is possible that p53 can only become immunogenic in association with the correct transplantation rejection antigen. The responses to p53 and TSTA are in general different, being humoral for p53 and cell mediated for TSTA, although there is also a humoral reaction to Meth A.

B. Control in SV40-Transformed Cells

One of the most surprising results in studies on p53 was the finding that the levels of translatable messenger RNA in untransformed mouse cells was the same as that in their SV40-transformed progeny (Oren *et al.*, 1981). The levels of incorporation into p53 in long pulses are very different in these cells, as are the amounts of p53 measured directly (Benchimol *et al.*, 1982). The difference in protein level was about 50-fold and yet the messenger RNA levels were less than 2-fold apart (Oren *et al.*, 1981). This evidence suggests that there is no regulation at the transcriptional level. With short

pulses of methionine (1 or 2 hours) the rates of p53 protein synthesis in normal and transformed cells were different but by much less than 50-fold, indicating that control there was not predominantly at the translational level. Evidence for control at the posttranslational level was obtained by comparing the rates of degradation of p53 in normal and transformed cells. The half-life of p53 in transformed cells is greater than 24 hours, whereas that in normal cells is less than 0.5 hours. This is so short a half-life that it is difficult to measure accurately, and the half-life difference could easily be 50-fold, similar to the difference between p53 levels in normal and transformed cells and accounting entirely for it. The protein in transformed cells is not markedly different from p53 in normal cells, possibly having three to five altered tryptic peptides (Maltzman *et al.*, 1981), but the association with large-T appears to be essential for the stabilization of p53. The temperature-sensitive function of large-T present in *tsA58* does affect the stability of p53 in the transformed cells (Linzer *et al.*, 1979; Greenspan and Carroll, 1981). Here again the messenger RNA level is similar in the *SVTERtsA58Cb* cells at high, nonpermissive temperature and at the low, permissive temperature, although there is a 100-fold difference in the amount of p53 labeled *in vivo* at the two temperatures after pulse and chase (Oren *et al.*, 1981). It is not clear if the tryptic peptide differences between *in vivo*- and *in vitro*-synthesized p53 from SV40-transformed and normal cells (Maltzman *et al.*, 1981) are a cause or a consequence of the association with large-T. They are most likely the result of some sort of posttranslational modification and not of phosphorylation. There is no evidence for glycosylation of p53 from tunicamycin experiments (Crawford *et al.*, 1981) or endoglycosidase H digestion, but other modifications are possible. The amount of phosphorylation on p53 may be different in its various associations. The large-T associated with p53 is more highly phosphorylated than free p53 (McCormick and Harlow, 1980), and the adenovirus E1b 58K which is p53-associated is less phosphorylated than the rest (Sarnow *et al.*, 1982a). The p53 in the complex in *tsA58*-transformed cells was phosphorylated to about the same extent at the high and low temperature (Greenspan and Carroll, 1981). In studies on p53 in infected monkey cells phosphorylation of p53 was increased much more than synthesis, perhaps implying an increased phosphorylation of the newly made p53 (Harlow *et al.*, 1981a).

C. Control in Differentiating Cells

In contrast to the situation in SV40-transformed cells, where control of p53 appears to be at the posttranslational level, control can take place at the transcriptional level. Teratocarcinoma cells such as F9 in culture undergo

various sorts of differentiation when exposed to retinoic and cyclic AMP. These changes seem to be accompanied by changes in the level of translatable messenger RNA (Oren *et al.*, 1982). The changes in rate of synthesis of p53 seen in the intact cells *in vivo* are more or less paralleled by the change in synthesis of the protein *in vitro*. It must be remembered that these are not necessarily changes in the level of p53, and these levels are in fact similar and low in both differentiated and undifferentiated teratocarcinoma cells (Table IV; S. Benchimol, unpublished results). The half-life of the p53 in differentiated and undifferentiated F9 cells appeared to be the same (3.5 hours), so that this cannot have contributed to the changes in apparent rates of synthesis (Oren *et al.*, 1982).

Another situation in which rates of p53 synthesis seem to be regulated at the transcriptional level is in concanavalin A-stimulated lymphocytes (Milner and Milner, 1981) Section X,E). It is clear from these studies that control of p53 synthesis can be exerted at the transcriptional level as well as, or in addition to, the control over p53 levels mediated by changes in the stability of the protein.

Chandrasekaran *et al.* (1982) also studied F9 differentiation and compared a pluripotent embryonal carcinoma cell, OTT6050, with PYS-2, a differentiated parietal endoderm cell. Here again there was a decrease in the incorporation of [^{35}S]methionine into p53 in the differentiated derivative. The changes in p53 synthesis did not seem to be a result of changes in growth rate as far as could be determined. The half-life determined for p53 in PYS-2 cells was 200 minutes, compared with much more than 10 hours in F9 cells and SV40-transformed cells (Chandrasekaran *et al.*, 1982).

IX. The Gene(s) for p53

A. Chromosomal Location of the Gene(s) for p53

Until recently, satisfactory immunochemical reagents for discriminating between mouse and human p53 have not been available so that studies on human p53 in human–mouse hybrid cells have relied on the physical separation of the two proteins on polyacrylamide gels. Using this technique Stitt and Mangel (1981) concluded that if the gene for human p53 was located on a single chromosome, the most probable location was chromosome 7. With the large number of human–mouse hybrid cells available containing selected human chromosomes and monoclonal antibody specific for human p53, PAb1005 (Levine, 1982), it should be possible to confirm and extend these observations. Since p53 is heterogeneous on two-dimensional gels there may be several genes for p53. When DNA clones of a p53 gene become

available it should be reasonably straightforward to define how many genes there are for p53 and how much of the heterogeneity of p53 is due to differences of amino acid sequence and how much to post-translational modification.

B. Cloning of the Gene(s) for p53

Many of the critical questions about control of p53 and its synthesis can only be answered by looking directly at such things as messenger RNA levels. Similarly, until it is known whether there is one or several genes for p53, and whether these genes code for p53 species with different roles, much of the discussion of possible functions for p53 will be clouded with uncertainty. The most direct approach to answering these and many other questions is to clone the gene(s) for p53. DNA sequence data can then be obtained and translated into a predicted protein sequence, which can in turn be checked against amino acid sequence data from authentic p53 to confirm that the gene does correspond to p53.

Because p53 is a minor protein and its messenger RNA is also not very abundant, cloning is technically difficult. Normally it would be possible to use "+/−" screening with RNA preparations derived from cells with high and low (or zero) levels of the relevant messenger RNA. However, as already discussed, the level of translatable mRNA for p53 can be very similar in cells with high and very low levels of p53 protein. One mouse cell line with a high level of p53 messenger was used by Chumakov *et al.* (1982) to isolate the first clone of p53 cDNA. The screening procedure was hybrid selection, making use of the ability of the cloned cDNA to hybridize with p53 messenger RNA. Translation of the selected mRNA *in vitro* followed by immunoprecipitation of the radioactive p53 protein with the monoclonal antibody PAb122 completed the proof that the correct cDNA had been obtained. Other attempts to clone p53 using "+/−" screening have been made in several laboratories. Although they have not yet been successful in getting p53 clones, several interesting genes have been picked up (Schutzbank *et al.*, 1982; Scott and Rigby, 1983). Another approach that has been used successfully is via immunoprecipitation of polysomes. In cells synthesizing p53 the polysome fraction will contain p53 mRNA carrying ribosomes with growing polypeptide chains of p53. By attaching anti-p53 monoclonal antibody to those polypeptide chains that have reached a size large enough to generate the required determinant, these polysomes can be bound to staphylococcal protein A. Oren and Levine (1983) used the monoclonal antibody RA3-2C2 for this polysome immunoselection step. After removal of unbound polysomes the mRNA was extracted from the bound polysomes and used to generate cDNA. In theory, the enrichment of polysomal RNA for p53 mRNA by immunoprecipitation could be as much as 10,000-fold but in practice a 100-fold enrichment is normal and a 1,000-fold enrichment exceptionally high, according to Oren and Levine (1983). Their first clone, pp53-208, was composed of about 300 nucleotides from the 3′ noncoding region of the gene. The isolation of these first few p53 clones does constitute a major advance. It opens up the way for isolation of many more clones covering the whole of the coding sequence and for isolation of genomic DNA clones complete with flanking sequences and any introns that the p53 gene may contain. In the next phase of p53 research, we can expect a rapid expansion of our knowledge of the number and anatomy of p53 gene(s) and the control of their expression and, once the intact gene has been cloned, large-scale production of the protein in bacteria and eucaryotic cells by introduction of the gene for p53 into expressing vectors. This will in turn make it much easier to purify the protein to study its function by techniques such as microinjection. Overall this is a very exciting prospect.

X. The Function of p53

We still do not know the function of p53 in the metabolism of transformed or normal cells but there have been several suggestions.

A. Tumorigenicity

It was clear some time ago that while elevated levels of p53 frequently accompanied tumorigenicity, this correlation was by no means complete even with cell lines from malignant human tumors. In HeLa cells no evidence could be found for the presence of p53 labeled with either $^{32}P_i$ or [^{35}S]methionine followed by immunoprecipitation with the monoclonal antibody PAb122 (Crawford *et al.*, 1981). We have subsequently gone back to HeLa cells several times with other monoclonal antibodies and with other approaches, such as looking for a T-associated protein in these cells after SV40 infection, but still without getting any convincing evidence for the presence of p53 in these carcinoma cells. This is not a peculiarity of cervical tumor lines since another cervical carcinoma cell line C33I (Auersperg, 1964) had high levels of p53 by $^{32}P_i$ incorporation and by direct assay (Benchimol *et al.*, 1982). It is also not a peculiarity of HeLa cells, a line which has been in culture for a very long time. Two other cell lines from bladder carcinomas, EJ and T24 (Marshall *et al.*, 1977; Bubenik *et al.*, 1973), also appear to be negative for p53. These are among the most potent donor cell lines for DNA transformation studies (Section X,D), and the oncogenes that they carry have been characterized in considerable detail (Krontiris and Cooper, 1981; Shih *et al.*, 1981; Perucho *et al.*, 1981; Pulciani *et al.*, 1982). In conjunction with M. Barbacid we looked at the level of p53 (human) in the T24 bladder carcinoma cell line and at the level of p53 (mouse) in the lines of

NIH/3T3 cells which had been transformed by T24 DNA. There was no significant elevation of the p53 in any of the first or second cycle transfer transformed NIH/3T3 cell lines.

All of these negative findings are dependent on the methods of detection used, the specificity of the antibodies or antisera, and the level of sensitivity. This is equally true of the work with mouse cells. The earliest indication that spontaneously transformed cells which were tumorigenic in mice might lack p53 was obtained by immunoprecipitating extracts of [^{35}S]methionine-labeled cells with anti-T sera from tumor-bearing hamsters (Chang *et al.*, 1979). The line of cells used was 210 from AL/N mice. This line had transformed properties and some of its subclones (210C) had low tumorigenicity both in syngeneic and in nude mice (Mora and Chandrasekaran, 1982). Parallel lines of mouse fibroblasts also apparently lacked p53 and were highly tumorigenic, e.g., 104C and 219CT (Mora *et al.*, 1980), and SV40-transformed derivatives of 210 still retained their low tumorigenicity and showed normal labeling of SV40 large-T and p53 after immunoprecipitation with the same anti-T serum (Chang *et al.*, 1979). The problem here is that the p53 in the transformed cells could be precipitated either by virtue of its association with large-T or directly by interaction with anti-p53 antibodies. Only if these antibodies were present at a sufficiently high level in the serum used, and if the volume of serum added was adequate, could the negative result obtained with 210C and the highly tumorigenic 104C and 219CT AL/N cell lines be interpreted as showing that synthesis of p53 was lacking in these cells. The antiserum used was tested against F9 teratocarcinoma cells and did precipitate p53 in the absence of SV40 large-T (Mora *et al.*, 1980). On this basis then it should have detected p53 in extracts of 210C, 104C, and 219CT, if there was any significant amount present. This result could also be confirmed with the array of anti-p53 monoclonal antibodies now available, although in some ways a polyclonal serum gives a wider cover for p53 molecules with diverse antigenic properties.

Because SV40 induces a strong transplantation rejection antigen, it can appear to be an agent which prevents rather than causes tumors. Some spontaneously transformed AL/N mouse cell lines such as 104C are tumorigenic, but when they are transformed by SV40, they become T-antigen positive and 100-fold less tumorigenic (Mora *et al.*, 1977). The subsequent loss of T antigen is then correlated with a return to high tumorigenicity when the cells are again passed through mice to cause tumors. This is a rather artificial system but it does illustrate the difficulty of correlating tumorigenicity with other properties of the cell such as the presence of p53 or the rate of synthesis of p53. It is clear that these are lines of spontaneously transformed cells with high or low tumorigenicity which have low p53 levels by the criteria applied here. Different inbred strains of mice have been found to differ in the amount of p53 that their untransformed cells contain, as judged by [^{35}S]methionine incorporation in culture. An interesting correlation has been observed between the rates of SV40 transformation of the cells and their p53 content (S. Shen, personal communication). This would imply that the efficiency of SV40 transformation of mouse cells is to some extent dependent on the amount of p53 present in the cell before exposure to the virus. The amount of p53 does rise after SV40 infection (S. Benchimol, unpublished results) (Section VIIA).

Another example of a low-p53 line of transformed mouse cells has been described by Rotter *et al.* (1980). One line of C57L bone marrow cells transformed by Abelson MuLV L1-2 had a low p53 content as judged by the rate of [^{35}S]methionine or [^{3}H]leucine incorporation. The tumors produced in mice by this cell line were unusual in that they came up at the usual time but later regressed. After exposure of L1-2 to the tumor promoter TPA (12-0-tetradecanoyl phorbol-13-acetate) cells showed higher incorporation into p53, together with increased tumorigenicity, i.e., the tumors produced did not regress but rapidly killed the recipient mice (V. Rotter, personal communication). It could be argued that this is an instance where there is a correlation of p53 with progressive growth of the tumor, but it is a complex situation and there may be other changes in the cells caused by exposure to the TPA.

B. Embryonic Antigen

It was suggested by Mora *et al.* (1980) that the high level of incorporation they observed in cultures of mouse embryo cells taken from embryos early but not late in gestation would be consistent with p53 being an embryo antigen. The high level in transformed cells would then be another example of an embryonic antigen appearing in cells that have undergone malignant transformation. Such antigens are more often located on the cell surface rather than in the nucleus as is the case for p53. Examples of embryonic antigens would be carcinoembryonic antigen (CEA) found in colon cancer and α-fetoprotein found in liver tumors. As we have seen the level of p53 is in fact low in embryos—it is only the rate of synthesis which is high—so whether we should still think in terms of analogies between embryos and transformed cells is debatable. In any case, the function of embryonic antigens in the growth and differentiation of the embryo is not in general well defined, so that we would not really be saying anything very significant about the function of p53 if we included it among the embryonic antigens expressed in transformed cells.

C. Protein Kinase

Many metabolic processes are controlled by phosphorylation of enzymes at critical points in the pathways of synthesis or degradation. There was therefore a good deal of excitement when it was shown that the transform-

ing protein of Rous sarcoma virus *src* was a protein kinase which acted on itself, on immunoglobulin in immune complexes, and on a variety of host proteins in the transformed cell (Collett and Erikson, 1978). It is now known that about half of the RNA tumor viruses have transforming proteins with kinase activity (reviewed by Bishop, 1982). The activity is unusual in that the site phosphorylated is tyrosine rather than (or in addition to) the more usual serine and threonine (Hunter and Sefton, 1980; Collett *et al.*, 1980). Protein kinase activity specific for tyrosine has also been reported in association with polyoma virus middle-T antigen (Eckhart *et al.*, 1979; Schaffhausen and Benjamin, 1979; Smith *et al.*, 1979b). Since SV40 does not have a middle-T antigen it was not unreasonable to think that p53 might perform a similar function for SV40. Kinase activity had been found associated with SV40 large-T (Tjian and Robbins, 1979), although this activity does not seem to be intrinsic (Tjian *et al.*, 1979) and it is directed toward serine and not tyrosine (Griffin *et al.*, 1980). Kinase activity has also been detected in adenovirus-transformed cell extracts associated with the E1b 58K protein already mentioned in the context of complex formation with p53 (Section IV,B) (Branton *et al.*, 1981). As p53 is associated with both the adenovirus 58K protein and large-T, it could contribute this T-associated kinase activity. Direct assay of immune complexes of p53 with monoclonal antibody 200.47 did indeed show that there was kinase activity associated with p53 (Jay *et al.*, 1981). The amino acids phosphorylated were serine and threonine and the activity was highest with Mn^{2+} as a divalent cation rather than Mg^{2+}. Other monoclonal antibodies which react with p53, such as PAb421 (Harlow *et al.*, 1981b), also show kinase activity in immune complexes with p53, but none of the anti-p53 antibodies we tested seemed to inhibit the kinase activity (our unpublished results). The activity is extremely weak and the most likely explanation would seem to be that one or more of the kinases present in substantial amount in the cell binds to p53 and is coprecipitated with it to a minor extent. The alternative explanation, that p53 is a kinase but that the assay conditions normally used are far from optimal or that the substrate is wrong, cannot be ruled out. Certainly the activity seen to far is not analogous to that of RNA tumor virus-transforming proteins or polyoma virus middle-T in its amount, specificity, or cellular location.

D. Transforming Gene

One of the most exciting results obtained in this field in recent years has been the detection and cloning of DNA oncogenes from tumors and tumor cell lines (reviewed by Weinberg, 1982a). DNA is extracted from malignant human cells and then used to transform NIH/3T3 mouse cells by the calcium phosphate coprecipitation method. The efficiency of transformation varies greatly and by no means all tumors and tumor cell lines are active as donors, but it is clear that human DNA sequences are acquired by the transformed mouse cells together with the morphological changes which comprise the transformed phenotype. Different oncogenes come from different types of tumor and can be distinguished by restriction-site mapping and by DNA sequencing in the cases where they have been cloned. The gene products of the various oncogenes have been identified in some cases and these are also different in size and cellular location. Although the number of oncogenes may be smaller than at first thought (Weinberg, 1982b) the number is still substantial. This type of oncogene is thus clearly distinguishable from p53 since alteration of p53 is found in a wide variety of different tumors and, in addition, cells such as EJ and T24, which are good sources of transforming DNA, do not contain detectable p53. It seems, therefore, that p53 is not a member of this family of DNA oncogenes.

E. Cell Cycle-Related Protein

Mouse lymphocytes stimulated with concanavalin A show greatly increased DNA synthesis (Milner and McCormick, 1980; Milner and Milner, 1981). After exposure to concanavalin A for a few hours the cells were stimulated to synthesize p53 and, later, DNA. The time of p53 synthesis seemed to coincide with commitment to leaving the G_0 and entering the S phase. Inhibition of transcription during concanavalin A stimulation delayed the onset of p53 synthesis so that new messenger RNA synthesis seemed to be involved. Whether this was the synthesis of p53 messenger RNA or of the messenger RNA for some protein which stabilized p53 was not resolved, but as it was p53 synthesis, rather than amount of protein which was followed, it seems more likely that the effect was directly on p53 messenger RNA. The antibody used here, PAb421, reacts with mouse p53 and the results show clearly that p53 synthesis is affected by this type of stimulation, but it is not known whether the level of p53 protein is similarly affected.

A different approach also using monoclonal antibody has given further support to the idea that p53 is involved in cell division and DNA replication. Microinjection of anti-p53 antibody into the nuclei of 3T3 cells about to undergo serum stimulation largely prevented the normal DNA synthesis response (Mercer *et al.*, 1982). The antibodies used here were PAb122 and 200.47 together with an anti-Lyt 2.2 antibody as a negative control. The use of two anti-p53 antibodies with different specificities makes it very unlikely that reaction with proteins other than p53 can account for the results obtained. Both anti-p53 antibodies inhibited the serum stimulation of DNA

synthesis when they were injected into the nuclei of 3T3 cells 2 hours before or 2 hours after serum stimulation. The amount of inhibition varied from 31 to 55%, on the basis of the number of nuclei labeled with [^{3}H]thymidine. Injection into cells 4 to 17 hours after serum stimulation had no significant effect on DNA synthesis, showing that the antibodies were not exerting a general inhibitory effect on DNA metabolism. The theory initially put forward to explain the effect of a pulse of cycloheximide on DNA synthesis was that a labile gene product required for initiation of DNA replication was synthesized in the first 3 to 4 hours after serum stimulation (Novi and Baserga, 1972). This would fit in well with p53 being the labile gene product postulated by these and other workers (Campisi *et al.,* 1982) since its half-life in 3T3 cells is short. Injection of anti-p53 antibody did not affect DNA synthesis in SV40-infected 3T3 cells (Mercer *et al.,* 1982). The effect of SV40 T antigen on DNA synthesis seems therefore to be quite different, as though T antigen overrides the other controls, and this would clearly be an advantage to the virus in its replication, making it less dependent on the state of the cell before infection.

The rate of synthesis of p53 has been found to vary during the cell cycle in mouse cells synchronized by serum starvation (N. Reich, R. Thomas, and A. Levine, personal communication). Serum-starved 3T3 cells accumulate in G_0 and then, in readdition of serum, set off through G_1, S, and mitosis as a relatively synchronous population. Incorporation of [^{35}S]methionine into p53 is initially low and increases as the cells enter the S phase. The rate of synthesis peaks during the S phase and then falls as the cells leaves the S phase and enter mitosis. During the S phase, when DNA replication is taking place, the labeled p53 behaved as a nuclear matrix-associated protein (N. Reich, R. Thomas, and A. Levine, personal communication). Since newly synthesized DNA is also found associated with the nuclear matrix this would all be consistent with p53 being intimately involved in control of DNA replication.

XI. Conclusions

We now have a great deal of information about the amount and rate of synthesis of p53 in different cells and under different conditions, and we are in a much better position to address the question of the role of p53 in transformation. Initially it seemed that the situation was simple and that the presence of p53 was correlated with transformation. Detection of p53 in normal cells and tissues meant that this could not be correct and that we must look in more detail at the amount and type of p53 present in normal and transformed cells. Quantitation of p53 has proved difficult and only recently have reliable values been obtained by making use in radioimmunoassays of the anti-p53 monoclonal antibodies that are now available. The data from these assays show clearly that there is a good correlation in one sense, in that cells containing high levels of p53 are transformed. However, the correlation is by no means complete and there are clear examples of highly transformed cell lines, of both mouse and human origin, which have little or no p53 as far as our present techniques of detection and assay go. It is still possible that we are looking in a much too general way at p53 and that we should rather be looking at one particular form of p53 in one location in the cell. Further information on the function of p53 would be invaluable here in directing our attention to the active form of p53 and to its site of action. An example of the importance of a particular form of p53 may be phosphorylated p53, although it should be emphasized that this is still going to be a heterogeneous collection of molecules phosphorylated to varying extents at several different sites. Even so the correlation of phosphorylated p53 with transformation is better than that of p53 in general. Normal cells, and in particular normal thymocytes, contain p53, but it is not phosphorylated. Clearly we need much more detailed information on the sequence and strcture of p53 from normal and transformed cells. None of the information available at present excludes quite substantial differences in amino acid sequence between normal- and transformed-cell p53, let alone modification of all sorts. Even so it seems unlikely that differences of this sort could account for the apparent lack of phosphorylated p53 in malignant human tumor cell lines such as HeLa, EJ, and T24.

Probably the reason why the correlation of p53 with transformation is so incomplete is that it is a secondary consequence of the change which constitutes transformation. Certainly the correlation is poor and there is no reason to think of p53 being implicated in tumorigenicity. The most likely role for p53 in normal cells is as a control protein required for the onset of cellular DNA synthesis and replication. The experiments described in Section X,E give strong support for this idea and hold out a good prospect of major advances in the near future in our understanding of the function of p53. Such a role for p53 would be consistent with its presence at low levels and with its short half-life in normal cells, as well as with its synthesis immediately before cells enter the S phase. Changes in p53 leading to increased half-life of the protein would then have the consequence that the p53 which initiated one S phase would persist for long enough to push the cell without delay from mitosis into the next S phase, and so on. The involvement of p53 in one of the major pathways by which cellular DNA replication is controlled would also account for its rapid synthesis in the tissue of developing embryos, and its relatively short half-life here would also fit with the limited duration of rapid growth in the embryo which is followed by differentiation.

Changes in the amount and rate of turnover of p53 may become a com-

mon way of obtaining uncontrolled cell growth, but it can clearly not be the only way. For example, SV40 T antigen seems able to overcome the defect in cells that show temperature-sensitive DNA replication, and injection of anti-p53 antibodies into SV40-transformed cells does not interfere with subsequent DNA synthesis (Mercer *et al.*, 1982). This would imply that there are p53-independent pathways leading to DNA replication. A similar explanation could account for the tumor cells that lack p53 but are transformed themselves and act as a source of transforming DNA. The oncogene in EJ and T24 cells (Section X,D) appears to be closely related to *ras*^{H} (Der *et al.*, 1982; Parada *et al.*, 1982; Pulciani *et al.*, 1982; Santos *et al.*, 1982). This gene, previously characterized as coding for a transforming protein with a molecular weight of 21,000, is carried by Harvey murine sarcoma virus. The function of p21 in transformed cells is not known, but although it binds GTP strongly, it does not appear to be a protein kinase. The difference between the normal cellular p21 sequence and the transforming p21 sequence appears to be a single amino acid change pinpointing the nature and location of the critical change (Tabin *et al.*, 1982; Reddy *et al.*, 1982). As neither the EJ human tumor cells nor the transformed mouse 3T3 cells carrying the human transforming gene from these cells showed altered p53, we conclude that the p21 is involved in a p53-independent pathway leading to malignancy and uncontrolled cell growth.

Interest in p53 has been high for several years and we may now be at the end of the first phase. We can take a more balanced view of the importance of p53 in transformation and again emphasize the importance of p53 in normal cells. This may prove to be another instance of working with transformed cells and finding out more about normal cells. In any case there is little prospect of understanding how control over cell growth breaks down in malignant cells unless we have a better understanding of how control works in the normal cells from which they arise. As we enter the next phase of work on p53 applying the techniques of recombinant DNA technology in conjunction with the immunochemical approaches already established, the prospects for a continued rapid advance in our understanding of p53 and its function seem very good.

ACKNOWLEDGMENTS

It is a pleasure to thank the members of my laboratory and my colleagues for their generous help in providing figures, data, and helpful comments for this review. In particular I should like to thank S. Benchimol, K. Leppard, E. Harlow and C. Makin for permission to quote unpublished data and Drs. D. Lane, G. Klein, P. Real, L. Old, R. Clark, S. Chen, V. Rotter, N. Reich, R. Thomas, and A. Levine for personal communications. I am greatly indebted to Gina Yiangou for her help with the manuscript and the Audrey Symons for the preparation of the figures.

REFERENCES

Anderson, J. L., Martin, R. G., Chang, C., Mora, P. T., and Livingston, D. M. (1977). *Virology* **76**, 420.
Auersperg, J. (1964). *J. Natl. Cancer Inst.* **32**, 135.
Benchimol, S., Pim, D., and Crawford, L. (1982). *EMBO J.* **1**, 1055.
Bishop, J. M. (1982). *Sci. Am.* **246**, 68.
Branton, P. E., Lassam, N. J., Downer, J. F., Yee, S. P., Graham, F. L., Mark, S., and Bayley, S. T. (1981). *J. Virol.* **37**, 601.
Bubenik, J., Baresova, M., Villicky, V., Jakoubkova, J., Sainerova, H., and Donner, J. (1973). *Int. J. Cancer* **11**, 765.
Campisi, J., Medrano, E. E., Morreo, G., and Pardee, A. B. (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79**, 436.
Carroll, R. B., Muello, K., and Melero, J. A. (1980). *Virology* **102**, 447.
Chandrasekaran, K., Winterbourne, D. J., Luborsky, S. W., and Mora, P. T. (1981a). *Int. J. Cancer* **27**, 397.
Chandrasekaran, K., McFarland, V. W., Simmons, D. T., Diadek, M., Gurney, E. G., and Mora, P. T. (1981b). *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6953.
Chandrasekaran, K., Mora, P. T., Nagarajan, L., and Anderson, W. B. (1982). *J. Cell. Physiol.* **113**, 134.
Chang, C., Pancake, S. J., Luborsky, S. W., and Mora, P. T. (1977). *Int. J. Cancer* **19**, 258.
Chang, C., Simmons, D. T., Martin, M. A., and Mora, P. T. (1979). *J. Virol.* **31**, 463.
Chaudry, F., Harvey, R., and Smith, A. E. (1982). *J. Virol.* **44**, 54.
Clark, R., Lane, D. P., and Tjian, R. (1981). *J. Biol. Chem.* **256**, 11854.
Chumakov, P. M., Jotsova, V. S. and Georgiev, G. P. (1982). *Proc. Natl. Acad. Sci. U.S.S.R.* **267**, 1272.
Clayton, C. E., Murphy, D., Lovett, M., and Rigby, P. W. J. (1982). *Nature (London)* **299**, 59.
Cleveland, D. W., Fischer, S. G., Kirschner, M. W., and Laemmli, U. K. (1977). *J. Biol. Chem.* **252**, 1102.
Coffman, R. L., and Weissman, I. L. (1981). *J. Exp. Med.* **153**, 269.
Colby, W. W., and Shenk, T. (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5189.
Collett, M. S., and Erikson, R. L. (1978). *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2021.
Collett, M. S., Purchio, A. F., and Erikson, R. L. (1980). *Nature (London)* **285**, 167.
Cosman, D. J., and Tevethia, M. J. (1981). *Virology* **112**, 605.
Crawford, L. V. (1982a). *Adv. Viral Oncol.* **2**, vii.
Crawford, L. V. (1982b). *Adv. Viral Oncol.* **2**, 3.
Crawford, L. V., Cole, C. N., Smith, A. E., Paucha, E., Tegtmeyer, P., Rundell, K., and Berg, P. (1978). *Proc. Natl. Acad. Sci. U.S.A.* **75**, 117.
Crawford, L. V., Lane, D. P., Denhardt, D. T., Harlow, E. E., Nicklin, P. M., Osborn, K., and Pim, D. C. (1979a). *Cold Spring Harbor Symp. Quant. Biol.* **44**, 179.
Crawford, L. V., Pim, D. C., and Lane, D. P. (1979b). *Virology* **100**, 314.
Crawford, L. V., Pim, D. C., Gurney, E. G., Goodfellow, P., and Taylor-Papadimitriou, J. (1981). *Proc. Natl. Acad. Sci. U.S.A.* **78**, 41.
Crawford, L. V., Pim, D. C., and Bulbrook, R. D. (1982a). *Int. J. Cancer* **30**, 403.
Crawford, L. V., Leppard, K., Lane, D. P., and Harlow, E. E. (1982b). *J. Virol.* **42**, 612.
DeLeo, A. B., Jay, G., Appella, E., Dubois, G. C., Law, L. W., and Old, L. J. (1979). *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2420.
Denhardt, D., and Crawford, L. V. (1980). *J. Virol.* **34**, 315.
Der, C. J., Krontiris, T. G., and Cooper, G. M. (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3637.

Dippold, W. G., Jay, G., DeLeo, A. B., Khoury, G., and Old, L. J. (1981). *Proc. Natl. Acad. Sci. U.S.A.* **78,** 1695.
Eckhart, W., Hutchinson, M. A., and Hunter, T. (1979). *Cell* **18,** 925.
Fogh, J., and Trempe, G. (1975). *In* "Human Tumor Cells in Vitro" (J. Fogh, ed.), p. 115. Plenum, New York.
Gaudray, P., Rassoulzadegan, M., and Cuzin, F. (1978). *Proc. Natl. Acad. Sci. U.S.A.* **75,** 4987.
Gluzman, Y., Davison, J., Oren, M., and Winocour, E. (1977). *J. Virol.* **22,** 256.
Greenspan, D. S., and Carroll, R. B. (1981). *Proc. Natl. Acad. Sci. U.S.A.* **78,** 105.
Griffin, J. D., Spangler, G., and Livingston, D. M. (1980). *Proc. Natl. Acad. Sci. U.S.A.* **76,** 2610.
Gurney, E. G., Harrison, R. O., and Fenno, J. (1980). *J. Virol.* **34** *752.*
Harlow, E., Pim, D. C., and Crawford, L. V. (1981a). *J. Virol.* **37,** 564.
Harlow, E., Crawford, L. V., Pim, D. C., and Williamson, N. M. (1981b). *J. Virol.* **39,** 861.
Hunter, T., and Sefton, B. M. (1980). *Proc. Natl. Acad. Sci. U.S.A.* **77,** 1311.
Hutchinson, M. A., Hunter, T., and Eckhart, W. (1978). *Cell* **15,** 65.
Ito, Y., Brocklehurst, J. R., and Dulbecco, R. (1977a). *Proc. Natl. Acad. Sci. U.S.A.* **74,** 4666.
Ito, Y., Brocklehurst, J., Spurr, N., Griffiths, M., Hurst, J., and Fried, M. (1977b). *Colloq.—Inst. Natl. Sante Rech. Med.* **69,** 145.
Jay, G., DeLeo, A. B., Appella, E., Dubois, G. C., Law, L. W., Khoury, G., and Old, L. J. (1979). *Cold Spring Harbor Symp. Quant. Biol.* **44,** 659.
Jay, G., Khoury, G. DeLeo, A. B., Dippold, W. G., and Old, L. J. (1981). *Proc. Natl. Acad. Sci. U.S.A.* **78,** 2932.
Jessel, D., Hudson, J., Landau, T., Tenen, D., and Livingston, D. M. (1975). *Proc. Natl. Acad. Sci. U.S.A.* **72,** 1960.
Jornvall, H., Luka, J., Klein, G., and Appella, E. (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79,** 287.
Kessler, S. W. (1975). *J. Immunol.* **115,** 1617.
Klockmann, U., and Deppert, W. (1983). *FEBS Lett.* **151,** 257.
Köhler, G., and Milstein, C. (1975). *Nature* (*London*) **256,** 495.
Kress, M., May, E., Cassingena, R., and May, P. (1979). *J. Virol.* **31,** 472.
Krontiris, T. G., and Cooper, G. M. (1981). *Proc. Natl. Acad. Sci. U.S.A.* **78,** 1181.
Kuypers, B. (1982). M.Sc. Thesis, University of London.
Lane, D. P., and Crawford, L. V. (1979) *Nature* (*London*) **278,** 261.
Lane D. P., and Crawford, L. V. (1980). *Proc. R. Soc. London, Ser. B* **210,** 451.
Lane, D. P., and Harlow, E. (1982) *Nature* (*London*) **298,** 517.
Lane, D. P., and Hoeffler, W. K. (1980). *Nature* (*London*) **288,** 167.
Lane, D. P., and Koprowski, H. (1982) *Nature* (*London*) **296,** 200.
Lane, D. P., and Robbins, A. K. (1978). *Virology* **87,** 182.
Lane, D. P., Gannon, J., and Winchester, G. (1982). *Adv. Viral Oncol.* **2,** 23.
Levine, A. J. (1982). *Adv. Cancer Res.* **37,** 75.
Linzer, D. I. H., and Levine, A. J. (1979). *Cell* **17,** 43.
Linzer, D. I. H., and Maltzman, W., and Levine, A. J. (1979). *Virology* **98,** 308.
Lowe, J., Bird, P., Hardie, D., Jefferis, R., and Ling, N. R. (1982). *Immunology* **47,** 329.
Luka, J., Jornvall, H., and Klein, G. (1980). *J. Virol.* **35,** 592.
Luka, J., Sternas, L., Jornvall, H., Klein, G., and Lerner, R. (1983). *Proc. Natl. Acad. Sci. U.S.A.* **80,** 1199.
McCormick, F., and Harlow, E. (1980). *J. Virol.* **34,** 213.
McCormick, F., Chaudry, F., Harvey, R., Smith, R., Rigby, P. W. J., Paucha, E., and Smith, A. E. (1979). *Cold Spring Harbor Symp. Quant. Biol.* **44,** 171.
McCormick, F., Clark, R., Harlow, E., and Tjian, R. (1981) *Nature* (*London*) **292,** 63.

Maltzman, W., Oren, M., and Levine, A. J. (1981). *Virology* **112,** 145.
Marshall, C. J., Franks, L. M., and Carbonell, A. W. (1977). *J. Natl. Cancer Inst.* **58,** 1743.
May, P., Kress, M., Lange, M., and May, E. (1979). *Cold Spring Harbor Symp. Quant. Biol.* **44,** 189.
May, P., May, E., and Kress, M. (1982). *Adv. Viral Oncol.* **2,** 59.
Melero, J. A., Stitt, D. T., Mangel, W. F., and Carroll, R. B. (1979a). *Virology* **93,** 466.
Melero, J. A., Greenspan, D. S., and Carroll, R. B. (1979b). *Cold Spring Harbor Symp. Quant. Biol.* **44,** 201.
Mercer, W. E., Nelson, D., DeLeo, A. B., Old, L. J., and Baserga, R. (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79,** 6309.
Milner, J., and McCormick, F. (1980). *Cell Biol. Int. Rep.* **4,** 663.
Milner, J., and Milner, S. (1981). *Virology* **112,** 785.
Mora, P. T., and Chandrasekaran, K. (1982). *Biomembranes* **11,** 259.
Mora, P. T., Chang, C., Couvillion, L., Kuster, J. M., and McFarland, V. W. (1977). *Nature* (*London*) **269,** 36.
Mora, P. T., Chandrasekaran, K., and McFarland, V. W. (1980). *Nature* (*London*) **288,** 722.
Novi, A., and Baserga, R. (1972). *Lab. Invest.* **26,** 540.
O'Farrell, P. Z., Goodman, H. M., and O'Farrell, P. H. (1977). *Cell* **12,** 1133.
Oren, M., and Levine, A. J. (1983). *Proc. Natl. Acad. Sci. U.S.A.* **80,** 56.
Oren, M., Maltzman, W., and Levine, A. J. (1981). *Mol. Cell Biol.* **1,** 101.
Oren, M., Reich, N. C., and Levine, A. J. (1982). *Mol. Cell Biol.* **2,** 443.
Parada, L. F., Tabin, C. J., Shih, C., and Weinberg, R. A. (1982) *Nature* (*London*) **297,** 474.
Perucho, M., Goldfarb, M., Shimizu, K., Lama, C., Fogh, J., and Wigler, M. (1981). *Cell* **27,** 467.
Pintel, D., Bouck, N., diMayorca, G., Thimmappaya, B., Swerdlow, B., and Shenk, T. (1979). *Cold Spring Harbor Symp. Quant. Biol.* **44,** 305.
Prehn, R. T., and Main, J. M. (1957). *J. Natl. Cancer Inst.* **18,** 769.
Pulciani, S., Santos, E., Lauver, A. V., Long, L. K., Robbins, K. C., and Barbacid, M. (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79,** 2845.
Reddy, E. P., Reynolds, R. K., Santos, E., and Barbacid, M. (1982) *Nature* (*London*) **300,** 149.
Reed, S. I., Ferguson, J., Davis, R. W., and Stark, G. R. (1975). *Proc. Natl. Acad. Sci. U.S.A.* **72,** 1605.
Reich, N. C., and Levine, A. J. (1982). *Virology* **117,** 286.
Rotter, V., Witte, O. N., Coffman, R., and Baltimore, D. (1980). *J. Virol.* **36,** 547.
Rotter, V., Boss, M. A., and Baltimore, D. (1981). *J. Virol.* **38,** 336.
Rotter, V., Abutbul, H., and Ben-Ze'ev, A. (1983). EMBO Journal **2,** 1041.
Rundell, K., Collins, J. K., Tegtmeyer, P., Ozer, H. L., Lai, C. J., and Nathans, D. (1977). *J. Virol.* **21,** 636.
Santos, E., Tronick, S. R., Aaronson, S. A., Pulciani, S., and Barbacid, M. (1982). *Nature* (*London*) **298,** 343.
Santos, M., and Butel, J. S. (1982). *Virology* **120,** 1.
Sarnow, P., Ho, Y. S., Williams, J., and Levine, A. J., (1982a). *Cell* **28,** 387.
Sarnow, P., Sullivan, C. A., and Levine, A. J. (1982b). *Virology* **120,** 510.
Schaffhausen, B. S., and Benjamin, T. L. (1979). *Cell* **18,** 935.
Schmidt-Ullrich, R., Kahn, S. J., Thomson, W. S., and Wallach, D. F. H. (1980). *J. Natl. Cancer Inst.* **65,** 585.
Schutzbank, T., Robinson, R., Oren, M., and Levine, A. J. (1982). *Cell* **30,** 481.
Scott, M. R. D., and Rigby, P. W. J. (1983). *Cell* (submitted for publication).
Shih, C., Padhy, C., Murray, M., and Weinberg, R. A. (1981). *Nature (London)* **290,** 261.
Simmons, D. T. (1980). *J. Virol.* **36,** 519.

Simmons, D. T., Martin, M. A., Mora, P. T., and Chang, C. (1980). *J. Virol.* **34,** 650.
Smith, A. E., Smith, R., and Paucha, E. (1979a). *Cell* **18,** 335.
Smith, A. E., Smith, R. Griffin, B., and Fried, M. (1979b). *Cell* **18,** 915.
Soeda, E., Arrand, J. R., Smolar, N., Walsh, J. E., and Griffin, B. E. (1980). *Nature (London)* **283,** 445.
Soule, H. R., and Butel, J. S. (1979). *J. Virol.* **30,** 523.
Soule, H. R., Lanford, R. E., and Butel, J. S. (1980). *J. Virol.* **33,** 887.
Stitt, D. T., and Mangel, W. F. (1981). *Virology* **114,** 149.
Stitt, D. T., Carroll, R. B., Melero, J. A., and Mangel, W. F. (1981). *Virology* **111,** 283.
Stringer, J. R. (1982). *J. Virol.* **42,** 854.
Tabin, C. J., Bradley, S. M., Bargmann, C. I., and Weinberg, R. A. (1982). *Nature (London)* **300,** 143.
Tegtmeyer, P., and Andersen, B. (1981). *Virology* **115,** 67.
Tegtmeyer, P., Schwartz, M., Collins, J. K., and Rundell, K. (1975). *J. Virol.* **16,** 168.
Tegtmeyer, P., Rundell, K., and Collins, J. K. (1977). *J. Virol.* **21,** 647.
Tjian, R., and Robbins, A. (1979). *Proc. Natl. Acad. Sci. U.S.A.* **76,** 610.
Tjian, R., Robbins, A., and Clark, R. (1979). *Cold Spring Harbor Symp. Quant. Biol.* **44,** 103.
Todaro, G., Green, H., and Swift, M. R. (1966). *Science* **153,** 1252.
Van Roy, F., Fransen, L., and Fiers, W. (1981). *J. Virol.* **40,** 28.
Weinberg, R. A. (1982a). *Adv. Cancer Res.* **36,** 149.
Weinberg, R. A. (1982b). *Cell* **30,** 3.
Yang, Y. -C., Hearing, P., and Rundell, K. (1979). *J. Virol.* **32,** 147.

INTERNATIONAL REVIEW OF EXPERIMENTAL PATHOLOGY, VOL. 25

Surface Antigens as Markers of Mouse Macrophage Differentiation

S. HIRSCH AND S. GORDON

Sir William Dunn School of Pathology, University of Oxford Oxford, England

I. Introduction 51
II. Stem Cells and Progenitor Cells 52
III. Colony-Stimulating Factors 54
A. Characteristics and Classes 54
B. Colony-Stimulating Factor Action 55
IV. The Use of Monoclonal Antibodies to Study the Differentiation, Physiology, and Heterogeneity of Macrophages 56
A. Antigens Defined by Monoclonal Antimacrophage Antibodies 57
B. Macrophage Activation and Changes at the Cell Surface 65
C. Macrophage Heterogeneity 66
V. Conclusion 70
References 71

I. Introduction

Hemopoiesis is central to the maintenance of homeostasis and the control and curtailment of many pathological insults. The cells of the hemopoietic system are involved in constant processes of differentiation, maturation, and turnover, and are relatively accessible for study. Consequently, hemopoiesis has become an increasingly important model for the understanding of mammalian cell differentiation. The different cellular components of this system, comprising erythroid, myeloid, and lymphoid lineages, are derived from a single population of pluripotent stem cells (reviewed by Till and McCulloch, 1980). The cells within these lineages turn over at different rates under both physiological and pathological conditions, placing complex demands on differential recruitment from the stem cell pool.

Copyright © 1983 by Academic Press, Inc.
All rights of reproduction in any form reserved.
ISBN 0-12-364825-0