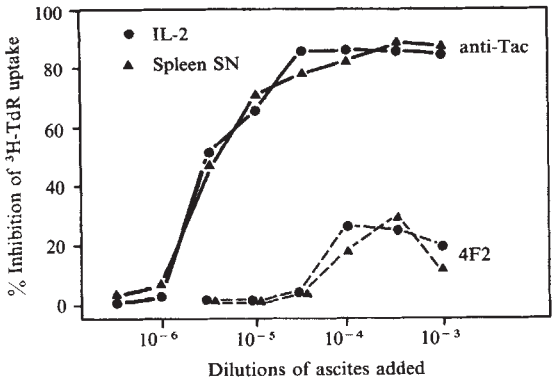

Fig. 4 Anti-Tac inhibits the IL-2-induced proliferation of *S. aureus*-activated B cells. Serial dilutions of anti-Tac or anti-4F2 were added to 5×10^4 spleen B cells that had been cultured for 3 days with optimal concentrations of *S. aureus* in round-bottomed 96-well microtitre plates. Spleen supernatant or recombinant IL-2 were added subsequently at a final concentration of 7 U ml^{-1} IL-2. Triplicate cultures were incubated for 72 h at 37 °C and 0.5 mCi of ^{3}H-TdR was added 18 h before collection (see also Fig. 1). Data are expressed as % inhibition of the response obtained in cultures receiving spleen supernatant or recombinant IL-2 alone (53,500 ± 3,400 c.p.m. and 46,800 ± 1,400 c.p.m., respectively).

We next investigated whether the IL-2-induced B-cell proliferative response could be inhibited by anti-Tac (in the absence of complement). The B-cell proliferative response to recombinant IL-2 or spleen supernatant was strongly inhibited by the addition of anti-Tac at a final dilution of 10^{-3} of anti-Tac ascitic fluid whereas anti-4F2 ascites, used as control, had minimal inhibitory effect even when added at final dilutions as high as 10^{-3} (Fig. 4). According to previous studies, supernatant of lectin-stimulated lymphocytes should contain lymphokines with BCGF activity in addition to IL-2. However, in our study, up to 90% inhibition of B-cell proliferation was achieved with the anti-Tac. Taken together, the present results suggest that IL-2 may be responsible for a large part of the BCGF activity generated by PHA-stimulated human spleen cells. Our data do not rule out the possibility that molecules other than IL-2 display BCGF activity. For example, supernatants of lectin-stimulated 72-h lymphocyte cultures have been reported to contain strong BCGF activity but little TCGF activity[2]; in addition, T-T-cell hybridomas have been described which released BCGF and not IL-2[13,14]. In any case, limiting dilution analysis of the precursors of IL-2-producing cells has previously indicated that as many as 60% of peripheral blood T cells have this functional potential[15], and it is therefore evident that a large fraction of human T cells can also influence B-cell responses via IL-2.

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Production of functional chimaeric mouse/human antibody

Gabrielle L. Boulianne*†‡, Nobumichi Hozumi*† & Marc J. Shulman*‡

* Department of Medical Biophysics, University of Toronto, and † Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario, Canada M4X 1K9
‡ Rheumatic Disease Unit, Wellesley Hospital, Toronto, Ontario, Canada M4Y 1J3

The availability of monoclonal antibodies has revived interest in immunotherapy. The ability to influence an individual's immune state by administering immunoglobulin of the appropriate specificity may provide a powerful approach to disease control and prevention. Compared with immunoglobulin from other species, human immunoglobulin (Ig) might be best for such therapeutic intervention; it might function better with the recipient's effector cells and should itself be less immunogenic. The success of the mouse hybridoma system suggests that immunoglobulin of virtually any specificity can be obtained from a properly immunized animal. In the human system, however, immunization protocols are restricted by ethical considerations, and it is not yet clear whether human antibody-producing cell lines of the required specificity can be obtained from adventitiously immunized individuals or from *in vitro* immunized cells. A method which might circumvent these difficulties is to produce antibodies consisting of mouse variable regions joined to human constant regions. Therefore, we have constructed immunoglobulin genes in which the DNA segments encoding mouse variable regions specific for the hapten trinitrophenyl (TNP) are joined to segments encoding human μ and κ constant regions. These 'chimaeric' genes are expressed as functional TNP-binding chimaeric IgM. We report here some of the properties of this novel IgM.

The variable regions used in the experiments described here are derived from the hybridoma cell line Sp6 which secretes IgM(κ) specific for TNP[1]. The specific κ gene[2] and μ gene[3] have been cloned and were used as a source of TNP-specific variable regions which were joined to cloned human μ and κ constant regions[4,5] in the vector pSV_2-neo[6] (Fig. 1).

To assay the chimaeric light- and heavy-chain genes independently of each other, we transferred these genes into appropriate mutant hybridoma cell lines derived from Sp6. The vector pN·χ-κTNP bearing the chimaeric κ light-chain gene, was transferred as described elsewhere[7–9] to the mutant cell line, igk14 (ref. 7). This cell line has lost the ability to produce the TNP-specific κ light chain (κ_{TNP}) but continues to synthesize the TNP-specific μ heavy chain (μ_{TNP}). In a similar manner, the vector pN·χ-μTNP bearing the chimaeric heavy-chain gene, was transferred to another mutant cell line, igm10 (ref. 3), which produces κ_{TNP} but no μ_{TNP}. To produce the totally chimaeric IgM, we transferred the vectors pN·χ-κTNP and pN·χ-μTNP together into the cell line, Sp2/0 (ref. 10), which produces neither heavy nor light immunoglobulin chains. Transformants were selected for resistance to the drug G418, then tested for their production of TNP-specific IgM, as measured by their ability to agglutinate TNP-coupled sheep red blood cells (TNP-SRBC). The frequency at which stable G418-resistant transformants were generated was found to be 10^{-3}. In experiments involving a single vector-transfer, approximately 30% of the resistant transformants produced detectable IgM. In the co-transfer experiment, where the chimaeric κ and μ were introduced into Sp2/0, we estimate that 0.1–1% of the transformants produced enough of both μ_{TNP} and κ_{TNP} to make detectable IgM. The selective advantage to co-transfer seems here to be significantly less than that reported by others using calcium phosphate co-precipitation[11] or protoplast fusion[12]. We have also transferred

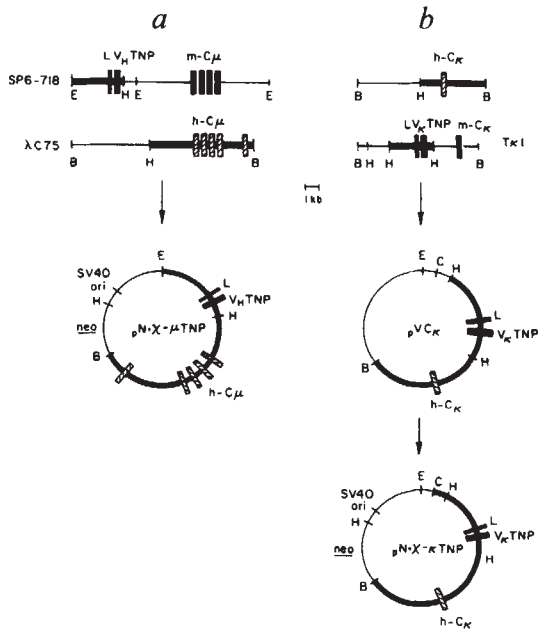

Fig. 1 *a*, Construction of the chimaeric heavy-chain gene. The 3.5-kilobase (kb) *Eco*RI–*Hin*dIII fragment containing the mouse TNP-specific heavy-chain variable region was obtained from the cloned TNP-specific heavy chain, Sp6-718 (ref. 3). This fragment, together with the 8.0-kb *Bam*HI–*Hin*dIII fragment containing the human constant region from the cloned segment λC75 (ref. 4), was introduced into pSV2-neo by ligation at the *Eco*RI–*Bam*HI site. *b*, Construction of the chimaeric light-chain gene. The 5.4-kb *Bam*HI–*Hin*dIII fragment containing the human C_κ gene was introduced into pBR322 by ligation at the *Bam*HI–*Hin*dIII site. The 4.2-kb *Hin*dIII–*Hin*dIII fragment containing the mouse TNP-specific light-chain variable region was obtained from the cloned TNP-specific light chain $T_\kappa 1$ (ref. 2) and introduced into the *Hin*dIII site of the pBR322 vector containing the human C_κ. From this plasmid, pVCκ, the 9.6-kb *Bam*HI–*Cla*I fragment containing the chimaeric light chain was obtained and introduced at the *Bam*HI–*Cla*I site of a modified pSV2-neo vector containing the *Bam*HI–*Eco*RI segment derived from pBR322. The chimaeric light-chain gene was also introduced into the vector pSV2-gpt[13] in the manner described above. Restriction enzymes: B, *Bam*HI; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII.

Sp2/0; in a subsequent step, transfer of the light-chain gene, carried in this case on the vector pSV2-gpt[13], was selected by resistance to mycophenolic acid. Stable transformants which produced the highest levels (~5 μg ml^{-1}) of IgM were selected for further study and cloned by limiting dilution. The vector pR-HLTNP, which bears in their entirety the mouse genes for TNP-specific μ and κ chains, was also transferred to the cell line Sp2/0, (refs 3, 14) and the IgM made by one such transformant, TSp2/mIgM12, is compared here with the chimaeric IgM made by the transformant TSp2/χ-IgM1. By using various antisera specific for antigenic determinants of the mouse and human μ and κ constant regions, we confirmed that the chimaeric genes encode chimaeric proteins, that is, the TNP-binding capacity of the mouse IgM is linked to antigenic determinants of human μ and κ chains (results not shown).

To determine whether the chimaeric IgM is pentameric, we analysed the biosynthetically labelled IgM secreted by these transformants using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The SDS-denatured chimaeric IgM from TSp2/χ-IgM1 migrates at nearly the same rate as pentameric mouse IgM produced by the hybridoma Sp6 and the transformant TSp2/mIgM12 (Fig. 2*A*), indicating that the chimaeric genes produce μ and κ chains which combine to form pentameric IgM. After reduction of the disulphide bonds, chimaeric μ and

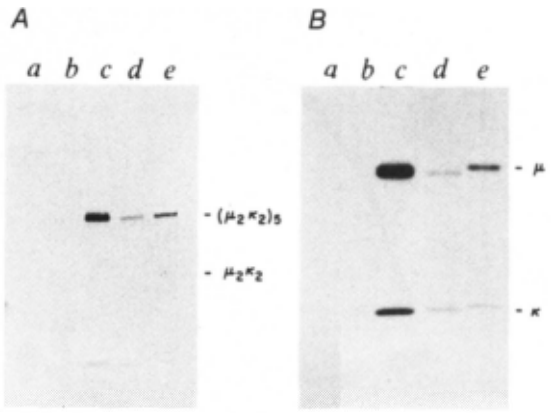

Fig. 2 Production of immunoglobulin heavy and light chains in transformants. The transformants expressing the chimaeric μ and κ genes are compared with two cell lines making TNP-specific murine IgM: the parental Sp6 hybridoma (Sp603 subclone) from which the μ_{TNP} and κ_{TNP} genes were cloned, and a transformant (TSp2/mIgM12) expressing the transferred murine μ_{TNP} and κ_{TNP} genes (denoted as cell line Sp2/T12 in ref. 14). The secreted IgM(κ) of the indicated cell lines was biosynthetically labelled with ^{14}C-leucine and subjected to SDS-PAGE as described[23]. Immunoglobulin from cell lines Sp2/0 (*a*), Sp6 (*c*) and TSp2/mIgM12 (*d*) was precipitated with anti-mouse IgM. Immunoglobulin from the cell lines Sp2/0 (*b*) and TSp2/χ-IgM (*e*) was precipitated with anti-human IgM. *A*, Material was not reduced so that the immunoglobulin disulphide bonds are intact. *B*, Material was treated with 2-mercaptoethanol to reduce disulphide bonds.

κ constant regions are nearly the same as the corresponding murine amino acid sequences[15]; therefore the difference in mobility does not reflect simply a difference in molecular weight. Work is in progress to determine the reason for these differences in mobility.

We used two methods to compare the binding sites of mouse and chimaeric IgM. First, we determined the affinity of these IgMs for TNP by measuring the ability of free hapten to inhibit the inactivation by these IgMs of TNP-coupled phage T4 (Fig. 3); the association constant of mouse and chimaeric IgM for the hapten TNP-cap (2,4,6-trinitrophenyl-ε-aminocaproic acid) was $1.3 \pm 0.5 \times 10^4$ M^{-1} and $1.5 \pm 0.5 \times 10^4$ M^{-1}, respectively. Within experimental error, we can detect no significant difference in affinity for TNP between the mouse and chimaeric IgMs.

We also compared the affinity of these IgMs for each of several trinitrophenyl-like compounds by measuring the ability of these compounds to block the agglutination of TNP-SRBC by IgM. Figure 4 illustrates the results obtained for TNP-cap and DNPy-Ala (3,5-dinitropyridine-β-alanine). The displacement of the inhibition curves indicates that the affinity of each IgM for DNPy-Ala is about threefold less than for TNP-cap. Table 1 summarizes the results obtained for other compounds. Again, these results suggest that the hapten binding sites are comparable in the mouse and chimaeric IgMs. The direct analysis of immunoglobulin structure predicted that immunoglobulin specificity should be unchanged when the same variable region is joined to different constant regions[16]. This prediction has been verified both in studies of IgM and IgD in normal cells[17] and in analyses of the binding specificity of the immunoglobulin made by hybridoma cell lines which have switched *in vitro*[18,19]. In addition, Sharon *et al.*[20] have shown that the substitution of a light-chain constant region for a heavy-chain constant region does not affect the affinity of the resulting protein. On the other hand, we can distinguish between the chimaeric and mouse IgMs using hapten inhibition of TNP-SRBC agglutination as a binding assay (Fig. 4). For each hapten, the curve for chimaeric

Table 1 Relative affinity of mouse and chimaeric IgMs for trinitrophenyl-like compounds

Hapten	mIgM	χ-IgM
TNP-cap	1.00	1.00
TNP-Lys	1.00	1.00
DNP-Lys	1.00	1.00
DNPy-Ala	3.00	3.00
NIP	>10	>25
NP	>10	>25

As described in Fig. 4 legend, we measured the ability of each hapten to inhibit agglutination of TNP-SRBC. TNP-cap was obtained from Dr G. D'Agostura; TNP-Lys (2,4,6-trinitrophenyl-L-lysine) and DNPy-Ala were obtained from Research Plus; NIP (4-hydroxy-3-iodo-5-nitrophenylacetic acid) and NP (4-hydroxy-3-nitrophenylacetic acid) were obtained from Sigma and Aldrich, respectively. The affinity of each IgM for the indicated haptens, relative to TNP-cap, was determined by the displacement of the inhibition curves. mIgM, murine IgM; χ-IgM, chimaeric IgM.

these IgMs have the same affinity for TNP-cap. In this context, the displacement of the agglutination curves suggests that the binding of the chimaeric IgM to TNP-SRBC is different in some way from that of the mouse IgM. The significance of this difference in molecular terms is unclear. Crystallographic analysis has suggested that there are several sites at which the variable region interacts with the first domain of the constant region[16]; such interactions may affect the binding site differently in the chimaeric and mouse IgMs. On the other hand, agglutination is a complex process involving the binding of multiple IgMs at each of the multiple sites. Subtle differences in aspects such as flexibility of the constant region may affect binding and thus the sensitivity to inhibition by free hapten. The molecular stress necessary to effect agglutination may distort the variable region in ways which would not usually occur while the immunoglobulin is free in solution.

We have also compared the chimaeric and mouse IgMs for their ability to activate complement. Culture supernatants were titred for their ability to promote lysis of TNP-SRBC in the presence of a source of complement, that is, guinea pig serum. Compared with the haemagglutination titre, the haemolysis titre for chimaeric IgM was about fourfold less than that for mouse IgM; we do not know whether this reflects a difference in the intrinsic ability of these IgMs to activate complement or the difference in TNP-SRBC binding discussed above.

Several therapeutic uses for specific antibodies have been proposed. The transfer of passive immunity by injecting specific immunoglobulins is a long-standing treatment. Other uses are more speculative. In animal models, anti-idiotype antibodies have been used both to elicit and to suppress the production of specific antibodies by the recipient animal[21]. These results might be extended to humans so that anti-idiotype antibodies could be used in some cases as a vaccine to enhance antibody production and in other cases to depress the destructive immune responses which cause autoimmune diseases. The identification of tumour-associated antigens might lead to the production of monoclonal antibodies that selectively destroy tumour cells *in vivo*[22]. However, as mentioned above, it may prove difficult to obtain human monoclonal antibodies having the specificities required for immunotherapy. Chimaeric immunoglobulins may provide a good compromise. For both monoclonal human immunoglobulin and chimaeric immunoglobulin, the constant region is expected to be non-immunogenic. We have no reason to expect the mouse variable region would in this form be more immunogenic than a human variable region of the same specificity.

In terms of DNA and protein, the chimaeric antibody system works well. The regulatory signals for RNA transcription, initiation, termination and splicing function, so that these genes express a high level of chimaeric μ and κ chains. The specificity

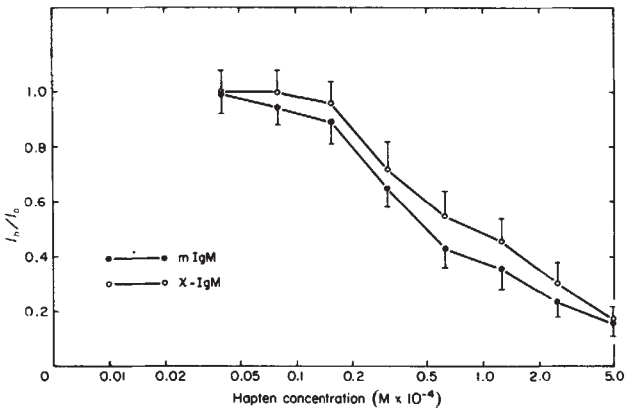

Fig. 3 Affinity of chimaeric and mouse IgMs for TNP. TNP was coupled to phage T4 as described elsewhere[24]. The affinity for hapten was calculated as follows. The rate-limiting step for the inactivation of phage T4 is the attachment of the first immunoglobulin binding site[25], that is, the concentration of surviving phage, Φ, is given by $\Phi = P \exp(-aB)$ where P = initial haptenated phage concentration before reacting with the anti-TNP, B = concentration of free binding sites and a = proportionality constant. By incubating free hapten in the reaction mix, the concentration of free binding sites can be manipulated according to the formula $K_a = [hB]/[h][B]$, where K_a is the association constant, $[h]$ the hapten concentration, $[B]$ the concentration of free binding sites and $[hB]$ the concentration of binding sites bound to hapten. The inactivation index, I_h, is defined as $\log \Phi(h)/P$ where $\Phi(h)$ is the phage concentration after incubation with anti-TNP in the presence of hapten at concentration h. The figure plots the ratio I_h/I_0 as a function of hapten concentration where I_0 is the inactivation index obtained in the absence of free hapten ($h = 0$). Each point was determined in triplicate. The affinity constant for the hapten can be calculated from any point as $K_a = 1/h[I_0/I_h - 1]$. The values of K_a given in the text represent the average of all points.

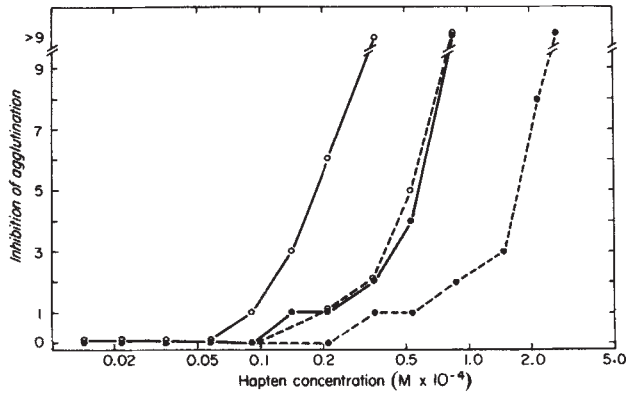

Fig. 4 Relative affinity of mIgM (●) and χ-IgM (○) for TNP (——) and DNPy (- - - -) haptens. Culture supernatants from the transformants TSp2/mIgM12 and TSp2/χ-IgM1 were serially diluted in twofold steps and incubated with either TNP-cap or DNPy-Ala at the indicated concentration in V-bottomed 96-well trays. TNP-SRBC were then added and the wells were scored for agglutination. The inhibition of agglutination, that is, the reduction in the number of wells with positive haemagglutination, is shown here for the indicated concentrations of each hapten. The displacement of the curves for the different haptens indicates the hapten concentrations required to yield the equivalent number of free binding sites, and thus measures the relative affinities of each IgM for the two haptens[26].

chains are covalently bound to form pentameric IgM which can activate complement. We have, nevertheless, detected differences in the binding to TNP-SRBC of the chimaeric and mouse IgMs. Further work is needed to assess whether these differences have significance for immunotherapy.

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Participation of p53 cellular tumour antigen in transformation of normal embryonic cells

Daniel Eliyahu, Avraham Raz*, Peter Gruss†, David Givol & Moshe Oren

Departments of Chemical Immunology and *Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel
†ZMBH, Universität Heidelberg, D-6900 Heidelberg 1, FRG

The cellular tumour antigen p53 is found at elevated levels in a wide variety of transformed cells (for reviews see refs 1, 2). Very little is yet known about the precise relationship of p53 to malignant transformation. Although the increase in p53 levels could be a secondary by-product of the transformed state, it is equally possible that p53 is actively involved in altering cellular growth properties, especially as it has been implicated in the regulation of normal cell proliferation[3-6]. We sought to test whether p53 could behave in a manner similar to known genes in a biological test system, and we demonstrate here that p53 can cooperate with the activated Ha-*ras* oncogene to transform normal embryonic cells. The resultant foci contain cells of a markedly altered morphology which produce high levels of p53. Cell lines established from such foci elicit tumours in syngeneic animals.

Recent findings have suggested certain similarities between p53 and the product of the oncogene *myc*. Both are DNA binding proteins (ref. 7 and D. Lane, personal communication) that accumulate in the nuclei of transformed cells[7,8]. Both are regulated with the cell cycle[6,9,10] and are induced at an early stage following the treatment of resting cells with mitogens[3,5,9,10]. Cycloheximide-treated cells accumulate p53[11] and show increased *myc* messenger RNA levels[9]. Detailed analysis of the amino acid sequences predicted for the two proteins shows weak similarities in both the overall molecular organization and the

Table 1 Transformation of rat and Chinese hamster embryo fibroblasts by various gene combinations

Transfected DNA	Foci per 10^6 cells: REF expt 1	REF expt 2	CHEF expt 3	Tumorigenicity
Carrier (BALB/c DNA)	0	0	0	
pEJ6.6	0	0	0	
pMSVp53G	0	0	ND	
pPyp53c	ND	ND	0	
pMSVp53G + pEJ6.6	13	5	ND	15/15
pPyp53c + pEJ6.6	ND	ND	20	
pLSVmyc + pEJ6.6	ND	21	ND	9/9
pLA8 + pEJ6.6	68*	ND	165*	
pMSVE + pEJ6.6	ND	0	ND	
None				0/8

Primary Fisher rat or Chinese hamster embryo fibroblasts were prepared[30] and maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 4 mM L-glutamine (maintenance medium). 10^6 cells were seeded per 90-mm dish, and were transfected with the indicated DNA combination after 1 day (10 μg of each plasmid, made up to a total of 25 μg with sheared BALB/c liver DNA). pLSVmyc was constructed linking the 5.6 kb *Bam*HI fragment of the murine c-*myc* gene (see ref. 14) to the SV40 early promoter. pLA8 contains the left-end 9.1% of the adenovirus12 genome, including the E1A and part of the E1B region[16]. pMSVE is a derivative of pMSVp53G, containing only the MSV enhancer inserted in the *Bam*HI site but no p53-specific sequences (compare with Fig. 1). The cells were transfected by the calcium phosphate procedure[31] for 16 h, glycerol-shocked (10% glycerol in maintenance medium for 90 s), replenished with maintenance medium, allowed to recover for an additional day, then split and reseeded at a density of 4×10^5 (rat fibroblasts) or 2×10^5 (Chinese hamster cells) per 90-mm dish. Medium was changed every 6-7 days. Distinct foci overgrowing the monolayer were scored 16 days (expt 1), 14 days (expt 2) or 24 days (expt 3) after replating of transfected cultures. REF, rat embryo fibroblasts; CHEF, Chinese hamster embryo fibroblasts. The tumorigenicity of cell lines established from corresponding foci was determined by injecting subcutaneously 5×10^6 cells into 5-8 day-old Fisher rats whole-body irradiated with 200 rads. As a non-transfected control (bottom line) we used REF propagated in culture to obtain a sufficient number of cells. Data are derived from two different p53 + Ha-*ras* lines and one *myc* + Ha-*ras* line. ND, not determined.
* Foci possessing a distinctly different morphology from that induced by pLA8 alone.

A biological test system demonstrating the involvement of the *myc* product in malignant transformation has been established recently[14,15]; primary rat embryo fibroblasts are transformed stably by the joint action of *myc* and another oncogene such as Ha-*ras*. Similar results are obtained when another nuclear oncogene, the adenovirus-2 E1A region, is assayed by co-transfection with Ha-*ras* in an analogous system[16]. In both cases, the transformation is visualized by the appearance of dense foci capable of overgrowing the monolayer of normal cells and are dependent on the presence of both oncogenes. This system is therefore a suitable test of the oncogenic properties of p53.

Two types of recombinant DNA constructs were used as templates for efficient p53 expression in transfected cells (Fig. 1). The plasmid pMSVp53G contains the 16 kilobase (kb) *Eco*RI fragment encompassing the functional murine p53 gene[12,17] juxtaposed to the enhancer portion of the Moloney murine sarcoma virus (MoMSV) long terminal repeat. This approach utilizes the presence of a functional promoter in the 16 kb fragment (B. Bienz, unpublished results). The second construct, pPyp53c, contains a stretch of p53 cDNA linked to the polyoma virus early promoter; this cDNA contains the intact coding region for p53[17] and directs the synthesis of authentic p53 in a heterologous system[18].