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# Isolation of Monoclonal Antibodies Specific for Human c-*myc* Proto-Oncogene Product

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**Six monoclonal antibodies have been isolated from mice immunized with synthetic peptide immunogens whose sequences are derived from that of the human c-*myc* gene product. Five of these antibodies precipitate p62$^{c\text{-}myc}$ from human cells, and three of these five also recognize the mouse c-*myc* gene product. None of the antibodies sees the chicken p110$^{gag\text{-}myc}$ protein. All six antibodies recognize immunoblotted p62$^{c\text{-}myc}$. These reagents also provide the basis for a immunoblotting assay by which to quantitate p62$^{c\text{-}myc}$ in cells.**

The human c-*myc* proto-oncogene is the human cellular homolog of the avian v-*myc* gene found in several leukemogenic retroviruses. Aberrant expression of the c-*myc* gene has been implicated in a number of neoplasms in a variety of avian and mammalian species.

The human c-*myc* gene has been sequenced (4, 5, 9, 25, 27). From the open reading frame in exons 2 and 3 of the gene it is possible to infer the existence of a 439-amino-acid gene product. From this sequence, synthetic peptide immunogens have been constructed. Antibodies raised against these peptides identify the human c-*myc* gene product as a protein with the mobility of a 62-kilodalton (kDa) protein on sodium dodecyl sulfate (SDS)–polyacrylamide gels, p62$^{c\text{-}myc}$ (13, 18, 20). Like the products of the avian v-*myc*-containing genes, p62$^{c\text{-}myc}$ is associated mainly with the nuclei of cells. Probably, then, it is within the cell nucleus that p62$^{c\text{-}myc}$ exerts its normal and oncogenic functions.

In an effort to derive better immunological reagents for the identification, assay, and study of mammalian c-*myc* gene products, we have isolated six monoclonal antibodies (MAbs), each specific for one of two synthetic peptides whose sequences are encoded within human p62$^{c\text{-}myc}$. In this report we describe the characterization of these reagents and outline their possible uses in the analysis of p62$^{c\text{-}myc}$ levels in cells.

## MATERIALS AND METHODS

**Cell lines.** The human colonic apudoma Colo 320 HSR (2), cervical carcinoma line HeLa, and the promyelocytic leukemia line HL60 (28) were all obtained from the American Type Culture Collection. The small cell lung carcinoma line N417 (17) was a gift from J. Minna (National Cancer Institute, Bethesda, Md.). The human foreskin fibroblast line Detroit 532, the human embryonic kidney line Flow 4000, and the human fibroblast line MRC-5 were obtained from Flow Laboratories. All cell lines with a finite life were used at passage numbers between 12 and 25. The human T-lymphoma line CCRF-CEM was a gift from D. Pauza (Medical Research Council, Cambridge, United Kingdom). The mouse fibrosarcoma line SEWA Rec2A, which also possesses an amplified c-*myc* gene, is derived from the SEWA1R osteosarcoma (16).

**Antibodies.** The monoclonal anti-HLA (public) antibody W6/32 (3) was a gift from C. Milstein (Medical Research Council). The rabbit anti-c-*myc* peptide G polyclonal antibody (20) and the anti-avian *myb* protein MAb Myb2-2.24 (11) have both been described previously.

**Synthetic peptides.** Two synthetic peptides, corresponding to residues 408 through 432 (C-terminus peptide G) and residues 171 through 188 (peptide D) of the human p62$^{c\text{-}myc}$ sequence were gifts from R. Lerner (Scripps Clinic, La Jolla, Calif.) and have been described previously (20). For use as immunogens, the peptides were conjugated to keyhole limpet hemocyanin (KLH) by linkage through their cysteine residues.

**Isolation of monoclonal MAbs.** Ten female BALB/c mice were each immunized subcutaneously with 20 μg of peptide (as KLH conjugate) in Freund complete adjuvant. After 2 and 4 weeks, the immunization was repeated with peptide-KLH conjugate in Freund incomplete adjuvant. At 6 and 4 days before cell fusion, the mice exhibiting the highest antipeptide titers were boosted intraperitoneally with a further 20 μg of peptide in the form of an acetone precipitate of the KLH conjugate. These mice were sacrificed, their spleens were diasaggregated, and the resultant splenocytes were fused with SP2 myeloma cells as described previously (12). Hybrid cells were dispersed among 1,000 microtiter wells and allowed to grow for 14 days. Culture supernatants were then assayed for antipeptide activity by enzyme-linked immunosorbent assay (ELISA). Hybridomas from positive wells were immediately cloned and recloned by limiting dilution, grown up, and inoculated intraperitoneally into syngeneic mice. Ascites fluids were tapped, made 5 mM with respect to sodium EDTA, clarified by low-speed centrifugation, and stored at −20°C.

**Purification of MAbs.** MAbs were purified from culture supernatants by protein A-Sepharose affinity chromatography where possible. MAbs were purified from ascitic fluids as follows. Ascitic fluids were adjusted to pH 5 with citrate buffer and made 5% with respect to octanoic acid. The mixture was stirred for 30 min at room temperature (RT) and clarified by centrifugation at 10,000 × *g*, and the immunoglobulin in the supernatant was precipitated with 40% saturated ammonium sulfate. The precipitate was redissolved in deionized water and dialyzed against several changes of Tris-buffered saline (TBS; 25 mM Tris hydrochloride, 144 mM NaCl, pH 8.0). Purified MAbs were stored at −20°C.

**ELISA.** A 100-μl sample of a peptide solution (50 pmol/ml) in 0.1 M sodium bicarbonate buffer (pH 9.6) was aliquoted

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into each well of a 96-well microtiter plate (Immulon 2; Dynatech Ltd.). Peptide was allowed to adsorb overnight at RT in a humid environment. Microtiter plates were then washed four times in TBS containing 0.1% Tween 20 and once in TBS, and 200 μl of TBS containing 5% fetal calf serum was then added to each well. After a further 20 min at RT, plates were shaken dry and either used or stored at −20°C.

A 100-μl sample of antibody, diluted in TBS-fetal calf serum, was added to each microtiter well and allowed to bind for 30 min at RT. Wells were washed in TBS, and 100 μl of a 1/150 dilution of rabbit anti-mouse immunoglobulin–horseradish peroxidase conjugate (Dakopatts Ltd.) in TBS-fetal calf serum was added to each well. After a further 30 min at RT, wells were shaken empty and washed as before, and then 100 μl of substrate solution (0.5 mg of 2,2′-azino-di-C3-ethylbenz-thiazolin sulfonic acid per ml of 0.1 M citrate buffer [pH 5] containing 1 μl of 30% hydrogen peroxide per ml) was added to each well. After color development (about 30 min at RT) the optical density at 406 nm of each well was read with a Titertek multiscan ELISA plate reader.

For reuse of plates, wells were incubated in 200 μl of 50 mM diethylamine hydrochloride (pH 11.5) for 10 min, rinsed once in the same buffer and twice in TBS, and reblocked with TBS-fetal calf serum as described above.

**Immunoprecipitation.** Immunoprecipitation from labeled cell lysates was performed as described previously (11). Briefly, cells were labeled for 1 h in lysine-free medium containing 250 μCi of [$^3$H]lysine (75 to 100 Ci/mmol; Amersham Ltd., United Kingdom) per ml. Cells were then washed three times in ice-cold phosphate-buffered saline containing 0.1% sodium azide, lysed in 25 mM Tris hydrochloride–50 mM NaCl–0.5% Nonidet P-40–0.5% sodium deoxycholate–0.1% SDS–0.1% sodium azide (pH 8), sheared through a fine-gauge needle until no longer viscous, and clarified by centrifugation at 10,000 × *g* for 30 min at 4°C. Samples of cell lysates were preadsorbed with preimmune immunoglobulin-agarose conjugates and then incubated with agarose- or protein A-Sepharose-bound antibody. Purified antigens were fractionated on SDS-polyacrylamide gels (15), fluorographed in 2,5-diphenyloxazole–dimethyl sulfoxide (DMSO), dried, and visualized by exposure to prefogged film as described previously (6).

***Staphylococcus aureus* V8 protease mapping of antigens.** Antigens were immunoprecipitated from [$^3$H]lysine-labeled Colo 320 HSR cells and fractionated on a 12.5% SDS–polyacrylamide gel. The gel was equilibrated in 0.5 M sodium salicylate, dried down, and exposed to prefogged film at −70°C. Bands were identified by autoradiography and cut out, and the sodium salicylate was removed by rehydrating and washing the gel slices in deionized water. Digestion of the antigens with V8 protease was then carried out exactly as described previously (8).

**Immunoblotting.** Cell lysates, prepared in SDS sample buffer, were fractionated on SDS-polyacrylamide gels and electroblotted for 150 V·h onto nitrocellulose paper in electroblotting buffer (25 mM Tris, 192 mM glycine, 0.01% SDS, 20% methanol) with a Bio-Rad Trans-Blot cell. After blotting, nitrocellulose filters were washed briefly in TBS and incubated for 30 min in TBS containing 2% nonfat dried milk (Marvel; Cadbury's Ltd.). Filters were then incubated in a solution (10 μg/ml) of antibody diluted in TBS-fetal calf serum containing 0.5% Tween 20 for 2 h at RT, washed in the same buffer, and incubated for 2 h in $10^5$ cpm of affinity-purified F(ab)$_2$ fragment of rabbit anti-mouse immunoglobulin (specific activity, 20 μCi/μg of immunoglobulin) per ml. Filters were washed, dried, and exposed to prefogged X-ray film at −70°C with an intensifier screen.

**Preparation and analysis of RNA.** Total cell RNA was prepared as described previously (7) from log-phase cultures. A 10-μg sample of total RNA from each cell type was glyoxylated (24), fractionated on a 1.4% agarose gel, and transferred to nitrocellulose paper (23). RNAs were hybridized to nick-translated probe (22). The c-*myc* probe PUC-cDNA was a gift from T. Rabbits (19).

## RESULTS

**Isolation of c-*myc* peptide-specific MAbs.** We selected the sequences of the two peptides D and G by inspection of a hydrophilicity plot (14) of the deduced p62$^{c\text{-}myc}$ amino acid sequence. Both peptides D and G are from regions of the c-*myc* protein which are fairly hydrophilic (Fig. 1) and therefore likely to be exposed in the intact c-*myc* protein. The sequences of peptides D and G and their homologies with murine (5) and chicken v-*myc* (26) and c-*myc* (21) genes are shown in Fig. 2.

The peptides were conjugated with KLH, and the conjugates were used as immunogens. After three immunizations, the mouse responding best to peptide G had a half-maximum titer of 1/1,000, and the best responder to peptide D had a half-maximum titer of 1/700. Two separate cell fusions were carried out, one with each of these mice. Both fusions yielded in excess of 900 viable hybridomas, and those secreting peptide-specific antibodies, as detected by ELISA, were immediately cloned and recloned by limiting dilution. Only six stable peptide-specific secretors were eventually isolated from both fusions, three specific for each peptide. However, the number of wells initially positive for antipeptide activities was about twice that number. The majority of other hybridomas from each cell fusion secreted KLH-specific antibodies (data not shown).

Antipeptide MAbs were subclassed by ELISA with a commercial kit (Boehringer Mannheim Biochemicals). All ascitic fluids from mice inoculated with the various hybridomas showed substantial titers. We observed no cross-reactivity of any of the MAbs when tested against the inappropriate peptide immunogen. Characteristics of these c-*myc* peptide-specific MAbs are summarized in Table 1.

**Recognition of native c-*myc* protein by anti-c-*myc* peptide MAbs.** To test whether any of our peptide-specific MAbs

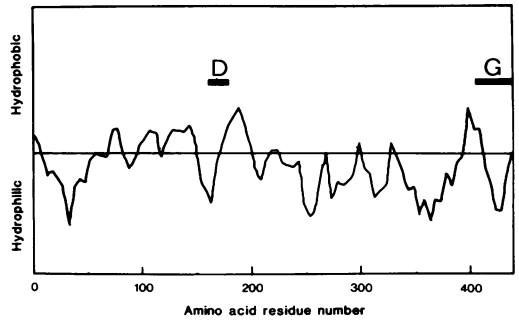


FIG. 1. Hydrophilicity plot of human c-*myc* product sequence. Hydrophilicity analysis of the human c-*myc* protein sequence was by the method of Hopp and Woods (14). The positions of the two synthetic peptides used as immunogens (peptides D and G) are indicated by the bars at the top of the figure.

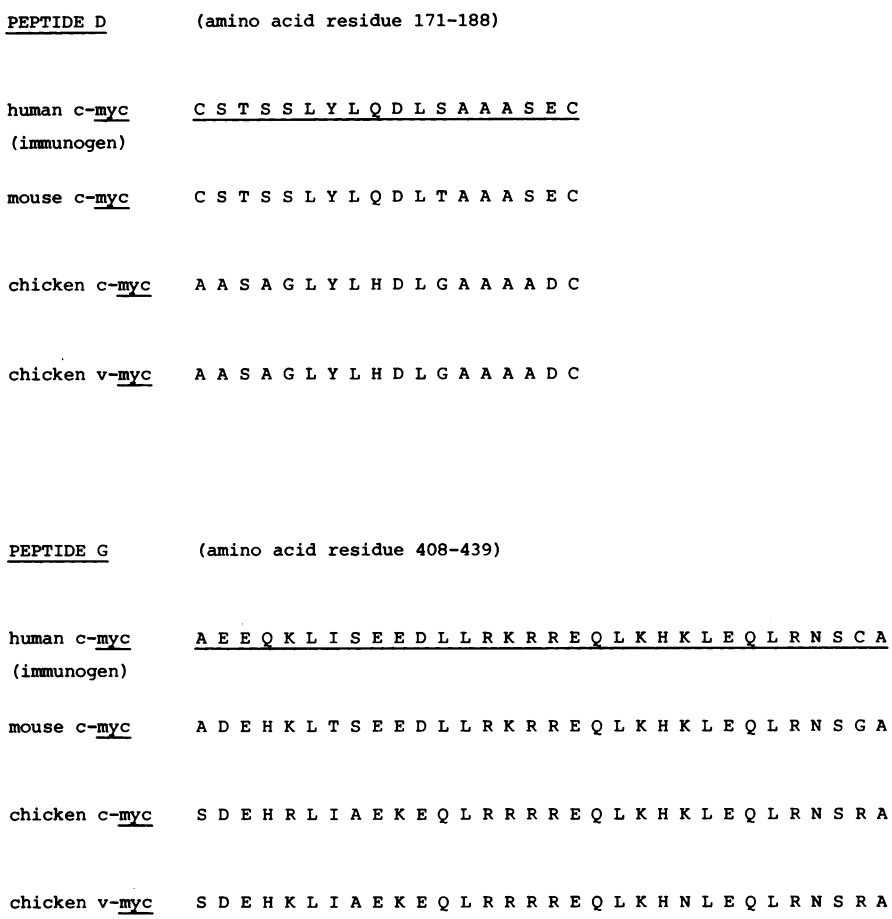


FIG. 2. Sequences of synthetic peptide immunogens and homologous sequences in mouse and chicken *myc* proteins.

recognized the intact native human c-*myc* protein, purified MAbs were coupled to agarose (Affi-gel 10; Bio-Rad Labs) at a concentration of 1 mg of antibody per ml of swollen gel, and the immobilized MAbs were assayed for their abilities to immunoprecipitate c-*myc* protein from labeled detergent extracts of Colo 320 HSR cells (Fig. 3B). Of the three MAbs specific for C-terminal peptide G, two (CT9-B7 and CT14-G4) precipitate a 62-kDa protein. All three of the MAbs specific for peptide D, Mycl-3C7, Mycl-8F9, and Mycl-6E10, immunoprecipitate a 62-kDa protein from cell lysates. A faint accompanying band at about 64 kDa can also be seen to be precipitated by all of these MAbs in some experiments. Following our earlier convention (20), we will henceforth refer to this 62-kDa–64-kDa doublet as p62. A 62-kDa protein of identical mobility is also recognized by a polyclonal rabbit antibody specific for peptide G which we have previously shown to recognize the human c-*myc* gene product, $p62^{c\text{-}myc}$ (20). The peptide G-specific MAb Mycl-9E10 does not immunoprecipitate any component from Colo 320 HSR cell extracts.

TABLE 1. Characteristics of anti-*myc* peptide MAbs

| MAb | Peptide specificity | IgG subclass | Binding to: | | |
|---|---|---|---|---|---|
| | | | Human $p62^{c\text{-}myc}$ | Mouse $p64^{c\text{-}myc}$–$p66^{c\text{-}myc}$ | Chicken $p110^{c\text{-}myc}$ |
| Mycl-8F9 | D | IgG1.k | + | + | – |
| Mycl-3C7 | D | IgG1.k | + | + | – |
| Mycl-6E10 | D | IgG1.k | + | + | – |
| CT14-G4 | G | IgG1.k | + | – | – |
| CT9-B7 | G | IgG1.k | + | – | – |
| Mycl-9E10 | G | IgG1.k | +[a] | – | – |

[a] Binds only immunoblotted $p62^{c\text{-}myc}$.

We tested the abilities of our $p62^{c\text{-}myc}$ MAbs to immunoprecipitate the $p110^{gag\text{-}myc}$ protein from MC29 virus-transformed quail fibroblasts. Of the four MABs tested, CT9-B7, CT14-G4, Mycl-8F9, and Mycl-6E10, none recognize $p110^{gag\text{-}myc}$ significantly, although both the rabbit polyclonal anti-peptide G antibody and an established anti-chicken v-*myc* protein-specific antibody (1) do so (Fig. 3A).

We next tested the abilities of our $p62^{c\text{-}myc}$-specific MAbs to recognize the mouse c-*myc* gene product. All three peptide D-specific MAbs immunoprecipitate a protein doublet of 64 kDa-66 kDA on an SDS gel from Rec2A cell lysates. Figure 3C shows a comparison of the protein recognized by one such MAb, Mycl-8F9, from Colo 320 HSR human and Rec2A mouse cells. An anti-HLA MAb is used as a control in this experiment. Clearly, the protein recognized by the peptide D-specific MAb Mycl-8F9 in mouse cells has a slightly lower apparent mobility than human $p62^{c\text{-}myc}$ on SDS-polyacrylamide gels and exhibits a more pronounced doublet nature. Our rabbit anti-peptide G-specific polyclonal antibody also recognizes this p64-p66 doublet in mouse cells (4; unpublished observations). We believe that this 64-kDa–66-kDa doublet is the product of the mouse c-*myc* gene. Neither of the two peptide G-specific

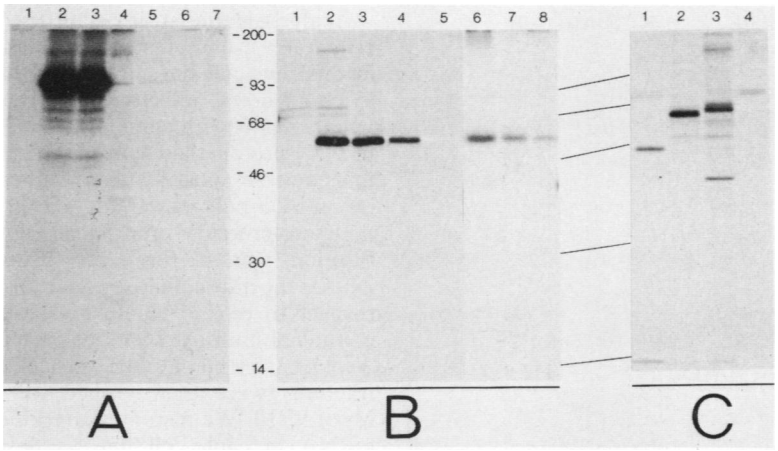


FIG. 3. Immunoprecipitation of *myc* proteins from human, mouse, and quail cells. MC29 virus-transformed quail fibroblasts (A), human Colo 320 HSR cells (B and C, tracks 1 and 2), and mouse Rec2A fibroblasts (C, tracks 3 and 4) were all labeled with [$^3$H]lysine for 1 h as described in Materials and Methods. Cells were lysed in 25 mM Tris hydrochloride–50 mM NaCl–0.5% Nonidet P-40–0.5% sodium deoxycholate–0.1% SDS–0.1% sodium azide (pH 8), and samples of each cell lysate were immunoprecipitated with antibodies. Purified antigens were fractionated on a 12.5% SDS–polyacrylamide gel and detected by autoradiography. Antibodies used were as follows. (A) Tracks: 1, preimmune rabbit serum; 2, rabbit anti-peptide G antibody; 3, rabbit antibody to chicken *myc* proteins; 4, MAb CT9-B7; 5, MAb CT14-G4; 6, MAb Mycl-8F9; 7, MAb Mycl-6E10. (B) Tracks: 1, preimmune rabbit serum; 2, rabbit anti-peptide G antibody; 3, MAb CT9-B7; 4, MAb CT14-G4; 5, MAb Mycl-9E10; 6, MAb Mycl-8F9; 7, MAb Mycl-3C7; 8, MAb Mycl-6E10. (C) Tracks: 1, W6/32 monoclonal anti-HLA antibody; 2, MAb Mycl-8F9; 3, MAb Mycl-8F9; 4, MAb W6/32 anti-HLA. Tracks 1 and 2 are immunoprecipitates from human cells, tracks 3 and 4 are from mouse cells. Molecular mass markers were myosin (200 kDa), phosphorylase *b* (93 kDa), bovine serum albumin (68 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), and lysozyme (14 kDa).

precipitating MAbs, CT14-G4 and CT9-B7, recognizes mouse $p64^{c\text{-}myc}$–$p66^{c\text{-}myc}$.

To ascertain whether or not the 62-kDa human antigens recognized by each of our MAbs are identical to each other and to $p62^{c\text{-}myc}$ recognized by our polyclonal rabbit anti-peptide G antibody, we subjected the purified antigens to partial *S. aureus* V8 protease fingerprinting (Fig. 4). Clearly, the peptide fingerprints of all of the 62-kDa antigens are identical. We have, in addition, carried out immunodepletion experiments in which we show that exhaustive immunoprecipitation of the 62-kDa antigen recognized by any one MAb concomitantly removes all of the antigen recognized by any of the other MAbs (data not shown). In conclusion, therefore, the same antigen is specifically recognized by a number of different antibodies which, between them, recognize at least two discontinuous sequences on the intact human c-*myc* protein. We feel this to be good evidence that the 62-kDa protein recognized by each of our peptide specific antibodies is the human c-*myc* protein, $p62^{c\text{-}myc}$.

**Immunoblotting analyses.** We next assayed the abilities of

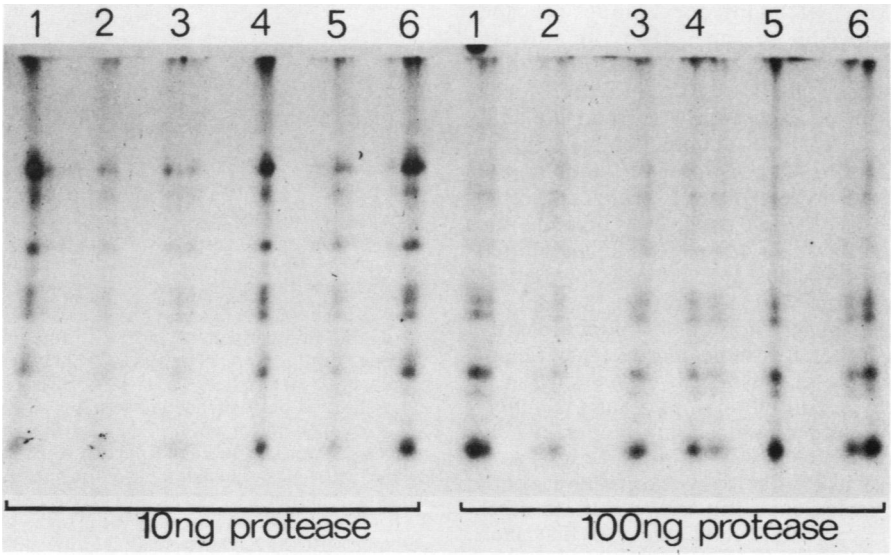


FIG. 4. *S. aureus* V8 protease fingerprints of antigens recognized by anti-c-*myc* peptide antibodies. [$^3$H]lysine-labeled antigens were precipitated from Colo 320 HSR cells and digested with one of two concentrations of *S. aureus* V8 protease per gel slot, 10 or 100 μg. Proteolyzed antigens were fractionated on a 15% SDS–polyacrylamide gel and detected by autoradiography. Tracks contained the following antigens: 1, rabbit polyclonal anti-peptide G; 2, CT9-B7; 3, CT14-G4; 4, Mycl-8F9; 5, Mycl-3C7; 6, Mycl-6E10.

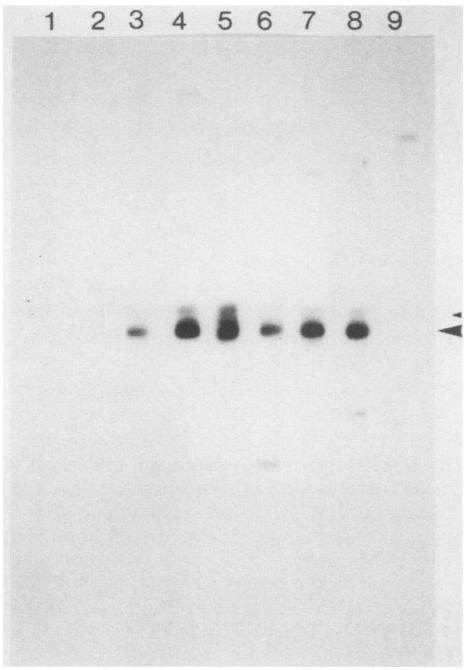


FIG. 5. Immunoblotting assay of anti-*myc* MAbs. Colo 320 HSR cells ($5 \times 10^7$) were dissolved in SDS sample buffer and fractionated on a 12.5% SDS–polyacrylamide gel. Fractionated proteins were electroblotted onto nitrocellulose paper, the blot was cut into longitudinal strips, and each strip was probed with a different antibody. Tracks contained the following MAbs: 1, normal mouse serum; 2, W6/32 anti-HLA; 3, CT9-B7; 4, CT14-G4; 5, Myc1-9E10; 6, Myc1-8F9; 7, Myc1-3C7; 8, Myc1-6E10; 9, Myb2-2.24 (anti-avian *myb* protein). Prestained marker proteins (Bethesda Research Laboratories) were run in tracks alongside cell lysates, and molecular weights of detected antigens were estimated by comparison with the positions of these markers.

our anti-peptide MAbs to recognize $p62^{c\text{-}myc}$ on immunoblots of Colo 320 HSR lysates fractionated on SDS-polyacrylamide gels. A total lysate of Colo 320 HSR cells was fractionated on a 10% SDS–polyacrylamide gel and electroblotted onto nitrocellulose paper. The blot was cut into longitudinal strips, and each strip was assayed with a different antibody. Approximately $5 \times 10^4$ Colo 320 HSR cell equivalents were present on each strip. All six of the anti-peptide MAbs recognized $p62^{c\text{-}myc}$ on immunoblots (Fig. 5). Interestingly, MAb Myc1-9E10, which fails to immunoprecipitate $p62^{c\text{-}myc}$, nonetheless recognizes the immunoblotted protein. Indeed, MAb Myc1-9E10 is, in our opinion, the most sensitive probe out of all six MAbs for immunoblotted $p62^{c\text{-}myc}$. Both components of the $p62^{c\text{-}myc}$–$p64^{c\text{-}myc}$ doublet are recognized by each of the MAbs. Therefore, the upper of the two components is probably not $p62^{c\text{-}myc}$ noncovalently associated with some other low-molecular-weight component in native cell lysates, because such an association is likely to be disrupted by denaturation in SDS sample buffer. In addition to $p62^{c\text{-}myc}$, other components recognized by some of the MAbs are faintly visible in Fig. 5 (tracks 4, 6, and 7). These are most likely due to weak cross-reactions and not indicative of any relationship between these other components and $p62^{c\text{-}myc}$. Nonetheless, the fact that such cross-reacting specificities are different for each MAb argues that the precise antigenic specificity of each MAb is different, even when directed against the same peptide immunogen.

**Analysis of the steady-state levels of $p62^{c\text{-}myc}$ in different cells.** We were interested in comparing the levels of $p62^{c\text{-}myc}$ in different cell lines to see whether they correlated with levels of c-*myc* mRNA. In particular, we wished to know whether cells with amplified c-*myc* genes accumulated more $p62^{c\text{-}myc}$ protein than cells without amplification. To examine this, we fractionated equal numbers of cell of various types on an SDS-polyacrylamide gel and electroblotted the fractionated proteins onto nitrocellulose paper. Appropriate titrations were performed to ensure that we were saturating neither the nitrocellulose paper nor the antibody probes with respect to $p62^{c\text{-}myc}$ (data not shown). Electroblotting was continued until over 95% of proteins under 150 kDa in size were transferred onto the nitrocellulose filter. Electroblotted fractions were then probed for $p62^{c\text{-}myc}$ with the MAb Myc1-9E10. We also measured the relative levels of c-*myc* mRNA in each cell line as described in Materials and Methods (Fig. 6). Clearly, the steady-state levels of $p62^{c\text{-}myc}$ protein (Fig. 6A) and c-*myc* mRNA (Fig. 6B) vary widely in different cells. Cells containing amplified c-*myc* genes (Colo 320 HSR, N417, and HL60) have the highest levels of protein and c-*myc* mRNA, although the levels of c-*myc* mRNA and protein do not always correlate exactly. However, such apparent differences between mRNA and protein levels may be due to the fact that protein levels are normalized with

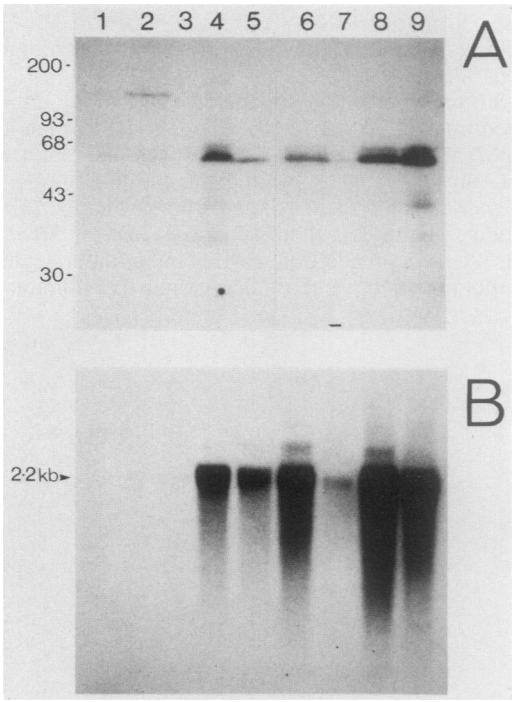


FIG. 6. Comparison of $p62^{c\text{-}myc}$ protein and mRNA levels in various cell lines. (A) Samples of $10^6$ cell equivalents of each cell type were fractionated on a 10% SDS–polyacrylamide gel and electroblotted onto nitrocellulose paper. The blot was probed with MAb Myc1-9E10 for $p62^{c\text{-}myc}$ as described in Materials and Methods. (B) Samples of 10 μg of total cell RNA from each cell type were probed with nick-translated c-*myc* probe. After hybridization and washing, the blot was exposed to prefogged X-ray film at −70°C for 12 h with an intensifier screen (6). Tracks contained the following cells: 1, MRC-5; 2, Detroit 532; 3, Flow 4000; 4, HeLa; 5, CCRF-CEM; 6, HL60 (before addition of DMSO to growth medium); 7, HL60 (3 h after addition of 1.2% DMSO to growth medium); 8, Colo 320 HSR; 9, N417. Molecular weights were estimated by reference to the positions of prestained protein markers.

respect to the number of cell equivalents fractionated on the gel, whereas c-*myc* RNA levels are normalized only with respect to the amount of total isolated RNA. The lowest levels of c-*myc* mRNA and protein are found in the nontransformed cell lines with finite in vitro lives, MRC-5, Detroit 532, and Flow 4000. Both HeLa and CCRF-CEM cells have relatively high levels of $p62^{c\text{-}myc}$ and c-*myc* mRNA (between 20 and 50% of the amount in Colo 320 HSR cells). It has been reported that DMSO-induced differentiation of HL60 cells results in a switching off of c-*myc* expression (28). Analysis of $p62^{c\text{-}myc}$ levels in HL60 cells shows that the within 3 h of the start of induction of differentiation by DMSO, c-*myc* mRNA and protein levels are already significant lower than in control cells (Fig. 6, lanes 6 and 7).

In summary, then, there is a fairly good correlation between c-*myc* mRNA and protein levels in the cell lines tested so far.

## DISCUSSION

Using synthetic peptides as immunogens, we have isolated six MAbs specific for the human c-*myc* gene product, $p62^{c\text{-}myc}$. The MAbs are all of the immunoglobulin of the G1.K (IgG1.k) subclass, and all accumulate to high levels in the ascitic fluid of syngeneic mice bearing the appropriate hybridomas as tumors. Of these six MAbs, five (CT9-B7, CT14-G.4, Mycl-3C7, Mycl-8F9, Mycl-6E10) immunoprecipitate native $p62^{c\text{-}myc}$, whereas all six recognize the protein on immunoblots. As a guide to the affinities of these MAbs for native $p62^{c\text{-}myc}$, we have assayed the dissociation rates of the antibody-antigen complexes for each of the five immunoprecipitating antibodies as previously described (10). We find the half-lives of dissociation of the immune complexes of all of these MAbs to be in excess of 24 h, suggesting that all possess high affinity for the $p62^{c\text{-}myc}$ antigen (data not shown).

Of the six MAbs isolated, three are specific for one peptide sequence (peptide D), and three are specific for another peptide sequence (peptide G). We have observed no cross-reactivity between any of the MAbs and the inappropriate peptide immunogen. The fact that six individual MAbs, recognizing between them two different and discontinuous $p62^{c\text{-}myc}$ sequences, all bind the same 62-kDa protein argues strongly that this protein is indeed the human c-*myc* gene product. Moreover, the 62-kDa protein in question is present at high levels in cells with elevated degrees of c-*myc* mRNA expression and almost undetectable in nontransformed cell lines with low levels of c-*myc* message.

The three peptide G-specific MAbs, CT9-B7, CT14-G4, and Mycl-9E10, fail to recognize the chicken $p110^{gag\text{-}myc}$ protein present in MC29 virus-transformed quail fibroblasts. This is despite there being substantial homology between peptide G and the C-terminal sequence of $p110^{gag\text{-}myc}$ (Fig. 2) and despite the fact that a polyclonal rabbit antibody specific for peptide G does recognize the v-*myc* protein. Moreover, none of the three peptide G-specific MAbs recognizes the mouse c-*myc* protein. We therefore conclude that these three MAbs recognize sequences in peptide G which are peculiar to the human protein.

We are not surprised that none of the peptide D-specific MAbs (Mycl-3C7, Mycl-8F9, and Mycl-6E10) recognizes $p110^{gag\text{-}myc}$, since there is little homology between $p62^{c\text{-}myc}$ and $p110^{gag\text{-}myc}$ in the region of this particular sequence (Table 1). However, all three peptide D-specific MAbs immunoprecipitate a 64-kDa to 66-kDa antigen from the Rec2A mouse cell line, which possesses an amplified mouse c-*myc* gene. The evidence is good that this 64- to 66-kDa antigen is the mouse c-*myc* gene product, because the same antigen is recognized by our polyclonal antibody to peptide G. In a previous report (18) the mouse c-*myc* gene product was described as being of higher mobility than its human homolog in SDS-polyacrylamide gels, which is in contradiction to our own findings. We have noted, however, that the mobility of all c-*myc* proteins in SDS-polyacrylamide gels is highly dependent on the ratio of acrylamide to *NN'*-methylenebisacrylamide used in the resolving gel. It is thus possible that different formulations of the polyacrylamide resolving gel are responsible for this difference in apparent mouse c-*myc* protein size. Possibly *myc* proteins fail to assume a standard globular conformation in SDS, which may explain why all tested *myc* gene products migrate with an aberrantly high mobility in SDS gels. The specificities and characteristics of the six anti-$p62^{c\text{-}myc}$ MAbs are summarized in Table 2.

All six of the $p62^{c\text{-}myc}$-specific MAbs recognize $p62^{c\text{-}myc}$ when it is electroblotted onto nitrocellulose filters. Perhaps surprisingly, one of the best immunoblotting probes for $p62^{c\text{-}myc}$ is the peptide G-specific MAb Mycl-9E10, which does not immunoprecipitate native $p62^{c\text{-}myc}$ from cell lysates. We have further tested the abilities of all six MAbs to immunoprecipitate $p62^{c\text{-}myc}$ after it had been denatured by boiling in SDS and 2-mercaptoethanol. All MAbs but Mycl-9E10 recognize the denatured protein (data not shown). Thus it is only the Mycl-9E10 antigenic determinant which is dependent upon the way the $p62^{c\text{-}myc}$ Ag is presented, in that the Mycl-9E10 determinant is only available in the electroblotted protein. Immunoblotted $p62^{c\text{-}myc}$ must therefore possess a comformation distinct from that of either native or denatured $p62^{c\text{-}myc}$.

Immunoblotting analysis of $p62^{c\text{-}myc}$ in cells gives a measure of the absolute levels of the protein at the steady state. This is not true of immunoprecipitation analyses in which only the antigen which is metabolically labeled over a given period is detected. Although the bulk of $p62^{c\text{-}myc}$ has been shown to have a short half-life in tumor cells (13), it is possible that the same is not true in all cells or of all $p62^{c\text{-}myc}$ protein in any one cell. Without detailed knowledge of the rates of $p62^{c\text{-}myc}$ metabolism in each cell type, it is therefore impossible to unambiguously deduce $p62^{c\text{-}myc}$ levels by immunoprecipitation analysis. Because of this, we have used immunoblotting to assess the levels of $p62^{c\text{-}myc}$ in different cell types and to compare it with c-*myc* mRNA levels. We find that cell lines with amplified c-*myc* genes (Colo 320 HSR, N417, HL60) also have high steady-state levels of $p62^{c\text{-}myc}$ and c-*myc* mRNA when compared with the very low levels found in nontransformed cell lines with finite in vitro lifetimes such as MRC-5, Flow 4000, and Detroit 532. We find that the levels of c-*myc* mRNA and protein in HeLa and CCRF-CEM cells are lower than, but comparable with, levels in cells with c-*myc* amplifications. This is somewhat surprising, because neither HeLa nor CEM cells possess c-*myc* gene amplifications (P. Hamlyn, personal communication). In a further story (Pauza and Evan, submitted for publication), we have found that steady-state levels of $p62^{c\text{-}myc}$ are high, relative to MRC-5 cells, in most leukemia and Burkitt lymphoma lines, even though most do not possess amplified c-*myc* genes. High steady-state levels of c-*myc* mRNA and protein may therefore be a common feature of highly transformed cell lines and not solely dependent upon c-*myc* gene amplification. We have investigated the levels of $p62^{c\text{-}myc}$ in HL60 cells which have been induced to differentiate by treatment with DMSO. We observed the rapid disappearance of both c-*myc* mRNA and $p62^{c\text{-}myc}$

within 3 h of the start of induction. We are at present examining the dynamics of this DMSO-induced disappearance of $p62^{c\text{-}myc}$ in detail.

In conclusion, we have isolated a set of six MAbs, between them specific for two separate regions of the human c-*myc* protein, $p62^{c\text{-}myc}$. These MAbs are of use in immunoprecipitation and immunoblotting analyses of $p62^{c\text{-}myc}$ in cells. Our preliminary data (manuscripts in preparation) also suggest that certain of these MAbs may be of use in the immunohistological analysis of $p62^{c\text{-}myc}$ in normal and neoplastic tissues. We believe that the availability of these and other similar reagents will facilitate investigations into the function and pathology of c-*myc* expression in normal and neoplastic cells.

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