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# Inhibition of DNA binding of purified p55$^{v\text{-}myc}$ *in vitro* by antibodies against bacterially expressed *myc* protein and a synthetic peptide

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**To identify viral *myc* proteins, we have prepared *myc*-specific antibodies: (i) against a synthetic peptide corresponding to the nine carboxy-terminal amino acids of the viral *myc* ($C_9$); (ii) against a bacterially expressed viral *myc* protein obtained by inserting the *SalI-BamHI* fragment of the viral MC29 DNA clone in the expression vector pPLc24. Both antisera recognize a protein of 55 000 mol. wt., p55$^{v\text{-}myc}$, in MH2- and OK10-transformed fibroblasts. The protein is located in the nucleus, as shown by indirect immunofluorescence and cell fractionation. Antibodies against the $C_9$ peptide were used to purify the p55$^{v\text{-}myc}$ by immunoaffinity column purification (3000-fold) from OK10- and MH2-transformed fibroblasts. p55$^{v\text{-}myc}$ binds to double-stranded DNA *in vitro* as does p110$^{gag\text{-}myc}$. DNA binding *in vitro* is inhibited by the immunoglobulin fraction of antibodies against the bacterially expressed *myc* protein. Furthermore, a synthetic peptide consisting of 16 amino acids ($C_{16}$) was used to isolate specific immunoglobulins which also inhibit DNA binding *in vitro*. OK10 codes, in addition to p55$^{v\text{-}myc}$, for a p200$^{gag\text{-}pol\text{-}myc}$ polyprotein. The majority of this protein is located in the cytoplasm (79%). The purified protein binds to single-stranded RNA *in vitro*, unlike other *gag-myc* or myc proteins.**

*Key words:* DNA binding site/fused and unfused *myc* proteins/MH2 and OK10/immunoaffinity purification/inhibition of DNA binding

## Introduction

The family of avian myelocytomatosis viruses comprises several isolates besides MC29, including CMII isolated in Germany, MH2 a British strain and OK10 originating from Finland (Graf and Beug, 1978). MC29 and CMII code for transformation-specific fusion proteins, p110$^{gag\text{-}myc}$ and p90$^{gag\text{-}myc}$, respectively. MH2 and OK10 encode *myc* sequences which are, however, not linked to *gag* (Saule *et al.*, 1982). The *gag*-linked protein of MH2 is not of the *gag-myc* type, since it is located in the cytoplasm instead of the nucleus and it binds *in vitro* to single-stranded RNA. The latter property is unprecedented amongst other known oncogene products (Bunte *et al.*, 1983). Analysis of the MH2 genome revealed that it has two oncogenes, a *gag-mil* and a *myc* gene (Jansen *et al.*, 1983). OK10 codes for a *myc*-specific sequence which is, however expressed in two ways, as a large fusion protein, p200$^{gag\text{-}pol\text{-}myc}$ and, in addition, as an unlinked *myc* protein. OK10 also expresses Pr76$^{gag}$ in large quantities (Ramsay *et al.*, 1980). The p110$^{gag\text{-}myc}$ protein is located in the nucleus of transformed cells (Donner *et al.*, 1982; Abrams *et al.*, 1982) and binds to double-stranded DNA *in vitro* (Donner *et al.*, 1982). The DNA-binding ability of the *gag-myc* protein correlates with transformation, since it is largely reduced in deleted proteins from transformation-defective mutants (Donner *et al.*, 1982). A sequence specificity of DNA-protein interaction has not yet been shown (Moelling *et al.*, 1984a).

The contribution of the *gag*-specific portions to the *gag-myc* proteins has so far been evaluated only indirectly, by comparing the fusion proteins with the *gag* precursor Pr76$^{gag}$, and was considered negligible. Recently, however, a dramatic effect of *gag* regions fused to another oncogene, *abl*, has been described. The *gag* region of the *gag-abl* protein is necessary for its oncogenic potential in lymphocytes (Prywes *et al.*, 1983). The role of *gag* sequences in the case of *gag-myc*, therefore, needs to be defined. To this end, we have purified the *gag*-free p55$^{v\text{-}myc}$ protein and the large polyprotein p200$^{gag\text{-}pol\text{-}myc}$ and analyzed their properties *in vitro*. While p55$^{v\text{-}myc}$ is a DNA-binding protein similar to p110$^{gag\text{-}myc}$, p200$^{gag\text{-}pol\text{-}myc}$ resides in the cytoplasm and exhibits RNA/DNA-binding properties which may be attributed to the presence of *pol* sequences. Synthetic peptide antibodies which inhibit DNA-protein interaction *in vitro* allow mapping of the domain of the *myc* protein involved in DNA-binding.

## Results

### *Preparation of myc-specific antibodies*

Three types of *myc*-specific antibodies were produced. The first type, prepared against a synthetic peptide corresponding to the nine carboxy-terminal amino acids of the viral *myc* gene (Leu-Glu-Gln-Leu-Arg-Asn-Ser-Arg-Ala, Alitalo *et al.*, (1983a), precipitate a p55$^{v\text{-}myc}$ protein from cells transformed by MC29 viruses (Moelling *et al.*, 1984a). A similar peptide has also been used for antibody production by Hann *et al.* (1983). A second type of *myc*-specific antibody was raised against bacterially expressed viral *myc* sequences. To express the viral protein in bacteria, an expression vector was chosen which carries a strong leftward promoter of the bacteriophage lambda. The activity of the promoter can be controlled by using host strains that carry the gene for a temperature-sensitive lambda repressor (*cI*λts). This expression vector, pPLc24, also carries information for the first 99 amino acids of the replicase gene of the bacteriophage MS2, followed by unique sites for restriction endonucleases (Remaut *et al.*, 1981). MC29 has been cloned as double-stranded DNA into pBR322 (Lautenberger *et al.*, 1981). Insertion of a *SalI/BamHI* fragment into a pPLc24 resulted in expression of a fusion protein of 35 000 daltons which represents ~10% of the bacterial protein content. The polypeptide consists of 99 amino acids of the MS2 replicase followed by 198 amino

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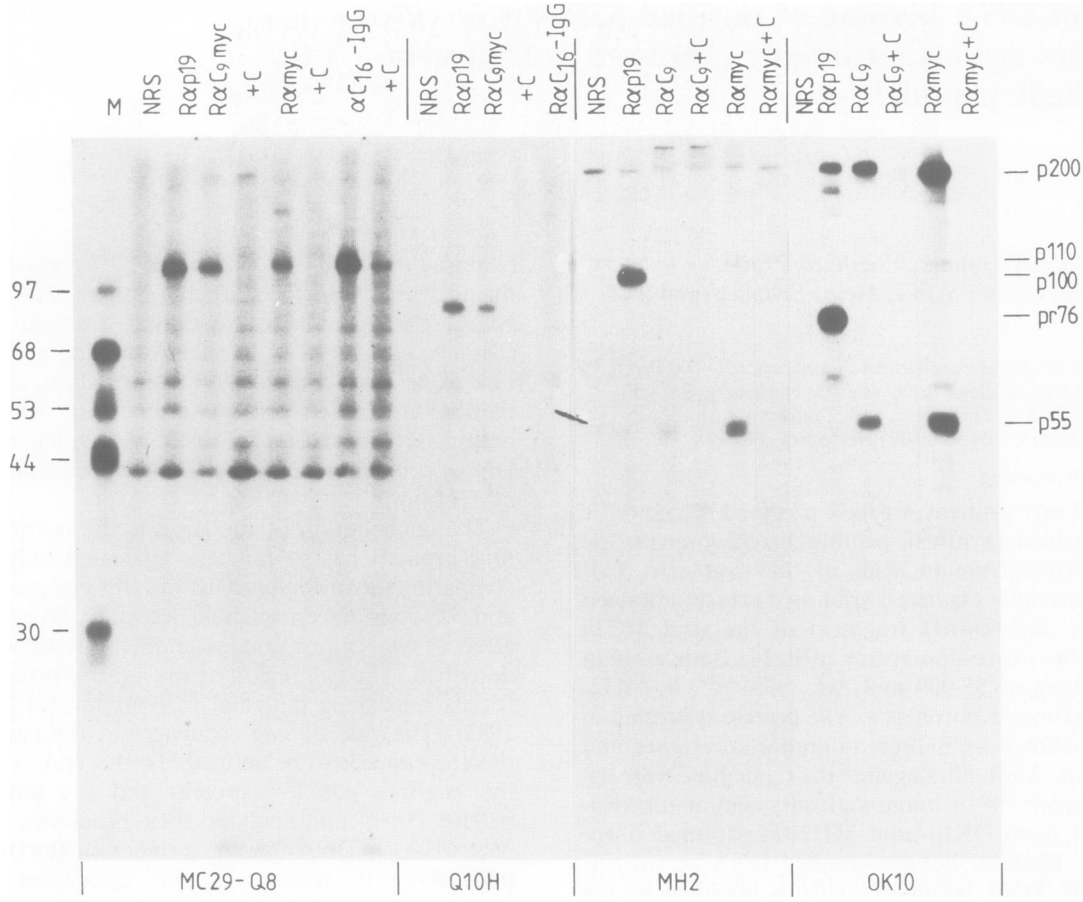


**Fig. 1.** Indirect immunoprecipitation of *myc* proteins from [$^{35}$S]methionine-labeled cellular extracts. The cells used were the MC29-transformed quail fibroblast line MC29-Q8, mutant infected quail cell line Q10H, MH2-transformed quail fibroblasts (MH2), and OK10-transformed fibroblasts (OK10). The sera used were: normal rabbit serum (NRS); rabbit antiserum against p19 (Rαp19); rabbit antiserum against the C-terminal viral *myc* nonapeptide (Rα$C_9$); rabbit antiserum against the bacterially expressed viral *myc* protein (Rαmyc); purified IgG against the $C_{16}$ peptide (α$C_{16}$IgG), + C indicates competition of the immunoprecipitation with an excess of $C_9$ peptide (10 μg) or an excess of the bacterially expressed *myc* protein (20 μg). M indicates $^{14}$C-labeled mol. wt. markers (from top to bottom): 97 K, 68 K, 53 K, 44 K, 30 K.

acids specific for the carboxy-terminal half of the viral *myc* gene (corresponding to amino acids 228–425) (Alitalo *et al.*, 1983a). The cloned region comprises those sequences which presumably are deleted from the three non-transforming deletion mutants (Bister *et al.*, 1982). The MS2-*myc* fusion protein was eluted from gels and used for immunization. Two other groups prepared antibodies against bacterially expressed v-*myc* while this work was in progress (Lautenberger *et al.*, 1983; Alitalo *et al.*, 1983b). A third type of *myc*-specific antibody was obtained by isolating a subpopulation of immunoglobulins from the serum against the bacterially expressed v-*myc* protein by using an immobilized synthetic peptide of 16 amino acids, $C_{16}$ (Tyr-Glu-Glu-Glu-Gln-Glu-Glu-Asp-Glu-Glu-Ile-Asp-Val-Val-Thr-Leu-Ala), corresponding to amino acid numbers 239–254 of the v-*myc* gene (Alitalo *et al.*, 1983a) to which Tyr was added.

### *Identification of the v-myc protein*

Figure 1 shows the analysis by indirect immunoprecipitation of MC29-, MH2- and OK10-transformed fibroblasts with the two *myc*-specific antibodies in comparison with the monoclonal antibody against p19. The *myc*-specific sera precipitate p110$^{gag\text{-}myc}$ from MC29 cells and proteins of ~55 000 mol. wt. from MH2- and OK10-transformed cells. The specificity of the precipitations is shown by competition with excess of synthetic peptide or bacterial *myc* protein, respectively. The p55 molecule is therefore considered to be the viral *myc* protein p55$^{v\text{-}myc}$. In the case of MH2 p55$^{v\text{-}myc}$ is a doublet. From OK10 cells we also precipitated a p200$^{gag\text{-}pol\text{-}myc}$ protein. The p110$^{gag\text{-}myc}$, p55$^{v\text{-}myc}$ and p200$^{gag\text{-}pol\text{-}myc}$ are phosphorylated (Hann *et al.*, 1983; Moelling *et al.*, 1984a). IgG specific for the $C_{16}$ peptide allows precipitation of the intact p110$^{gag\text{-}myc}$ protein but fails to recognize the deleted mutant protein p90 of the mutant virus Q10H (Ramsay *et al.*, 1980).

### *Cellular localization of p55$^{v\text{-}myc}$*

Indirect immunofluorescence analysis was performed with normal quail fibroblasts and MC29-, OK10- and MH2-transformed fibroblasts, using $C_9$ peptide antibodies and monoclonal antibodies against p19 as described previously. The results are shown in Figure 2. *myc*-specific sera give rise to nuclear fluorescence similar to the *gag-myc* protein in MC29 cells (Donner *et al.*, 1982). The *gag*-linked fusion proteins in the case of OK10- and MH2-transformed fibroblasts result in total-cell fluorescence (Figure 2). Antisera against the bacterially expressed *myc* protein gave identical results (not shown). Competition with synthetic peptide or bacterial *myc* protein abolished the fluorescence (not shown). Similar data were obtained by Hann *et al.* (1983).

### *Isolation of p55$^{v\text{-}myc}$ from MH2- and OK10-transformed cells and p200$^{gag\text{-}pol\text{-}myc}$*

MH2- and OK10-transformed fibroblasts were labeled with

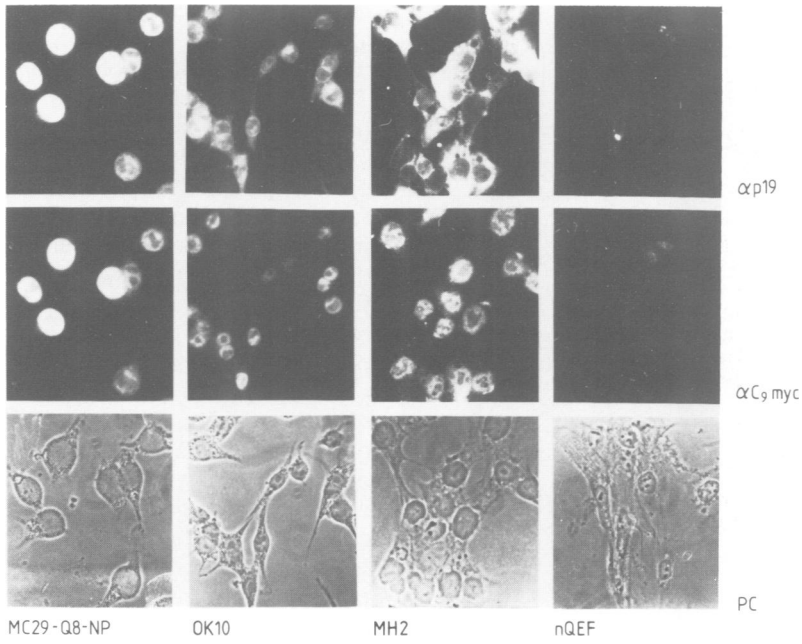


**Fig. 2.** Double-indirect immunofluorescence was performed with IgG of monoclonal antibodies against p19 (αp19) as described (Donner *et al.*, 1982) and rabbit antibodies against the C-terminal viral *myc*-specific nonapeptide ($\alpha C_9$myc). The second antibody against the mouse monoclonal IgG was rhodamine-coupled rabbit antimouse IgG, and the one against the rabbit serum was fluorescein-labeled goat anti-rabbit IgG. The cell types used were the same as those described in Figure 1. PC indicates the identical cells by phase contrast microscopy. Magnification is 360-fold.

[$^{35}$S]methionine (250 μCi/ml) for 2 h and applied to an immunoaffinity column. The column material was prepared by covalently coupling the *myc*-specific immunoglobulin (IgG) fraction of the antibodies against the synthetic C-terminal peptide $C_9$. The IgG fraction was isolated using the $C_9$ peptide coupled to Sepharose. The purified p55$^{v\text{-}myc}$ from MH2 cells is shown in Figure 3A. OK10 cells contain two *myc*-specific proteins. To separate the p200$^{gag\text{-}pol\text{-}myc}$ from the p55$^{v\text{-}myc}$, an immunoaffinity column containing anti-p19 monoclonal IgG was used first to absorb p200$^{gag\text{-}poly\text{-}myc}$ and Pr76$^{gag}$, which is also expressed. The flow-through material of this column was directly applied to the anti-$C_9$ immunoaffinity column which bound p55$^{v\text{-}myc}$. After extensive washings, both columns were eluted separately (Figure 3B).

To identify the purified proteins they were subsequently precipitated with the antibody against the bacterial *myc* protein. The results are shown in Figure 3A and B. Since both antibodies were isolated by independent approaches, this protein is considered to be p55$^{v\text{-}myc}$.

### *Interaction of purified p55$^{v\text{-}myc}$ and p200$^{gag\text{-}pol\text{-}myc}$ with nucleic acids in vitro*

The DNA-binding properties *in vitro* of p55$^{v\text{-}myc}$ and p110$^{gag\text{-}myc}$ were compared in a filter-binding assay. The result is shown in Figure 4A. The amounts of the purified proteins were standardized to equal molar inputs. The purified p200$^{gag\text{-}pol\text{-}myc}$ binds to RNA as well as DNA *in vitro* (Figure 4B,C). Binding to RNA is a property shared by the reverse transcriptase which was therefore tested in parallel (Figure 4C). This result suggests an effect of the *pol* region on properties of the viral *myc* protein when it is fused to *pol*. Cell fractionation analysis confirms the differences between p55$^{v\text{-}myc}$ and p200$^{gag\text{-}pol\text{-}myc}$. While 78% of the former is nuclear, the majority of the fusion protein, 79%, is cytoplasmic (data not shown).

### *Inhibition of DNA-binding of p55$^{v\text{-}myc}$ by myc-specific antibodies*

The bacterially expressed MS2-*myc* fusion protein contains the *Sal*I-*Bam*HI region of the MC29 DNA clone (Lautenberger *et al.*, 1981). It therefore comprises the complete $exon_3$ and only a small part of $exon_2$. Antibodies against the bacterially expressed protein should allow us to determine whether the DNA-binding domain of the *myc* protein is coded for by $exon_2$ or $exon_3$. Therefore, a DNA-p55$^{v\text{-}myc}$ filter-binding assay was performed in the presence of purified IgG against the bacterial protein. Binding of p55$^{v\text{-}myc}$ to DNA was strongly inhibited by this IgG. The site of the *myc* protein involved in DNA-binding was specified further by using $C_{16}$-peptide specific IgG which also interfered with the *in vitro* DNA binding reaction. In contrast, the IgGs of the $C_9$ synthetic peptide antibodies and the p19 monoclonal antibodies showed no significant inhibitory effects (Figure 5), neither did normal rabbit serum (not shown).

## Discussion

The viral *myc* gene product is a protein of 55 000 mol. wt., p55$^{v\text{-}myc}$, which is recognized by two types of antisera which were obtained by independent approaches. The p55$^{v\text{-}myc}$ purified by means of one type of antibody is recognized after purification by the other, thus proving the specificity of the purification. The p55$^{v\text{-}myc}$ is located in the nucleus and after purification binds to double-stranded DNA *in vitro*. According to these parameters p55$^{v\text{-}myc}$ closely resembles the *gag*-linked p110$^{gag\text{-}myc}$. As was shown recently, p55$^{v\text{-}myc}$ is a phosphoprotein (Hann *et al.*, 1983; Moelling *et al.*, 1984a).

Using the filter-binding assay for DNA-protein interaction *in vitro* we attempted to locate the site of the protein molecule involved in DNA binding, by inhibition with the immunoglobulin fractions of various sera. Antibodies against the

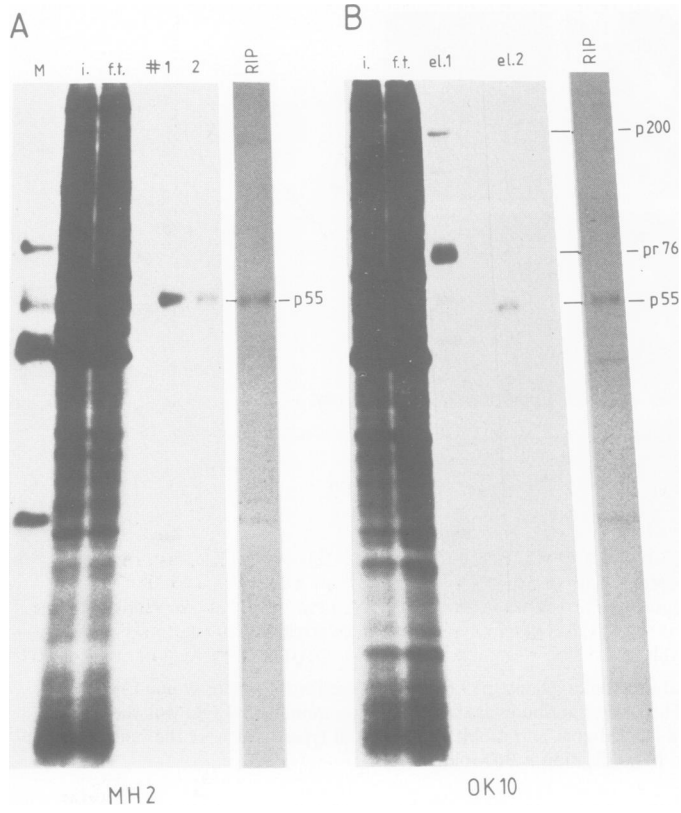


**Fig. 3.** Autoradiogram of transformation-specific proteins purified from MH2-QEF **(A)** and OK10-QDP 9c **(B)** cells which were [$^{35}$S]methionine-labeled. 40 ml lysate (corresponding to 20 Petri dishes of $10^9$ cells) were applied to an immunoaffinity column consisting of rabbit IgG against the $C_9$ peptide. After extensive washing, the bound proteins were eluted in fractions of 2 ml. Details of this procedure have been described elsewhere (Donner *et al.*, 1982). **(B)** The OK10 lysate was first applied to a column containing monoclonal αp19 IgG. Subsequently the flow-through was directly layered onto the $\alpha C_9$myc IgG immunoaffinity column used in **(A)**. Both columns were washed and eluted separately. i: input (5 μl of 40 ml). In the case of MH2 **(A)**, the eluted fractions are #1 and #2 (75 μl out of 2 ml each). In the case of OK10 **(B)**, 75 μl of the eluted material from the first column (el.1, 2 ml total) and 75 μl of the eluted material from the second column (el.2, 2 ml total) were applied to the gel. M represents $^{14}$C-labeled protein markers (see legend to Figure 1). RIP indicates radioimmunoprecipitation with antibodies against the bacterially expressed *myc* protein using 1 ml of fraction 2 in **(A)** and 1 ml of fraction 2 of the second column in the case of **(B)**. f.t. indicates flow through.

bacterially expressed viral *myc* were quite effective and located the domain of DNA binding to the carboxy-terminal half of the protein. Since antibodies against the so-called $C_{16}$ peptide also inhibit DNA binding, this region may be particularly important. Two control antibodies, one against the carboxy- and the other one against the amino-terminal ends did not inhibit DNA binding significantly. The $C_{16}$ peptide was selected for synthesis since the three known transformation-defective deletion mutants map in this domain (Enrietto and Hayman, 1982). Their transforming proteins exhibit reduced DNA binding *in vitro* (Donner *et al.*, 1983). The inhibition of DNA binding by the $C_{16}$ peptide antibodies supports the notion that this region is important in DNA binding. While this work was in progress, Ralston and Bishop (1983) published a *myc* sequence which by computer analysis appears structurally related to E1A of adenovirus 12. Seven of the 12 aminoacids, which they describe, overlap with amino acids of the $C_{16}$ peptide. The *myc* protein consists of two domains (Reddy *et al.*, 1983), one of them, the carboxy-terminal half which is more hydrophilic and histone-like, is involved in tumorigenicity in birds but is of low relevance to transformation of fibroblasts *in vitro* (Ramsay *et al.*, 1980). The amino-terminal portion may be important for transformation of fibroblasts.

The virus OK10 codes for a $p55^{v\text{-}myc}$ protein and a large protein, $p200^{gag\text{-}pol\text{-}myc}$, with an identical *myc* portion. This fusion protein behaved differently from the unlinked *myc* protein and resided predominantly in the cytoplasm and furthermore bound to RNA *in vitro*. This difference between a fused and an unfused *myc* protein was attributed to the viral replication gene *pol* whose product apparently directs the $p200^{gag\text{-}pol\text{-}myc}$ protein to the cytoplasm. The total amount of $p200^{gag\text{-}pol\text{-}myc}$ in the cell is lower than that of $p55^{v\text{-}myc}$ and spread over a larger area, so that the cytoplasmic distribution of the p200 still allows the detection of nuclear fluorescence of p55 by *myc*-specific antibodies. So far the role of fused viral replicating genes has been considered negligible. In the case of OK10 the role of the $p200^{gag\text{-}pol\text{-}myc}$ is unknown. However the *gag* portion of a *gag-abl* gene plays an important role in transformation (Prywes *et al.*, 1983).

Recently, Hann *et al.* (1983) described the association of the *myc* protein with the nuclear matrix. Our own observations agree with this finding (Moelling *et al.*, 1984b). We furthermore observed the association of 14% of the *myc* protein with chromatin (Bunte *et al.*, 1982). The properties of the *myc* protein may resemble those of actively transcribed ovalbumin genes which involve nuclear matrix association as well as DNA interaction (Ciejek *et al.*, 1983). One property does not exclude another one.

While this work was in progress, similar *myc*-specific antibodies were obtained by other groups. A carboxy-terminal synthetic peptide, 12 amino acids in length, was used to identify a protein of 62 000 daltons in MH2- and also OK10-transformed cells (Hann *et al.*, 1983). Differences in mol. wt. determinations may be due to the gel systems and markers used. Our designation of p55 was based on a commercially available mol. wt. marker set (see Figure 1). A protein similar in size to the one described here has been reported by Alitalo *et al.* (1983b). They prepared antibodies against a chimeric bacterial protein consisting of $pp60^{src}$ (151 amino acids) and *myc*-specific sequences (116 amino acids of *myc* from $exon_2$), and identified a protein of 57 000 daltons. The carboxy-terminal half of the *myc* gene, spanning the *Cla*I to the *Bam*HI sites, which is slightly smaller than the one described here (*Sal*I-*Bam*HI), has recently been successfully cloned for protein expression by Lautenberger *et al.* (1983).

Our *myc*-specific antibodies allowed us to identify *myc* gene products in other cell types, e.g., in RP9, an avian leukosis virus-induced lymphoma cell line (Hayward *et al.*, 1981) and in HeLa cells. In RP9 cells a molecule of 55 000 daltons was identified, whereas in HeLa cells a molecule of 64 000 mol. wt. was detected (unpublished observation). How such a molecule relates to the ones published by others (Hann *et al.*, 1983; Giallongo *et al.*, 1983), is not yet clear.

## Materials and methods

*Cells*

The MC29-Q8-NP fibroblastic cell line (Bister *et al.*, 1977) MH2-transformed quail embryo fibroblasts (MH2-QEF) originating from C.Moscovici, Gainsville, Florida, and the OK10-transformed cell line OK10-QDP-9C from S.Pfeifer-Ohlsson and A.Vaheri, Helsinki, Finland, were used. OK10 cells produce $Pr76^{gag}$, the other cell lines are not virus-producing. Cells were grown

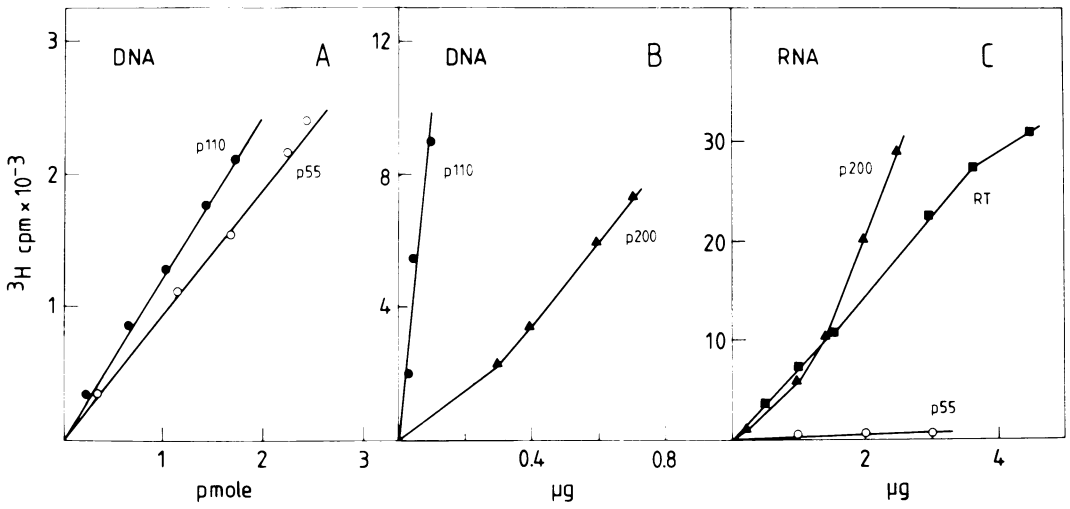


**Fig. 4.** Filter-binding assays of purified proteins. **(A)** Equal molar amounts of purified p110$^{gag\text{-}myc}$ and P55$^{v\text{-}myc}$ from MC29- and MH2-transformed cells, respectively, were analyzed *in vitro* for DNA binding using [$^3$H]thymidine-labeled normal chicken cell DNA ($10^5$ c.p.m./µg). **(B)** Purified p110$^{gag\text{-}myc}$ and p200$^{gag\text{-}pol\text{-}myc}$ from MC29- and OK10-transformed cells were tested for their abilities to bind to DNA *in vitro*. Since the preparation of p200 contains large amounts of Pr76$^{gag}$ and other p19-related viral proteins, no quantitative comparison with p110 is possible. **(C)** Binding of purified p200 and purified RNA-dependent DNA polymerase from avian myeloblastosis virus were tested for their abilities to bind to radioactively labeled poly(A)-containing normal cell RNA ($1.8 \times 10^4$ c.p.m./µg). p55$^{v\text{-}myc}$ is shown for comparison.

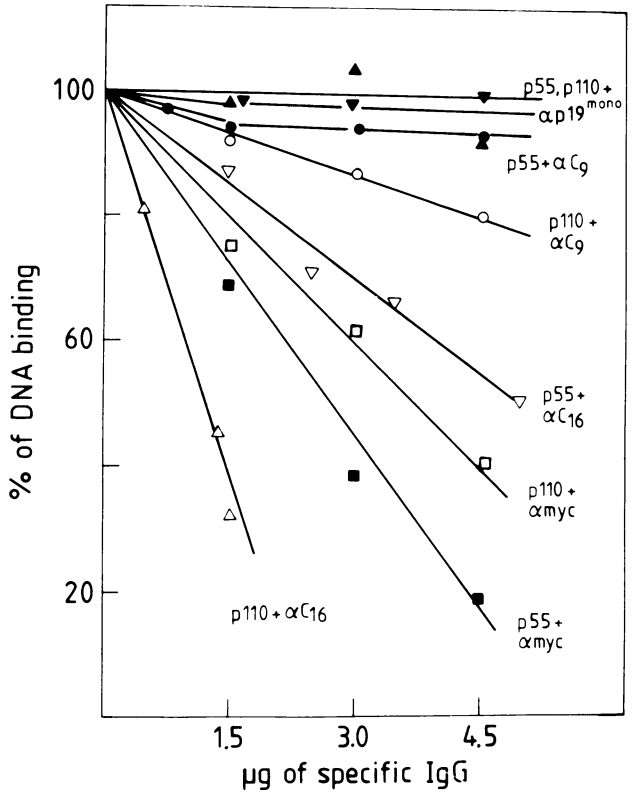


**Fig. 5.** Inhibition of DNA binding of purified p110$^{gag\text{-}myc}$ and p55$^{v\text{-}myc}$ by specific IgGs of various sera. The purified *myc*-specific proteins were pre-incubated for 15 min at 4°C with increasing amounts of various IgGs as indicated. Subsequently a DNA filter-binding assay was performed as described (Donner *et al.*, 1982). Radioactive DNA bound by antibodies in the absence of *myc* proteins was considered background and therefore subtracted. αp19$^{mono}$: monoclonal IgG directed against viral p19; αC$_9$: IgG against the C$_9$ viral *myc* peptide; αmyc: IgG from antibodies raised against the cloned and bacterially expressed v-*myc* protein; αC$_{16}$: IgG against the C$_{16}$ peptide mapping close to the *Cla*I site.

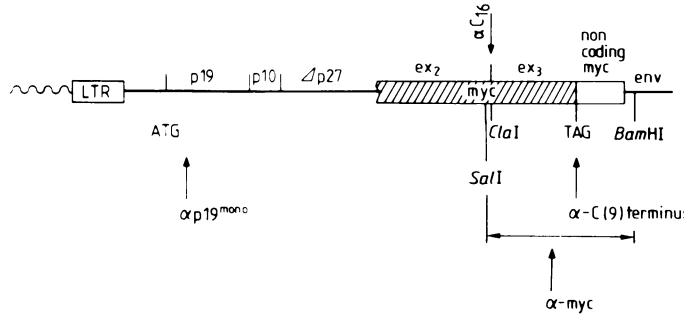


**Fig. 6.** Diagram of the *myc* gene.

in 1/2 x Dulbecco's modified Eagle's medium (DMEM), 1/2 x RPMI, 1 mM Hepes, 5% tryptose phosphate broth (TPB), 0.5% dimethylsulfoxide (DMSO), 1% heat-inactivated (30 min, 56°C) chicken serum and 5% newborn calf serum, except for OK10 cells which contained 5% fetal calf serum. All other procedures have been previously described (Donner *et al.*, 1982).

*Indirect immunofluorescence*

Double-label technique was used. Mouse anti-p19 monoclonal antibodies were diluted 1:40 and rabbit anti-C$_9$ peptide serum was diluted 1:20. Second antibodies (1:100) were either labeled with rhodamine (anti-mouse IgG) or fluorescein (anti-rabbit IgG). Further details have been described (Donner *et al.*, 1982).

*Molecular cloning of the viral myc gene*

To express viral *myc* sequences in bacteria, an expression vector was chosen which carries a strong leftward promoter of the bacteriophage lambda. The activity of the promoter can be controlled by using host strains that carry the gene for a temperature-sensitive lambda repressor (*c*Λts). This expression vector, pPLc24, also carries information for the first 99 amino acids of the replicase gene of the bacteriophage MS2, followed by unique restriction sites for the restriction endonucleases *Bam*HI and *Hind*III (Remaut *et al.*, 1981). The MC29 viral RNA genome has been cloned as double-stranded DNA into pBR322 (Lautenberger *et al.*, 1981). Several efforts of cloning sequences of this DNA into pPLc24 were undertaken (see Figure 6 for diagram of the *myc* gene).

(i) The cloned *Bam*HI-*Bam*HI fragment comprised part of p19, p10, the deleted p27 (αp27), and the complete *myc* exons $_2$ and $_3$ (ex$_2$, ex$_3$) and a portion of the envelope (*env*) sequence. The protein expressed had a mol. wt. of

75 000 daltons and contained a small *myc*-specific region of ~5000 daltons, only. The amount of protein corresponded to ~3% of the bacterial lysate. Antibodies obtained were directed against p19, whereas no *myc*-specific serum was obtained.

(ii) The clone described in (i) was restricted with *SstI*, a linker was inserted and expression in all three reading frames performed. Strong expression was obtained in a wrong frame resulting in a molecule of ~30 000 daltons. Detectable expression in the correct reading frame was not achieved.

(iii) The *BclI* site which includes 20 amino acids of p27 was also used for *myc* cloning. Even though sequencing analysis indicated that the *myc* gene was inserted in the correct reading frame, no significant protein expression was obtained.

(iv) Cloning of the *SalI-BamHI* fragment resulted in expression of an MS2-*myc* fusion protein of 35 000 mol. wt. It comprises $exon_3$ and a small part of $exon_2$. The polypeptide consists of ~99 amino acids of MS2 polymerase and 198 amino acids out of the carboxy-terminal *myc*-specific amino acids. The total *myc* protein corresponds to ~10% of the total bacterial protein 2 h after induction. The protein was highly immunogenic but is rather insoluble. The specific region was sliced out of a gel, the protein eluted and injected after mixing with adjuvants.

The protein expressed as described in (i) served to absorb out antibodies against the MS2-specific portion of the serum obtained in (iv).

*Preparation of antibodies against synthetic peptides and bacterial myc protein*

Synthetic peptides were synthesized by Bachem AG, Bubendorf, Switzerland. Two peptides were selected: the nine carboxy-terminal amino acids of the viral *myc* to which a tyrosine was added at the amino end $C_9$: Tyr-Leu-Glu-Gln-Leu-Arg-Asn-Ser-Arg-Ala. Furthermore, a peptide designated as $C_{16}$ peptide was selected consisting of 16 amino acids and a tyrosine corresponding to the v-*myc* amino acid 239–254: Tyr-Glu-Glu-Glu-Gln-Glu-Glu-Asp-Glu-Glu-Ile-Asp-Val-Val-Thr-Leu-Ala.

Rabbit serum against the $C_9$ peptide was obtained after several injections of a complex consisting of glutaraldehyde-treated peptide and keyhole limpet hemocyanine (KLH) according to Pfaff *et al.* (1982). In order to isolate specific anti-$C_9$ peptide antibodies, 12 mg of peptide were coupled covalently to N-hydroxy-succinimide-activated Sepharose with a 6-carbon spacer arm according to the procedure described by the supplier (Pharmacia, activated CH Sepharose 4B). 10 ml of antiserum was applied to the peptide column. The column was washed extensively with phosphate-buffered saline (PBS, 17 mM $Na_2HPO_4$, 2.6 mM $KH_2PO_4$, pH 7.4, 120 mM NaCl) and the specific antibodies were eluted with 1.5 M NaSCN in PBS and dialyzed against PBS.

To isolate *myc*-specific protein from the sera against the bacterial *myc* protein, 5 g of bacteria were lysed with lysozyme, treated with DNase I, dialyzed against PBS and cross-linked with 2.5% glutaraldehyde (Avremeas and Ternynck, 1969). This material was treated with 5 ml of serum to absorb the *myc*-specific antibodies and wash away the unspecific ones. Elution was performed with 1.5 M NaSCN as described above. The procedure was performed twice, once with the protein expressed as described in (ii), to absorb MS2 specificity and subsequently with the bacterial myc protein described in (iv). The $C_{16}$ peptide did not yet give rise to high-titer antibodies. To speed up the isolation of specific antibodies, the $C_{16}$ peptide was immobilized on Sepharose as described for the $C_9$ peptide. Serum against the bacterial *myc* protein was applied to this column, the specific antibodies against the $C_{16}$ peptide were bound and subsequently eluted for further use. There were defined as $C_{16}$ peptide-specific immunoglobulins.

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