The EMBO Journal Vol.1 No.8 pp.919-927, 1982

Association of gag-myc proteins from avian myelocytomatosis virus wild-type and mutants with chromatin

Thomas Bunte, Irene Greiser-Wilke, Peter Donner, and Karin Moelling*

Max-Planck-Institut für Molekulare Genetik, Ihnestrasse 63, D-1000 Berlin-Dahlem (33), FRG

Communicated by R. Rott
Received on 23 June 1982

The localization of the transformation-specific proteins was analyzed in quail embryo fibroblast cell lines transformed by wild-type avian myelocytomatosis virus MC29 and by three of its deletion mutants, Q10A, Q10C, and Q10H, with altered transforming capacities, and in a chicken fibroblast cell line transformed by the avian erythroblastosis virus (AEV). These viruses code for polyproteins consisting of part of the *gag* gene and of a transformation-specific region, *myc* for MC29 and *erb* A for AEV. Analysis by indirect immunofluorescence using monoclonal antibodies against p19, the N-terminal region of the polyprotein, showed that the gag-myc proteins in cells transformed by the wild-type MC29 as well as by the three deletion mutants are located in the nucleus. In contrast, cells transformed by AEV, which express the gag-erb A protein, give rise to cytoplasmic fluorescence. Fractionation of cells into nuclear and cytoplasmic fractions and analysis by immunoprecipitation and gel electrophoresis confirmed these results. About 60% of the gag-myc proteins of wild-type as well as of mutant origin were found in the nucleus, while 90% of the gag-erb A protein was present in the cytoplasm. Also, pulse-chase analysis indicated that the gag-myc protein rapidly accumulates in the nucleus in just 30 min. Further, it was shown that the wild-type and also mutant gag-myc proteins are associated with isolated chromatin. Association to chromatin was also observed for the gag-myc protein from MC29-transformed bone marrow cells, which are believed to be the target cells for MC29 virus *in vivo*.

Key words: acute oncornaviruses/fibroblasts and bone marrow/chromatin/mutants/nuclear fluorescence

Introduction

Avian acute leukemia viruses code for transformation-specific proteins which rapidly induce various types of leukemias as well as carcinomas and sarcomas (for review, see Graf and Beug, 1978). The known strains can be classified into three groups, the myelocytomatosis viruses, MC29-type, the avian erythroblastosis viruses, AEV-type, and the avian myeloblastosis viruses, AMV-type, which probably code for three different viral oncogenes (for review, see Hayman, 1981). Since antibodies against the transformation-specific proteins are not available at present, only those oncogenes that are fused to viral structural proteins can be studied. While AMV-type viruses express their tumor gene unlinked to viral structural proteins (Gonda *et al.*, 1980), the transformation-specific protein from MC29-type viruses is fused to p19, the N-terminal portion of the gag protein. In contrast, AEV-transformed cells synthesize two putative transforming proteins, erb A, a gag-fusion protein, and erb B, which is unlinked to gag proteins. Their roles in transformation are not yet understood (Bister and Duesberg, 1979, Lai *et al.*, 1980). Monoclonal antibodies against p19 (Greiser-Wilke *et al.*, 1981) allow identification of the transforming protein from MC29, gag-myc, and one of the AEV-specific proteins, gag-erb A.

Both MC29 and AEV viruses can transform fibroblasts as well as bone marrow cells in *in vitro* systems (for review, see Graf and Beug, 1978). In fact, MC29- and AEV-transformed quail or chicken fibroblastic cell lines, which express the virus-specific proteins, have been established. These lines, MC29-Q8-NP and AEV-cl 23 are so-called non-producer cell lines since they do not express any helper virus and thus are devoid of gag-related structural polyprotein precursors.

One MC29-transformed producer cell line, MC29-Q10, spontaneously produced mutant viruses after a short time in culture, which became evident as these cells synthesized smaller gag-related proteins besides the original wild-type proteins. This led to the isolation of three non-producer quail cell clones, Q10A, Q10C, and Q10H, which continuously synthesize the smaller gag-fusion proteins. These mutants have an altered ability to transform bone marrow cells, but they transform fibroblasts as efficiently as the wild-type (Ramsay *et al.*, 1980).

We have recently shown that the gag-myc protein from MC29-transformed fibroblasts is located in the nucleus and after purification binds to double-stranded DNA (Donner *et al.*, 1982). We have extended these studies by investigating further the presence of the gag-myc protein in isolated chromatin to obtain biological evidence for the significance of the *in vitro* DNA-binding capacity. Accumulation of the gag-myc protein in the nucleus was also shown by pulse-chase analysis. Furthermore, we investigated the localization of the gag-myc proteins of the three deletion mutants in fibroblasts. In addition, MC29-transformed bone marrow cells were analyzed since the localization of the gag-myc protein in bone marrow cells was unknown. In parallel, studies were performed with the gag-erb A protein from AEV-transformed fibroblasts.

Results

Cellular localization of gag-myc and gag-erb A polyproteins by immunofluorescence

The established cell line of MC29-transformed quail fibroblasts MC29-Q8-NP (Bister *et al.*, 1977) was analyzed for the presence of the 110 K gag-myc fusion protein using monoclonal antibodies as well as rabbit sera directed against p19 in an indirect immunoprecipitation. MC29-Q8-NP is a non-producer cell line that does not produce any viral polyprotein precursors unless superinfected with the replication-competent helper virus RAV60 (MC29-Q8 x RAV 60) (Figure 1). The non-producer MC29-Q8-NP cells contain the gag-myc protein of mol. wt. 110 K, which is also expressed in the virus-producing superinfected cells in addition to the

*To whom reprint requiests should be sent.

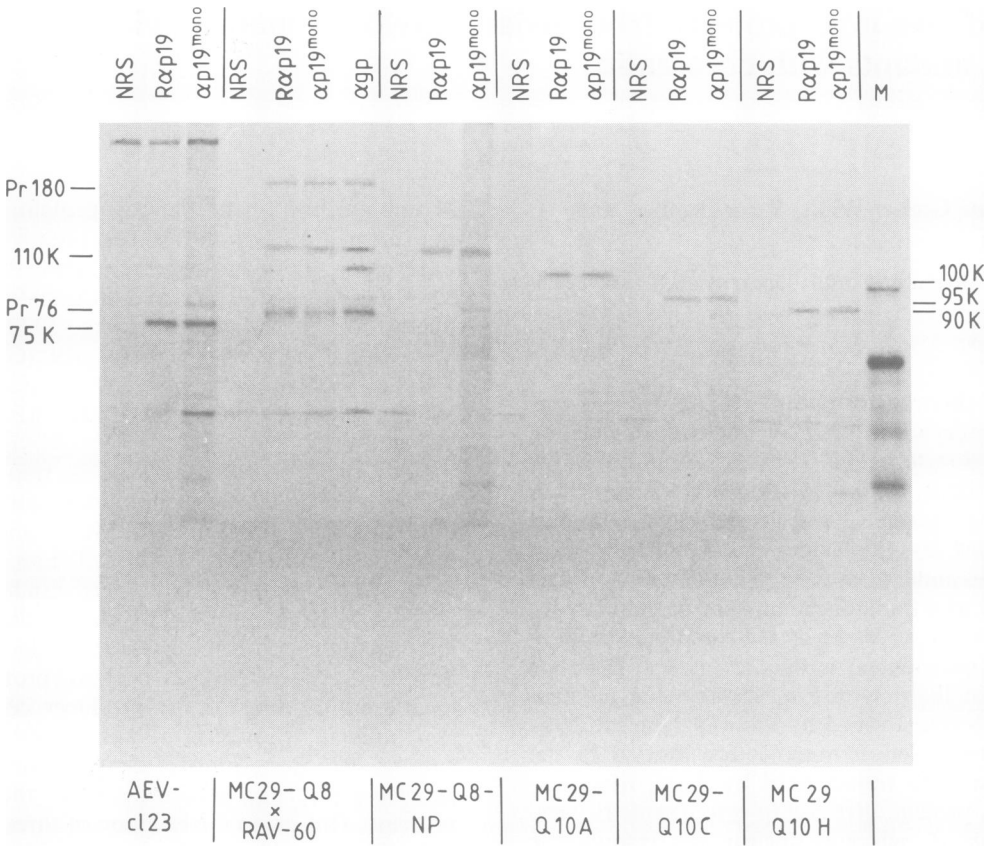


Fig. 1. Immunoprecipitation of wild-type and mutant gag-myc and gag-erb A polyproteins. Transformed cells were labeled with [^{35}S]methionine for 4 h (250 μCi/ml), lysed and treated with antibodies and *S. aureus* for indirect immunopreciptation. The cells used were: AEV-cl 23, MC29-Q8 x RAV60, MC29-Q8-NP, MC29-Q10A, -Q10C, and -Q10H. NRS: normal rabbit serum. Rαp19: rabbit anti-p19 antiserum, αp19^{mono}: purified monoclonal anti-p19 IgG (this IgG precipitates a protein from MC29-Q8-NP cells which is similar but not identical to Pr76). αgp: rabbit serum against the glycoprotein and p27. The immune precipitates were analyzed on 10% polyacrylamide gels, dried, and exposed for autoradiography. M: ^{14}C marker with mol. wts. from top: 97 K, 68 K, 44 K, 35 K.

Pr180$^{gag\text{-}pol}$ and Pr76^{gag} (Bister *et al.*, 1977). The three cell lines transformed by the MC29 deletion mutants, designated Q10A, Q10C, and Q10H, produce deleted gag-myc proteins with mol. wts. of 100 K, 95 K, and 90 K, respectively (Ramsay *et al.*, 1980) as shown in Figure 1.

AEV-cl 23 is an AEV-transformed non-producer chicken fibroblast cell line (Pawson and Martin, 1980). Antibodies directed against p19 immunoprecipitate the 75 K gag-erb A fusion protein (Hayman *et al.*, 1979). Monoclonal antibodies directed against p19 (Greiser-Wilke *et al.*, 1981), the N-terminal region of the gag-protein, were used for further analysis of the gag-linked transforming proteins synthesized by MC29-Q8-NP wild-type, Q10A, Q10C, and Q10H deletion mutants, and AEV-cl 23 cells. The cellular localization of the proteins was studied by indirect immunofluorescence using as a second antibody, fluorescein-labeled anti-mouse IgG (Donner *et al.*, 1982). The MC29-Q8-NP as well as the three cell lines transformed with the mutant MC29 viruses exhibit a strong nuclear fluorescence which excludes the nucleoli (Figure 2A, B). Unambiguous identification of nuclei, nucleoli, and cytoplasm was achieved by using Nomarski differential interference contrast microscopy (Khalaf and Bainbridge, 1981) on the same cells that were analyzed by fluorescence (Figure 2A, B, bottom pictures). In contrast, AEV-cl 23 cells show a clear cytoplasmic fluorescence (Figure 2C). Dark areas inside these cells represent vacuoles. Cytoplasmic fluorescence with the same monoclonal antibodies was also observed in helper virus superinfected cells, MC29-Q8 x RAV60, while normal quail fibroblasts were absolutely negative with these monoclonal antibodies as was shown previously (Donner *et al.*, 1982).

Isolation of chromatin from wild-type and mutant transformed fibroblasts

MC29-Q8-NP cells, the superinfected MC29-Q8 x RAV60 cells, and the mutant-infected Q10A and Q10C cells were labeled with [^{35}S]methionine, lysed by treatment with detergent-containing hypertonic buffers and fractionated by differential centrifugation for separation of cytoplasmic material and nuclei and subsequent isolation of chromatin. The amounts of the transformation-specific wild-type and mutant proteins, 110 K, 100 K, and 95 K, in isolated chromatin (chr) and soluble fraction (S) were determined by immunoprecipitation with antiserum against p19 and are shown in Figure 3A and Table I, which also includes the nuclei (N). In all cases the gag-myc proteins were found to be associated with the chromatin. As control for the effective separation of nuclear and cytoplasmic fractions, viral proteins known to be cytoplasmic or membrane-associated such as Pr76^{gag}, Pr92^{env}, and p27, were monitored in fractionated MC29-Q8 x RAV60 producer cells. They were mainly found in the cytoplasmic fraction (Table I, S) while ~60% of the gag-myc proteins from the various cell types were found in the nuclear fraction (Table I, N). The purity of the chromatin

fractions was determined by electron microscopy, which gave evidence that there was no contamination by ribosomes or membrane material (Figure 3B, a). Biochemical analysis showed that the chromatin preparation consisted of various sized DNAs (Figure 3B, c) to which histone proteins were bound (Figure 3B, b). The quantitative evaluation of the chromatin-associated protein given in Table I indicates that ~10–20% of the total cellular gag-myc protein is bound to the chromatin both in wild-type MC29-Q8 and in mutant virus-transformed Q10C cells. In comparison, only 1–3% of p27 and Pr76^{gag} are found in the chromatin fraction. Preparation of chromatin involves some loss of material, therefore it is difficult to account for the residual amount of protein. The binding of the 110 K protein to chromatin proved to be strong, since a high salt concentration was required to allow its elution. Most effective was the presence of the detergent SDS (data not shown).

Determination of the total cellular [^{35}S]methionine radioactivity and the radioactivity incorporated into the gag-myc protein (Table I) gives an estimate of the amount of 110 K protein present in MC29-transformed cells. The 110 K protein accounts for ~0.01% of the total cellular protein.

Analysis of MC29-transformed bone marrow cells.

MC29, virus, if injected into animals, predominantly affects bone marrow cells, which are considered to be the natural target cells (Ivanov *et al.*, 1962). In contrast, the MC29-Q8-NP cell line was derived from MC29-transformed quail fibroblasts. Therefore, it was of interest to analyze and compare the distribution of the 110 K protein in both cell systems. Bone marrow cells were infected *in vitro* with various dilutions of a virus stock of MC29 with RAV-1 as helper virus. After soft-agar cloning, two clones, the non-producer clone MC29-BM cl 10 and the virus producer clone MC29-BM cl 9, were selected and chromatin analysis was performed as described for the fibroblast cell lines. Immunoprecipitation of the chromatin preparation and the soluble fraction showed that the distribution of the 110 K protein is similar in both cell types (Figures 3A and 4A). Effective isolation of the chromatin preparation is evident since the viral proteins Pr92^{env} and p27 synthesized in the producer bone marrow cells were found in the cytoplasmic fractions only (Figure 4A). The radioactivity recovered from the 110 K protein was too low to be determined and is therefore only indicated qualitatively in Table I.

Similar analysis was performed with the AEV-transformed fibroblastic cell line AEV-cl 23. As expected from the fluorescence results (Figure 2C), only ~10% of the gag-erb A protein was present in the nucleus, while the majority, 90%, was found associated with the cytoplasmic fraction (Figure 4B and Table I).

Pulse-chase experiment with MC29-transformed fibroblasts

To obtain further evidence for the nuclear localization of the gag-myc protein, cells were radioactively labeled for 20 min and subsequently the distribution of the radioactivity was followed after chase with complete medium for various times. The numbers presented in Table II were derived from immune precipitates analyzed on slab gels and elution of the radioactivity from the gels. While the total radioactivity present in the cellular lysate remained constant during the chase periods, the amount of 110 K protein present in the nucleus increased. In a 20 min pulse, ~30% of the 110 K was detected in the nucleus. This amount increased during a 60 min chase to 50% and during a 120 min chase to ~60%. This amount was also detected in the nucleus after a 4 h pulse (Table I). MC29-transformed producer and non-producer cells behaved similarly. The distribution of the Pr180$^{gag\text{-}pol}$, which under these conditions was not processed (in contrast to Pr76^{gag}), served as a control for cytoplasmic contamination.

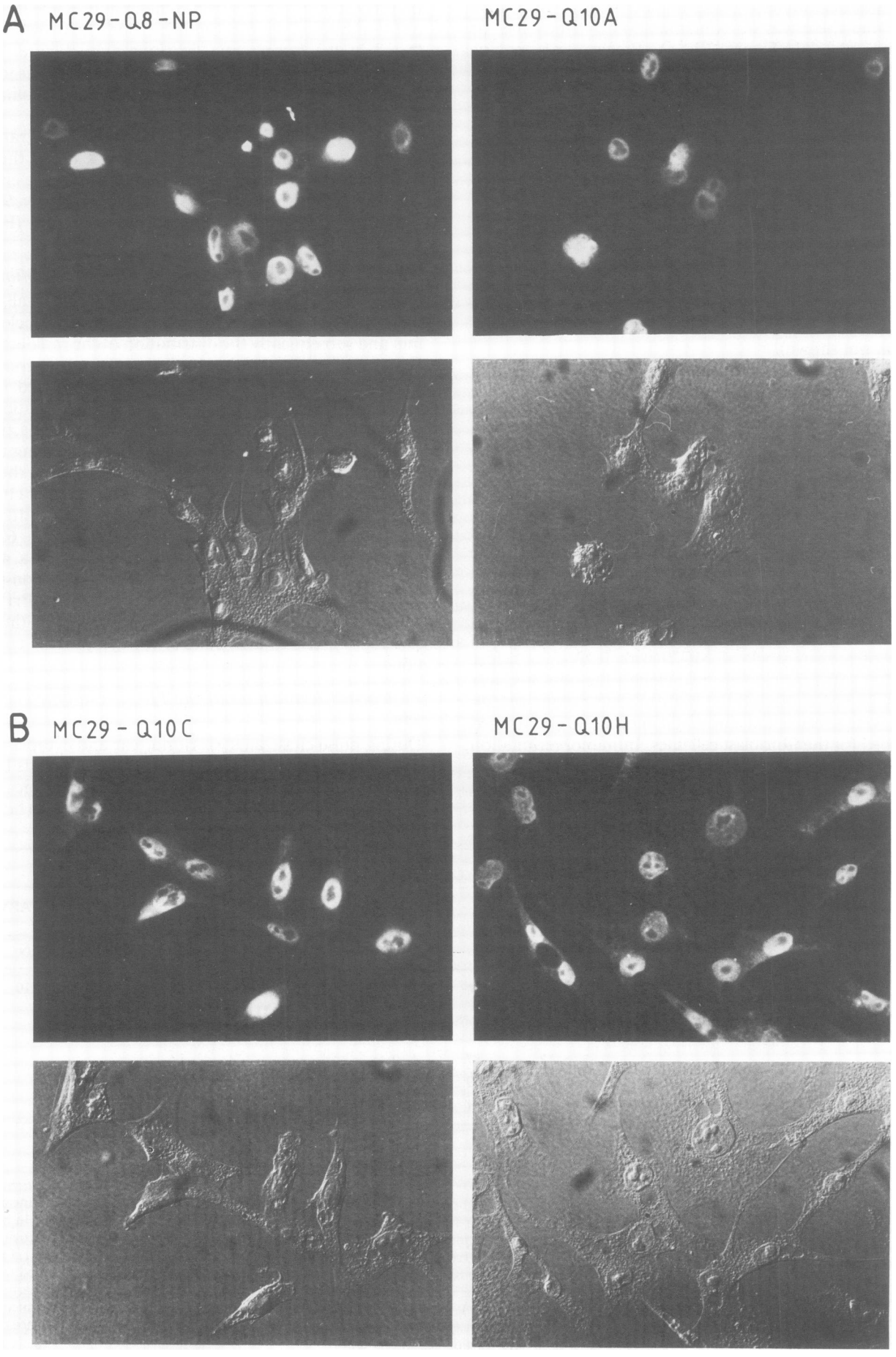
Proteolytic degradation of the 110 K wild-type gag-myc rotein

During pulse-chase analysis of nuclear and cytoplasmic fractions a heterogeneous population of protein bands below the 110 K protein was detected. In the cytolasmic fractions smaller proteins of ~100 K and 95 K appeared to be enriched

Table I. Cellular distribution of proteins

	MC29-Q8-NP			MC29-Q8 x RAV60			MC29-Q10C			MC29-BM cl 10			AEV cl 23	
	chr	N	S	chr	N	S	chr	N	S	chr	S		N	S
p110$^{gag\text{-}myc}$	19	62	38	15	60	40				+++	–	p75$^{gag\text{-}erb}$	A10	90
[^{35}S]methionine c.p.m.	6300	20 300	13 100											
p95$^{gag\text{-}myc}$							11	55	45					
Pr76^{gag}				3	12	87								
Pr92^{env}				<1	nd	>99								
p27				1	8	91								
Amount of protein (%)	5	20	80											
[^{35}S]methionine c.p.m.	8 x 10^7	4 x 10^8	1.5 x 10^9											

MC29-Q8-NP cells, helper virus superinfected MC29-Q8 x RAV60 cells, and one mutant-infected cell line Q10C were labeled with [^{35}S]methionine and fractionated into a cytoplasmic soluble (S) and a nuclear fraction (N) which was further processed for the isolation of chromatin (chr) as described in Materials and methods. From defined aliquots of these fractions the proteins were immunoprecipitated, processed on polyacrylamide gels and the radioactivity determined. These numbers were corrected for total volumes and indicated as percentages. For example, the MC29-Q8-NP cellular lysate contained 1.5 x 10^9 c.p.m. in S and 4 x 10^8 c.p.m. in N, representing 80 and 20% of the whole cell, respectively. The 110 K protein in these two fractions amounted to 13 100 and 20 300 c.p.m., respectively, corresponding to 38 and 62%. Furthermore, the amount of chromatin corresponded to 8 x 10^7 c.p.m. which harbored 6300 c.p.m. of 110 K. This amount corresponds to 19% of the total cellular 110 K protein which is chromatin-bound. The same analysis was performed with the other cell lines presented. MC29-BM cl 10 cells contained too low radioactivity after fractionation for quantitative evaluation. Therefore, presence of 110 K is indicated by (+ + +) and absence by (–). AEV-cl 23 fibroblasts were fractionated into N and S only.


A
MC29-Q8-NP
MC29-Q10A
B
MC29-Q10C
MC29-Q10H

at the expense of the 110 K (Figure 5, left). This effect seemed to be a consequence of proteolytic degradation. To allow integration of the three bands for quantitation of nuclear and cytoplasmic distribution of the gag-myc protein, it was important to prove that they were related. A partial proteolytic digest of the three proteins was therefore performed using V8 protease. The result, which is shown in Figure 5, indicates that indeed the three proteins are related and most likely arise by proteolysis predominantly in the soluble fraction.

Discussion

We have recently described that the transformation-specific protein, $p110^{gag\text{-}myc}$, from the acute avian leukemia virus MC29 binds to double-stranded DNA after 3700-fold purification by immune-affinity column chromatography. Furthermore, it was shown by immunofluorescence micro-

Table II. Nuclear distribution of $p110^{gag\text{-}myc}$ during pulse-chase

	pulse/chase	min/min	20/0	20/60	20/120
MC29-Q8-NP	$p110^{gag\text{-}myc}$	% c.p.m. in nucleus	25	48	55
MC29-Q8 x RAV60	$p110^{gag\text{-}myc}$	% c.p.m. in nucleus	31	50	63
	$Pr180^{gag\text{-}pol}$		3.4	7.2	7

MC29-Q8-NP and superinfected MC29-Q8-RAV60 cells were labeled for 20 min with [^{35}S]methionine (500 μCi/ml). Subsequently the cells were chased with complete medium for 60 and 120 min. The amount of p110 in the nucleus was determined by immunoprecipitation from fractionated cellular lysates and elution from polyacrylamide gels. The amount is indicated as a percentage. The amount of $Pr180^{gag\text{-}pol}$ which is not processed under these labeling conditions was also determined in the nuclear fraction.

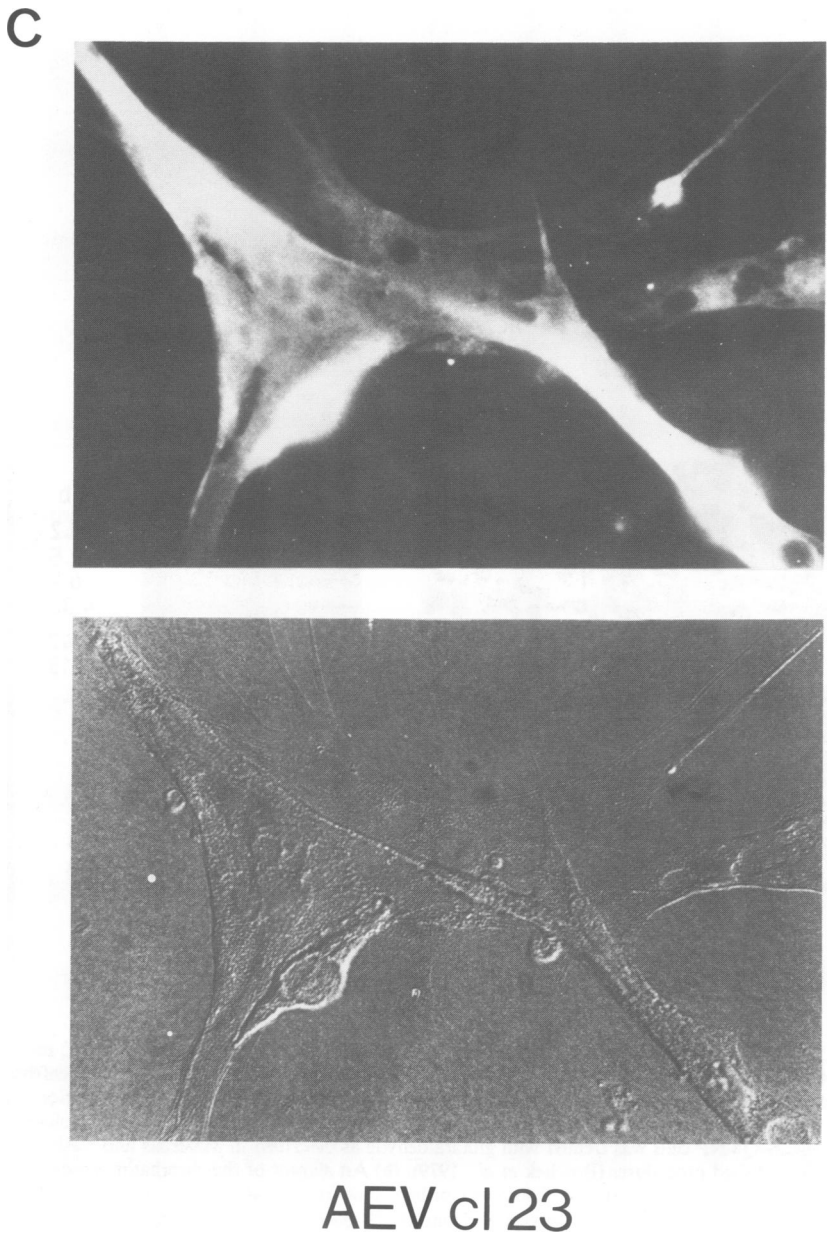


Fig. 2. Localization of gag-fusion proteins by indirect immunofluorescence. Indirect immunofluorescence with monoclonal antibodies against p19 and fluorescence-labeled second antibody was performed with the following cells: **(A)** MC29-Q8-NP, MC29-Q10A, **(B)** -Q10C, -Q10H, and **(C)** AEV-cl 23. Normarski optic analysis of the identical cells is shown for identification of cellular structures. Magnification of MC29 cells: 600 x, AEV cells: 1000 x.

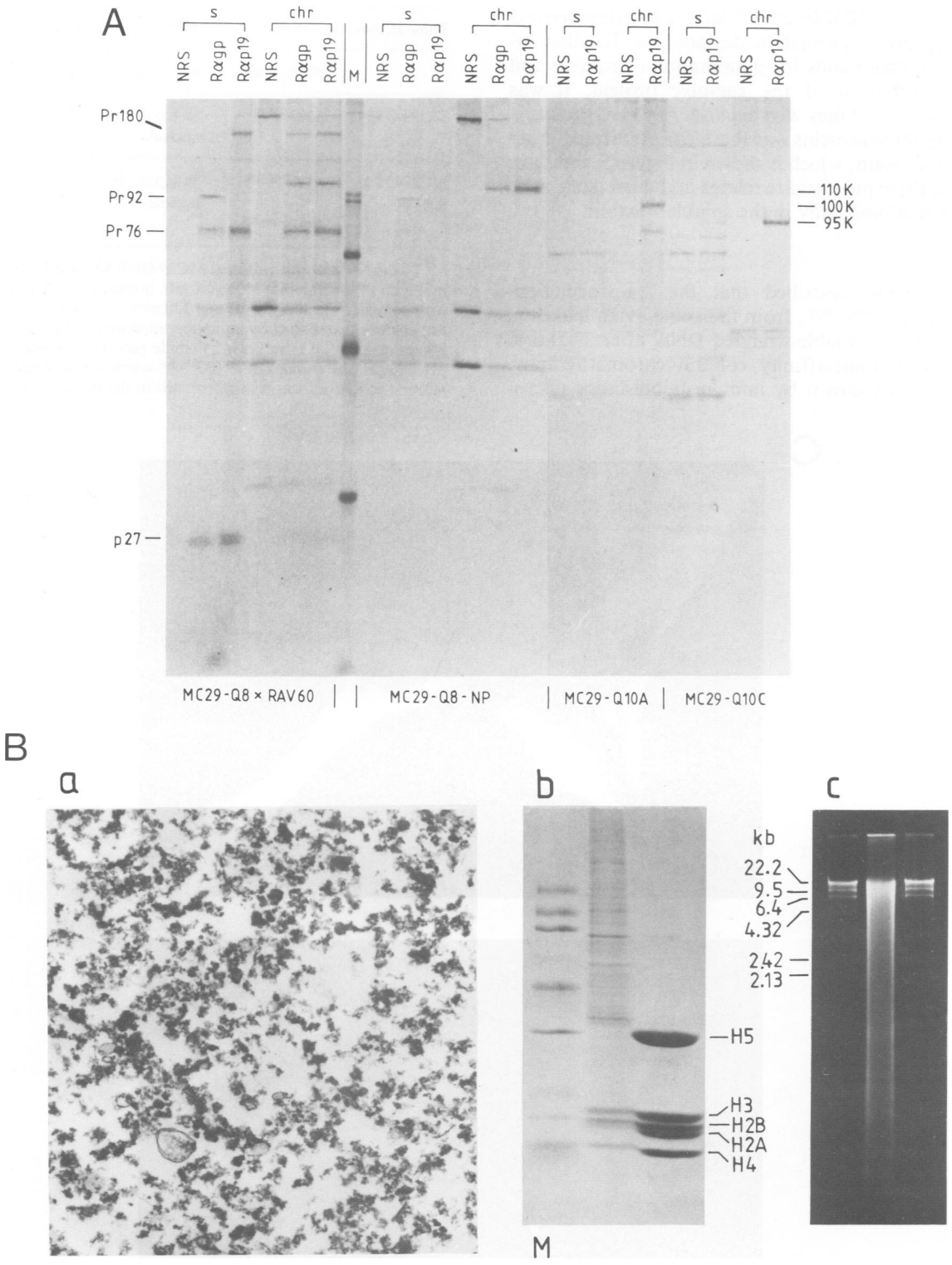


Fig. 3. Isolation of chromatin. **(A)** MC29-Q8-NP, MC29-Q8 x RAV60 superinfected cells, and MC29-Q10A and -Q10C mutant cell lines were labeled with [^{35}S]methionine (250 μCi/ml) for 4 h. The cells were disrupted by a Dounce homogenizer and submitted to low speed centrifugation. The supernatant (S) was stored at -20°C until needed and the pellet was processed for the isolation of chromatin (chr). Each immunoprecipitation was performed with a standardized amount of radioactive material (4 x 10^6 c.p.m.) which corresponds to 5% of the chromatin fraction and 0.2% of the soluble fraction. For nomenclature see Figure 1. **(B) (a)** Chromatin from MC29-Q8-NP cells was treated with glutaraldehyde as described in Materials and methods. The fixed sample was analyzed by electron microscopy according to published procedures (Boschek *et al.*, 1979). **(b)** An aliquot of the chromatin preparation of MC29-Q8-NP cells was analyzed by polyacrylamide gel electrophoresis and stained with Coomassie briliant blue (middle slot). Purified chicken histones served as marker proteins H5, H3, H2B, H2A, H4 (right). M indicates mol. wt. markers. **(c)** Chromatin from MC29-Q8-NP cells was analyzed on a 1.4% agarose gel which was stained with ethidium bromide (1 μg/ml). A photograph was taken in the presence of u.v. light (254 nm). λ DNA restricted by *Hind*III was used as mol. wt. standard.

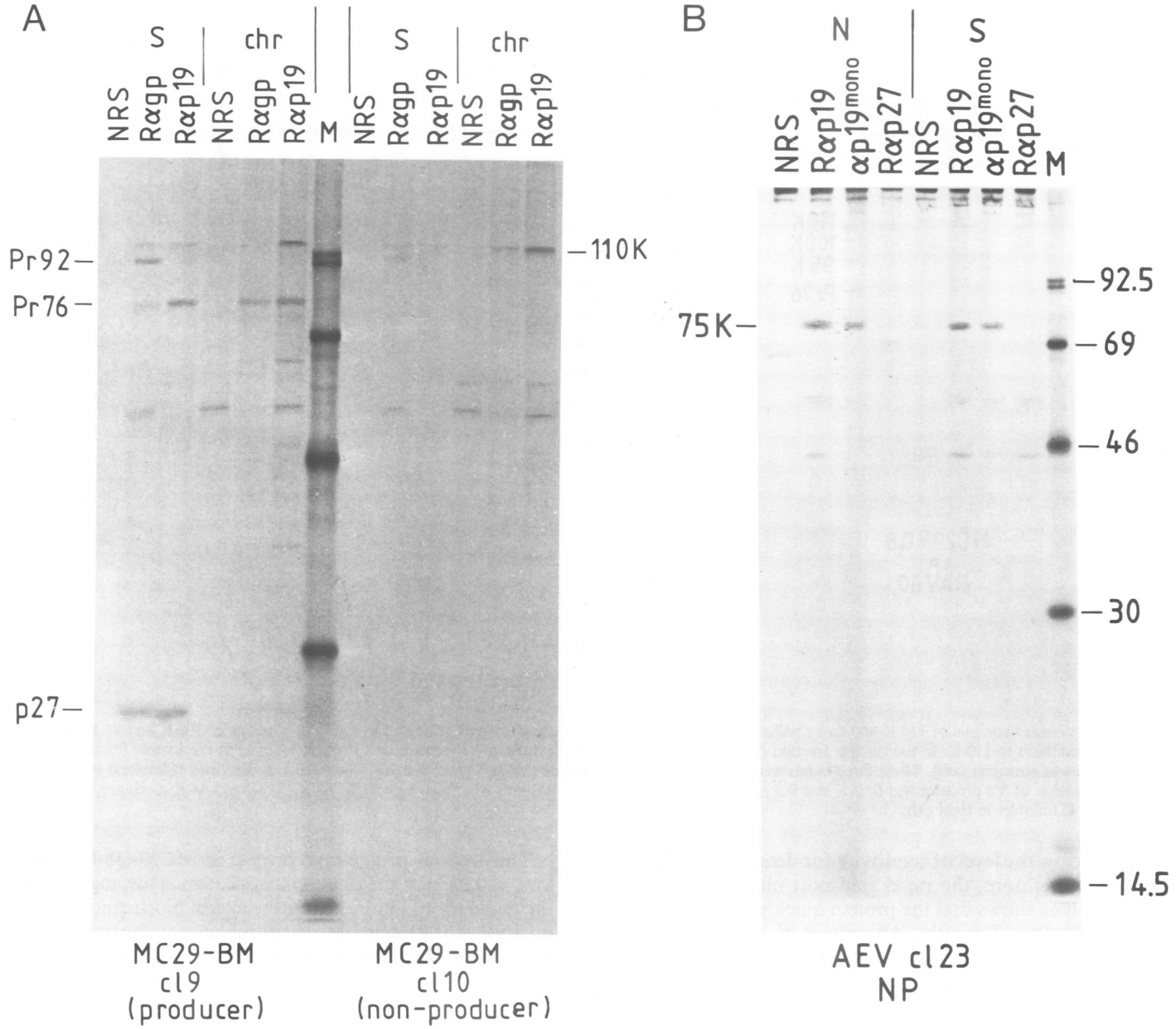


Fig. 4. Fractionation of MC29 bone marrow cells and AEV-cl 23 cells. **(A)** Two MC29-transformed bone marrow clones were isolated by soft-agar cloning, a virus producing clone (MC29-BM cl 9) and a non-producer clone (MC29-BM cl 10). The cells were labeled with [^{35}S]methionine for 4 h. A soluble (S) fraction and chromatin (chr) were prepared as described in Figure 3 and Materials and methods. Indirect immunoprecipitation was performed with 1 x 10^6 c.p.m. of radioactivity as input material reflecting 0.4% of the soluble fraction and 10% of the chromatin fraction. For nomenclature see Figure 1. **(B)** The AEV-transformed chicken fibroblast cell line AEV-cl 23 was labeled with [^{35}S]methionine for 4 h and then fractionated into a nuclear (N) and soluble (S) fraction as described previously (Donner *et al.*, 1982). A standardized amount of radioactive material was used as input (1 x 10^6 c.p.m.) which corresponds to 11% of the nucledar and 1% of the soluble fraction. Indirect immunoprecipitation was performed with the sera described in Figure 1. Rαp27: rabbit serum against p27. 75 K indicates the gag-erb A fusion protein.

scopy with monoclonal antibodies against the gag portion of the protein that the p110 is located in the nucleus of a MC29-transformed fibroblast cell line. To prove that binding of the protein to DNA is of biological significance, and not a consequence of cleavage or conformation, we have extended our previous studies by isolating chromatin from transformed cells. The gag-myc protein of MC29-transformed fibroblasts was found associated with the chromatin. The binding appears to be quite strong since it can only be reverted by high salt and detergent. Detergent is of practical importance during manipulations of the gag-myc containing material since it improves the recovery of the protein, which otherwise adsorbs to plastic and glass surfaces. This is probably the reason for the incomplete recovery of the gag-myc protein during chromatin isolation.

Furthermore, previous localization studies by indirect immunofluorescence have been improved. Simultaneous application of fluorescence and Nomarski interference techniques allow us to identify clearly the exclusion of the gag-myc protein from nucleoli. The protein appears to be located inside the nucleus and not enriched at the nuclear membrane. Almost none of the protein was detected in the cytoplasm by fluorescence analysis, in spite of the fact that the gag-myc protein is synthesized in the cytoplasm. However, its amount

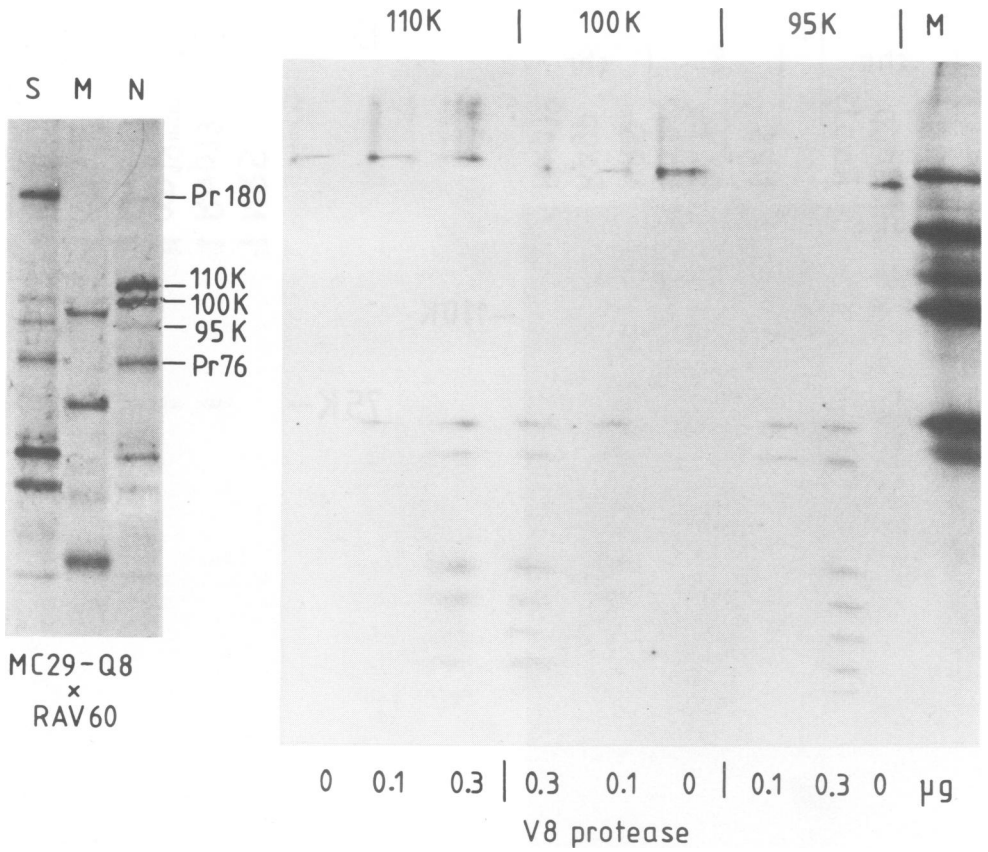


Fig. 5. V8-cleavage analysis of 110 K and two smaller proteins. During the pulse-chase analysis presented in Table II, proteins of 100 K and 95 K were observed in addition to 110 K. In the nuclear fraction (N) of MC29-Q8 x RAV60 cells the 110 K protein was stronger whereas in the soluble fraction (S) the 95 K protein was strongest (left). These three bands were eluted from polyacrylamide gels and processed for V8-cleavage as described (Cleveland *et al.*, 1977). The concentration of V8 protease was 0, 0.1, and 0.3 µg/sample. M: ^{14}C marker with mol. wts. from top: 97 K, 68 K, 53 K, and 44 K (both are weak in left gel), and 35 K (doublet in right gel).

may be below the level of sensitivity for detection by fluorescence. Furthermore, the rapid transport observed by pulse-chase analysis shows that the protein quickly accumulates inside the nucleus. Recently, Abrams *et al.* (1982) have also described the nuclear localization of the wild-type v-myc protein using polyvalent rabbit sera.

MC29-transformed bone marrow cells which are assumed to be the natural target cells *in vivo* were also analyzed. These cells exhibit a limited life-span in culture and therefore only a qualitative analysis of the localization of the gag-myc protein could be performed by cellular fractionation. Clearly, the gag-myc protein is also associated with the chromatin in bone marrow cells. Transformation of fibroblasts and bone marrow cells has recently been described to be uncoupled in the case of three deletion mutants from MC29. The deletions have been located in the transformation-specific myc portion (Ramsay and Hayman, 1982; Bister *et al.*, 1982). These mutants transform fibroblasts, but transform bone marrow cells only to a significantly lesser extent. Since only fibroblasts transformed by the mutants are available, they were analyzed for localization of the deleted gag-myc proteins and compared to the wild-type. No difference in fluorescence and chromatin analyses was so far detectable. The comparative studies performed, however, may not be sufficiently sensitive to reveal minor differences in strength or specificity of binding. Further studies, in particular with the purified gag-myc proteins from wild-type and mutants, are required to resolve this question.

The amount of gag-myc protein in MC29-Q8-NP cells is low, $<0.01\%$ of the total cellular protein. This, together with the tendency to bind to surfaces, makes biochemical analysis difficult. The protein bears a resemblance to the transforming protein coded for by simian virus 40 (SV40), the T-antigen, which is also a nuclear protein. The T-antigen has, however, been studied much more extensively since techniques have become available to produce it in larger quantities (for review, see Tijan, 1981).

For comparison with the gag-myc protein, we have analyzed the gag-erb A protein from AEV. Its role in transformation is unclear. The gag-erb A protein behaves differently in every respect analyzed here; it gives rise to cytoplasmic fluorescence and after cell fractionation is located predominantly in the cytoplasm. No accumulation of fluorescent dye was observed in the end of cytoplasmic protrusions, a phenomenon we observed with the same technique using sarcoma-virus transformed fibroblasts, which are assumed to harbor their transforming protein in the cytoplasmic membrane (unpublished observation). The gag-erb A analyses presented here support the specificity of the observations with the gag-myc proteins. Much, however, remains to be learnt about its function and properties.

At present, it seems that three different mechanisms can give rise to fibroblastic transformation, the nuclear gag-myc protein, the cytoplasmic gag-erb A protein possibly in concert with the erb B protein, and the recently well-studied, membrane-bound sarcoma-specific protein which appears to be a

protein kinase (for review, see Erikson *et al.*, 1980). Whether all these mechanisms are basically similar remains to be seen. The immune-affinity column with monoclonal antibodies against p19 represents a universal approach for purification of all gag-related transforming proteins and may help to elucidate their functions.

Materials and methods

Cells and viruses

MC29-Q8-NP is an established quail fibroblast cell line which did not produce any virus. It was kindly supplied by Klaus Bister. MC29-Q8 x RAV60 cells were obtained by superinfection with RAV60 helper virus. The non-producer cell lines Q10A, Q10C, Q10H, transformed by three deletion mutants of MC29, were generously supplied by Michael Hayman. AEV-cl 23 is an AEV-transformed established chicken fibroblast cell line kindly supplied by Steve Martin. MC29-infected bone marrow cells were obtained from Hartmut Beug and further analyzed for producer and non-producer clones in this laboratory. MC29-Q8-NP and MC29-Q8 x RAV60 cells were grown in 1/2 Dulbecco's modified Eagle's Medium (DMEM), 1/2 RPMI, 1 mM Hepes, 5% calf serum, 1% heat-inactivated chicken serum and 0.5% dimethyl sulphoxide. The mutants and bone marrow cells as well as AEV-cl 23 were grown in 5% fetal calf serum instead of in calf serum.

Radioactive labeling procedure

Cells were grown on 10 cm Petri dishes near to confluency, starved in medium without methionine for 2–4 h and then labeled with the methionine-free medium supplemented with 250–500 μCi/ml of [^{35}S]methionine for 4 h. After washing in phosphate buffered saline the cells were processed immediately for the isolation of nuclei and chromatin or stored at −70°C until used for immunoprecipitations. Bone marrow cells that grow in suspension were labeled in Eppendorf tubes.

Immunoprecipitation

Details of the procedure have been published previously (Kessler, 1975; Brugge and Erikson, 1977). Briefly, cells, nuclei, or isolated chromatin were treated with RIPA buffer (50 mM Tris-HCl, pH 7.2, 150 mM NaCl, 0.1% SDS, 1% deoxycholate, 1% Triton X-100) supplemented with 5 μg/ml of Trasylol (Boehringer, Mannheim) for 15 min at 4°C. The suspension was then centrifuged at high speed (Beckman SW60 rotor, 55 000 r.p.m., 6 h, 4°C). 4 x 10^6 c.p.m. of acid-precipitable material from the supernatant was incubated with 5 μl of serum or 10 μg of purified IgG isolated from monoclonal antibody-containing ascites fluid for 60 min at 4°C. Then *Staphylococcus aureus* was added for indirect precipitation. The precipitates were collected, washed extensively, and analyzed on 10% polyacrylamide slab gels. The gels were dried, exposed for autoradiography, and then the radioactivity of the excised bands determined after solubilization in a liquid scintillation counter. For the numbers given in the tables a correction for total volume of the individual fractions was performed.

Chromatin isolation

Chromatin was isolated according to Lapeyre and Bekhor (1974). [^{35}S]methionine-labeled cells from 4 to 6 Petri dishes were collected by low-speed centrifugation and suspended in 10 ml of 10 mM NaCl, 1.5 m M $MgCl_2$, 10 mM Tris-HCl, pH 7.0, 0.2% Nonidet P40 and disrupted by 30 strokes in a tight-fitting Dounce homogenizer. Efficiency of disruption was controlled for by phase contrast microscopy. The suspension was centrifuged at 1000 *g* to collect the nuclei. The supernatant (S) was stored frozen. The nuclei were washed twice with 10 ml of 10 mM Tris-HCl, pH 7.0, 10 mM NaCl and 1.5 mM $MgCl_2$ (TNM). This fraction was designated as nuclei (N). Subsequently the nuclei were disrupted in a Branson cell disruptor using a microtip at 25 W, 20 KHz, five times for 10 s. The sonicated material was centrifuged through 10 ml of 30% sucrose in 2.5 mM Tris-HCl, pH 7.0, 10 mM NaCl at 5200 r.p.m. for 15 min, 4°C in a Sorvall HB4 rotor to remove nucleoli. The material which remained on top of the sucrose was diluted to 10 ml in TNM and centrifuged through 27 ml 60% sucrose-containing 2.5 mM Tris-HCl, pH 7.2, 10 mM NaCl, and 24 mM EDTA. Centrifugation was in a Beckman SW27 rotor at 27 000 r.p.m., for 90 min at 4°C. The pellet consisted of chromatin which was washed twice by resuspension in 10 mM Tris-HCl, pH 7.2, 1 mM EDTA (T-E) and was repelleted by centrifugation for 20 min at 27 000 r.p.m. in a SW27 rotor. This chromatin preparation (chr) was stored in 2 ml T-E at −20°C until further use.

The purity of the chromatin preparation was analyzed by electron microscopy. Chromatin was repelleted, suspended in 0.1 M sodium cacodylate, pH 7.0, and treated with 2.5% glutaraldehyde for 60 min at room temperature. After fixation the sample was analyzed by electron microscopy according to published procedures (Boschek *et al.*, 1979).

Immunofluorescence

Monoclonal anti-p19 IgG was purified with protein A-Sepharose (Pharmacia). Fluoresceine isothiocyanate-labeled anti-mouse IgG was purchased from Cappel (Cochranville, USA). Details of the procedure have been published (Donner *et al.*, 1982).

Acknowledgements

We are grateful to Dr. M. Hayman, Imperial Cancer Research Fund Laboratories, London, for Q10A, Q10C, and Q10H; Dr. S. Martin, University of California, Berkeley, for AEV-cl 23; Dr. K. Bister, Max-Planck-Institut für Molekulare Genetik, Berlin, for MC29-Q8-NP; Dr. H. Beug, Deutsches Krebsforschungszentrum, Heidelberg, for infected bone marrow cells. The electron microscopic analysis of chromatin by Dr. B. Boschek, Universität Giessen, is gratefully acknowledged. Dr. A. Ruiz-Carrillo, Institut Pasteur, Paris, generously supplied the histone proteins used as markers.

References

Abrams,H.D., Rohrschneider,L.R., and Eisenman,R.N. (1982) *Cell,* **29**, 427-439.
Bister,K., and Duesberg,P.H. (1979) *Proc. Natl. Acad. Sci. USA,* **76**, 5023-5027.
Bister,K., Hayman,M.J., and Vogt,P.K. (1977) *Virology,* **82**, 431-448.
Bister,K., Ramsay,G.M., and Hayman,M.J. (1982) *J. Virol.,* **41**, 754-766.
Boschek,C.B., Jockusch,B.M., Friis,R.R., Back,R., and Bauer,H. (1979) *Direktabb. Oberfl.,* **12**, 47-54.
Brugge,J.S., and Erikson,R.L. (1977) *Nature,* **269**, 346-348.
Cleveland,D.W., Fischer,S.G., Kirschner,M.W., and Laemmli,U.K. (1977) *J. Biol. Chem.,* **252**, 1102-1106.
Donner,P., Greiser-Wilke,I., and Moelling,K. (1982) *Nature,* **296**, 262-266.
Erikson,R.L., Purchio,A.F., Erikson,E., Collett,M.S., and Brusse,J.S. (1980) *J. Cell Biol.,* **87**, 315-325.
Gonda,T.J., Shiness,D.K., Fanshere,L., Bishop,J.M., and Moscovici,M.G. (1980) *Cell,* **23**, 279-290.
Graf,T., and Beug,H. (1978) *Biochim. Biophys. Acta,* **516**, 269-299.
Greiser-Wilke,I., Owada,K.M., and Moelling,K. (1981) *J. Virol.,* **39**, 325-329.
Hayman,M.J. (1981) *J. Gen. Virol.,* **52**, 1-14.
Hayman,M.J., Royer-Pokora,B., and Graf,T. (1979) *Virology,* **92**, 31-45.
Ivanov,X., Mladenov,Z., Nedyalkov,S., and Todorov,T.G. (1962) *Bull. Inst. Pathol. Comp. Animaux,* **9**, 5-36.
Kessler,S.W. (1975) *J. Immunol.,* **115**, 1617-1624.
Khalaf,R.M.J., and Bainbridge,D.R. (1981) *Eur. J. Immunol.,* **11**, 816-824.
Lai,M.M.C., Neil,J.C., and Vogt,P.K. (1980) *Virology,* **100**, 475-483.
Lapeyre,J.-N., and Bekhor,I. (1974) *J. Mol. Biol.,* **89**, 137-162.
Pawson,T., and Martin,G.S. (1980) *J. Virol.,* **34**, 280-284.
Ramsay,G., Graf,T., and Hayman,M.J. (1980) *Nature,* **288**, 170-172.
Ramsay,G.M., and Hayman,M.J. (1982) *J. Virol.,* **41**, 745-753.
Tijan,R. (1981) *Curr. Top. Microbiol. Immunol.,* **53**, 5-20.