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DNA-Binding Ability of Transforming Proteins from Avian Erythroblastosis Virus and Mutant Avian Myelocytomatosis Virus, MC29, in Comparison with MC29 Wild Type

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A. Introduction

Oncogenic RNA tumor viruses code for oncogenes, which induce tumors in the animal and malignant transformation of cells in culture. The oncogenes are derived from normal cellular genes and their expression is controlled by viral regulatory elements which results in a roughly 100-fold amplification of the gene in comparison to its normal expression [9]. Since several viral oncogenes are expressed as hybrid proteins consisting of viral structural elements fused to transformation-specific regions [7], analysis of transforming proteins can be experimentally approached by antibodies against the viral structural part which allow the identification of the transforming proteins. We have analyzed such proteins from representatives of different classes of acute leukemia viruses, the acute avian myelocytomatosis viruses, MC29, and the acute avian erythroblastosis viruses, AEV, both of which affect the hematopoietic system of the infected animal at various stages of differentiation and cause rapid death [5]. Furthermore, they transform fibroblasts in culture. The transformation-specific protein from the third class of viruses, from avian myeloblastosis viruses, AMV, cannot be analyzed by this approach, as it is not expressed as *gag-onc* fusion protein [9]. We have recently identified the MC29 transforming protein *v-myc*, a molecule of 110K, as a DNA-binding protein [3]. Here we demonstrate that the *v-myc* protein, if purified from a transformation-defective MC29 deletion mutant, Q10C, which has a molecular weight of 95K instead of 110K and has reduced transformation ability [11], no longer binds to DNA. Furthermore, the purified AEV-specific protein, a molecule of 75K [8], also does not bind to DNA. Nothing is so far known about its function. Our results indicate that avian oncornaviruses carry at least three different types of oncogenes which cause transformation in vivo and in vitro, probably by different mechanisms. One of these mechanisms involves a DNA-binding protein, and a second one a protein kinase, while nothing is known about the third mechanism.

B. Methods

All methods have been described by Donner et al. [3] and Moelling et al. [10].

C. Results

Fluorescence microscopy of MC29-Q8-NP cells, which is a MC29-transformed quail fibroblast cell line [1], and Q10C cells, from a quail fibroblast cell line transformed by a deletion mutant of MC29 [11], was performed with monoclonal antibodies against the structural protein p19 [6], which represents the N-terminal portion of the 110K and 95K *v-myc* proteins. The procedure for indirect immunofluorescence with fluorescein-labeled second antibodies has been described previously [3]. The cells (Fig. 1) show strong nuclear fluorescence in both cases. In contrast, AEV-transformed fibroblasts exhibit cytoplasmic fluorescence.

The transformation-specific proteins from ^{35}S-methionine-labeled MC29-Q8-

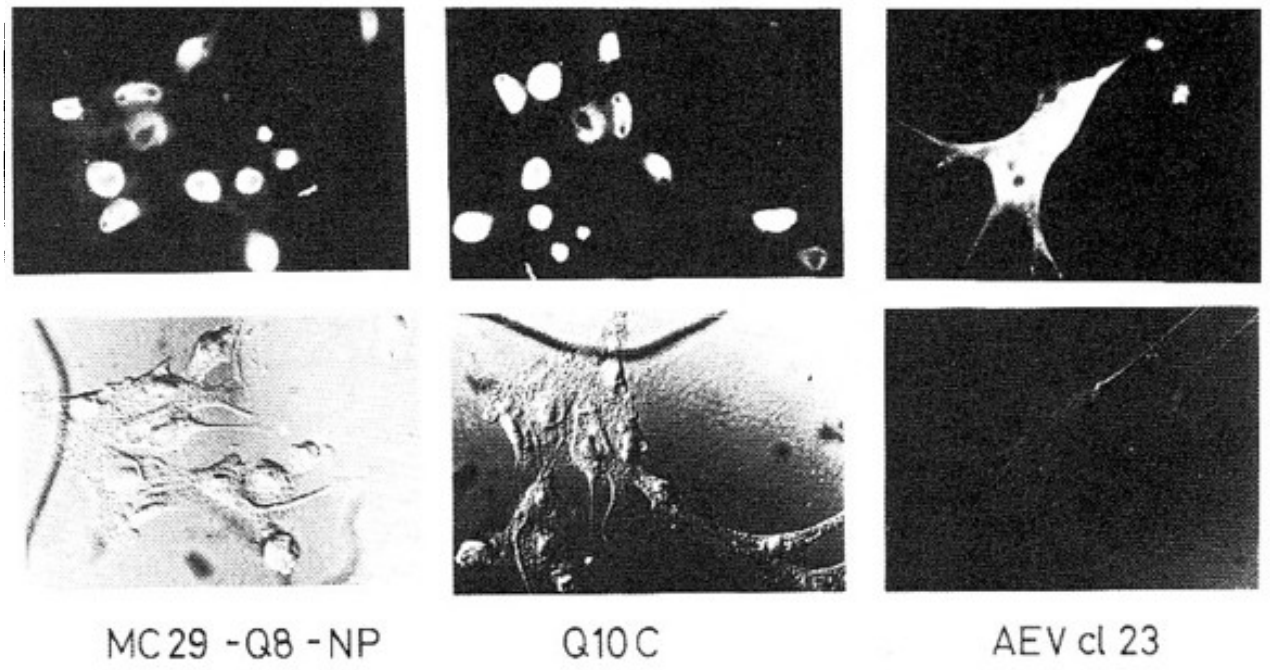

Fig. 1. Immunofluorescence of MC29-transformed quail fibroblasts (MC29-Q8-NP), of MC29 deletion mutant-infected quail fibroblasts Q10C, and of AEV-transformed chicken fibroblasts. All cells were nonproducers. Indirect immunofluorescence was performed using monoclonal antibodies against p19 [6], the N-terminus of the transforming proteins, and FITC-labeled anti-mouse IgG (Miles-Yeda, Miles Labs, Elkhart, Indiana/USA). The method has already been described [3]. *Bottom pictures:* Nomarski interference contrast of the same field. ×515

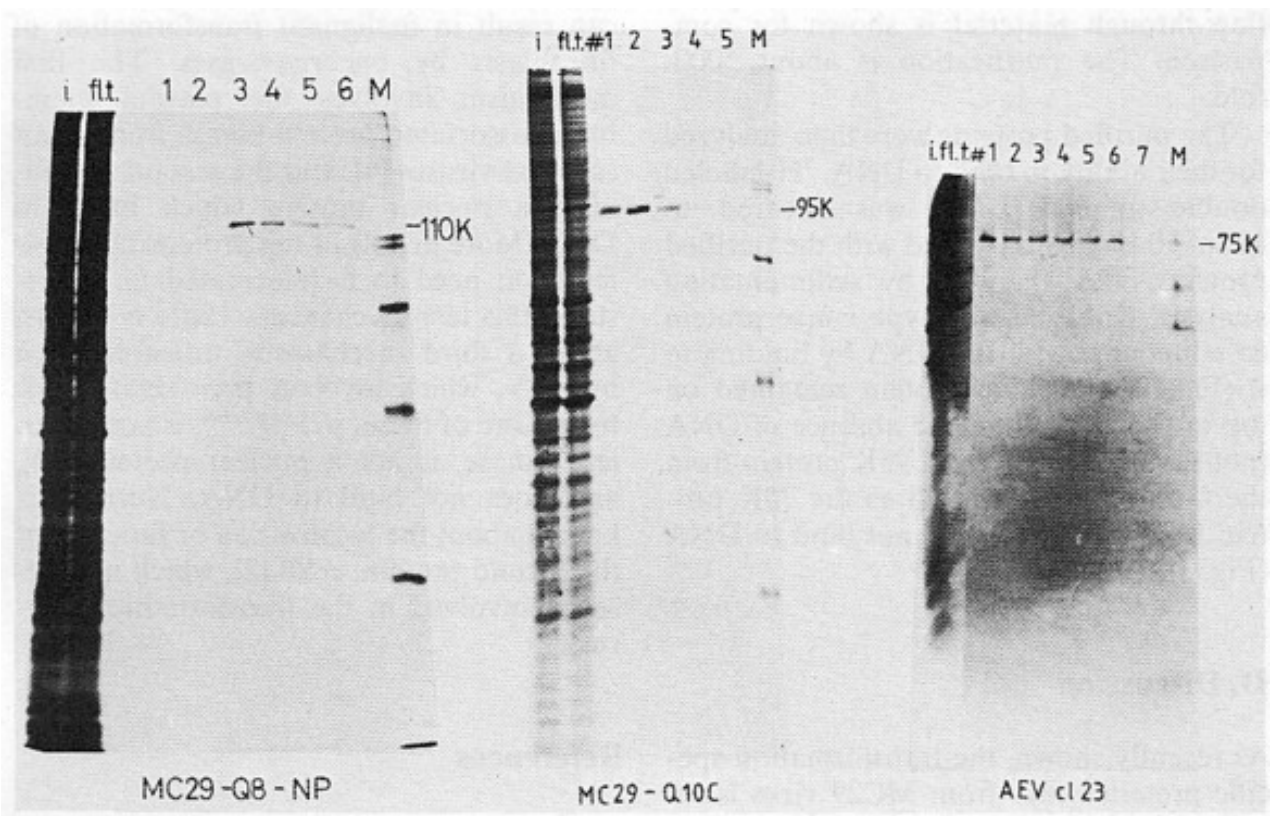

Fig. 2. Purification of 110K proteins from MC29-Q8-NP, 95K proteins from Q10C, and 75K proteins from AEV cl 23 using immunoaffinity column chromatography with monoclonal IgG against p19. Details have been described [3, 6]. Cells were labeled with ^{35}S-methionine for 2 h and lysed. Aliquots of the input (5 µl out of 15 ml), flow-through (5 µl out of 15 ml), and eluted material (25 µl out of 1 ml) were applied to a 10% polyacrylamide gel which was exposed for autoradiography. *M* indicates marker proteins

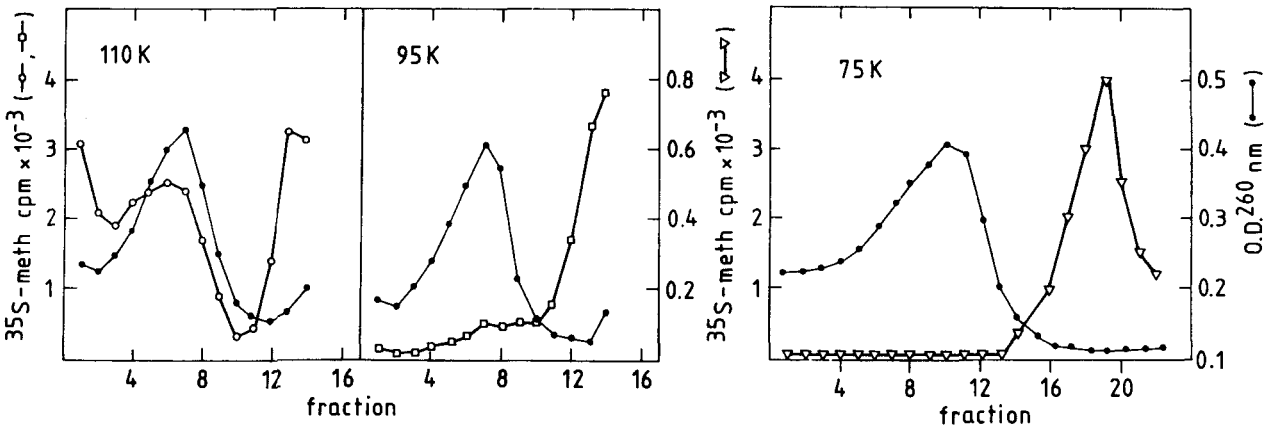

Fig. 3. DNA-binding of purified transforming proteins. ^{3}H-labeled chicken DNA sheared to about 10 kb was mixed with the ^{35}S-methionine-labeled purified proteins in the presence of 50 m*M* NaCl, sedimented through a 10%–30% glycerol gradient in 50 m*M* Tris-HCl, pH 8, and fractionated. The radioactivity of the fractions was determined by liquid scintillation counting

NP, Q10C, and AEV cl 23 cells were purified by immunoaffinity column chromatography with monoclonal antibodies against p19. The proteins were eluted with a low pH buffer and aliquots from the eluted fractions were analyzed by polyacrylamide gel electrophoresis. The result of the purification is shown in Fig. 2. The input and flow-through material is shown for comparison. The purification is about 3000-fold.

The purified proteins were then analyzed for their ability to bind to DNA. ^{3}H-labeled double-stranded DNA was sheared to about 10 kb in size, mixed with the purified proteins, and analyzed by sedimentation analysis. Only the wild-type *v-myc* protein cosedimented with the DNA by binding to it (Fig. 3), while the protein remained on top of the gradient in the absence of DNA (not shown). The deleted 95K protein from the Q10C mutant as well as the 75K protein from AEV cl 23 did not bind to DNA (Fig. 3).

D. Discussion

As recently shown, the transformation-specific protein *v-myc* from MC29 virus is localized in the nucleus and binds to double-stranded DNA [3]. Our results indicate that the DNA-binding correlates with transformation at least of bone marrow cells, as the protein from Q10C cells transformed by the MC29 deletion mutant lost its DNA-binding property together with part of its transformation capacity. While the MC29 deletion mutants have a markedly reduced transformation potential for bone marrow cells in vivo and in vitro, the transformation ability of fibroblasts in vitro remains unaffected [11]. It can be concluded that apparently three unrelated mechanisms can result in malignant transformation of fibroblasts by oncornaviruses. The first mechanism involves the plasma membrane-associated protein kinase from avian sarcoma viruses [4], and the second mechanism a nuclear protein which binds to DNA. More details of the protein-DNA interaction need to be elucidated to understand this last mechanism. Little is known about a third mechanism, transformation by AEV, which involves two specific proteins. One of these, p75$^{gag\text{-}erbA}$, is not a protein kinase, is not a nuclear protein [10], and does not bind to DNA. Nothing is known about the localization or function of the second protein, *erbB* [2], which may also be involved in the transformation process.

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