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# Expression of a truncated *v-myb* product in transformed chicken embryo fibroblasts

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Transformed cells have been isolated after transfection of chicken embryo fibroblasts (CEF) with the DNA of a recombinant clone (KXA 3457) in which the *v-myb* sequences are flanked by the two AMV-LTRs. Abnormal *myb*-specific RNA species and *myb*-related polypeptides were found to be expressed in these cells, suggesting that transformation of CEF by *v-myb* might require alterations of the oncogene product.

Transformation; Myeloblastosis; *V-myb* oncogene; Fibroblast; (Avian)

## 1. INTRODUCTION

Although the in vitro transforming potential of AMV has been reported to be restricted to hematopoietic cells [1], we have isolated recently a fibroblastoid cell line transformed by AMV proviral DNA. These cells both contained and expressed rearranged AMV proviral sequences [2], suggesting that an alteration of the *v-myb* oncogene might be responsible for the new transforming properties of its product. An analysis of the *myb*-related polypeptides expressed in these transformed cells reinforced this possibility [3] and prompted us to determine whether a transformation of chicken embryo fibroblasts could be induced without the participation of any infectious viral particles by the product of *v-myb* itself. For this purpose, we have constructed a recombinant clone (KXA 3457) in which the expression of the *v-myb* sequences is under the control of the AMV-LTRs [3]. Here, we report that the transformed cells obtained following transfection of Brown Leghorn chicken embryo fibroblasts with KXA 3457 DNA express both abnormal *myb*-specific RNA species, and *myb*-related polypeptides.

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## 2. MATERIALS AND METHODS

### 2.1. *Chicken strain, cell cultures and transfections*

Fertile chicken eggs were C/E Brown Leghorn, Edinburgh strain (gs+ chf+ V−) from Station de Pathologie Aviaire et de Parasitologie, Institut National de la Recherche Agronomique, Nouzilly, France. Preparations of chicken embryo fibroblasts and yolk sac cells were performed as described [2]. All cultures were performed in Eagle medium (MEM) supplemented with newborn calf serum (Gibco Laboratories, Paisley, Scotland) at the indicated concentrations. DNA-calcium phosphate coprecipitates [4] were used to transfect chicken embryo fibroblasts in suspension as described [2].

### 2.2. *Origin and structure of probes*

The different steps in the preparation of KXA 3457 recombinant clone have already been described [3]. The pBR322/SES3 and pBR322/SX12 clones contain respectively 350 and 450 base-pairs of *myb*-specific sequences [5].

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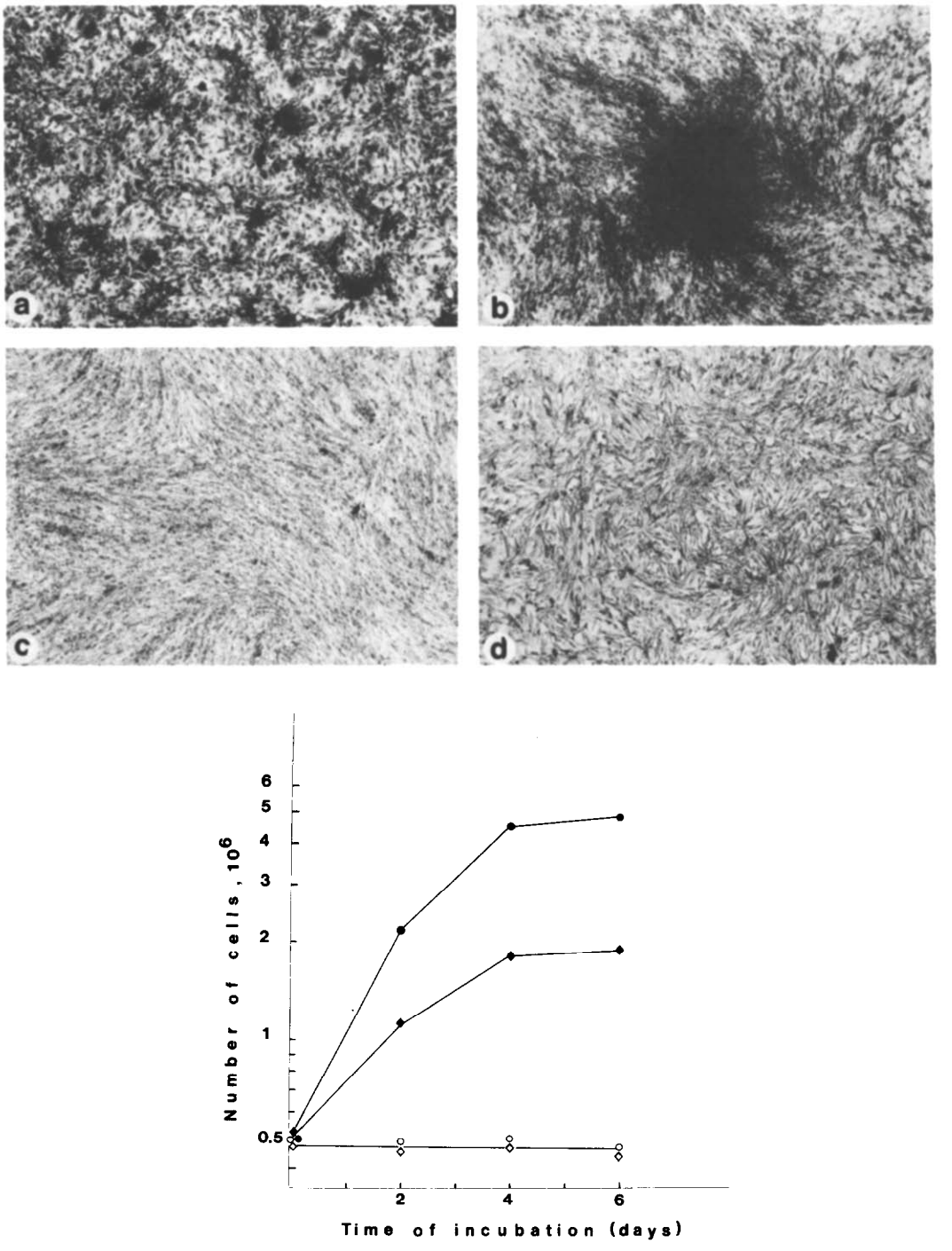


Fig.1. Overgrowth of transformed cells. Top: (a) foci of transformed cells after 10 days following transfection of CEF with KXA 3457; (b) isolated focus at higher magnification. Middle: monolayers of normal CEF (c), and transformed 25S2 cells (d) in the presence of 5% calf serum. Bottom: growth curves for normal CEF (◆) and 25S2 transformed cells (●) in the presence of 5% calf serum. No growth is observed in the presence of 0.5% calf serum (open symbols). Each point represents an average of 3 independent counts.

### 2.3. *RNA purification and analysis*

Polyadenylated RNA species from normal and transformed cells were purified by guanidine thiocyanate extraction, selected on oligodeoxythymidylic acid-cellulose columns and analyzed by electrophoresis in 1% agarose-formaldehyde gels, transfer to nitrocellulose and hybridization to radioactive probes [6].

### 2.4. *Labeling of cells, preparation of cell extracts, immunoprecipitation and polyacrylamide-SDS gel electrophoresis*

Normal and transformed chicken embryo fibroblasts were first incubated for 4 h in MEM deprived of methionine and supplemented with 2% dialyzed calf serum. The culture medium was then changed to MEM containing 3 $\mu$g/ml of methionine (1/5 of the regular methionine concentration) and 70 $\mu$Ci/ml of L-[$^{35}$S]methionine (New England Nuclear, spec. act. 1000 Ci/mmol). After 18 h labeling, the cells were lysed in 500 $\mu$l of RIPA per 50 mm plate, and immune precipitation of *myb*-related polypeptides present in extracts of transformed cells was carried out under the conditions described elsewhere [6]. Briefly the labeled cell extracts were incubated with 5 $\mu$l of *myb*-specific antiserum [7] for 1 h at 4°C and with 20 $\mu$l of CL4B protein A Sepharose (Pharmacia) for 30 min at 4°C. The immune complexes were run on 8.5% polyacrylamide gels in the presence of SDS.

## 3. RESULTS

A plating of chicken embryo fibroblasts grown for 10 days after transfection with KXA 3457 DNA revealed the presence of transformants able to pile up and form dense foci on monolayers of normal cells (fig.1a,b). Several of these foci were trypsinized and grown out for further characterization. In this paper we report the results obtained with clone 25S2. As already described in other systems [8], the 25S2 cells expressed an intermediate transformation phenotype. When assayed for their capacity to grow without anchorage in semi-solid medium (0.5% Bacto agar) they gave rise to abortive colonies of about 100–150 cells. Also, the 25S2 cells were not able to grow in the presence of low concentration (0.5%) of serum (fig.1) and did not produce an increased level of plasminogen activator as compared to that of normal cells. However, the 25S2 cells grew in a more disorganized way than their normal counterpart (fig.1b,c) and reached higher saturation densities than normal CEF in the presence of 5% serum (fig.1), this last property being most certainly correlated with their ability to form foci.

These cells were found to express a new *myb*-specific 4.8 kb polyadenylated RNA species which

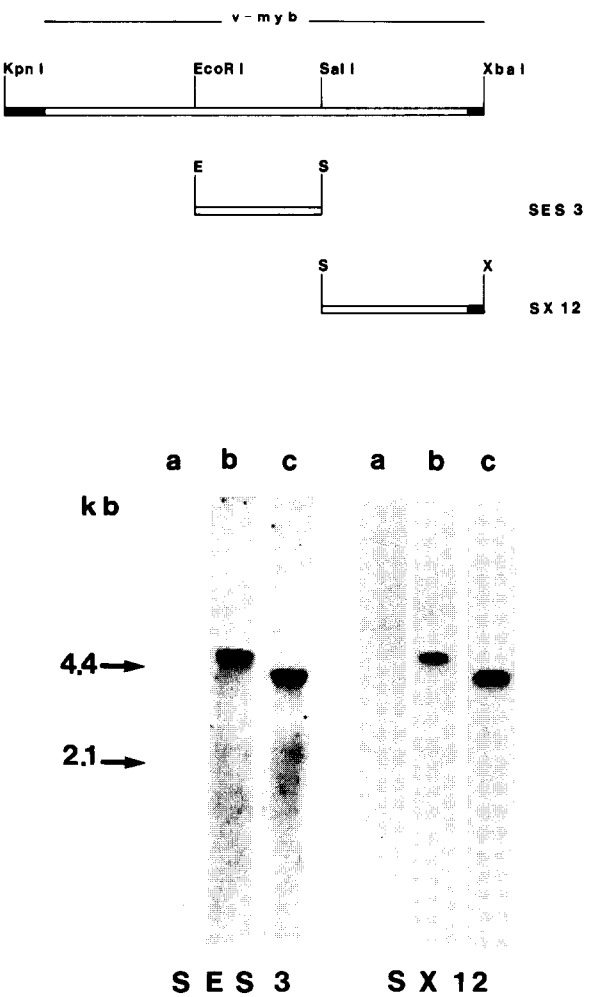


Fig.2. *myb*-specific RNA species expressed in 25S2 transformed cells. Samples (1 $\mu$g) of polyadenylated RNA from normal CEF (a), 25S2 transformed cells (b) and yolk sac cells (c) were electrophoresed on formaldehyde-agarose (1%) gels and hybridized to the SES3 and SX12 *v-myb*-specific probes. Molecular mass markers (4.4 and 2.1 kb) were chicken ribosomal RNA species run in parallel.

is clearly larger than the 4.0 kb *c-myb* mRNA expressed in yolk sac cells from the same embryo and which is not detected in control CEF under similar conditions (fig.2). Since these RNA transcripts were also revealed after hybridization with the SX12 probe, they must contain sequences located beyond the unique *Sal*I site present in *v-myb* [5]. The size of the *myb*-specific RNA species is larger than that corresponding to the transcription of intact KXA 3457 DNA, which gives rise to a 2.0 kb *myb*-specific message [3]. This observation suggests that a rearranged KXA 3457 DNA was expressed in the transformed cells.

Two kinds of *myb*-specific antisera were used to search for *myb*-related polypeptides expressed in 25S2 transformed cells. These antisera were raised

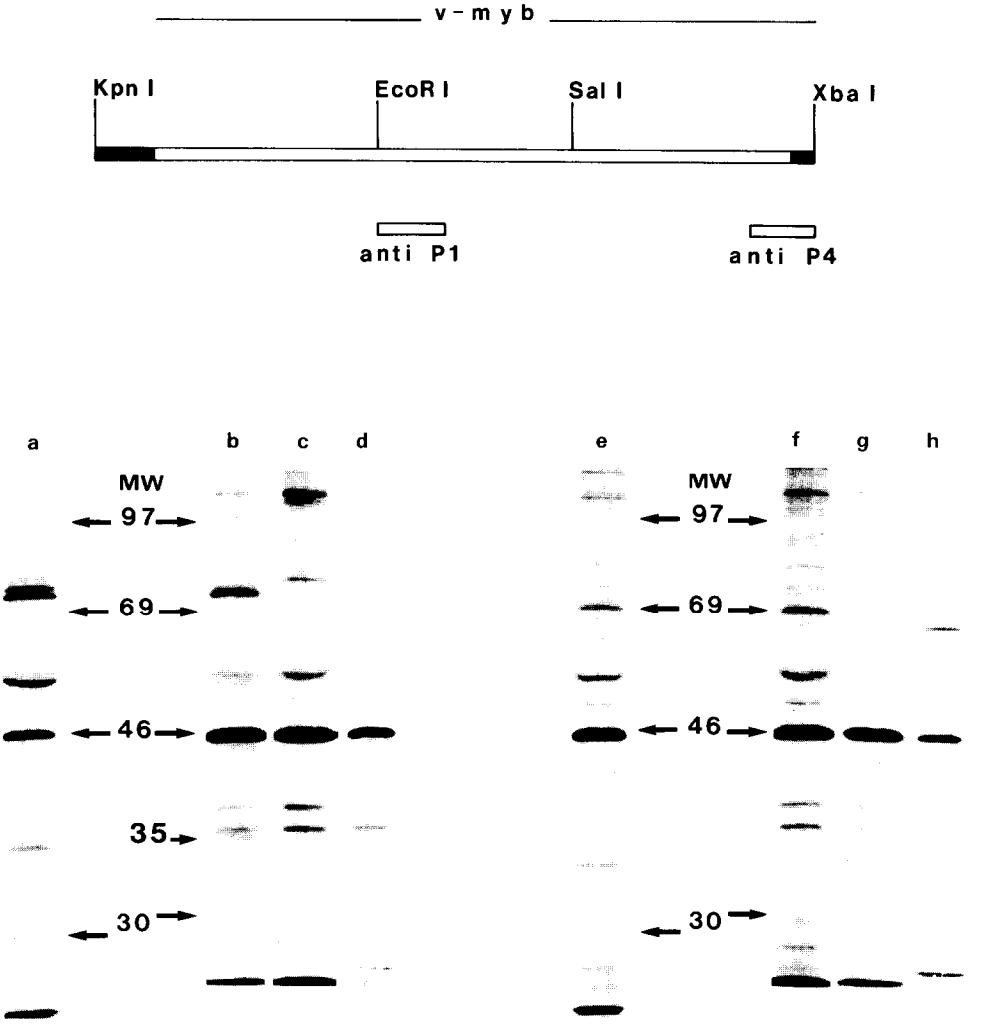


Fig.3. Immunoprecipitation of *myb*-related polypeptides expressed in 25S2 transformed cells. Labeled lysates ($1.6 \times 10^6$ cpm) of trichloroacetic acid precipitable material from normal CEF (a,e) or 25S2 transformed cells (b–d,f–h) were immunoprecipitated with 5 $\mu$l of: anti-P1 (a,b); preimmune serum from the same rabbit (c); anti-P1 preincubated with 1 $\mu$g of P1 peptide (d); anti-P4 (e,f); preimmune serum from the same rabbit (g); and anti-P4 preincubated with 1 $\mu$g of P4 peptide (h). $M_r$ markers expressed as $M_r \times 10^{-3}$ were: phosphorylase *b* (97), bovine serum albumin (69), ovalbumin (46) and carbonic anhydrase (30).

against two synthetic peptides of 15–19 amino acids representing different regions of the $P48^{v\text{-}myb}$ polypeptide [7]. Anti-P1 is specific for the peptide encoded by the sequence between nucleotide positions 546 and 590 from the 3′-proximal *Kpn*I site in AMV while anti-P4 corresponds to the carboxy-terminal portion of the *myb* product, located between nucleotide positions 1254 and 1310. Both antisera allow specific immunoprecipitation of the $P48^{v\text{-}myb}$ polypeptide expressed in AMV-transformed myeloblasts and infected fibroblasts [3,7].

Transformed cells (25S2) and the corresponding normal CEF were labeled with [$^{35}$S]methionine. Cellular extracts were incubated with either: (i) anti-P1; anti-P1 preincubated with P1 peptide; and the preimmune serum from the same rabbit; or (ii) anti-P4; anti-P4 preincubated with P4 peptide; and the preimmune serum.

The results obtained after immunoprecipitation revealed that a 35 kDa *myb*-related polypeptide was expressed specifically in 25S2 transformed cells, as demonstrated by the positive reaction with anti-P1 and the inhibition of precipitation observed with anti-P1 preincubated with P1 peptide (fig.3). However, this polypeptide was not detected after incubation with anti-P4 which is specific for the carboxy-terminal portion of *v-myb*. The other major bands detected in 25S2 cells with either anti-P1 (53–55 and 72–74 kDa) or anti-P4 (50–54 and 69 kDa) were also detected in control CEF (fig.3, lanes a,e) and probably result from non-specific precipitation since no *myb*-specific product was ever detected in normal CEF under similar conditions [3].

## 4. DISCUSSION

In a previous paper we reported the properties of a clone of transformed cells (25CUP) which expressed a 3.9 kb *myb*-specific mRNA, clearly different from *c-myb* RNA species, and a 70 kDa polypeptide larger than the $P48^{v\text{-}myb}$ [3].

Herein, we show that another set of transformed cells (25S2) also obtained after transfection with KXA 3457 DNA expressed a new 4.8 kb *myb*-specific mRNA and a *myb*-related 35 kDa polypeptide shorter than the $P48^{v\text{-}myb}$ product.

The use of antisera raised against different portions of the *v-myb* protein enabled us to show that the *myb*-related polypeptides expressed in these two clones of transformed cells correspond to altered *v-myb* products.

Since the accumulation of the normal *v-myb* protein in the nucleus of AMV infected fibroblasts does not appear to modify their physiology [9] the results reported here reinforce the possibility that expression of a truncated *myb* polypeptide is required to induce transformation of chicken embryo fibroblasts.

The construction of recombinant clones harboring in vitro-induced deletions has been undertaken to determine the extent of the alterations needed to induce transformation.

It is particularly tempting to relate the loss of growth control exhibited by the different transformed fibroblasts isolated thus far with the existence of three different functional domains in the *myb* product [10]. One of them which is thought to be involved in DNA-binding activity, includes the *v-myb* sequences located between nucleotide positions 900 and 960 [11] downstream to the unique *Sal*I site. Positive hybridization of the new *myb*-related RNA species (3.9 and 4.8 kb) with the SX12 probe indicated that at least part of this region is expressed in the transformed cells. However, the region corresponding to the P4 synthetic peptide is not detected in the corresponding *myb*-related polypeptides. It would therefore be conceivable that alterations occurring in the domain involved in the DNA binding activity of the *v-myb* product could modify its properties, in such a way as to enable it to induce fibroblastic transformation. This model might also apply to the oncogene product of another acute leukemia virus (E26), able to transform fibroblasts in vitro, and expressing *v-myb* sequences in the form of a fusion polypeptide in which neither the 5′- nor the 3′-proximal *v-myb* sequences are represented [12,13].

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