Identification of *c*-myb (chicken), *c*-myb (mouse) and *v*-myb (AMV) protein products by immunoprecipitation with antibodies directed against a synthetic peptide

Antonín Maly+ and Viktor Krchňák

+*Institute of Molecular Genetics, Czechslovak Academy of Sciences, 166 37* and *Léčiva-Pharmaceuticals, 143 10 Praha, Czechoslovakia*

Received 9 June 1986

A synthetic nonadecapeptide (IL 19) derived from a sequence of *v*-myb was covalently bound to haemocyanin and used for immunization. Anti-IL 19 serum immunoprecipitated a 75 kDa protein in the lysate of metabolically labelled chicken and murine thymus cells. Presaturation of the serum with IL 19 abolished this immunoprecipitation, thus indicating that the product of *c*-myb in both chicken and murine thymuses is the 75 kDa protein ($p75^{c\text{-}myb}$). Anti IL 19 serum also precipitated $p48^{v\text{-}myb}$ in the lysate of nonproducer myeloblasts.

Anti-peptide antibody *Immunoprecipitation* c-*myb protein* $p48^{v\text{-}myb}$ *(Chicken, Mouse)*

1. INTRODUCTION

Over 20 different *v*-onc have been explored and corresponding *c*-onc have been isolated and, in most cases, structurally analysed. The genomes of a broad spectrum of vertebrate species contain one or more copies of *c*-myb [1–3], whose transcription is restricted to hematopoietic organs [4–6]. This contrasts sharply with the ubiquitous expression of many other *c*-onc. There is apparently an inverse correlation between *c*-myb expression and the initiation of terminal differentiation [7,8]. A large portion of chicken *c*-myb [9], and the *c*-myb cDNA [10], have been molecularly cloned and sequenced. The avian *c*-myb protein product has been identified as $p75^{c\text{-}myb}$ by immunoprecipitation of the lysate of AEV-transformed erythroblasts with antibodies directed against a part of *v*-myb expressed in a procaryotic vector [11] and has been found to be located in the nucleus [12]. The protein product of *c*-myb, however, has not been unequivocally detected in non-transformed avian cells.

Murine *c*-myb cDNA has also been molecularly cloned and sequenced [13]. However, the protein product of *c*-myb (mouse) was not detected.

AMV is an oncogenic retrovirus which transforms myelomonocytic cells in vitro and causes myeloblastic leukaemia in chicks [14]. The gene responsible for this transformation has been designated *v*-myb. Apparently, it has emerged through transduction of several *c*-myb exons into a retroviral genome [2,9]. The product of *v*-myb is a protein of 45–48 kDa ($p48^{v\text{-}myb}$) [11,15,16]. It is located in the nucleus [12,17] and binds to DNA [18].

Abbreviations: KLH, keyhole limpet haemocyanin; AEV, avian erythroblastosis virus; AMV, avian myeloblastosis virus; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; *c*-onc, cellular oncogene; *v*-myb, transforming sequence of avian myeloblastosis virus; $Pr76^{gag}$, precursor of gag proteins; cDNA complementary DNA; BDB, bis-diazotized benzidine; p48, protein of 48 kDa; *v*-onc, viral transforming sequence; *c*-myb, cellular homologue of the transforming sequence of avian myeloblastosis virus

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

In this study, we identified the *c*-myb protein product in avian and murine thymus cells by means of antibodies directed against a synthetic nonadecapeptide hapten. The $p48^{v\text{-}myb}$ was also detected in the lysate of nonproducer transformed myeloblasts.

2. MATERIALS AND METHODS

KLH was from Calbiochem, [^{35}S]methionine (>30 TBq/mmol) from Amersham and tryptose phosphate broth from Difco.

The AMV-transformed GM 727 line of chicken nonproducer myeloblasts (producers of only noninfectious virions) [19] was kindly provided by T. Graf; the cells were grown at 37°C in Eagle's MEM [20] supplemented with 10% tryptose phosphate broth, 5% heat-inactivated foetal calf serum and 1% chicken serum. A short-term tissue culture of chicken thymus cells was prepared from the freshly explanted thymi of 1–3 weeks old leukosis-free Brown Leghorns [21]. Short-term tissue cultures of mouse thymus cells were prepared from 2–4-week-old Balb/c mice. For labelling, cells were suspended (1×10^7 myeloblasts/ml or 2.5×10^7 thymus cells/ml) in Eagle's MEM (minus methionine) with 1% dialysed foetal calf serum and 1% dialysed chicken serum and incubated for 20 min. [^{35}S]Methionine was then added (9.25 MBq/ml) and the incubation continued for 120 min. The average incorporations (in 10^6 cpm) per 10^6 cells were as follows: myeloblasts, 4.5; chicken thymocytes, 1.5; mouse thymocytes, 1.

The synthetic peptide used for immunization was composed of 19 amino acids (IL 19). The position of IL 19 is: in *v*-myb, 789–845 bp [22]; V812–V868 bp [9]; in *c*-myb, C3715–C3771 bp [9]; 1075–1131 bp [10]; 874–930 bp [13]. IL 19 was covalently bound to KLH with BDB [23] (9 mol IL 19 per mol KLH) and 5 rabbits were immunized with the IL 19-KLH conjugate. The 6th rabbit was immunized with BDB-treated KLH without peptide. The final sera from rabbits immunized with IL 19-KLH were designated 1–5, serum from the 6th rabbit was control. Details concerning the choice of IL 19, its synthesis and covalent binding to KLH will be published separately. The procedures of immunization, immunoprecipitation, SDS-PAGE and processing of gels by fluorography are described in [16]. In a typical experiment, lysates of 1×10^6 myeloblasts or 1×10^7 thymus cells were preimmunoprecipitated with 5 μl of control serum (2 h, 18°C). The supernatant was then immunoprecipitated with 5 μl of immune serum. Presaturation of 5 μl of serum 4 was done with 1.5 μg of a peptide or 50 μg of KLH.

3. RESULTS

The synthetic peptide (IL 19) used as hapten to elicit myb-specific antibodies was composed of 19 amino acids in the following sequence: Ile-Gln-Arg-His-Tyr-Thr-Asp-Glu-Asp-Pro-Glu-Lys-Glu-Lys-Arg-Ile-Lys-Glu-Leu. For immunization, IL 19 was covalently bound to carrier protein KLH. We used all the final antisera for immunoprecipitation of metabolically labelled lysates of GM 727 cells, and p48 was immunoprecipitated (fig.1). Immunoprecipitation was the most effective by serum 4 (lane 4). Control serum did not precipitate p48 (lane 6) while anti-p27 serum immunoprecipitated $Pr76^{gag}$ (lane 7). The specificity of p48 immunoprecipitation by anti-IL 19 serum (fig.2, lane 3) was proved by abolition following presaturation of the serum with IL 19 (lane 4). Presaturation of the serum with peptide SV 17 (lane 5), also derived

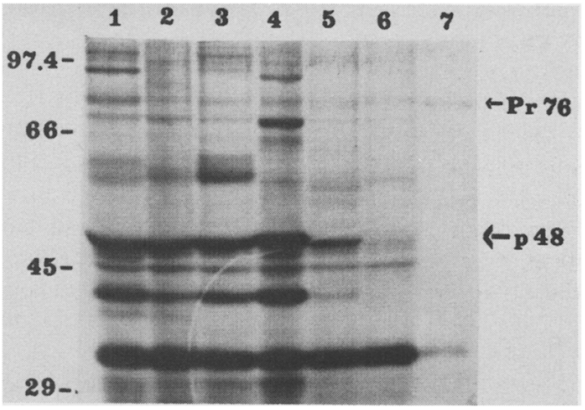

Fig.1. Fluorogram of a gel slab after electrophoresis of proteins from AMV myeloblasts immunoprecipitated by sera 1–5. The lysates were immunoprecipitated with sera 1–5 (lanes 1–5, respectively), control serum (lane 6) and 1.5 μl of anti-p27 serum (lane 7). The immunoprecipitated proteins were subjected to SDS-7.5% PAGE. On the left are the positions and sizes of molecular mass markers. Arrows on the right indicate the positions of $Pr76^{gag}$ and $p48^{v\text{-}myb}$.

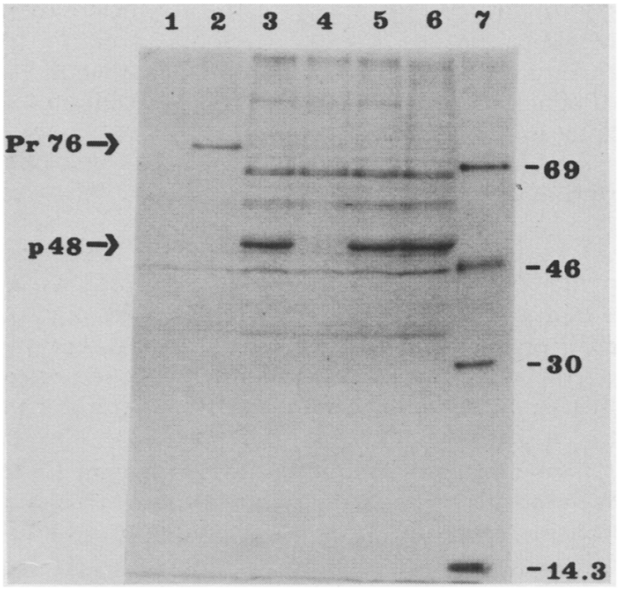

Fig.2. Fluorogram of a gel slab after electrophoresis of proteins from AMV myeloblasts immunoprecipitated by presaturated serum 4. Lysates of GM 727 cells were immunoprecipitated with: control serum (lane 1), anti-p27 serum (lane 2), serum 4 (lane 3), serum 4 presaturated with IL 19 (lane 4), serum 4 presaturated with SV 17 (lane 5), serum 4 presaturated with KLH (lane 6). Immunoprecipitated proteins and molecular mass markers (lane 7) were subjected to SDS-10% PAGE. Arrows on the left indicate the positions of $Pr76^{gag}$ and $p48^{v\text{-}myb}$.

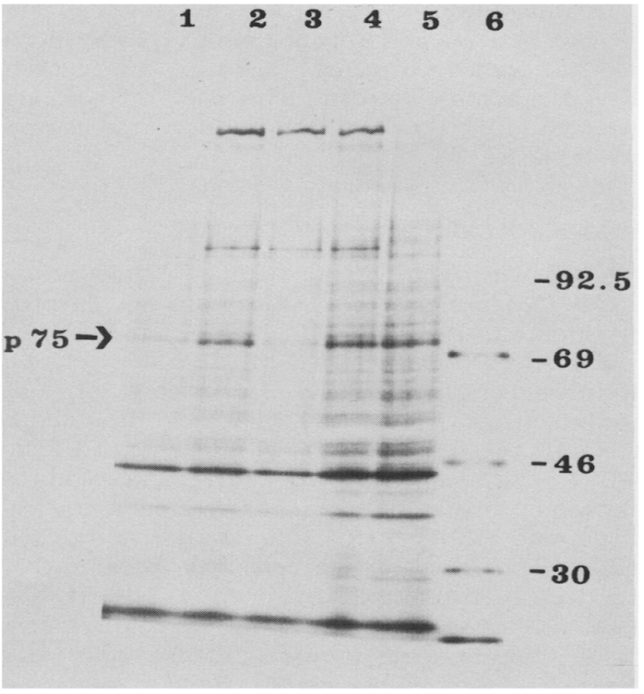

Fig.3. Fluorogram of a gel slab after electrophoresis of immunoprecipitated proteins from chicken thymus. The lysates were immunoprecipitated with: control serum (lane 1), serum 4 (lane 2), serum 4 presaturated with IL 19 (lane 3), serum 4 presaturated with SV 17 (lane 4), serum 4 presaturated with KLH (lane 5). Immunoprecipitated proteins and molecular mass markers (lane 6) were subjected to SDS-7–15% gradient PAGE. Arrow on the left indicates the positions of $p75^{c\text{-}myb}$.

from a *v*-myb sequence but different from IL 19 [16], was without any effect on immunoprecipitation of p48; so was presaturation with KLH (lane 6). Thus, the immunoprecipitation of p48 in the lysate of AMV-transformed myeloblasts by anti-IL 19 serum was specific and the protein precipitated was therefore $p48^{v\text{-}myb}$. Control serum did not precipitate it (lane 1). Anti-p27 serum immunoprecipitated $Pr76^{gag}$ (lane 2).

We next metabolically labelled a short-term chicken thymus tissue culture and lysates of it were immunoprecipitated by anti-IL 19 serum (fig.3). It followed from the pattern of immunoprecipitated proteins that there was specific precipitation of p75 by anti-IL 19 serum and, hence that the chicken *c*-myb protein product $p75^{c\text{-}myb}$ was detected in non-transformed chicken cells. Of sera 1–5, only serum 4 immunoprecipitated p75. This was in agreement with the precipitation of the *v*-myb protein (fig.1, lane 4). On some gels, $p75^{c\text{-}myb}$ migrated as a doublet. Neither band was recognized by IL 19 presaturated anti-IL 19 serum.

The nucleotide sequence from which IL 19 was derived is contained in mouse mRNA but its translation product is not known [13]. We prepared a short-term tissue culture from the murine thymus and immunoprecipiated metabolically labelled lysates of it with control serum and serum 4 (fig.4). p75 was only precipitated by serum 4 (cf. lanes 1 and 2). Presaturation of serum 4 with IL 19 abolished this immunoprecipitation (lane 3). It therefore seems that in murine thymus *c*-myb is expressed and that its product, $p75^{c\text{-}myb}$ (mouse), does not substantial-

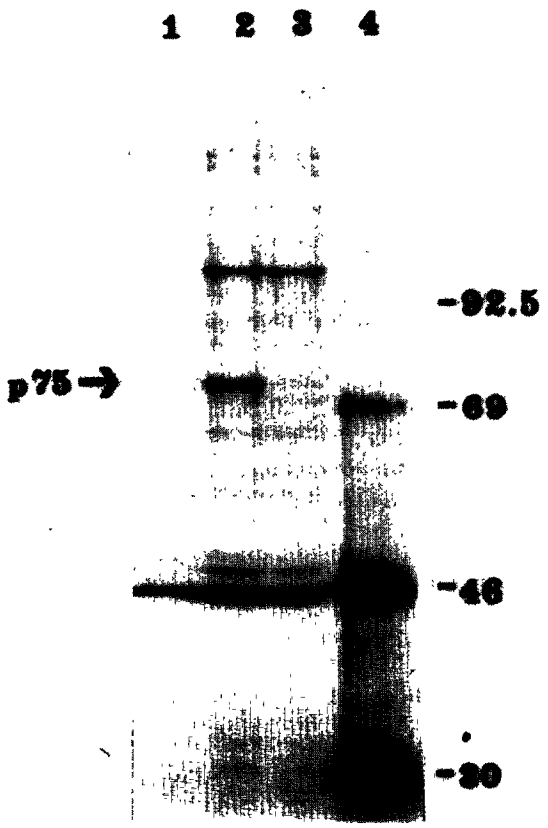

Fig.4. Fluorogram of a gel slab after electrophoresis of immunoprecipitated proteins from mouse thymocytes. The lysates were immunoprecipitated with control serum 4 (lane 1), serum 4 (lane 2) and serum 4 presaturated with IL 19 (lane 3). Immunoprecipitated proteins and molecular mass markers (lane 4) were subjected to SDS-7.5% PAGE. Arrow on the left indicates the position of $p75^{c\text{-}myb}$.

ly differ in molecular mass from the chicken *c*-myb protein product.

4. DISCUSSION

The synthetic nonadecapeptide hapten IL 19 was derived from a sequence of cloned *v*-myb [22]. It is also contained in the *c*-myb sequence, but surprisingly in *c*-myb (both avian and murine) there is Asn instead of Thr in position 6. We do not know if the Thr substituting the more polar Asn in this sequence could be of any importance for tumorigenicity by $p48^{v\text{-}myb}$, but the change is apparently of little importance for the specificity of antibodies.

We had 5 final anti-IL 19 serum samples at our disposal. They precipitated $p48^{v\text{-}myb}$ at different intensity, with serum 4 being the most effective.

The expression of *c*-myb in chicken tissues on the level of mRNA has been estimated [6]. Maximal expression is in AEV-transformed erythroblasts and in thymus cells. The translation product in transformed cells is p75 [11]. We attempted to detect the *c*-myb protein product in the chicken thymus with anti-IL 19 serum. The p75 was immunoprecipitated by anti-IL 19 serum; by this serum after presaturation with a heterologous peptide or the carrier protein; but not after its presaturation with peptide IL 19. This demonstrated that the *c*-myb protein product in the chicken thymus and erythroblasts is one of 75 kDa.

The sequence of IL 19 is contained in the presumptive protein product of *c*-myb (mouse), and *c*-myb transcripts are found in mice at much higher levels in thymic cells and cells of the erythroid lineage than elsewhere [4]. We also labelled mouse thymus cells in tissue culture and immunoprecipitated their lysate with anti-IL 19 serum. p75 was detected on the fluorogram positioned in good agreement with its predicted size [13].

ACKNOWLEDGEMENTS

We thank Dr J. Bubeník for providing us with murine thymus cells and Dr V. Karafiát for cultivation of chicken cells. Dr P. Kašpar kindly prepared the gradient gels for us.

ADDENDUM

When the experimental part of this work was finished we learned [24] that AMV-transforming proteins were detected with antibodies directed against a peptide with a sequence partially overlapping with ours.

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