Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 1714–1718, March 1986
Cell Biology

Identification and preferential expression in thymic and bursal lymphocytes of a c-*ets* oncogene-encoded M_r 54,000 cytoplasmic protein

(E26 leukemia virus oncogenes/*ets*-specific antibodies/lymphocytes differentiation)

J. GHYSDAEL, A. GEGONNE, P. POGNONEC, D. DERNIS, D. LEPRINCE, AND D. STEHELIN

Institut National de la Santé et de la Recherche Médicale Unite 186, Centre National de la Recherche Scientifique UA 04 1160, Institut Pasteur, 1 Rue Calmette, 59019 Lille Cedex, France

Communicated by J. Brachet, October 21, 1985

ABSTRACT **The avian retrovirus E26 is unique among acute leukemia viruses in its ability to induce transformation of cells belonging to either the myeloid or erythroid lineage. The genome of E26 carries two oncogenes, v-*myb* and v-*ets*, that are derived from distinct cellular loci, c-*myb* and c-*ets*. We have constructed a plasmid vector that allows expression of part of the coding region of v-*ets* in a bacterial host. Antisera to the bacterially synthesized ets protein specifically precipitated the E26-encoded P135$^{gag\text{-}myb\text{-}ets}$ transforming protein. These antisera permitted us to identify a chicken c-*ets*-encoded protein of M_r 54,000 (P54$^{c\text{-}ets}$) that shares 7 out of 10 of its major [^{35}S]methionine-containing tryptic peptides with the v-*ets*-encoded domain of P135$^{gag\text{-}myb\text{-}ets}$. Unlike P135$^{gag\text{-}myb\text{-}ets}$ and the M_r 75,000 translation product of c-*myb* (P75$^{c\text{-}myb}$), which are nuclear proteins, P54$^{c\text{-}ets}$ was found to be predominantly cytoplasmic. P54$^{c\text{-}ets}$ is expressed at low levels in most cell lines and tissues tested, including bone marrow cells and circulating lymphocytes. P54$^{c\text{-}ets}$, together with a minor but closely related M_r 56,000 protein, was found to be expressed at high levels in chicken thymocytes and bursal lymphocytes.**

The replication-defective avian leukemia virus E26 induces a mixed erythroid/myeloid leukemia in chickens and transforms cells belonging to either the myeloid or erythroid lineage in tissue culture (1–3). The genomic RNA of E26 contains, in addition to the v-*myb* oncogene also found in the strictly myeloid transforming retrovirus avian myeloblastosis virus (AMV) (4), a second transformation-specific sequence, v-*ets* (5, 6). It is generally believed that the broader oncogenic potential of E26 compared to that of AMV results at least in part from the expression of the v-*ets* oncogene. The v-*myb* and v-*ets* oncogenes are expressed as part of a unique M_r 135,000 transforming polyprotein (P135$^{gag\text{-}myb\text{-}ets}$) containing retroviral *gag* determinants at its amino terminus (7). P135$^{gag\text{-}myb\text{-}ets}$ is localized in the nucleus of transformed cells (8) and exhibits DNA binding activity *in vitro*, a property that appears instrumental in its ability to transform myeloblasts (9). The v-*myb* oncogene apparently arose by transduction of portions of a cellular c-*myb* protooncogene, which is present in the genome of all vertebrate animals (10) and encodes a M_r 75,000 nuclear protein (11). High level expression of c-*myb* appears restricted to hematopoietic tissues and cell lines and is particularly prominent in thymocytes (12, 13). The v-*ets* oncogene is also derived from cellular DNA sequences (c-*ets*) clearly distinct from the c-*myb* locus (5).

To gain some insight on the physiological role(s) of c-*ets* and the mode of oncogenic activation of v-*ets*, we undertook to identify the protein encoded by c-*ets* and to study its pattern of expression in various tissues and cell lines.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

We describe here the production of a rabbit antiserum specific for the *ets*-encoded domain of P135$^{gag\text{-}myb\text{-}ets}$ and report the expression of high levels of a c-*ets*-encoded M_r 54,000 cytoplasmic protein (P54$^{c\text{-}ets}$) in avian thymocytes and bursal lymphocytes.

MATERIALS AND METHODS

Cells and Viruses. E26-transformed chicken fibroblasts and myeloblasts were obtained from T. Graf (European Molecular Biology Laboratory, Heidelberg). Other cell lines used in this study were described (14) and included erythroblasts transformed by avian erythroblastosis virus (AEV-Ebl); myeloblasts transformed by AMV (AMV-Mbl); HD11, a macrophage cell line transformed by the avian myelocytomatosis virus MC29; OK10 BM, a macrophage-like cell line obtained from an OK10 virus-infected chicken; RP9 and TLT, B-lymphoblastic cell lines derived from avian leukosis virus-induced leukemia; TV1, a non-B non-T lymphoblastoid cell line derived from an avian reticuloendotheliosis virus-induced leukemia; MSB1 and RP1, T-lymphoblastoid cell lines obtained from chickens infected with Marek disease virus. Chicken embryo cells were obtained by trypsinization of decapitated 9-day-old embryos; cell suspensions from thymus and bursa were prepared by squeezing the organ through a plastic mesh to obtain single cells; bone marrow cells were obtained by flushing the femurs of 3-week-old chickens with Dulbecco's modified Eagle's medium; peripheral leukocytes were obtained by Ficoll/Hypaque centrifugation of whole blood.

Construction of a v-*ets* Expression Vector. The E26 molecular clone used has been described (5). A fragment of the v-*ets* coding region was inserted into the bacterial expression vector pPLc24 (15) as described in Fig. 1*A*.

Purification of the Bacterial Protein and Preparation of Antisera. To prepare large amounts of the bacterial fusion protein bP17$^{MS2\text{-}ets}$ for immunization, induced bacterial cells from a 1-liter culture were centrifuged (8000 × *g*, 15 min) and resuspended in 0.1 vol of 50 mM Tris·HCl (pH 8.0), 1 mM EDTA and 2 mg of lysozyme/ml. After 5 min at 4°C, the crude lysate was adjusted to 0.5 M NaCl, 5 mM $MgCl_2$, 1% Nonidet P-40 and 50 μg of DNase I/ml and incubated for 60 min at 4°C. bP17$^{MS2\text{-}ets}$ is insoluble under these conditions and was recovered by centrifugation at 10,000 × *g* for 10 min. The pellet was washed several times in 10 mM Tris·HCl (pH 8.0), 1 mM EDTA, and 1% Nonidet P-40, and proteins were solubilized by boiling in $NaDodSO_4$ as described above and electrophoresed on a preparative $NaDodSO_4$/polyacrylamide gel. bP17$^{MS2\text{-}ets}$ was recovered by electroelution (16). Rabbits were immunized with 100–200 μg of purified bP17$^{MS2\text{-}ets}$ emulsified in complete Freund adjuvant by intra-

Abbreviation: AMV, avian myeloblastosis virus.

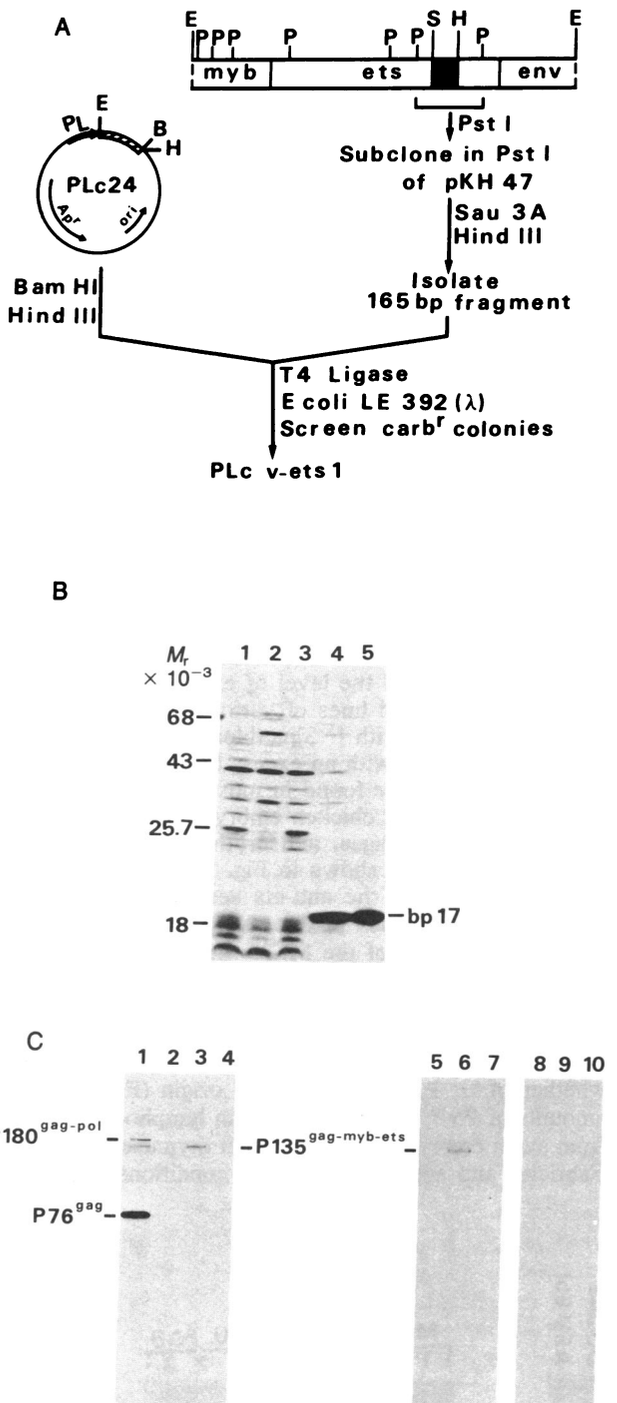

FIG. 1. Expression of a portion of v-*ets* in bacteria and specificity of an antiserum to the bacterially expressed v-*ets* product. (*A*) Schematic outline describing the construction of pPLc24-v-ets-1. A *Sau*3A–*Hin*dIII fragment (nucleotides 1603–1765 in ref. 6) was isolated from a plasmid containing a biologically active E26 provirus. The purified fragment was inserted between the *Bam*HI and *Hin*dIII sites of pPLc24 (15), an expression vector that contains the P_L promoter of bacteriophage λ, the ribosome binding site, and sequences for the first 99 amino acids of the polymerase of phage MS2. This results in the in-frame fusion of the sequence for 56 v-*ets*-encoded amino acids to the MS2 polymerase sequences. Recombinant plasmids were cloned into an *Escherichia coli* LE 392 (λ) host. For expression, plasmids were transferred into an *E. coli* K12 Δ H1 Δ Trp host (23) that supplies a temperature-sensitive repressor coded by a resident defective prophage. In this host, repression is complete at 28°C and full induction obtained at 42°C. Restriction enzymes are as follows: B, *Bam*HI; H, *Hin*dIII; P, *Pst* I; S, *Sau*3A; E, *Eco*RI. (*B*) Expression of a v-*ets* polypeptide (bP17$^{MS2-ets}$) in *E. coli*. Cultures of exponentially growing bacteria carrying pPLc24-v-ets-1 or the parental pPLc24 vector were incubated at 28°C or 42°C for 3 hr. Aliquots containing bacterial cells were removed from each culture, the cells were disrupted by boiling in a $NaDodSO_4$-containing buffer (18), and the lysates were electrophoresed in a 15% $NaDodSO_4$/polyacrylamide gel. Proteins were visualized by staining with Coomassie brilliant blue. Transformants are as follows: (lane 1) pPLc24, 28°C; (lane 2) pPLc24, 42°C; (lane 3) pPLc24-v-ets-1, 28°C; (lane 4) pPLc24-v-ets-1, 42°C; (lane 5) purified bP17$^{MS2-ets}$. (*C*) Immunoprecipitation analysis of E26-transformed cells. Myeloblasts transformed by E26 were grown in either the presence (lanes 5–7) or absence (lanes 8–10) of chicken myelomonocytic growth factor and E26-transformed fibroblasts (lanes 1–4) were labeled for 60 min with L-[^{35}S]methionine (100 μCi/ml; 1 Ci = 37 GBq), lysates were prepared, viral-encoded proteins were immunoprecipitated, and immunoprecipitates were analyzed by electrophoresis on a 10% $NaDodSO_4$/polyacrylamide gel followed by fluorography. Sera used were as follows: (lanes 2, 5, and 8) nonimmune; (lane 1) antiserum to avian retroviral gag structural proteins; (lanes 3, 6, and 9) anti-ets serum; (lanes 4, 7, and 10) anti-ets serum blocked by preincubation with an excess of bP17$^{MS2-ets}$.

dermal injections at 30 different spots along the back. Booster injections were done subcutaneously and intramuscularly in incomplete Freund adjuvant at intervals of 3 weeks.

Cell Labeling, Immunoprecipitation, and Tryptic Peptide Analyses. Labeling of cells with L-[^{35}S]methionine, preparation of cell lysates, immunoprecipitation, $NaDodSO_4$/polyacrylamide gel electrophoresis, and two-dimensional tryptic peptide analyses have been described (17, 18).

Subcellular Fractionation. We followed essentially the method described by Abrams *et al.* (19) for subcellular fractionation. Labeled cells were rinsed twice in phosphate-buffer saline (Na_2HPO_4, 8 mM; KH_2PO_4, 1.5 mM; NaCl, 0.136 M; KCl, 2 mM; pH 7.2), pelleted, and resuspended at 10^7 cells/ml in lysis buffer [20 mM Hepes (pH 7.1), 5 mM KCl, 1 mM $MgCl_2$, and 1% Trasylol]. After 15 min at 0°C cells were broken by 20–30 strokes of a tight fitting pestle in a Dounce homogenizer. The homogenate was adjusted to 150 mM NaCl and centrifuged at 2000 × *g* for 10 min to pellet nuclei. The 2000 × *g* supernatant was further separated into soluble and membrane fractions by centrifugation at 100,000 × *g* for 30 min. The pelleted nuclei were resuspended in 1 ml of lysis buffer adjusted to 0.5% Nonidet P-40, centrifuged at 2000 × *g*, and then washed twice in N buffer (19). The pooled supernatants of these centrifugations represented the nuclear-wash fraction. Nuclei were finally centrifuged through a 1.8 M sucrose cushion at 2500 × *g* for 15 min. All subcellular fractions were adjusted to 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% $NaDodSO_4$, and 1% Trasylol and centrifuged at 100,000 × *g* for 30 min to pellet debris. Integrity of each fraction was monitored on parallel fractionations of MSB1 cells infected with Rous-associated virus, type 1. *gag*-encoded proteins served as markers of the cytoplasmic fraction and *env*-encoded glycoproteins as markers of the membrane fraction. Since MSB1 cells express increased levels of c-*myc*, P61$^{c\text{-}myc}$ was used as nuclear marker.

RESULTS

To obtain antisera against the *ets*-encoded proteins, a v-*ets*-specific *Sau*3A–*Hin*dIII fragment (nucleotides 1603–1765 in ref. 6) of a plasmid containing a biologically active E26 provirus (5) was inserted between the *Bam*HI and *Hin*dIII sites of the expression vector pPLc24 (15), which contains the thermoinducible P_L promoter from phage λ. In the resultant plasmid (pPLc-v-ets-1), the sequence coding for 56 v-*ets*-encoded amino acids is fused in frame with that coding for 99 amino acids of the polymerase of phage MS2, as diagrammed in Fig. 1*A*. Upon temperature induction, bacte-

rial cells synthesized large amounts of a polypeptide with an apparent M_r 17,000 (bP17$^{MS2\text{-}ets}$; Fig. 1*B*, lane 4). Antisera directed against *ets*-encoded proteins were obtained by immunizing rabbits with NaDodSO$_4$/PAGE purified bP17$^{MS2\text{-}ets}$. The reactivity and specificity of these antisera were tested by their ability to immunoprecipitate the E26-encoded P135$^{gag\text{-}myb\text{-}ets}$ protein. As shown in Fig. 1*C*, lanes 3 and 6, an antiserum obtained from an immunized rabbit specifically immunoprecipitated a M_r 135,000 polypeptide from lysates of [^{35}S]methionine-labeled E26-transformed fibroblasts or myeloblasts. Several lines of evidence indicate that this protein is the E26-encoded P135$^{gag\text{-}myb\text{-}ets}$. First, the same protein (as judged by tryptic peptide analysis, data not shown) is precipitated by a serum to *gag* virion proteins (Fig. 1*C*, lane 1). Second, it is absent from lysates of various uninfected cells or lysates of helper virus-infected cells precipitated in similar conditions (Fig. 2). Third, its level in E26-transformed myeloblasts is enhanced in the presence of chicken myelomonocytic growth factor (ref. 20; Fig. 1*C* compare lanes 6 and 9), a property of P135$^{gag\text{-}myb\text{-}ets}$ (21). The immunoprecipitation of P135$^{gag\text{-}myb\text{-}ets}$ from lysates of E26-transformed cells with our anti-ets serum can be completely suppressed in the presence of purified bP17$^{MS2\text{-}ets}$ thus demonstrating that the antibodies responsible for the precipitation of P135$^{gag\text{-}myb\text{-}ets}$ are directed against bP17$^{MS2\text{-}v\text{-}ets}$ (Fig. 1*C*).

To identify products encoded by c-*ets*, we screened various avian cell lines for the presence of proteins reactive with our anti-ets serum. As shown in Fig. 2*A*, lane 2, the anti-ets serum specifically immunoprecipitated a protein of apparent M_r 54,000 from lysates of [^{35}S]methionine-labeled MSB1 cells, a T-lymphoid cell line. The reaction of the antiserum with this protein is abolished in the presence of an excess of bP17$^{MS2\text{-}v\text{-}ets}$ (Fig. 2*A*, compare lane 1 and 2), suggesting that the M_r 54,000 protein might be encoded by c-*ets* (P54$^{c\text{-}ets}$). To substantiate this point, we compared the two-dimensional maps of the [^{35}S]methionine-containing peptides obtained by trypsin digestion of P54$^{c\text{-}ets}$ and P135$^{gag\text{-}myb\text{-}ets}$. As shown in Fig. 3 *B*, *C*, and *E*, 7 out of 10 of the major peptides obtained from P54$^{c\text{-}ets}$ are shared by P135$^{gag\text{-}myb\text{-}ets}$. Most of the other peptides of P135$^{gag\text{-}myb\text{-}ets}$ are contributed by the *gag*-encoded domain of P135$^{gag\text{-}myb\text{-}ets}$, since they were also found in the tryptic peptide map of Pr76^{gag}, the translation product of the *gag* gene of replication-competent retroviruses (Fig. 3 *A* and *B*). The remaining peptides unique to P135$^{gag\text{-}myb\text{-}ets}$ presumably derived from the v-*myb*-encoded domain of P135$^{gag\text{-}myb\text{-}ets}$. These results are summarized in Fig. 3*F*. All of the seven peptides shared between P54$^{c\text{-}ets}$ and P135$^{gag\text{-}myb\text{-}ets}$ were found to be present in a M_r 80,000 fragment generated from the carboxyl-terminal domain of P135$^{gag\text{-}myb\text{-}ets}$ by cleavage with virion protease p15 (result not shown). We conclude from these experiments that the M_r 54,000 protein precipitated by the anti-ets serum is closely related to the carboxyl-terminal domain of P135$^{gag\text{-}myb\text{-}ets}$ and thus represents a protein encoded by c-*ets*. Polypeptides with similar size and structurally related to the avian P54$^{c\text{-}ets}$ were also identified by the anti-ets serum in lysates of murine, bovine, and human cells, indicating the high conservation of c-*ets* in vertebrates (results not shown).

To determine the subcellular location of P54$^{c\text{-}ets}$, we fractionated lysates of [^{35}S]methionine-labeled MSB1 cells into cytoplasmic, membrane, and nuclear fractions and immunoprecipitated an equivalent amount of each fraction with an excess of anti-ets serum. As shown in Fig. 4, the majority of P54$^{c\text{-}ets}$ was found to be present in the cytoplasmic fraction. Similar fractionation experiments using E26-transformed myeloblasts showed that, in accordance with previous results (10), P135$^{gag\text{-}myb\text{-}ets}$ is localized predominantly in the nucleus of transformed cells (data not shown).

We next compared the level of expression of P54$^{c\text{-}ets}$ in avian tissues and cell lines of various histological origins. Cells were labeled with [^{35}S]methionine, and lysates were immunoprecipitated with an excess of anti-ets serum. Low levels of P54$^{c\text{-}ets}$ were found in most tissues and cell lines tested including total chicken embryo, bone marrow cells, fibroblasts, macrophages, and erythroblasts (Fig. 2*A* and data not shown). As shown in Fig. 2*A*, in addition to low amounts of P54$^{c\text{-}ets}$, the anti-ets serum precipitates three additional polypeptides of M_r 60,000, M_r 62,000, and M_r 64,000 from lysates of the MC29-transformed macrophage cell line HD11. These polypeptides are highly related to each other but show only a limited domain of sequence homology to P54$^{c\text{-}ets}$ (unpublished results). Conspicuous levels of P54$^{c\text{-}ets}$ were found in most lymphoid cell lines tested, whether of B, T, or non-B/non-T origin (Fig. 2*A*). Large amounts of P54$^{c\text{-}ets}$ were detected in lymphocytes obtained from avian embryonic and postnatal thymuses and bursa of Fabricius and analyzed in similar conditions (Fig. 2*B*). In

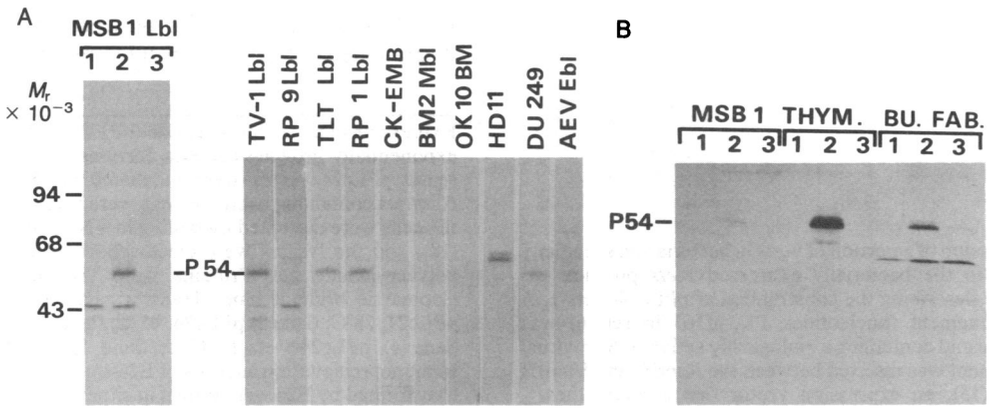

FIG. 2. Identification of a protein encoded by c-*ets*. Approximately 10^7 cells were labeled for 60 min with 250 μCi/ml of L[^{35}S]methionine, lysates were prepared and immunoprecipitated, and immunoprecipitates were electrophoresed on a 15% NaDodSO$_4$/polyacrylamide gel. The amount of radioactivity incorporated into total cellular proteins of each lysate was determined from trichloroacetic acid precipitates. Equal amounts of acid-insoluble radioactivity of each lysate were used in all immunoprecipitations. Cell types used are listed across the top of the figure. (*A*) MSB1 cell line lysate immunoprecipitated with the following: (lane 1) anti-ets serum adsorbed with an excess of bP17$^{MS2\text{-}ets}$; (lane 2) anti-ets serum; (lane 3) nonimmune serum. Other lysates were immunoprecipitated with anti-ets serum. (*B*) Cell suspensions from thymuses of 16-day-old embryos and bursa of Fabricius (B.F.) from 2-week-old chickens immunoprecipitated with the following: (lane 1) nonimmune serum; (lane 2) anti-ets serum; (lane 3) anti-ets serum absorbed with an excess of bP17$^{MS2\text{-}ets}$. Exposure time of fluorograms of *A* and *B* were 10 days and 3 days, respectively.

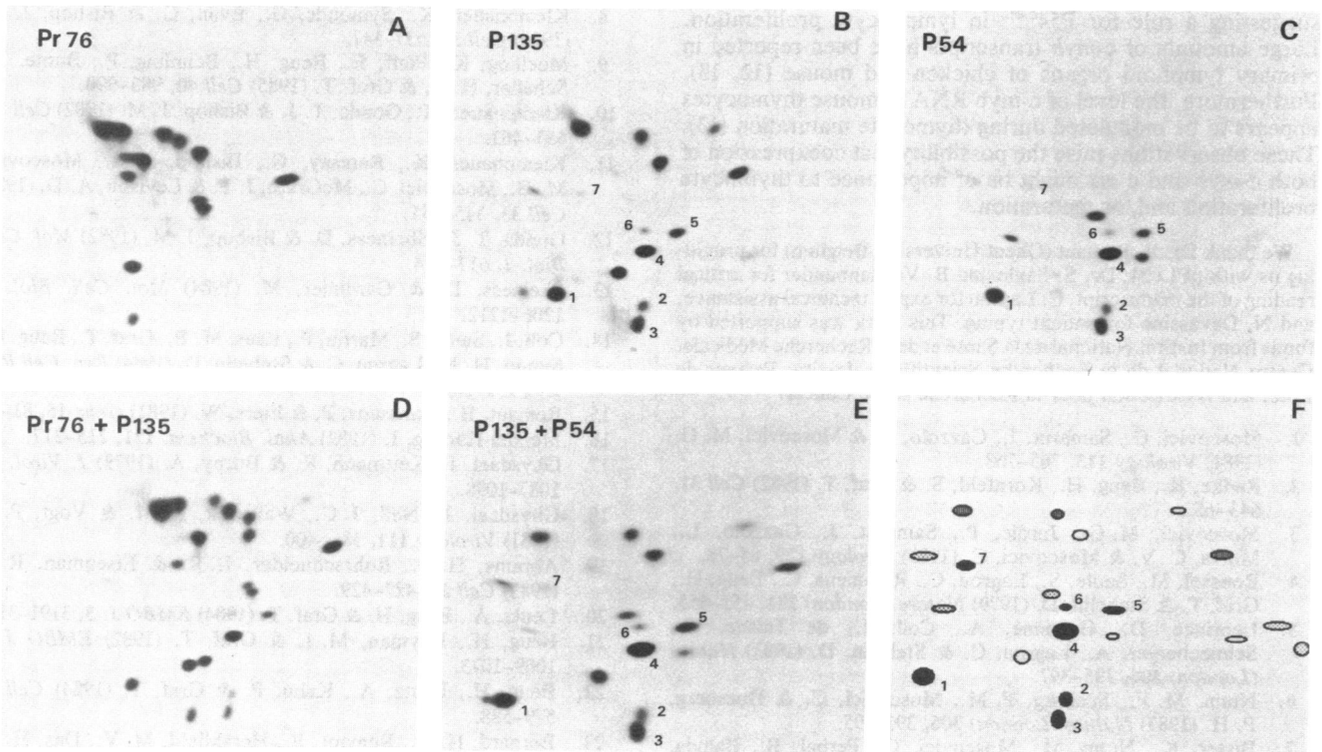

FIG. 3. Two-dimensional tryptic peptide maps of *ets*-encoded polypeptides. [^{35}S]methionine labeled proteins were eluted from gels similar to those of Figs. 1 and 2, oxidized with performic acid, digested with L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin, applied to cellulose thin layer plates, and separated in the first dimension by electrophoresis at pH 4.5 and by ascending chromatography in the second dimension. The origin of each map is at the lower left. Electrophoresis toward the cathode is from left to right and chromatography from bottom to top. (*A*) Pr76^{gag}. (*B*) P135$^{gag\text{-}myb\text{-}ets}$. (*C*) P54$^{c\text{-}ets}$. (*D* and *E*) Mixing experiments where similar amounts of radioactivity of tryptic digests (as indicated) are combined prior to two-dimensional separation. (*F*) Schematic drawing of *E*. Peptides shared by P135$^{gag\text{-}myb\text{-}ets}$ and P54$^{c\text{-}ets}$ are numbered and represented as solid spots. (Hatched spots) Peptides shared by P135$^{gag\text{-}myb\text{-}ets}$ and Pr76^{gag}. (Stippled spots) Peptides unique to P54$^{c\text{-}ets}$. (Open spots) Peptides unique to P135$^{gag\text{-}myb\text{-}ets}$.

contrast, expression of P54$^{c\text{-}ets}$ was found to be below the limit of detection in circulating lymphocytes and myeloblasts (Fig. 2*A* and results not shown). In addition to P54$^{c\text{-}ets}$, small amounts of a M_r 56,000 protein are specifically precipitated by the anti-ets serum from thymocyte lysates (Fig. 2). This protein is closely related to P54$^{c\text{-}ets}$ by two-dimensional tryptic peptide analysis (data not shown) and might result from posttranslational modifications of P54$^{c\text{-}ets}$.

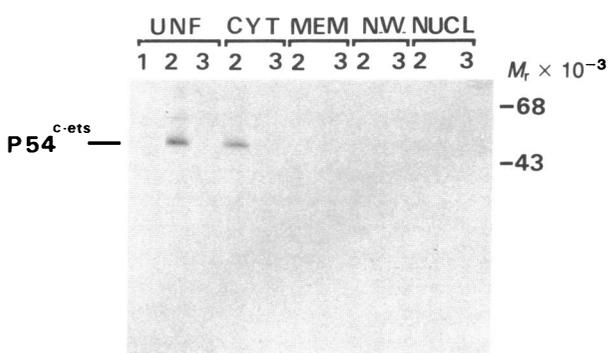

FIG. 4. Subcellular fractionation of MSB1 cells. Approximately 3×10^7 MSB1 cells were labeled with 500 μCi of L-[^{35}S]methionine for 2 hr as described in Fig. 1. Labeled cells were fractionated into nuclear, membrane, cytoplasmic, and nuclear wash fractions. Aliquots of the unfractionated lysate (UNF) and of each fraction corresponding to the same number of cells were immunoprecipitated, and immunoprecipitates were analyzed by electrophoresis on 15% $NaDodSO_4$/polyacrylamide gels.

DISCUSSION

In this study we identified a c-*ets*-encoded protein of M_r 54,000 (P54$^{c\text{-}ets}$) on the basis of its immunological and structural relatedness to the v-*ets*-encoded domain of P135$^{gag\text{-}myb\text{-}ets}$. From the comparison of its apparent size and the alleged size of the E26 v-*ets*-encoded domain (475 amino acids, unpublished data) and from the fact that P54$^{c\text{-}ets}$ contains unique peptides in addition to those shared with P135$^{gag\text{-}myb\text{-}ets}$, we conclude that v-*ets* includes most but not all of the coding sequence of c-*ets*.

Unlike the avian c-*myb* protein and E26 P135$^{gag\text{-}myb\text{-}ets}$, which are nuclear, P54$^{c\text{-}ets}$ was found to be cytoplasmic. This result strongly suggests that the features that determine the nuclear location of P135$^{gag\text{-}myb\text{-}ets}$ are encoded by the E26 v-*myb* gene. The oncogenic potential of E26 differs markedly from that of another v-*myb*-containing retrovirus, AMV, since myeloblasts transformed by E26 appear to be more tightly blocked in their differentiation capacity than AMV-transformed myeloblasts (22) and since only E26 induces erythroid and fibroblastic transformation (1–3). Although the exact contribution of v-*ets* to the unique oncogenic potential of E26 remains to be formally established by the study of appropriate deletion mutants, it is tempting to speculate that the abnormal (nuclear) location of the v-*ets*-encoded domain of P135$^{gag\text{-}myb\text{-}ets}$ might be of importance to the oncogenic activation of v-*ets*.

We found P54$^{c\text{-}ets}$ to be expressed at high levels (similar to those of P135$^{gag\text{-}myb\text{-}ets}$ in E26-transformed cells) in lymphocytes of both the thymus and bursa of Fabricius. In contrast, only low levels of P54$^{c\text{-}ets}$ were found in circulating blood lymphocytes as well as in all other tissues or cell lines tested,

suggesting a role for $P54^{c\text{-}ets}$ in lymphocyte proliferation. Large amounts of c-*myb* transcripts have been reported in primary lymphoid organs of chicken and mouse (12, 13). Furthermore, the level of c-myb RNA in mouse thymocytes appears to be modulated during thymocyte maturation (13). These observations raise the possibility that coexpression of both c-*myb* and c-*ets* might be of importance to thymocyte proliferation and/or maturation.

We thank Dr. E. Remaut (Ghent University, Belgium) for providing us with pPLc24, Dr. S. Saule and B. Vandenbunder for critical reading of the manuscript, C. Lagrou for expert technical assistance, and N. Devassine for patient typing. This work was supported by funds from Institut National de la Santé et de la Recherche Médicale, Centre National de la Recherche Scientifique, Institut Pasteur de Lille, and Association pour la Recherche sur le Cancer.

1. Moscovici, C., Samarut, J., Gazzolo, L. & Moscovici, M. G. (1981) *Virology* **113**, 765–768.
2. Radke, K., Beug, H., Kornfeld, S. & Graf, T. (1982) *Cell* **31**, 643–653.
3. Moscovici, M. G., Jurdic, P., Samarut, J., Gazzolo, L., Murra, C. V. & Moscovici, C. (1983) *Virology* **129**, 65–78.
4. Roussel, M., Saule, S., Lagrou, C., Rommens, C., Beug, H., Graf, T. & Stehelin, D. (1979) *Nature (London)* **281**, 452–455.
5. Leprince, D., Gegonne, A., Coll, J., de Taisne, C., Schneeberger, A., Lagrou, C. & Stehelin, D. (1983) *Nature (London)* **306**, 395–397.
6. Nunn, M. F., Seeburg, P. M., Moscovici, C. & Duesberg, P. H. (1983) *Nature (London)* **306**, 391–395.
7. Bister, K., Nunn, M., Moscovici, C., Perbal, B., Baluda, M. A. & Duesberg, P. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3677–3681.
8. Klempnauer, K., Symonds, G., Evan, G. & Bishop, J. M. (1984) *Cell* **37**, 537–547.
9. Moelling, K., Pfaff, E., Beug, H., Beimling, P., Bunte, T., Schaller, H. E. & Graf, T. (1985) *Cell* **40**, 983–990.
10. Klempnauer, K., Gonda, T. J. & Bishop, J. M. (1982) *Cell* **31**, 453–463.
11. Klempnauer, K., Ramsay, G., Bishop, J. M., Moscovici, M. G., Moscovici, C., McGrath, J. P. & Levison, A. D. (1983) *Cell* **33**, 345–355.
12. Gonda, T. J., Sheiness, D. & Bishop, J. M. (1982) *Mol. Cell. Biol.* **2**, 617–624.
13. Sheiness, D. & Gardinier, M. (1984) *Mol. Cell. Biol.* **4**, 1206–1212.
14. Coll, J., Saule, S., Martin, P., Raes, M. B., Graf, T., Beug, H., Simon, H. E., Lagrou, C. & Stehelin, D. (1984) *Exp. Cell Res.* **149**, 151–162.
15. Remaut, E., Stanssens, P. & Fiers, W. (1981) *Gene* **15**, 81–93.
16. Mendel-Hartvig, I. (1982) *Anal. Biochem.* **121**, 215–217.
17. Ghysdael, J., Kettmann, R. & Burny, A. (1979) *J. Virol.* **29**, 1087–1098.
18. Ghysdael, J., Neil, J. C., Wallbank, A. M. & Vogt, P. K. (1981) *Virology* **111**, 386–400.
19. Abrams, H. D., Rohrschneider, L. R. & Eisenman, R. N. (1982) *Cell* **29**, 427–429.
20. Leutz, A., Beug, H. & Graf, T. (1984) *EMBO J.* **3**, 3191–3197.
21. Beug, H., Hayman, M. J. & Graf, T. (1982) *EMBO J.* **1**, 1069–1073.
22. Beug, H., Leutz, A., Kahn, P. & Graf, T. (1984) *Cell* **39**, 579–588.
23. Bernard, H. H., Remant, E., Hershfield, M. V., Das, H. K., Helinski, D. R., Yanofsky, C. & Franklin, N. (1979) *Gene* **5**, 59–76.