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Identification in chicken macrophages of a set of proteins related to, but distinct from, the chicken cellular c-*ets*-encoded protein p54$^{c\text{-}ets}$

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Using an antiserum to a bacterially expressed polypeptide corresponding to 56 amino acids of v-*ets*, we previously identified in chicken tissues a protein of 54 kd (p54$^{c\text{-}ets}$) which shares extensive sequence homology to the v-*ets*-encoded domain of the E26-transforming protein p135$^{gag\text{-}myb\text{-}ets}$ and is thus apparently encoded by the c-*ets* proto-oncogene. We report here that the anti-*ets* serum specifically identifies in chicken cells a second set of proteins of 60 kd (p60), 62 kd (p62) and 64 kd (p64) which appear to be highly related to each other but display only a limited domain of homology with p54$^{c\text{-}ets}$ and p135$^{gag\text{-}myb\text{-}ets}$ and are thus probably encoded by a gene(s) partially related to, but different from c-*ets*. In contrast to p54$^{c\text{-}ets}$ which is expressed at high levels in chicken lymphoid tissues, prominent syntheses of p62 and p64 were found in both normal and transformed chicken macrophages but not in avian cells corresponding to immature stages of the myeloid differentiation pathway. These observations together with the fact that differentiation of avian myeloblastosis virus-transformed myeloblasts into macrophage-like cells after treatment with 12-*O*-tetradecanoylphorbol-13-acetate is accompanied by the synthesis of p62 and p64 suggest a role for these proteins in chicken macrophage differentiation or function. Induction of differentiation of human leukemia cell lines HL60 and U937 into macrophages is also accompanied by the increased synthesis of c-*ets*-encoded 68 kd, 62 kd and 58 kd proteins.

Key words: c-*ets*/proto-oncogene product/macrophage/differentiation

Introduction

The extensive phylogenetic conservation of proto-oncogenes, the cellular progenitors of retroviral transforming genes, has led to the notion that these genes participate in fundamental cellular processes such as proliferation and/or differentiation. This idea has been supported by the demonstration that several proto-oncogenes are expressed in a tissue-specific manner and differentially at various stages of mouse development (Müller and Verma, 1984). Also, the expression of specific proto-oncogenes is induced in response to several growth factors (Kelly *et al.*, 1983; Armelin *et al.*, 1984; Müller *et al.*, 1984) and diminished or induced during certain differentiation processes (Westin *et al.*, 1982a, b; Gonda *et al.*, 1984; Mitchell *et al.*, 1985; Müller *et al.*, 1985). Finally, the cellular progenitors of the retroviral oncogenes v-*sis*, v-*erb*-B and v-*fms* have been shown to be highly related to genes encoding the B chain of platelet-derived growth factor (PDGF), the epidermal growth factor (EGF) receptor and the macrophage colony-stimulating factor [M-CSF (CSF-1)] receptor, respectively (Waterfield *et al.*, 1983; Downward *et al.*, 1984; Scherr *et al.*, 1985).

The acute leukemia virus E26 induces a mixed erythroid/myeloid leukemia in chickens and transforms myeloblasts and erythroblasts in tissue culture (Graf *et al.*, 1979; Moscovici *et al.*, 1981, 1983; Radke *et al.*, 1982). The genome of E26 contains, in addition to the v-*myb* oncogene also found in avian myeloblastosis virus (AMV), a second cell-derived oncogene v-*ets* (Leprince *et al.*, 1983; Nunn *et al.*, 1983). The v-*myb* and v-*ets* oncogenes are expressed, together with a partial viral *gag* gene, as a 135-kd polyprotein (p135$^{gag\text{-}myb\text{-}ets}$), localized in the nucleus of transformed cells (Beug *et al.*, 1982; Bister *et al.*, 1982; Klempnauer *et al.*, 1984). Previous studies have shown that expresion of the c-*myb* oncogene is prominent in immature cells of the lymphoid, erythroid and myeloid lineages (Chen, 1980; Westin *et al.*, 1982a, b; Gonda *et al.*, 1982; Coll *et al.*, 1984) and appears to correlate with the proliferative status of these cells (Westin *et al.*, 1982b). To understand the physiological role of the cellular progenitor (c-*ets*) of the v-*ets* oncogene, we recently derived antisera to part of the amino acid sequence encoded by the v-*ets* oncogene. These antisera allowed us to identify in various chicken tissues and cell lines a major 54-kd cytoplasmic protein (p54$^{c\text{-}ets}$) and a minor 56-kd (p56$^{c\text{-}ets}$) component that show extensive sequence homology with the v-*ets*-encoded domain of p135$^{gag\text{-}myb\text{-}ets}$ and which appear to be expressed at high levels in chicken thymocytes and bursal lymphocytes as well as in most avian lymphoid cell lines (Ghysdael *et al.*, 1986).

We describe here the identification in chicken cells of three additional proteins that share a limited domain of homology with both p54$^{c\text{-}ets}$ and p135$^{gag\text{-}myb\text{-}ets}$. These proteins are expressed at high levels in transformed and normal macrophages and their synthesis is specifically induced when AMV-transformed myeloblasts are induced with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) to differentiate into macrophages. TPA-induced differentiation of the human promyelocytic cell line HL60 (Collins *et al.*, 1978) and the human histiocytic cell line U937 (Sundström and Nilsson, 1976) into non-dividing macrophages is accompanied by the increased synthesis of human c-*ets*-encoded proteins of 68, 62 and 58 kd.

Results

Using an antiserum directed against a bacterially synthesized polypeptide corresponding to 56 v-*ets*-encoded amino acids (anti-*ets* serum), we previously identified in chicken lymphoid cells a major c-*ets*-encoded polypeptide of mol. wt 54 kd (p54$^{c\text{-}ets}$) and a minor component of 56 kd (p56$^{c\text{-}ets}$). During these studies we also found the anti-*ets* serum to precipitate three additional proteins of 60 kd (p60), 62 kd (p62) and 64 kd (p64) from lysates of the MC29-transformed macrophage cell line HD11 (Ghysdael *et al.*, 1986; and Figure 1, lanes C and I). The precipitation of p60, p62 and p64 is specific of the anti-*ets* serum since none of these proteins were found to be precipitated by a non-immune control serum (Figure 1, lane A) and since their precipitation

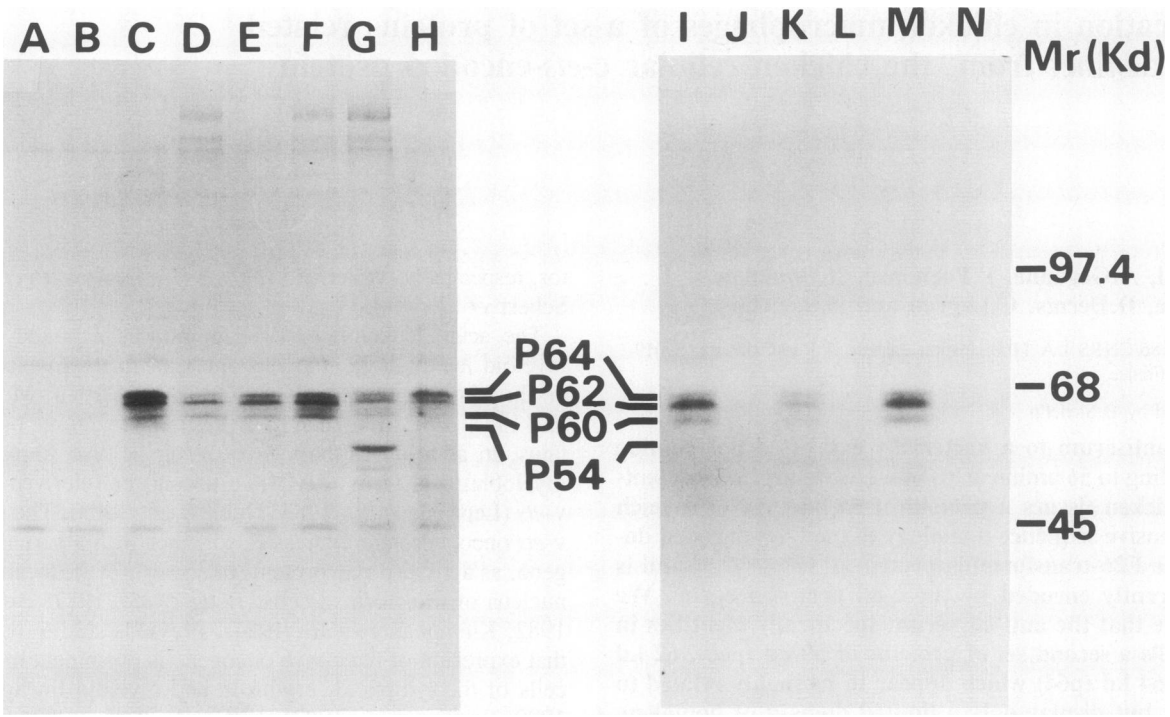

Fig. 1. Expression of *ets*-related polypeptides in transformed macrophages and normal bone marrow-derived macrophages. Cells were labelled for 60 min with 250 μCi/ml of L-[^{35}S]methionine, lysed and the immunoprecipitations were carried out on 10^7 radioactive counts of each lysate with: **A,** non-immune serum; **B, J, L, N,** anti-*ets* serum blocked by pre-incubation with an excesss of the bacterially expressed *ets*-antigen; **C–H, I, K, M,** anti-*ets* serum. Immunoprecipitates were analyzed by electrophoresis on a 10% polyacrylamide slab gel followed by fluorography. Cells analyzed were as follows: **A–C, I, J,** MC29-transformed macrophage cell line HD11; **D, E,** OK10-transformed macrophages, clones 1 and 2; **F,** MH2- E1 mil$^-$ myc$^+$-transformed macrophages; **G, H,** MH2-transformed macrophages, clones 1 and 2; **K, L,** 3-day-old culture of chicken bone marrow cells, non-adherent cells; **M, N,** 3-day-old culture of chicken bone marrow cells, adherent macrophages.

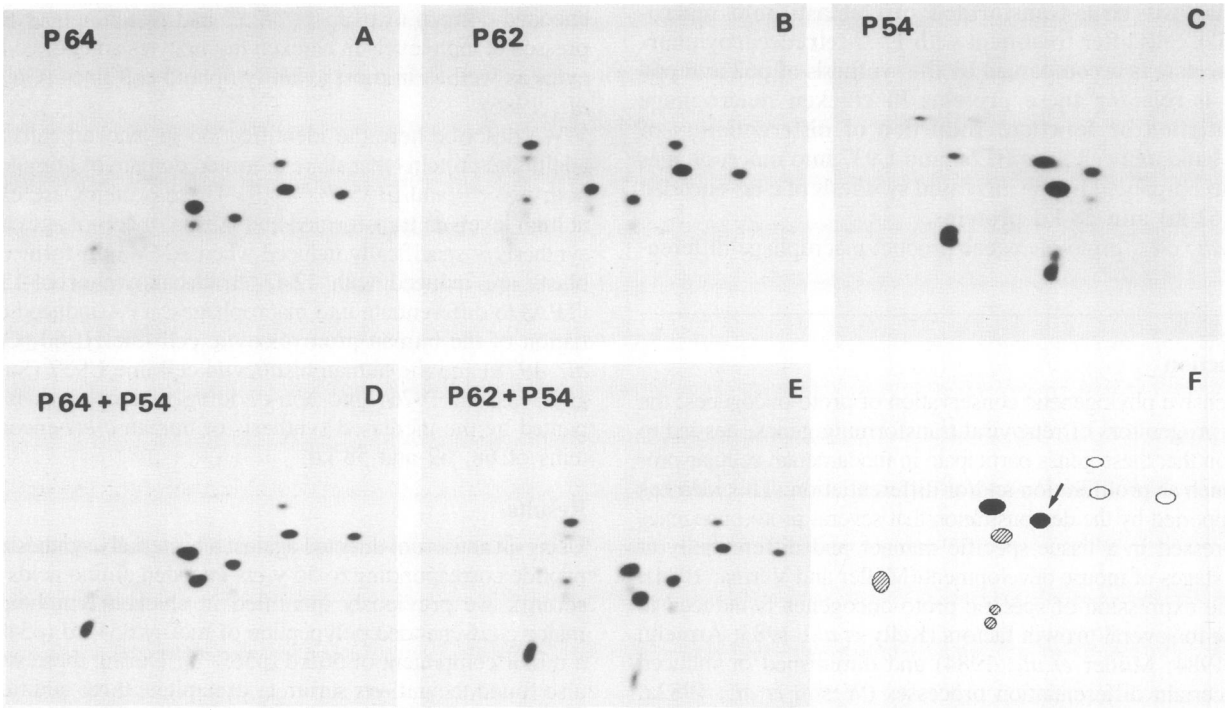

Fig. 2. Two-dimensional tryptic peptide maps of *ets*-related proteins. [^{35}S]methionine-labelled proteins were excised from gels similar to those of Figure 1, eluted, oxidized with performic acid and digested with TPCK-treated trypsin. The resulting peptides were separated by electrophoresis in the first dimension (origin at the left, cathode at the right) and chromatography in the second dimension. **Panel A,** p 64; **panel B,** p62; **panel C,** p54$^{c\text{-}ets}$; **panel D,** mixture of p64 and p54$^{c\text{-}ets}$; **panel E,** mixture of p62 and p54$^{c\text{-}ets}$; **panel F,** summary of *ets*-specific peptides. Solid spots, peptides shared between p54$^{c\text{-}ets}$ and p60–p64; hatched spots, peptides unique to p54$^{c\text{-}ets}$; open spots, peptides unique to p60–p64. Arrow points to the peptide shared between p135$^{gag\text{-}myb\text{-}ets}$, p54$^{c\text{-}ets}$ and p60–p64.

by the anti-*ets* serum was completely abolished in the presence of an excess of the bacterial immunogen (Figure 1, lanes B and J). To test whether the synthesis of these polypeptides was particular to that cell line or was a general characteristic of chicken macrophages, we first analyzed several independent clones of macrophages freshly obtained by infection of chicken bone marrow cells with several v-*myc*-containing retroviruses. Two classes of transformed macrophages were analyzed. The first class included macrophages transformed by retroviruses carrying only the v-*myc* oncogene and which require the continuous presence of chicken myelomonocytic growth factor (cMGF, Beug *et al.*, 1982; Leutz *et al.*, 1984) for growth and survival. The second class included macrophages transformed by MH2, a retrovirus containing both the v-*myc* and v-*mil* oncogenes. These macrophages produce cMGF or cMGF-related factor(s) and grow in an autocrine fashion (Graf *et al.*, 1986). The results of Figure 1, lanes C−H show that all transformed macrophage clones analyzed synthesized levels of p60, p62 and p64 similar to those detected in the HD11 cell line. The synthesis of these proteins is not restricted to transformed macrophages since they were also detected in normal chicken macrophages. In the presence of cMGF, culture of cell suspensions derived from chicken bone marrow resulted within 3−4 days in the emergence of a population of non-adherent proliferating macrophages which progressively differentiated into adherent slowly proliferating macrophages. The experiment of Figure 1, lanes I−N, shows that the level of synthesis of p60, p62 and p64 in adherent macrophages was similar to that observed in the MC29-transformed macrophage cell line HD11 (Figure 1, compare lanes I and M). The lower levels of these proteins detected in the non-adherent cell population as compared with adherent macrophages (Figure 1, compare lanes K and M) probably reflect the fact that this population is heterogeneous and includes other cell types in addition to macrophages.

To analyze the structural relationship between p64, p62, p60 and p54$^{c\text{-}ets}$, we compared the two-dimensional maps of the [^{35}S]-methionine-containing peptides obtained after trypsin digestion of each of the purified proteins. As shown in Figure 2 (panels A and B) the peptide maps of p64 and p62 are very similar since all the peptides of p64 were also found in the map of p62. Two peptides are present in the map of p62 which appear to be absent in that of p64. The tryptic peptide map of p60 is indistinguishable from that of p62 (result not shown). The duplication of some of the peptides in these maps results from partial oxidation of the peptides during their preparation. Comparison of the tryptic peptide maps of p54$^{c\text{-}ets}$ with that of either p64 or p62 shows that only two out of the six major peptides of p54$^{c\text{-}ets}$ are also found in p64 and p62 (Figure 2, panels A−C, and mixing experiments in panels D and E). All other peptides are unique to either p54$^{c\text{-}ets}$ or p64/p62. The results of these comparisons are summarized in Figure 2, panel F. As previously described, most of the peptides of p54$^{c\text{-}ets}$ are found in the v-*ets*-encoded domain of the E26-transforming protein p135$^{gag\text{-}myb\text{-}ets}$ (Ghysdael *et al.*, 1986). It also appears that none of the peptides unique to p64, p62 and p60 are found in the peptide map of p135$^{gag\text{-}myb\text{-}ets}$ whereas only one of the peptides shared between p54$^{c\text{-}ets}$ and p60−p64 is found in the peptide map of p135$^{gag\text{-}myb\text{-}ets}$ (indicated by an arrow in Figure 2, panel F). We conclude from these experiments that p64, p62 and p60 are highly related to each other but probably exhibit only a limited domain of homology with p54$^{c\text{-}ets}$. To further substantiate this point, we compared the one-dimensional cleavage patterns obtained from these polypeptides after partial digestion with *Staphylococcus aureus* V8 protease,

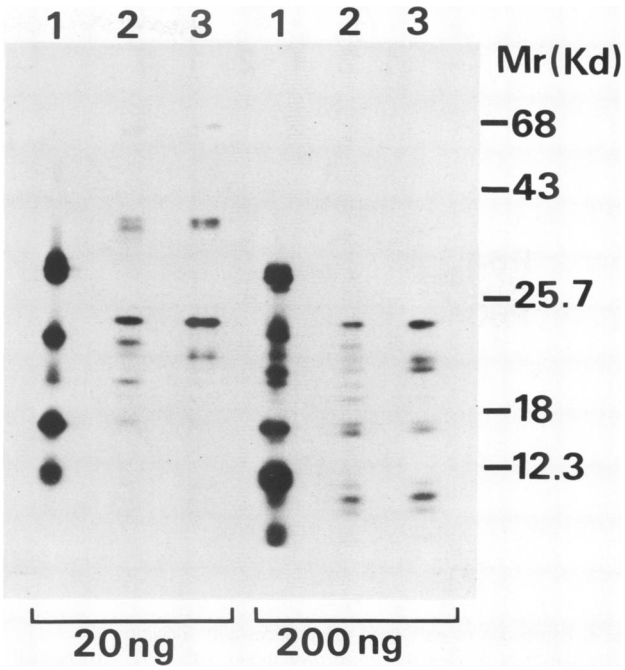

Fig. 3. One-dimensional V8 protease mapping of *ets*-related proteins. [^{35}S]methionine proteins were excised from gels similar to those of Figure 1 and run on a 15% polyacrylamide gel after partial digestion for 30 min with either 200 or 20 ng of *S. aureus* V8 protease. **Lane 1,** p54$^{c\text{-}ets}$; **lane 2,** p64; **lane 3,** p62.

a procedure particularly suited to demonstrate extensive sequence homology between polypeptides (Cleveland *et al.*, 1977). The results of Figure 3 show that the V8 partial digests of p64 and p62 are very similar although not identical since one of the major peptides obtained after digestion of p62 with low doses of V8 protease is not observed in the digest of p64. This result is in agreement with those obtained after digestion to completion of p64 and p62 with trypsin (Figure 2). In contrast, no common peptides could be detected between the V8 partial digests of p54$^{c\text{-}ets}$ and that of either p64 or p62. We conclude that p54$^{c\text{-}ets}$ and p60−p64 are different in most of their sequences and that these two sets of proteins share only a limited domain of homology.

The experiments described in Figure 1 together with our previous observations (Ghysdael *et al.*, 1986) showed the synthesis of p62 and p64 to be most prominent in macrophages but low or below the limit of detection in other avian cells including immature cells of the myeloid lineage. These observations suggest that these proteins might be of importance to macrophage differentiation or function. To lend support to this hypothesis we analyzed the synthesis of p60, p62 and p64 during induced macrophage differentiation.

AMV-transformed myeloblasts differentiate into cells with the phenotype and functional properties of mature macrophages following treatment by phorbol esters (Pessano *et al.*, 1979). This induced differentiation is accompanied by the translocation of the p45$^{v\text{-}myb}$ AMV-transforming protein from its original location in the nucleus to the perinuclear cytoplasm (Klempnauer *et al.*, 1984). The experiment of Figure 4 shows that, while exponentially growing cells of the AMV-transformed myeloblasts cell line 5YS synthesized low levels of p60 and barely detectable levels of p62 and p64 (Figure 4, panel A), treatment of these cells with 20 nM TPA induced both differentiation into macrophage-like cells and a considerable increase in the level of syn-

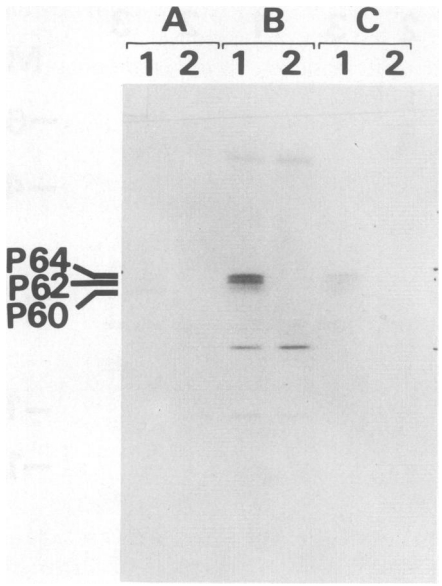

Fig. 4. Expression of *ets*-related polypeptides after induction of differentiation of AMV-transformed myeloblasts with TPA. Exponentially growing AMV-transformed myeloblasts (5YS cell line) were incubated at 10^6 cells/ml in differentiation medium without **(panel A)** or with 20 nM TPA **(panel B)** for 18 h and labelled for 60 min with 250 μCi/ml of L-[^{35}S]methionine. **Panel C,** cells were treated as in panel B and then shifted to standard growth medium for 24 h before labelling. Lysates were prepared and immunoprecipitations carried out on 5×10^6 acid-insoluble radioactive count. **Lane 1,** anti-*ets* serum and **lane 2,** anti-*ets* serum blocked by an excess of the bacterially expressed *ets*-antigen. Immunoprecipitated proteins were separated by electrophoresis on a 12.5% polyacrylamide slab gel followed by fluorography.

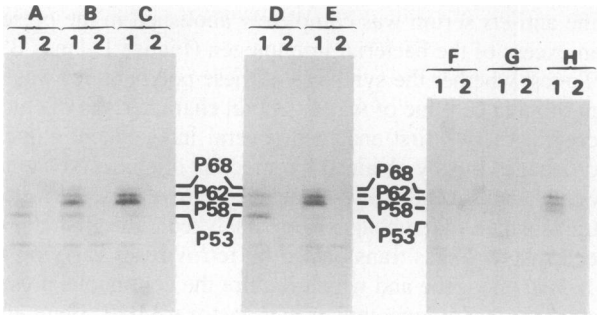

Fig. 5. Expression of *ets*-related polypeptides after induction of differentiation of HL60 with TPA. Exponentially growing HL60 cells were either untreated **(A** and **F)**, or treated with 40 nM TPA for 1 day **(B)** or 2 days **(C** and **H)** or treated with 400 nM TPA for 15 min **(D)** or treated with 400 nM TPA for 15 min, washed extensively and further incubated in complete growth medium without TPA **(E)**, or treated with 1 μM retinoic acid **(G)**, were labelled for 30 min with 500 μCi/ml of L-[^{35}S]methionine, lysed and immunoprecipitates were prepared on the same amount of acid-insoluble radioactive count with (1) anti-*ets* serum and (2) anti-*ets* serum blocked by pre-incubation with an excess of bacterially expressed *ets* antigen. Immunoprecipitated proteins were separated by electrophoresis on a 10% polyacrylamide slab gel followed by fluorography.

thesis of p62 and p64 (Figure 4, panel B). This increase appears to be specific since no detectable increase in the synthesis of p54$^{c\text{-}ets}$ was observed in differentiated cells as compared with untreated cells (Figure 4, panels A and B). Similar results were obtained with another AMV-transformed cell line BM2 (data not shown). TPA-induced differentiation of AMV-transformed myeloblasts requires the continuous presence of TPA and can be reversed upon TPA removal (Pessano *et al.*, 1979). As shown in Figure 4, panel C, reversion of differentiation by further culture of differentiated cells in the absence of TPA is accompanied by a decrease in the synthesis of p64 and p62.

Several human immature hematopoietic cell lines can differentiate terminally following induction with a wide range of different substances. For example, treatment of both human promyelocytic cell line HL60 and histiocytic cell line U937 by TPA induces these cells to differentiate into macrophages (Rovera *et al.*, 1979; Nilsson *et al.*, 1981), whereas treatment of HL60 by dimethylsulfoxide or retinoic acid induces differentiation into granulocytes (Collins *et al.*, 1978; Breitman *et al.*, 1980). As shown in Figure 5, exponentially growing HL60 cells only synthesize an *ets*-related protein of 53 kd as well as low amounts of immunologically related 58 kd, 62 kd and 68 kd proteins (Figure 5, panels A and F). TPA-induced differentiation of this cell line into macrophages is accompanied by a considerable increase in the level of synthesis of the 58 kd, 62 kd and 68 kd (Figure 5, panels B and C). Differentiation of HL60 cells can be induced by continuous treatment with low amounts of TPA or by short treatment with a high TPA concentration followed by incubation in regular growth medium (Collins *et al.*, 1978). The results of Figure 5, panels D and E, show that the synthesis of the 58 kd, 62 kd and 68 kd *ets*-related proteins is rapidly induced after a 15-min TPA pulse and that their synthesis at high levels does not require the continuous presence of TPA. These observations, together with the fact that increased synthesis of these proteins does not occur when HL60 cells are induced to differentiate into granulocytes by retinoic acid treatment (Figure 5, panel G), suggest that the induction of these proteins is specific of macrophage differentiation. The significance of the decrease in the synthesis of the 53-kd protein observed after 48 h of TPA treatment in the experiment of Figure 5, panels A−C, is unclear since it is not consistently observed in other experiments (see Figure 5, panels F−G). We conclude from these experiments that the induction of differentiation of various progenitor cells of avian and human origin into macrophages is accompanied by the synthesis of cellular proteins related to the v-*ets*-encoded domains of p135$^{gag\text{-}myb\text{-}ets}$.

Discussion

We previously identified a major 54-kd protein in chicken tissues (p54$^{c\text{-}ets}$) and a minor 56-kd component (p56$^{c\text{-}ets}$) which are apparently encoded by the cellular progenitor (c-*ets*) of the v-*ets* oncogene of avian leukemia virus E26. These proteins are precipitated by an antiserum to a bacterially expressed polypeptide corresponding to a portion of v-*ets* (anti-*ets* serum) and share most (five out of six) of their [^{35}S]methionine-containing tryptic peptides with those derived from the v-*ets*-encoded domain of p135$^{gag\text{-}myb\text{-}ets}$, the transforming protein of E26 (Ghysdael *et al.*, 1986).

We report here that the anti-*ets* serum identifies a second set of three proteins of 60 kd (p60), 62 kd (p62) and 64 kd (p64) in chicken cells. Comparative analyses of the [^{35}S]methionine-containing tryptic peptides and V8 protease digestion of these two sets of immunologically cross-reactive proteins showed p60, p62 and p64 to be highly related to each other and to share only a limited domain of homology with p54$^{c\text{-}ets}$. These results, together with the fact that these two sets of polypeptides do not appear to display any precursor-to-product relationship (personal observations of our laboratory) and that their synthesis is differential and independently regulated in chicken tissues and cell lines (see below), strongly suggest that each set originates from the translation of distinct mRNA species. Northern blot analyses

of normal chicken cell, poly(A)-containing, RNAs have identified three RNA species of 7.5 kb, 2.2 kb and 2.0 kb that hybridize with a v-*ets*-specific probe (Leprince *et al.*, 1983; Chen, 1985; Watson *et al.*, 1985). Although the precise genetic content of these RNAs still needs to be formally established by *in vitro* translation experiments, several assumptions can be made. Since chicken lymphoid tissues have been shown to contain both high levels of the 7.5-kb transcript and to synthesize high levels of p54$^{c\text{-}ets}$ (Chen, 1985; Ghysdael *et al.*, 1986), it is reasonable to hypothesize that the 7.5-kb RNA species encodes p54$^{c\text{-}ets}$. In this context and since the v-*ets*-encoded domain of p135$^{gag\text{-}myb\text{-}ets}$ shares extensive amino acid sequence homology with p54$^{c\text{-}ets}$ and only a limited domain of homology with p64, p62 and p60, it is probable that most of the v-*ets* oncogene of E26 derived from retrotranscription of part of the mRNA encoding p54$^{c\text{-}ets}$. Experiments are in progress to characterize further the structural relationship between p60, p62 and p64 and to determine whether these proteins are encoded by the 2.0−2.2-kb size class RNAs or by mRNA species different from those detected so far by the probes used.

Evidence has been provided recently for the existence of two distinct c-*ets* chromosomal loci (c-*ets*-1 and c-*ets*-2) in man and other vertebrate species (Watson *et al.*, 1985). In man, these loci map on chromosome 11 (11q23; de Taisne *et al.*, 1984; Watson *et al.*, 1985) and 21 (21q22; Watson *et al.*, 1985), respectively, and share at least one common domain of homology of 14 amino acids (Watson *et al.*, 1985). We do not believe that the two sets of *ets*-related polypeptides identified in this study are the translation products of chicken genes corresponding to the human c-*ets*-1 and c-*ets*-2 proto-oncogenes. Indeed, from the comparison of the tryptic peptide maps of p135$^{gag\text{-}myb\text{-}ets}$, p54$^{c\text{-}ets}$, p64−p60 (Figure 2; Ghysdael *et al.*, 1986) and of bacterially synthesized proteins corresponding to various portions of v-*ets* (data not shown) it appears that the domain of homology between p54$^{c\text{-}ets}$ and p64−p60 is more extended than that described for the putative human c-*ets*-1 and c-*ets*-2 gene products (Watson *et al.*, 1985) and, in fact, includes a peptide (indicated by an arrow in Figure 2, panel F) corresponding to a domain defined as unique (Watson *et al.*, 1985) to the human c-*ets*-2 proto-oncogene product. Furthermore, p54$^{c\text{-}ets}$ shares tryptic peptides with p135$^{gag\text{-}myb\text{-}ets}$ (peptides No. 2 and No. 5 of Figure 2 in Ghysdael *et al.*, 1986) corresponding to coding regions defined by Watson *et al.* (1985) as unique to the human c-*ets*-1 and c-*ets*-2 proto-oncogenes, respectively (A.Gegonne *et al.*, submitted). These data suggest that the mode of expression and organization of the cellular sequences related to v-*ets* are probably different in birds and mammals. Whether the mRNA species corresponding to the two sets of *ets*-related proteins we have identified in chicken tissues either derived from different loci or are translated from differentially spliced RNA transcripts derived from a single locus is presently under study.

We and others previously found p54$^{c\text{-}ets}$ to be expressed at high levels in chicken thymocytes and bursal lymphocytes but not in quiescent circulating lymphocytes, suggesting a role for p54$^{c\text{-}ets}$ in lymphocyte proliferation (Ghysdael *et al.*, 1986; Chen, 1985). Several lines of evidence suggest that p62 and p64 may play a role in either macrophage differentiation or function. First, these proteins were found to be synthesized at low or undetectable levels in chicken bone marrow cells and in chicken cells corresponding to immature stages of the myeloid lineage (Ghysdael *et al.*, 1986). Second, increased synthesis of these proteins was found in both normal chicken bone marrow-derived macrophages as well as in macrophage clones obtained after infection of bone marrow cells with various v-*myc*-containing retroviruses. Third, the synthesis of p64 and p62 is considerably increased during TPA-induced differentiation into macrophages of AMV-transformed myeloblasts. The synthesis of these proteins does not appear to be a non-specific response of transformed chicken myeloblasts to TPA since treatment of E26-transformed myeloblasts with TPA failed to induce their synthesis (personal observations of our laboratory) as well as macrophage differentiation (Beug *et al.*, 1984). Several human leukemic cell lines believed to be the neoplastic derivatives of committed progenitor cells of the myelomonocytic lineage can be induced to differentiate terminally by a variety of inducers. Differentiation of both the promyelocytic cell line HL60 (Figure 5) and the histiocytic cell line U937 (results not shown) into adherent non-proliferating macrophages by TPA is accompanied by the increased synthesis of proteins of 58 kd, 62 kd and 68 kd that share immunological cross-reactivity and structural homology with each other and with the avian p54$^{c\text{-}ets}$ and p60−p64 (unpublished data). Experiments are in progress to determine whether these proteins are encoded by either the human c-*ets*-1 or c-*ets*-2 loci. Increased synthesis of these proteins occurs rapidly after TPA treatment and, once induced, their synthesis at high levels no longer requires the continuous presence of TPA. Furthermore, increased syntheses of these proteins are not observed when HL60 cells are induced to differentiate into granulocytes.

Taken together these observations show that expression of cellular gene(s) related to the v-*ets* oncogene of avian leukemia virus E26 accompanies macrophage differentiation, suggesting a role for products encoded by the c-*ets* proto-oncogene or gene(s) related to c-*ets* in the induction or maintenance of macrophage differentiation or function.

Materials and methods

Cells and viruses

Chicken bone marrow-derived macrophages were obtained by culturing bone marrow cells for 4 days in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum and 2% chicken serum (standard growth medium) in the presence of 4 U/ml of cMGF (Leutz *et al.*, 1984). The MC29-transformed macrophage cell line HD11 and macrophages transformed by MH2, OK10 and various v-*mil* deletion mutants of MH2 were provided by Dr T.Graf (EMBL) and grown in standard growth medium (HD11 cell line and MH2-transformed macrophages) or growth medium supplemented with 5 μg/ml of bacterial lipopolysaccharide (macrophages transformed by OK10 and v-*mil* deletion mutants of MH2). The AMV-transformed myeloblast lines BM2 and 5YS were obtained from C.Moscovici and grown in standard growth medium. Induction of differentiation of AMV-transformed myeloblasts was obtained by culturing the cells in Iscove's modified DMEM supplemented with 30 μg/ml transferrin, 5 μg/ml insulin, 20 μM ethanolamine, 2% fetal calf serum, 1.5% chicken serum, 2 U/ml cMGF and 20 mM TPA (Sigma), essentially as described by Beug *et al.* (1984).

Cell labelling and immunoprecipitation analyses

Cells were labelled with L-[^{35}S]methionine (as indicated in figure legends) in methionine-free Eagle's medium supplemented with 5% dialyzed calf serum and lysed by boiling for 5 min in a 1% solution of sodium dodecylsulfate (SDS). After dilution with 10 vol of Tris−HCl 10 mM, pH 7.4, Triton X-100 1%, Na deoxycholate 0.5%, Trasylol 1%, the lysates were centrifuged at 100 000 *g* for 60 min and immunoprecipitations carried out as described previously (Ghysdael *et al.*, 1979). Immunoprecipitates were analyzed by electrophoresis on 10% or 12.5% polyacrylamide slab gels (acrylamide:bisacrylamide 30:0.4) in the presence of SDS, followed by fluorography. Cellular *ets*-related polypeptides were precipitated using a rabbit antiserum to a bacterially expressed protein corresponding to a portion of the v-*ets* oncogene of E26 (Ghysdael *et al.*, 1986).

Peptide mapping analyses

[^{35}S]methionine-labelled proteins were eluted from gel slices, precipitated, oxidized with performic acid and digested to completion with L-1-Tosylamide-2-phenylethyl chloromethyl ketone (TPCK)-treated trypsin. Tryptic peptides were separated in the first dimension by electrophoresis at 600 V for 100 min at pH 4.5 on thin-layer cellulose plates (Merck) and by chromatography in *n*-butanol:acetic acid:water:pyridine (75:15:60:60) in the second dimension as

described previously (Ghysdael *et al.*, 1979, 1981). Plates were prepared for fluorography by treatment with a 7% solution of diphenyloxazole in ether and exposed to Kodak XAR film at −70°C.

Partial digestion in gel slices containing [^{35}S]methionine-labelled proteins by *S. aureus* V8 protease was essentially as described by Cleveland *et al.* (1977).

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