Nucleic Acids Research, 1993, Vol. 21, No. 7 1601–1606

Cell-specific expression of helix-loop-helix transcription factors encoded by the E2A gene

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Received January 4, 1993; Revised and Accepted February 26, 1993

ABSTRACT

The E2A gene encodes transcription factors of the helix-loop-helix family that are implicated in cell-specific gene expression as part of dimeric complexes that interact with E box enhancer elements. It has previously been shown that transcripts of the E2A gene can be detected in a wide range of cell types. We have now examined expression of the mouse E2A gene at the protein level using polyclonal antisera directed against distinct portions of the E2A protein to probe blots of cellular extracts. A 73 kDa protein was identified by this analysis: this protein is highly enriched in cell lines of B lymphoid origin as compared to pancreatic β-cells and fibroblast cells. The detection of this protein selectively in extracts of lymphoid cells correlates with the presence of the E box-binding activity LEF1/BCF1 in these cells; this binding activity was previously shown to be efficiently recognized by antiserum directed against E2A gene products. Transfection of cells with full length E2A cDNA leads to appearance of protein co-migrating with the 73 kDa protein on SDS gel electrophoresis and co-migrating with LEF1/BCF1 on mobility shift analysis. Our results are consistent with the view that the DNA-binding activity LEF1/BCF1 is a homodimer of E2A proteins; the selective appearance of this putative cell-specific transcription factor in B lymphoid cells seems to be attributable, at least in part, to the elevated E2A protein concentrations in these cells.

INTRODUCTION

E boxes (consensus sequence CAxxTG) are cis-acting DNA elements important for transcription of a number of cell-specifically expressed genes (1–5). Proteins of the helix-loop-helix (HLH) family are capable of sequence-specific recognition of E boxes (6, 7), and are believed to mediate transcriptional activation *in vivo* through these sequences (8). The human E2A gene encodes at least two HLH proteins, E12 and E47 (6, 9), resulting from alternate splicing events (10); the respective cDNAs were originally isolated by expression screening of a lymphoid library using a DNA probe corresponding to κE2, an E box located in the immunoglobulin κ enhancer (6). Corresponding E2A homologs have been cloned from rodents by screening of pancreatic endocrine (11, 12) and exocrine (13) libraries using E box probes derived from the control regions of the insulin and chymotrypsin genes respectively. E2A RNA is detectable in a variety of cell types (6, 11, 13, 14) consistent with a broad role in transcriptional regulation. Indeed, genetic evidence from studies of daughterless (da), the presumptive Drosophila homolog of the E2A gene, have revealed that da mutations lead to severe phenotypes in several cell types (15). In mammalian systems, the *in vivo* role of E2A gene products has been most thoroughly explored in muscle cells; the cell-specifically expressed gene MyoD (16) and its close relatives myogenin, Myf-5 and MRF4 (17–20) heterodimerize *in vitro* with E2A proteins to form complexes that bind efficiently to E boxes (21). Such heterodimers apparently are also present *in vivo*: antibodies directed against MyoD or E2A proteins were shown to recognize muscle specific E box binding complexes using electrophoretic mobility shift analysis (EMSA) (22). Furthermore, fibroblasts expressing anti-sense E2A RNA showed diminished ability to undergo MyoD-induced differentiation, underscoring the importance of E2A proteins in myogenesis (22).

In pancreatic β cells, E boxes have been shown to play a key role in controlling cell-specific expression of the insulin gene (3, 23, 24). Endocrine cells contain a nuclear complex, IEF1, capable of specific E box recognition (25–27). IEF1 is recognized by antisera directed against E2A proteins indicating that these proteins are a component of the complex (12, 27–29). An additional HLH component of IEF1, absent from non-endocrine cells, has been defined by *in vitro* mixing experiments using nuclear extracts and *in vitro* translated HLH proteins (27, 30). Thus, in at least two mammalian cell types, functional cell-specific transcription factors appear to be generated by dimerization of cell-specific HLH proteins with apparently ubiquitously-expressed HLH proteins, the E2A gene products (21).

In lymphoid cells, a cell-specific E box binding complex (LEF1/BCF1) has also been identified (27, 31, 32); antibody experiments indicate the presence of E2A protein in the complex, yet a lymphoid-specific HLH partner has not been reported. In fact, current evidence indicates that LEF1/BCF1 may be a

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homodimer of E2A proteins (31, 33). However, this conclusion cannot explain the absence of the LEF1/BCF1 complex in other cell types, where E2A RNA is present.

To further elucidate the role of the E2A gene in cell-specific gene expression, we used two types of polyclonal antibodies, directed against the C-terminus and N-terminus region of E2A protein, to investigate protein levels in different cell types. Although comparable levels of steady state RNA of the E2A gene have been reported for multiple cell types, including lymphoid cells and fibroblasts (14), we observed a dramatic difference at the protein level: immunoblot analysis identified a prominent 73 kDa protein in extracts of B lymphoid cells. The protein was barely detectable in non-lymphoid lines tested (at least 15-fold lower levels). Several lines of evidence indicate that this protein is a product of the E2A gene. The data therefore indicate that cell-specific mechanisms, perhaps involving post-transcriptional control, lead to preferential accumulation of E2A gene products in lymphoid cells.

MATERIALS AND METHODS

Cell culture

The following established cell lines were used: HIT M2.2.2—hamster pancreatic β cells (34, 35); βTC1—mouse pancreatic β cells (36); Ltk$^-$ cells—mouse fibroblast cell; BHK—hamster fibroblast cells; S194, L10AC2, and WEHI 231 cells—mouse B lymphoid lineage: S194 cells are a plasmacytoma line, WEHI 231 and L10AC2 are mature B cell lines. Raji is a human lymphoma line. All cells except WEHI 231 and L10A-C2 were grown in Dulbecco's modified Eagles medium in the presence of 10% fetal calf serum, penicillin (200 I.U./ml) and streptomycin (100 μg/ml). For WEHI 231 cells, RPMI medium was used, supplemented as above, together with 50 μM β-mercaptoethanol.

Generation of polyclonal antibodies

The E2A gene encodes two closely related proteins, products of alternative splicing events: the alternate cDNAs have been designated E47 and E12 (human), A1 and A7 (mouse), Pan-1 and Pan-2 (rat), and shPan-1 and shPan-2 (hamster) (6, 11–13). We have used the mouse cDNA A1 to generate polyclonal antisera; fusion proteins were produced by subcloning of appropriate cDNA fragments to the expression vector pATH (37). pATH-A1(C) (11) contains the sequences encoding the C-terminal 281 amino acids of A1 (amino acids 369–649), pATH-A1(N) encodes 336 amino acids from the N-terminus of A1 (amino acids 50–385). Bacteria containing the appropriate plasmid were induced to over-express the fusion protein and bacterial extract was fractionated by electrophoresis on 10% polyacrylamide sodium dodecyl sulphate gels (SDS-PAGE) (38). Gels were lightly stained with Coomassie brilliant blue (2 g/l in H_2O). The band corresponding to fusion protein was excised from the gel, and 100 μg of protein was injected subcutaneously into rabbits as a suspension in incomplete Freund's Adjuvant. After three injections at two week intervals, serum was obtained and tested.

Immunoblot analysis

Proteins fractionated by SDS–PAGE were electrophoretically transferred to nitrocellulose sheets (39). Sheets were soaked for 2 hours in blocking buffer (phosphate-buffered saline (PBS) containing 10% (v:v) low fat milk and 0.05% Tween-20). All further incubation steps and washes were performed in the above buffer. Sheets were then incubated for two hours at room temperature in the presence of a 1/2,000 dilution of appropriate antibody. Following three washes in blocking buffer, protein A-horseradish peroxidase (Amersham) (1/10,000 dilution) was added, and incubation allowed to proceed for an additional two hours. For visualization of antibody–antigen complexes, the ECL detection system (Amersham) was employed, according to the manufacturer's instructions.

Immunoprecipitation

Protein-A-sepharose beads (Sigma) were pre-incubated with the appropriate anti-sera in 150 μl PBS for 90 min. Following three washes with PBS, nuclear extract was added (300 μg of protein) and incubated for two hours at 4°C. Subsequently beads were washed three times with 1 ml PBS and then sample buffer (38) was added. Samples were heated at 100°C for 3 min and the supernatant solutions were fractionated by SDS-PAGE. Immunoprecipitates were analyzed by immunoblot analysis as described above.

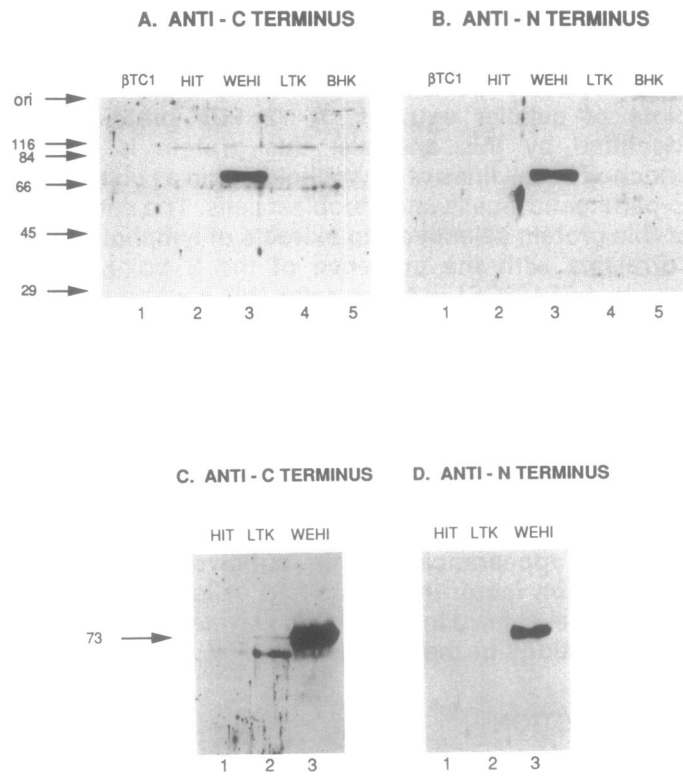

Figure 1. A, B: Immunoblot analysis of nuclear extracts (100 μg protein) derived from βTC1, HIT M2.2.2, WEHI 231, BHK, and Ltk$^-$ cell lines. Extracts were fractionated on 10% SDS–PAGE and electrophoretically transferred to nitrocellulose filters. Proteins were detected using antibodies directed against either C-terminus (Fig 1 A) or N-terminus (Fig 1 B) fragments of the A1 protein. The filters were developed using the ECL protein-A-HRP detection procedure as described. Molecular weights indicated are derived from the mobility of standard proteins (Rainbow markers—Amersham). **C, D:** Immunoblot analysis of whole cell lysates (200 μg protein) from HIT M2.2.2, Ltk$^-$, and WEHI 231 cells (C and D). Extracts were fractionated on 10% SDS-PAGE and electrophoretically transferred to nitrocellulose filters. Proteins were detected using antibodies directed against either C-terminus (Fig 1C) or N-terminus (Fig 1D) fragments of the A1 protein. The filters were developed using the ECL protein-A-HRP detection procedure as described. The migration of the 73 kDa protein is indicated.

Preparation of cell extracts and electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared as previously described (27). For whole cell lysate extracts, cells were harvested and pelleted as above. Subsequently, 100 μl of PBS and 100 μl of sample buffer (6.25 mM Tris−HCl pH 6.8, 10% glycerol, 1% lithium dodecyl sulfate, and 0.005% bromophenol blue) were added. The mixture was sonicated for 2 min in a sonicator bath (Elma transsonic 310), and then heated at 100°C for 5 min. Protein content of the extract was determined by the Lowry procedure (40), following precipitation of proteins by addition of trichloroacetic acid (10%) in presence of sodium deoxycholate (2 mg/ml). EMSA analysis of nuclear extracts was performed as previously described (27).

Transfection

HIT M.2.2.2 (35) cells (2×10^6) were transfected with a total of 15 μg DNA using the calcium phosphate procedure (41). Cells were harvested after 36 hours and nuclear extracts were prepared as described previously (27, 42).

In vitro transcription−translation

The plasmid pBS-ATG-#267 contains a near full-length A1 protein fused in frame to a portion of the β lactamase gene to generate a protein (A267) of almost identical molecular weight to full length A1 (30). *In vitro* transcription was performed using 0.5 μg of DNA in the presence of T7 RNA polymerase. Typically, 20% of the transcription reaction was used for *in vitro* translation with rabbit reticulocyte lysate (Promega) according to the manufacturer's instructions. Lysate product was used for EMSA reactions.

Densitometry

Autoradiograms were scanned and evaluated using a Molecular Dynamics 300A Computing Densitometer.

RESULTS

To determine whether E2A proteins, like E2A transcripts, are present at comparable concentrations in different cell types, we performed immunoblot analysis of nuclear extracts using anti-A1(C) antibodies (serum raised against the carboxy terminal part of A1 (27)). A 73 kDa protein was efficiently identified in WEHI 231 nuclear extract; this protein was barely detectable in extracts from fibroblasts or β cells (Fig. 1 A). In order to confirm that the 73 kDa protein represents an E2A gene product, and to exclude the possibility that the recognition is towards an HLH structural determinant, we raised polyclonal antibodies against an amino terminal segment of A1 (see Methods), which we designate A1(N). The serum directed against A1(N) detected a protein of identical electrophoretic mobility (73 kDa) in extracts from WEHI 231 cells but not fibroblasts or β cells (Fig. 1 B). The reactivity to the 73 kDa protein was not observed using the corresponding preimmune sera or anti-trpE antiserum (data not shown—see also Fig 5 A). The anti-A1(N) antiserum also efficiently recognized LEF1/BCF1 binding activity upon EMSA analysis (not shown).

Since the above analyses were performed using nuclear extracts, they could conceivably reflect differences in subcellular localization of the E2A proteins among the different cell types. We therefore performed immunoblot analysis using extracts of total cell lysates from fibroblasts, β cells and B lymphoid cells. The results obtained with both anti-sera were similar to those obtained with nuclear extracts (Figs. 1 C,D), indicating that the 73 kDa protein is present at substantially higher levels in lymphoid cells compared to fibroblast and β cells and that the differential signals observed using nuclear extracts are not attributable to differential subcellular localization.

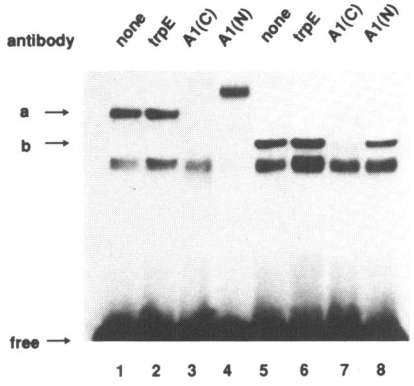

Figure 2. EMSA analysis of *in vitro* translated A1 proteins to determine specificity of antisera directed against A1 proteins. Clone A267 (encoding near full length A1) and clone A131 (encoding the A1(C) protein) were subjected to *in vitro* transcription-translation reactions. Translation products were incubated with IEB1 (25) probe in the absence of added IgG (lanes 1,5), and in the presence of IgG directed against trpE (lanes 2,6), A1(C) (lanes 3,7) or A1(N) (lanes 4,8). Lanes 1−4 contain translation products derived from clone A267, lanes 5−8 contain translation products derived from clone A131. The complexes marked 'a' result from the A267-derived protein. The complexes marked 'b' result from the A1(C) protein. The complex migrating slightly faster than 'b' corresponds to a non-specific DNA-binding activity.

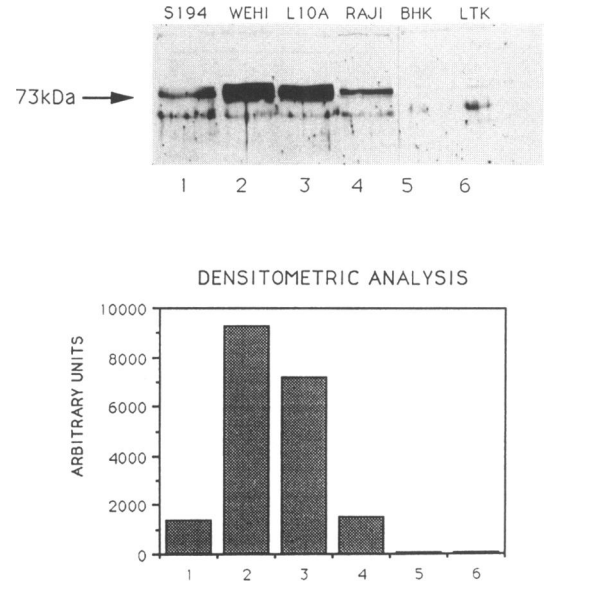

Figure 3. Immunoblot analysis of nuclear extracts (100 μg protein) derived from S194, WEHI 231, L10AC2, Raji, Ltk$^-$ and BHK cell lines. Top panel−extracts were fractionated on 10% SDS−PAGE and electrophoretically transferred to nitrocellulose filters. Proteins were detected using antibodies directed against C-terminus of the A1 protein. The filters were developed using the ECL protein-A-HRP detection procedure as described. The migration of the 73 kDa protein is indicated. Bottom panel—quantitative densitometric analysis of above immunoblot.

To confirm that the two antisera recognize distinct epitopes on E2A proteins, we tested their ability to interact with *in vitro* translated E2A proteins using EMSA analysis. As expected, anti-A1(C) recognizes both *in vitro* translated A267 (encoded by a near-full length A1 cDNA (pBS-ATG-#267) spanning both A1(N) and A1(C) proteins) and *in vitro* translated A1(C) protein (Fig 2, lanes 3,7). Anti-A1(N) antisera recognized efficiently the A267 protein (Fig 2, lane 4) but showed essentially no recognition of the A1(C) protein (Fig 2, lane 8). Thus the epitopes recognized by A1(N) antisera are distinct from those on the A1(C) protein; the efficient cell-specific recognition of the 73 kDa protein by both antisera is a strong indication that the 73 kDa protein is an E2A gene product.

We further analyzed whether other lymphoid cells exhibit increased levels of the 73 kDa protein using immunoblot analysis probed with anti-A1(C)(Fig 3). Indeed, nuclear extracts derived from S194, L10AC2 and Raji cells showed increased levels of the 73 kDa protein compared to fibroblast cell extracts. This indicates that increased levels of the 73 kDa is a more general characteristic of B-lymphoid cells; moreover the ability to detect the E2A protein product in these cells extract correlates with the LEF1/BCF1 binding activity detected using EMSA analysis (27, 32).

The detection system employed (ECL, Amersham) is based on enzymatic activity (horseradish peroxidase conjugated to protein-A). Using a purified glutathione S-transferase fusion protein containing the HLH domain of E2A protein, a linear response was obtained in the range of 0−50 μg of purified protein (data not shown). In order to quantitatively compare the expression in B lymphoid cells to that in fibroblasts, we performed immunoblot analysis using serial dilutions of WEHI 231 nuclear extract (Fig 4). Based on the analysis, the 73 kDa protein is at least 15-fold more abundant in WEHI 231 (Fig 4B) and L10AC2 extracts (Fig 3) as compared to fibroblast (Ltk$^-$) extracts.

The relationship between the 73 kDa protein, LEF1/BCF1 and E2A gene products was further examined using an immunoprecipitation procedure. Anti-A1(C) antiserum but not pre-immune serum was able to efficiently immunoprecipitate the 73 kDa protein (Fig 5 A, lanes 1, 2). The supernatant solution remaining following immunoprecipitation was tested for the presence of LEF1/BCF1 binding activity using EMSA (Fig 5 B). Both the untreated nuclear extract (lane marked '−') and the extract treated with pre-immune serum (lane 1) contained LEF1/BCF1 activity; the extract treated with anti-A1(C) was completely depleted of LEF1/BCF1 activity (lane 2). Thus, immunoprecipitation by anti-A1(C) of the 73 kDa protein from nuclear extracts correlates with depletion of LEF1/BCF1 binding activity from the extracts.

The observed size of the 73 kDa protein corresponds fairly well with the calculated molecular weight of E2A protein (67−68 kDa) based on cDNA sequences (13, 43) and with the mobility of in vitro translated E2A proteins on SDS and EMSA gel electrophoresis (30). To compare more directly the size of the 73 kDa lymphoid cell protein with the protein product of the full length cloned cDNA of A1 and A7, we expressed these cDNAs *in vivo*. Expression plasmids containing cDNAs encoding full length A1 and A7 under the control of a viral promoter (Rous sarcoma virus) were transiently transfected into HIT cells, a pancreatic β cell line chosen for this experiment because of its relatively high transfection efficiency and low basal levels of endogenous 73 kDa protein (Fig 1). Nuclear extracts were prepared, and immunoblot analysis was performed using anti-A1(C) antibodies. Cells transfected with the full length cDNA showed a band absent in non-transfected control cells and that co-migrated with the 73 kDa lymphoid cell protein (Fig. 6 A). Moreover, when the extracts from transfected cells were used in EMSA with an E box probe (μE4, which is efficiently recognized by LEF1/BCF1 (27)), a protein−DNA complex was observed with identical migration compared to LEF1/BCF1

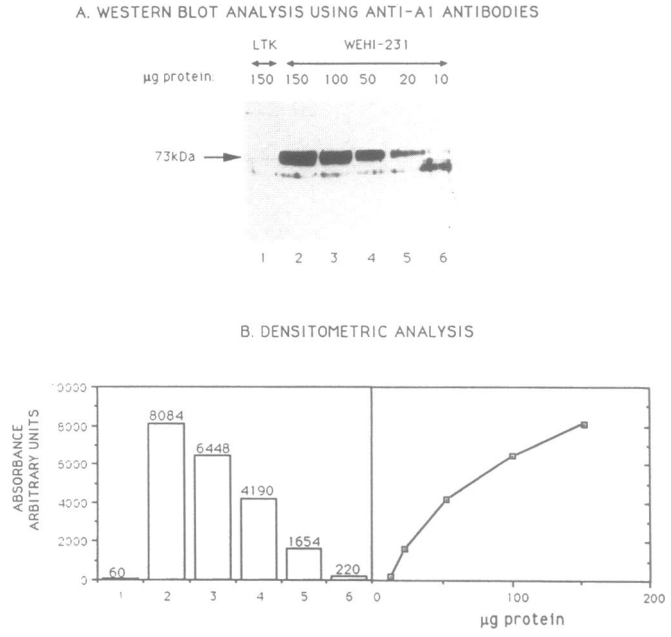

Figure 4. A: Immunoblot analysis of WEHI-231 nuclear extract. The indicated amounts of Ltk$^-$ and WEHI-231 nuclear extracts were subjected to immunoblot analysis using anti-A1(C) antibodies. **B:** The autoradiogram was analyzed using quantitative densitometric analysis. Left panel−absorbance measurement of 73 kDa band from each lane of Figure 4A. Right panel−absorbance measurement of 73 kDa band from serial dilutions of WEHI 231 extract (Figure 4A, lanes 2−6)

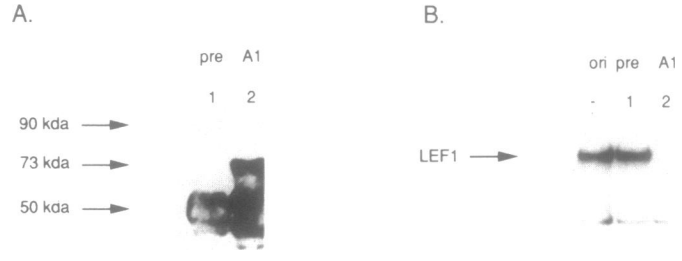

Figure 5. Immunoprecipitation of nuclear extracts of WEHI 231 cells using anti-A1(C) antiserum. Immunoprecipitates (Fig 5A) using pre-immune serum (lane 1) or anti-A1 (C) (lane 2) were subjected to SDS-PAGE and immunoblot analysis using anti A1(C) antisera. Molecular weights indicated are derived from the mobility of standard proteins (Rainbow markers—Amersham). The prominent band at 50 kDa corresponds to IgG heavy chain present in the immunoprecipitates and recognized by protein A-HRP. Figure 5B shows EMSA analysis of the supernatant solution remaining following treatment with antiserum. Nuclear extracts following treatment with no antiserum (lane marked '−'), preimmune serum (lane 1) or anti A1 (C) (lane 2) were analyzed by EMSA with the E box probe IEB1 (25). The mobility of LEF1/BCF1 is indicated.

(Fig. 6 B compare lane 2 and lane 3, 4) and also identical to the complex obtained with *in vitro* translated protein corresponding in size to the full length cDNA (Fig. 6 B lane 5). Therefore, the data strongly suggest that the 73 kDa protein identified by immunoblot using anti-E2A antibodies represents the cellular E2A protein.

DISCUSSION

Cell-specific expression of genes derived from diverse cell lineages requires the cis elements known as E boxes. The significance of these sequences appears to be their ability to be recognized by transcription factors of the HLH family. This rapidly growing family of proteins has been classified into 3 groups designated basic-HLH domain (bHLH) and bHLH-zip (basic HLH−leucine zipper) and dnHLH (dominant negative) (44). Within the bHLH proteins, a further sub-classification has been made according to tissue distribution: cell-specifically expressed bHLH proteins such as MyoD and achaete scute appear to homodimerize inefficiently, although they can heterodimerize with 'constitutively' expressed bHLH proteins such as E2A to produce an efficient DNA binding complex (21). The proteins from the bHLH family can also heterodimerize with dnHLH to produce a protein complex incapable of DNA binding.

Enhancers of immunoglobulin genes contain multiple E boxes whose importance was originally recognized by *in vivo* footprint experiments: the E boxes showed lymphoid selective footprinting (1, 45). Mutagenesis experiments revealed that the E boxes play an important role in the *in vivo* activity of immunoglobulin enhancers (2). More recently, an E box binding nuclear complex (LEF1/BCF1) was identified in lymphoid cells (27, 31). This complex was efficiently recognized by antibodies directed against E2A proteins, indicating the presence of E2A proteins in the complex (27, 31). Based on the preferential recognition of this complex as compared to heterodimeric complexes between E2A proteins and MyoD, and on the co-migration of the complex with *in vitro* and *in vivo* expressed E2A gene products, it has been suggested that LEF1/BCF1 corresponds to a homodimer of E2A proteins (31). This conclusion however cannot explain the apparent absence of LEF1/BCF1 in extracts of non-lymphoid cells given the presence of E2A RNA and protein in such cells.

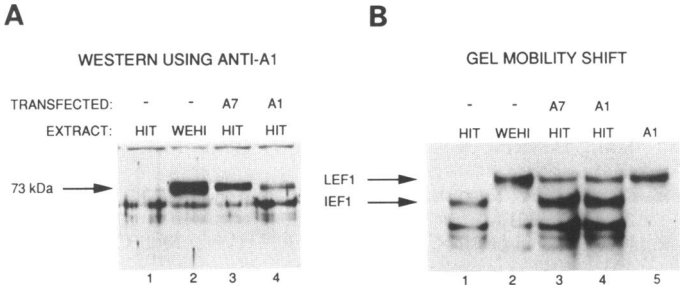

Figure 6. Analysis of E2A protein products expressed in transfected insulin producing cells. HIT M2.2.2 cells were transfected with 15 μg of plasmid expressing the complete coding sequence of A7 or A1 under the control of the Rous Sarcoma Virus (RSV) enhancer promoter. 36h following the transfection, nuclear extract was prepared and subjected to immunoblot analysis using antibodies directed against the C terminus of A1 (Fig 6 A) and EMSA analysis using the E box sequence μE4 (Fig 6 B). In both panels, the lanes contain nuclear extracts from the following cells: HIT M2.2.2 (lane 1), WEHI 231 (lane 2), HIT M2.2.2 transfected with full length A7 (lane 3), HIT M2.2.2 transfected with full length A1 (lane 4). HIT and WEHI 231 nuclear extracts were used as size references for the E2A protein product (73 kDa), and the IEF1 and LEF1/BCF1 binding activities. Panel B lane 5 contains the *in vitro* translation product generated from full length A1 cDNA of the E2A gene (A267 protein).

We and others have previously shown that polyclonal antiserum directed against E2A gene products recognize the cell-specific complexes IEF1 (12, 27−29) and LEF1/BCF1 (27, 31−33) suggesting that these complexes contain E2A gene products. We used two independently derived antisera, one directed towards the N-terminal portion of A1 and the other to the C-terminal portion of A1, to determine the relative abundance of E2A gene products in different cell types using immunoblot analysis. With both antibodies, we observed a prominent protein of 73 kDa in nuclear extracts of B lymphoid cells; the protein was barely detectable (at least 15-fold lower levels) in extracts from fibroblast or β cells. This result was also obtained using whole cell lysates. The lymphoid cell preference and the recognition of the 73 kDa protein by 2 distinct anti-A1 antisera indicates strongly that the protein corresponds to a product of the E2A gene and is a component of LEF1/BCF1. The Jurkat cell line (human T lymphoid) also shows substantial levels of the 73 kDa protein (data not shown). Several additional lines of evidence support the notion that the 73 kDa protein indeed corresponds to the cellular E2A protein product:

1. Transfection experiments, in which full length E2A gene products were transiently expressed in cultured β cells: upon transfection, extracts from such cells showed proteins which co-migrated with the 73 kDa protein on immunoblot analysis following SDS−PAGE and generated protein−DNA complexes *in vitro* which co-migrated with LEF1/BCF1 on EMSA (Fig 6).

2. Immunoprecipitation, using anti-E2A protein antibodies, of the 73 kDa protein from lymphoid extracts correlates with the loss of LEF1/BCF1 binding activity of this extract (Fig. 5).

3. In a parallel series of experiments, we have screened a λgt11 expression library from a late pre-B lymphoid cell line 70Z (which contain both LEF1/BCF1 and the 73 kDa protein—data not shown) using antibodies directed against the C-terminus of A1. Of 7 clones identified, sequencing analysis has shown that all were derived from the E2A gene (data not shown); this underscores the specificity of the anti-A1 antibodies towards the E2A gene product, and argues against the possibility that the 73 kDa protein may be a product of a related gene, eg. ITF2 (9) or HEB (46), recognized by anti-A1 antibodies because of immunological cross-reactivity.

In this work, we present data which can explain the fact that LEF1/BCF1 binding activity is restricted to lymphoid cells: lymphoid cells have dramatically elevated levels of E2A gene products as compared with other cells. This can account for the ready detection of homodimeric E2A complexes (LEF1/BCF1), capable of DNA binding. On the other hand, in non-lymphoid cells, E2A gene products are present at substantially lower levels (at least 15-fold less) and presumably insufficient homodimer can form to produce detectable binding activity. In endocrine cells or muscle cells, where E box binding activity is detected, the binding complexes appear to be composed of heterodimers of E2A gene products together with cell specific HLH proteins such as MyoD. The ability to detect such DNA binding complexes, despite the apparently very low levels of E2A proteins, is consistent with the known dimerization and DNA-binding properties of E2A proteins: for example, E47 forms heterodimers with MyoD much more readily than homodimers with itself; E12 binds DNA very poorly as a homodimer (10). The above

explanation is consistent with our ability to generate an LEF1/BCF1-like binding activity in β cells upon transfection with an E2A expression plasmid.

The precise role of LEF1/BCF1 in control of lymphoid cell gene expression has not been determined (47). Its preferential appearance in cells where immunoglobulin enhancers are active (27, 31), its ability *in vitro* to bind several of the key E boxes of immunoglobulin enhancers (27, 31), together with the ability of E2A proteins to trans-activate reporter plasmids containing multiple E box sequences (9) and activate germ line immunoglobulin heavy chain gene transcription (48) are consistent with a role as a lymphoid cell-specific transcriptional activator of immunoglobulin enhancers. Interestingly, this cell-specific factor appears to be composed of proteins which had hitherto been considered to be 'constitutive'. In fact, the data presented in this report indicates that there are substantially higher concentrations of E2A proteins in cells of B lymphoid lineage than in fibroblasts or β cells.

It appears therefore that distinct cell lineages use different strategies to generate the characteristic HLH transcription complexes that are necessary for generating unique patterns of cell-specific gene expression. In pancreatic β cells and muscle cells, heterodimeric complexes are formed between a cell-specific HLH protein (eg IESF1, MyoD) and E2A proteins (present at low concentrations in non-lymphoid cells). In B lymphoid cells, the accumulation of elevated concentrations of E2A proteins leads to formation of the homodimeric DNA binding activity (LEF1/BCF1), a presumptive lymphoid cell transcription factor. The precise mechanisms mediating the preferential accumulation of E2A gene products in lymphoid cells remain to be established.

ACKNOWLEDGEMENTS

We thank Dr Y.Citri and C.W.Park for valuable discussions. The work was supported by grants from the Juvenile Diabetes Foundation International, the Israel Academy of Sciences and Humanities, the Henry and Francoise Glasberg Foundation, and the Forchheimer Center for Molecular Genetics. MDW is the incumbent of the Maurice M.Bookstein Career Development Chair.

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