Lymphoid-specific Expression of the Id3 Gene in Hematopoietic Cells

SELECTIVE ANTAGONISM OF E2A BASIC HELIX-LOOP-HELIX PROTEIN ASSOCIATED WITH Id3-INDUCED DIFFERENTIATION OF ERYTHROLEUKEMIA CELLS

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Among the diverse family of eukaryotic transcriptional regulatory proteins characterized by the presence of the helix-loop-helix domain, an expanding subgroup of basic helix-loop-helix (bHLH)1 proteins has become recognized which play a pivotal role in the regulation of cell growth, commitment, and differentiation (1, 2). This distinct family of regulatory HLH proteins of the bHLH protein partner (24). As functional antagonists of bHLH proteins inhibit bHLH functions at a further level since Id-bHLH heterodimerization results in destabilization of the target DNA-binding domain, such Id-bHLH heterodimers are thought to be functionally inert since they are unable to transactivate E box regulatory sequences (11, reviewed in Refs. 1 and 2). Because Ids lack a basic DNA-binding domain, such Id-bHLH heterodimers are thought to be functionally inert since they are unable to transactivate E box regulatory sequences (11, 13, 19–23). In addition, Id proteins inhibit bHLH functions at a further level since Id-bHLH heterodimerization results in destabilization of the target bHLH protein partner (24). As functional antagonists of bHLH transcription factors, Id proteins act at a general level as positive regulators of cell growth and negative regulators of cell differentiation (11, 16, 19, 21, 23, 25–30).

In several studies where the functional properties of different Id proteins have been evaluated either biochemically or biologically, they display a high level of similarity, implying at
least some degree of functional redundancy as would be expected for a multigene family of this type (16, 18, 19, 26, 27, 31). Consistent with this, a number of cell types express multiple Id genes both in vitro and in vivo (Refs. 16, 19, and 32–34 and references therein). However, in other instances, expression of individual Id genes appears to be highly cell-type/lineage restricted. One example of this is provided by the hematopoietic system. The Id1 gene is widely expressed in both lymphoid and myeloid cell lineages (20, 35, 36) and its enforced ectopic expression has been shown to arrest differentiation in monocyte-macrophage (28), erythroid (29, 30), and B lymphocyte precursors (20, 27). In the latter case, inhibition of B lymphopoiesis can be accounted for solely on the basis of Id1 interaction with E2A-encoded E protein(s) since functional ablation of this bHLH E protein gene by gene targeting results in an identical phenotype to that observed in Id1 transgenic mice (27, 37). The Id2 gene is similarly highly promiscuous in its hematopoietic expression pattern (20, 35, 36), but Id3 appears to be expressed exclusively within the lymphoid compartment of hematopoietic cells (21, 35, 36) despite the fact that, like Id1/Id2, it can also function to promote cell growth/block differentiation (19) and to inhibit E box reporter gene activity at least in studies in other model cell types (14, 19, 21, 22, 24).

In evaluating the functional significance of the restricted expression pattern of Id3 in hematopoiesis we report here that repression of transcription of the Id3 gene in myeloid cells is correlated with hypermethylation of the 5′ region of the gene, implying a strict developmental control of Id3 expression in lymphoid versus non-lymphoid hematopoietic cells. Enforced ectopic expression of Id3 in K562 erythroid progenitor cells promotes erythroid differentiation and is correlated with a quantitative/qualitative shift in the profile of interacting TAL1 and E protein heterodimers that bind to a consensus E box promoter as described above. Hybridization, post-hybridization washes, and autoradiography were carried out as described by Murphy and Norton (15).

### Experimental Procedures

#### Cell Culture—All cell lines were maintained in either Dulbecco’s modified Eagle’s medium or RPMI 1640 medium supplemented with 10% fetal calf serum and 2 mM l-glutamine. Retroviral transduction of K562 cells was carried out essentially as described previously (38). The vector pBabe puro Id3, (19, 39) which was transfected into PA317 cells to generate high titer amphotropic virus was introduced into target cells by co-cultivation. Following selection with puromycin (2.5 μg/ml) for 10 days, individual clones were isolated by limiting dilution and subjected to further characterization. Differentiation of K562 cells was induced by treatment of cells for 4–5 days with cytokine arabinoside (2 × 10⁻⁶ M) and monitored by staining for hemoglobin-positive cells essentially as described previously (40).

#### DNA Extraction and Southern Hybridization—Genomic DNA was isolated from cells by SDS lysis, proteinase K digestion, and phenol extraction as described previously (14). 5–10 μg samples of total DNA were digested to completion by using a 5-fold excess ofMspI or HpaII using conditions recommended by the suppliers (Boehringer Mannheim). Digested DNA was electrophoresed on 1% agarose gels and transferred to nitrocellulose membranes by capillary blotting. A 275-base fragment corresponding to nucleotides 1–375 (12) was generated by PCR and radiolabeled with [α-³²P]dCTP by a random primed reaction as described above. Hybridization, post-hybridization washing, and autoradiography was carried out as described by Murphy and Norton (15).

#### RNA Extraction and Analysis—Total RNA was prepared from cell lines using the RNAZol extraction technique (CinnaBiotec). RNA concentration was estimated by absorption at 260 nm. Samples of 10 μg of total cellular RNA were analyzed on 1.2% agarose, 2.2 M formaldehyde gels and stained with ethidium bromide to monitor the equivalence of loading by fluorascence intensity of rRNA bands. After blotting onto “GeneScreen Plus” nylon membranes (NEN Life Science Products Inc.), hybridization conditions were as described by Murphy and Norton (15). Probes were labeled to a specific activity of 5 × 10⁶ cpm using a random primed labeling kit (Boehringer Mannheim), with [α-³²P]dATP (specific activity 3000 Ci/mmol; Amersham, United Kingdom). Blots were washed at 65 °C for successive 30-min periods first in 2 × SSC, 0.1% SDS, and then in 0.2 × SSC, 0.1% SDS and exposed to x-ray film at −70 °C with an intensifying screen.

#### Western Blotting—Analysis of proteins by Western blots was performed essentially as described by Harlow and Lane (41). Briefly, cells were detached by gentle scraping and were washed once in phosphate-buffered saline. The resultant cell pellet was re-suspended in SDS sample buffer (0.1 M Tris-HCl, pH 6.8, 2% SDS, 40% glycerol, 0.004% bromphenol blue) and boiled for 5 min. Cellular proteins were separated on 12% polyacrylamide-SDS gels and transferred to nitrocellulose membranes. After blotting for 1 h at room temperature in 15 mM NaCl, 10 mM Tris-HCl, pH 8, 0.05% Tween 20 (containing 5% dried milk) and then incubating with either control sera or with anti-Id3 antibody RD1 (diluted 1–100 in the above buffer) for a further hour at room temperature, the membrane was washed extensively in the same buffer and blocked for at least 1 h, followed by overnight incubation with the primary anti-rabbit (Dako, diluted 1–4000) in conjunction with the enhanced chemiluminescence detection system (Amersham). A rabbit polyclonal Id3 antisera (RD1) was raised by a standard immunization regime using a synthetic C-terminal 15-amino acid Id3 peptide as immunogen. Immunopurified antibody cross-reacted with mouse and human Id3 proteins but did not detect Id2 or Id1 proteins in control experiments.

**In Vitro Protein Binding Studies—** A PCR product was generated using the primers, 5′-ATGGATCCCATGAACTGCTGAAGCGCGG- GTG-3′ and 5′-AGGGTGTCAGGAGACGAGCAGGCTCA-3′ corresponding to the nucleotides 368–388 and 727–748 in the human Id3 cDNA template (14), which resulted in the addition of a BamHI site at the 5′ end of the Id3 coding sequence. PCR amplification was performed on plasmid DNA template (1 ng). Reaction conditions were 94 °C (1 min), 54 °C (1 min), and 72 °C (1.5 min) for 30 cycles. PCR reactions comprised 100 μM dATP, dCTP, dGTP, and TTP together with primers (1 μM), Tq polymerase (1 unit, Promega, Madison, WI) in reaction buffer (100 μl). PCR products were subcloned into the PCR II vector (Invitrogen) and subsequently as a BamHI-EcoRI fragment into the PGE2XZ vector (Phar- macia), sequenced and expressed in the Escherichia coli strain INVαF’. Bacterial protein purification was carried out essentially as described by Kaelin et al. (42) with protein harvest at 6 h post-isopropyl-1-thio-β-D-galactopyranoside induction. Purified GST fusion protein was analyzed for relative protein content and purity by SDS-polyacrylamide gel electrophoresis.

**Plasmids encoding full-length E47 and an E47 variant containing only the bHLH domain, E47a (40), Id1 (44), TAL1 (5), and MyoD (13) were used as templates for in vitro transcription and translation using a commercial kit according to the manufacturer’s instructions (Promega). Equimolar concentrations of translated protein were generated using γ-³²P]methionine and analyzed by SDS-polyacrylamide gel electrophoresis. Purified GST fusion protein bound to glutathione-Sepharose beads were preincubated with 1.0 ml of 25 mM HEPES, pH 7.5, 12.5 mM MgCl₂, 150 mM NaCl, 1 mM dithiothreitol supplemented with 20% glycerol, 0.1% Nonidet P-40, and 0.5 μg/ml bovine serum albumin, at 20 °C for 10 min and protein interactions were assessed by the addition of each reticulocyte lysate mixture (10-μl aliquots) containing in vitro translated protein. This was incubated with constant mixing at 20 °C for 1 h and then washed 3 times in 20 mM Tris-HCl, pH 8, 100 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40. Radiolabeled proteins bound to the Sepharose beads were reconstituted with sample buffer (25 μl), heated to 95 °C for 5 min, and analyzed by SDS-polyacrylamide gel electrophoresis. Following electrophoresis the gel was dried and autoradiographed.

**DNA Mobility Shift Assays—** A 20-base pair double-stranded oligonucleotide containing the wild type E box recognition sequence: ACCCTGAAGATGTCGCG or mutant, ACCCTGGA GGATGTCGCTT was synthesized. A single E box element (underlined), was used in all reactions to assay for HLB binding. Competition reactions with either wild type or mutant probe (above) established the specificity of the reaction. DNA mobility shift assays were carried out essentially as described by Murre et al. (46). Oligonucleotides were self-anneled, 5′-end labeled using [γ-³²P]ATP (specific activity 3000 Ci/mmol; Amersham) with T4 polynucleotide kinase and were subse-
FIG. 1. Methylation analysis of the 5’ region of the human Id3 gene in lymphoid and myeloid cell lines. A, simplified diagram of the human Id3 genomic locus (12) showing relevant *MspI/HpaII* sites (M). Approximately 4.0 kb of the genomic locus is shown where exons are depicted by boxes and protein-coding regions are stippled. The probe used for Southern analysis (generated as a 375-base pair PCR fragment) is indicated by a solid box. The *MspI/HpaII* site marked with an asterisk is differentially methylated in hematopoietic cell lines. nt, nucleotide. B, Southern analysis of DNA from cell lines. 10-μg samples of genomic DNA were digested to completion using a 5-fold excess of either *MspI* or *HpaII* then electrophoresed and transferred to nitrocellulose filters for analysis by hybridization with the 5’ Id3 probe indicated in A. The resulting digest patterns did not change on addition of further enzyme (data not shown). The analysis of NALM6 DNA was performed on a separate gel and is shown aligned with other samples for comparison.

Id3 Function in Hematopoiesis

A simplified diagram of the human Id3 genomic locus is shown where exons are depicted by boxes and protein-coding regions are stippled. The probe used for Southern analysis (generated as a 375-base pair PCR fragment) is indicated by a solid box. The *MspI/HpaII* site marked with an asterisk is differentially methylated in hematopoietic cell lines. nt, nucleotide. B, Southern analysis of DNA from cell lines. 10-μg samples of genomic DNA were digested to completion using a 5-fold excess of either *MspI* or *HpaII* then electrophoresed and transferred to nitrocellulose filters for analysis by hybridization with the 5’ Id3 probe indicated in A. The resulting digest patterns did not change on addition of further enzyme (data not shown). The analysis of NALM6 DNA was performed on a separate gel and is shown aligned with other samples for comparison.

RESULTS

Lymphoid-specific Expression of Id3 in Hematopoietic Cell Lines—To investigate the lymphoid restricted expression of Id3 in the hematopoietic system, we analyzed the methylation status of the human Id3 gene in the immediate vicinity of its upstream regulatory sequences (12) by using a combination of methylation-sensitive/insensitive enzyme isoschizomers, *HpaII/MspI*. In general, transcriptional activity, particularly of developmentally regulated genes involved in control of cell fate in metazoan, is correlated with a more open chromatin conformation which in turn is inversely correlated with the extent of methylation of cryptic cytosine residues, typically in the context of CpG dinucleotides (50). As shown in Fig. 1A, the human Id3 gene possesses several *MspI/HpaII* sites within 2 kb of the 5’ cap site. Digestion with *MspI* (methylation insensitive) generated fragments of 0.87 and 0.18 kb which could be detected with a probe overlapping the 5’ cap site in Southern analysis (Fig. 1, A and B). This fragment pattern was invariant among different lymphoid and non-lymphoid cell line DNAs examined (Fig. 1B). In contrast, *HpaII* digestion (methylation sensitive) of myeloid HL60 and K562 DNA generated, in addition, a fragment of approximate size 2.0 kb, with a corresponding reduction in intensity of the 0.87-kb fragment (Fig. 1B). None of the lymphoid cell lines tested generated this additional 2.0-kb fragment (shown for MA42L-mature B; Nalm 6 and Ramos-immature B cells in Fig. 1B). Thus, we conclude that the *MspI/HpaII* site bounding the 0.87- and 1.4-kb fragments (marked with an asterisk in Fig. 1A) is differentially methylated in myeloid versus lymphoid cell types, consistent with cis-repression of the Id3 gene in myeloid lineage cells.

Effect of Enforced, Unscheduled Expression of Id3 in K562 Erythroleukemia Cells—To assess the possible functional significance of the lymphoid restricted expression of Id3 in the hematopoietic system, we investigated the functional consequences of enforced unscheduled expression of this gene on the erythroid differentiation program of K562 cells. Following retroviral transduction of the Id3 cDNA into K562 cells using the vector, pBabepuroId3 (19, 39), a number of clones expressing a range of Id3 mRNA levels were obtained (Fig. 2A), in parallel with control clones expressing pBabe vector without insert. In contrast to the rather variable Id3 expression levels seen at the RNA level, Western analysis revealed relatively invariant Id3 protein levels among Id3 transductants (shown for clones K2 and K4 in Fig. 2B). The expression of Id3 protein in these K562 clones was also very low, being near the lower limit of detection with available antisera.

As shown in Fig. 2C, the extent of spontaneous erythroid differentiation in Id3-expressing K562 clones was consistently elevated when compared with 4 control clones evaluated in parallel in two separate experiments. Following induced differentiation with cytokine arabinoside, the Id3-transduced clones also underwent a more rapid terminal differentiation, and at...
lower drug concentrations than controls. In further experiments, we noted no significant differences in proliferative potential between Id3-expressing K562 clones and control clones (as assessed by cell numbers in liquid culture and from enumeration of colony formation in soft agar).

**Id3 Does Not Abrogate Id1 Function**—In addition to forming stable heterodimers with bHLH proteins such as E47 (but not Tal1), Id3 also heterodimerizes with Id1. Since Id1 is known to negatively regulate erythroid (and myeloid) differentiation (28–30), we reasoned that enforced expression of Id3 in K562 cells might promote erythroid differentiation by interacting with and effectively neutralizing the functions of Id1. To directly test this hypothesis, we designed a simple experiment which exploited the shared properties of Id1 and Id3 in their abilities to heterodimerize with bHLH target E proteins (11, 13, 14, 19–22, 24), thereby preventing the association of E protein with cognate E box recognition sequence in *in vitro* band shift analyses. As shown in Fig. 3, increasing inputs of *in vitro* synthesized Id1 and Id3 proteins both efficiently inhibited the formation of E47 homodimer band shift complexes when added separately. However, when equimolar equivalents of the two proteins were added in combination, the effect on inhibition of E box binding by E47 was not significantly different from that seen with each Id separately (Fig. 3).While the two Id proteins are evidently capable of physical association at least *in vitro*, this experiment shows that there is no detectable functional antagonism between these Ids which might explain the differentiation-promoting effect seen with Id3 in K562 cells.

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2 M. Jasiok, R. Deed, and J. Norton, unpublished observations.
Id3 Selectively Ablates E Box Binding of E2A Proteins in Vitro and in Vivo—We next examined the ability of Id3 to prevent heterodimers comprising TAL1 and E protein-containing heterodimers from binding to E box DNA in in vitro band shift assays. As shown by the experiment in Fig. 4A, exogenous Id3 but not control GST protein readily abrogated E47 homodimer binding to E box sequence as seen with the in vitro synthesized Id protein in Fig. 3. In contrast, binding of TAL1-E47 heterodimer to its preferred consensus sequence was only partially titratable upon addition of exogenous Id3 (Fig. 4A). These results extend previous data for Id1 and Id2 (49, 51–53) which demonstrate that TAL1-E2A heterodimers are relatively resistant to the actions of Id proteins.

To determine whether Id3 selectively antagonizes E box E2A binding of homodimers in vivo, we used the same E box probe with nuclear extracts from K562 cells as shown in Fig. 4B. In wild type cells, several specific complexes were detected which could be competed by unlabeled homologous oligonucleotide but not by heterologous mutant probe. The retarded complexes designated I and II in Fig. 4B each comprise only a single band, whereas complex III comprises 3–4 poorly resolved species which, in addition showed some variation in intensity in different experiments. As shown in Fig. 4B (right panel) an almost identical band pattern was generated with extract from Jurkat T cells, consistent with previously published data (45, 52, 53). A comparison between extracts from wild type K562 cells and extracts from Id3 overexpressing clones (shown for clones K2 and K4 in Fig. 4B, right panel) revealed selective loss of complex I. In addition, the fastest migrating band within complex III appeared more intense in the Id3-expressing K562 clones. To determine which (if any) of these changes was likely to be attributable to the presence of exogenous Id3 in these cells, the effect of adding bacterially synthesized Id3 protein to wild type cell extracts was examined as shown in Fig. 4C (left panel). While addition of control GST protein had no effect, GST-Id3 totally abrogated complexes I and II with only a minor effect on the relative intensity of bands within complex III.

The experiment depicted in Fig. 4C (right panel) revealed the identities of complexes detected in our extracts; complex I was selectively supershifted by E2A-specific antiserum while complexes II and III were abrogated by anti-TAL1 antiserum leaving complex I unaffected.

The E47 E2A Protein Is Selectively Targeted by Id3 in Vitro and in Vivo—The preceding experiments demonstrate that ectopic expression of Id3 in K562 cells is associated with selective loss of E2A homodimer-containing complexes as detected by binding to DNA and would be consistent with this E protein being selectively targeted by Id3 in vivo. However, the levels of endogenous Id1 (and exogenous Id3) proteins in K562 cells were found to be prohibitively low for direct analysis of their in vivo association with E protein (see Fig. 2B and data not shown). Therefore, to determine whether the selective loss of E2A homodimer binding reflects differences between Id1 and Id3 in their in vivo association with E proteins, exemplified by E2A, we compared the magnitude of different E2A (E47)-Id interactions in vitro by a GST pull-down assay, and in vivo by a Gal4-Vp16 two-hybrid assay. As shown in Fig. 5, in vitro translated E2A protein, E47, preferentially bound to Id3 in an in vitro pull-down assay. For comparison, the class B bHLH protein, MyoD, showed preferential binding to Id1 in this assay (Fig. 5), consistent with previous data (54). To evaluate Id-E2A interaction in vivo, we used a quantitative VP16-Gal4 two-hybrid assay in transiently transfected cells. In preliminary experiments, however, we found no significant differences between the ability of Id1 and Id3 to associate with E47 using VP16-Id and Gal4-E47 gene fusions in direct interaction assays, consistent with previous findings (54). Therefore, as an alternative more discriminating assay, we employed a competitive two-hybrid approach in which the E2A homodimer interaction was challenged with increasing inputs of each of the wild type Id proteins, as shown in Fig. 6. At the highest input of Id competitor, both Id proteins almost totally abrogated E2A homodimer formation (Fig. 6). However, at progressively decreasing inputs, the Id3 protein appeared to be significantly more potent in competing for E2A homodimer formation than Id1. To determine whether this apparent difference in the ability of Id1 and Id3 to interact with E2A protein is reflected by differences in their abilities to antagonize E2A-dependent gene expression, we examined the effect of increasing inputs of each Id on E2A-dependent trans-activation of an E-box reporter as shown in Fig. 7. As with the two-hybrid assay, Id3 inhibited E2A function more efficiently than did Id1. Thus, in cells expressing Id1 and Id3 (such as would be the case for K562 clones manipulated to express exogenous Id3) Id3 would selectively target E2A protein.

DISCUSSION

Although the Id3 gene is widely expressed in cell types of multiple (non-hematopoietic) lineages both in vitro and in vivo (13, 14, 34, 55, 56), cells of the myelopoietic compartment are notable for the absence of detectable expression of this gene (35, 36). Moreover, this transcriptional inactivity is correlated with hypermethylation of sequences in the immediate vicinity of the upstream regulatory region of the Id3 gene, suggestive of a strict developmental control of Id3 gene expression in lymphoid versus non-lymphoid hematopoietic cells. To investigate the possible biological significance of this regulated expression pattern, we evaluated the functional consequences of enforced expression of the Id3 gene in progenitor erythroid cells in which

![Diagram](http://www.jbc.org/Downloaded from http://www.jbc.org/ by guest on July 23, 2018)
Id3 is not normally expressed. Ectopic expression of the related Id1 protein in erythroid precursors is known to lead to an arrest in the differentiation program (29, 30), as occurs in several other cell types, including those of myeloid cell lineage (28). Since Id1 is normally expressed in such non-lymphoid hematopoietic cells, at least in their undifferentiated counterparts, this has led to the suggestion that the Id1 family member normally serves as a negative regulator of myeloid cell differentiation, in addition to its proposed similar role in regulation of early B lymphopoiesis (27–30). However, in our studies, all Id3-expressing K562 clones that were examined displayed an enhanced propensity to differentiate either spontaneously or in response to chemical induction when compared with controls. This observation demonstrates that the unscheduled expression of Id3 in the “non-permissive” erythroid environment interferes with the normal differentiation program and implies that at least in this cellular context, the functions of different Ids (Id1 versus Id3) are biologically distinguishable. We note in passing that despite relatively high (although variable) levels of the exogenous Id3 retroviral mRNA observed among different transduced clones, expression of the Id3 protein was only just detectable using available antisera and, moreover, this expression level displayed little variation between different Id3-transduced K562 clones. This phenomena has been previ-
ously reported in studies on ectopic Id gene expression (23),(291,80),(992,179) including Id3 (19), in cell line models and may well reflect a limited tolerance of particular cell types to unscheduled Id protein expression.

The observed enhancement in differentiation of K562 cells in response to transduction of Id3 is very reminiscent of the effect reported to occur in response to enforced overexpression of the TAL1 bHLH protein in these and other primitive erythroid precursor cell lines (8, 9). The TAL1 protein, whose functions are essential for normal erythropoiesis (6) interacts avidly with bHLH proteins, such as E47 (45, 51–53). TAL1 also associates in vivo with members of the LIM domain protein family, specifically LM02(RBTN2/TTG2) which is also indispensable for erythropoiesis (57–59). However, beyond this, little is known about the mechanisms/gene targets through which TAL1-E47/LM02-mediated differentiation occurs during erythropoiesis.

Although the Id3 gene is not normally expressed in erythroid progenitors and is therefore not a normal physiological regulator of erythropoiesis, an understanding of the mechanisms underlying this differentiation-promoting effect of Id3 might provide some insight into how candidate interacting regulatory partners function during normal erythropoiesis. To date some non-bHLH proteins have been described as potential Id targets, such as the retinoblastoma susceptibility gene product, Rb (and related pocket proteins) for Id2 (25, 31) and the MIDA1 protein for Id1 (60), thus far only bHLH proteins (essentially of the E protein type) have been reported to associate with Id3 (13, 14, 19, 21, 22, 24, 61). However, in addition to the E47 and TAL-1 bHLH proteins, we also examined the possibility of Id1-Id3

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FIG. 5. Enhanced affinity of Id3 for E protein in GST pull-down assays. The [35S]methionine-labeled in vitro translated proteins E47, and MyoD were incubated with Sepharose beads bound to GST, GST Id1, or GST Id3 protein. Bound proteins were eluted and analyzed on a 12.5% Tris glycine/SDS gel. A sample of control in vitro translate representing 50% of the total lysate used in the GST pull-down assay was analyzed in the first lane of each gel.

FIG. 6. Enhanced inhibition of Gal4E47-Vp16E12 interaction in vivo by Id3 compared with Id1. Cells were transfected with 2.5 μg each of the Gal 4 luciferase reporter, p5E1bLuc, the CMV driven Renilla luciferase plasmid pRLCMV, the pGal4E47 and pVp16E12 fusion vectors together with each of the CMV driven Id expression constructs indicated plus pcDNA3 to normalize for DNA input. 24 h post-transfection, luciferase activities were measured. Results are shown from two independent experiments and immunoprecipitation data are shown from parallel transfections to monitor for Id protein expression levels.

FIG. 7. Enhanced activity of Id3 compared with Id1 on E2A-dependent gene expression in vivo. Fibroblasts were transfected with 2.0 μg of E box driven CAT reporter plasmid (E1bCat) in combination with expression vectors encoding E47 and Id1 or Id3 as indicated. Transfected DNA was normalized to a total of 15 μg with pcDNA3 as carrier plasmid together with 1 μg of luciferase reporter driven by an heterologous CMV promoter as a control for transfection efficiency. Twenty-four hours post-transfection, cells were harvested and relative CAT activities determined by using [14C]chloramphenicol and thin layer chromatography with quantitation by PhosphorImager analysis. The data shown were determined from two independent experiments.
interactions since such Id homo-heterodimers are predicted to occur from three-dimensional modeling studies (18). Although Id3 did appear to associate with Id1 in vitro, albeit to a lesser extent than with E47,2 we could find no evidence for functional antagonism between these proteins in a functional assay of abrogation of E protein binding to an E box oligonucleotide. A simplistic explanation for Id3 promotion of erythroid differentiation based on antagonism of Id1 function therefore seems unlikely.

As with other Ids, Id3 was found to associate avidly with the E47 E protein in vitro (confirming previous data, see Refs. 13, 14, 19, 21, 22, 24, and 61) and although binding to the TAL1 bHLH protein was detectable, the ability of Id3 to abrogate E47-TAL1 heterodimer binding to an E box sequence was considerably less than that seen for E47 homodimers. Such resistance of TAL1-E protein heterodimers to Id antagonism has previously been reported for the Id1 and Id2 proteins (49, 52, 54, 14, 19, 21, 22, 24, and 61) and although binding to the TAL1 protein was detectable, the ability of Id3 to abrogate interaction since such Id homo-heterodimers are predicted to occur from three-dimensional modeling studies (18). Although Id3 did appear to associate with Id1 in vitro, albeit to a lesser extent than with E47,2 we could find no evidence for functional antagonism between these proteins in a functional assay of abrogation of E protein binding to an E box oligonucleotide. A simplistic explanation for Id3 promotion of erythroid differentiation based on antagonism of Id1 function therefore seems unlikely.

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55. Ellmeier, W., Aguzzi, A., Kleiner, E., Kurzbaur, R., and Weith, A. (1992) EMBO J. 11, 2563–2571
56. Ellmeier, W., and Weith, A. (1995) Dev. Dyn. 203, 163–173
57. Warren, A. J., Colledge, W. H., Carlton, M. E. L., Evans, M. J., Smith, A. J. H., and Rabbits, T. H. (1994) Cell 78, 45–58
58. Valge-Archer, V. E., Osada, H., Warren, A. J., Forster, A., Li, J., Baer, R., and Rabbits, T. H. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 18617–18621
59. Wadman, I., Li, J., Bash, R. O., Forster, A., Osada, H., Rabbits, T. H., and Baer, R. (1994) EMBO J. 13, 4831–4839
60. Shoji, W., Inoue, T., Yamamoto, T., and Obinata, M. (1995) J. Biol. Chem. 270, 24818–24825
61. Deed, R. W., Armitage, S., Brown, M., and Norton, J. D. (1996) Biochem. Soc. Trans. 24, 5
62. Goldfarb, A. N., and Lewandowska, K. (1995) Blood 85, 465–471
63. Condorelli, G. L., Tocci, A., Botta, R., Facchiano, F., Testa, U., Vitelli, L., Valtieri, M., Croce, C. M., and Peschle, C. (1997) Mol. Cell. Biol. 17, 2954–2969
64. Bain, G., Engel, I., Mandaag, E., TeReile, H., Voland, J., Sharp, L., Chun, J., Huey, B., Pinkel, D., and Murre, C. (1997) Mol. Cell. Biol. 17, 4782–4791
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