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Biochemistry

Association of erythroid transcription factors: Complexes involving the LIM protein RBTN2 and the zinc-finger protein GATA1

(erythropoiesis/transcription/leukemia/translocation)

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ABSTRACT **The RBTN2 LIM-domain protein, originally identified as an oncogenic protein in human T-cell leukemia, is essential for erythropoiesis. A possible role for RBTN2 in transcription during erythropoiesis has been investigated. Direct interaction of the RBTN2 protein was observed *in vivo* and *in vitro* with the GATA1 or -2 zinc-finger transcription factors, as well as with the basic helix–loop–helix protein TAL1. By using mammalian two-hybrid analysis, complexes involving RBTN2, TAL1, and GATA1, together with E47, the basic helix–loop–helix heterodimerization partner of TAL1, could be demonstrated. Thus, a molecular link exists between three proteins crucial for erythropoiesis, and the data suggest that variations in amounts of complexes involving RBTN2, TAL1, and GATA1 could be important for erythroid differentiation.**

Hematopoiesis is the process by which cells from the pluripotential bone marrow stem cell pool differentiate into terminally differentiated blood cells. Erythropoiesis creates enucleated red blood cells which express hemoglobin and function to transport oxygen and carbon dioxide. Like other developmental pathways which emanate from stem cells, specific transcription factors are required for erythroid cellular differentiation and commitment. The *RBTN2/TTG2* gene, originally identified by its association with the chromosomal translocation t(11;14)(p13;q11) in T-cell leukemias (1, 2), has been shown to be essential for erythroid differentiation since null mutations of this gene cause blockage of erythropoiesis (3). Further, experiments in which null mutations have been introduced into other mouse genes first showed that the zinc-finger-like proteins GATA1 (4–7) and GATA2 (8) and later that the basic helix–loop–helix (bHLH) protein TAL1/SCL (9) are necessary for erythroid development since mutations of these genes block erythropoiesis. These results imply a close functional relationship among RBTN2, TAL1, and GATA1 or -2 in transcription during erythroid differentiation.

It is interesting that both RBTN2 and TAL1 are important for erythropoiesis and both are activated by distinct chromosomal translocations in T-cell leukemias (10). While neither *RBTN2* or *TAL1* genes are normally expressed in $CD3^+$ T cells, they are coexpressed in a number of sites, including erythroblasts (3). In addition, a heterocomplex of RBTN2 and TAL1 has been found in erythroid cells (11, 12). Finally, the similarity of the erythroid-deficient phenotype resulting from *rbtn2*, *GATA1*, or *tal1* null mutations suggests that a molecular relationship may exist between the three encoded proteins, in addition to the RBTN2/TAL1 heterodimerization already reported.

The RBTN2 protein consists of two cysteine-rich LIM domains (1, 13, 14) which bind zinc atoms (15–19) and form finger-like structures with some similarity to those of the DNA-binding finger of the GATA family (18, 20). However, a function for the LIM domain in protein interactions has been postulated (21) and such a role has recently been shown (11, 12, 22, 23). Indeed, it appears to be the LIM domain(s) of RBTN2 which binds to the bHLH domain of the TAL1 protein (12). The various properties of *GATA1, rbtn2*, and *tal1* genes suggest that the proteins encoded by these three genes may play a related role in erythropoiesis and that they might interact at certain stages of erythroid development. Such a role for RBTN2 has now been investigated and evidence obtained that a complex can form among RBTN2, GATA1 (or -2), and TAL1.

MATERIALS AND METHODS

Immunoprecipitation Assays: Endogenous Proteins. Murine erythroleukemia (MEL) cells ($1–2 \times 10^7$) were labeled with [^{35}S]cysteine for 4 h, and labeled proteins were immunoprecipitated essentially as described (11). Anti-RBTN2 (3) and anti-TAL (25) antisera have been described. Polyclonal rabbit anti-GATA1 antiserum was raised by immunization with peptides derived from published sequences (26), coupled to thyroglobulin. The N-terminal GATA1 peptide was GLGALGTSEPLPQFVD, and the C-terminal GATA1 was VSHLMPFPGPLLGSPT.

Transient Transfections. COS cells (1×10^7) were either mock transfected or transfected with 5 μg of expression vectors for RBTN2 and/or GATA by electroporation. After 2 days, transfectants were labeled with 500 μCi (1 Ci = 37 GBq) of [^{35}S]cysteine for 4 h. Single and two-step immunoprecipitation was carried out as described (11) and precipitates were analysed by SDS/12.5% PAGE. For peptide inhibition, immunoprecipitation was carried out as above in presence of 50 μg of the RBTN2 or GATA2 peptides used for raising the respective antisera. The rabbit polyclonal antiserum recognising GATA2 was prepared by using the peptide PFSHSGHILPTPTPIH, obtained from published sequences (27), coupled to thyroglobulin for immunization. Expression plasmids were constructed in the pEF-BOS vector (28). pEF-BOS-RBTN2 contained the full-length *rbtn2* sequence cloned as the *Not* I restriction fragment from the mouse *rbtn2* cDNA clone pMR2A12N (1), pEF-BOS-TAL1 contained the full-length *tal1* sequence cloned as the *Eco*RI–*Hin*dIII restriction fragment from clone ptalM1 (25), pEF-BOS-GATA-1 contained a PCR product of full-length mouse GATA1 amplified from MEL cell cDNA and verified by sequence analysis, and pEF-BOS-GATA-2 was prepared by subcloning the *Nco* I restriction fragment from pDT3A (27) that encodes the complete GATA2 sequence but lacks the last residue.

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Abbreviations: CAT, chloramphenicol acetyltransferase; GST, glutathione *S*-transferase; MEL, murine erythroleukemia; bHLH, basic helix–loop–helix; MBP, maltose-binding protein.

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***In Vitro* Protein-Binding Assays.** Bacterial cultures were induced to produce fusion proteins by standard methods, and soluble proteins were prepared by sonication of cells followed by centrifugation at 3000 rpm. Supernatants were bound to glutathione-agarose beads or amylose-agarose beads (New England Biolabs), and the bound proteins were washed four times with lysis buffer (20 mM Hepes, pH 7.4/200 mM NaCl/50 μM ZnOAc/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride/1 mg each of pepstatin A, aprotinin, and leupeptin per ml). The equivalent of 0.6 ml of bacterial culture was used per binding reaction. *In vitro* translation was carried out with either reticulocyte or wheat germ cell-free systems (Promega). *In vitro* binding reactions were carried out as described.

The clones for *in vitro* translation were transcribed in T7βTAG (29) or pBluescript (Stratagene), and *in vitro* translation with reticulocyte or wheat germ extracts was carried out with the TNT system (Promega). T7βTAG-rbtn2 contains the complete coding region of mouse *rbtn2*, which was amplified by PCR from pMR2A12N (1), sequenced, and inserted in frame in T7βTAG. T7βTAG-GATA-1 contains a 1.24-kb fragment comprising the complete coding region of mouse GATA1, which was amplified by reverse transcription–PCR from MEL cDNA, sequenced, and inserted into T7βTAG. T7βTAG-GATA-2, containing a 740-bp fragment extending from codon 230 to the C terminus of human GATA2 [amplified by PCR from clone pDT3A (27) and sequenced], was inserted in frame in T7βTAG. pBS-TAL1 contains the *TAL1* cDNA from the clone ptalM1 (25) inserted into pBluescript. *Fos* and gelsolin clones have been described (30).

Mammalian Two-Hybrid Assays. GAL4-TAL1, VP16-TAL1, GAL4-E47, and VP16-E47 plasmids were described previously (31). To construct GAL4-RBTN2 and VP16-RBTN2, the complete coding region of RBTN2 was amplified by PCR and inserted into *Eco*RI/*Xba* I restriction sites of pM3 (32) or *Sal* I/*Xba* I sites of pNLVP16 (33), respectively. VP16-GATA-1 was constructed by PCR amplification of mouse GATA1 (by using MEL cDNA as template) and insertion in the *Xho* I/*Xba* I restriction sites of the pNLVP16 vector. The VP16-GATA-2 clone was made by inserting the *Nco* I fragment of pDT3A in the *Xho* I restriction site of the pNLVP16 vector. C3H10T½ cells were transfected with 2.5 μg of G5E1bCAT reporter, 4 μg of GAL4-hybrid plasmid (or pM3 parent plasmid), 4 μg of VP16-hybrid plasmid (or pNLVP16 parent plasmid), 4 μg of pEF-BOS-TAL1, and/or pEF-BOS-RBTN2 expression plasmids (or pEF-BOS parental plasmid) by using DEAE-dextran. Additionally, cells were cotransfected with 2 μg of pEF-BOS-βGAL plasmid to standardize transfection efficiency. The pM3, pNLVP16, or pEF-BOS parental vectors were introduced to adjust the DNA amount. Two days after transfection, cells were harvested, proteins were prepared, and chloramphenicol acetyltransferase (CAT) or β-galactosidase activity was measured.

RESULTS

***In Vivo* Interactions of RBTN2 with GATA1 or -2 and TAL1.** An association of RBTN2 and GATA1 was investigated by immunoprecipitation of labeled proteins from extracts of MEL cells. ^{35}S-labeled cells were lysed, and proteins were immunoprecipitated with anti-GATA1 serum and analyzed by SDS/PAGE. This showed labeled GATA1 protein of about 48 kDa associated with a small amount of a band corresponding to the size of RBTN2 (≈20 kDa) (Fig. 1*A*). To confirm that this was RBTN2, a two-step immunoprecipitation protocol was followed (11). Labeled proteins were first precipitated with anti-GATA1 serum, and the precipitate was suspended, denatured, reprecipitated with anti-RBTN2 serum, and analyzed by SDS/PAGE. In these experiments, a RBTN2 band could be visualized clearly after long exposures of the gel (Fig. 1*A*, starred lane 2). Therefore, some RBTN2 protein is bound to

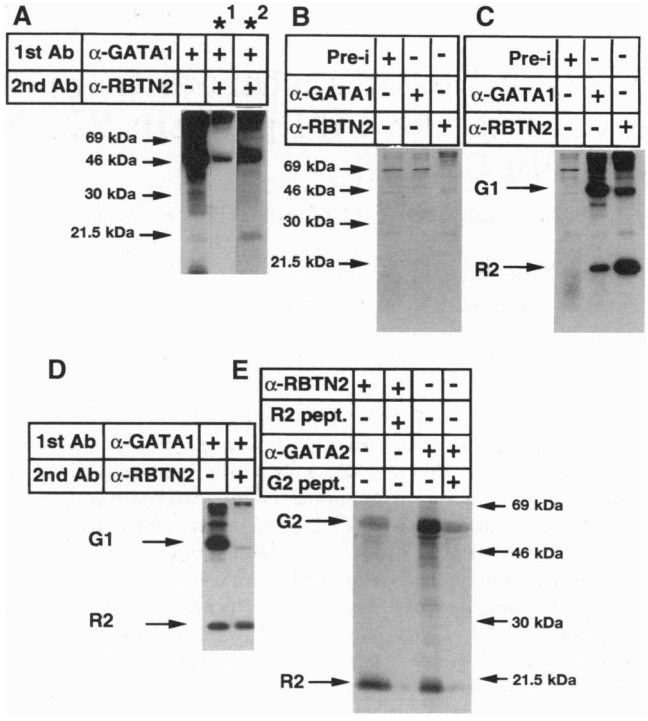

FIG. 1. RBTN2 interacts with TAL1, GATA1, and GATA2 *in vivo*. The association of RBTN2 with TAL1, GATA1, or GATA2 was studied by coimmunoprecipitation of proteins from MEL cells (*A*) or from transfected Cos cells (*B–E*). (*A*) MEL cells were labeled with [^{35}S]cysteine, and labeled proteins were immunoprecipitated as indicated. Two-step immunoprecipitations (first precipitation with anti-GATA1 serum, suspending the pellet, and reprecipitating with anti-RBTN2 serum) are shown in the starred lanes, which are the same gel track exposed for different times: lane *^{2} was exposed for 30 days compared with 30 h for lane *^{1}. (*B–E*) Cos cells were mock transfected (*B*), transiently cotransfected with GATA1 and RBTN2 expression clones (*C* and *D*), or transiently cotransfected with GATA2 and RBTN2 expression clones (*E*). Lysates from cells labeled with [^{35}S]cysteine were immunoprecipitated with preimmune sera, anti-GATA serum, or anti-RBTN2 serum. Two-step immunoprecipitations (11) are shown (*D*) in which labeled cell extracts were immunoprecipitated with only anti-GATA1 serum or first with anti-GATA1 and then the precipitated material was reprecipitated with anti-RBTN2 serum. In *E*, inhibition of precipitation was carried out by including either the RBTN2 or GATA2 peptide (pept.) used to raise the respective polyclonal antiserum in the precipitation reaction mixture. G1, GATA1; R2, RBTN2; G2, GATA2, Pre-i, preimmune serum; + represents presence of antiserum; − represents absence of antiserum; Ab, antibody.

GATA1 in MEL cells, but the amount appears low relative to the amount of free, unbound GATA1.

The complex between RBTN2 and GATA1 was investigated by using coimmunoprecipitation of labeled proteins from transfected cells. Transfection of Cos cells with *rbtn2* and *tal1* expression vectors results in a complex between the two proteins (12). The association of GATA and RBTN2 proteins *in vivo* was analyzed by cotransfection of Cos cells with expression vectors, followed by protein labeling, immunoprecipitation, and SDS/PAGE analysis. When cells were cotransfected with *GATA1* and *rbtn2* expression clones, immunoprecipitates made with both anti-RBTN2 and anti-GATA1 sera showed similar patterns and contained proteins of the sizes expected for GATA1 and RBTN2 indicating an association of these proteins *in vivo* (Fig. 1*C*). To verify that the RBTN2 and GATA1 proteins exist in a complex, the two-step immunoprecipitation procedure was used. Lysates were prepared from cells transfected with *GATA1* and *rbtn2* expression clones. First, anti-GATA1 serum was used for immunoprecipitation,

revealing GATA1 and an associated ≈20-kDa protein (Fig. 1*D*; R2). When the precipitate made with the anti-GATA1 serum was suspended, boiled, and reprecipitated with anti-RBTN2 serum, the 20-kDa band (R2) was again observed (Fig. 1*D*), demonstrating that RBTN2 is present in the anti-GATA1 immunoprecipitate. Thus RBTN2 and GATA1 are present in a complex in these transfection experiments.

The GATA2 protein is homologous to GATA1, particularly in the DNA-binding region (27, 34). The possibility that GATA2 and RBTN2 could associate was investigated. Labeled proteins from cells transfected with *GATA2* and *rbtn2* expression clones were immunoprecipitated with anti-GATA2 serum, revealing the GATA2 protein of about 55 kDa and a coprecipitated 20-kDa RBTN2 protein (Fig. 1*E*). Immunoprecipitation of both bands was inhibited by the presence of the peptide used to raise the anti-GATA2 serum, indicating association between the two precipitated proteins. Similarly, the use of anti-RBTN2 antiserum (3) coprecipitated RBTN2 (20 kDa; Fig. 1*E*) and associated an ≈55-kDa GATA2 band. Precipitation of both bands was inhibited by the peptide used to prepare anti-RBTN2 serum. Thus, RBTN2 can associate with GATA1 and with GATA2 *in vivo*.

RBTN2 Can Bind Directly to TAL1 and GATA Proteins. The nature of the interactions occurring via RBTN2 was investigated by *in vitro* binding assays and by mammalian two-hybrid assays. Glutathione *S*-transferase (GST) fusion with TAL1 (GST-TAL1) or a mutant version lacking the bHLH region (GST-δTAL1) (25) and a fusion of maltose-binding protein (MBP) with the GATA2 DNA-binding domain (MBP-GATA2) were used to test the binding of radioactively labeled *in vitro* translated proteins. RBTN2, GATA1, or GATA2 protein was produced by *in vitro* translation, and binding to either GST-TAL1 or GST-δTAL1 was examined by eluting bound material and separating it by SDS/PAGE (Fig. 2*A*). RBTN2 bound efficiently to TAL1 (and not to δTAL1, which lacks the bHLH region), consistent with previous data demonstrating that the bHLH region of TAL1 is necessary for RBTN2–TAL1 binding (12). Only a very small proportion of GATA1 or -2 protein was bound to the GST-TAL1 columns, and the significance of this is unclear because very low proportions of input label could also be visualized binding to GST resins lacking fusion protein.

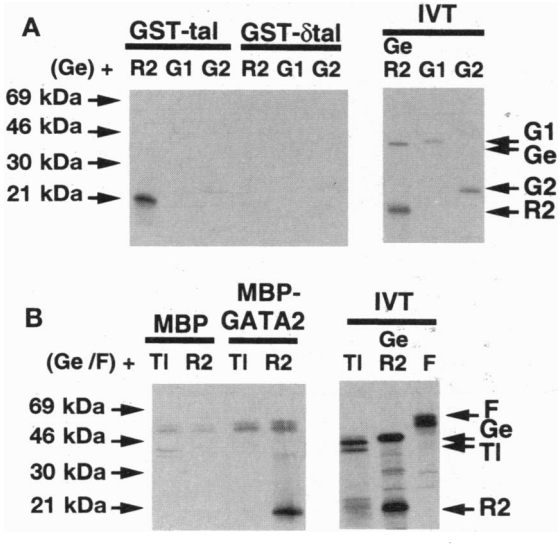

FIG. 2. *In vitro* protein-binding studies. Bacterial fusion proteins GST-TAL1 (GST-tal) or GST-δTAL1 (GST-δtal) (25), MBP, or a fusion of MBP with GATA2 was linked to affinity resins and incubated with ^{35}S-labeled proteins translated *in vitro* by using wheat germ extracts. Bound protein was eluted from the resin and fractionated by SDS/12.5% PAGE. (*A*) Indicated *in vitro* translated proteins were mixed with *in vitro* translated gelsolin as an internal control. (*B*) Indicated *in vitro* translated proteins were mixed with *in vitro* translated gelsolin and FOS as internal controls. The horizontal bars indicate those gel lanes pertaining to material bound to each fusion protein or the *in vitro* translated (IVT) material. The latter are included as markers for size only, and the positions of standard size markers are indicated for reference. R2, RBTN2; T1, TAL1; G1, GATA1; G2, GATA2; Ge, gelsolin; and F, FOS.

In similar experiments with MBP-GATA2 fusion protein, *in vitro* translated RBTN2 bound, but TAL1 did not (Fig. 2*B*). In addition, MBP alone does not bind appreciable RBTN2. Since MBP protein often resulted in nonspecific binding, experiments were conducted with internal controls [FOS (30) and gelsolin], and binding to resins containing only MBP was tested for each labeled protein analyzed. Specific binding of RBTN2 to MBP-GATA2 fusion was observed. Furthermore, the binding of RBTN2 to the GATA2 fusion protein occurs in 1 mM or 10 mM dithiothreitol (data not shown), favoring the view that the binding is due to specific protein–protein interaction and not to disulfide bridge formation.

It has previously been shown that RBTN2 can associate with TAL1 (11, 12) and that this in turn can complex with E47 (25, 31). Further, a trimer of RBTN2, TAL1, and E47 can occur, at least as judged by a two-hybrid assay (12). We have used a similar two-hybrid assay in C3H10T½ cells (32) to assess the binding of the three proteins in conjunction with GATA1 and -2. A GAL4-TAL1 fusion was able to bind to a VP16-E47 fusion and cause transcriptional activation of a CAT reporter construct (GAL4-CAT; Fig. 3). Similarly, the GAL4-TAL1 fusion binds to VP16-RBTN2, causing transactivation (Fig. 3*A*), but no marked transactivation was found when GAL4-TAL1 fusion was coexpressed with either VP16-GATA1 or VP16-GATA2 fusion protein (Fig. 3), suggesting that at least the C-terminal portion of TAL1 does not interact directly with GATA1 or -2. On the other hand, GAL4-RBTN2 activated transcription by dimerization with either VP16-GATA1 or VP16-GATA2 but not with VP16-E47, consistent with RBTN2 binding GATA1 or -2.

These data suggest that a tetramer transcription complex can form, comprising E47, TAL1, RBTN2, and GATA1 or

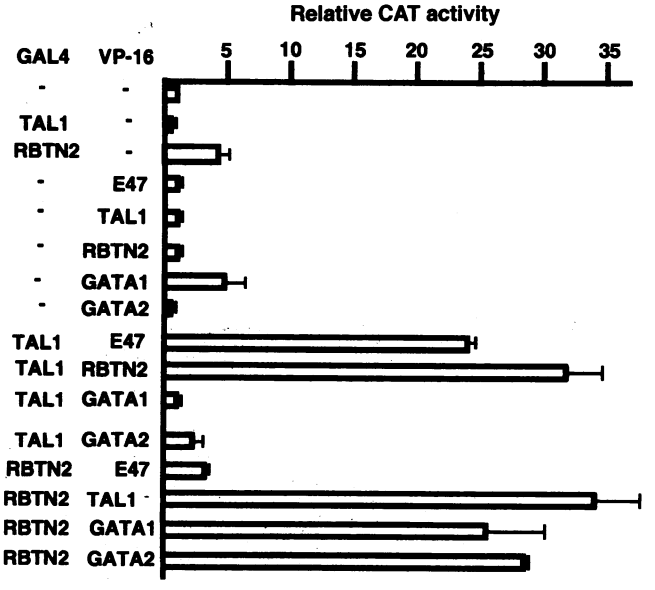

FIG. 3. Two-hybrid assays demonstrating the ability of RBTN2 and GATA to form a complex in C3H10T½ cells. C3H10T½ cells were transfected with the indicated GAL4 or VP16 fusion expression constructs together with the G5E1bCAT reporter clone (35). Relative CAT activity was measured by reference to β-galactosidase synthesized as a result of cotransfecting pEF-BOS-βGAL. GAL4-TAL1 or GAL4-RBTN2 was cotransfected with VP16 fusion of E47, GATA1, GATA2, TAL1, or RBTN2.

GATA2. Reconstruction of this complex was investigated in a modified two-hybrid system in which a GAL4-TAL1 fusion was expressed with either VP16-GATA1 or VP16-GATA2 fusion protein, together with a vector expressing normal full-length RBTN2. In the absence of either VP16-GATA1 or -2, GAL4-TAL1 with RBTN2 or GAL4-TAL1 with either VP16-GATA1 or -2 caused very little activation in this system (Fig. 4*A*). However, when the GAL4-TAL1 and either VP16-GATA1 or -2 were introduced with the *rbtn2* expression clone, strong transactivation was found (Fig. 4*A*). Thus, a trimeric complex of TAL1, RBTN2, and either GATA1 or GATA2 can form in which RBTN2 appears to form a bridge between TAL1 and the GATA proteins.

The possible involvement of E47 in a tetrameric complex with TAL1, RBTN2, and GATA was investigated in two-hybrid assays. In these tests, a GAL4 DNA-binding E47 fusion was coexpressed with VP16-GATA1 or VP16-GATA2 in the presence or absence of expression vectors encoding RBTN2 and TAL1 (Fig. 4*B*). GAL4-E47 combined with VP16-GATA1 or -2 does not result in activation, indicating no direct association between E47 and GATA1 or -2 (Fig. 4*B*). However, transcriptional transactivation does occur if the GAL4-E47 and VP16-GATA clones were coexpressed with both TAL1 and RBTN2 (Fig. 4*B*). Since E47 does not bind GATA1, -2, or RBTN2 (Fig. 3) but does bind TAL1 (25, 31) (Fig. 3), and TAL1 binds to RBTN2 (11, 12) but not to GATA1 or -2 (Figs. 2*B* and 3), the complex which causes activation in the assay appears to comprise GAL4-E47/TAL1/RBTN2/VP16-GATA, in which TAL1 and RBTN2 form bridges between the E47 and GATA components.

DISCUSSION

Complex Interactions of RBTN2, TAL1, and GATA1 Proteins. There is a crucial role for lineage-restricted transcription factors in hematopoiesis in which self-renewing stem cells produce progenitor cells which undergo a program of terminal differentiation to produce hematopoietic lineages. These factors include RBTN2, GATA1 and -2, and TAL1. The phenotype resulting from the null mutants of these genes have features in common, suggesting that the proteins may function at similar times in the red cell differentiation pathway. This seems likely to occur, at least partly, by the interaction of the RBTN2 LIM protein with the bHLH region of the TAL1 protein (11, 12). The data now presented show in addition the formation of a complex of RBTN2 and either GATA1 or -2.

The amount of RBTN2 bound to GATA1 in MEL cells appears to be less than that in the transfected Cos cells. This is presumably due to the relative concentrations of the two proteins in the different cellular contexts and to other cellular factors present in MEL cells but perhaps not in Cos cells. Thus, in erythroid cells, it is possible that complex equilibria occur which may vary with stages of erythropoiesis. Presumably there is an equilibrium among monomers of GATA1 (or -2), RBTN2, and TAL1, as well as among TAL1/E47 heterodimers, E47 homodimers, or RBTN2 homodimers (24) and

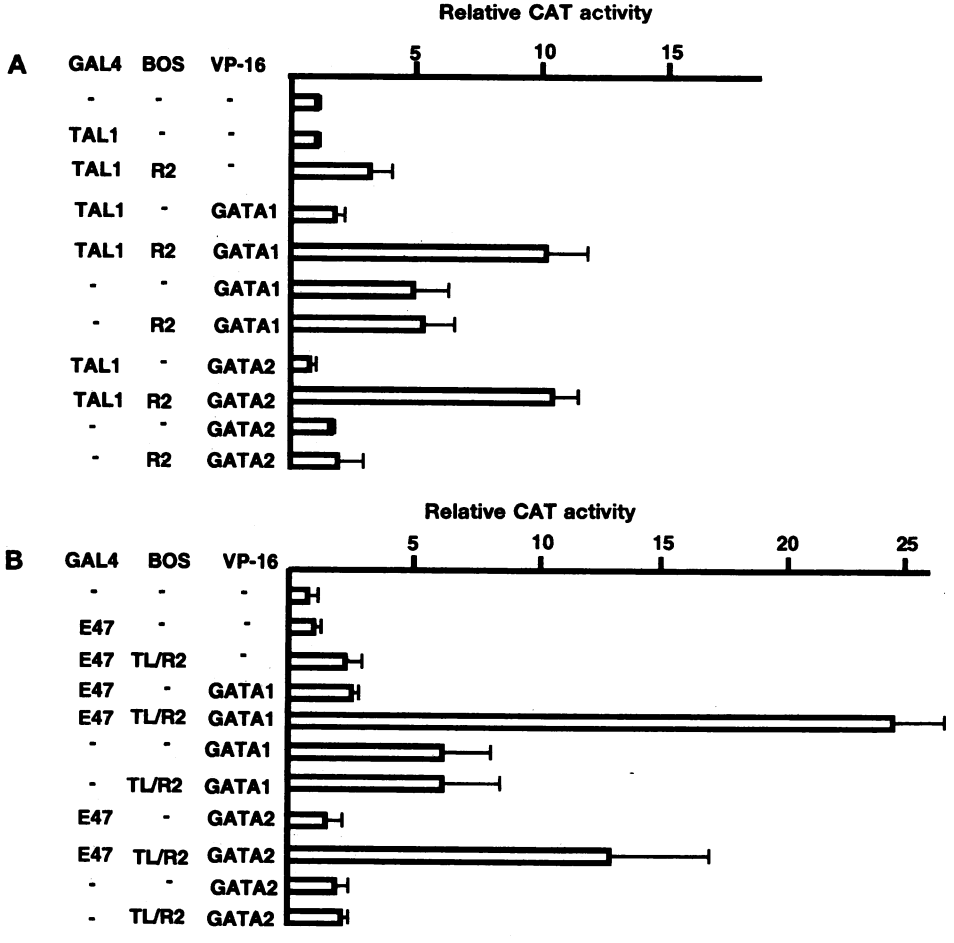

FIG. 4. Two-hybrid assays demonstrating the ability of RBTN2, E47, TAL1, and GATA to form a complex in C3H10T½ cells. C3H10T½ cells were transfected with the indicated GAL4 or VP16 fusion expression constructs together with the G5E1bCAT reporter clone (35). Relative CAT activity was measured by reference to β-galactosidase synthesized as a result of cotransfecting pEF-BOS-βGAL. (*A*) GAL4-TAL1 was transfected with VP16 fusions of GATA1 or GATA2 in the presence or absence of an RBTN2 clone in the pEF-BOS expression vector. (*B*) GAL4-E47 was transfected with VP16 fusions with GATA1 or GATA2 together with combinations of pEF-BOS expression clones producing TAL1 and RBTN2.

a putative tetramer of RBTN2/GATA1/TAL1/E47. The amounts of each complex will depend on relative concentrations of the proteins and relative affinities for each. It is therefore possible that the structures of the various complexes will vary according to the status of a particular erythroid cell.

Separately, TAL1 and E47 can bind the E-box motif as a heterodimer (25) and GATA proteins bind to GATA sites (6). No direct evidence of RBTN2 binding to DNA has been obtained, as yet (20). Therefore, a complex involving GATA1 (or -2) and/or TAL1/E47 heterodimer might occur with the RBTN2 protein bridging the two by contacting both TAL1 and GATA1 proteins. Our data do not rule out a direct interaction between TAL1 and GATA1 proteins. Further, weak interactions would not necessarily be clearly evident in the *in vitro* binding assay. Thus, a complex network of interactions involving RBTN2, TAL1, and GATA is possible. The role of the RBTN2 molecule(s) in the complex may be DNA binding or it may be to interact with other proteins creating a more powerful transcriptional transactivation machinery since a weak transcriptional transactivation domain occurs in RBTN2 (24). This might enhance transactivation over the similarly weak activator in TAL1 (36, 37). Nonetheless, the association of GATA1 and RBTN2 in MEL cells (Fig. 2) suggests that a natural complex comprising RBTN2, TAL1, and GATA1 can occur.

The formation of the complex among RBTN2, GATA1, and TAL1 could effectively alter the binding specificity of GATA and TAL1 proteins, and this may affect the expression of downstream target genes. An intriguing problem is the consequence of the translocation t(11;14)(p13;q11) in T-cell tumors by which *RBTN2* is activated and the translocation t(1;14)(p32;q11) by which *TAL1* is activated. It seems likely that some of the target genes activated (or repressed) by RBTN2 or TAL1 in T-cell leukemias are also normally activated in erythropoiesis. However, T cells do not express GATA1 or -2, but they do express GATA3 and perhaps other members of the GATA family, and sometimes abnormalities of both *RBTN2* and *TAL1* genes occur in a single T-cell tumor (38). It will be of interest to see if these various proteins are in a complex with each other and/or with different proteins in T-cell tumor cells with the respective chromosomal translocations and also to assess the spectrum of genes whose transcription is affected (positively or negatively) by these complexes.

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1. Boehm, T., Foroni, L., Kaneko, Y., Perutz, M. P. & Rabbitts, T. H. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4367–4371.
2. Royer-Pokora, B., Loos, U. & Ludwig, W.-D. (1991) *Oncogene* **6,** 1887–1893.
3. Warren, A. J., Colledge, W. H., Carlton, M. B. L., Evans, M. J., Smith, A. J. H. & Rabbitts, T. H. (1994) *Cell* **78,** 45–58.
4. Pevny, L., Simon, M. C., Robertson, E., Klein, W. H., Tsai, S.-F., D'Agati, V., Orkin, S. H. & Costantini, F. (1991) *Nature (London)* **349,** 257–260.
5. Simon, M. C., Pevny, L., Wiles, M. V., Keller, G., Costantini, F. & Orkin, S. H. (1992) *Nat. Genet.* **1,** 92–98.
6. Orkin, S. H. (1992) *Blood* **80,** 575–581.
7. Weiss, M. J., Keller, G. & Orkin, S. H. (1994) *Genes Dev.* **8,** 1184–1197.
8. Tsai, F.-Y., Keller, G., Kuo, F. C., Weiss, M., Chen, J., Rosenblatt, M., Alt, F. W. & Orkin, S. H. (1994) *Nature (London)* **371,** 221–226.
9. Shivdasani, R. A., Mayer, E. & Orkin, S. H. (1995) *Nature (London)* **373,** 432–434.
10. Rabbitts, T. H. (1994) *Nature (London)* **372,** 143–149.
11. Valge-Archer, V. E., Osada, H., Warren, A. J., Forster, A., Li, J., Baer, R. & Rabbitts, T. H. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8617–8621.
12. Wadman, I., Li, J., Bash, R. O., Forster, A., Osada, H., Rabbitts, T. H. & Baer, R. (1994) *EMBO J.* **13,** 4831–4839.
13. Boehm, T., Foroni, L., Kennedy, M. & Rabbitts, T. H. (1990) *Oncogene* **5,** 1103–1105.
14. Foroni, L., Boehm, T., White, L., Forster, A., Sherrington, P., Liao, X. B., Brannan, C. I., Jenkins, N. A., Copeland, N. G. & Rabbitts, T. H. (1992) *J. Mol. Biol.* **226,** 747–761.
15. Crawford, A. W. & Beckerle, M. C. (1991) *J. Biol. Chem.* **266,** 5847–5853.
16. Hempe, J. M. & Cousins, R. J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9671–9674.
17. Michelsen, J. W., Scheichel, K. L., Beckerle, M. C. & Winge, D. R. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 4404–4408.
18. Perez-Alvarado, G. C., Miles, C., Michelsen, J. W., Louis, H. A., Winge, D. R., Beckerle, M. C. & Summers, M. F. (1994) *Nat. Struct. Biol.* **1,** 388–398.
19. Archer, V., Breton, J., Sanchez-Garcia, I., Osada, H., Forster, A., Thomson, A. J. & Rabbitts, T. H. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 316–320.
20. Sanchez-Garcia, I. & Rabbitts, T. H. (1994) *Trends Genet.* **10,** 315–320.
21. Rabbitts, T. H. & Boehm, T. (1990) *Nature (London)* **346,** 418.
22. Schmeichel, K. L. & Beckerle, M. C. (1994) *Cell* **79,** 211–219.
23. Wu, R.-Y. & Gill, G. N. (1994) *J. Biol. Chem.* **269,** 25085–25090.
24. Sanchez-Garcia, I., Axelson, H. & Rabbitts, T. H. (1995) *Oncogene* **10,** 1301–1306.
25. Hsu, H.-L., Cheng, J.-T., Chen, Q. & Baer, R. (1991) *Mol. Cell. Biol.* **11,** 3037–3042.
26. Zon, L. I., Tsai, S.-F., Burgess, S., Matsudaira, P., Bruns, G. A. P. & Orkin, S. (1991) *Proc. Natl. Acad. Sci. USA* **87,** 668–672.
27. Lee, M.-E., Temizer, D. H., Clifford, J. A. & Quertermous, T. (1991) *J. Biol. Chem.* **266,** 16188–16192.
28. Mizushima, S. & Nagata, S. (1990) *Nucleic Acids Res.* **18,** 5322.
29. Pollock, R. & Treisman, R. (1990) *Nucleic Acids Res.* **18,** 6197–6204.
30. Hagemeier, C., Bannister, A. J., Cook, A. & Kouzarides, T. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1580–1584.
31. Hsu, H.-L., Wadman, I. & Baer, R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3181–3185.
32. Sadowski, I., Bell, B., Broad, P. & Hollis, M. (1992) *Gene* **118,** 137–141.
33. Dang, C. V., Barrett, J., Villa-Garcia, M., Resar, L. M. S., Kato, G. J. & Fearon, E. R. (1991) *Mol. Cell. Biol.* **11,** 954–962.
34. Dorfman, D. M., Wilson, D. B., Bruns, G. A. P. & Orkin, S. H. (1992) *J. Biol. Chem.* **267,** 1279–1285.
35. Lillie, J. W. & Green, M. R. (1989) *Nature (London)* **338,** 39–44.
36. Sanchez-Garcia, I. & Rabbitts, T. H. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 7869–7873.
37. Wadman, I. A., Hsu, H.-L., Cobb, M. H. & Baer, R. (1994) *Oncogene* **9,** 3713–3716.
38. Baer, R. (1993) *Semin. Cancer Biol.* **4,** 341–347.