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Transcriptional activation by TAL1 and FUS–CHOP proteins expressed in acute malignancies as a result of chromosomal abnormalities

(T-cell acute lymphoblastic leukemia/fusion proteins/DNA binding/solid tumors/activation domain)

I. SÁNCHEZ-GARCÍA AND T. H. RABBITTS

Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, United Kingdom

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ABSTRACT **Proteins that appear to participate in transcriptional control of gene expression are increasingly implicated in leukemias and malignant solid tumors. We report here that the N-terminal domains of the proteins TAL1 (ectopically activated in T-cell acute leukemias after chromosomal abnormalities caused by V–D–J recombinase error) (V, variable; D, diversity; J, joining) and FUS–CHOP (a liposarcoma tumor-specific fusion protein that is produced as a result of a chromosomal translocation) can function as transcription activators of specific responsive reporter genes. The result with TAL1 provides evidence that transcriptional activation can be mediated by a gene activated by translocation in T-cell acute leukemias. In the case of the liposarcoma, transactivation by the FUS–CHOP protein occurs because the FUS transcriptional activation domain is added to the DNA-binding CHOP protein normally lacking such activity. Therefore, the association of transcriptional activation and DNA-binding elements is a common consequence in proteins activated or newly created as fusion proteins after chromosomal translocations in acute leukemias and in malignant solid tumors.**

Chromosome abnormalities are a frequent and consistent feature of hematopoietic malignancies, and increasingly large numbers of solid tumors are also being found to carry such abnormalities (1). Molecular cloning of the breakpoints of chromosomal translocations in acute leukemias and solid tumors has led to the identification of a number of different classes of putative oncogenes thought to contribute to tumor phenotype (2).

The majority of these activated genes seem to be transcription factors because of structural features they share with known transcriptional factors (reviewed in ref. 2). Thus, frequently transcription factors are converted to oncogenic proteins after chromosomal translocations in certain cancers including leukemias and malignant solid tumors (3, 4). These include basic helix–loop–helix (bHLH) proteins like CMYC activated in Burkitt lymphoma (5); homeodomain proteins such as HOX11 in T-cell acute leukemia (6–9) or PBX1, which can be fused to E2A in pre-B-cell leukemias (10, 11); and LIM proteins like RBTN2/Ttg-2 in T-cell leukemia (12, 13). The HOX11 protein has been shown to be a nuclear protein capable of specific DNA binding and of supporting transcriptional transactivation (14). This protein, activated by the T-cell acute leukemia translocation t(10;14)(q24;q11), typifies a master gene (4) that encodes a specific transcription factor activated by chromosomal translocation, which causes target responder genes to be aberrantly activated in the tumor cells.

In this paper, the association of transcriptional transactivation domains with DNA-binding elements has been investigated by using, respectively, TAL1 and FUS–CHOP protein fragments as examples from a leukemia and a sarcoma. TAL1 (SCL/TCL5) represents an example of a protein product that is ectopically activated in T-cell acute leukemias as a result of chromosomal abnormalities apparently occurring by V–D–J recombinase error (V, variable; D, diversity; J, joining) (15). Disruption of the *TAL1* gene is the most common genetic alteration associated with T-cell acute lymphoblastic leukemia (T-ALL) (16). The main site of *TAL1* expression is in erythrocyte precursors and not T cells, but after chromosomal translocation it is inappropriately expressed in T cells as a consequence of chromosomal deletion or translocation. TAL1 is a member of the bHLH family of transcription factors, in which the N-terminal region of the protein contains a proline-rich region similar to that found in transcriptional activation domains (15). DNA binding via the basic region of TAL1 occurs when the protein forms a heterodimer with either of the *E2A* gene products through the HLH dimerization region (17). Transcriptional transactivation by TAL1, however, has not been demonstrated.

FUS–CHOP is, in contrast, a fusion protein resulting from the chromosomal translocation t(12;16)(q13;p11) (18, 19), which is characteristic of the human myxoid liposarcomas (20). The molecular characterization of this chromosomal abnormality revealed a fusion between the *CHOP* gene and a gene called *FUS* (18, 19), in which the putative RNA-binding domain of FUS is replaced by the basic leucine zipper domain of CHOP, which confers the ability to form protein dimers (18, 21) and presumably can specifically bind DNA through the basic region. However, CHOP has not been shown to have a transcriptional activation domain and has no sequence characteristic of such regions, while the portion of FUS that is present in the FUS–CHOP fusion protein contains a Gln-Tyr-Ser-rich region reminiscent of some transcriptional activation domains.

We report here that the N-terminal domains of both TAL1 and FUS–CHOP protein products confer *in vivo* transcriptional transactivation capacity when linked to specific DNA-binding domains in reporter transfection assays, thus demonstrating that these proteins can function as positive regulators of transcription. These data suggest that the activation of TAL1 most likely results in activation of downstream target genes and, in the FUS–CHOP fusion, transcriptional activation is specifically conferred on the chimeric protein after the translocation event.

EXPERIMENTAL PROCEDURES

Plasmid Constructions. DNA fragments to be inserted into the different plasmids were generated by reverse transcriptase PCR with primers that included initiation and termina-

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Abbreviations: bHLH, basic helix–loop–helix; CAT, chloramphenicol acetyltransferase.

tion codons and restriction sites for cloning. Constructs were made after the cloned PCR product was sequenced to confirm the product. The reporter plasmid pHAT-CAT (22) has six Isl-1 homeodomain-binding sites upstream of the metallothionein minimal promoter linked to the chloramphenicol acetyltransferase (CAT) gene. pHA-Isl-1 contains the homeodomain and activation domain of Isl-1 (22) inserted in the pESP-SVTEXP expression vector (23). Plasmid pG5EC has five consensus 17-mer GAL4-binding sites upstream from the minimal promoter of the adenovirus E1b TATA box (24). pM1VP16 vector encodes an in-frame fusion between the DNA-binding domain of GAL4 (aa 1–147) and the activation domain of herpes simplex virus VP16 (24).

TAL-1 full-length cDNA (aa 1–331) and mutant TAL1 cDNAs were fused to the DNA-binding domain of GAL4 using the pM1 expression vector (24). pTALFL contains residues 1–331, p4TAL contains residues 25–331, p5TAL contains residues 159–331, and p6TAL contains residues 175–331. pTALGAL1 contains residues 1–175, pTALGAL2 contains residues 1–159, and pTALGAL3 contains residues 1–117 from the TAL-1 mRNA.

FUS cDNA (aa 1–252) was placed into the *Hin*dIII/*Xho* I sites of the mammalian expression vector pESP-SVTEXP (pFUS). pΔIsl-1 expression vector encodes a truncated form of HA-Isl-1 that does not contain activation domain. Plasmid pHD-FUS is an expression vector in which the activation domain of Isl-1 (aa 244–350) has been replaced by the FUS cDNA (aa 1–252).

Transfection and CAT Expression. Cells were transiently cotransfected with 10 μg of reporter plasmid (HAT-CAT) together with 5 μg of expression vector RSVL (25) containing the Rous sarcoma virus long terminal repeat linked to luciferase for standardization as described (22). Sonicated denatured salmon sperm DNA was used to adjust the total transfected DNA to 20 μg. C3H 10T½ and 3T3.L1 cells were transfected by the calcium phosphate precipitation method and Ba/F3 and Molt-4 cells were transfected by electroporation. All CAT assays were done as duplicate independent transfections. Relative CAT activities were determined by comparing the ratios of acetylated/unacetylated [^{14}C]chloramphenicol present in spots cut from the thin-layer chromatographs. Equivalent amounts of protein (15–25 μg as determined with the Bio-Rad protein kit and bovine serum albumin as a standard) were used in each case. A reaction time of 1 hr was used in all CAT assays, which kept all values within the linear range of the assay.

RESULTS

The N-Terminal Domain of TAL1 Protein Can Act as a Transcriptional Transactivator *in Vivo*. The *TAL1* gene is involved in chromosomal abnormalities in around a quarter of all childhood T-cell acute leukemias (15, 16), resulting in the ectopic expression of two phosphoproteins, pp42 and pp22 (26). Since TAL1 belongs to the bHLH family of proteins (15, 16) and can bind DNA (17), it implies that the protein can participate in specific gene expression by transcriptional activation of target genes. The ability of a part(s) of the TAL1 protein to recruit factors necessary for the transcription of target genes was investigated by using reporter transcription assays, which exploit the DNA-binding property of GAL4 (27). Residues 1–147 of GAL4 bind to a specific 17-bp nucleotide sequence but cannot activate transcription unless provided with a transcriptional activating function (27).

Various expression vectors were constructed that encode segments of the *TAL1* gene fused to the GAL4 DNA-binding domain, using the vector pM1, which allows fusion of exogenous segments to the C terminus of GAL4 (aa 1–147) (24). Vectors expressing GAL4–TAL1 fusions were transfected into the human T-cell line Molt-4 or the mouse interleukin 3-dependent pre-B-cell line Ba/F3 along with G5EC, a GAL4-responsive CAT reporter plasmid. Transfection of the reporter alone failed to induce significant CAT activity in either transfected cell line (Fig. 1), nor did transfection of G5EC plus pM1 (the GAL4 cloning vector) (data not shown), while GAL4 linked to the potent transcription activation domain VP16 (24) caused a strong stimulation of CAT activity (Fig. 1). Transfection of GAL4–TAL1 fusion constructs gave similar results in both cell lines. pTALFL (whole TAL1), p4TAL (TAL1 aa 25–331), and p5TAL (aa 159–331) expression vectors generated a significant increase in CAT activity (15- to 20-fold above the reporter clone alone) while p6TAL (aa 175–331) failed to induce any CAT activity (Fig. 1). Therefore, the N terminus of the TAL1 protein, when fused to the GAL4 DNA-binding domain, is able to activate transcription. In addition, this function is carried out in both a pre-B-cell line and in human T-cell leukemia line Molt-4. Since TAL1-associated abnor-

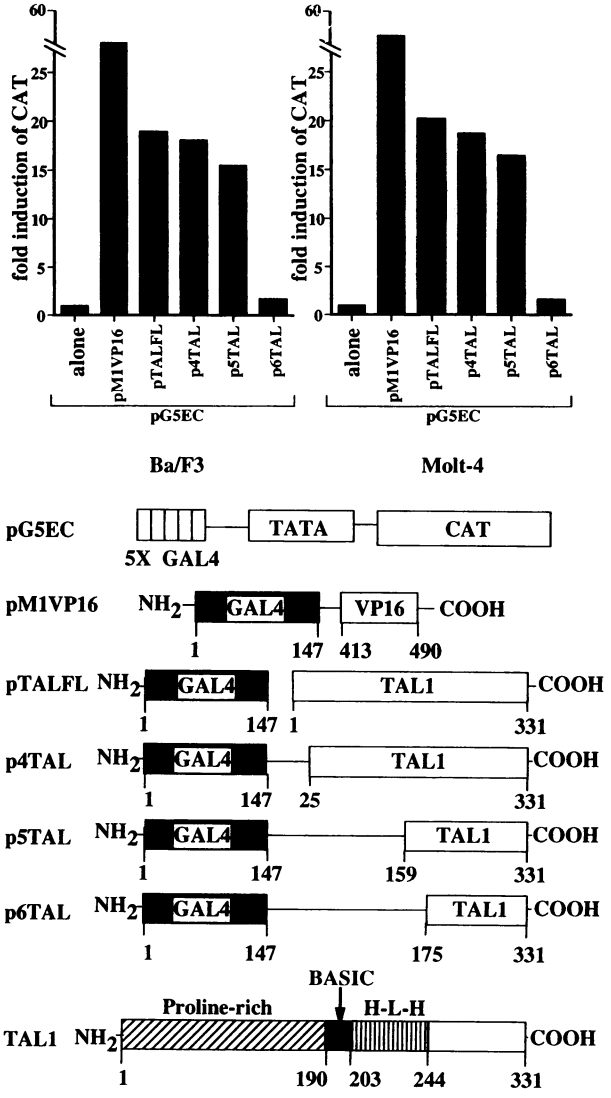

FIG. 1. Transcriptional transactivation reporter assays of TAL1 fragments linked to a heterologous DNA-binding domain. Ba/F3 and Molt-4 cells were transfected with the CAT reporter clone pG5EC, which contains tandem GAL4 DNA-binding sites (24), and various expression constructs, which consist of the GAL4 DNA-binding domain linked to parts of the TAL1 protein (as indicated). After 36 hr of culture, CAT activity was measured as percentage conversion of substrate. The fold activation relative to the basal promoter level is given. Values are means of at least two experiments. (*Upper*) Histogram of relative CAT activities for the indicated combinations of plasmid. (*Lower*) Diagrammatic representation of the different constructs and of the TAL1 protein.

malities are T-cell specific in pediatric leukemias, these observations indicate that the TAL1 protein can act as a positive regulator of transcription in leukemic T cells.

The data in Fig. 1 show that the transcriptional function of the GAL4–TAL1 chimeric proteins is conferred in p5TAL but not p6TAL, delineating residues 159–175 of TAL1 as having the capability of transcriptional transactivation. To establish whether this is the sole region of TAL1 with this ability, further constructs were made with truncations of TAL1 fused to GAL4 DNA-binding domain and transfected into the two lymphoid cell lines. Stimulation of CAT activity was observed with the clone pTALGAL1, which has aa 1–175 of TAL1 (again stimulation was lower than the control GAL4-VP16 plasmid) (Fig. 2). A truncated GAL4 fusion clone encoding only TAL1 aa 1–159 displayed stimulation of CAT levels but about half the stimulation of pTALGAL1, while a clone with TAL1 aa 1–117 had detectable but markedly reduced CAT stimulation (Fig. 2). Therefore, the main part of TAL1 that can function in this assay for transactivation of transcription is between aa 117 and 175.

Typically, transcriptional activation domains, even those derived from cell-specific transcription factors, are not themselves cell specific (28). The nonselectivity of the TAL1 transactivation domain was shown by its function in nonlymphoid cells. C3H 10T½ fibroblasts were cotransfected with the G5EC reporter construct plus the various expression plasmids used with Ba/F3 and Molt-4 (Fig. 2). Qualitatively CAT stimulation in the fibroblasts was similar to that in the lymphoid cells and the region of TAL1 defined as a functional transactivation domain was also similar. Therefore, TAL1 has a non-cell-specific transcriptional transactivation domain. However, the quantitative results are different since the levels of CAT stimulation in the C3H 10T½ was reproducibly ≈50% of that in the lymphoid cells tested, indicating that part of the activity may require sequestration of lymphoid-specific factors.

The FUS Segment in the FUS–CHOP Fusion Protein Can Activate Transcription. The liposarcoma solid tumor has a consistent t(12;16) associated with the generation of a fusion protein (Fig. 3 *Lower*) made from part of the FUS protein and all of the CHOP protein (18, 19), the latter possessing a potential DNA-binding domain (21). The FUS segment in the fusion protein is Gln-Tyr-Ser-rich (18, 19) and, therefore, might have the ability to function in transcriptional transactivation. This possibility was examined by transcription of reporter genes. In these assays, a CAT reporter (designated pHAT-CAT) was used that contains tandem copies of a sequence previously shown to be bound by the homeodomain of the insulin enhancer binding protein (Isl-1) and to facilitate transactivation by the Isl-1 activation domain in similar assays (22).

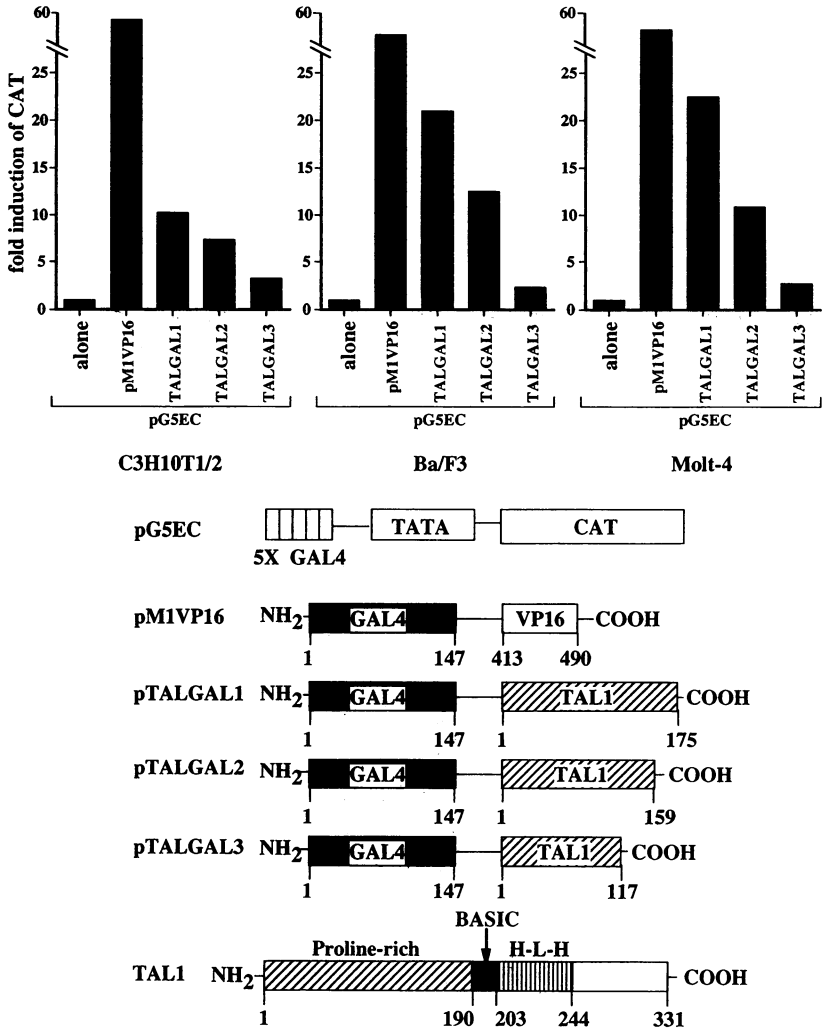

FIG. 2. Transcription reporter assays using GAL4–TAL1 N-terminal domain fusion proteins. Ba/F3, Molt-4, and C3H 10T½ cells were transfected with pG5EC reporter and each expression GAL4 DNA-binding domain TAL1 fusion construct (as indicated). CAT activity was measured as described. The fold stimulation relative to the control is given in the form of a histogram (*Upper*) and values are means of at least three transfections. Structures of the GAL4–TAL1 fusion proteins and of the complete TAL1 protein are shown (*Lower*).

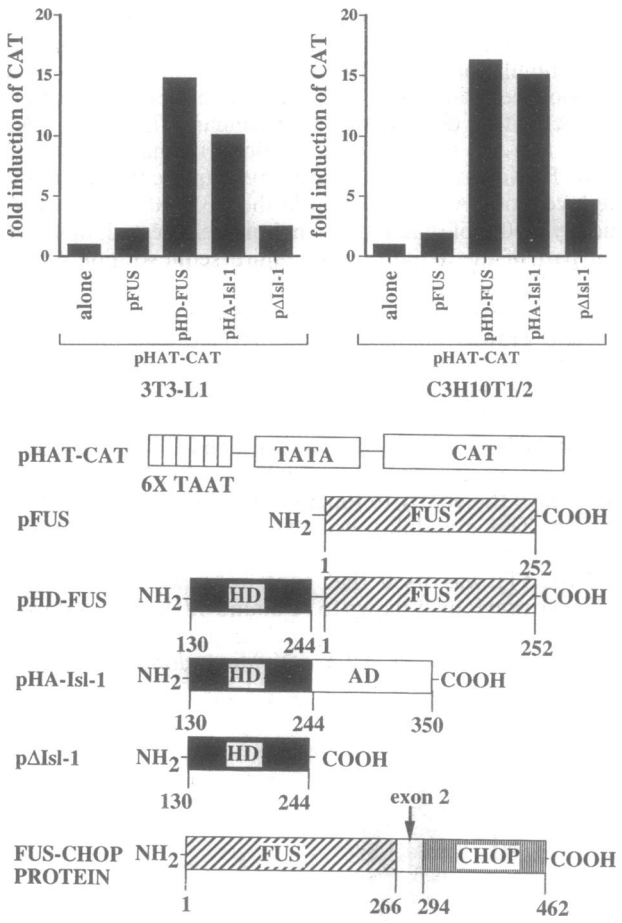

FIG. 3. Transactivation reporter assays using FUS–homeodomain chimeras. C3H 10T½ and 3T3.L1 cells were transiently cotransfected with a CAT reporter plasmid containing six copies of the TAATGG motif [recognized by the Isl-1 homeodomain (22)] immediately upstream of a metallothionein promoter–CAT reporter gene (HAT-CAT) together with expression vectors coding for homeodomain of Isl-1 alone (pΔIsl-1) or fused to the activation domain of Isl-1 (pHA-Isl-1, REF) or to the part of FUS involved in the FUS–CHOP liposarcoma-specific fusion protein (the particular constructs used in each transfection are indicated below the histogram). Data represent the fold activation with respect to a sample in which reporter alone was transfected and represent an average obtained from four separate experiments.

The pHAT-CAT reporter has minimal transcriptional activity when transfected alone into either C3H 10T½ fibroblasts or 3T3.L1 preadipocytes (Fig. 3), whereas cotransfection of the reporter with an expression construct encoding the homeodomain and transcriptional transactivation domain of Isl-1 [designated HA-Isl-1 (22)] results in ≈15-fold stimulation of CAT activity (Fig. 3). Conversely, the pΔIsl-1 expression construct (which encodes the Isl-1 DNA-binding domain without the activation domain) poorly activates transcription with this reporter (≈5-fold less than the pHA-Isl-1 expression vector). The ability of a vector encoding the Isl-1 DNA-binding homeodomain fused to the FUS coding sequence (pHD-FUS) to mediate transactivation was assessed in the two fibroblast cell lines. This clone stimulated CAT synthesis with this reporter gene (Fig. 3), yielding an activity ≈15-fold above basal activity and just greater than the transactivation effect by the control pHA-Isl-1 plasmid. No significant difference can be seen between the two cell lines used in these assays. Very low levels of CAT induction resulted from cotransfection of the reporter with a construct that encodes the FUS portion but lacking the DNA-binding domain of Isl-1 (Fig. 3). No transactivation was observed when a control expression plasmid (pESP-SVTEXP without cDNA) was cotransfected (data not shown). These studies show that the N-terminal domain of FUS promotes transcriptional activity of a specific reporter gene by transactivation.

DISCUSSION

N-Terminal Domains of FUS–CHOP and TAL1 Are Transcriptional Activators. In general, genes activated by chromosomal translocations in acute leukemias are transcription regulators, which have been for convenience termed master genes (4) to explain their probable role in the activation of downstream responsive target genes (3, 4). The likelihood that the majority of translocations in malignant solid tumors have parallel consequences has recently been highlighted (2, 29). The direct demonstration that the proteins activated or fused by translocations actually fulfill the description of DNA-binding transcriptional transactivators has been restricted to a few representative examples such as the T-cell oncogene HOX11 (4). The current data define a transcriptional activation domain in the N terminus of two diverse proteins expressed after chromosomal abnormalities in distinct tumor types—namely, TAL1 activated in T-cell acute leukemias and FUS, which is fused to the CHOP protein after the t(12;16) in liposarcoma solid tumors.

Although superficially a difference in mode of action is operative in the two instances, the basic features of both are transcriptional activation and specific DNA binding. The CHOP protein has a potential DNA-binding region in the form of a basic ZIP motif but may not be able to activate transcription; therefore the translocation t(12;16) seems to convert the DNA-binding protein (CHOP) into a transcriptional activator upon acquiring the N-terminal region of FUS by gene fusion. Thus, CHOP target responder genes could be positively activated in cells with the FUS–CHOP fusion. Conversely, while the *TAL1* coding region is unaltered by the chromosomal translocation t(1;14), a key feature is that the protein is not normally expressed in mature T cells. Thus, transcriptional activation by TAL1 can occur only in mature T cells with the chromosomal translocation (29). Therefore, by activating the *TAL1* gene, the chromosomal translocation might alter the special differentiation control mechanism by altering the pattern of gene expression, thereby promoting T-cell leukemogenesis.

A Recurring Theme of Transcriptional Activation and Specific DNA Binding in Translocation-Activated Oncogene Proteins. The results presented in this communication are a functional demonstration that TAL1 and FUS–CHOP proteins, which are critical to the development of distinct tumor types, share the common structural composition of transactivation domains fused to DNA-binding elements, which points to an important mechanism of oncogenic activation by chromosomal translocations in acute malignancies. This observation concurs with data on other proteins, such as HOX11, MYC, E2A-PBX/HLF, EWS-ATF1/FLI-1, and PML-RARα proteins (14, 30–33). In addition, many other proteins activated by chromosomal abnormalities in both acute leukemias and malignant solid tumors also contain potential transcriptional activation domains linked to potential DNA-binding domains (reviewed in refs. 2 and 29).

It is clear that a major outcome of chromosomal translocation/inversion in tumors is direct target gene recognition (by specific DNA-binding domains) and activation of these target genes (by nonspecific transcriptional activation domains). Thus, gene activation by association with immunoglobulin or T-cell receptor genes (generically grouped as "recombinase error"; Fig. 4) or gene fusion (both acute leukemias and malignant solid tumors) can have the same outcome (Fig. 4). Variations on this theme might be activation by translocation of proteins with only DNA-binding or

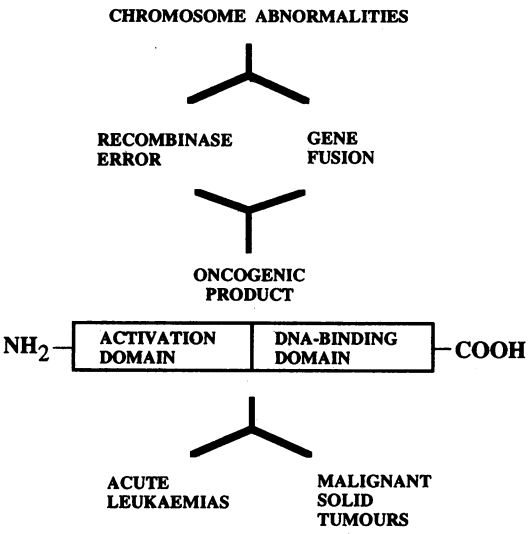

FIG. 4. Schematic diagram of a recurrent molecular consequence of chromosomal translocations in acute leukemia and malignant solid tumors. The hallmark of tumors in general and leukemia in particular is the presence of specific chromosomal abnormalities. The creation of a chromosomal abnormality by either recombinase error (as often occurs in acute leukemias) or gene fusion (acute leukemias and malignant solid tumors) results in an oncogenic protein in the mutant cell. A common structural composition of such proteins is transcription activation capacity (frequently N terminal) associated with a DNA-binding element. The oncogenic potential of these proteins often seems dependent on both characteristics in acute malignancies.

transactivation domains, with the second domain being supplied by a heterodimerization partner.

A major problem in tumorigenesis is whether the translocation-activated proteins carry out the same function as in the normal cell. The finding that proteins with different structural motifs can generate the same type of leukemia, when misdirected via chromosome translocation (e.g., in T-ALL LIM, bHLH and homeodomains are involved), opens the possibility that proteins other than those identified via chromosomal abnormalities could carry out a similar function in tumorigenesis if the protein has the same structural composition as that illustrated in Fig. 4. The recent demonstration that PAX3 can cause tumors in an NIH 3T3 cell assay supports this general proposal (34).

The fact that we can draw many analogies between the proteins identified via different chromosomal translocations, involved in acute leukemias and malignant solid tumors, illustrates that we are beginning to understand the principles that underlie the molecular basis of cancer via chromosomal translocations, which may ultimately allow specific therapies to be devised.

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