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LIM Domain-Containing Protein Trip6 Can Act as a Coactivator for the v-Rel Transcription Factor

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The retroviral oncoprotein v-Rel is a transcriptional activator in the Rel/NF-κB family of eukaryotic transcription factors. v-Rel malignantly transforms a variety of cell types in vitro and in vivo, and its transforming activity is dependent on the ability of v-Rel to bind to DNA and activate transcription. In this report, we used the yeast two-hybrid assay to identify proteins that interact with C-terminal sequences of v-Rel that are needed for transcriptional activation and transformation. One protein, Trip6, that we identified in this screen was previously identified as a thyroid hormone receptor-interacting protein. Trip6 is a member of a subfamily of LIM domain-containing proteins that are thought to transport intracellular signals from the cell surface to the nucleus. By several criteria, we show that sequences from Trip6, which include the LIM domains, behave as a coactivator for transcriptional activation by v-Rel. That is, a GAL4-Trip6 fusion protein can activate transcription in yeast and chicken cells, Trip6 can enable C-terminal sequences of v-Rel to activate transcription in yeast, and Trip6 can enhance activation by v-Rel from a κB site reporter plasmid in yeast. Although full-length Trip6 localizes to adhesion plaques, deletion of N-terminal sequences allows human Trip6 to enter the nucleus of chicken cells. Lastly, Northern blotting shows that Trip6 mRNA is expressed in many human tissues. Coexpression of Trip6 does not affect the transforming activity of v-Rel. Taken together, our results indicate that Trip6 may be a protein that is important for the ability of v-Rel to activate transcription and transform cells, and may represent a potential target for blocking Rel-mediated oncogenesis and transcriptional activation.

v-Rel Retroviral oncogene Trip6 Coactivator LIM domain Transcription factor
Malignant transformation NF-κB Two-hybrid assay

THE v-Rel oncoprotein, encoded by the highly oncogenic avian Rev-T retrovirus, can transform a variety of hematopoietic cells in vitro and in vivo [reviewed in (14)]. v-Rel is a member of the Rel/NF-κB family of eukaryotic transcription factors. Like other members of this family, v-Rel has an N-terminal domain of approximately 300 amino acids, the Rel Homology (RH) domain, that contains sequences important for DNA binding, the formation of homo- and heterodimers, and nuclear localization. Residues located C-terminal to the RH domain in v-Rel are important for transcriptional activation in vertebrate cells (28,29, 34).

Several lines of evidence suggest that v-Rel must form homodimers, bind to DNA, and activate transcription to effect malignant transformation of chicken spleen cells. First, a v-Rel mutant that cannot form homodimers, but can still form heterodimers with cellular Rel family proteins, cannot transform cells (32). Second, v-Rel mutants that are temperature

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sensitive for transformation are also temperature sensitive for DNA binding (31). Third, deletions that remove the C-terminal sequences involved in transcriptional activation by v-Rel also render it transformation defective (28,29). However, the C-terminal sequences of v-Rel cannot be replaced by a heterologous transcriptional activation domain (16) and not all v-Rel mutants that can activate transcription can likewise transform cells (5). Thus, the C-terminal sequences of v-Rel represent a transformation-specific domain that is likely to participate in transformation by activating transcription of specific genes, and the ability of v-Rel to activate transformation-specific transcription may require a distinct molecular pathway.

The C-terminal sequences of v-Rel contain multiple transcriptional activation domains that can act in a cell type-specific manner (13,19,21,28,34). For example, although GAL4 or LexA fusion proteins containing C-terminal sequences of v-Rel can activate transcription in chicken cells (26,28,29), such proteins cannot activate transcription in yeast (21). Therefore, C-terminal sequences of v-Rel may require cell type-specific coactivators to activate transcription. Moreover, the ability of sequences in v-Rel to interact directly with general transcription factors, such as TFIID and TFIIB, is controversial (22,34).

In this report, we demonstrate that Trip6, a LIM domain-containing protein originally identified as an interacting partner with the thyroid hormone receptor (23), can also interact with C-terminal sequences of v-Rel in a yeast two-hybrid screen. Moreover, Trip6 fulfills certain requirements of a transcriptional adaptor or coactivator for v-Rel.

MATERIALS AND METHODS

Yeast Two-Hybrid Screening

Yeast two-hybrid screening using C-terminal sequences of v-Rel fused to the GAL4 DNA binding domain (pGB-3′v-Rel) was performed in Y190 cells, which contain GAL4 site *HIS3* and *lacZ* reporter loci as described (11). The human B-cell two-hybrid cDNA library was kindly provided by Steven Elledge (11). Yeast transformants (8×10^5) were plated and selected for histidine auxotrophy in the presence of 25 mM 3-amino-triazole (3-AT). Colonies that grew on the initial plates were picked and streaked on plates containing 35 mM 3-AT and were then subjected to a second screen for *lacZ* expression by performing a β-galactosidase overlay assay (2). Positive cell clones were grown in the presence of tryptophan for several passages, and were screened for loss of the *TRP1*-containing pGB-3′v-Rel plasmid. Cells containing only the cDNA library plasmids were mated to cells containing pGB-3′vRel, pGBT9 (GAL4 DNA binding domain alone), or expression plasmids for GAL4 fused to p53, lamin, SNF1, or CDK. β-Galactosidase overlay assays were performed on the diploid yeast cells to confirm the specificity of the interactions.

Plasmids

Plasmid GBT9, containing codons 1–147 of GAL4, was used for the expression of GAL4 fusion proteins in yeast. To create pGB-3′v-Rel, a *Hin*cII fragment containing 3′v-Rel sequences encoding aa 332–503 was subcloned into plasmid GBT9 that had been digested with *Bam*HI and treated with Klenow. To create pGB-Trip6, a *Bgl*II fragment containing Trip6 sequences encoding aa 116–476 was excised from the cDNA two-hybrid library plasmid and was subcloned into the *Bam*HI site of pGBT9. To create pGB-3′c-Rel, an *Eco*RI fragment from pSG-3′mC (28) containing 3′ sequences from mouse c-Rel was subcloned into the *Eco*RI site of pGBT9.

To create pSG5-FLAG-ΔNTrip6, Trip6 sequences were recovered from the two-hybrid cDNA library plasmid by digestion with *Eco*RI, treatment with Klenow, and subsequent digestion with *Bgl*II. The Trip6 fragment was then subcloned into pSG5-FLAG (18) that had been digested with *Eco*RV and *Bam*HI. The expression plasmid for FLAG-tagged full-length Trip6 9 (aa 2–476) was created by PCR using an upstream adaptor primer (5′-GACGAATTCCGGGC CCACCTGGCTTC-3′) corresponding to the 5′ end of human *trip6* and containing a flanking *Eco*RI site and a downstream T3 primer. The PCR product was digested with *Eco*RI and subcloned into the *Eco*RI site of pcDNA3-FLAG (30).

To create pJK-Trip6 for the expression of FLAG-ΔNTrip6 in yeast, an *Eco*RI to *Bgl*II fragment from pSG5-FLAG-Trip6 was first subcloned into pGEM-4 that had been digested with *Eco*RI and *Bam*HI to create pGEM4-FLAG-Trip6. This plasmid was then digested with *Eco*RI, treated with Klenow, and digested with *Sal*I. The fragment containing FLAG-Trip6 was subcloned into vector pJKTPI (a kind gift of Joanne Kamens) that had been digested with *Sma*I and *Sal*I. Yeast plasmids BC102, BC-v-Rel, and reporter plasmid IgK6 have been described previously (20).

Plasmid SG424, encoding aa 1–147 of GAL4, was used to express GAL4 fusion proteins in chicken cells (27). GAL4 producer plasmids SG424, SG-3′c-Rel (mouse c-Rel), and SG-3′v-Rel and GAL4 site reporter plasmid G5BCAT have been described previously (28). pSG-Trip6 for the expression of GAL4-Trip6 was made by subcloning a *Hin*dIII to *Sal*I frag-

ment containing the GAL4-Trip6 fusion from pGB-Trip6 into pSG424 digested with *Hin*dIII and *Sal*I.

Plasmid MH105 is a spleen necrosis virus vector for the expression of v-*rel* and *neo* (32). To create pMH-v-Rel/FLAG-Trip6, an *Nco*I to blunt-ended *Bgl*II fragment from pSG5-FLAG-Trip6 was subcloned into pMH105 that had been digested with *Rsr*I, treated with Klenow, and then digested with *Nco*I, thus replacing the 3′ *neo* gene with FLAG-Trip6. To create pMH-FLAG-Trip6, pMH-v-Rel/FLAG-Trip6 was digested with *Xba*I and religated to remove the v-*rel* sequences.

Reporter Gene Assays in Yeast and Tissue Culture Cells

lacZ reporter gene assays were performed in yeast by using either Y190 cells with an integrated *lacZ* reporter gene driven by GAL4 DNA binding sites (11) or EGY40 cells transformed with IgK6, which is a *lacZ* reporter plasmid containing six upstream κB DNA binding sites (20). The yeast cells were transformed with the indicated producer plasmids and *lacZ* expression in liquid cultures was determined as described previously (20).

CAT reporter gene assays were performed in CEF using 5 μg of pSG424-based GAL4 expression plasmids and 5 μg of GAL4 site reporter plasmid G5B-CAT as described (30). For normalization, cells were cotransfected with 1 μg of pCMV-β-gal. Forty-eight hours posttransfection, cells were harvested and liquid β-galactosidase assays were performed to determine transfection efficiency. CAT assays were then performed on extracts containing equal β-galactosidase activity. Thin-layer plates were analyzed and quantitated using a Bio-Rad Molecular Imager.

Indirect Immunofluorescence and Western Blotting

Indirect immunofluorescence with anti-Rel antiserum was performed as described previously (31). Indirect immunofluorescence and Western blotting with the M2 anti-FLAG antibody (Sigma) were performed as described (30). For indirect immunofluorescence, cells were washed with PBS, fixed for 10 min in 4% paraformaldehyde, treated for 2 min with 0.2% Triton X-100, and blocked for 30 min with 20% goat serum in PBS before incubating with 1–2.5 μg/ml M2 antibody for 1 h in the presence of 20% goat serum. After washing, cells were incubated with FITC-conjugated goat anti-mouse antiserum for 1 h at a dilution of approximately 1:80. Cells were then washed with PBS, mounted on microscope slides, and imaged with an Olympus inverted confocal microscope. Western blotting on lysates of transformed spleen cells was performed with anti-v-Rel as described previously (31).

Chicken Spleen Cell Transformation Assays

Chicken spleen cell transformation assays were performed as described previously (31) by electroporating fresh spleen cells from 19-day-old chickens (Spafas, Inc.) with 10 μg of SW253 Rev-A helper virus plasmid and 20 μg of pMH105 or pMHv-rel/FLAG-ΔN-Trip6. Cells were then plated for 4 days in DMEM containing 20% fetal calf serum, after which cells were suspended in soft agar, and colonies were scored 10–14 days later. Colony numbers were normalized to the number of colonies obtained with pMH105.

Northern Blotting

Multiple human tissue RNA filters were purchased from Clontech. A probe for Trip6 was prepared by random primer ^{32}P-labeling of an *Xho*I fragment encompassing the Trip6 sequences from the cDNA library plasmid isolated in the yeast two-hybrid screen.

RESULTS

Trip6 Interacts With C-Terminal Sequences of v-Rel in a Yeast Two-hybrid Assay

We previously showed that C-terminal sequences of v-Rel, which can activate transcription in chicken cells, cannot activate transcription in yeast (21,26, 28). Therefore, we used a GAL4 fusion protein (GAL4-3′v-Rel) containing the C-terminal sequences of v-Rel as bait in a yeast two-hybrid screen to identify interacting proteins. Because v-Rel primarily transforms lymphoid cells, a human B-cell cDNA library was screened. After screening 8×10^5 yeast transformants, we identified three positive clones. Restriction enzyme digestions and preliminary DNA sequencing indicated that two of these cDNAs were identical to one another and contained approximately 1.4 kbp of sequences encoding a portion of Trip6, a protein initially identified in a two-hybrid screen as interacting with the thyroid hormone receptor (23).

The GAL4 activation domain-Trip6 fusion protein (GAD-Trip6) that interacted with GAL4-3′v-Rel encoded aa 116–476 of the full-length human Trip6 protein (35) (Fig. 1A) and included the three C-terminal LIM domains. The interaction of v-Rel sequences with Trip6 is specific: in two-hybrid assays Trip6 interacted with GAL4-3′v-Rel, but not with the GAL4 DNA binding domain alone or unrelated GAL4 fusion proteins, including GAL4-p53, GAL4-lamin, GAL4-SNF1, and GAL4-CDK2 (data not shown).

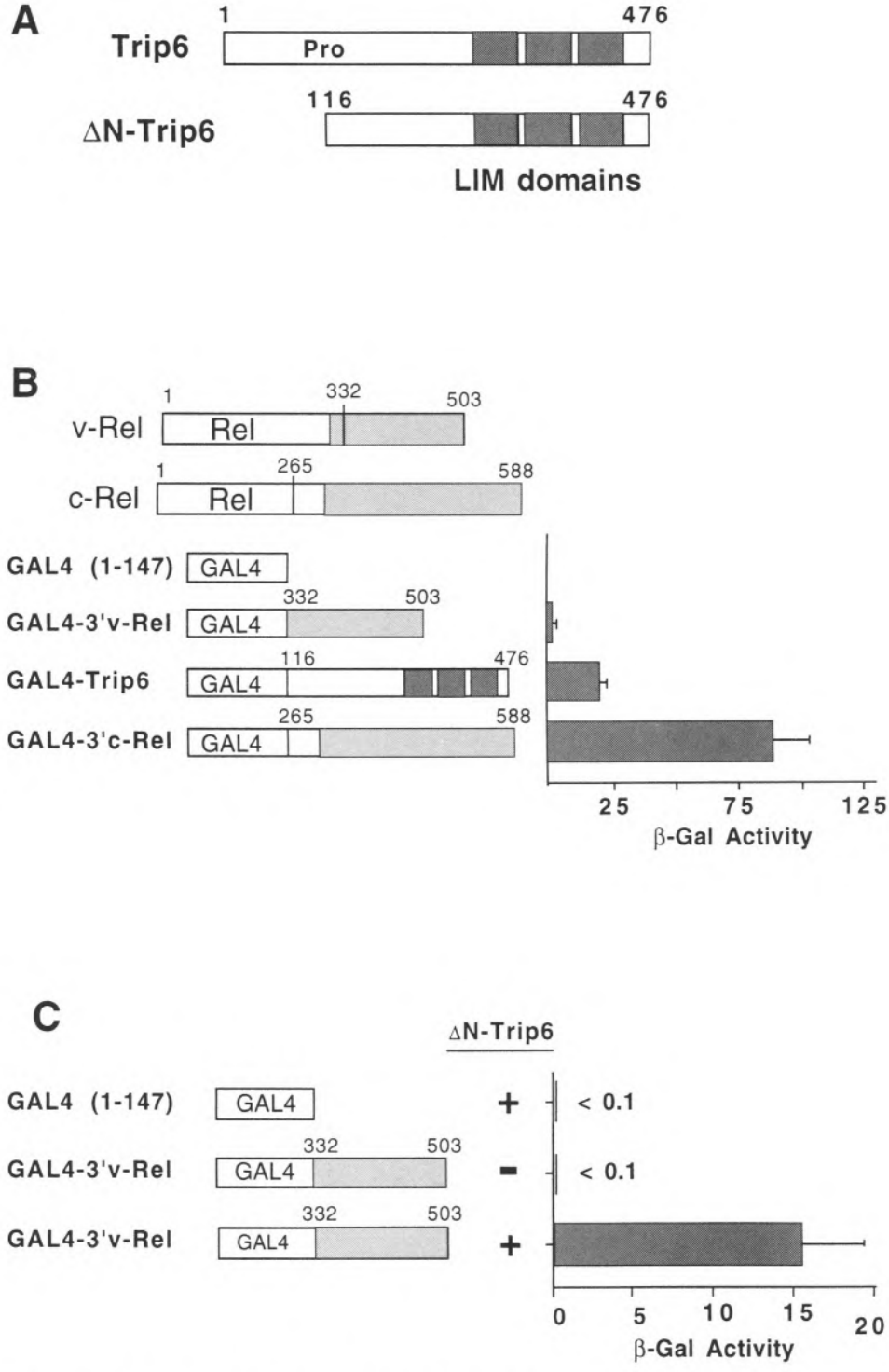

FIG. 1. Trip6 can enable GAL4-3′v-Rel to activate transcription in yeast. (A) Schematic of full-length Trip6 (which contains an N-terminal proline-rich domain and three C-terminal LIM domains) and the N-terminally deleted portion of Trip6 encoded by the cDNA that was isolated in the two-hybrid screen. (B) Yeast strain Y187 cells, containing an integrated GAL4 *lacZ* reporter locus, were transformed with plasmids encoding the indicated GAL4 proteins. Liquid β-galactosidase assays were performed in triplicate, and the values were then averaged and normalized to the value obtained for GAL4(1–147). Shown at the top of (B) are the generalized structures of v-Rel and c-Rel. In these figures, the white box with Rel indicates the approximate extent of the Rel Homology DNA binding/dimerization domain, while the gray shaded areas indicate the C-terminal transactivation domains. Numbers above the figures indicate the aa sequences included in all proteins. (C) Yeast strain Y190 cells, containing integrated *lacZ* and *HIS3* GAL4 site reporter loci, were cotransformed with plasmids for the expression of the indicated GAL4 proteins and with a plasmid for the expression of FLAG-ΔNTrip6 (+) or the corresponding empty expression vector (–). Cells were grown in the presence of 15 mM 3-amino-triazole to ensure continued expression of the *HIS3* gene. Liquid β-galactosidase assays were performed in duplicate and values are expressed as average units of β-galactosidase activity.

Based on these results, we embarked on a series of experiments to determine whether Trip6 could influence the ability of v-Rel to activate transcription in yeast and chicken cells. Except where noted (i.e., Fig. 4), we assayed the effect of Trip6 proteins containing aa 116–476 (ΔN-Trip6) because these sequences can efficiently localize to the nucleus and contain the three LIM domains that are essential for transactivation [see below and (30)].

Trip6 Can Restore the Ability of C-Terminal Sequences of v-Rel to Activate Transcription in Yeast

During the course of our characterization of human Trip6, we noted that a GAL4 DNA binding domain-Trip6 fusion protein could activate transcription from a GAL4 site reporter plasmid in yeast (Fig. 1B). Moreover, similar results were reported by Lee et al. (23). Therefore, we wished to determine whether expression of the Trip6 sequences by themselves (i.e., not as a GAD fusion protein) could restore the ability of GAL4-3′v-Rel to activate transcription in yeast. As shown in Fig. 1C, coexpression of GAL4-3′v-Rel and a FLAG-ΔN-Trip6 fusion protein (containing aa 116–476 of Trip6) resulted in specific activation of transcription from a GAL4 site reporter locus in yeast. As controls, expression of the GAL4-3′v-Rel protein alone did not activate transcription and the FLAG-ΔN-Trip6 protein did not enable GAL4 sequences (aa 1–147) alone to activate transcription. These results suggest that Trip6 can, directly or indirectly, bridge an interaction between the C-terminal sequences of v-Rel and the general transcription apparatus in yeast.

Trip6 Can Enhance the Ability of v-Rel to Activate Transcription From a κB Site Reporter Plasmid

We next wished to determine whether Trip6 could also enhance the ability of full-length v-Rel to activate transcription from a promoter containing DNA binding sites for v-Rel. Therefore, we determined whether FLAG-ΔN-Trip6 could affect the ability of v-Rel to activate transcription from a *lacZ* reporter plasmid (IgK6) containing six upstream κB sites from the mouse kappa light chain gene. Because vertebrate cells have cellular Rel/NF-κB proteins that could influence the interpretation of these results, this reporter gene experiment was performed in yeast. As reported previously (20), full-length v-Rel activated transcription from reporter plasmid IgK6 approximately 10-fold compared to the vector control. Coexpression of FLAG-ΔN-Trip6 resulted in an approximately twofold further increase in the ability of v-Rel to activate transcription from IgK6 (Fig. 2). Therefore, Trip6 is able to enhance transcriptional activation by full-length v-Rel from a κB site-containing promoter.

Trip6 Contains a Transcription Activation Domain That Functions in Chicken Fibroblasts

To characterize the activity of Trip6 in vertebrate cells, we first determined whether a GAL4-Trip6 fusion protein could activate transcription in chicken embryo fibroblasts (CEF). As shown in Fig. 3, GAL4-Trip6 activated transcription from a GAL4-site CAT reporter plasmid in CEF. As positive controls for these experiments, we also measured transcriptional activation by GAL4-3′v-Rel, which does activate transcription in CEF, and GAL4-3′c-Rel, which contains a very strong activation domain (28). These results indicate that the ability of human Trip6 sequences to activate transcription is conserved between yeast and chicken cells.

An N-Terminal Deletion Allows Trip6 to Localize to the Nucleus in CEF

For Trip6 to function as a coactivator of transcription, it must localize to the nucleus of cells under some conditions. We have recently shown that full-length mouse Trip6 localizes primarily to adhesion plaques in CEF, whereas an N-terminal deletion allows mouse Trip6 to enter the nucleus (30). To determine the subcellular localization of human Trip6, CEF were transfected with a retroviral expression

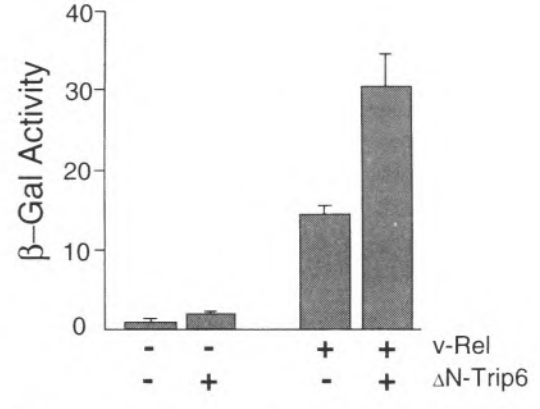

FIG. 2. Trip6 potentiates transcriptional activation by full-length v-Rel from a κB site-containing reporter locus in yeast. Yeast containing κB site reporter plasmid IgK6 was cotransformed with a v-Rel expression vector (+) or the corresponding empty vector (−) and with a FLAG-ΔNTrip6 expression vector (+) or the corresponding empty vector (−). Liquid β-galactosidase assays were performed in triplicate in two separate experiments, which were then averaged. Relative β-galactosidase activities were determined by normalizing values to those obtained with double vector (−,−) control cells (value = 1.0).

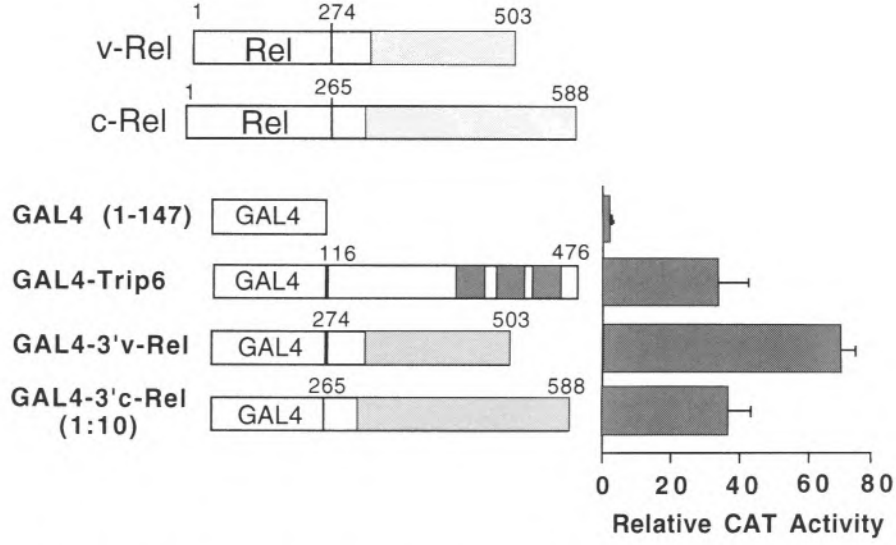

FIG. 3. Trip6 sequences can activate transcription in chicken embryo fibroblasts. CEF were cotransfected with a GAL4 site CAT reporter plasmid and expression plasmids for the indicated GAL4 fusion proteins. Forty-eight hours later, CAT activity was determined using cell lysates that were normalized by an internal *lacZ* control plasmid. In the case of GAL4-3′c-Rel, which contains a very strong transactivation domain, a 10-fold dilution of cell extract was used. Relative CAT activity was calculated by setting the value for GAL4 to 1.0. As in Fig. 1B, the generalized structures of the Rel proteins are shown above the figure, and the numbers above the figures indicate the aa residues included in the proteins.

vector for FLAG-ΔN-Trip6 (aa 116–476) or with one for both FLAG-ΔN-Trip6 and v-Rel, and indirect immunofluorescence was performed with anti-FLAG primary antiserum. Much FLAG-ΔN-Trip6 localizes to the nucleus of CEF, whereas full-length human Trip6 localizes to adhesion plaques and is excluded from the nucleus (Fig. 4, panels 1 and 2). As a control, wild-type v-Rel is shown to localize to the nucleus of CEF (15) (Fig. 4, panel 3). Furthermore, FLAG-ΔN-Trip6 also localized to the nucleus when coexpressed with v-Rel (Fig. 4, panel 4). These results demonstrate that deletion of N-terminal sequences allows human Trip6 to localize to the nucleus.

Coexpression of Nuclear Trip6 Does Not Affect the Transforming Activity of v-Rel in Chicken Spleen Cells

To determine whether Trip6 could affect the transforming activity of v-Rel, we first created an IRES-containing spleen necrosis virus vector for the coexpression of v-Rel and FLAG-ΔN-Trip6. The ability of the v-Rel/FLAG-ΔN-Trip6 vector to transform chicken spleen cells was compared to a control vector that encoded v-Rel and Neo. As shown in Fig. 5A, the transforming activities of these two viral vectors were nearly identical. Moreover, cells transformed by the v-Rel/FLAG-ΔN-Trip6 vector expressed both v-Rel and FLAG-ΔN-Trip6 (Fig. 5B). Taken together, these results indicate that coexpression of a nuclear form of human Trip6 does not affect the transforming activity of v-Rel.

Trip6 mRNA Is Expressed in Multiple Tissues

To determine the tissue-specific expression pattern of Trip6 mRNA, Northern blotting of mRNA from multiple human tissues was performed using a Trip6 probe (Fig. 6). Although the level of expression varies among cell types, Trip6 is expressed as an approximately 1.8 kb mRNA in every tissue tested. Similar results have been reported by Lee et al. (23) and Murthy et al. (24) in a more limited survey of human tissues. Of note, even though Trip6 mRNA appears to be expressed at quite low levels in lymph node and bone marrow, we initially isolated the Trip6 cDNA from a human B-cell two-hybrid cDNA library. In short, Trip6 appears to be a ubiquitously expressed mRNA.

DISCUSSION

In this report, we have identified Trip6 as a likely coactivator for transcriptional activation by the v-Rel retroviral oncoprotein. Trip6 was initially identified in a two-hybrid assay using a portion of the thyroid hormone receptor (TR) as bait (23). In those experiments, a region of the rat TR subunit containing the ligand binding, dimerization, and transcription activation domains was used; however, the precise region of the TR that interacted with Trip6 was not deter-

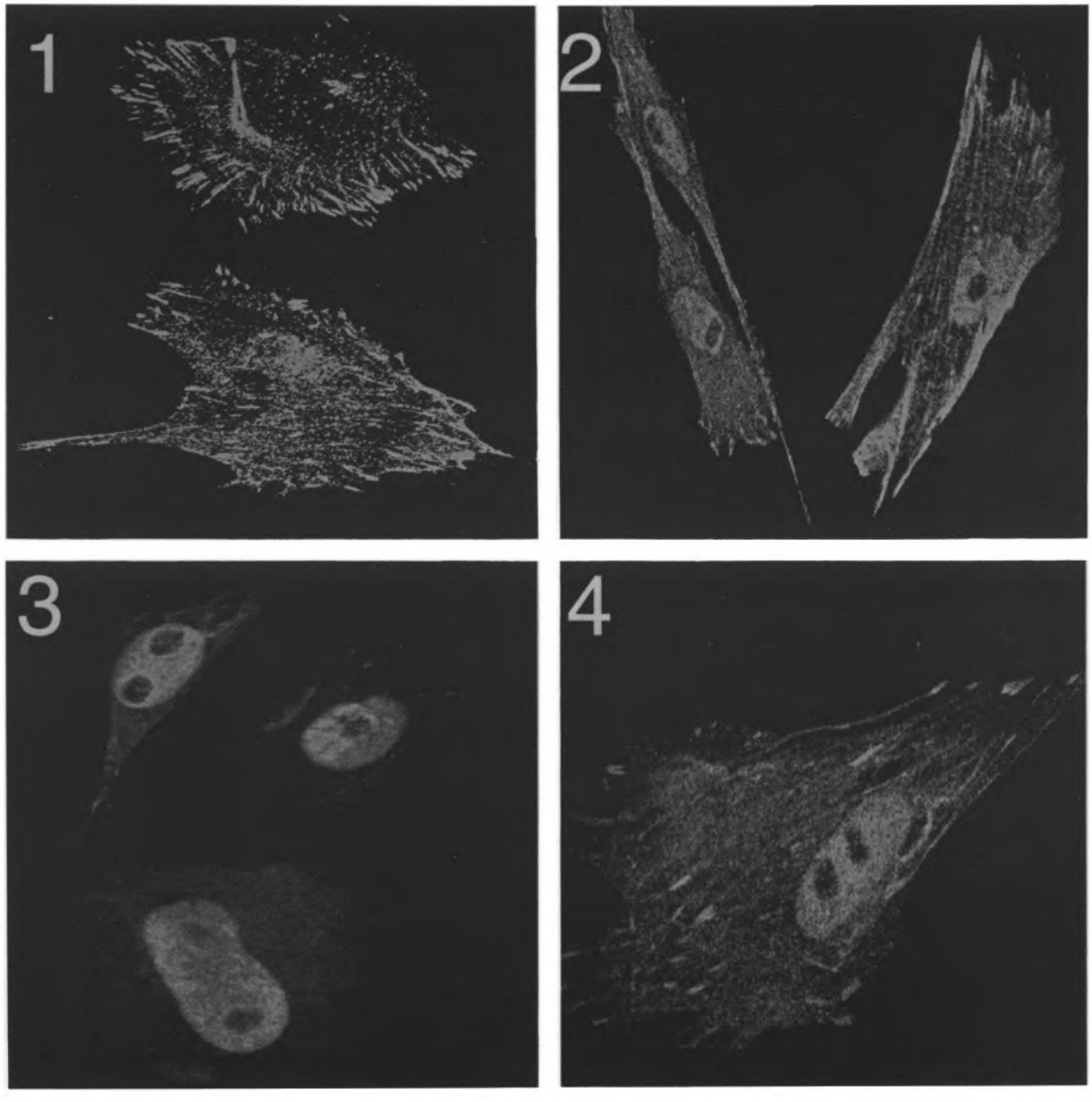

FIG. 4. Deletion of N-terminal sequences enables Trip6 to localize to the nucleus of CEF. CEF were transfected with expression vectors for a FLAG-tagged full-length human Trip6, a FLAG-tagged N-terminally deleted form of human Trip6 (FLAG-ΔN-Trip6), or both v-Rel and FLAG-ΔN-Trip6. Indirect immunofluorescence was then performed using anti-FLAG or anti-v-Rel antibodies as warranted. (1) FLAG-human Trip6 (anti-FLAG), (2) FLAG-ΔN-Trip6 (anti-FLAG), and (3 and 4) FLAG-ΔN-Trip6/v-Rel (anti-v-Rel, 3; anti-FLAG, 4). Several control (nonexpressing) cells are also visible as nonstaining cells in these panels.

mined. Thus, it is possible that Trip6 interacts with the transcription activation domain of the TR, as it does with v-Rel. A comparison of the TR aa sequence with the C-terminal sequence of v-Rel does not reveal any obvious similarities to explain why both proteins interact with Trip6. Trip6 has also been identified as interacting with three other proteins in two-hybrid screens, namely, the tyrosine phosphatase PTP1E, the adaptor protein RIL, and the OpaP protein of *Neisseria gonnorrheae* (7,24,33).

Trip6 has three LIM domains located towards its C-terminus. LIM domains are important in several settings for mediating protein–protein interactions (9). The region of Trip6 that interacts with TR, v-Rel, PTP1E, and the OpaP protein in two-hybrid screens contains these LIM domains. Thus, it is reasonable to suppose that Trip6 interacts with these diverse proteins through its LIM domains.

Trip6 has been proposed to be one of a subfamily of LIM domain-containing proteins, which also includes the highly related protein zyxin, that function as intracellular signaling proteins, relaying informa-

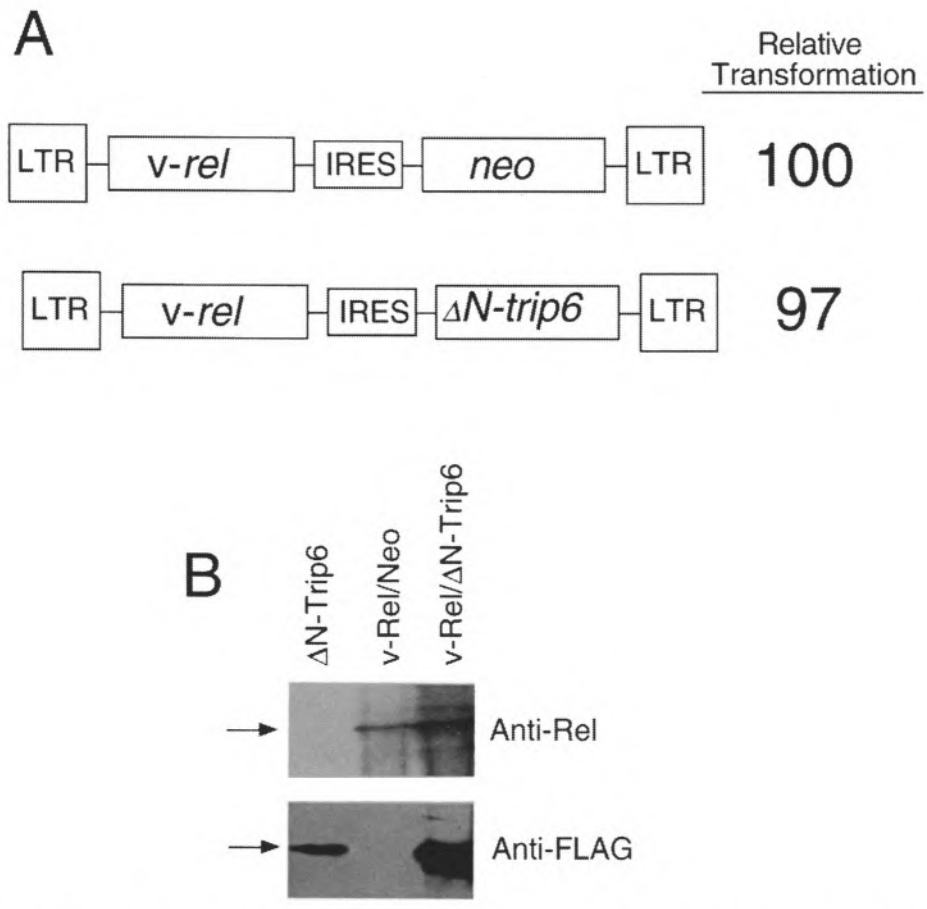

FIG. 5. Coexpression of v-Rel and Trip6 in chicken spleen cells. (A) The abilities of the depicted viral vectors to transform chicken spleen cells were determined as described in Materials and Methods. Relative transforming activity is relative to the transforming activity of the pMH105 vector (100). Values are the average of 15 independent assays, which were performed in 4 separate experiments. (B) Western blot of extracts from spleen cells transformed by pMH105 (v-Rel/Neo) or pMH-FLAG-ΔN-Trip6 (v-Rel/FLAG-ΔN-Trip6). Filters were first probed with anti-FLAG antiserum, then stripped and reprobed with anti-v-Rel antiserum. The lane on the left (ΔN-Trip6) contains in vitro-translated FLAG-ΔN-Trip6.

tion between adhesion plaques and the nucleus (1). Thus, the interaction of Trip6 with plasma membrane proteins, such as a tyrosine phosphatase, and with nuclear proteins, such as v-Rel and TR, need not be surprising. That is, Trip6 may have specific and functional protein–protein interactions at the cell surface and in the nucleus. A number of transcription factors, including STAT proteins (8), Rel/NF-κB proteins (14), sterol regulatory element binding proteins (SREBP) (4), and Notch (3), show highly regulated cytoplasmic/nuclear shuttling. In each of these cases, specific extracellular signals induce nuclear translocation of the transcription factor. There is, however, no physiological signal that is known to cause Trip6 to enter the nucleus of cells. Nevertheless, our results [(30) and herein] demonstrate that an N-terminal deletion can allow both human and murine Trip6 proteins to localize to the nucleus of CEF, and Nix and Beckerle (25) have shown that an internal deletion can cause zyxin to accumulate in the nucleus. Therefore, a cytoplasmic proteolytic processing event (or other posttranslational modification) could allow a C-terminal fragment of Trip6 to enter the nucleus, where it could participate in transcriptional activation, probably via its LIM domains [(30); Fig. 2 herein]. This type of proteolytic control is used in the SREBP and Notch signaling pathways [reviewed in (3,4)].

Only a limited number of transcriptional coactivators have been identified [reviewed in (17)]. In some cases, such as the JAB1 coactivator of c-Jun, coactivators appear to enhance the DNA binding activity of a specific transcription factor (6); of note, Jab1 also appears to be able to enhance the formation of Bcl-3–NF-κB p50 complexes on DNA (10). In other cases, such as what is likely to occur with Trip6 and v-Rel, coactivators have their own "activation domains" and bridge interactions between specific tran-

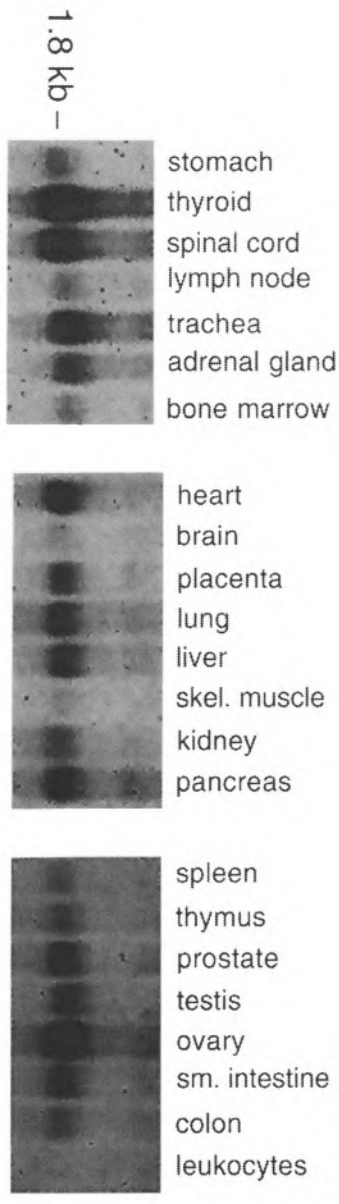

FIG. 6. Trip6 is expressed in multiple human tissues from a 1.8 kb mRNA. A Northern blot was performed on filters containing 10 μg of poly(A)-selected mRNA from the indicated tissues (multiple tissue blots from Clontech). Longer exposures show expression in all tissues.

scription factors and the general transcription machinery. Interestingly, a LIM domain-containing protein, ACT, has recently been identified in a two-hybrid screen as an enhancer of transcription activation by the related CREM and CREB transcription factors (12). Like Trip6, ACT has an independent activation domain, and ACT associates with CREM via its LIM domains (12).

The use of a coactivator does not, however, exclude the possibility of direct interactions between a given transcription factor and the general transcription apparatus. For example, C-terminal sequences of v-Rel have also been reported to interact weakly with TBP and TFIIB (34). In this sense, v-Rel may make direct contacts with subunits of the general transcription apparatus (e.g., TBP and TFIIB) and indirect interactions (e.g., through coactivators such as Trip6). In cases where multiple interactions occur, the interaction between a transcription factor and any isolated transcription complex component may be quite weak. Indeed, the two-hybrid interaction between v-Rel and Trip6 is weaker than most other interactions we have tested, and we have been unable to identify conditions under which we can detect a direct interaction between Trip6 and v-Rel in vitro (data not shown). Therefore, a posttranslational modification or additional factor may be necessary for the Trip6–v-Rel interaction to occur. Along these lines, the two-hybrid interaction of Trip6 with the TR is dependent on the presence of thyroid hormone (23). Alternatively, Trip6 may interact indirectly with v-Rel in cells.

Our results are consistent with Trip6 acting as a coactivator for v-Rel in vivo. First, Trip6 contains a transcriptional activation domain that can function when fused to GAL4 (30) (Figs. 1B and 3) or LexA (23) in yeast and in chicken fibroblasts. Second, Trip6 can enhance transcriptional activation by v-Rel (Figs. 1C and 2). Third, an N-terminally deleted form of Trip6 can localize to the nucleus of cells (Fig. 4). Fourth, as with Rel/NF-κB proteins, Trip6 mRNA is expressed in many human cell types, including hematopoietic cells (Fig. 6).

Our experiments do not, however, address whether Trip6 is required for transcriptional activation and/or transformation by v-Rel. In the most obvious scenario, Trip6 could be necessary for v-Rel to activate transcription of transformation-specific target genes. In this model, overexpression of Trip6 might enhance transformation by v-Rel. Alternatively, it is possible that Trip6 is a suppressor of transformation, and that Trip6 activity must be titrated out by v-Rel for transformation to ensue. In this second model, overexpression of Trip6 would be expected to suppress transformation by v-Rel. However, our results show that overexpression of an N-terminally deleted, nuclear form of human Trip6 does not affect the transforming activity of v-Rel (Fig. 5). Nevertheless, this result must be interpreted cautiously; for example, we do not know whether there is a specific form of Trip6 that functionally interacts with v-Rel in cells or whether there is sufficient functional homology between chicken and human Trip6 for human Trip6 to affect the transforming activity of v-Rel. Future experiments will address the role, if any, of Trip6 in transformation by v-Rel. It will also clearly be of interest to determine directly whether Trip6 can func-

tion as a coactivator for cellular Rel/NF-κB proteins, because Trip6 could represent an additional target for therapeutics aimed at disrupting the Rel/NF-κB pathway.

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