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Transcriptional activation of a conserved sequence element by *ras* requires a nuclear factor distinct from c-fos or c-jun

(oncogenes/trans-acting factors/signal transduction/transforming growth factor β1)

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ABSTRACT **The expression of transforming growth factor β type 1 mRNA was increased by conditional expression of *ras*. A 31-base-pair sequence found ≈420 base pairs upstream of the gene encoding human transforming growth factor β1 acted as a *ras*-responsive enhancer element in transient transfection assays. The human sequence contains the element TGACTCT that also is found in a murine *ras*-responsive enhancer. Analysis of nuclear factors present in cells stably transformed by *ras* indicated that both human and murine sequences were recognized by the same nuclear factor. The role of *fos* and *jun* in *ras* transcriptional activation was analyzed in transfection assays using murine elements that contained either TGACTCT or TGAGTAA. These experiments showed that while both elements are activated by *fos/jun* expression to nearly the same event, only the former element responded to *ras*. In addition, activation of reporters containing TGACTCT is 6-fold higher by *ras* than by *fos/jun*. Gel retention experiments revealed that the nuclear factor present in cells transformed by *ras* exhibited the same sequence preference as demonstrated in the transient transfection assays. UV-crosslinking experiments identify a protein of apparent molecular mass 120 kDa that recognizes the *ras*-responsive element. This work identifies a persistent signal transduction pathway that links *ras* to nuclear transcription and indicates that a 120-kDa protein is a target of this pathway.**

Nuclear oncoproteins probably transform cells by directly altering gene expression. For example, c-fos and c-jun proteins are transcription factors activated by tumor promoters (1, 2). These protooncogene products are believed to play an important role in cell-cycle control (1, 2). Uncontrolled expression of such gene products could be expected to alter the fate and growth potential of cells. Regulation of gene expression may also be important in cellular transformation by oncogene products that are located in nonnuclear compartments: v-src and v-mos products can alter expression of cellular genes (3, 4). Our lab has identified genes that are up-regulated when ras p21 is conditionally expressed (5). Recent work demonstrates that transformation by *ras* can also down-regulate muscle-specific genes, with important consequence on the differentiation of myoblasts in culture (6).

Activated *ras* oncogenes are involved in the formation of many types of malignant tumors in animals and also have been linked to human carcinogenesis (7). The *ras* gene products are 21-kDa proteins located at the inner surface of the cell plasma membrane (7). These proteins, like the well-characterized guanine nucleotide-binding proteins (G-proteins), are believed to be involved in signal-transduction mechanisms (7). However, *ras* signal-transduction pathways have remained elusive. One approach to identifying such signal-transduction pathways is to identify a physiological endpoint of the pathway and to use that endpoint to biochemically or genetically define other parts of the pathway. For example, such an approach was used to identify cAMP as an intracellular second messenger (8).

Transcriptional activation of genes in transformed cells is a physiological consequence of *ras* oncogene action that we have chosen to study. We have defined a *ras*-responsive, cis-acting element present in *NVL-3*, a member of the family of mouse genomic virus-like 30S elements designated VL30 (9). Our results show that the activation of transcription by *ras* is linked to cellular transformation. In addition, several lines of evidence are consistent with the hypothesis that *ras* nuclear signal transduction is distinct from the phorbol ester/protein kinase C pathway (9). This published work defines a signal-transduction pathway that is rapidly activated by conditional expression of v-*ras* (5) and that persists in cells stably transformed by *ras* oncogenes (9).

In the present work, the amount of transforming growth factor β type 1 (TGF-β1) mRNA is observed to increase after conditional expression of v-Ha-*ras*. A 31-base-pair (bp) sequence located 420 bp upstream of the first exon encoding human TGF-β1 is shown to serve as a *ras*-responsive enhancer element. The human sequence is demonstrated to be recognized by the same nuclear factor that recognizes the mouse *NVL-3* *ras*-responsive sequence (9). Evidence provided by DNA-mediated gene transfer and gel mobility shift experiments demonstrates that *fos* and *jun* protooncogenes are not sufficient for persistent *ras*-responsive transcriptional activation. Finally, UV-crosslinking experiments demonstrate that a 120-kDa protein present in transformed cells recognizes the *ras*-responsive enhancer element.

MATERIALS AND METHODS

Cell Culture and RNA Analysis. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 9.5 mM L-glutamine, and 100 μg of mezlocillin per ml. The normal rat kidney NRK B1 and Rat-1 A1 fibroblast lines were obtained by introducing a mouse mammary tumor virus/v-Ha-*ras* chimeric gene (5) into the parent cells. Colonies that contained the gene were cloned and characterized as described for similar NIH 3T3 cell lines (5). Dexamethasone (0.1 μM) was added to the culture medium to induce expression of the v-Ha-*ras* gene.

Total cellular poly $(A)^+$ RNA was isolated from hormone-treated and untreated cells as described (5). RNA was analyzed by gel electrophoresis and blot hybridization analyses (Northern blotting) (5). The probe for TGF-β1 RNA was cloned from a human placental cDNA library by using a synthetic oligonucleotide as probe. The oligonucleotide was based on published sequence information (10). The sequence

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Abbreviations: TGF-β1, transforming growth factor β type 1; AP-1, HeLa cell activator protein 1.

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of a 1500-bp cDNA clone obtained in this way matched the published human cDNA sequence.

Construction of Plasmids. The construction of pRDO51 and pRDO53 has been described (9). These plasmids contain short deletions in the *NVL-3* enhancer sequences. The former lacks the sequence TGACTCT found in the wild-type U3 region of *NVL-3*, while the latter lacks TGAGTAA (9). The plasmid pRDO52 was constructed in the same manner as the other two plasmids, but it contains a deletion of both sequences (see Fig. 2*A*). A double-stranded oligonucleotide, TTGTTTCCCAGCCTGACTCTCCTTCCGTTCT (sense strand), was cloned into the unique *Pst* I restriction site of pRDO52 so that both possible orientations relative to luciferase transcription were obtained (Fig. 2*A*). The oligonucleotide sequence corresponds to base pairs −430 to −400 upstream of the human gene for TGF-β1 (10). The plasmids were sequenced with the Sequenase kit (United States Biochemical) to confirm the orientation and copy number of the cloned oligonucleotides. The expression vector pHO6T1 (11) that contains the human c-Ha-*ras* EJ bladder carcinoma gene was used for expression of *ras*. For expression of c-*fos* and v-*jun*, the expression vector pHβAPr-1-gpt, which contains the human β-actin gene promoter region (12), was used. Genomic c-*fos* (13) or v-*jun* (14) sequences were inserted into the *Bam*HI site located downstream of the β-actin promoter.

Transfection Experiments. Transfections were performed exactly as described (9). As an internal control, 1 μg of the secreted placental alkaline phosphatase gene driven by the Rous sarcoma virus long terminal repeat (RSV-SEAP; ref. 15) was included in the DNA precipitates. Preparation of extracts and assays for both luciferase and alkaline phosphatase were performed as described (9). Luciferase activity is presented as luminescence per μg of protein extract divided by secreted alkaline phosphatase activity per ml of culture medium. In experiments to determine basal levels of enhancer activity, the expression plasmids Homer 6 (11) and pHβAPr-1-gpt (12) that lack external coding sequences were included in the DNA precipitates.

Gel Retention Assays and UV-Crosslinking of DNA to Protein. Nuclear extracts were prepared from *ras*-transformed DT cells, and gel retention assays were conducted as described (9). Double-stranded oligonucleotides were used as competitors and probes in the experiments. The sequences of the sense strand of the oligonucleotides used were: TTGTTTCCCAGCCTGACTCTCCTTCCGTTCTGCAG (TGF-β1 gene; ref. 10), CCAGATGTATTGCCAAACACAGGATATGACTCTTGGT (*NVL-3* box 1), CCCTGCAGTGGTTGAGTAAATTTGTGGT (*NVL-3* box 2), and GATCCTAGGAATAACGGAAT (putative *myb* binding site; ref. 16). Complementary oligonucleotides at 100 μg/ml were adjusted to contain 100 mM NaCl, heated to 95°C for 1 min, and slowly cooled to room temperature to ensure that they were double-stranded.

UV-crosslinking analysis was performed as described (17). Briefly, the complement of the *NVL-3* box 1 oligonucleotide shown above was used as template for primed second-strand synthesis by using Klenow polymerase (Boehringer Mannheim). The primer used had the sequence CCAGATGTA. Bromodeoxyuridine triphosphate, [α-^{32}P] ATP, and [α-^{32}P]CTP were incorporated into the newly synthesized strand. The double-stranded, labeled probe was purified on a nondenaturing gel, and 10^6 dpm were used per gel shift assay. After electrophoresis, the protein–DNA complex was visualized by autoradiography and excised from the gel. Crosslinking was accomplished by using a Foto/Prep I light source (Fotodyne, New Berlin, WI). Labeled protein was analyzed by sodium dodecyl sulfate (SDS) gel electrophoresis.

RESULTS

We have previously constructed NIH 3T3 cell lines that contained steroid hormone-regulated v-Ha-*ras*, and reported on the kinetics of ras p21 induction in such cells (5). For the experiments presented here, we constructed similar cell lines by introducing the steroid hormone-responsive v-Ha-*ras* chimeric gene into NRK fibroblasts and Rat-1 fibroblasts. The kinetics of ras mRNA and p21 induction in the rat cell lines was identical to that found for NIH 3T3, with ras p21 levels peaking 4 hr after addition of steroid to the cell culture media (data not shown). Two such cell lines, NRK B1 and Rat-1 A1, were treated for 4 hr with hormone, at which time total cell RNA was prepared.

Fig. 1 *Upper* shows Northern analysis of the NRK B1 and Rat-1 A1 cells with a human TGF-β1 cDNA probe. The data in Fig. 1 show that TGF-β1 2.2-kilobase (kb) mRNA is increased 5-fold in both NRK B1 (lane 3 vs 5) and Rat-1 A1 (lane 4 vs 6) cell lines after steroid stimulation of ras p21 expression. Steroid treatment has no effect on wild-type NRK and Rat-1 cells (lanes 1 and 2). However, the basal levels of TGF-β1 mRNA are 10-fold higher in cells harboring v-*ras* genes than in wild-type cells (compare lanes 1 and 2 to lanes 3 and 4). To demonstrate that the amounts of RNA loaded in each gel lane were the same, parallel Northern blots with the same RNA samples were hybridized to a c-*myc* probe (Fig. 1 *Lower*).

Our previous work implicated a 7-bp sequence, TGACTCT, as a *ras*-responsive enhancer element (9). The sequence of the rat TGF-β1 gene has not been published. However, analysis of the human TGF-β1 5′ flanking gene region (10) showed that the sequence TGACTCT is located 420 bp upstream of the first exon for the gene (Fig. 2*A*). Since many protooncogenes and growth-regulating genes are highly conserved between humans and rodents, including noncoding regulatory regions (e.g., refs. 18 and 19), we decided to test whether the human sequence was regulated by *ras*. A 31-bp double-stranded oligonucleotide that contains the 7-bp sequence and the flanking regions found in the human sequence was synthesized and cloned into a luciferase reporter vector, pRDO52 (pRDO52 lacks the *ras*-responsive sequences, Fig. 2*A*). Both sense and antisense orientations of the human sequence were obtained.

The TGF-β1–luciferase gene constructs were introduced into NIH 3T3 with a *ras*-expression vector. The luciferase

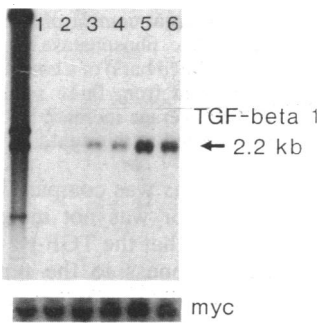

FIG. 1. The effect of *ras* expression on the abundance of TGF-β1 mRNA. Total cell poly(A)$^+$ RNA was fractionated by denaturing agarose gels. Lanes contained 5 μg of RNA. Identity of RNA samples in lanes: 1, dexamethasone-treated NRK cells; 2, dexamethasone-treated Rat-1 cells; 3, NRK B1 cells; 4, Rat-1 A1 cells; 5, dexamethasone-treated NRK B1 cells; 6, dexamethasone-treated rat-1 A1 cells. Hormone treatment was for 4 hr. (*Upper*) Filter was hybridized with a random primer-labeled TGF-β1 probe, and the autoradiogram was exposed for 72 hr. The size of the transcript is indicated to the right of the blot. (*Lower*) Filter was hybridized with a random primer-labeled c-*myc* probe (exons 2 and 3), and the autoradiogram was exposed for 7 days.

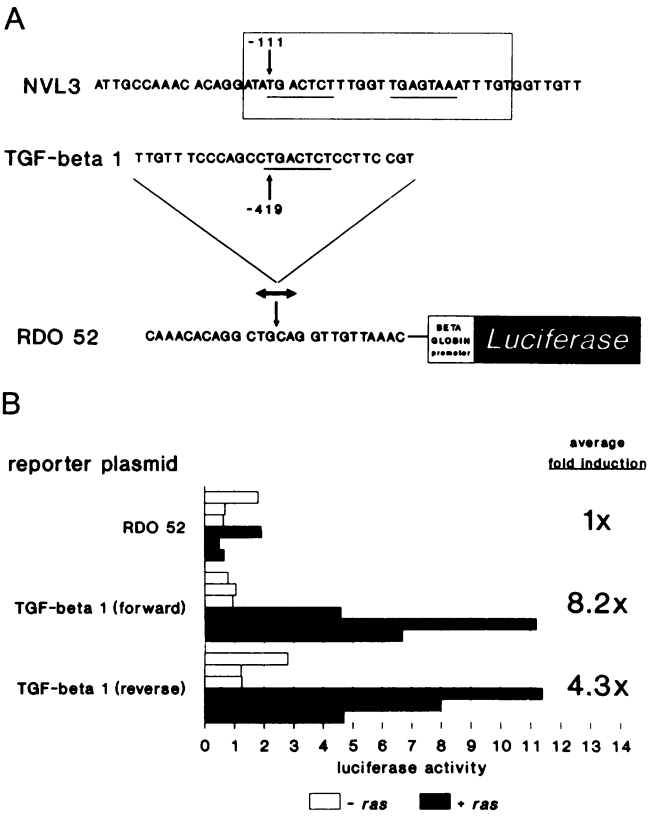

FIG. 2. The effect of *ras* expression on the transcriptional activity of a TGF-β1 gene upstream element. (*A*) The wild-type *NVL-3* sequence from −129 to −80 (sense strand) is shown at the top. The *ras*-responsive enhancer element and another AP-1-like binding sequence are underlined. The sequences enclosed in the box are deleted in the mutant *NVL-3* U3 region contained in pRDO52. Directly under the *NVL-3* sequence are the human TGF-β1 gene sequences located at −405 to −430 (sense strand). Underlined is the sequence in this region that is the same as the *ras*-responsive region of *NVL-3*. A double-stranded, 35-bp oligonucleotide identical to this TGF-β1 genomic sequence was cloned into the *Pst* I site of pRDO52. Both orientations of the oligonucleotide with respect to transcription were obtained to create "TGF-β1 (forward)" plasmid and "TGF-β1 (reverse)" plasmid. At the bottom is shown the general structure of pRDO52, including the sequences that border the *Pst* I cloning site. (*B*) NIH 3T3 cells were transiently transfected with pRDO52, TGF-β1 (forward) plasmid, or TGF-β1 (reverse) plasmid. Each plasmid was cotransfected with an internal control plasmid containing the gene encoding secreted alkaline phosphatase as well as either a c-Ha-*ras* expression plasmid (filled bars) or a control plasmid lacking the *ras* gene (open bars). Data from three separate transfection experiments are shown. The average increase in luciferase expression produced by *ras* is indicated at the right.

activity in the presence of *ras* was compared to the enzyme activity when the *ras* vector was not included (Fig. 2*B*). These experiments showed that the TGF-β1 oligonucleotide could confer *ras*-responsiveness to the reporter pRDO52 (Fig. 2*B*). Both the sense (8.2-fold increase) and antisense (4.3-fold increase) orientation of the TGF-β1 oligonucleotide function as *ras*-activated elements (Fig. 2*B*).

The sequence TGACTCT is similar to the consensus binding site for heterodimers of fos and jun peptides (1, 2). To test whether fos/jun protein complexes participate in stimulation of transcription by *ras*, we compared the ability of *ras* versus *fos* and *jun* to stimulate expression of reporter plasmids that contain HeLa cell activator protein 1 (AP-1) binding site-like sequences. As previously reported, the *NVL-3* long terminal repeat contains two such sequences, TGACTCT and TGAGTAA; only the former responds to *ras* (9). Luciferase reporter constructs that contain only the sequence TGACTCT (pRDO53) or TGAGTAA (pRDO51) were cotransfected with either *ras* expression vector or with vectors for *fos* and *jun* expression. The results of these cotransfection experiments are shown in Fig. 3. In three independent experiments, expression from pRDO51 (TGAGTAA) is increased 2-fold by activated *ras*, while cotransfection of *fos* and *jun* with this vector results in a 10-fold increase in activity. In contrast, *ras* increases expression from pRDO53 (TGACTCT) 40-fold, while *fos* and *jun* coexpression increases luciferase levels only 6-fold. A reporter plasmid that lacked all *NVL-3* sequences does not respond to *ras* or *fos/jun* expression (data not shown).

The 31-bp TGF-β1 oligonucleotide was used in gel retention assays along with a 38-mer that contains the murine *NVL-3 ras*-responsive element (Fig. 4*A*; see *Materials and Methods* for the exact *NVL-3* sequence used). Extracts were prepared from *ras*-transformed DT cells. Both TGF-β1 and *NVL-3* oligonucleotide probes form a complex with similar mobility with factors present in DT nuclear extracts (Fig. 4*A*, compare lanes 1 and 5). In competition experiments, a 40-fold molar excess of either unlabeled TGF-β1 or *NVL-3* oligonucleotides blocks the formation of this complex, irrespective of which labeled probe is used (Fig. 4*A*, lanes 2, 3, 6, and 7).

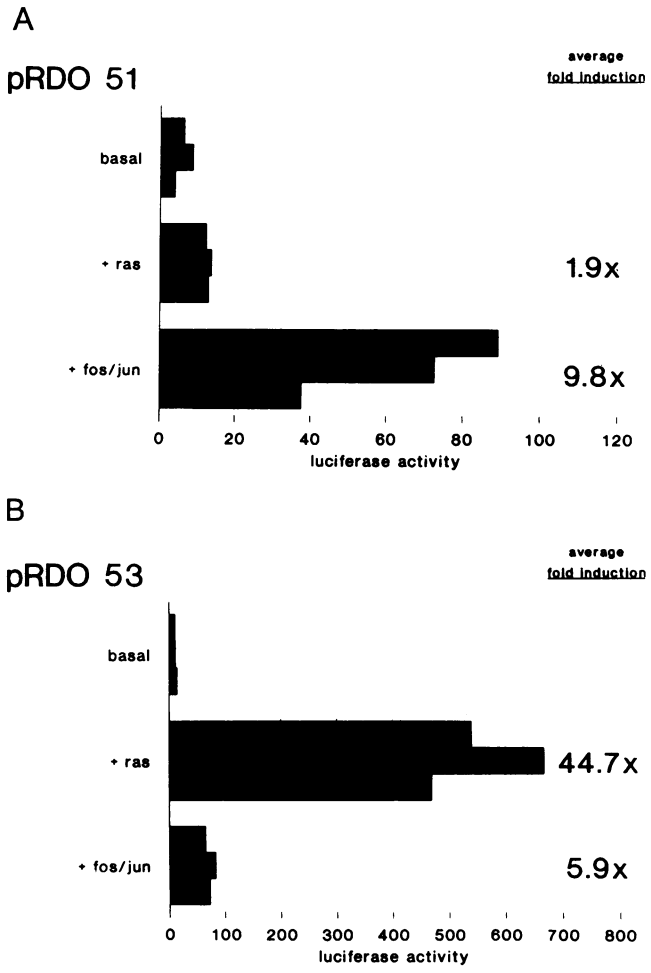

FIG. 3. Differential effect of *ras* versus *fos/jun* on luciferase reporter plasmids that contain *NVL-3* site-specific internal deletion mutations. NIH 3T3 cells were transiently transfected with pRDO51 (*A*) and pRDO53 (*B*). Each plasmid was cotransfected with an internal control plasmid containing the gene encoding secreted alkaline phosphatase or either control plasmids (promoters but no oncogene), a c-Ha-*ras* expression plasmid, or a combination of c-*fos* and v-*jun* expression plasmids. Data from three separate transfection experiments are shown. The average increase in luciferase activity produced by oncogene expression is indicated to the right.

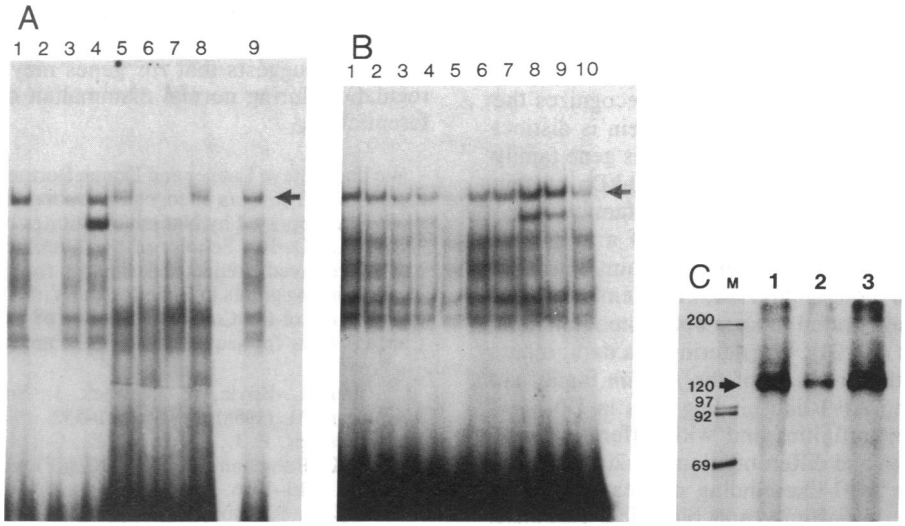

FIG. 4. A nuclear factor in *ras*-transformed cells recognizes *NVL-3* and TGF-β1 oligonucleotide sequences. Nuclear extract was prepared from DT cells. The exact sequences for oligonucleotides used are given in *Materials and Methods*. (*A*) Crude nuclear extract (5 μg) was incubated with a radiolabeled oligonucleotide probe corresponding to the *NVL-3* U3 box 1 sequence (lanes 1–4 and 9) or a radiolabeled oligonucleotide probe corresponding to the TGF-β1 sequence (lanes 5–8). Unlabeled oligonucleotides in lanes: 2 and 6, 40-fold molar excess of unlabeled *NVL-3* box 1 oligonucleotide; 3 and 7, 40-fold molar excess of unlabeled TGF-β1 oligonucleotide; 4 and 8, 40-fold molar excess of an unlabeled oligonucleotide corresponding to the binding site of the *myb* oncogene; 9, 200-fold molar excess of unlabeled *myb* binding site oligonucleotide. The mobility of the specific protein–DNA complex is indicated by an arrow to the right of the autoradiogram. (*B*) Crude nuclear extract (5 μg) was incubated with radiolabeled *NVL-3* box 1 oligonucleotide. Lanes: 2–5, unlabeled *NVL-3* box 1 oligonucleotide in 5-, 10-, 25-, and 50-fold molar excess, respectively; 6–10 contained unlabeled oligonucleotide corresponding to the *NVL-3* box 2 site in 5-, 10-, 25-, 50-, and 200-fold molar excess, respectively. The mobility of the specific protein–DNA complex is indicated by an arrow to the right of the autoradiogram. (*C*) Crude nuclear extract (5 μg) was incubated with the *NVL-3* box 1 probe that was labeled with bromodeoxyuridine. The complex designated with an arrow in *A* and *B* was identified by wet autoradiography, excised from the gel, and exposed to UV light. The cross-linked protein component was analyzed on a SDS/7.5% polyacrylamide denaturing gel. Lanes: 1, no competitor added to the gel retention assay; 2, a 40-fold molar excess of unlabeled *NVL-3* box 1 included; 3, a 40-fold molar excess of *myb* oligonucleotide included; M, ^{14}C-labeled protein size markers.

In contrast, an unrelated oligonucleotide that contains a putative binding site for the *myb* oncogene (16) does not abolish formation of the complex (Fig. 4*A*, lanes 4 and 8, 40-fold molar excess; lane 9, 200-fold molar excess).

The affinity of the nuclear factor present in transformed DT cells for oligonucleotides that contained either TGACTCT or TGAGTAA was analyzed by gel retention assays (Fig. 4*B*). In these experiments an oligonucleotide that contains the *ras*-specific site TGACTCT was used as probe. Increasing the amount of unlabeled homologous oligonucleotide resulted in competition for specific complex formation (Fig. 4*B*, lanes 2–5). Increasing the amount of heterologous oligonucleotide that contains only the sequence TGAGTAA had little effect on complex formation (Fig. 4*B*, lanes 6–9), although some competition was observed when high molar ratios of this competitor were included in the binding reactions (Fig. 4*B*, lane 10, 200-fold molar excess). The bands with higher electrophoretic mobility present in Fig. 4 *A* and *B* represent nonspecific complexes formed with single-stranded oligonucleotides (data not shown).

The size and number of proteins present in the specific complex demonstrated in Fig. 4 *A* and *B* was analyzed by UV-crosslinking (17). Such experiments reveal a single protein with an apparent molecular mass of 120 kDa in the complex (Fig. 4*C*, lane 1). If unlabeled competitor that contains the *ras*-responsive element is included in the gel retention reaction, crosslinking of DNA to the 120-kDa protein is decreased (Fig. 4*C*, lane 2), while including the *myb* competitor has no effect on crosslinking (lane 3). Other gel shift bands seen in Fig. 4 *A* and *B* do not contain the 120-kDa protein; extracts prepared from nontransformed cells also lack this protein (data not shown).

DISCUSSION

TGF-β1 mRNA is up-regulated by conditional expression of v-Ha-*ras* (Fig. 1). As was the case in NIH 3T3 cells (5), the conditional phenotype in NRK and Rat-1 cells is "leaky," indicated by the 10-fold higher levels of TGF-β1 RNA in unstimulated cells that contain exogenous oncogenes compared with wild type (Fig. 1). We assumed that the regulation of expression of rat and human TGF-β1 genes would be conserved, thus implying conservation of the sequence elements responsible for regulation. The conservation of both coding and regulatory sequences between human and rodent protooncogenes and growth-regulating genes (e.g., refs. 18 and 19) lends credence to this assumption. Consequently, we have demonstrated that a human TGF-β1 gene sequence located at base pair −420 acts as a *ras*-responsive enhancer element in transient transfection assays (Fig. 2*B*). The enhancer contains the sequence TGACTCT (Fig. 2*A*). This sequence is recognized by a nuclear factor present in *ras*-transformed cells (Fig. 4*A*). The same factor also recognizes the identical sequence present in the *NVL-3 ras*-responsive element (Fig. 4*A*). We conclude that transcriptional activation of the two genes by *ras* is mediated by a nuclear factor that recognizes the sequence TGACTCT.

The sequence TGACTCT is related to sequences activated by the transcription factor AP-1 (1, 2, 20). Since AP-1 is a heterodimer composed of c-fos and c-jun protooncoproteins (2, 20), we wished to directly test the role of the *fos* and *jun* genes on the *ras*-responsive enhancer. The data in Fig. 3 show that *fos/jun* in combination activate two target sequences almost equally well, while *ras* activates only one of them. Activation of the sequence TGACTCT by *ras* is more efficient than activation by *fos/jun* (Fig. 3*B*). Therefore, activation of the cis-acting elements by *ras* depends on a sequence specificity that is distinct from activation by AP-1.

In vitro analysis of DNA binding provides additional evidence that supports this conclusion. Nuclear extracts prepared from transformed cells contain a factor that has the same sequence preference as demonstrated by the transient transfection assays (Fig. 4*B*). The nuclear factor recognizes

oligonucleotides containing the consensus TGACTCT with higher affinity than those containing TGAGTAA (Fig. 4*B*).

Analysis of the protein component present in the gel shift complex indicates that a protein of 120 kDa recognizes the *ras*-responsive element. The size of this protein is distinct from fos or jun or other known products of this gene family (20, 21). However, we speculate that the 120-kDa protein may be the product of a member of this gene family.

The c-*fos* and c-*jun* genes are members of a multigene family that encode proteins with related domains for DNA binding specificity and dimerization (20, 21). Members of the family have been demonstrated to form both homodimer and heterodimer products (20, 21). Considering this data, others have suggested that the products of the *fos/jun* family are capable of recognizing AP-1-like binding sites in DNA sequences with different affinities and with different consequences for cell growth and differentiation (19, 20). Our data provide evidence that AP-1-like binding sites can indeed be recognized with differing affinity and biological response. Further purification and characterization of the 120-kDa nuclear factor may lend additional insight into how this may be accomplished.

Work by other groups has indicated that activated *ras* genes can rapidly increase expression of the c-*fos* (22, 23) and c-*jun* or *junB* genes (24). A consequence of this activation would be activation of genes that contain AP-1 binding sites (23, 25). However, the activation of *fos* and *jun* genes by *ras* is transient, lasting only a few hours (22–24). It has clearly been demonstrated that c-fos protein is not expressed constitutively in *ras*-transformed mouse 3T3 cells but remains inducible by serum (22). Likewise, recent evidence suggests that c-jun is absent in *ras*-transformed cells (26). We interpret this data to indicate that *ras* transiently activates a signal-transduction pathway that results in short-term increases in expression of fos and jun proteins as well as increases in expression of genes regulated by these proteins.

In contrast, our results indicate that *ras* can also persistently activate gene expression in transformed cells (9). This persistent pathway is rapidly activated following the expression of *ras* genes (5). Further, fos–jun complexes are not sufficient for transcriptional activation, but a 120-kDa protein is required for *ras*-responsiveness. Thus, the 120-kDA protein is a target of the persistent signaling pathway but should provide a focus for studies aimed at defining the persistent signaling pathway and its role in the transformation process.

A human sequence data base was analyzed by using the GCG programs (27) to determine if other genes contain the *ras*-responsive consensus sequence in 5′ noncoding positions. Examples revealed by this analysis include growth factor receptors and growth factors. For instance, both the human c-*neu* and c-*sis* protooncogenes contain a *ras*-responsive element in their 5′ flanking sequences. Genes that are expressed in a tissue-specific manner also contain this element (e.g., involucrin and enkephalin B). We hypothesize that the sequence TGACTCT identifies a set of *ras*-responsive genes. Activation of the expression of a subset of the genes may be relevant to tumor progression. For example, TGF-β1 has been described to play a key regulatory role in angiogenesis (28). Expression of such a gene during carcinogenesis might increase vascularization of a developing tumor, aiding tumor growth. That genes expressed in a very strict tissue-specific manner may also be subject to *ras* regulation suggests that *ras* genes may be involved in gene regulation during normal mammalian development and differentiation.

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