The heat-stable enterotoxins (ST) elaborated by enterotoxicogenic Escherichia coli are a family of small cysteine-rich peptides that bind to specific epithelial receptors in the mammalian intestine, causing a secretory diarrhea. The expression of ST receptors is tightly regulated; they are found primarily in intestine, and their expression is developmentally modulated. One receptor for ST has been cloned, and its cDNA encodes a ~120-kDa particulate guanylyl cyclase (guanylyl cyclase-C). Recent studies suggest that there are additional ST receptors that are not homologous to guanylyl cyclase-C. We used an expression cloning strategy to identify intestinal mRNAs that lead to expression of ST receptor activity in transfected cells. Using an ST-specific affinity panning system, we identified a novel 1891-base pair cDNA that does not encode a receptor protein, but instead, consists primarily of untranslated sequence. This cDNA induced receptor activity in both COS and 293 embryonic kidney cells. Northern analysis of the T84 human intestinal cell line, from which this cDNA was cloned, suggests that it is part of a 7.8-kilobase mRNA transcript. This transcript was also identified in human small intestine and colon, as well as in several extra-intestinal tissues. Functional analysis of subcloned fragments reveals that ST binding activity is induced by a 457-base pair human Alu repetitive sequence within the cDNA and that the phenotype is independent of orientation. These findings suggest that a human Alu element induces expression of a unique ST receptor by a trans-acting mechanism. An unrelated Alu-rich genomic clone did not confer ST binding, suggesting that there may be structural and functional specificity within individual Alu sequences.

Alu sequences are 300-bp repetitive elements that comprise ~6% of the human genome, where they are interposed between single copy genes. They are also present in the untranslated regions of ~10% of hnRNAs and mRNAs (1). Despite the abundance of Alu sequences, surprisingly little is known about their biological effects. There is some evidence implicating Alu sequences as regulators of cellular differentiation, and of developmental and tissue-specific gene expression (2–6).

The heat-stable enterotoxins (ST) are a family of small cysteine-rich peptides that are secreted by enterotoxicogenic Escherichia coli, a major etiologic agent of diarrhea for travelers and infants in the Third World. These peptide toxins bind to specific intestinal receptors. The toxin-receptor interaction causes increased chloride secretion, resulting in a secretory diarrhea (7). Recently, the mammalian intestinal peptide guanylin has been isolated and its cDNA cloned (8–10). The amino acid sequence, receptor specificity, and pharmacologic properties of guanylin are similar to ST, which suggests that guanylin is an endogenous ligand for ST receptors.

Although the physiologic role of ST receptors is unknown, their expression is tightly regulated. ST binding sites are sparse in intestinal crypt cells; they increase in abundance as these proliferating crypt cells become terminally differentiated villus enterocytes (11–13). ST receptors are also developmentally regulated, with maximal expression in embryonic and early infant life (14). The molecular basis for this cell specific and developmental expression has not yet been determined.

One ST receptor has been cloned, and its cDNA encodes a ~120-kDa particulate guanylyl cyclase (GC-C) (15, 16). However, biochemical studies of brush-border membranes suggest that there may be multiple intestinal ST-binding proteins, ranging in size from 45 to 160 kDa, some of which lack guanylyl cyclase activity. Some of these lower molecular weight species may be post-translationally processed fragments of GC-C (17, 18). Other ST-binding proteins may represent unique classes of receptors (19–21) that are not homologous to GC-C (22). This hypothesis is especially relevant, because another member of the particulate guanylate cyclase family (atrial natriuretic peptide receptor-A, molecular mass 130 kDa) has a cyclase independent, nonhomologous counterpart (atrial natriuretic peptide clearance receptor) that is a 70-kDa protein (23).

In the study described below, we used an expression cloning strategy to identify mRNAs that either encode novel ST receptors, or induce ST receptor expression in transfected cells. A unique 1.9-kb cDNA that induces ST binding activity when transfected into COS and human embryonic 293 cells was cloned and characterized. We show that the ST binding phenotype can be conferred by an Alu sequence within this cDNA. These findings demonstrate that an Alu sequence can induce expression of ST receptor activity and may do so by a trans-acting mechanism.

**EXPERIMENTAL PROCEDURES**

**cDNA Library**—The T84 human colon carcinoma cell line was obtained from ATCC and maintained as previously described (12). Total RNA was prepared from ~70% confluent cells (24); poly(A) RNA was then selected with two rounds of oligo(dT) chromatography. First strand synthesis was performed using 5 µg of poly(A) RNA, oligo(dT) primers, and avian myeloblastosis virus reverse transcriptase. After second strand synthesis, the resulting cDNA was ligated (25) to BstXI adapters
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**Results**

In order to identify novel ST receptors or genes inducing ST receptor expression, we used an SV40 based expression cloning strategy (25, 29). COS cells were transfected with a human intestinal cDNA library from the T84 cell line, a human colon carcinoma-derived cell line that expresses ST receptor activity. The transfected COS cells were then subjected to affinity panning using BST, a biotinylated derivative of ST, the preparation and pharmacologic properties of which we have previously described (12). Transfected cells expressing ST receptor activity were then captured onto plastic coated with an antibiotic antibody. Plasmids from the captured cells were extracted, and the process of transfection followed by affinity panning was repeated several times, thereby creating an enriched cDNA pool (25, 29). Ligand-specific capture of cells was ascertained by lack of adhesion in the presence of biotin alone or ST alone, and by competitive inhibition studies using ST.

**Identification of a cDNA Clone Exhibiting ST Receptor Activity**—Three iterative cycles of panning and transfection were performed (25). After the first cycle, <1% of cells were adherent to antibiotin-coated plates. When panning was done with the enriched plasmid pools (i.e. second and third cycles), ~20–30% of COS cells were adherent to the antibiotin-coated plates (not shown). Several clones from the third cycle, with inserts ranging in size from 2 to 4 kb, were selected from the enriched pool and individually tested for ST binding activity.

Three of these clones were tested by the ST-specific affinity panning assay described above. Clones 1 and 2 exhibited negligible adhesion (not shown). In contrast, clone 3 showed marked ST-specific adhesion (38.5 ± 1.3% S.E., where n = the number of plates analyzed, Fig. 1). Its adhesion was similar in magnitude to transfectants expressing GC-C-pSVL (guanylyl cyclase-C cDNA in the SV40 based expression vector pSVL), the positive control (34.3 ± 3.7%) (12). These values are consistent with the transfection efficiency of ~35–45% that we obtain using pCH110, an SV40 based expression vector encoding lacZ (12). The adherence of clone 3 transfectants was ~5-fold greater than the level of adhesion seen with the transfected vectors pCEV4 and pSVL (without insert), which were used as negative controls. Adherence was ST specific in that it was reduced to 3.0 ± 0.6% in the presence of excess unlabeled ST (Fig. 1), but not affected by equimolar concentrations of an unrelated ligand, atrial natriuretic peptide (α human 1–28) (33.0 ± 0.8%, n = 4; not shown). Furthermore, adhesion did not occur in cells transfected with clone 3, if BST was replaced with native ST or with biotin (<1%, not shown).

To further validate these findings, clone 3 was also transfected into human embryonic kidney 293 cells as described under "Experimental Procedures." Like COS cells, 293 cells are easily transfected with DEAE-dextran, and do not express endogenous ST receptors. Data using the ST-specific affinity panning assay are shown in Fig. 1B. Human 293 cells transfected with clone 3 showed BST-dependent adhesion to antibiotin plates (43.5 ± 3.8% S.E.), and this adhesion was markedly inhibited by the presence of excess unlabeled ST (5.3 ± 4.0%). 293 cells transfected with GC-C (positive control) were adherent (54.3 ± 2.4%) when incubated with BST, and this adherence was also inhibited in the presence of excess unlabeled ST (5.3%, n = 2). Vectors transfected 293 cells (negative controls) showed minimal BST-dependent adhesion (6.9 ± 2.5%). These findings demonstrate that ST receptor activity is inducible in human (293) as well as in monkey (COS) cells.

Six clones obtained from the enriched library, including clone 3, were individually transfected. The transfected cells were tested for ST-receptor activity using 125I-STAs in a radioligand filter-binding assay (12, 28). No specific binding was detected in
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Fig. 1. A, affinity panning of clone 3. In brief, transfected COS cells were incubated with 10 μM BST for 1 h (25 °C), washed, seeded onto antibiotin-coated plates, and the percent adherent cells determined after washing as previously described (12). In the experiment labeled clone 3, 10 μM antibiotin was substituted for BST. In experiments labeled competition, the cells were preincubated for 30 min with 3 μM ST1b followed by addition of BST (10 μM), and incubation continued for 1 h. pCEV4 corresponds to cells transfected with vector. N represents the total number of plates analyzed, and error bars show the S.E. for all plates tested with a given clone. GCC corresponds to COS cells transfected with clone GC-C-pSVL. GCC competition was carried out by incubating transfected cells with excess unlabeled ST1b as above. pSVL is the SV40 expression vector without insert, into which GC-C-pSVL was cloned. The three experiments with GC-C-pSVL have been previously published (12), and are presented here as a positive control.

B, human embryonic kidney 293 cells were transfected using DEAE-dextran with chloroquine as described under “Experimental Procedures.” Affinity panning experiments were performed as described in A. these transfectants, in contrast to GC-C-pSVL transfectants, which exhibited ~10% specific binding. Cells transfected with the positive control GC-C-pSVL showed a ~25–50-fold increase in intracellular cGMP (16) in the presence of 4 μM BST or ST1b. In contrast, clone 3 showed no guanylyl cyclase stimulation in response to these ligands (not shown). This indicates that although COS cells transfected with either clone 3 or with GC-C will bind ST specifically in the affinity panning assay, the ST binding phenotypes induced by these two cDNAs are different. Affinity panning has been demonstrated to enrich for low affinity interactions through multiplicative binding (30). We hypothesize that transfection with clone 3 induced expression of ST-specific low affinity binding that was detectable by affinity panning, but not by the less-sensitive radioligand binding assay.

The ~2.0-kb insert (clone 3) was used as a probe to identify additional hybridizing species in the T84 cDNA library by Southern blotting (Fig. 2). A prominent band, approximately the same size as the clone 3 insert, was seen in the enriched cDNA library (Fig. 2, lane 2), suggesting that iterative panning had enriched for this clone.

Sequence Analysis—The nucleotide (nt) sequence of clone 3 (1891 bp) is shown in Fig. 3A, and a schematic representation of its organization is presented in Fig. 3B. The sequence orientation is presented with respect to the SV40 promoter of pCEV4. Analysis of this sequence revealed an incomplete open reading frame extending from nt 1 to 449, that was in the reverse complement orientation to the SV40 promoter. The deduced polypeptide sequence of this potential reading frame was interrupted after amino acid 149, where it rejoined the vector. The Kozak consensus sequence neighboring the ATG at nt 449 was unfavorable (31). The hydropathy profile of this putative polypeptide was not suggestive of a transmembrane protein, and it had no significant similarities to protein sequences in
gested with XhoI-SstI, all other DNA was digested with uncharged nylon, and cross-linked (W-Stratalinker, Stratagene). The inserts. Samples were electrophoresed (1% agarose), capillary blotted to T84 cDNA library before selection.

Alu cluster, with a mid-poly(A) tract. Both of these sequence motifs are highly abundant in cellular mRNA.

Hybridization was performed at 65 °C in rapid hybridization buffer (Amersham); this was followed by washing at a final stringency of 0.7 x SSC, 0.1% SDS (65 °C). Lane 1 contains 2 µg of plasmid DNA from the T84 cDNA library before selection. Lane 2 contains 2 µg of DNA from the enriched plasmid library recovered after 3 rounds of panning. Lane 3 contains GC-C p-SVL (1 µg). Lane 4 contains vector pCEV4 (1 µg). A smear of hybridizing signal was seen in both the Lanes 1 and 2, but was absent in the lanes containing vector alone (Lane 3) or GC-C-pSVL (Lane 4). In view of the sequence (Fig. 3), it is likely that this diffuse pattern of lane-specific hybridization is due to the presence of a 638-bp Alu cluster, with a mid-poly(A) tract. Both of these sequence motifs are highly abundant in cellular mRNA.

GenBank. Based on this analysis, we conclude that this open reading frame is unlikely to encode a protein with structural features typical of a cell surface receptor.

Analysis at the nucleotide level revealed that the clone contains an Alu cluster (nt 516-1153). This cluster is comprised of two human-specific Alu repeats from different subfamilies, that are arranged in a nested configuration (Fig. 3B). Fig. 3C shows both Alu repeats aligned to their consensus sequence in the reverse complementary orientation relative to their original presentation in Fig. 3A. The evolutionarily younger of the two, classified as Alu-Sx (651-1007), is flanked by 7-bp tandem repeats (CCAGGTA, Fig. 3A, lower case letters) that were found in both the Lanes 1 and 2, but was absent in the lanes containing vector alone (Lane 3) or GC-C-pSVL (Lane 4). In view of the sequence (Fig. 3), it is likely that this diffuse pattern of lane-specific hybridization is due to the presence of a 638-bp Alu cluster, with a mid-poly(A) tract. Both of these sequence motifs are highly abundant in cellular mRNA.

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Functional Analysis of Subclones for ST Binding Activity—In order to determine the sequence requirements needed for expression of the ST-binding phenotype, various regions of clone 3 were subcloned and then tested for ST binding activity using the affinity panning assay described above (Fig. 4). In order to establish whether the ST binding activity was attributable to the partial open reading frame, subclone pS604 was constructed. This construct contains the partial reading frame in the same orientation as the SV40 promoter, plus 85 bp of the right Alu-J monomer. Transfection of pS604 resulted in a small amount of cellular adhesion (6.4 ± 1.8%) that was only slightly greater than the vector background (3.1 ± 0.1%), and markedly less than the full-length cDNA (38.5 ± 1.3%). Next, we tested pS1891 (nt 605-1891), which contains the remaining sequence of clone 3 (most of the Alu cluster as well as the AT-rich 3' end). Transfectants exhibited ligand-dependent adhesion that was similar in magnitude (33.8 ± 5.6%) to that of the full-length clone (38.5 ± 1.27%). This adhesion was specific in that it was competitively inhibited by unlabelled ST to a background level (3.2 ± 1.3%).

We next asked whether this phenotype was conferred by a more circumscribed region of the noncoding sequences of clone pS1891. To study this, we tested a construct containing the region that was 3' to the Alu cluster (pS1243 (nt 1243-1891); Fig. 4). Cells transfected with clone pS1243 did not exhibit BST binding activity (3.24 ± 0.7%).

The remaining region to be tested was the Alu cluster. A 632-bp fragment containing nt 605-1237 was subcloned (pS1237). It contained the truncated right Alu-J monomer, the full S dimer, and the complete left J monomer, and in addition, 83 bp of a non-Alu flanking sequence (nt 1154-1237). When transfected into COS cells, pS1237 was found to be highly active, with 33.6 ± 3.4% of cells adherent to antibiotin. Adherence was inhibited by excess unlabeled toxin (6.9 ± 3.1%), indicating that it was ST-specific.

Sequences that flank certain Alu elements have been shown to influence their cellular effects (4). Therefore, a construct containing sequences within the Alu cluster, but excluding flanking sequences was tested. This construct (pS1062, in the same orientation as Clone 3) contained the intact Alu-Sx dimer, flanked on both sides by truncated J monomers. When COS cells were transfected with pS1062, BST binding was preserved and in fact slightly increased (44.6 ± 2.0%), compared to the full-length clone. Ligand-dependent adherence was reduced to 3.6 ± 0.8% by excess unlabeled toxin, again demonstrating specificity for ST. Thus, the Alu sequence was sufficient to influence this phenotype.

Finally, we tested the effect of orientation with respect to the SV40 promoter, on expression of the ST binding phenotype. Two constructs (pS1679 forward and reverse), corresponding to nt 605-1679, were transfected and tested. The cellular adhesion was 37.9 ± 4.3% for construct pS1679-forward, which was in the same orientation as clone 3, and only slightly lower (31.9 ± 3.1%) for the subclone in the opposite orientation (pS1679-reverse).

Transfection of COS Cells with HSAG-1, An Alu-rich Genomic Clone—Having established that an Alu sequence induces this ST-binding phenotype, we tested a different Alu-rich gene known as HSAG-1 (human surface antigen). This 3.4-kb genomic clone is derived from a human-Chinese hamster ovary
Nu-J sequence, and splits it into two parts (Alu-J-Right, cluster, and an AT-rich untranslated region. Alu sequences were human-specific, and consist of an Alu dimer was divided into three general domains. These consist of the 5' region flanking Nu, which contains a truncated potential reading frame, the Alu cluster 516-643, and flanking repeats are in the left and right arms of Alu-J. To human transfected into HeLa cells (35-37). It was therefore of interest to human and the transfectants tested for ST receptor activity by affinity panning. The transfected cells showed negligible adhesion of these repeats are rodent specific, and others have homology to human Alu (J-family). HSAG has been shown to induce expression of a leukemia-associated cell surface antigen in stable transfectants, and also to inhibit cell proliferation when transfected into HeLa cells (35-37). It was therefore of interest to test the biologically active HSAG clone in our system, to address the question of whether other Alu sequences could also induce ST binding activity. HSAG-1 (under the same SV40 promoter as GC-C-pSVL, Fig. 1) was transfected into COS cells, and the transfectants tested for ST receptor activity by affinity panning. The transfected cells showed negligible adhesion (1.5 ± 0.7%, n = 5) to antibiotic after incubation with BST.

Northern Analysis of Transfected Cells, the T84 Cell Line, and Human Tissues—In order to study the expression of clone 3-specific RNAs in transfected COS cells, probes corresponding

Fig. 3. Sequence analysis of clone 3. A, complete nucleotide sequence. Double stranded sequencing was performed (Sequenase 2, U. S. Biochemical Corp.). The sequence was analyzed using IntelliGenetics Suite (IntelliGenetics Corp., Mountainview, CA). The Alu cluster is underlined, and flanking repeats are in lower case letters. B, overview of sequence domains. For the purposes of structure-function analysis, the clone was divided into three general domains. These consist of the 5' region flanking Alu, which contains a truncated potential reading frame, the Alu cluster, and an AT-rich untranslated region. Alu sequences were human-specific, and consist of an Alu dimer (851-1005, flanked by 7-bp tandem repeats) with a 79-bp mid-poly(A) tract, belonging to the Sx evolutionary family. The dimer is inserted into the middle of the evolutionarily older Alu-J sequence, and splits it into two parts (Alu-J-Right, 1015-1153) (32, 33). C, alignment of Alu-Sx and both halves of Alu-J to the Alu consensus. The regions containing "..." identify to the consensus. The symbol "-" shows alignment gaps, and "xx" separates the left and right arms of Alu-J.
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FIG. 4. Affinity panning with subcloned fragments of clone 3. Transfections and panning were performed as described in the legend to Fig. 1 using plasmids containing various fragments of clone 3. Transfections were performed at least twice for each plasmid construct; n represents the total number of plates analyzed. Constructs shown with the symbol (+) represent transfectants incubated with BST alone. The symbol (−+) represents a competitive inhibition control containing saturating concentrations of ST in the presence of BST.

to the regions directly flanking Alu were prepared. These probes contained the non-Alu flanking sequences of clone 3 (nt 1–517, 5' probe and nt 1243–1891, 3' probe). Although these flanking sequences do not confer receptor activity to transfected cells, they were used for Northern analysis in order to avoid nonspecific hybridization with the many Alu-like sequences that are present in human RNA (Fig. 2). As expected, Fig. 5A (5' probe) demonstrates that COS cells transfected with clone 3 produce an abundant ~2.2-kb transcript that is easily detectable in total RNA (Lane 2). This is consistent with RNA polymerase II transcription of the cDNA insert originating at the SV40 promoter of pCEV4. No hybridization was detected in COS cells transfected with vector alone (Lane 1).

Fig. 5B shows a Northern blot containing 5 µg of poly(A) RNA from the T84 cell line, probed with the 5'-flanking sequence. A single ~7.8-kb transcript was detected, suggesting that clone 3 is part of an expressed message in the T84 cell line. The transcript could not be detected in 35 ug of total RNA from T84 cells (not shown), indicating that it is of relatively low abundance. Fig. 5C demonstrates that there is a single ~7.8-kb transcript detectable in poly(A) RNA from other human tissues including spleen, thymus, testes, ovary, small intestine, and colon (3' flanking probe). The 5' probe showed faint bands in the same region of the human tissue blot (not shown). In contrast, when this human tissue blot was reprobed with GC-C-specific sequences, transcripts (~4 kb) were detected only in small intestine and colon (not shown), consistent with previously published work (15).

DISCUSSION

In this report, we describe a novel cDNA from the T84 human intestinal cell line that induces heat-stable enterotoxin binding activity in transfected COS cells and in human embryonic kidney 293 cells. The phenotype seen in these transfected cells fulfills criteria for receptor activity in that it is ST-mediated, and specifically inhibited by excess unlabeled ligand. Furthermore, incubation of transfected cells with biotin alone, or with unlabeled toxin does not promote their adherence in this affinity panning system. Blocking the immobilized antibody with biotin abolishes this ligand-dependent cellular adherence. Taken together, these data indicate that transfection of cells with this cDNA leads to the expression of specific ST binding activity by the transfected cells. By testing subcloned fragments of the cDNA, we established that a 457-bp fragment composed of Alu repetitive sequences within the cDNA is sufficient to induce expression of this phenotype, and that regions of the cDNA that did not contain Alu were inactive. This Alu sequence is somewhat unusual; it is a cluster of two nested Alu dimers of different evolutionary ages containing a mid-poly(A) tract that is preserved without mutations expected in Alu sequences of this evolutionary age. The locations of both the poly(A) tract and the flanking repeats of Alu conform to the consensus. This supports the view that this is a naturally occurring sequence, and raises the possibility that the mid-poly(A) tract may be a conserved region.

This cDNA was selected by affinity panning. The ST-binding phenotype of the clone reported here was the same as the GC-C receptor, as determined by the affinity panning assay used here. However, it differs from GC-C in that it neither induces specific binding to 125I-STa, nor activates guanylyl cyclase in response to toxin. Because the panning assay entails multiplicative ligand-receptor interactions, it is inherently more sensitive than solution-phase radioligand binding. Thus, it is likely that our use of iterative panning led to selection of a low affinity ST binding phenotype, that was not detectable with radioligand binding, but only detectable in the more avid affinity panning system. Indeed, it has been demonstrated that affinity panning strongly selects for low affinity interactions (30). Many ligands, including nerve growth factor, interleukin-7, and peptide-major histocompatibility complexes participate in biologically important low-affinity, ligand-specific, receptor-mediated events (38–40). Recently low-affinity cyclase-independent ST binding sites have been identified in an intestinal crypt cell line (22); this activity may be relevant to the phenotype described here.

A concern about the Alu-induced phenotype reported here is that it has been identified using transient expression systems, where transfected plasmids containing SV40 promoters are replicated to high copy number, and could potentially cause nonspecific effects. To address this issue, we transfected COS cells with HSAG-1, an Alu-rich genomic clone (driven by the same SV40 promoter as the GC-C-pSVL clone), and it did not induce ST binding. Although the high level of replication of clone 3 in transfected COS cells may have enhanced detection of ST binding, the negative result with HSAG indicates that there may be structural and functional specificity within individual Alu elements.

Northern analysis of COS cells transfected with clone 3 demonstrates the presence of an abundant ~2-kb RNA transcript,
consistent with RNA polymerase II transcription initiated at the SV40 promoter (Fig. 5A). Northern analysis in the T84 intestinal cell line, from which this cDNA was derived, as well as other human tissues, suggests that it is part of a 7.8-kb mRNA transcript (Figs. 5, B and C). This 7.8-kb mRNA was found in spleen, thymus, prostate, ovary, and testis, as well as in small intestine and colon (Fig. 5C). This is in contrast to the ~4-kb GC-C transcript, which is found is detected only in small intestine and colon (15). Differences in the expression of these two mRNA species are not surprising considering that, 1) the nucleotide sequences of clone 3 and of GC-C are unrelated, and that 2) the pharmacological properties of cells transfected with clone 3 are distinct from those transfected with GC-C. Furthermore, unlike GC-C the Alu sequence does not itself encode a receptor protein, but instead induces expression of ST binding activity. From these observations we hypothesize that this Alu sequence might regulate other cellular functions in tissues that do not express ST binding activity.

Other Alu-rich mRNAs have also been associated with specific cellular effects. Garret et al. (41) described a gene that induces expression of tumor necrosis factor receptor activity. This gene had no significant open reading frames, but contained many Alu-rich regions. Similarly, Koga et al. (42) identified 12 unique mRNAs that were specifically expressed by T cells in response to human immunodeficiency virus infection. These mRNAs were all Alu-rich untranslated sequences. In both reports, however, the relationship of the Alu sequences to their biologic effects was not investigated. Here we specifically demonstrate that the 457-bp Alu element is both necessary and sufficient for induction of ST binding activity.

The precise mechanism of this induction is unknown. Several models, based on current knowledge of the biology of Alu sequences can be proposed. Alu sequences are found in ~10% of mRNAs, and are usually located within the untranslated regions of these RNA polymerase II transcripts (1). Alu sequences have been shown to act in cis, as both transcriptional or post-transcriptional regulatory elements, through RNA-RNA interactions (see Ref. 1, for review). In addition, Alu family members are ~90% homologous to the translational arrest domain of 7SL RNA. Because of this homology, Alu sequences in mRNAs have the potential to modulate gene expression by interacting with cellular proteins of the signal recognition particle, as well as other cellular proteins (43, 44). In the present study, Alu sequences in both orientations can induce the ST binding phenotype, and thus may be working through a trans-acting mechanism. One possible mechanism for these results is that the transfected Alu sequence is exerting its effect by titrating specific cellular inhibitors that normally regulate transcription or translation (45). Whether the mechanism involves RNA-RNA interactions, or RNA-protein interactions, is presently unknown.

In summary, we have cloned an 1891-bp cDNA that induces ST binding activity in transfected cells, and have demonstrated that a 457-bp Alu sequence within this cDNA is sufficient to induce expression of a unique cellular receptor. Our data suggest that this Alu sequence is part of an expressed message in human tissue, and that it may work through a trans-acting mechanism. We propose that this sequence may belong to a newly emerging class of mRNAs that are untranslated and yet play a regulatory role (46) in eukaryotic cell biology.

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