Decreased Stability of Transforming Growth Factor β Type II Receptor mRNA in RER$^+$ Human Colon Carcinoma Cells†

Wen Jiang, Manoranjani P. M. Tillekeratne, Michael G. Brattain, and Sunandita S. Banerji*

Department of Biochemistry and Molecular Biology, Medical College of Ohio, Toledo, Ohio 43699

Received July 23, 1997; Revised Manuscript Received October 6, 1997⊗

ABSTRACT: Transforming growth factor β (TGF-β) is a potent inhibitor of cell growth and tumor progression. Previous work has shown that loss of functional TGF-β type II receptor (RII) due to a frameshift mutation in the 5′ half of the RII gene leads to TGF-β resistance in a highly progressed, RER$^+$ human colon carcinoma cell line designated HCT116. Expression of this mutated RII gene was highly repressed in RER$^+$ cell lines such as HCT116 and RKO, as analyzed by RNase protection assays. Nuclear run-on and RII promoter−reporter (CAT) assays showed that the transcriptional levels of the RII gene in these RER$^+$ cells were not reduced, compared to RII-expressing cells. However, the half-lives of the RII mRNA, as analyzed by RNase protection assays following actinomycin D treatment, were significantly decreased. This suggested that the decreased expression of the RII gene mutant was due to decreased mRNA stability. Furthermore, RII mRNA from HCT116 transfected with wild-type RII had a longer half-life than the endogenous mutated RII mRNA. A dominant negative RII mutant, which encodes a similarly truncated RII protein as HCT116 but lacks the extensive 3′ untranslated region of RII mRNA, gave the same half-life as endogenous wild-type RII mRNA. We conclude that the frameshift mutation which results in a premature stop codon in the 5′ half of the mRNA transcript accounts for the reduced RII mRNA levels in RER$^+$ cells.

Transforming growth factor β (TGF-β)[1] has been shown to regulate many cellular processes including cell proliferation, differentiation, and extracellular matrix formation in multiple cell types (*1*, *2*). TGF-β acts as a potent inhibitor of cell growth and tumor progression, and loss of this negative regulation of TGF-β can contribute to tumor development. This growth factor exerts its effects through specific binding to cell surface receptors. Three distinct cell surface receptors, type I (RI), type II (RII), and type III (RIII), have been cloned and characterized. Both RI and RII are transmembrane serine/threonine kinase receptors, and the presence of both receptor types appears to be necessary for TGF-β signal transduction (*3*, *4*, *5*). After direct binding of TGF-β to RII, whose kinase activity is constitutively active, the TGF-β/RII complex is recognized by RI. RI is then recruited into the complex and becomes phosphorylated by RII. Phosphorylation of RI initiates signal propagation to downstream targets (*6*). RIII is a transmembrane proteoglycan lacking any intrinsic signaling ability, but may be involved in presenting TGF-β ligands to RI and RII (*7*).

The direct involvement of RI and RII in TGF-β signal transduction indicates that the loss of functional RI and/or RII expression could contribute to the loss of TGF-β responsiveness, resulting in a growth advantage that, in turn, contributes to tumor progression. In colon cancer cells, RII is of particular interest, because RII inactivation is common in tumor cells exhibiting microsatellite instability (termed RER) (*8*). RER$^+$ is associated with mismatch repair defects and characterized by genetic alteration of simple repeated sequences (*9*, *10*, *11*). Previous work in our laboratory has found that reconstitution of TGF-β autocrine activity by re-expression of RII can reverse malignancy in the RER$^+$ colon cancer cells HCT116, thus verifying that malignancy of hereditary nonpolyposis colorectal cancer is directly associated with loss of TGF-β RII expression (*12*). Inactivation of RII appears to be a critical step in tumor progression rather than a simple correlation of the RER$^+$ phenotype. Thus, RII mutation links DNA repair defects with a known pathway of tumor progression (*8*).

The FET colon carcinoma cell line was derived from an early stage human colon cancer and as such reflects the properties of early malignant cells rather than highly progressed cells (*13*, *14*). FET is highly differentiated but poorly invasive. It has normal RII mRNA levels and functional RII protein. In contrast, HCT116, a highly progressed and poorly differentiated late stage cell line, has very low RII mRNA levels and nondetectable cell surface RII protein (*8*). Sequence analysis of the 5′ half of the RII cDNA from the HCT116 cells showed a homozygous loss of 1 adenine within a sequence of 10 repeating adenines at nucleotides 709−718 (*12*). This one base pair deletion gives rise to a premature stop codon at nucleotide 820, and, thus, encodes a truncated protein of 161 amino acids instead of the 565 amino acids of wild-type RII. Similar RII mutations resulting from one or two base pair deletions within this polyadenine tract have been detected in other RER$^+$ colon cancer cells and primary RER$^+$ colon cancer tissues (*8*). Earlier reports demonstrated that there was no detectable truncated RII in the FET cells (*8*). In this report, we

† This work was supported by National Institutes of Health Grants CA38173, CA50457, and CA72001.

* To whom correspondence should be addressed. Telephone: (419)-381-4137. Fax: (419)382-7395.

⊗ Abstract published in *Advance ACS Abstracts,* November 15, 1997.

[1] Abbreviations: TGF-β, transforming growth factor β; RI, RII, and RIII, transforming growth factor β receptor types I, II, and III, respectively; RER, replication error(s); (k) bp, (kilo) base pair(s); PAGE, polyacrylamide gel electrophoresis; CAT, chloroamphenicol acetyltransferase.

S0006-2960(97)01789-3 CCC: $14.00 © 1997 American Chemical Society

investigated the mechanism for the reduced RII levels in RER^+ cells. Our results showed that reduced truncated RII expression in RER^+ cells is a result of decreased RII mRNA stability. Furthermore, the premature stop codon generated by frameshift mutation in the polyadenine tract was involved in this message instability.

MATERIALS AND METHODS

Cell Culture. The FET, CBS4, HCT116, and RKO human colon cancer cell lines were established *in vitro* from primary tumors and maintained as described previously (*14*, *15*). VACO481 cells were maintained in MEM 2+ medium as previously described (*16*). A typical limiting dilution clone of HCT116 and stable transfected lines, HCT116-RII37, HCT116-Neo (*12*), and CBS4-DN (Ye and Brattain et al., unpublished), were maintained as previously described.

RNA Analysis. Total RNA from subconfluent cells was isolated by guanidine thiocyanate homogenization and purified through a cesium gradient (*17*). To determine TGF-β RII mRNA levels, RNase protection assays were performed as previously described (*18*, *19*). Briefly, 40 μg of total RNA was hybridized with ^{32}P-labeled RII riboprobes at room temperature overnight. The hybridization mixture was then digested with RNase A and RNase T1 to remove free probes and nonduplexed RNA followed by precipitation. The protected fragments were resolved on a 6% PAGE gel containing 7 M urea and visualized by autoradiography. The antisense probes used to detect RII mRNA expression were previously described (*19*; Ko and Brattain et al., unpublished). The actin-protected fragment was used to normalize sample loading as described previously (*18*).

Nuclear Run-On Assays. Isolation of nuclei was performed as described previously (*20*). Briefly, cells were homogenized in lysis buffer [0.05% Nonidet P-40, 300 mM sucrose, 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 8.0), 3 mM $MgCl_2$, 0.25 mM EDTA, 0.5 mM dithiothreitol, 10 μg/mL leupeptin, 10 μg/mL chymostatin, 25 μg/mL soybean trypsin inhibitor, and 0.1 mM phenylmethanesulfonyl fluoride] with a Wheaton tissue homogenizer. Nuclei were then pelleted by centrifugation, resuspended in storage buffer (50 mM Tris-HCl, pH 8.3, 40% glycerol, 5 mM $MgCl_2$, and 0.2 mM EDTA), and quick-frozen in dry ice−ethanol. To obtain radioactive nascent RNA transcripts, nuclei were thawed and transcripts were elongated at 30 °C for 30 min in the presence of 0.4 mM each ATP, CTP, and GTP, 4 μM UTP, and 100 μCi of [α-^{32}P]UTP (3000 Ci/mmol, Dupont, NEN). Chromatin and protein were then removed from this reaction mixture by DNase I and proteinase K digestion, followed by phenol/chloroform extraction and ethanol precipitation. ^{32}P-labeled incorporated material was purified away from unincorporated radioactivity using a NACS-52 column (Gibco BRL). An equal amount of radioactivity was used for each hybridization reaction. Eight micrograms of linearized and denatured RII cDNA-plasmid and pBluescript SK (pBSK) was immobilized on nitrocellulose membranes before hybridization at 42 °C for 72 h with the ^{32}P-labeled RNA. After hybridization, filters were washed twice in 2× SSC solution with 0.1% SDS for a total of 60 min, and then incubated in 2× SSC containing RNase A (10 μg/mL) for 30 min at 37 °C. Transcripts were visualized by autoradiography.

Transient Transfections and CAT Assays. Ten micrograms of pSV-β-galactosidase vector (Promega) and 30 μg of RII promoter-CAT chimeras (−1.8 kb RII/CAT and −274 bp RII/CAT; gift of Drs. Seong-Jin Kim and Anita B. Roberts) or a promoterless-CAT expression plasmid were transiently transfected into subconfluent cells by electroporation at 300 V and 960 μF in a Biorad Gene Pulser. Cells were harvested 48 h after electroporation, and total RNA was extracted. Lysate was prepared from a portion of the cells for use in β-galactosidase assays performed. The levels of CAT mRNA expressed by the transient transfectants were measured by RNase protection assays as described above. Sample loading was normalized by β-galactosidase assays (*21*). All transfections were performed a minimum of 3 times.

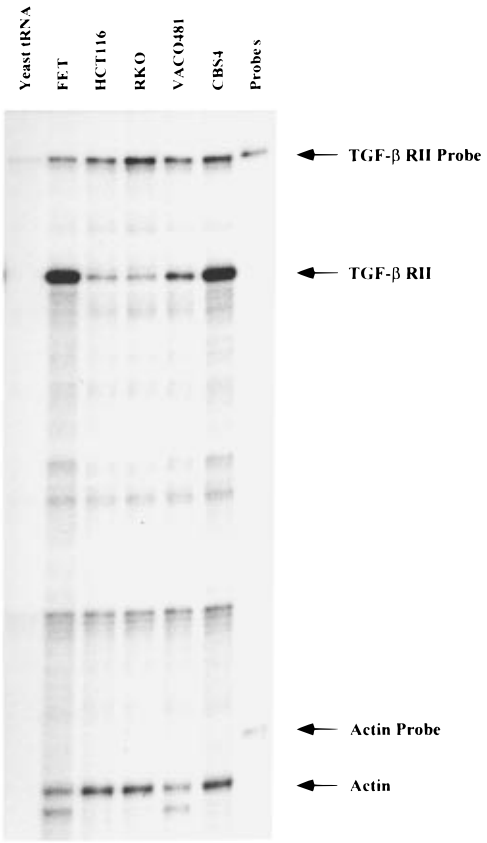

FIGURE 1: Expression of TGF-β RII mRNA levels in human colon cancer cell lines. Forty micrograms of total RNA from each cell line was hybridized with the ^{32}P-labeled RNA probes indicated. The hybridization mixture was then digested with RNase A and RNase T1 to remove free probes and nonduplexed RNA. Protected fragments were resolved on a 6% PAGE gel containing 7 M urea and visualized by autoradiography. RII mRNA levels in each cell line are shown. Actin mRNA levels were used for normalization of sample loading.

Determination of RII mRNA Half-Life. Subconfluent cells were treated with 5 μg/mL actinomycin D, and total RNA was harvested at 0, 0.5, 1, 2, 4, and 6 h after treatment. RNase protection assays were performed as described above. Quantitation was performed by densitometry using the Ambis system after an optical scan of the respective autoradiographs. Material in RII bands was normalized to actin within the same lane. All half-life experiments were performed a minimum of 3 times.

RESULTS

Expression of TGF-β RII mRNA in HCT116 and FET Cells. HCT116, a RER^+ cell line, has been previously shown

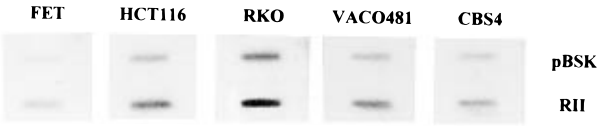

FIGURE 2: Transcriptional activity of TGF-β RII in human colon cancer cell lines. Eight micrograms of linearized RII cDNA-plasmid and pBluescript SK (pBSK) was immobilized on nitrocellulose membranes before hybridization with ^{32}P-labeled transcription products from isolated nuclei. pBSK plasmid was used as a negative control.

to have low levels of RII transcripts and nondetectable cell surface RII protein (*8*). RNase protection assays were performed to determine the steady-state RII mRNA levels in HCT116 cells and other colon cancer cell lines. HCT116 cells had substantially lower RII mRNA levels than FET cells (Figure 1). No difference was found in RI mRNA levels in FET and HCT116 cells (data not shown).

Transcriptional Activity of TGF-β RII in HCT116 and FET Cells. The basis for decreased RII mRNA levels in HCT116 cells relative to FET cells was examined. Nuclear run-on assays were carried out to determine whether the low levels of RII mRNA in HCT116 were caused by reduced transcriptional activity of RII. As shown in Figure 2, transcription of RII in HCT116 cells was not reduced compared to that in FET cells. As an alternative approach to assess transcription of RII and to confirm the results of nuclear run-on assays, RII promoter−reporter (CAT) transient transfections were performed. The CAT assay reflects the availability of the transcription factors necessary for transcription of the RII promoter-CAT chimeras. A promoterless-CAT construct and two RII promoter-CAT chimeras with different lengths of RII promoter were transiently transfected into subconfluent cells. The −1.8 kb RII/CAT chimera, consisting of 1.8 kb from the 5′ region of the RII gene, and the −274 bp RII/CAT chimera, containing the proximal −274 bp of this region, have been shown to be well expressed in HepG2 cells (*22*). RNase protection assays were done to measure CAT mRNA levels, which, in turn, reflect RII promoter activity. No significant differences in promoter activity were observed between the HCT116 and FET cell lines for either the −1.8 kb RII/CAT or the −274 bp RII/CAT construct (Figure 3). A slightly higher promoter activity was observed in cells transfected with the −1.8 kb RII/CAT construct in all cell lines tested. Taken together, nuclear run-on assays and CAT assays both indicate that decreased RII levels in HCT116 cells are not due to decreased transcriptional activity of the RII gene.

Stability of TGF-β RII mRNA in HCT116 and FET Cells. Transcriptional rates of RII in FET and HCT116 cells appeared similar, despite the obvious difference in steady-state levels of RII mRNA. Therefore, we next tested the stability of RII messenger RNA in FET and HCT116 cells. Subconfluent cells were treated with 5 μg/mL actinomycin D, and total RNA was extracted at different times following treatment. RNase protection assays were then performed to measure the levels of RII mRNA without the effects of new synthesis. Representative data of half-life experiments in FET and HCT116 cells are shown in Figure 4. The

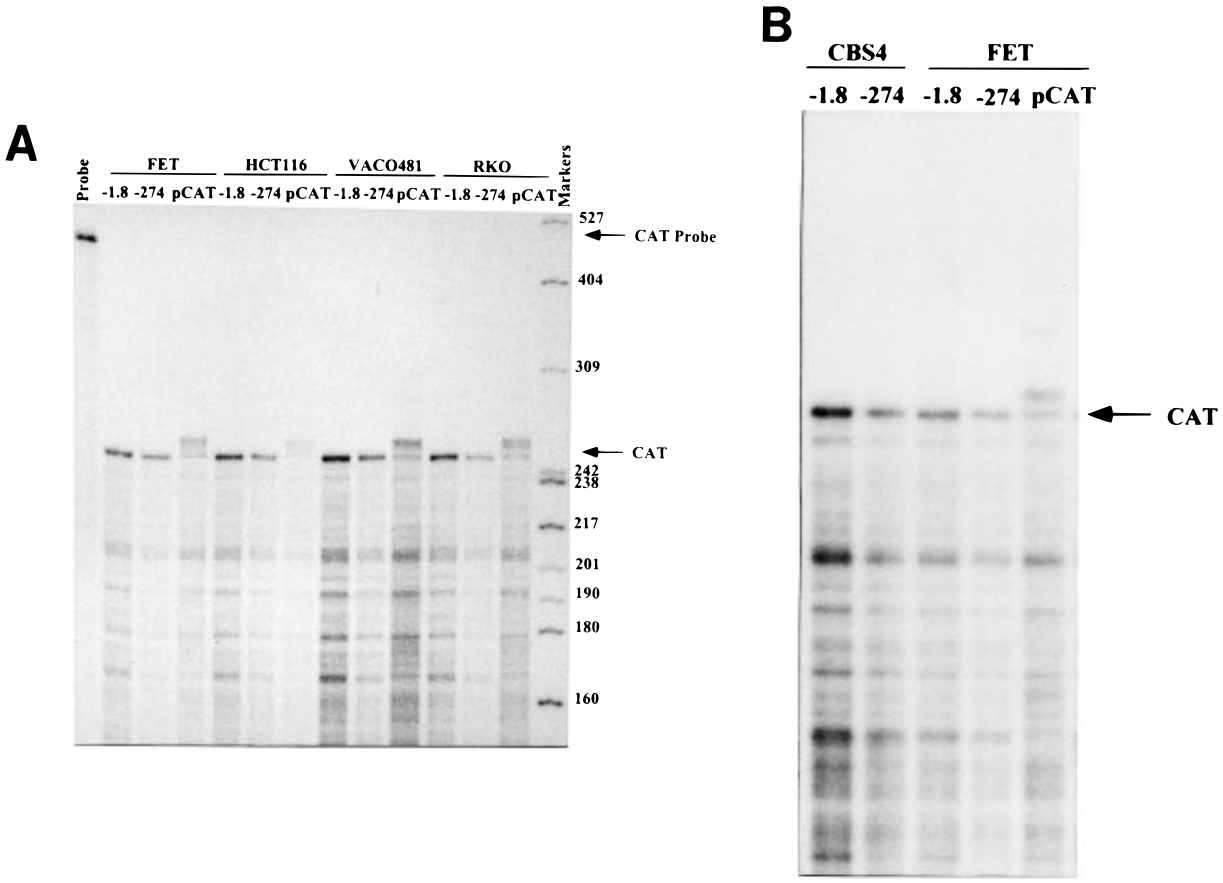

FIGURE 3: Promoter activity of TGF-β RII in human colon cancer cell lines. RII promoter (−1.8 kb and −274 bp) CAT chimeras and a promoterless-CAT (pCAT) construct were transiently transfected into FET, HCT116, VACO481, and RKO cells (A) and into CBS4 cells (B). The levels of CAT mRNA (which reflect promoter activity) were measured by RNase protection assays. Sample loading was normalized by β-galactosidase assays.

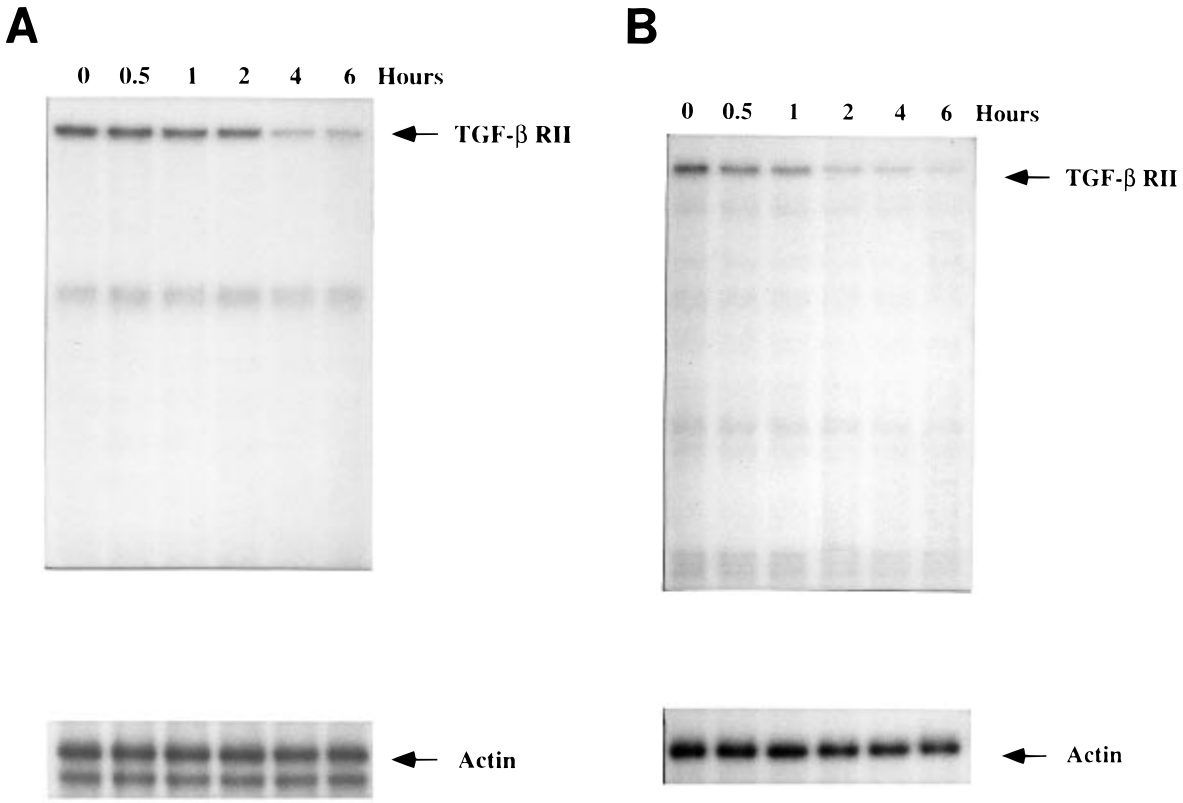

FIGURE 4: Stability of TGF-β RII mRNA in FET (A) and HCT116 (B) cell lines. Subconfluent cells were treated with 5 μg/mL actinomycin D and harvested at 0, 0.5, 1, 2, 4, and 6 h after treatment. RNase protection assays were performed as described under Materials and Methods.

experiments were performed a minimum of 3 times with different sets of RNA from independent actinomycin D treatments. RI mRNA decreased slowly with time in both FET and HCT116 cells compared to RII mRNA, and the half-life of RI mRNA in HCT116 was similar to that in FET cells (>6 h, data not shown). The half-life of RII mRNA was consistently measured to be 4−5 h in FET cells and 1−2 h in HCT116 cells. These data suggest that the low levels of RII mRNA found in HCT116 cells are due to decreased message stability.

TGF-β RII mRNA Levels and Transcriptional Activity in RKO and VACO481 Cells. HCT116 cells are homozygous for a frameshift mutation in the RII gene which causes the insertion of a premature stop codon. Studies concerning message stability suggest that frameshift mutations which result in premature termination of translation in the 5′ half of the mRNA molecule are associated with low message levels (*23*). Two other RER$^+$ cell lines, RKO and VACO481, possess mutations in the RII gene which are different from the mutation in HCT116. We examined RII mRNA levels and stability in these cell lines to determine the effects of the different RII gene mutations on RNA stability. One of the RII alleles in RKO cells (a RER$^+$ cell line) contains the wild-type sequence which is not expressed. The second allele contains a 2 base pair deletion within the 10 base polyadenine tract (*8*). This frameshift mutation results in a premature stop codon at nucleotide 725, thus encoding a truncated RII protein of 129 amino acids (Figure 5). If a premature stop codon is responsible for decreased half-life, we predict that RII mRNA in RKO cells should exhibit decreased stability similar to that in HCT116 cells. The VACO481 RER$^+$ cell line harbors two different types of frameshift mutations (Figure 5). One of the alleles has a

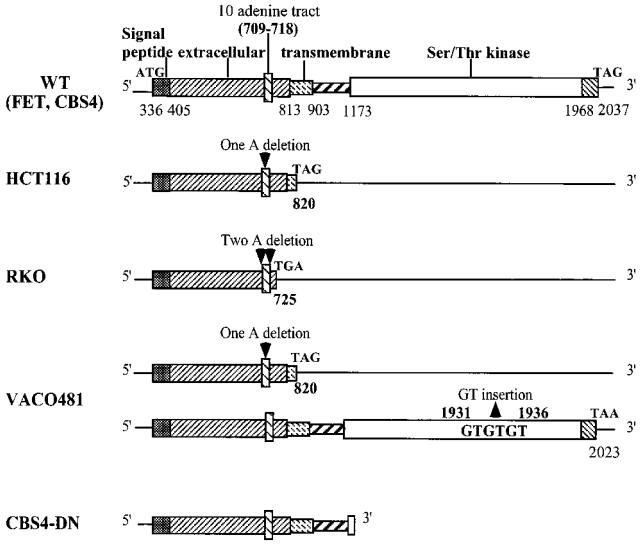

FIGURE 5: Schematic representation of TGF-β RII and its mutants in human colon cancer cell lines. Mutations were characterized by sequence analysis of cDNAs from cellular RNA (*8*). The frameshift mutations also change the coding region downstream of the deletion or insertion. Construction of the dominant negative mutant was previously described (*27*). Gene regions are labeled for the wild-type construct. Numerals represent nuclear acid numbers for the wild-type sequence, with 1 as the start of transcription.

deletion in the RII polyadenine tract identical to the HCT116 cells, while the second allele has an additional GT repeat in a region which normally contains three GT repeats at nucleotides 1931−1936. This GT insertion truncates RII by only 4 amino acids and substitutes a highly basic carboxyl terminus (29 amino acids) for the moderately acidic wild-type C-terminal region (33 amino acids) (*8*). Thus, RII mRNA from VACO481 cells represents a mixed population

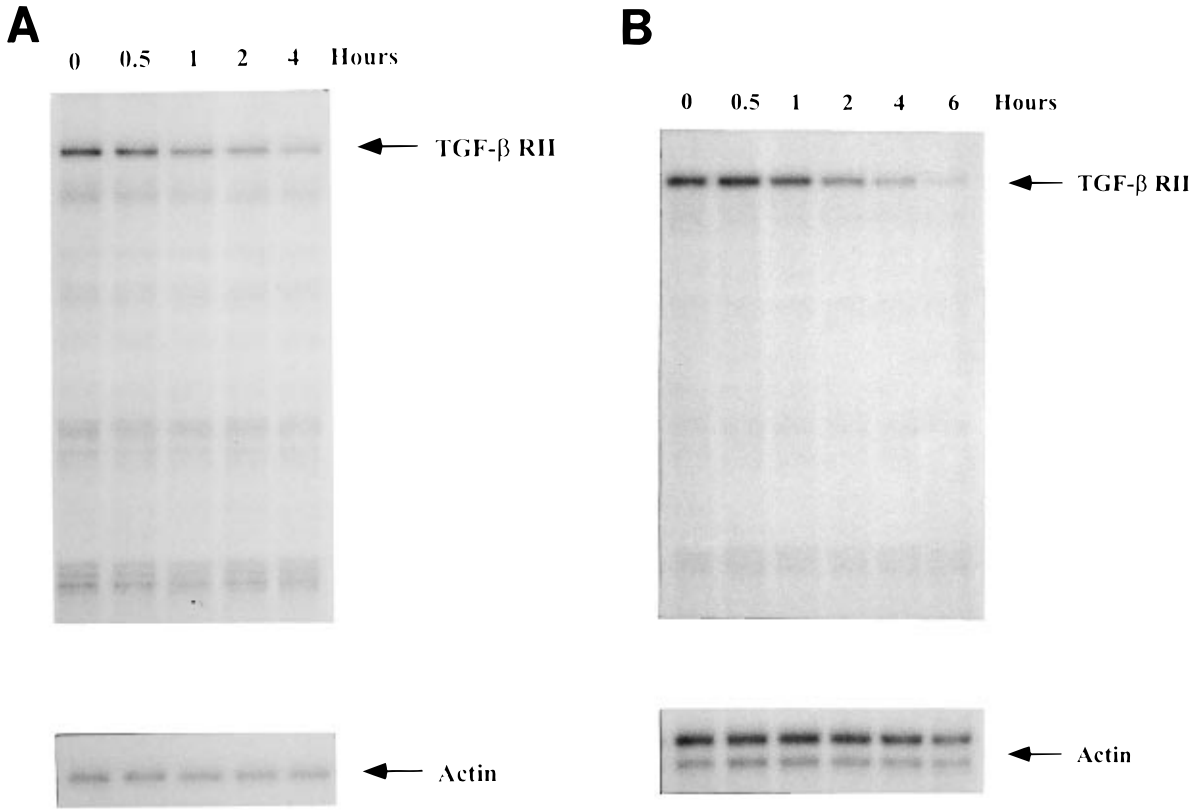

FIGURE 6: Stability of TGF-β RII mRNA in RKO (A) and VACO481 (B) cell lines. Subconfluent cells were treated with 5 μg/mL actinomycin D and harvested at 0, 0.5, 1, 2, 4, and 6 h after treatment. RNase protection assays were performed as described under Materials and Methods.

of two messages. Studies on frameshift mutations and message stability in other systems (*23*, *24*, *25*, *26*) have shown that only frameshift mutations which lead to premature translation termination in the 5′ half of the message are associated with decreased steady-state levels of RNA due to increased message instability. Thus, we hypothesized that the message from the allele with the GT insertion would be as stable as the wild-type message since this mutation does not severely truncate the protein. However, the message from the allele with the adenine deletion should show a decreased stability as in the case of the HCT116 cells. The overall levels of RII derived from a mixed population of two messages may fall in between the high wild-type levels and the low levels seen for HCT116 cells.

RII mRNA levels and RII transcriptional activity in RKO and VACO481 cells were compared with those of HCT116 and FET cells. As predicted, RII mRNA levels in RKO cells are similar to those of HCT116 cells (Figure 1). Levels of RII mRNA from VACO481 cells fall between those in FET and HCT116 cells (Figure 1). This may be explained by the presence of two species of frameshift mutations as described above. RII gene transcription activity, as measured by nuclear run-on (Figure 2) and promoter activity assays (Figure 3), showed no significant differences among these cell lines, suggesting that as in HCT116 cells, the different RII mRNA levels are not caused by differences in the transcription of the RII gene.

TGF-β RII mRNA Stability in RKO and VACO481 Cells. We measured RII mRNA in RKO and VACO481 cells after actinomycin D treatment to test whether the decreased mRNA levels were due to decreased mRNA stability. As expected, the TGF-β RII transcripts in RKO cells, which harbor a premature stop codon in the 5′ half as described above, had a half-life of 1−2 h, like that in HCT116 cells (Figure 6A). Consistent with their steady-state levels, the TGF-β RII transcripts in VACO481 cells had a half-life (3 h) falling between those of FET and HCT116 cells (Figure 6B).

RII Stability in HCT116-RII37, HCT116-Neo Cells, CBS4, and CBS4DN. To further examine whether the frameshift mutations in HCT116 and RKO cells cause decreased RII message stability, we measured the RII mRNA half-life in HCT116-RII37 and CBS4DN cells. HCT116-RII37 is a HCT116 limiting dilution clone stably transfected with a wild-type RII cDNA in a cytomegalovirus-driven expression vector. If the message stability is determined by a *cis* mechanism of the message rather than a *trans* mechanism, the wild-type transfected message will have a longer half-life than the endogenous mutant RII mRNA in HCT116 cells. Measurement of the half-life of the transfected wild-type RII message showed that the wild-type RII mRNA in HCT116-RII37 cells had a RII mRNA half-life twice as long as the endogenous mutant RNA (Figure 7A). Another previously described RII riboprobe (*19*) was used in these RNase protection assays in attempts to distinguish the transfected RII transcripts from endogenous RII transcripts. This probe routinely results in doublet protection, most probably due to secondary structure in the region of the protection. However, the transfected mRNA product is overly abundant compared to the endogenous; thus, we were unable to evaluate the products on the same experiment. Therefore, we measured the half-life of RII mRNA in HCT116-Neo cells as a control. HCT116-Neo cells, stably transfected with the control vector only, had a RII mRNA half-life of 1−2 h, similar to HCT116 cells (Figure 7B); thus, the RII mRNA in HCT116-Neo cells represents the endogenous mutant

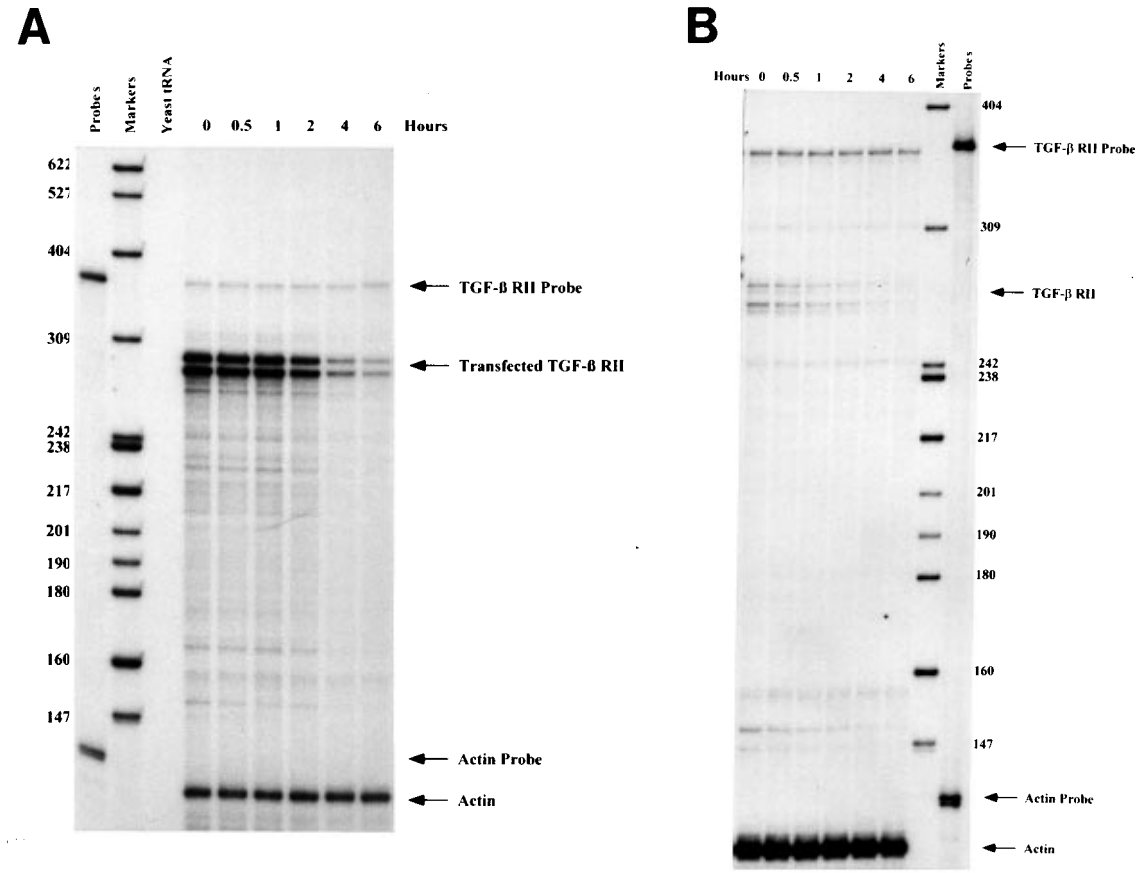

FIGURE 7: Stability of TGF-β RII mRNA in HCT116-RII37 (A) and HCT116-Neo (B) cell lines. Subconfluent cells were treated with 5 μg/mL actinomycin D and harvested at 0, 0.5, 1, 2, 4, and 6 h after treatment. RNase protection assays were performed as described under Materials and Methods.

mRNA. These results suggest that the frameshift mutations resulting in a premature stop codon in the 5′ half of TGF-β RII transcripts account for decreased message stability of RII mRNA in the colon carcinoma cell lines tested.

CBS4-DN is a limiting dilution clone of CBS (a RER$^-$ cell line) which has been stably transfected with a RII dominant negative cDNA lacking much of the receptor's cytoplasmic domain including the serine/threonine kinase active site (Figure 5). While it produces a similarly truncated RII protein as predicted for mutated RII of 161 amino acids in HCT116 cells, the recombinant dominant negative message is different. The premature stop codon in the recombinant RII dominant negative mutant is located at the similar site in the protein as in the HCT116 RII; however, the recombinant mutant lacks the extensive 3′ untranslated region of RII mRNA. The recombinant message, therefore, does not contain a translation termination signal within the 5′ half of the message. Accordingly, we hypothesize that the recombinant truncated message will appear as stable as the wild-type RII message. RNase protection assays were done with these cell lines to compare the levels of RII message. The same riboprobe was used as in Figure 7. In these experiments, the overexpressed RII mRNA transfected gene was smaller than the endogenous, allowing for the separation and evaluation of the two types of message. CBS4 has RII mRNA levels equivalent to FET (Figure 1), and RII mRNA in CBS4 had a half-life (4−5 h) (Figure 8A) similar to that in FET cells. When CBS4 is stably transfected with the dominant negative mutant RII cDNA lacking the serine/threonine kinase domain (designated CBS4-DN) (*27*) (Figure 5), the mutant RII mRNA had a similar half-life (4−5 h) (Figure 8B) as wild-type. These results suggest that not just the presence of truncating mutation itself, but also the location of a premature stop codon in the 5′ half of the message is responsible for decreased RII mRNA stability.

DISCUSSION

The direct involvement of RI and RII in TGF-β signal transduction indicates that the loss of functional RI and/or RII expression could contribute to the loss of TGF-β responsiveness and tumor progression. Previous work in our laboratory has compared receptor and ligand expression in RER$^-$ versus RER$^+$ colon carcinoma cells. The results indicated that RI and TGF-β were expressed in all cell lines. However, RER$^+$ cell lines uniformly lacked either RII mRNA or functional cell surface RII protein (*8*). Reintroduction of RII back into RER$^+$ HCT116 cells by stable transfection reconstituted TGF-β signaling and significantly inhibited the tumorigenicity of the cell line (*12*). A similar result was reported in a breast cancer cell line (*19*). Therefore, TGF-β RII appears to be a target for defects during tumor progression. Analysis of the mechanism of RII loss may prove to have clinical use in defining the clinical course for subsets of different types of malignancies, and in addition, it may result in the identification of new therapeutic targets and approaches for some subsets of cancers.

In this paper, we studied the mechanism for the reduced RII mRNA level in late malignant colon carcinoma cells. Despite the obvious difference in steady-state levels of RII mRNA, somewhat surprisingly, RII transcriptional activity, as measured by nuclear run-on and RII promoter-CAT

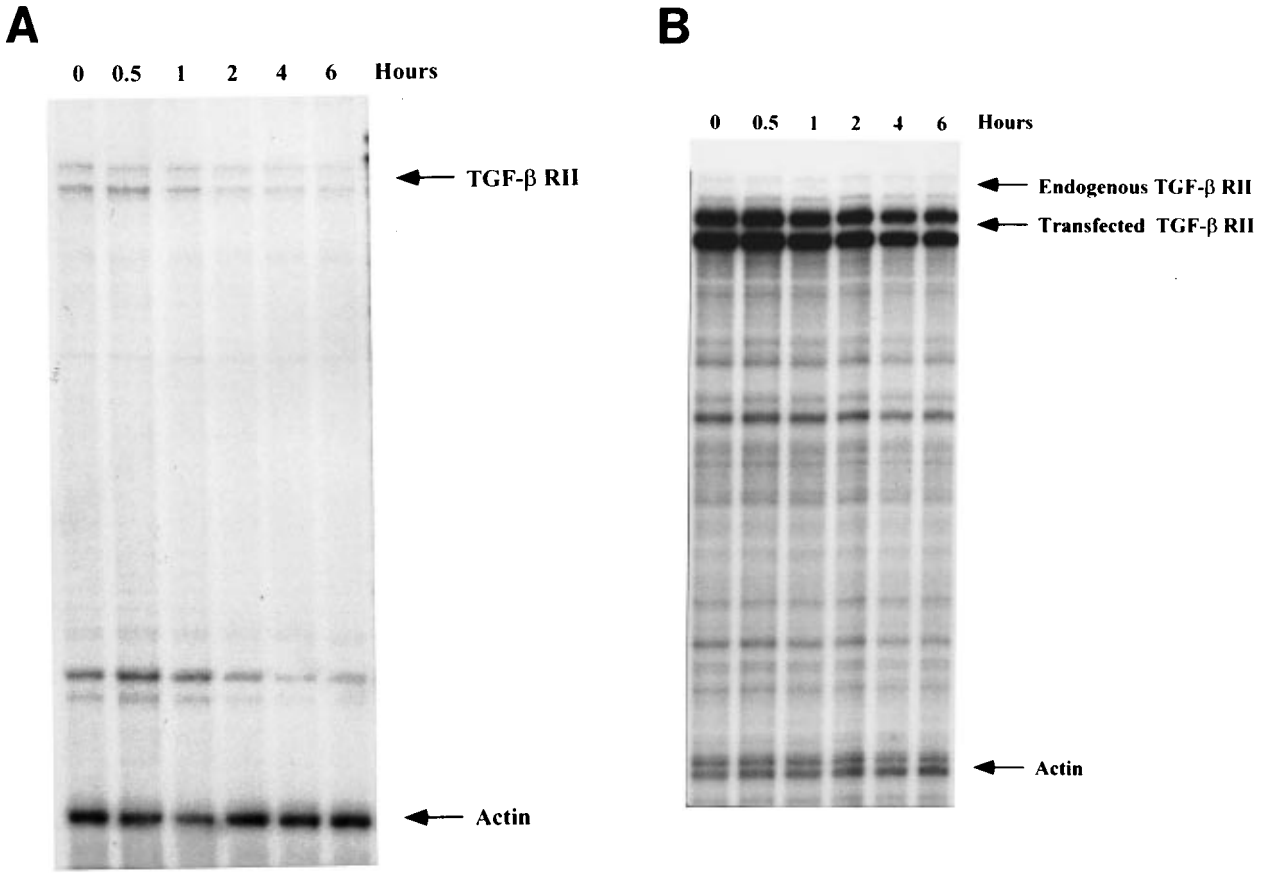

FIGURE 8: Stability of TGF-β RII mRNA in CBS4 (A) and CBS4-DN (B) cell lines. Subconfluent cells were treated with 5 μg/mL actinomycin D and harvested at 0, 0.5, 1, 2, 4, and 6 h after treatment. RNase protection assays were performed as described under Materials and Methods.

assays, showed no significant difference among cell lines. This indicated that the reduction of RII mRNA level was not regulated at the transcriptional level. Therefore, the mechanism was associated with the mRNA itself or with the alteration of mRNA degradation in the late malignant tumor cells.

The polyadenine tract and the six base pair GT repeats within the RII coding region provide ideal targeting sites for the so-called "microsatellite instability" or "replication errors (RER)" phenotype (*28*). As described above, these two regions have been shown to be mutated in RER^+ colon carcinoma cells which showed either low RII mRNA or low functional RII protein (*8*). In HCT116 and RKO cells, the mutations occur in the polyadenine tract and result in a premature stop codon within the 5′ half of the RII message. One possibility for decreased RII mRNA levels in HCT116 cells could be that the mutation within the RII gene results in reduced mRNA stability and, in turn, a lower steady-state level. The work reported here showed that mutant RII transcripts in HCT116 and RKO cells had significantly shorter half-lives than wild-type RII transcripts in FET and CBS4 cells, suggesting that the reduced RII mRNA levels in RER^+ cells are due to decreased message stability. Another possibility is that an additional mutation in HCT116 and RKO cells is *trans* acting for destabilizing RII mRNA. This factor would have to be specific because RI mRNA levels and stability in HCT116 cell are quite similar to those in FET cells. When HCT116 cells were transfected with a wild-type RII cDNA, wild-type RII mRNA had a longer half-life than endogenous mutated RII mRNA, thus supporting the hypothesis for a *cis* acting regulation by the nonsense mutation resulting in premature termination of translation.

An unsolved issue is that the exogenous wild-type RII in HCT116 RII transfectants showed a half-life falling between endogenous mutant RII transcript in HCT116 and wild-type RII transcript in FET and CBS4 cells. Three possible explanations are as follows: (1) The rapid degradation of mutant RII transcripts also accelerates the degradation of wild-type transcripts. The RNA degradation system in HCT116 cells may not distinguish the two with high fidelity. (2) The transfected RII cDNA is not exactly same as the wild-type transcript: It lacks introns and therefore is not subjected to any additional controls possibly involving splicing. (3) A portion of wild-type transfected alleles of stable insertion may be mutated in culture due to the presence of the mismatch repair defects in these cells. A satisfactory explanation of this issue awaits a better understanding of the mechanism of mRNA degradation in mammalian cells.

When the CBS4 cells were stably transfected with a TGF-β RII dominant negative mutant, the mutant RII mRNA showed a similar half-life to wild-type mRNA. This suggested that not just the presence of the premature stop codon, but also the location of the stop codon within the mRNA transcript which has a long 3′ untranslated region, influences the mRNA stability. Low levels of RNA from genes in which frameshift mutations result in translation termination in the 5′ half of a messenger RNA have been reported in a number of mammalian systems including human β-globin (*23*, *29*), murine immunogloblin μ (*30*), Rous sacroma virus (*31*), and human triosephosphate isomerase (*24*, *32*). While it is tempting to evoke ribosomal involvement in protecting a message from degradation, it is not clear

what role translation has in determining message stability, since effects on both nuclear and cytoplasmic RNA metabolism have been reported (*23*, *24*, *25*, *31*, *33*). Such mechanisms may have been preserved during evolution to prevent production of severely truncated proteins from mutated genes. In the case of TGF-β RII, this may provide protection for surrounding tissues. A recombinant truncated TGF-β RII encoding the entire extracellular domain has been expressed in COS-7 cells and was found to be a secreted, soluble receptor which binds TGF-β1 and TGF-β3 with high affinity (*34*). Thus, if the truncated mutant encoded in HCT116 were expressed, it would be released into the environment where it could neutralize its ligands by binding to them. The disruption of the paracrine function of TGF-βs would interfere with the cell growth and differentiation of the surrounding tissue. A cellular mechanism which degrades mutant mRNA encoding severely truncated proteins may have evolved to protect cells from possible negative effects of mutant proteins.

ACKNOWLEDGMENT

We thank Drs. Seong-Jin Kim and Anita B. Roberts at NCI, National Institutes of Health, for the −1.8 kb RII/CAT and −274 bp RII/CAT constructs.

REFERENCES

1. Massagué, J. (1990) *Annu. Rev. Cell Biol. 6*, 597−641.
2. Roberts, A. B., and Sporn, M. B. (1990) in *Peptide Growth Factors and Their Receptors* (Sporn, M. B., and Roberts, A. B., Eds.) pp 419−472, Springer-Verlag, Heidelberg.
3. Lin, H. Y., Wang, X.-F., Ng-Eaton, E., Weinberg, R. A., and Lodish, H. F. (1992) *Cell 68*, 775−785.
4. Franzén, P., ten Dijke, P., Ichijo, H., Yamashita, H., Schulz, P., Heldin, C.-H., and Miyazono, K. (1993) *Cell 75*, 681−692.
5. Bassing, C. H., Yingling, J. M., Howe, D. J., Wang, T., He, W. W., Gustafson, M. L., Shah, P., Donahoe, P. K., and Wang, X.-F. (1994) *Science 263*, 87−89.
6. Wrana, J. L., Attisano, L., Wieser, R., Ventura, F., and Massagué, J. (1994) *Nature 370*, 341−347.
7. Wang, X.-F., Lin, H. Y., Ng-Eaton, E., Downward, J., Lodish, H. F., and Weinberg, R. A. (1991) *Cell 67*, 797−805.
8. Markowitz, S., Wang, J., Myeroff, L., Parsons, R., Sun, L.-Z., Lutterbaugh, J., Fan, R. S., Zborowska, E., Kinzler, K. W., Vogelstein, B., Brattain, M. G., and Willson, J. K. V. (1995) *Science 268*, 1336−1338.
9. Fishel, R., Lescoe, M. K., Rao, M. R. S., Copeland, N. G., Jenkins, N. A., Garber, J., Kane, M., and Kolodner, R. (1993) *Cell 75*, 1027−1038.
10. Leach, F. S., Nicolaides, N. C., Papadopoulous, N., Liu, B., Jen, J., Parsons, R., Peltomäki, P., Sistonen, P., Aaltonen, L. A., Nyström-lahti, M., Guan, X.-Y., Zhang, J., Meltzer, P. S., Yu, J.-W., Kao, F.-T., Chen, D. J., Cerosaletti, K. M., Fournier, R. E. K., Todd, S., Lewis, T., Leach, R. J., Naylor, S. L., Weissenbach, J., Mecklin, J.-P., Järvinen, H., Petersen, G. M., Hamilton, S. R., Green, J., Jass, J., Watson, P., Lynch, H. T., Trent, J. M., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. (1993) *Cell 75*, 1215−1225.
11. Parsons, R., Li, G.-M., Longley, M. J., Fang, W.-H., Papadopoulos, N., Jen, J., de la Chapelle, A., Kinzler, K. W., Vogelstein, B., and Modrich, P. (1993) *Cell 75*, 1227−1236.
12. Wang, J., Sun, L.-Z., Myeroff, L., Wang, X.-F., Gentry, L. E., Yang, J., Liang, J., Zborowska, E., Markowitz, S., Willson, J. K. V., and Brattain, M. G. (1995) *J. Biol. Chem. 270*, 22044−22049.
13. Brattain, M. G., Brattain, D. E., Fine, W. D., Khaled, F. M., Marks, M. E., Kimball, P. M., Arcolano, L. A., and Danbury, B. H. (1981) *Oncodev. Biol. Med. 2*, 355−366.
14. Brattain, M. G., Levine, A. E., Chakrabarty, S., Yeoman, L. C., Willson, J. K. V., and Long, B. (1984) *Cancer Metastasis Rev. 3*, 177−191.
15. Boyd, D., Levine, A. E., Brattain, D. E., McKnight, M. K., and Brattain, M. G. (1988) *Cancer Res. 48*, 2469−2474.
16. Willson, J. K. V., Bittner, G., Oberley, T., Meisner, G., and Weese, J. (1987) *Cancer Res. 47*, 2704−2713.
17. Glisin,V., Cvkvenjakov, R., and Byus, C. (1974) *Biochemistry 13*, 2633−2637.
18. Wu, S. P., Theodorescu, D., Kerbel, R., Willson, J. K. V., Mulder, K. M., Humphrey, L. E., and Brattain, M. G. (1992) *J. Cell Biol. 116*, 187−196.
19. Sun, L.-Z., Wu, G., Willson, J. K. V., Zborowska, E., Yang, J., Rajkarunanayake, I., Wang, J., Gentry, L. E., Wang, X.-F., and Brattain, M. G. (1994) *J. Biol. Chem. 269*, 26449−26455.
20. Sun, L.-Z., Wu, S. P., Coleman, K., Fields, K. C., Humphrey, L. E., and Brattain, M. G. (1994) *Exp. Cell Res. 214*, 215−224.
21. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning*, pp 16.66−16.67, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
22. Bae, H. W., Geiser, A. G., Kim, D. H., Chung, M. T., Burmester, J. K., Sporn, M. B., Roberts, A. B., and Kim, S.-J. (1995) *J. Biol. Chem. 270*, 29460−29468.
23. Maquat, L. E., Kinniburgh, A. J., Rachmilewitz, E. A., and Ross, J. (1981) *Cell 27*, 543−553.
24. Cheng, J., Fogel-Petrovic, M., and Maquat, L. E. (1990) *Mol. Cell. Biol. 10*, 5215−5225.
25. Belgrader, P., Cheng, J., and Maquat, L. E. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 482−486.
26. Hagan, K. W., Ruiz-Echevarria, M. J., Quan, Y., and Peltz, S. W. (1995) *Mol. Cell. Biol. 15*, 809−823.
27. Koli, K. M., Ramsey, T. T., Ko, Y., Dugger, T. C., Brattain, M. G., and Arteaga, C. L. (1997) *J. Biol. Chem. 272*, 8296−8302.
28. Eshlemen, J. R., and Markowitz, S. D. (1995) *Curr. Opin. Oncol. 7*, 83−89.
29. Baserga, S. J., and Benz, E. J., Jr. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 2056−2060.
30. Baumann, B., Potash, M. J., and Köhler, G. (1985) *EMBO J. 4*, 351−359.
31. Barker, G. F., and Beemon, K. (1991) *Mol. Cell. Biol. 11*, 2760−2768.
32. Daar, I. O., and Maquat, L. E. (1988) *Mol. Cell. Biol. 8*, 802−813.
33. Urlaub, G., Mitchell, P. J., Ciudad, C. J., and Chasin, L. A. (1989) *Mol. Cell. Biol. 9*, 2868−2880.
34. Lin, H. Y., Moustakas, A., Knaus, P., Wells, R. G., Henis, Y. I., and Lodish, H. F. (1995) *J. Biol. Chem. 270*, 2747−2754.

BI9717892