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Transcriptional repression of the *neu* protooncogene by the adenovirus 5 E1A gene products

(gene regulation/c-*erbB-2*/*HER-2*)

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ABSTRACT **Amplification/overexpression of the human *neu* protooncogene has been frequently found in human primary breast and ovarian cancers and is correlated with the number of axillary lymph nodes positive for metastasis in breast cancer patients. Identification of the factors controlling transcription of the *neu* gene is essential for understanding the mechanisms of *neu* gene regulation and its role in tumorigenicity. The adenovirus early region 1A (E1A) gene products are pleiotropic transcription regulators of viral and cellular genes and have been identified as a viral suppressor gene for metastasis. Here we demonstrate that transcription of *neu* can be strongly repressed by the E1A gene products. The 13S and 12S products of E1A gene are effective at repressing *neu* transcription and the transcriptional repression requires the conserved region 2 of the E1A proteins. The target for E1A repression was localized within a 139-base-pair DNA fragment in the upstream region of the *neu* promoter. In addition, competition experiments suggest that the sequence TGGAATG, within the 139-base-pair fragment, is an important element for the E1A-induced repression. These results indicate that E1A negatively regulates *neu* gene expression at the transcriptional level by means of a specific DNA element.**

The *neu* (also called murine c-*erbB-2*) oncogene was first identified by transfection studies in which NIH 3T3 cells were transformed with DNA from ethylnitrosourea-induced rat neuro/glioblastomas (1). Structural and functional analysis between the transforming *neu* oncogene and its normal cellular counterpart, *neu* protooncogene, revealed that a subtle structural alteration—namely, a single point mutation—is sufficient to convert the *neu* protooncogene into a transforming *neu* oncogene (2, 3). The *neu* gene encodes a 185-kDa transmembrane protein (p185) that is related to, but distinct from, the epidermal growth factor receptor (4). The transforming p185 is associated with an increased tyrosine kinase activity (4–6). Interestingly, a structurally divergent group of oncogenes encoding protein kinases has been shown to induce the metastatic phenotype (7). Evidence linking this kinase oncogene to the induction or progression of human malignancies comes from recent observations that the human homologue of the rat *neu* oncogene (human gene symbol *NGL* for neuro/glioblastoma-derived; has been called *ERBB2*, *HER-2*, human c-*erbB-2*, or *TKR1*) is amplified/overexpressed in 25–30% of human primary breast cancers and ovarian cancers, notably in breast cancer patients with more than three axillary lymph nodes positive for metastasis (8–10). It has also been noted that some human breast cancer cell lines overexpress human *neu* mRNA, while the *neu* gene is not amplified (11). Together, these studies suggest that regulation of the *neu* gene may play an important role in malignant transformation and metastasis.

The primary function of the adenovirus early region 1A (E1A) gene is to activate other adenoviral genes during a permissive viral infection by modifying the host cell transcriptional apparatus, thereby resulting in host cell immortalization or transformation by the whole adenoviral early region (12). Although transcriptional activation and transcriptional repression of nonadenoviral genes by the E1A proteins have been reported (13–20), their functional significance and physiological impact are unclear in many cases. Recently, it has been shown that exogenously added E1A gene can reduce the metastatic potential of *ras*-transformed rat embryo cells by activating the cellular NM23 gene that is associated with a lower metastatic potential, thus identifying it as nonimmunologically related metastasis-inhibitory gene (21–24). As E1A proteins are multifunctional transcription regulators and metastasis suppressors and overexpression of the human *neu* gene correlates with number of axillary lymph nodes positive for metastasis in breast cancer patients, we investigated the potential effects of the E1A products on the promoter activity of *neu*. We found that the transcriptional activity of the *neu* promoter can be strongly repressed by E1A products and that the 13S and 12S products of the E1A gene are effective at repressing transcription. We identified the conserved region 2 (CR2) of the E1A proteins as required for the repression. Furthermore, our data indicate that a cis-acting DNA element in the upstream region of *neu* promoter is responsible for the trans inhibition by the E1A gene products.

MATERIALS AND METHODS

Plasmids. The recombinants used in this study have been described. pE1A (25, 26) is a plasmid expressing only the E1A region gene; pE1A12S and pE1A13S (26) express 12S E1A protein and 13S E1A protein, respectively; pE1A-dl343 (26) contains a 2-base-pair (bp) frameshift deletion in the E1A coding sequences (adenovirus nucleotide sequence positions 621 and 622); pE1A-dl346 (26) contains an in-frame deletion of nucleotides 859–907 (48 bp), resulting in the deletion of 16 amino acids inside the CR2 of the E1A proteins; pE1Apr contains only the E1A promoter (−499 to +113 relative to the E1A cap site); pRSV-CAT is a reporter plasmid containing the CAT gene under the control of the Rous sarcoma virus (RSV) long terminal repeat (LTR); pE1B, pE2, and pE3 are plasmids expressing E1B, E2, and E3 genes, respectively. pNeuEcoRI-CAT contains the 2.2-kilobase (kb) rat *neu* promoter and upstream sequences linked to the CAT gene. The deletion mutant of the *neu* promoter (T.-C.S. and M.-C.H., unpublished data) used in this study are described in the legends to Fig. 3 and 4*a*. pRSV-β-gal contains the RSV LTR

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Abbreviations: CAT, chloramphenicol acetyltransferase; Ad5, adenovirus 5; E1A, early region 1A; CR, conserved region; RSV, Rous sarcoma virus; LTR, long terminal repeat.

linked to β-galactosidase gene used as an internal control for transfection efficiency.

Cell Cultures. Cell cultures were performed as described (27, 28).

DNA Transfection. All transfections were carried out with the calcium phosphate precipitation technique of Graham and Van der Eb as modified by Anderson *et al.* (29).

CAT Assays. Cell extracts were prepared 40 hr after transfection. Portions of cell lysates were assayed for β-galactosidase activity from the cotransfected pRSV-β-gal plasmid. All CAT assays (31) were normalized to the internal transfection efficiency control. The CAT assay monitors acetylation of [^{14}C]chloramphenicol in cell extracts; [^{14}C]-chloramphenicol and its products are separated by thin-layer chromatography (TLC) and visualized by autoradiography. Individual spots on TLC paper were cut, their radioactivities were assayed by liquid scintillation spectrometry, and the relative CAT activities were calculated accordingly. Each experiment has been reproducibly repeated at least three times and a representative of several experiments is shown.

Immunoblot. SK-BR-3 cell lysates were made 40 hr after transfection and immunoblots were performed as described (28). The mAb-3 monoclonal antibody against the human *neu* gene product—p185 protein—was purchased from Oncogene Science (Manhasset, NY).

RESULTS

Transcriptional Repression of *neu* by the Adenovirus 5 (Ad5) E1A Products. A DNA segment of 2.2 kb containing the *neu* promoter and upstream sequences was fused with the CAT expression vector to generate the pNeuEcoRI-CAT plasmid. In transient-expression assays using Rat-1 cells (Fig. 1*a*), a cotransfection of pNeuEcoRI-CAT with pE1A, a plasmid expressing the E1A gene, led to a significant decrease of CAT activity. Cotransfection with pSP64, a plasmid vector, had no effect on CAT activity. To rule out the possibility that decreased transcription from *neu* promoters could be due to the titration of cellular transcription factors by the cotransfected E1A promoter, a deletion mutant, pE1Apr, which contains only the E1A promoter, was cotransfected with pNeuEcoRI-CAT. No effect on CAT activity was observed. A reporter plasmid containing the CAT gene under the control of the RSV LTR was not E1A responsive, indicating that decreased CAT expression was not due to a general decrease of transcription by E1A. In parallel experiments, stimulation of transcription from the E2A transcription unit by the E1A products was assayed by cotransfecting pE1A and pE2A-CAT (CAT gene driven by E2 early promoter). The results showed that repression of *neu* and transactivation of E2A promoter occur in the same range of pE1A concentration (data not shown). To see if other adenovirus early genes can repress the *neu* promoter, plasmids expressing the early genes of adenovirus individually were cotransfected with pNeuEcoRI-CAT (Fig. 1*b*). No change in CAT activity was observed with E1B, E2, or E3 alone, indicating that among these early genes of adenovirus, only the E1A gene can function as a repressor of the *neu* promoter.

Repression of *neu* Is E1A Concentration Dependent and Requires the E1A Conserved Region 2. To further study the interactions of E1A gene products with the *neu* promoter, increasing amounts of pE1A were cotransfected with pNeuEcoRI-CAT in ratios of 1:1, 2:1, 3:1, and 4:1 (Fig. 2*a*). Inhibition of the gene expression directed by the *neu* promoter was dependent on pE1A concentration, and 50% repression can be observed at as low as a 1:1 ratio of pE1A: pNeuEcoRI-CAT. The Ad5 E1A gene produces two major spliced products, the 12S and 13S mRNAs, that encode proteins 243 and 289 amino acids long, respectively (32). To determine which E1A gene product is responsible for the

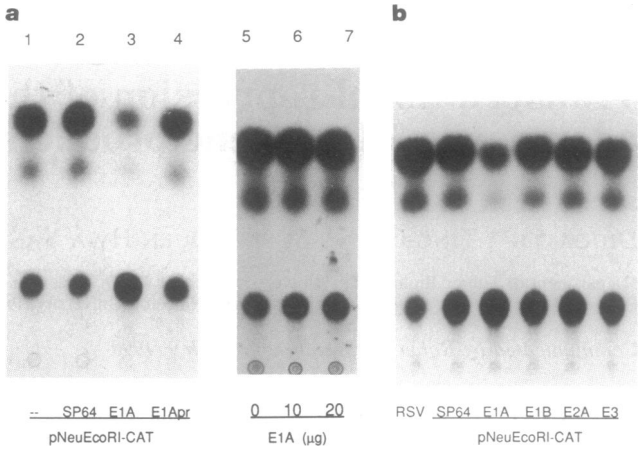

FIG. 1. (*a*) Transcriptional repression of *neu* promoter by E1A gene products. Rat-1 cells were transfected with 5 μg of the pNeuEcoRI-CAT construct, which contains the CAT gene driven by *neu* oncogene promoter containing 2.2-kb upstream DNA sequences. Lane 1, basal *neu* promoter activity (its relative CAT activity is defined as 100%); lanes 2–4, CAT activity after cotransfection with 10 μg of carrier DNA pSP64 vector (102%, lane 2); E1A-expressing plasmid pE1A (34%, lane 3); pE1Apr, a plasmid containing only the E1A promoter (98%, lane 4). The CAT activities of a reporter plasmid, RSV-CAT, containing the CAT gene under the control of RSV LTR (100%, lane 5) were not significantly changed by cotransfection of 10 μg of pE1A (98%, lane 6) or 20 μg of pE1A (96%, lane 7). (*b*) Effect of various adenovirus early genes on *neu* promoter activity. The pNeuEcoRI-CAT was cotransfected with pSP64 vector or plasmid expressing various adenovirus early genes, E1A, E1b, E2A, and E3, as indicated. The relative CAT activities are as follows: SP64, 100%; E1A, 35%; E1B, 97%; E2A, 99%; E3, 102%. RSV-CAT was used as a positive control.

observed repression, we performed the same experiments with recombinant plasmids expressing either 12S or 13S E1A gene product (pE1A-12S and pE1A-13S). As shown in Fig. 2 *b* and *c*, 12S and 13S products were effective at repressing *neu* transcription in a concentration-dependent manner. The E1A gene products contain three highly conserved regions: CR1, CR2, and CR3 (32, 33). CR1 and CR2 exist in the 12S and 13S, whereas CR3 is unique to the 13S product. Since 12S itself can repress *neu* efficiently, we reasoned that the CR3 is dispensable for transcriptional repression of *neu* by E1A. To further localize whether the CR1 or the CR2 in the E1A protein is required for efficient repression of *neu*, parallel experiments were performed using deletion mutants pE1Adl343 and pE1Adl346 (26). The pE1Adl343 mutant contains a 2-bp deletion in the E1A coding sequence, resulting in a frame shift in all three conserved regions of the E1A products and leaving only the N-terminal 40 amino acids intact. No effect on CAT activity was observed when pE1Adl343 mutant was cotransfected with pNeuEcoRI-CAT (data not shown). The pE1Adl346 mutant containing an in-frame deletion, which removed 16 amino acids within the CR2 but reserved the CR1, failed to express *neu* transcription (Fig. 2*d*). We conclude that the CR2 of E1A gene products is required for efficient transcriptional repression of *neu* (Fig. 2*e*).

Localization of Target DNA Element in the *neu* Promoter Responding to E1A Repression. To localize the DNA element in the *neu* promoter that mediates the transcriptional repression by the E1A products, a series of 5′ deletion constructs containing portions of the *neu* promoter linked to a functional CAT gene were cotransfected with pE1A into Rat-1 cells (Fig. 3*a*). The transient expression of the CAT gene driven by each of these promoter fragments after transfection with control plasmid vector pSP64 or with pE1A in a ratio of 1:2 is shown in Fig. 3*b*. Only the pNeuXhoI-CAT containing the smallest promoter fragment was not repressed by E1A.

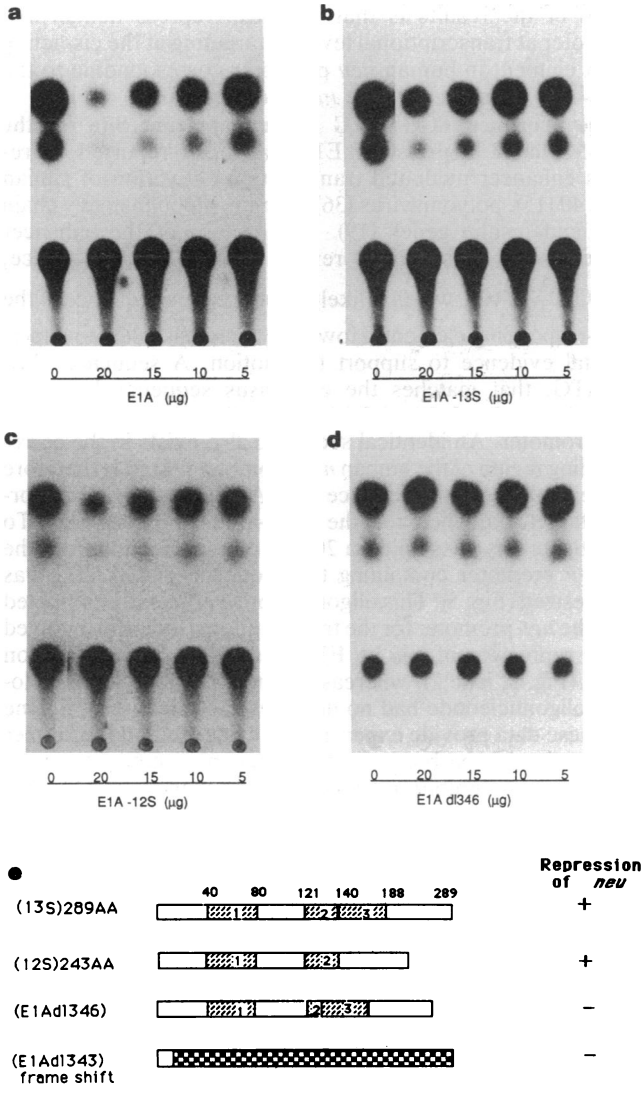

FIG. 2. Transient expression from *neu* promoter with cotransfection of increasing amounts of pE1A (*a*), pE1A-13S (*b*), pE1A-12S (*c*), and pE1Adl346 (*d*). A constant amount (5 μg) of the pNeuEcoRI-CAT construct was cotransfected into Rat-1 cells with 5, 10, 15, and 20 μg of the test constructs. The total amount of the transfected DNA was kept constant by adding the appropriate amount of carrier DNA pSP64. The relative CAT activities without E1A (lanes 0 in *a–d*) are defined as 100%. The relative CAT activities with 5, 10, 15, and 20 μg of test constructs are as follows: E1A, 68%, 35%, 26%, 17%; E1A-13S, 72%, 48%, 36%, 24%; E1A-12S, 66%, 46%, 28%, 21%; E1Adl346, 102%, 103%, 99%, 102%. (*e*) Summary of the effects of different E1A mutants on transient expression from the *neu* promoter. Schematic structures of the proteins encoded by different E1A mutants are shown on the bar diagram. Hatched areas represent the conserved protein regions of the E1A products. Bar diagrams are not drawn to scale.

Clearly the activity of a site within the *Stu* I–*Xho* I restriction fragment is sensitive to E1A repression. This *Stu* I–*Xho* I fragment is located between −198 and −59 with respect to the transcriptional start site of *neu* (T.-C.S. and M.-C.H., unpublished data). We conclude that the target DNA element responding to E1A repression resides inside this 139-bp *Stu* I–*Xho* I fragment.

Evidence for the Involvement of Trans-Acting Factor(s). To examine whether this repression by the E1A products is a trans-acting process, we attempted to remove the repression by cotransfecting a third recombinant, pSP64/Stu-Xho, containing only the *Stu* I–*Xho* I restriction fragment cloned in pSP64. Increasing amounts of pSP64/Stu-Xho, in cotransfections in which transcription of pNeuEcoRI-CAT was repressed by pE1A, relieved the repression of *neu* transcription in a concentration-dependent manner (Fig. 4*a*). In contrast, no derepression was observed when pSP64/RI-Xba containing the *Eco*RI–*Xba* I restriction fragment cloned in pSP64 was cotransfected. The derepression was effective at a 4:1 ratio of pSP64/Stu-Xho:pNeuEcoRI-CAT (Fig. 4*a*, lane 6), indicating that the *Stu* I–*Xho* I fragment can efficiently compete with the *neu* promoter for the transcription factor(s) involved in the repression of *neu* by E1A. These results confirm that the target for the E1A repression in the *neu* promoter is a cis DNA element within the *Stu* I–*Xho* I fragment of this promoter. Furthermore, this repression of transcription may involve an interaction between the DNA element and either the E1A products or some cellular transcription factor(s) interacting with or induced by the E1A products.

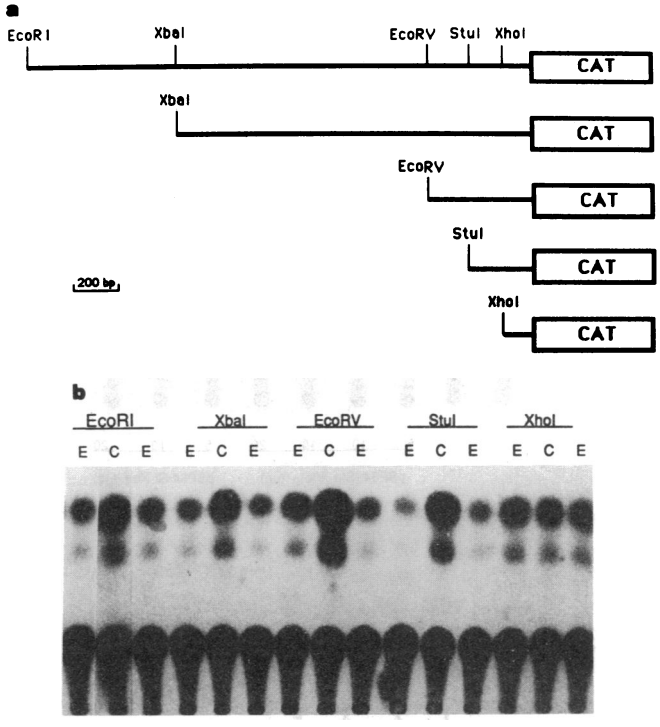

FIG. 3. Localization of E1A-responsive DNA element in the upstream region of *neu* promoter. (*a*) Schematic maps of the *neu* promoter 5′ deletion constructs that were fused individually to the CAT gene to create the plasmids as indicated by the names of the restriction enzymes used for generating the constructs. (*b*) Level of expression of the CAT gene directed by each of the promoter fragment constructs after transfection of 5 μg of the plasmids into Rat-1 cells with 10 μg of cotransfected pE1A (E) or carrier DNA pSP64 (C). The names of restriction enzymes above each triplet assay refer to the constructs indicated in the maps.

Repression of Human *neu* Expression in SK-BR-3 Cells. Comparison of the *Stu* I–*Xho* I fragment of rat *neu* promoter sequence (T.-C.S. and M.-C.H., unpublished data) with its counterpart sequence in human *neu* promoter (34) reveals >86% homology. We suspected that the human *neu* gene might also be repressed by E1A at transcriptional level by way of similar mechanisms. If this is the case, cotransfection of the *Stu* I–*Xho* I fragment of rat *neu* promoter might be able to relieve the repression of human *neu* incurred by E1A. To test this possibility, cotransfection experiments were carried out by using as recipient cells human breast cancer cell line SK-BR-3, which is known to overexpress human *neu* mRNA and p185 proteins (11). Immunoblotting experiments with SK-BR-3 cell lysates showed that the expression of human *neu* gene products, the p185 protein, was reduced by intro-

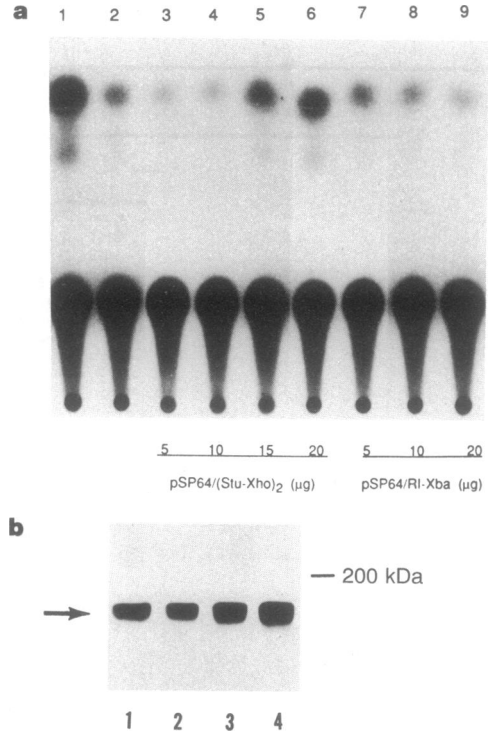

FIG. 4. Derepression of *neu* by cotransfection of competing amounts of *Stu* I–*Xho* I *neu* promoter fragments. (*a*) Rat-1 cells were transfected with 5 μg of the pNeuEcoRI-CAT plasmids giving basal *neu* promoter activity (lane 1); the repressed CAT activity after cotransfection with 5 μg of the pE1A is shown in lane 2. Plasmids pSP64/Stu-Xho containing the *Stu* I–*Xho* I *neu* promoter fragment cloned in pSP64 were cotransfected with pNeuEcoRI-CAT and pE1A. Lanes 3–6 show the competitive effects of increasing amounts (5, 10, 15, and 20 μg, respectively) of pSP64/Stu-Xho. Plasmids pSP64/R1-Xba containing the *Eco*RI–*Xba* I *neu* promoter fragment were also cotransfected with pNeuEcoRI-CAT and pE1A. Lanes 7–9 show CAT activities from *neu* promoter by cotransfecting 5, 10, and 20 μg of pSP64/R1-Xba, respectively. The relative CAT activities of lanes 1–9 are as follows: 100%, 32%, 27%, 31%, 58%, 79%, 38%, 31%, 24%. (*b*) Immunoblot for p185 protein in the cell lysates of SK-BR-3 cells transfected by pNeuEcoRV-CAT. Seventy-five micrograms of protein from each sample was electrophoresed on 7% SDS/PAGE gels prior to transfer on nitrocellulose. Filters were blotted with the primary antibody mAb-3. Lane 1, lysates of SK-BR-3 cells transfected with 5 μg of pE1A; lane 2, cotransfected with 5 μg of E1A and 20 μg of pSP64/R1-XbaI; lane 3, cotransfected with 5 μg of E1A and 20 μg of pSP64/Stu-Xho; lane 4, lysates of SK-BR-3 cells after mock transfection. The protein size marker is shown on the right. The arrow indicates the position of p185 protein. The p185 protein bands were scanned by Bio-Rad video densitometer model 620 to determine the relative p185 protein level. The p185 protein level in the mock transfection sample is defined as 100% and the relative amounts of p185 proteins in lanes 1–3 are 57%, 54%, and 89%, respectively.

duction of E1A (Fig. 4*b*, compare lane 1 with lane 4). Cotransfection of pSP64/R1-Xba plasmids with pE1A at a 4:1 ratio was ineffective in removing the repression of p185 expression by E1A, whereas cotransfection of pSP64/Stu-Xho with pE1A at the same ratio relieved the repression by E1A. It is known that the maximum efficiency of transient transfection can reach only 50% (35); the other 50% of nontransfected SK-BR-3 cells should still produce high levels of p185 proteins, which can result in high background in the E1A-mediated repression of p185. Therefore, the repression effect on the endogenous *neu*-encoded p185 by transiently transfected E1A in the immunoblotting assay was not as dramatic as that observed in CAT assays. However, we could detect the small difference reproducibly. The best interpretation of the results is that E1A can repress human *neu* promoter at transcriptional level by targeting at the cis-acting DNA element in human *neu* promoter corresponding to the *Stu* I–*Xho* I fragment of rat *neu* promoter.

The Sequence TGGAATG Is an Important Site for the E1A-Mediated Repression. E1A has been reported to repress enhancer mediated transcription activation of simian virus 40 (15), polyomavirus (36), immunoglobulin heavy chain (17), and insulin genes (19). Comparison of the enhancer sequences of these genes reveals a consensus sequence, (G)TGG$\frac{AAA}{TTT}$(G), which is likely to be the core sequence of the E1A-responding element. However, there has been no experimental evidence to support this notion. A sequence, TGGAATG, that matches the consensus sequence has been found in the *Stu* I–*Xho* I E1A-responding element of the rat *neu* promoter. An identical sequence also exists in the corresponding region of the human *neu* promoter (34). It is therefore conceivable that the sequence TGGAATG may be an important target sequence for the E1A-induced repression. To investigate this possibility, a 20-mer oligonucleotide from the rat *neu* promoter containing the sequence TGGAATG was synthesized (Fig. 5). This oligonucleotide efficiently competed with the *neu* promoter for the transcriptional factor(s) involved in the repression of *neu* by E1A, resulting in a derepression effect (Fig. 5, lane 2), whereas a 22-mer random nonhomologous oligonucleotide had no derepression effect (Fig. 5, lane 3). These data provide experimental evidence that the 20-mer oligonucleotide harbors a critical sequence required for the E1A-induced inhibition. Since the sequence TGGAATG within this 20-mer oligonucleotide resembles the consensus sequence in the enhancer sequences of other genes that can be repressed by E1A, it is likely that this 7-bp sequence is the critical sequence that is mediating the E1A effect.

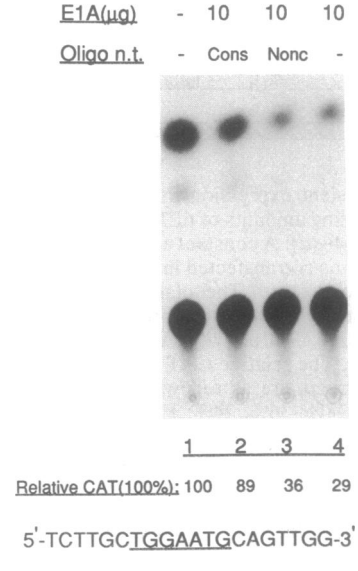

FIG. 5. Removal of the E1A-mediated repression of *neu* by cotransfection of a 20-mer oligonucleotide containing the consensus sequence. Rat-1 cells were transfected with 3 μg of pNeuEcoRV-CAT plasmids, giving basal *neu* promoter activity (lane 1); CAT activity after cotransfection with 10 μg of pE1A is shown in lane 4. Two micrograms of the 20-mer double-stranded oligonucleotide containing the consensus sequence (lane 2, Cons) was cotransfected with pNeuEcoRV-CAT and pE1A (molar ratio of oligomer:pNeuEcoRV-CAT = 35:1), resulting in significant derepression; cotransfection of 2 μg of a 22-mer random nonhomologous oligonucleotide with pNeuEcoRV-CAT and pE1A had no significant derepression effect (lane 3, Nonc). The values for relative CAT activity are the average of three experiments. The upper strand sequence of the synthetic 20-mer oligonucleotide is shown at the bottom; the proposed E1A-responding sequence is underlined.

DISCUSSION

The *neu* protooncogene is known to play an important role in tumorigenicity in animal models and human cancer (1–6, 8–10, 27); thus it is critical to understand mechanisms modulating *neu* expression. Our results show that in a cotransfection system, the E1A gene products repress the *neu* expression at transcriptional level. E1A products have been shown to repress several viral and cellular genes (15, 17, 19, 33, 36, 38). The conserved regions CR1 and CR2 have been reported to have repressor activity (32, 33, 38). Here we demonstrate that the repressive effect on *neu* expression is lost in E1A products when part of the CR2 (amino acids 120–136) is deleted. Notably, a structure motif in this deleted part of the adenoviral E1A CR2 region is shared among the papovaviral large tumor antigens, the v- and c-*myc* oncoproteins, the E7 transforming proteins of human papilloma viruses, and the yeast mitotic regulator CDC25 gene product (39, 40). This region encoding the shared motif is also required by E1A, simian virus 40 large tumor antigen, and human papilloma viruses 16 E7 for their specific binding to the human retinoblastoma gene product, RB protein (41–44). Determination of whether the binding of E1A to RB protein is involved in the repression of *neu* awaits further investigation. Additionally, this same region of E1A, simian virus 40 large tumor antigen, and human c-*myc* were known to be required for their transformation activity (40). Furthermore, polyoma large tumor antigen as well as the *myc* gene products can substitute for the E1A proteins in the *ras* cotransformation assay of primary embryo fibroblasts (30, 45). Therefore this shared motif is associated with similar biological functions in these proteins. It will be interesting to see if these proteins that share the common structure motif with E1A can also repress *neu* gene expression.

We have defined the oligonucleotide sequence mediating E1A-induced repression in the upstream region of *neu* promoter. The sequence TGGAATG is perfectly conserved between rat and human *neu* promoter, which is indicative of functional importance. In addition, this sequence matches the consensus sequence of other genes that can also be repressed by E1A at transcriptional level. Taken together, these findings suggest that there may be common mechanisms involved in this type of E1A-mediated repression. It has been proposed that E1A may form a complex with cellular transcription factor(s) and thereby modulate the specific binding of the transcription factor(s) to enhancer elements that are important for transcription (37). Identification of the defined DNA sequences responsible for the E1A-mediated inhibition of *neu* transcription will allow us to identify the transcription factor(s) involved in this process.

The *neu* protooncogene is notably amplified in patients with metastatic breast cancer (8–10). Expression of the E1A gene can inhibit experimental metastasis of *ras* oncogene-transformed rat embryo cells (21–24). Here we show that *neu* transcription can be repressed by E1A products in an established rat embryo fibroblast cell line, Rat-1. Furthermore, we have also found that in SK-BR-3 human breast cancer cells expression of the p185 protein, the human *neu* gene product, was reduced by introduction of E1A gene. The derepression effect observed in the cotransfection experiment with the *Stu* I–*Xho* I fragment has demonstrated that this reduction of p185 proteins is likely due to the similar transcriptional repression mechanisms. These findings raise a remote but interesting possibility that the E1A-mediated *neu* repression might confer a reduced metastatic potential or lower degree of malignancy. It is our hope that by elucidating the mechanisms that regulate *neu* expression, such as control by E1A, we can further our understanding of the role that *neu* plays in malignant transformation.

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