The adenovirus 55 residue E1A protein is a transcriptional activator and binds the unliganded thyroid hormone receptor

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The early region 1A (E1A) of human adenovirus types 2 and 5 is differentially spliced to yield five distinct mRNAs that encode different proteins. The smallest E1A RNA transcript encodes a 55 residue (55R) protein that shares only 28 amino acid residues with the other E1A proteins. Even though it is the most abundant E1A transcript at late times post-infection, little is known about the functions of this E1A isoform. In this study, we show that the E1A 55R protein interacts with, and modulates the activity of the unliganded thyroid hormone receptor (TR). We demonstrate that E1A 55R contains a signature motif known as the CoRNR box that confers interaction with the unliganded TR; this motif was originally identified in cellular corepressors. Using a system reconstituted in the yeast Saccharomyces cerevisiae, which lack endogenous TR and TR coregulators, we show that E1A 55R nonetheless differs from cellular corepressors as it functions as a strong co-activator of TR-dependent transcription and that it possesses an intrinsic transcriptional activation domain. These data indicate that the E1A 55R protein functions as a transcriptional regulator.

INTRODUCTION

Early region 1A (E1A) is the first viral gene expressed after human adenovirus (HAdV) infection (Nevins et al., 1979). It has been most extensively characterized in species C HAdV, specifically types 2 (HAdV-2) and 5 (HAdV-5), which are virtually identical in sequence and are typically treated interchangeably (Berk, 2005; Frisch & Mymryk, 2002; Pelka et al., 2008; Yousef et al., 2012). The primary E1A transcript is extensively spliced to produce the 13S, 12S, 11S, 10S and 9S mRNAs, which encode proteins of 289, 243, 217, 171 and 55 residues (R), respectively, in species C (Perricaudet et al., 1979; Stephens & Harlow, 1987) (Fig. 1a). Splicing preserves the translational reading frame in all cases except the 9S mRNA, which utilizes an alternative reading frame in exon 2. As a result, the 9S-encoded E1A 55R protein shares 26 amino acids also present in the other four E1A proteins, with the remaining 29 amino acid residues derived from the alternative reading frame in exon 2. Interestingly, the first two of the amino acid residues encoded by exon 2 in the 9S are identical to residues 27 and 28 in the 13S- and 12S-encoded E1A proteins, but not the 11S and 10S products (Fig. 1b). During the early stages of infection, splicing favours the production of the 13S and 12S E1A mRNAs. However, splicing at late times during infection favours the production of the 9S E1A mRNA, which then predominates. Despite the fact that the 9S mRNA is the major E1A product at late times and was originally identified in 1978 (Berk & Sharp, 1978), little is known about the 55R E1A protein other than that it can promote viral gene expression and replication (Miller et al., 2012).

Much more is known about the larger E1A proteins, which are responsible for activating expression of other viral early genes and reprogramming cell growth to provide an optimal environment for viral replication (Berk, 2005; Frisch & Mymryk, 2002; Pelka et al., 2008; Yousef et al., 2012). These changes result from the ability of these E1A proteins to interact with a variety of cellular regulatory...
proteins. Interaction of E1A with many of these proteins requires sequences that are highly conserved throughout the different serotypes, which are referred to as conserved regions 1–4 (Avvakumov et al., 2004). The E1A 55R protein does not contain any of these conserved regions and is therefore unlikely to be able to target most of the cellular regulators bound by the larger E1A isoforms.

The nuclear receptor (NR) superfamily is a diverse group of transcriptional regulators (McEwan, 2009). Many NRs are targeted by viruses to regulate expression of their own genes and to optimize cellular gene expression to facilitate viral propagation (Miller & Mymryk, 2011). The thyroid hormone receptors (TRs) are hormone-regulated NRs that control growth, development and homeostasis by binding to thyroid hormone response elements (TREs) in target promoters and altering transcription (Cheng, 2000; Mangelsdorf et al., 1995; Yen, 2001). Thyroid hormone, predominantly tri-iodothyronine (T3), activates transcription by enhancing packing of TR C-terminal helix 12 over the lower part of the corepressor’s binding surface, simultaneously promoting corepressor release and creating a binding site for co-activators (Glass & Rosenfeld, 2000).

We previously determined that E1A 289R and 243R proteins behave as co-activators of unliganded TRs that bind in the typical corepressor mode (Meng et al., 2003, 2005; Sato et al., 2009). We used the budding yeast Saccharomyces cerevisiae to reconstruct individual TR transcription complexes and analyse their activities as yeast contains conserved eukaryotic general transcription factors and chromatin remodelling enzymes, but lacks endogenous
NRs and NR coregulators that can complicate the interpretation of experiments. We found that the HAdV-2/5 E1A 289R and 243R proteins enhanced activity of unliganded TRs in yeast (Meng et al., 2003, 2005). Activation was suppressed by T3 and was dependent upon a consensus CBM spanning E1A residues 20–28 that binds TR similarly to coexpressors (Fig. 1b).

In this study, we report that the CBM is preserved in the E1A 55R proteins of HAdV-2 and -5, despite the presence of an intron/exon boundary in the portion of the transcript encoding this motif, and bind and co-activate unliganded TR. Further, the E1A 55R proteins function as transcriptional activation domains when recruited directly to a reporter gene construct via fusion to a heterologous DNA-binding domain. These results further support a role for the E1A 55R proteins in transcriptional regulation.

RESULTS

The HAdV-2 E1A 55R protein can activate transcription by the unliganded TR

We previously determined that HAdV-2 E1A 289R and 243R proteins function as co-activators of unliganded TRs in yeast (Meng et al., 2003, 2005). This activity requires the CBM between residues 20 and 28. Alternative splicing of the primary HAdV-2 E1A transcript occurs within the region encoding the CBM to generate the 11S, 10S and 9S mRNA products (Fig. 1a, b). Interestingly, while the signature motif of the CBM is destroyed in the 11S- and 10S-encoded proteins, it is regenerated in the 9S-encoded E1A 55R protein by the use of an alternative reading frame in exon 2 (Fig. 1b). To assess whether the E1A 55R protein could function as a co-activator of TR-dependent transcription, each of the five HAdV-2 E1A products was tested in a yeast model system.

In accordance with previous results, E1A 289R and 243R functioned as co-activators for the unliganded TR, and this effect was downregulated by the addition of T3 (Fig. 1c). The E1A 217R and 171R proteins, which do not contain a complete CBM, failed to activate transcription by the unliganded TR. In contrast, the E1A 55R protein functioned as a strong co-activator of the TR and this was downregulated by T3. Thus, alternative splicing appears to regenerate a functional CBM in the E1A 55R, allowing it to function as a co-activator of unliganded TRs similar to the 289R and 243R proteins.

The 55R E1A proteins of HAdV-2 and -5 contain a CBM and activate transcription by the unliganded TR

Alignment of the sequences of the HAdV-2 and the closely related HAdV-5 55R E1A proteins revealed that both contain a consensus CBM, but differ by three amino acids located near the C terminus (Fig. 2a). The HAdV-5 E1A 55R E1A protein also functioned as a co-activator of the unliganded TR, albeit at a lower level than the closely related HAdV-2 E1A 55R (Fig. 2b). This suggests that the three amino acid residues that differ between these two proteins affect function, despite the fact that they are not part of the CBM (Fig. 2a). Inspection of the amino acid sequences of the smallest E1A proteins expressed by the more distantly related HAdV-3 (species B) and 12 (species A) indicates that neither contains a consensus CBM (Fig. 2a). Not surprisingly, neither functioned as a co-activator (Fig. 2b).

The presence of the CBM in the HAdV-2 and -5 E1A 55R proteins suggested that their ability to co-activate TRs in the absence, but not in the presence, of T3 results from their ability to only bind unliganded TR. To confirm this, we examined interactions of TR/β with bacterially expressed E1A 55R proteins in glutathione S-transferase (GST) pulldown assays in vitro. TR/β bound to HAdV-2 and -5 E1A 55R in the absence of T3, and these interactions were decreased in the presence of T3 (Fig. 2c). This T3-dependent decrease in interaction by E1A parallels what is observed in TR–corepressor interactions, confirming that the E1A 55R proteins bind the TR like a cellular corepressor (Glass & Rosenfeld, 2000). GST pulldowns using recombinant HAdV-3 and -12 E1A did not detect binding of TR/β, as expected based on their lack of a consensus CBM and lack of function in the yeast co-activation assay (data not shown).

Mapping the regions of the E1A 55R protein needed for co-activation

To determine the regions of the HAdV-2 E1A 55R protein necessary for co-activation of transcription by unliganded TR, we constructed and tested a panel of mutants in the yeast system for their ability to activate TR-dependent gene expression (Fig. 3a). L23A/I24A, a double point mutation within the CBM or deletion of most of the CBM (Δ4–25) abrogated the ability of the E1A 55R protein to activate transcription, as did an N-terminal deletion of amino acids 2–9 and C-terminal truncation at residue 28. Thus, activation requires the CBM and flanking regions from residue 5 to residue 32.

Interestingly, the N- and C-terminal residues that flank the region of E1A 55R that is required for TR co-activation seemed to inhibit E1A activity. Indeed, removal of amino acids 1–6 enhanced activity, and truncations removing the C-terminal region greatly improved co-activation. Furthermore, the Δ1–4/Δ33–55R mutant, lacking both N- and C-terminal residues, functioned as a strong co-activator for the unliganded TR.

As mentioned above, the HAdV-2 and HAdV-5 E1A 55R proteins differ only by three amino acid residues located in the C-terminal region (Fig. 2a), and HAdV-5 E1A 55R is a weaker activator of the TR than HAdV-2 E1A 55R (Fig. 2b). Replacement of two of these residues with those found...
in HAdV-2 E1A 55R (HAdV-5 55R E1A K50N/R52C) increased activity substantially (Fig. 3a). Notably, C-terminal truncations of the E1A 55R protein that delete this region also increase activity, suggesting that the C-terminus negatively regulates the ability to activate TR-dependent transcription (Fig. 3a). We noted that two of the three changes in HAdV-5 55R E1A introduce basic amino acids, which are generally rare in the acidic E1A proteins. Conversion of these residues to aspartic acid (K50D/R52D) increased function dramatically (Fig. 3a).

In GST pulldown experiments, the D33–55 and D1–4/D33–55 E1A mutants exhibited enhanced interaction with the TR in the absence of T3 (Fig. 3b). In contrast, the L23A/I24A double point mutant, which is predicted to compromise binding of the CBM to the TR, no longer bound the TR under any conditions. Thus, amino acids 33–55 of the E1A 55R protein function to negatively regulate TR co-activation in yeast and reduce TR binding in vitro.

**E1A 55R binds the TR in mammalian cells**

Our results suggest that the species C E1A 55R proteins bind the TR in vitro and in vivo in yeast (Figs 2 and 3). We tested whether such an interaction could occur in mammalian cells using a mammalian two-hybrid test in human U2OS osteosarcoma cells. U2OS cells were transfected with a Gal4-responsive luciferase reporter, a vector expressing the hTR/β fused to the VP16 transcriptional activation domain, and vectors expressing the Gal4 DBD fused to the wt HAdV-2 or -5 55R E1A proteins or the C-terminal mutants that exhibit improved binding and activity in vivo in yeast and in vitro (Fig. 4). The results of these experiments exactly reproduce our observations in yeast and in vitro. Virtually no interaction was seen between the wt HAdV-5 55R E1A and the TR, a weak interaction was seen with the wt HAdV-2 55R E1A protein, and the C-terminal mutants interacted very strongly with the TR in the absence of T3. As observed in vitro and in yeast, these interactions in mammalian cells were abolished by the addition of T3. These results confirm that the HAdV-2 55R E1A protein can interact with the TR in the context of mammalian cells and also suggest that the C-terminal region inhibits this interaction.

**Mutations in the TR corepressor binding surface influence activation by E1A 55R**

We examined the requirement for the TR/β corepressor binding surface in co-activation by HAdV-2 E1A 55R (Fig. 5a) using a panel of TRβ mutants previously characterized for their effects upon NCoR interaction (Marimuthu et al., 2008).
We compared this with our previous data generated using a C-terminal fragment of NCoR (NCoRC), which also functions as a co-activator of unliganded TR (Meng et al., 2005). A number of these TRβ mutants also inhibited co-activation by E1A 55R in yeast. Most notably, mutations within residues that lie under the usual position of helix 12 in liganded TRβ and form an essential part of the corepressor binding surface (I280K, C309W) impaired TR co-activation by both E1A and NCoRC. However, there are three subtle differences. Specifically, (i) E1A 55R co-activation was not as significantly affected by the C309W mutation as was NCoRC; (ii) co-activation by E1A 55R was strongly reduced using the V284 mutant, which does not substantially affect co-activation by NCoRC; and (iii) the I302A mutant functioned better than wt hTRβ1 with E1A 55R, whereas it was slightly impaired with NCoRC. This is consistent with previous results (Hu et al., 2001; Marimuthu et al., 2002), which suggest that different NCoR and SMRT CBM sequences contact the same TR surface but exhibit subtle variations in this binding mode. Thus, E1A 55R interacts with an hTRβ1 surface that is similar, but not identical, to the one that binds NCoR.
Selective TR mutants were also tested for their ability to bind the 55R E1A protein in mammalian cells using co-immunoprecipitation assays (Fig. 5b). U2OS cells were transfected with plasmids expressing FLAG epitope tagged wt TR or selected TR mutants and the HAdV-2 55R E1A protein fused to EGFP. The HAdV-2 E1A 55R protein was co-immunoprecipitated with wt TR in the absence, but not in the presence of T3 (Fig. 5b). The F431X TR mutant, which contains a C-terminal truncation removing helix 12, is unable to bind ligand and associated with the E1A 55R in both the presence and absence of T3. The I280K mutation, which disrupts corepressor binding by the TR (Marimuthu et al., 2002), also greatly reduced interaction of the TR with the HAdV-2 E1A 55R protein, further suggesting that the CBM in E1A mediates interactions with the TR that are similar to that observed for various NR corepressors. These data agree fully with the yeast co-activation data obtained in Fig. 5(a).

To further confirm that HAdV-2 55R E1A interacts with hTR/β1 in the typical coresspor expression mode, we tested if a short peptide that overlaps NCoR CBM 3 could interfere with the ability of E1A 55R to bind unliganded TR in GST pulldowns (Fig. 5c). As expected, this peptide functioned in a dose-dependent fashion to inhibit E1A 55R wt as well as the Δ1–4/A33–55 mutant from interacting with the TR. Together, our observations indicate that E1A 55R is a TR co-activator that binds the TR in a manner similar to cellular NR corepressors.

**HAdV-2 E1A 55R contains an intrinsic transcriptional activation domain**

Given that HAdV-2 E1A 55R binds the unliganded TR and functions as a co-activator, it seemed likely that this E1A protein functions in some way to recruit components of the transcriptional apparatus to the transcriptional template. We tested the E1A 55R protein to determine if it contains a transcriptional activation domain by fusing it to the heterologous LexA DNA-binding domain (DBD) and expressing it in yeast containing a β-galactosidase reporter gene under the control of a minimal promoter containing LexA binding sites. If the E1A 55R protein contained a transcriptional activation domain, it would be expected to activate expression of the reporter gene when tethered by the LexA DBD. Intriguingly, the wt E1A 55R protein did not activate transcription in this assay. However, removal of the C-terminal 11 residues resulted in a strong activation of reporter gene expression, as did the mutation in which the two basic residues in the HAdV-5 55R E1A were replaced with acidic residues (Fig. 6a). Similar results were seen in transient transfection assays in human U2OS osteosarcoma cells using Gal4 DBD fusions and a Gal4-dependent luciferase reporter construct (Fig. 6b, top). The higher level of transcriptional activation by the E1A 55R mutants seen in U2OS cells did not result from large changes in protein expression as determined by Western blot analysis (Fig. 6b, bottom). These results demonstrate that both the HAdV-2 and -5 E1A 55R proteins contain a transcriptional activation domain, but that it is somehow masked by the C-terminal region. Interestingly, mutations that unmask this transcriptional activity also improve co-activation of unliganded TR (Fig. 3). This correlation suggests that these two activities are indeed related and also that interaction of the E1A 55R protein with the TR results in a conformational change that exposes this otherwise hidden transcriptional activation domain.

**Transcriptional activation by E1A 55R requires SAGA and SWI/SNF**

The yeast Gcn5 acetyltransferase serves as a highly conserved catalytic subunit of the multi-component SAGA (SPT, ADA, GCN5, Acetylation) transcriptional adaptor complex (Timmers & Tora, 2005). In addition, the yeast SWI/SNF chromatin remodelling complex is a highly conserved eukaryotic multi-component adaptor complex that provides an important ATP-dependent function that is required for transcription of a subset of yeast genes (Martens & Winston, 2003).

We assessed the effect of disrupting the genes encoding the Gcn5 or Ada2 component of the SAGA complex and the Swi2 component of the SWI/SNF complex on the ability of HAdV-2 E1A 55R to activate transcription when tethered to a reporter gene by the LexA DBD (Fig. 6a). Disruption of genes encoding SAGA components, as well as SWI2, reduced E1A 55R-dependent activation. These transcriptional defects appear specific as our previous studies...
indicate that these yeast strains retain the ability to support activation by other portions of E1A or p53 (Yousef et al., 2008, 2009). These results indicate that E1A 55R requires both SAGA and SWI/SNF for activation of transcription.

**DISCUSSION**

Despite its original identification as the major mRNA product of the HAdV E1A gene during the late phase of virus infection, little is known about the function of the 9S-encoded E1A 55R protein. In large part, this lack of information is due to the fact that this protein lacks the four regions of highly conserved sequence present in the E1A proteins of all the different HAdV types (Avvakumov et al., 2004). Our previous analysis of regions of the E1A 289R protein required to co-activate the unliganded TR implicated a CBM consensus motif (LDQLIEEVL) spanning residues 20–28 (Meng et al., 2003, 2005). We also determined that the same motif is conserved in E1A 55R. Therefore, we investigated whether E1A 55R and other E1A proteins produced by alternative splicing could potentiate transcriptional activation by unliganded TRs.

Our data confirm that the E1A 55R protein of species C, like the E1A 289R and 243R proteins, functions as a transcriptional coregulator of the TR. This appears to be a function not present in other HAdV species, indicating that this may be of secondary importance for infection. Mutational analysis has defined distinct regions of the E1A 55R that contribute to this process (Fig. 7a). The first of these is the CBM motif, which is reconstituted in the E1A 55R protein in spite of the presence of an intron/exon boundary within the region encoding this sequence (Fig. 1b). The CBM in the E1A 55R protein is required for co-activation of unliganded TR (Fig. 3a) and binding (Fig. 3b). The E1A 55R CBM binds the TR similarly to CBM containing cellular corepressors. Firstly, the E1A 55R–TR interaction was blocked by T3, a characteristic feature of corepressor binding. Secondly, a CoRNR box peptide derived from NCoR could compete in vitro with E1A 55R for interaction with the TR. Thirdly, mutations in the TR corepressor binding surface greatly impair the ability of the E1A 55R protein to co-activate unliganded TR in a yeast model system. Thus, the E1A 55R isoform is a viral TR interacting protein that binds in the corepressor mode.

In addition to the CBM, our data also indicate that the E1A 55R protein contains an intrinsic transcriptional activation domain and that the SAGA and SWI/SNF complexes are required for both the intrinsic activation by E1A 55R and TR-dependent co-activation (Fig. 6). Interestingly, co-activation by both the HAdV-2 and HAdV-5 E1A 55R
proteins appears to be negatively regulated by the C terminus, as mutants lacking this region bind and co-activate TR better than their wt counterparts (Figs 3 and 4). Removal or mutation of the C-terminal negative regulatory region also exposes the activation function of the E1A 55R protein when recruited directly to DNA by the Gal4 or LexA DBD in yeast and mammalian systems (Fig. 6). In addition, the C-terminal region of the HAdV-5 E1A 55R appears to be a more effective inhibitor. In the HAdV-5 E1A 55R protein, two of the three amino acid differences in the C-terminal region are basic residues which could potentially interact with the acidic residues present in the CBM or surrounding N-terminal region, stabilizing an inactive state. Mutation of these basic residues to acidic residues produces a hyperactive co-activator with improved ability to bind the TR, supporting this conjecture (Figs 3 and 4). Intriguingly, there are a number of serines located in the C-terminal region of the 55R E1A proteins. Phosphorylation of these residues and the resulting negative charges could similarly regulate 55R E1A function by affecting potential N- and C-terminal interactions. Binding of the E1A CBM to the unliganded TR may also

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**Fig. 6.** E1A 55R protein functions as a transcriptional activator. (a) Results of yeast one-hybrid analysis performed in *S. cerevisiae* wt yeast BY4741 and the indicated gene deletion strains. Yeast were cotransformed with plasmids expressing the indicated LexA DBD fused E1A bait proteins and β-galactosidase reporter gene under the control of a minimal promoter containing LexA binding sites. Both the HAdV-2 E1A 55R Δ45–55 and the HAdV-5 E1A 55R R52D/K50D mutants contain an intrinsic activation domain, whose function relies on components of the SNF/SWI and SAGA complex. (b) E1A 55R functions as a transcriptional activator in human cells. Human U2OS osteosarcoma cells were transiently transfected with a Gal4-dependent luciferase reporter construct and plasmids expressing the indicated E1A 55R E1A products fused to Gal4 DBD. Extracts were analysed for luciferase activity (top) and expression of Gal4 fusions (bottom). Removal or mutation of the C-terminal region of the E1A 55R protein unmasks a transcriptional activation function in mammalian cells. Error bars in (a) and (b) indicate ± SD.

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**Fig. 7.** Summary of E1A 55R activity. (a) E1A 55R contains a CBM-like sequence spanning residues 20–28 that confers interaction with the TR. The minimal region required for activation of TR-dependent gene expression spans residues 7–32 and flanking regions appear to repress the activity of the minimal activation domain. (b) Model of E1A-dependent activation of TR-dependent gene expression. E1A 55R interacts with the TR and requires the SWI/SNF and SAGA complexes for activation of gene expression.
destabilize the interaction of the E1A 55R C terminus with its N terminus, exposing its intrinsic transcriptional activation domain.

Taken together, we propose a model in which the E1A 55R protein enhances unliganded TR activity by acting as a bridge that permits TR to recruit and or stabilize chromatin modification and remodelling complexes, enhancing transcription (Fig. 7b). Most importantly, these data suggest that the E1A 55R protein has the potential to act as a transcriptional coregulator, much like the larger E1A splice variants. There are analogies between the properties of alternatively spliced HAdV E1A and another differentially spliced factor encoded by a small DNA tumour virus. Alternative splicing of the SV40 early gene is directly modulated by the TR through a hormone receptor (V. D. Arulsundaram and others, 1998). Mutant and wild-type E1As were expressed as LexA DBD fusions in L40 strain yeast (Clontech) and grown overnight in 2 % galactose and minimal media containing all essential amino acids except leucine. Intrinsic transcriptional activation abilities were monitored using the lacZ reporter gene and assayed as Miller units (mg protein)\(^{-1}\) (Meng et al., 2003).

Mammalian two-hybrid and one-hybrid assays. Human U2OS osteosarcoma cells were cultured at 37 °C with 5 % CO\(_2\) in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) supplemented with 10 % FBS (Sigma), penicillin (50 U ml\(^{-1}\)) and 100 μg ml\(^{-1}\) streptomycin (Invitrogen). Cells were transfected using Lipofectamine 2000 (Invitrogen). Following transfections, all samples were grown in DMEM containing no antibiotics and 5 % charcoal/dextran-treated FBS (HyClone). For the one-hybrid studies, 800 ng Gal4 DBD fusions of bait plasmid were prepared with 100 ng luciferase reporter plasmid and 100 ng of a plasmid constitutively expressing β-galactosidase. For the two-hybrid studies, cells were co-transfected with 300 ng Gal4 DBD bait plasmid, 500 ng VP16 prey plasmid, 100 ng luciferase reporter plasmid and 100 ng of the β-galactosidase expressing plasmid. Forty-eight hours after transfection, cells were harvested for luciferase assay (Promega). Photon production was detected as relative light units (RLU) and values were normalized to β-galactosidase activity. The A\(_{420}\) β-galactosidase reading was also normalized with respect to total protein present in extract, which was determined using the Bradford DC Protein Assay kit (Bio-Rad).

Immuno precipitation and Western blotting. U2OS cells were transfected with plasmids expressing either wild type or mutant TRs using X-tremeGENE transfection reagent (Roche Applied Science). Transfected cells were grown in medium containing 5 % stripped FBS for 3 days. Cells were treated or not treated with T3, and lysates were prepared 2 h later. TR and associated proteins were precipitated with anti-FLAG antibody and Western blots were performed using an anti-EGFP antibody to detect E1A. Levels of E1A 55R and TR expression were determined by Western blot of the lysate with anti-EGFP and anti-FLAG antibodies, respectively.

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REFERENCES


