THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 32, Issue of August 8, pp. 29954–29962, 2003
Printed in U.S.A.

Adenomatous Polyposis Coli (APC)-independent Regulation of β-Catenin Degradation via a Retinoid X Receptor-mediated Pathway*

Received for publication, May 7, 2003
Published, JBC Papers in Press, May 27, 2003, DOI 10.1074/jbc.M304761200

Jia-Hao Xiao‡§, Corine Ghosn‡, Cory Hinchman‡, Chad Forbes‡, Jenny Wang‡, Nonna Snider‡, Allison Cordrey‡, Yi Zhao‡, and Roshantha A. S. Chandraratna‡¶‖

From Retinoid Research, the Departments of ‡Biology and ¶Chemistry, Allergan, Inc., Irvine, California 92623

β-catenin is a component of stable cell adherent complexes whereas its free form functions as a transcription factor that regulate genes involved in oncogenesis and metastasis. Free β-catenin is eliminated by two adenomatous polyposis coli (APC)-dependent proteasomal degradation pathways regulated by glycogen synthase kinase 3β (GSK3β) or p53-inducible Siah-1. Dysregulation of β-catenin turnover consequent to mutations in critical genes of the APC-dependent pathways is implicated in cancers such as colorectal cancer. We have identified a novel retinoid X receptor (RXR)-mediated APC-independent pathway in the regulation of β-catenin. In this proteasomal pathway, RXR agonists induce degradation of β-catenin and RXRα and repress β-catenin-mediated transcription. *In vivo*, β-catenin interacts with RXRα in the absence of ligand, but RXR agonists enhanced the interaction. RXR agonist action was not impaired by GSK3β inhibitors or deletion of the GSK3β-targeted sequence from β-catenin. In APC- and p53-mutated colorectal cancer cells, RXR agonists still inactivated endogenous β-catenin *via* RXRα. Interestingly, deletion of the RXRα A/B region abolished ligand-induced β-catenin degradation but not RXRα-mediated transactivation. RXRα-mediated inactivation of oncogenic β-catenin paralleled a reduction in cell proliferation. These results suggest a potential role for RXR and its agonists in the regulation of β-catenin turnover and related biological events.

β-catenin is a key mediator in Wnt regulation of multiple cellular functions in embryogenesis and tumorigenesis (1). In adult tissues, β-catenin is a component of stable cell adherent complexes whereas its free form functions as a co-activator for a family of transcription factors called T cell factor/lymphoid enhancer factor (TCF/LEF).[1] Levels of free β-catenin are tightly regulated by two APC-dependent proteasomal degradation pathways, namely a GSK3β-regulated pathway involving the APC/Axin complex (2) and a p53-inducible pathway involving Siah-1 (3, 4). In the GSK3β-regulated pathway, β-catenin associates with the APC/Axin complex and undergoes a two-step phosphorylation by casein kinase I (CKI) and GSK3β at serine/threonine residues within the first 50 N-terminal amino acids (2, 5). β-catenin interacts with an ubiquitylation complex through the phosphorylated N terminus and undergoes proteasome-catalyzed degradation (6). Wnt inactivation of GSK3β leads to translocation of β-catenin to the nucleus, where it enables TCF/LEF to activate genes involved in embryogenesis and oncogenesis (1, 7). In the second pathway, p53-up-regulated Siah-1 interacts with the N-terminal region of APC, recruits an ubiquitylation complex to the N terminus of β-catenin, and targets it for proteasome-mediated degradation. Thus, both pathways require the intact N terminus of β-catenin. In cancers such as colorectal and hepatocellular cancers and melanoma, mutations in the key components of the two pathways, such as APC, p53, and Axin, or β-catenin itself, lead to dysregulation of β-catenin turnover and, consequently, high levels of nuclear β-catenin and abnormal activation of TCF/LEF-regulated genes that are involved in oncogenesis and metastasis (8, 9).

Retinoids, which are natural and synthetic derivatives of vitamin A, regulate gene transcription through two families of nuclear receptors, *i.e.* retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (10) and have significant anti-cancer effects (11, 12). These receptors are ligand-dependent DNA binding transcription factors. Each receptor has an N-terminal A/B region that harbors the ligand-independent activation function-1 (AF-1), a central DNA-binding domain (the C region), and a C-terminal E region containing a ligand binding domain and a ligand-dependent activation function-2 (AF-2). RARs and RXRs bind to target genes as RAR-RXR heterodimers or RXR homodimers. In the absence of ligands, retinoid receptors are associated with co-repressors and repress gene transcription (13). Once associated with agonists, RARs and RXRs undergo conformational changes, recruit co-activators, and activate target gene transcription. Interestingly, instances of crosstalk between the Wnt/β-catenin- and retinoid-signaling pathways have been reported recently. For example, RAR was found to interact with β-catenin *in vitro* and inhibits β-catenin-mediated gene transcription *in vivo* (14). Retinoic acid, an RAR agonist, was shown to synergize with Wnt signaling in the up-regulation of gene transcription (15, 16). Unlike the APC-dependent pathway, RAR signaling does not regulate β-catenin protein levels (14). On the other hand, RXR agonists have been shown to cause degradation of RXRα and also its receptor heterodimerization partners, including RARs and TR (17, 18). However, the biological consequences of such degradation phenomena have not been well understood. Here, we have investigated the role of RXR and its ligands in the

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence may be addressed: Allergan, Inc., RD-2C, 2525 Dupont Dr., Irvine, CA 92612. Tel.: 714-246-4692; Fax: 714-246-6207; E-mail: xiao_jia-hao@allergan.com.

‖ To whom correspondence may be addressed. Tel.: 714-246-6748; E-mail: chandraratna_rosh@allergan.com.

[1] The abbreviations used are: TCF/LEF, T cell factor/lymphoid enhancer factor; APC, adenomatous polyposis coli; GSK3β, glycogen synthase kinase 3β; RAR, retinoic acid receptor; RXR, retinoid X receptor; TR, thyroid hormone receptor; AF-1/2, activation function 1 or 2; HRP, horseradish peroxidase; HEK293, human embryonic kidney 293 (cells); DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; DSP, dithiobis(succinimidyl propionate).

regulation of β-catenin activity and identified a novel RXR-mediated APC-independent pathway. We show that RXR agonists reduce β-catenin-mediated activation of gene transcription and cell proliferation through a protein degradation mechanism.

EXPERIMENTAL PROCEDURES

Retinoids—The RAR antagonist AGN194310 and the RXR agonist AGN194204 have been described previously (19, 20). The RXR-specific agonists AGN195362, AGN195456, AGN195741, AGN196060, and AGN196459 and the RXR-specific antagonist AGN195393 were synthesized at Allergan. Me_2SO was used as a solvent for the compounds.

Plasmids—TOPFLASH, which contains TCF/LEF binding sites placed in front of the TK-Luc reporter gene, was purchased from Upstate Biotechnology. The β-catenin expression vector, Gene Storm clone H-X87838 M in pcDNA3.1/GS, was purchased from Invitrogen. ΔNβ-catenin, a β-catenin mutant with an N-terminal deletion (amino acid residues 1–50), was made by PCR amplification from wild type β-catenin using the following pair of primers: 5′-AGG GAT CCA ACC ATG AAT CCT GAG GAA GAG-3′ and 5′-AGT CTA GAT TAC AGG TCA GTA TCA AAC CAG-3′. The resulting fragment was cloned into expression vector pcDNA3.1+ (Invitrogen Corp) between *Bam*H1 and *Xba*I and confirmed by DNA sequencing. Finally, the N-terminal coding region with the deletion was released by digestion with *Mun*I and *Xho*I and used to replace the 5′-end of wild type β-catenin in pcDNA3.1/GS.

Human RXRα cDNA in a human keratinocyte cDNA library (21) was identified in a yeast two-hybrid system using RARγ as a bait. The RXRα coding region was amplified from this clone by PCR using a pair of following primers: 5′-AG GAA TTC ATG GAC ACC AAA CAT TTC CTG CCG-3′ and 5′-AG CTG CAG CTA AGT CAT TTG GTG CGG CGG CTC-3′. The resulting fragment was subcloned into pEGFP-N2 (Clontech) between the *Eco*RI and *Pst*I sites and then released by *Eco*RI and *Kpn*I digestion. The released RXRα coding region was then cloned into a modified pCMV-FLAG vector (Sigma) containing the FLAG epitope DYKDDDDK. The RXRα deletion mutants (see Fig. 6*A*) were constructed by PCR amplification of hRXRα cDNA using primers as follows: 5′-AGG AAT TCT GCG CCA TCT GCG GGG ACC GC-3′ and 5′-AGG GTA CCC TAA GTC ATT TGG TGC GGC GCC TCC-3′ for RXRαCDE; 5′-AGG AAT TCA AGC GGG AAG CCG TGC AGG AGG AGC GG-3′ and 5′-AGG GTA CCC TAA GTC ATT TGG TGC GGC GCC TCC-3′ for RXRαDE; 5′-AGG AAT TCT CGC CGA ACG ACC CTG TCA CC-3′ and 5′-AGG GTA CCC TAA GTC ATT TGG TGC GGC GCC TCC-3′ for RXRαE; and 5′-AGG AAT TCA TGG ACA CCA AAC ATT TCC TGC CG-3′ and 5′-AGG GTA CCC TAG ATG AGC TTG AAG AAG AAG AG-3′ for RXRα ΔAF2. The resulting PCR fragments were cut by *Eco*R1 and *Kpn*I and cloned into the pCMV-FLAG vector. For construction of RXRαΔC and RXRαΔCD, the *Eco*R1 fragment containing the A/B region of RXRα was obtained by PCR amplification with primers 5′-AGG AAT TCA TGG ACA CCA AAC ATT TCC TGC CG-3′ and 5′-AGG AAT TCG ATG TGC TTG GTG AAG GAA GCC-3′ and inserted into constructs RXRαDE and RXRαE at the *Eco*R1 site in front of the DE and E regions of RXRα, respectively. For making RXRαΔD, the EcoR1 fragment containing the ABC region of RXRα was prepared by PCR amplification using the following primers: 5′-AGG AAT TCA TGG ACA CCA AAC ATT TCC TGC CG-3′ and 5′-AGG AAT TCC ATG CCC ATG GCC AGG CAC TTC-3′ and inserted into the *Eco*R1 site in front of the E region in construct RXRαE.

Antibodies—Native or horseradish peroxidase (HRP)-conjugated mouse monoclonal antibodies against the FLAG tag in RXRα, M2, and HRP-M2 were purchased from Sigma. Native or HRP-conjugated mouse monoclonal antibodies against the V5 tag in β-catenin, V5, and HRP-V5, respectively, were purchased from Invitrogen. Rabbit polyclonal antibodies against the N terminus of RXRα (D20), the C terminus of β-catenin (H102), poly(ADP-ribose) polymerase (PARP, H-250), GSK3β (H76), and a mouse monoclonal antibody against β-tubulin (D-10) were purchased from Santa Cruz Biotechnology.

Cell Lines—HEK293, HeLa, CV1, and SW480 cells were purchased from the American Type Culture Collection and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 10 μg/ml streptomycin at 37 °C in 5% CO_2.

To generate the cell lines CAT and mCAT that stably expresses β-catenin or ΔNβ-catenin, respectively, pGS-β-catenin or pGS-ΔNβ-catenin was transfected into HEK293 cells using LipofectAMINE. Twenty-four hours later, the cells were subjected to selection in the presence of zeocin (Invitrogen) at 400–500 μg/ml. The selection medium was changed every 3 days, and individual zeocin-resistant clones were isolated. Clones stably expressing β-catenin or ΔNβ-catenin were identified by Western blotting.

To produce CATXα and mCATXα cell lines that stably express RXRα with wild type β-catenin or mutant ΔNβ-catenin, pGS-β-catenin and pGS-ΔNβ-catenin were transfected into cell line Xα that stably expresses FLAG-tagged RXRα. These cell lines were established as described above.

Inhibitors—Proteasome inhibitors MG132 and MG262 were purchased from Biomol Research Laboratories and Calbiochem, respectively. Lysosome inhibitors, bafilomycin, leupeptin, E-64, and ammonium chloride were purchased from Sigma-Aldrich.

Reporter Gene Assays—For measuring the TOPFLASH activity, cells were seeded at 50,000 cells per well in 24-well plates coated with poly-D-lysine (BD Biosciences). Twenty-four hours later, TOPFLASH and expression vectors were co-transfected into cells using FuGENE (Roche Applied Science) in DMEM containing 10% charcoal-treated FBS. To monitor transfection efficiency, either 15 ng of phRG-TK renilla or 100 ng of CMX-LacZ DNA were co-transfected. Five hours later, vehicle or retinoids were added. The cells were treated for 16 h before harvest. Luciferase activity was measured using the Dual luciferase reporter 100 assay system (Promega). Control Renilla activity was determined using the same kit. A second control, β-galactosidase activity, was measured by colorimetric assays. The reporter activity was normalized against either β-galactosidase or Renilla activity.

Analysis of RXRα and its mutants in transactivation was performed as follows. 3.5×10^3 CV-1 cells per well of a 96-well opaque plate (Falcon) were transiently transfected using LipofectAMINE with the reporter plasmid CRBPII-TK-Luc together with 0.04 μg of RXRα mutants. Five hours later, DMEM containing 10% charcoal-treated FBS and retinoids were added to the wells. Cells were grown for a further 18 h and lysed for determination of luciferase activity.

Protein Expression and Western Blotting Analysis—Cells were seeded at 2×10^6 per plate into 100-mm dishes in DMEM containing 10% FBS and, on the next day, transfected with 1–4 μg of various cDNA or parental expression vectors (8 μg of DNA in total) using LipofectAMINE (Invitrogen). Five hours later, the cells were fed with fresh DMEM medium containing 10% charcoal-treated FBS and vehicle or retinoids. After the retinoid treatment, the total cell lysates were prepared using a buffer containing 1% Nonidet P-40, 30 mM Tris-HCl (pH 7.4), 0.5 mM EDTA (pH 8.0), 150 mM NaCl, 10% glycerol, 1 mM sodium orthovanadate, 40 mM NaF, 0.5 mM phenylmethylsulfonyl fluoride, and a mixture of protease inhibitors (Merck). The cell lysates were homogenized by passing through a QIAshredder (Qiagen) and cleared from insoluble materials by centrifugation at 12,000 rpm in a bench top Eppendorf centrifuge. Protein concentrations were determined using the Bradford kit (Bio-Rad). Proteins were resolved on 4–12% SDS-PAGE and transferred to either nitrocellulose or polyvinylidene difluoride membranes. The membranes were blocked with 10% nonfat milk in phosphate-buffered saline (PBS, Invitrogen) containing 0.1% Tween 20 (PBST). The membranes were incubated with primary antibodies at room temperature for 2 h or at 4 °C overnight. After the removal of unbound antibodies, membranes were incubated with HRP-conjugated secondary antibodies for 1 h at room temperature and washed five times with PBST. The antibody-associated protein bands were revealed using the ECL plus system (Amersham Pharmacia Biotech).

Pulse-Chase Analysis—Cells in growth phase were washed with RPMI 1640 medium without cysteine, methionine, and glutamine (Cellgro), starved in the same medium for 60 min, and pulse-labeled using 200 μCi of ^{35}S Promix (Amersham Biosciences) for 1 h. Then, the cells were washed with RPMI 1640 three times and chased with the medium supplemented with cysteine (50 μg/ml), methionine (15 μg/ml), and glutamine (2 mM) in the presence or absence of AGN194204. Total cell extracts were prepared as described above. RXRα and β-catenin were immunoprecipitated using the M2 and V5 antibodies, respectively. The immunoprecipitated proteins were resolved on 4–12% SDS-PAGE. Gels were stained with Coomassie Blue and treated with the reagent Amplify (Amersham Biosciences) for 15 min, vacuum-dried, and subjected to autoradiography.

In Vivo Protein Crosslinking and Immunoprecipitation—HEK293 cells at ~80% confluence in 150-mm poly-D-lysine-coated plates (BD Biosciences) were transfected with expression vectors for β-catenin (8 μg), ΔN-β-catenin (8 μg), and RXRα (4 μg) and cultured overnight in DMEM containing high glucose and 10% activated charcoal-treated fetal bovine serum. The cells were treated with vehicle or 1 μM AGN194204 for 20 min in the same medium and then with 1 mM dithiobis(succinimidyl propionate) (DSP; Pierce), a reversible crosslinking reagent, in phosphate-buffered saline for 15 min. The reaction was quenched for 15 min by Tris-HCl buffer (pH 7.5) at 20 mM. The cells

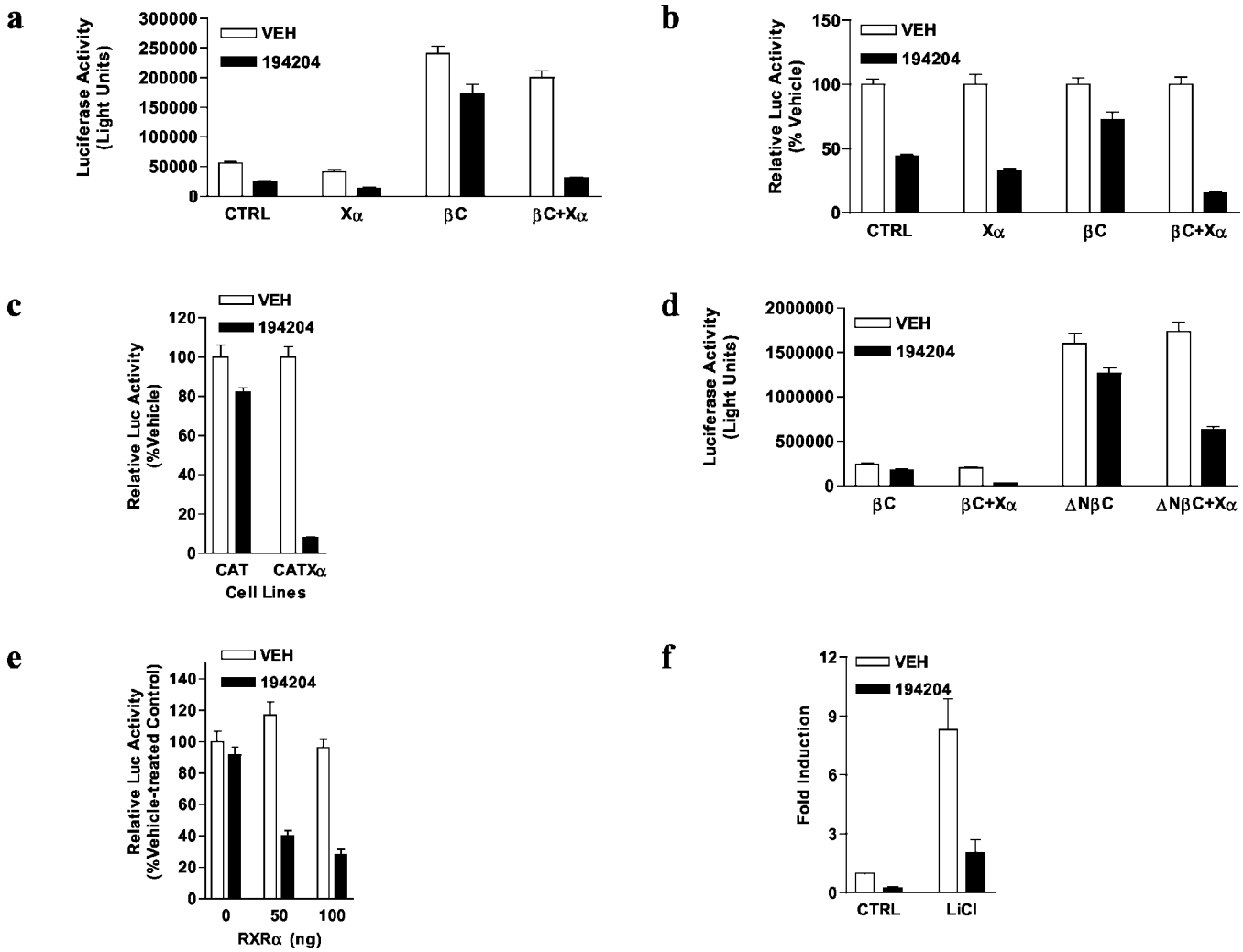

FIG. 1. **β-Catenin-mediated transcription is inhibited by RXR agonists *via* RXRα.** Cells were transiently transfected with 100 ng of TOPFLASH reporter gene with or without expression vectors as indicated. Cells were treated with vehicle (*VEH*) or 0.1 μM AGN194204 for 17 h, and reporter activity was measured. All reporter activity is expressed as mean ± S.E. from 6–8 samples. The total amount of DNA in all transfections was kept constant using appropriate parental empty expression vectors. *a* and *b*, HEK293 cells transfected with expression vectors for β-catenin (*βC*, 200 ng) and/or RXRα (*Xα*, 20 ng). The reporter activity is expressed as light units (*a*) or a percentage of the vehicle-treated cells transfected with the same plasmids (*b*). *c*, HEK293-derived cell lines stably expressing the indicated proteins. *CAT*, β-catenin; *CATXα*, β-catenin and RXRα. The reporter activity is expressed as percentage of the vehicle-treated cells of the same line. *d*, HEK293 cells transfected with expression vectors for β-catenin (*βC*, 200 ng) or mutant ΔNβ-catenin (*ΔNβC*, 200 ng) with or without RXRα (*Xα*, 20 ng). The reporter activity is expressed as light units. *e*, SW480 cells transfected with increasing amounts of RXRα as indicated. The reporter activity is expressed as percentage of the vehicle-treated control cells transfected with the empty expression vectors. *f*, CATXα cells treated with AGN194204 and LiCl (10 mM) as indicated. The reporter activity is expressed as fold induction over the vehicle-treated control (*CTRL*).

were lysed in ice-chilled radioimmune precipitation assay buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS in phosphate-buffered saline) containing a mixture of protease inhibitors (Sigma) and homogenized by QIAshredder (Qiagen). The extracts (1.5 mg per immunoprecipitation) were incubated overnight with specific antibodies and protein G-agarose beads with constant shaking at 4 °C. Immunoprecipitated materials were washed with the ice-cold radioimmune precipitation assay buffer and dissolved in SDS-PAGE loading dye containing β-mercaptoethanol by heating at 100 °C for 5 min. This procedure frees the DSP-crosslinked molecules pulled down by the antibodies. Proteins were resolved on 4–12% SDS-polyacrylamide gels followed by Western blotting.

Cell Proliferation Assay—Cell proliferation assays were performed in 96-well microtiter plates. HEK293, mCAT (HEK293-derived cells stably expressing ΔNβ-catenin), Xα (HEK293-derived cells stably expressing RXRα), and mCATXα cells were seeded at 200–400 cells/well in regular growth medium. The next day, vehicle or retinoids were added. Cell proliferation was measured after 6 days of treatment using a cell proliferation kit purchased from Chemicon International.

RESULTS

RXR Agonists Inactivate β-Catenin-mediated Transcription via Endogenous and Transfected RXRs—We investigated the effect of RXR-specific agonists on β-catenin-mediated TCF/LEF transcriptional activity, a surrogate marker for the oncogenic activity of β-catenin, using the TOPFLASH reporter gene, which contains TCF/LEF binding sites (22, 23). In HEK293 cells, the significant reporter activity produced by endogenous β-catenin was reduced by AGN194204, an RXR-specific agonist (20), in the absence (~50%) or presence (~70%) of transfected RXRα (Fig. 1, *a* and *b*). The significantly increased reporter activity obtained with β-catenin transfection was still very effectively (~80%) reduced by AGN194204 treatment in the presence of cotransfected RXRα (Fig 1, *a* and *b*). Similar inhibition was observed in CATXα cells that were stably transfected with both β-catenin and RXRα, whereas the AGN194204 effect was less pronounced in CAT cells that were stably transfected with only β-catenin (Fig. 1*c*).

RXR Agonists Induce β-Catenin Degradation via Endogenous and Overexpressed RXRs—Because APC-dependent protein degradation pathways regulate β-catenin-mediated transcription (6, 22, 23) and RXR agonists induce degradation of

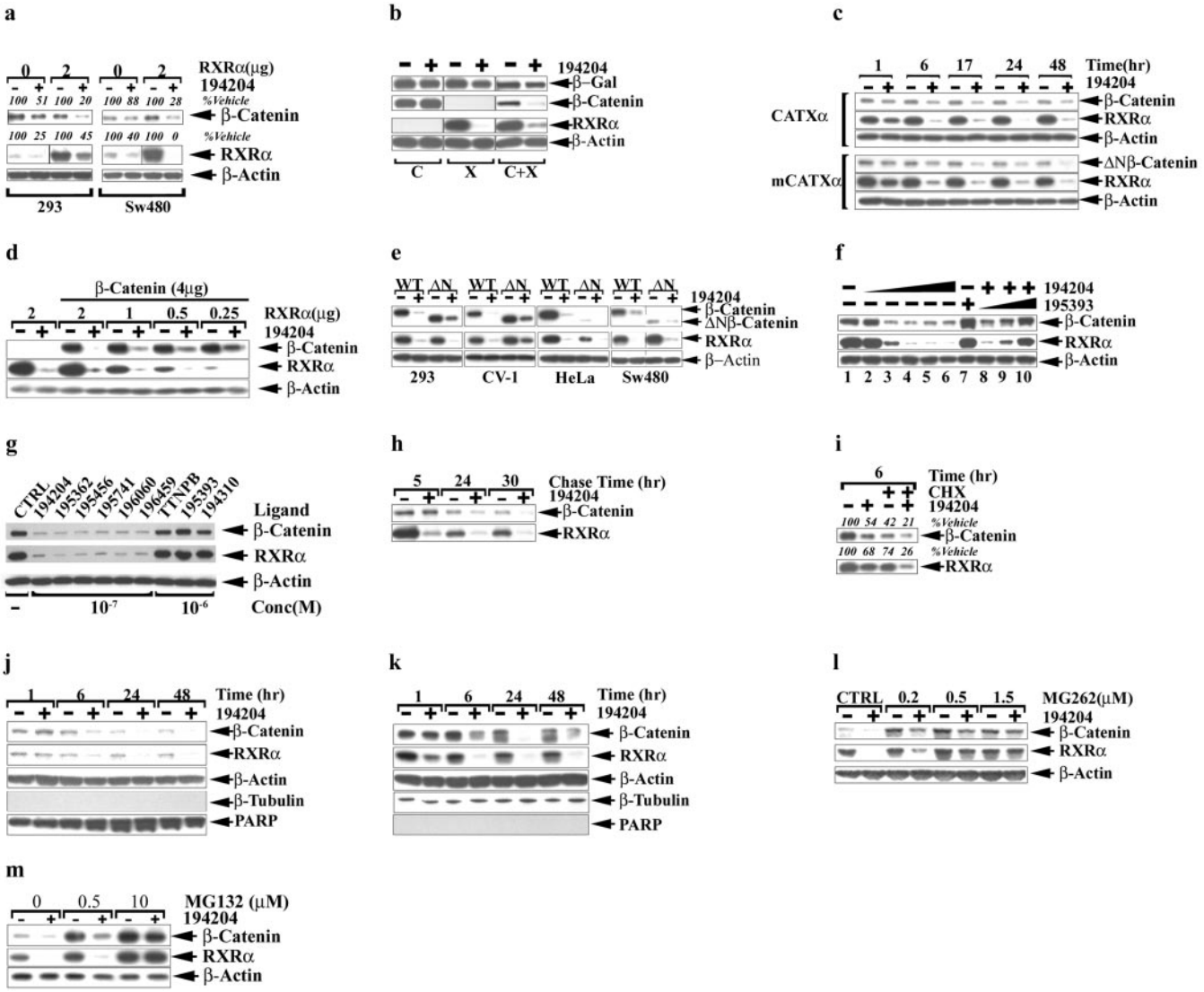

FIG. 2. ***β*-Catenin protein is degraded by RXR agonists in an RXR*α*-dependent manner.** Cells were treated with vehicle (−) or 0.1 μM RXR agonist AGN194204 (+) for 17 h unless otherwise indicated. Protein levels were determined by Western blotting using antibodies HRP-V5 and HRP-M2 for transfected V5-tagged β-catenin and FLAG-tagged RXRα, respectively, unless otherwise indicated. β-actin was used as an endogenous control. *a*, effects of AGN194204 on endogenous β-catenin and endogenous and transfected RXRα. Cell lines HEK293 and SW480 were transfected with 2 μg of RXRα expression vector or its parental vector. Endogenous β-catenin was immunoprecipitated using rabbit polyclonal antibody H12 and revealed by Western blotting using mouse monoclonal antibody E5. Endogenous and transfected RXRα was analyzed by Western blotting using rabbit polyclonal antibody D20. The relative levels of β-catenin and RXRα are expressed as a percentage of their levels in vehicle-treated cells as indicated on the *top* of the blots. *b*, effects of RXR agonist on transfected β-catenin and RXRα proteins. HEK293 cells were transfected with expression vectors for LacZ (2 μg), β-catenin (*C*, 4 μg), and RXRα (*X*, 2 μg) as indicated below the blots and treated for 15 h. *c*, effects of RXR agonist on RXRα and β-catenin proteins in the HEK293-derived cell lines CATXα and mCATXα (stably expressing ΔNβ-catenin and RXRα). *d*, effect of RXRα levels on β-catenin degradation. HEK293 cells were transfected with β-catenin plus increasing amounts of RXRα as indicated. *e*, RXRα-mediated β-catenin degradation in different cell lines transfected with RXRα and wild type (*WT*) or mutant β-catenin (Δ*N*). *f* and *g*, specificity of RXR ligands in inducing β-catenin degradation. HEK293 cells were transfected with RXRα and β-catenin. In *panel f*, cells were treated with AGN194204 at 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} (*lanes 2–6*), and 10^{-8} M (*lanes 8–10*) and/or RXR antagonist AGN195393 at 10^{-6} (*lane 7*), 10^{-8}, 10^{-7}, and 10^{-6} M (*lanes 8–10*), respectively. In *panel g*, cells were treated with vehicle or various synthetic retinoids at the indicated concentrations. *h*, pulse-chase analysis of the effects of RXR agonist on stability of β-catenin and RXRα in CATXα cells. AGN194204 was included in the chase media as indicated. ^{35}S-labeled β-catenin and RXRα were detected by autoradiography after immunoprecipitation and SDS-PAGE gel separation. *i*, effects of cycloheximide on the RXR agonist-induced β-catenin and RXRα degradation. HEK293 cells were transfected with RXRα and β-catenin. Seventeen hours later, the cells were treated with vehicle or AGN194204 for 6 h in the absence or presence of cycloheximide (10 μg/ml). The relative β-catenin and RXRα levels are expressed as a percentage of their levels in vehicle-treated cells, as indicated on *top* of the blots. *j* and *k*, RXRα-mediated reduction of β-catenin in nuclear and cytosolic fractions of CATXα cells. Nuclear extracts (*j*) were prepared as described previously (42). Cytosolic components (*k*) were isolated using a digitonin-based buffer (*26*). *l* and *m*, inhibition of RXR ligand-induced degradation of RXRα and β-catenin by proteasome inhibitors. HEK293 cells transfected with β-catenin and RXRα were pretreated with the proteasome inhibitor MG262 or MG132 for 1 h followed by a further 6-hour treatment with AGN194204.

RXR and associated receptor partners (17, 18), we sought to determine whether AGN194204 inhibited TOPFLASH reporter activity by reducing β-catenin protein levels. HEK293 cells were treated with vehicle or the RXR agonist, and total cell lysates were analyzed by immunoprecipitation and Western blotting. As shown in Fig. 2*a*, AGN194204 decreased endogenous β-catenin (~50%) and RXRα levels in HEK293 cells in the absence of transfected RXRα. AGN194204 further reduced endogenous β-catenin (~80%) in the presence of transfected RXRα. In HEK293 cells transfected with β-catenin alone, AGN194204 had no effect on β-catenin because of the low levels of endogenous RXRα relative to transfected β-catenin (Fig. 2*b*).

However, it dramatically reduced β-catenin protein levels concurrent with RXRα protein levels in cells transfected with both RXRα and β-catenin. Similar AGN194204 effects were obtained in stably transfected HEK293 cells (CATXα; Fig. 2*c*) or transiently transfected CV1, HeLa, and SW480 cells (Fig. 2*e*), indicating the ubiquitous nature of this phenomenon. The AGN194204 effects on reducing β-catenin protein levels were time- (Fig. 2*c*) and dose-dependent (Fig. 2*f*), and the efficiency of the reduction depended on RXR protein levels (Fig. 2, *a* and *d*). AGN194204 readily caused a significant decrease of β-catenin at a dose as low as 1 nM (Fig. 2*f*), reflecting its high affinity for RXRα. Several different RXR agonists, including AGN195362, AGN195456, AGN195741, AGN196060, AGN196459, and 9-*cis* retinoic acid, similarly reduced β-catenin protein levels (Fig. 2*g*, and data not shown). An RXR-specific antagonist, AGN195393 (24), dose-dependently inhibited the AGN194204 effects on β-catenin and RXR protein levels (Fig. 2*f*). An RAR agonist, TTNPB, or an RAR antagonist, AGN194310, showed no effects (Fig. 2*g*). Pulse-chase analysis was performed to ascertain whether the RXR agonist effect occurs at the level of protein degradation. AGN194204 accelerated degradation of both ^{35}S-labeled RXRα and β-catenin (Fig. 2*h*). In the presence of cycloheximide and the absence of AGN194204, β-catenin is readily subjected to degradation by the active APC-pathways in HEK293 cells (compare *lane 3* to *lane 1* in Fig. 2*i*) as expected. However, cycloheximide did not block the AGN194204-induced degradation of β-catenin and RXRα (comparing *lane 4* to *lane 3* in Fig. 2*i*), indicating that induction of transcriptional activity is not required for this effect. Together, these data indicate that RXR agonists reduce β-catenin protein levels by an RXR-mediated protein degradation pathway, which is independent of the RXR-mediated gene transcription activation pathway.

In cells where the Siah- and GSK3β-regulated APC pathways are impaired by mutations or GSK3β is inhibited by Wnt signaling, levels of β-catenin are increased in the cytoplasmic compartment, and, ultimately, β-catenin is translocated to the nucleus where it transactivates the TCF/LEF-targeted genes. Our transactivation data indicated that nuclear β-catenin-related transcriptional activity was reduced by RXR agonists. We further examined whether inhibition of β-catenin-mediated gene transcription by AGN194204 is due to a reduction of β-catenin protein levels in the nucleus with an analysis of nuclear and cytosolic fractions of CATXα cells. Poly(ADP-ribose) polymerase and β-tubulin were used as nuclear and cytoplasmic markers, respectively, for monitoring the efficiency of separation of the two fractions. Decreases in β-catenin protein levels as a result of AGN194204 treatment were observed in both nuclear and cytosolic compartments (Fig. 2, *j* and *k*).

The APC-dependent degradation of β-catenin and the agonist-dependent degradation of RXR proceed by proteasomal pathways (6, 17, 18). To determine whether the RXR agonist-induced degradation of β-catenin involves this pathway, we treated cells with the proteasome inhibitors MG262 and MG132. As shown in Fig. 2, *l* and *m*, these two inhibitors dose-dependently blocked AGN 194204-induced degradation of both β-catenin and RXRα. However, lysosomal inhibitors such as bafilomycin, E64, NH_4Cl, and leupeptin had no effect (data not shown). These data indicate that RXR agonist-induced degradation of β-catenin also proceeds by a proteasomal pathway.

The RXR-regulated β-Catenin Degradation Pathway Is Independent of the p53/Siah-1- and GSK3β-regulated APC Pathways—To determine whether the GSK3β- or p53/Siah-regulated APC pathways are involved in the RXR agonist effects, a β-catenin mutant (ΔNβ-catenin) with a deletion of the N-terminal sequence (50 amino acids) that is targeted by the two APC-dependent pathways was prepared (6, 23). Although this mutant, which is resistant to APC-mediated degradation pathways, showed higher TOPFLASH reporter gene activity than wild-type β-catenin, its increased activity was very effectively inhibited by AGN194204 in the presence of RXRα (Fig. 1*d*). High levels of TOPFLASH reporter activity associated with elevated β-catenin levels have been reported in SW480 colorectal cancer cells wherein both APC and p53 genes contain loss-of-function mutations (22, 23, 25). However, AGN194204 effectively inhibited reporter activity in SW480 cells containing cotransfected RXRα (Fig. 1*e*), which is consistent with the observed decrease in endogenous β-catenin levels in these cells (Fig. 2*a*). Similarly, whereas LiCl, a GSK3β inhibitor that is known to elevate free β-catenin levels (26) significantly increased TOPFLASH reporter activity, AGN194204 still effectively reduced this elevated activity (Fig. 1*f*). At the protein level, AGN194204 induced degradation of ΔNβ-catenin as effectively as that of wild-type β-catenin in transiently (Fig. 2*e*) or stably transfected cells (Fig. 2*c*). Together, these data clearly indicate that the RXR-mediated degradation of β-catenin does not involve the APC-dependent pathways.

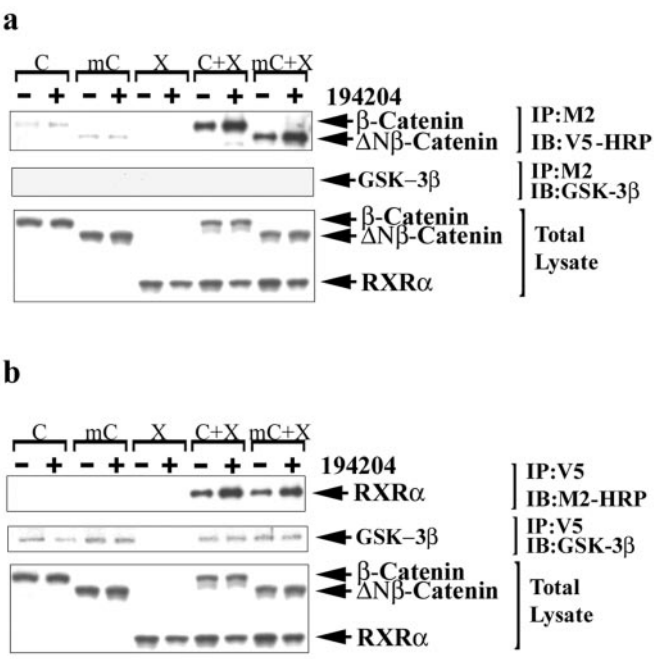

FIG. 3. **Interaction of β-catenin with RXRα *in vivo*.** HEK293 cells were transfected with expression vectors for β-catenin (*C*, 8 μg), Δβ-catenin (*mC*, 8 μg), and RXRα (*X*, 4 μg) as indicated. Cells were treated with 1 μM AGN194204 for 20 min and incubated with DSP for 15 min. After crosslinking, total cell lysates were prepared and subjected to immunoprecipitation (*IP*) using antibodies against FLAG in RXRα (*a*) or V5 in β-catenin and ΔNβ-catenin (*b*). Immunoprecipitates were dissociated by β-mercaptoethanol reduction and subjected to Western blotting (*IB*) using antibodies against V5 (*a*) or GSK3β (*a* and *b*) or FLAG antibodies (*b*). Total cell lysates were analyzed by direct Western blotting after β-mercaptoethanol reduction (*Total Lysate*, *bottom* gels in each *panel*).

β-Catenin Interacts with RXRα in Vivo—Our data showed that RXR agonist-induced new protein synthesis was not required for β-catenin degradation (Fig. 2*i*), whereas RXR protein degradation appeared to be essential for the process (Fig. 2*d* and Ref. 2l). These observations suggested that RXRα and β-catenin proteins interacted directly or were present in the same degradation complex. To test this hypothesis, HEK293 cells were transfected with a combination of expression vectors for RXRα and wild type β-catenin or ΔNβ-catenin. The cells were treated with AGN194204 and subjected to cross-linking

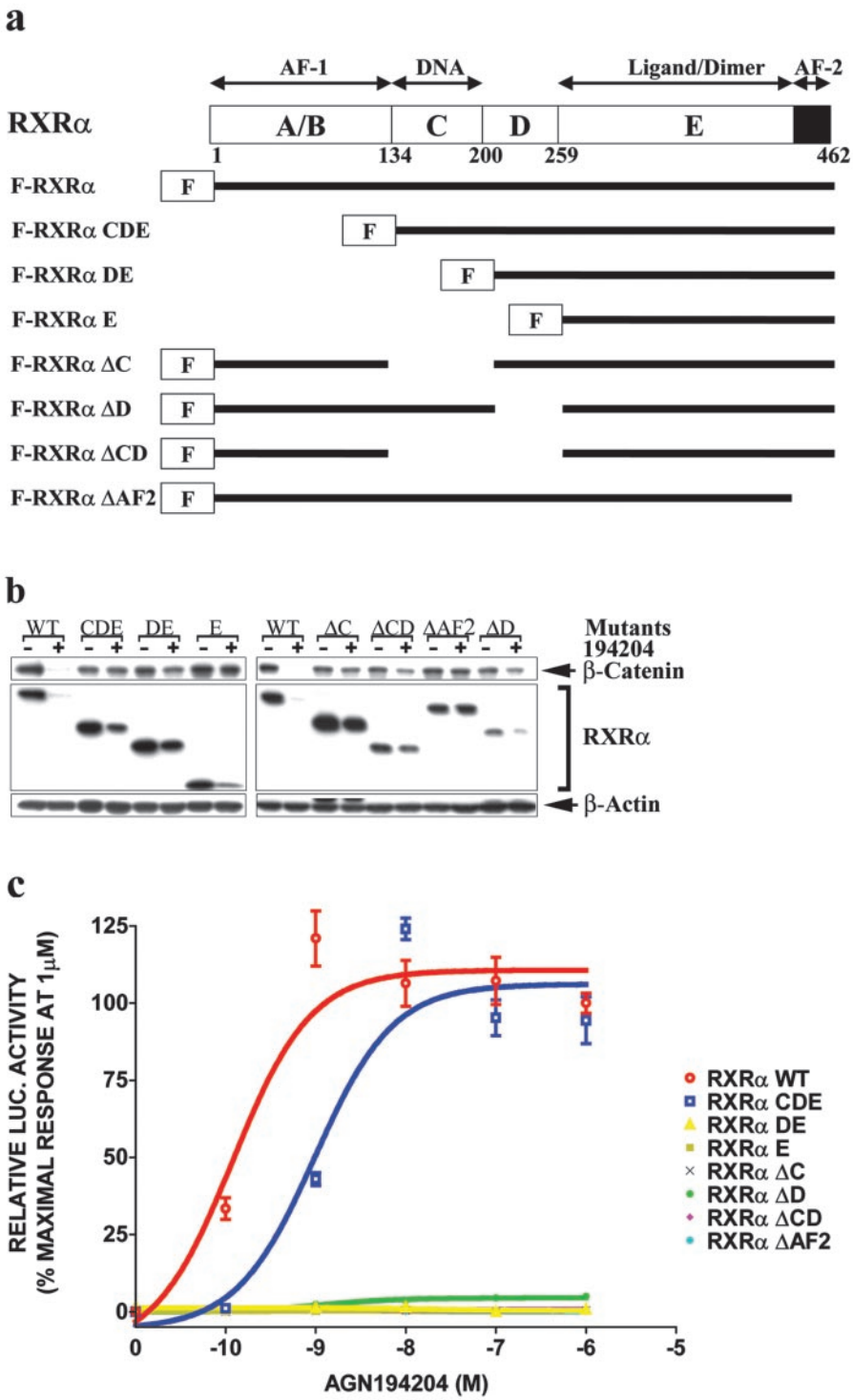

FIG. 4. **RXR agonist-induced β-catenin degradation and transactivation of RXR-responsive genes are two separate functions of RXRα.** *a*, diagram shows functional domains deleted in RXRα mutants. These include the AF-1 domain, the DNA binding domain, the ligand binding domain, the dimerization domain (*Dimer*), and the AF-2 domain. *Solid bars* indicate regions retained in the mutants. *F*, FLAG tag. *b*, effects of AGN194204 on HEK293 cells transfected with β-catenin (2 μg) and RXRα mutants (4 μg). Cells were treated with vehicle (−) or 0.1 μM AGN194204 (+) for 17 h. *c*, the dose-dependent effects of AGN194204 on luciferase reporter activity in cells transfected with CRBPII-TK-Luc and the RXRα mutants.

using the reversible cross-linker DSP prior to cell lysis and immunoprecipitation. Both β-catenin and ΔNβ-catenin were pulled down by the FLAG antibody only in cells cotransfected with FLAG-RXRα (Fig. 3*a*), and, conversely, RXRα was pulled down by V5 antibody only in cells cotransfected with V5-tagged β-catenin proteins (Fig. 3*b*). Under the same conditions, endogenous GSK3β was co-immunoprecipitated with β-catenin but not with RXR, thereby indicating that the RXRα/β-catenin interaction is specific and does not involve GSK3β. Although AGN194204 enhanced the effect, RXRα interacted with β-catenin even in the absence of an RXR agonist.

RXR Agonist-induced β-Catenin Degradation and Transactivation Are Two Separable Functions of RXRα—To determine the functional domains of RXRα associated with degradation of β-catenin, various deletions were introduced into the receptor (Fig. 4*a*). Helix 12 (AF-2) of RXRα was required for both self and β-catenin degradation as would be expected for agonist-induced functions (Fig. 4*b*). RXRα mutants carrying C and/or D region deletions were able to mediate agonist-induced degradation of β-catenin, albeit with reduced efficiency relative to the wild-type receptor. A/B region deletion mutants were particularly ineffective in mediating β-catenin degradation, although the mutant receptors themselves underwent degradation in response to AGN194204. Interestingly, RXRα CDE, an A/B region deletion mutant, was fully effective in agonist-induced gene transcription (Fig. 4*c*), indicating that β-catenin

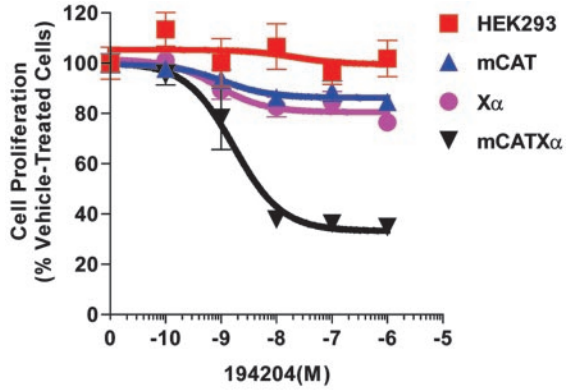

FIG. 5. **RXR agonists inhibit growth of ΔNβ-catenin-expressing cells *via* RXRα.** The stable cell lines mCAT, Xα, and mCATXα and their parental cell line HEK293 were treated with vehicle or AGN194204 for 6 days. Cell proliferation rate was measured using a cell proliferation kit (Chemicon). Data are mean ± S.E. from more than six samples.

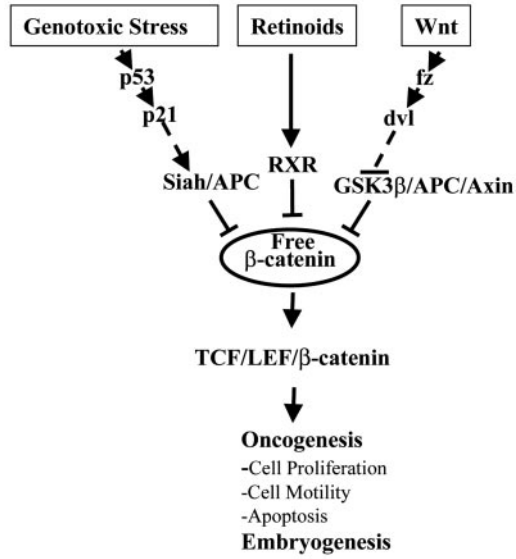

FIG. 6. **A schematic model of three pathways in regulation of β-catenin turnover.** Pathway-specific components are listed. *fz*, frizzled; *dvl*, dishevelled.

degradation and transcriptional activation are two separable functions of RXRα.

RXR Agonists Inhibit Growth of ΔNβ-Catenin-expressing Cells via RXR—Dysregulation of β-catenin by mutations in the N-terminal GSK3β-targeted sites is associated with cancer cell growth. Specific reduction of β-catenin protein levels by antisense oligonucleotides or small interference RNA in APC mutant colon cancer cells inhibited cell proliferation, anchorage-independent growth, and cellular invasiveness *in vitro* (27, 28). We have investigated whether the RXRα-mediated degradation of ΔNβ-catenin affected the cell proliferation rate. The growth of mCATXα cells, which stably express both ΔNβ-catenin (mCAT) and RXRα, was effectively inhibited by AGN194204 in a dose-dependent manner, whereas HEK293-cells that stably express either ΔNβ-catenin (mCAT) or RXRα (Xα) alone are not substantially impaired by AGN194204 treatment (Fig. 5). The IC_{50} for the growth inhibitory effect of AGN194204 was ~1 nM, which was consistent with the affinity of this ligand for RXRα and also its potency in inducing β-catenin degradation (Fig. 2*f*).

DISCUSSION

A Novel APC-independent and RXR-dependent Pathway Regulating β-Catenin Turnover—Several lines of evidence suggest that the RXR-dependent pathway of β-catenin regulation is distinct from the APC-dependent pathways. A common recognition target for the two APC-dependent pathways regulated by GSK3β and p53/Siah is the N-terminal region of β-catenin. In this study, we first showed that removal of the N terminus did not block RXR agonist-induced degradation of β-catenin *via* RXRα (Fig. 2). Second, in SW480 cells wherein both GSK3β- and Siah-mediated APC pathways are impaired because of loss-of-function mutations in APC and p53 (3, 4, 22, 23, 25), RXR agonists were still able to inactivate β-catenin through degradation (Figs. 1 and 2). Third, although inhibition of GSK3β by LiCl elevated β-catenin activity, it did not block the RXR agonist action. Finally, deletion of the GSK3β-targeted N terminus of β-catenin gave a mutant protein that was still efficiently degraded and whose transactivational activity was still significantly reduced by an RXR agonist. These features distinguish the RXR-mediated pathway from the two APC-dependent pathways. Thus, the RXR-dependent pathway clearly represents a novel regulatory pathway for controlling β-catenin turnover (Fig. 6).

Mechanism by Which RXR Agonists Induce Degradation of β-Catenin—Our data clearly indicated that stoichiometric levels of RXRα were required for the efficient degradation of β-catenin (Fig. 2, *a* and *d*), indicating that RXRα and β-catenin are very likely present in the same degradation complex. This was confirmed by our observation that β-catenin interacted with RXRα in intact cells (Fig. 3). The interaction appears to be transient, because we were not able to detect it under conventional immunoprecipitation conditions and also because GST-β-catenin had previously been shown not to interact with *in vitro* translated RXRα (14). Examination of the β-catenin protein sequence revealed the presence of five consensus L*XX*LL receptor-interacting motifs, which are usually found in co-activators and co-repressors that interact with nuclear receptors. Whether these serve as RXR-interacting motifs in β-catenin remains to be determined.

RXR agonists have been reported previously to cause RXR-mediated degradation of RXR dimerization partners such as RAR and TR. The degradation of these receptor heterodimers is achieved by the ubiquitin-mediated proteasomal system (17, 18). Heterodimeric partners of RXRs such as RARs and TR have been found to be co-degraded with RXRs in the presence of RXR agonists (17, 18). When each member of the RAR family was co-transfected with β-catenin, treatment with RAR agonist TTNPB or RXR agonist AGN194204 did not significantly alter β-catenin protein level (data not shown). However, when RXRα was included in the co-transfection, the RXR agonist induced degradation of β-catenin efficiently (data not shown). Under the same condition, all three members of the RAR family and RXRα were degraded when AGN194204 was added. These observations suggest that the RXR agonist induced β-catenin degradation via RXR and that heterodimeric partners such as RARs are not required in the regulation of β-catenin by RXR agonists. We showed that proteasome inhibitors blocked RXR agonist-induced degradation of both β-catenin and RXR, indicating that the process involves a proteasomal pathway (Fig. 2, *l* and *m*). Thus, it appears that a broad spectrum of RXR-interacting proteins, exemplified by RAR, TR and β-catenin, can be targeted for degradation by RXR agonists. It should be noted that RXR interacts with these various proteins in the absence of a ligand, although the interaction may be enhanced by agonists. However, dimerization partners were not required for RXRα degradation by RXR agonists (17, 18), and our results are consistent with these observations. Upon ligand binding, RXRα itself changes conformation, is subjected to modification, and becomes a target of the ubiquitin-proteasome machinery (17, 18). Two distinct mechanisms can be envisaged for the

degradation of RXR-targeted proteins. In the first scenario, RXRα serves as a docking and regulatory protein for its targets, which undergo parallel changes in response to RXR agonists and become substrates of the proteasome machinery. In the second scenario, RXRα serves as a targeting molecule that carries interacting proteins to certain cellular compartments for modification and degradation. Given the complexity of the APC-dependent β-catenin regulatory machinery, the RXRα-mediated pathway may also similarly involve multi-protein complexes and multi-step reactions. Further studies will be required to elucidate the details of this intriguing pathway.

Two Separate Functions of RXRα Are β-Catenin Protein Degradation and Transcriptional Activation—In this study, we have compared the effects of deletion of different RXR functional domains on the degradation of β-catenin and RXRα and on RXR-mediated transactivation (Fig. 4). This comparison revealed several key differences. First, the A/B region of RXRα was required for agonist-induced β-catenin degradation but not for transactivation. Second, the integrity of RXRα was required for efficient degradation of β-catenin, whereas degradation of RXRα itself was less sensitive to the loss of certain functional regions as illustrated by the sensitivity of RXRαE, which contains only the ligand-binding domain, to AGN194204-induced degradation. In addition, our experiment using cycloheximide indicated that the RXR agonist effects on β-catenin and RXRα degradation do not require *de novo* protein synthesis (Fig. 2*i*). In other words, transactivation of RXR-regulated genes was not required for β-catenin degradation. These observations suggest that the requirements for β-catenin degradation are different than those for transactivation, indicating that ligand-mediated protein degradation and transcriptional activation are two separable functions. In support of this view, Osburn *et al.* (18) have shown that ligand-induced degradation of RXRα is independent of its transcriptional activity and does not require interaction with a co-activator. On the other hand, helix 12 (AF-2) of RXRα is essential for all agonist-mediated biological activities. This is not unexpected, because repositioning of helix 12 is largely responsible for the changes in RXRα conformation caused by agonist binding (29). Although conformational changes of this type are necessary for both β-catenin degradation and transactivation functions, it is possible that the optimal conformation of RXRα for inducing β-catenin degradation may be different from that for transactivation. In summary, our results have identified a novel ligand-dependent function for RXRα, namely that of targeting oncogenic proteins for degradation, which is distinct from its role in the regulation of gene transcription.

β-Catenin and Cell Growth—Dysregulation of β-catenin turnover by mutations in the N-terminal GSK3β-targeted sites is associated with cancer cell growth. For example, expression of β-catenin with gain-of-function mutations such as mutations or deletions of its N-terminal casein kinase I/ glycogen synthase kinase 3β phosphorylation sites caused tissue neoplastic growth in animals (30–35). Overexpression of similar β-catenin mutants leads to neoplastic transformation of E1A-immortalized epithelial cells and stimulated proliferation of p53- or ARF-null mouse embryo fibroblasts (36, 37). Consistent with these results, specific reduction of β-catenin in APC-mutant colon cancer cells by antisense oligonucleotides or small interference RNA inhibited the proliferation, anchorage-independent growth, and cellular invasiveness *in vitro* and neoplastic growth of xenografts in animals (27, 28, 38). In keeping with the role of β-catenin in cell growth, activation of the RXR-mediated pathway reduced cell proliferation (Fig. 5) in parallel with the reduction in ΔNβ-catenin protein levels and transcriptional activity (Figs. 1 and 2).

The Potential Role of the RXRα Pathway in Cancer Therapy—There are four major pathological events that cause a slow turnover of β-catenin in cancer as follows: 1) mutations of the Axin- and β-catenin-interacting motifs in APC; 2) mutations of the N-terminal GSK3β phosphorylation sites in β-catenin; 3) mutations in Axin; and 4) inactivation of GSK3β by the Wnt signaling pathway (39). These events are believed to be the major factors underlying pathogenesis of cancers such as colorectal cancer and melanoma. Mechanistically, these events lead to the escape of β-catenin from cellular surveillance and subsequently to the pathogenic activation of genes involved in tumorigenesis and metastasis. Thus, in cancers involving dysregulation of β−catenin turnover, the following two therapeutic approaches could be considered: 1) restoration of the regulatory machinery; or 2) activation of a pharmacological pathway to reduce free β−catenin. The first approach is not practical, because the high frequency of mutations in key genes constituting the APC pathways, such as APC, Axin, p53, and β-catenin itself are the origin of the problem (Fig. 6). In the second approach, manipulation of the p53 and the Wnt signaling pathways have not been successful for various reasons, including the aforementioned genetic problem. However, the RXR-mediated pathway, which can be regulated by small molecule hormones, has the potential of being a powerful pharmacological approach to treating Wnt/β-catenin-related cancers. Our results suggest that β-catenin-associated tumors that concurrently express high levels of RXR will be the most responsive to RXR agonist therapy. Furthermore, the use of RXR agonists in conjunction with pharmacological (40) or genetic (41) approaches to elevating RXRα protein levels in target tumors may be effective therapies for cancers such as colorectal cancer. We are currently investigating these possibilities using *in vivo* model systems.

Acknowledgments—We are grateful to G. Sachs for critical comments, A. Nuyen and D. Kahler for technical assistance, and L. Atangan for technical advice.

REFERENCES

1. Peifer, M., and Polakis, P. (2000) *Science* **287,** 1606–1609
2. Polakis, P. (2002) *Curr. Biol.* **12,** R499—R501
3. Liu, J., Stevens, J., Rote, C. A., Yost, H. J., Hu, Y., Neufeld, K. L., White, R. L., and Matsunami, N. (2001) *Mol. Cell* **7,** 927–936
4. Matsuzawa, S. I., and Reed, J. C. (2001) *Mol. Cell* **7,** 915–926
5. Schwarz-Romond, T., Asbrand, C., Bakkers, J., Kuhl, M., Schaeffer, H. J., Huelsken, J., Behrens, J., Hammerschmidt, M., and Birchmeier, W. (2002) *Genes Dev.* **16,** 2073–2084
6. Polakis, P. (2001) *Cell* **105,** 563–566
7. Bienz, M., and Clevers, H. (2000) *Cell* **103,** 311–320
8. Conacci-Sorrell, M. E., Ben-Yedidia, T., Shtutman, M., Feinstein, E., Einat, P., and Ben-Ze'ev, A. (2002) *Genes Dev.* **16,** 2058–2072
9. Wong, N. A., and Pignatelli, M. (2002) *Am. J. Pathol.* **160,** 389–401
10. Chambon, P. (1996) *FASEB J.* **10,** 940–954
11. Altucci, L., and Gronemeyer, H. (2001) *Nat. Rev Cancer* **1,** 181–193
12. Sun, S. Y., and Lotan, R. (2002) *Crit. Rev. Oncol. Hematol.* **41,** 41–55
13. Westin, S., Rosenfeld, M. G., and Glass, C. K. (2000) *Adv. Pharmacol.* **47,** 89–112
14. Easwaran, V., Pishvaian, M., Salimuddin, and Byers, S. (1999) *Curr. Biol.* **9,** 1415–1418
15. Tice, D. A., Szeto, W., Soloviev, I., Rubinfeld, B., Fong, S. E., Dugger, D. L., Winer, J., Williams, P. M., Wieand, D., Smith, V., Schwall, R. H., Pennica, D., and Polakis, P. (2002) *J. Biol. Chem.* **277,** 14329–14335
16. Liu, T., Lee, Y.-N., Malbon, C. C., and Wang, H.-y. (2002) *J. Biol. Chem.* **277,** 30887–30891
17. Kopf, E., Plassat, J. L., Vivat, V., de Thé, H., Chambon, P., and Rochette-Egly, C. (2000) *J. Biol. Chem.* **275,** 33280–33288
18. Osburn, D. L., Shao, G., Seidel, H. M., and Schulman, I. G. (2001) *Mol. Cell. Biol.* **21,** 4909–4918
19. Johnson, A. T., Wang, L., Standeven, A. M., Escobar, M., and Chandraratna, R. A. S. (1999) *Bioorg Med. Chem.* **7,** 1321–1338
20. Vuligonda, V., Thacher, S. M., and Chandraratna, R. A. S. (2001) *J. Med. Chem.* **44,** 2298–2303
21. Nagpal, S., Ghosn, C., DiSepio, D., Molina, Y., Sutter, M., Klein, E. S., and Chandraratna, R. A. S. (1999) *J. Biol. Chem.* **274,** 22563–22568
22. Korinek, V., Barker, N., Morin, P. J., van Wichen, D., de Weger, R., Kinzler, K. W., Vogelstein, B., and Clevers, H. (1997) *Science* **275,** 1784–1787
23. Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K. W. (1997) *Science* **275,** 1787–1790
24. Dussault, I., Beard, R., Lin, M., Hollister, K., Chen, J., Xiao, J. H.,

Chandraratna, R., and Forman, B. M. (2003) *J. Biol. Chem.* **278,** 7027–7033
25. Abarzua, P., LoSardo, J. E., Gubler, M. L., and Neri, A. (1995) *Cancer Res.* **55,** 3490–3494
26. Playford, M. P., Bicknell, D., Bodmer, W. F., and Macaulay, V. M. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 12103–12108
27. Roh, H., Green, D. W., Boswell, C. B., Pippin, J. A., and Drebin, J. A. (2001) *Cancer Res.* **61,** 6563–6568
28. Verma, U. N., Surabhi, R. M., Schmaltieg, A., Becerra, C., and Gaynor, R. B. (2003) *Clin. Cancer Res.* **9,** 1291–1300
29. Egea, P. F., Mitschler, A., Rochel, N., Ruff, M., Chambon, P., and Moras, D. (2000) *EMBO J.* **19,** 2592–2601
30. Harada, N., Tamai, Y., Ishikawa, T., Sauer, B., Takaku, K., Oshima, M., and Taketo, M. M. (1999) *EMBO J.* **18,** 5931–5942
31. Gat, U., DasGupta, R., Degenstein, L., and Fuchs, E. (1998) *Cell* **95,** 605–614
32. Chan, E. F., Gat, U., McNiff, J. M., and Fuchs, E. (1999) *Nat. Genet.* **21,** 410–413
33. Kajino, Y., Yamaguchi, A., Hashimoto, N., Matsuura, A., Sato, N., and Kikuchi, K. (2001) *Pathol. Int.* **51,** 543–548
34. Saadi-Kheddouci, S., Berrebi, D., Romagnolo, B., Cluzeaud, F., Peuchmaur, M., Kahn, A., Vandewalle, A., and Perret, C. (2001) *Oncogene* **20,** 5972–5981
35. Cadoret, A., Ovejero, C., Saadi-Kheddouci, S., Souil, E., Fabre, M., Romagnolo, B., Kahn, A., and Perret, C. (2001) *Cancer Res.* **61,** 3245–3249
36. Kolligs, F. T., Hu, G., Dang, C. V., and Fearon, E. R. (1999) *Mol. Cell. Biol.* **19,** 5696–5706
37. Damalas, A., Kahan, S., Shtutman, M., Ben-Ze'ev, A., and Oren, M. (2001) *EMBO J.* **20,** 4912–4922
38. Green, D. W., Roh, H., Pippin, J. A., and Drebin, J. A. (2001) *J. Surg. Res.* **101,** 16–20
39. Polakis, P. (2000) *Genes Dev.* **14,** 1837–1851
40. Celli, G., Darwiche, N., and De Luca, L. M. (1996) *Exp. Cell Res.* **226,** 273–282
41. Chen, R. H., and McCormick, F. (2001) *Cancer Res.* **61,** 4445–4449
42. Xiao, J. H., Davidson, I., Ferrandon, D., Rosales, R., Vigneron, M., Macchi, M., Ruffenach, F., and Chambon, P. (1987) *EMBO J.* **6,** 3005–3013

Adenomatous Polyposis Coli (APC)-independent Regulation of β-Catenin Degradation via a Retinoid X Receptor-mediated Pathway

Jia-Hao Xiao, Corine Ghosn, Cory Hinchman, Chad Forbes, Jenny Wang, Nonna Snider, Allison Cordrey, Yi Zhao and Roshantha A. S. Chandraratna

J. Biol. Chem. 2003, 278:29954-29962.
doi: 10.1074/jbc.M304761200 originally published online May 27, 2003
