tion proteins ZO-1 and ZO-2 and erythroid p55 (*18*), it is possible that APC plays various physiologic roles by forming complexes with these DHR proteins.

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19. [^{35}S]methionine-labeled truncated DLGs, containing the indicated regions, were synthesized by in vitro transcription-translation (Promega) with the use of templates obtained by linearizing the *dlg* cDNA constructs with restriction enzymes or by polymerase chain reaction. We generated GST fusion proteins by subcloning the cDNAs into pGEX5X-1 (Pharmacia), expressing the fusion proteins in *Escherichia coli*, and isolating them by absorption to glutathione-Sepharose [D. B. Smith and K. S. Johnson, *Gene* **67**, 31 (1988)]. GST fusion proteins immobilized to glutathione-Sepharose were mixed with in vitro–translated proteins in buffer A [50 mM tris-HCl (pH 7.5), 140 mM NaCl, 2 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, leupeptin (5 μg/ml), and aprotinin (5 μg/ml)] containing 0.1% Triton X-100 for 1 hour at 4°C and then were washed extensively with buffer A containing 0.5% NP-40.
20. Antibodies to APC were prepared as in (*8*). Antibodies to the NH_2-terminus and the DHR region of DLG were prepared by immunization of rabbits with peptides containing amino acids 6 to 205 and 203 to 518 of DLG, respectively. Antibody to β-catenin was obtained from Transduction Laboratories. Embryonic mouse brain (embryonic day 18) was lysed with a dounce homogenizer in buffer A (*19*) containing 1% Triton X-100, and the lysates were incubated with indicated antibodies for 1 hour at 4°C. The immunocomplexes were adsorbed to protein A–Sepharose 4B and washed extensively with buffer A containing 0.1% Triton X-100. Samples were resolved by 5% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and subjected to immunoblotting.
21. Cell cultures were prepared from the hippocampus of 18-day-old fetal rats [T. L. Fletcher, P. Cameron, P. De Camilli, G. Banker, *J. Neurosci.* **11**, 1617 (1991)]. Staining patterns obtained with anti-APC and anti-DLG were visualized with fluorescein isothiocyanate (FITC)–labeled secondary antibodies to rabbit immunoglobulin G (IgG) (Cappel); those obtained with anti-synaptotagmin (Wako) were visualized with Texas Red–labeled secondary antibodies to mouse IgG (Amersham).
22. For fluorescence immunohistochemistry, serial frozen sections of rat colon were stained with anti–APC-COOH or anti-DLG-NH_2 and then with FITC-conjugated goat antibody to rabbit IgG (Seikagaku Kogyo) (*5*).
23. For double-labeling immunoelectron microscopy. Lowicryl ultrathin sections were incubated with anti-DLG-NH_2 and then with 5-nm colloidal gold-conjugated goat antibody to rabbit IgG (Amersham). After blocking with a goat antibody to rabbit IgG, the sections were stained with anti–APC-COOH and then with 10-nm colloidal gold-conjugated goat antibody to rabbit IgG (Amersham) (*5*) [H. J. Geuze, J. W. Slot, P. A. van der Ley, R. C. Scheffer, *J. Cell Biol.* **89**, 653 (1981)].
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Binding of GSK3β to the APC–β-Catenin Complex and Regulation of Complex Assembly

Bonnee Rubinfeld, Iris Albert, Emilio Porfiri, Carol Fiol, Susan Munemitsu, Paul Polakis*

The adenomatous polyposis coli gene (*APC*) is mutated in most colon cancers. The APC protein binds to the cellular adhesion molecule β-catenin, which is a mammalian homolog of ARMADILLO, a component of the WINGLESS signaling pathway in *Drosophila* development. Here it is shown that when β-catenin is present in excess, APC binds to another component of the WINGLESS pathway, glycogen synthase kinase 3β (GSK3β), a mammalian homolog of *Drosophila* ZESTE WHITE 3. APC was a good substrate for GSK3β in vitro, and the phosphorylation sites were mapped to the central region of APC. Binding of β-catenin to this region was dependent on phosphorylation by GSK3β.

The *APC* tumor suppressor gene is mutated in most human colon tumors and in the germ line of individuals with familial adenomatous polyposis coli (*1*, *2*). How *APC* dysfunction contributes to colon cancers is unknown but may involve its ability to interact with β-catenin. Both wild-type (WT) and truncated mutant forms of APC associate with β-catenin (*3*, *4*), but only WT APC down-regulates β-catenin when expressed ectopically in colon cancer cells (*5*). β-Catenin was originally identified as a cell adhesion protein by virtue of its association with cadherins (*6*). In addition, in both *Drosophila* and *Xenopus* β-catenins have been implicated in cell fate determination through a mechanism that is apparently independent of their interaction with cadherins (*7*). In both of these systems, interference with the serine-threonine kinase GSK3β results in a cell fate determination identical to that resulting from overexpression of β-catenin (*8*, *9*), which suggests that GSK3β is required for β-catenin down-regulation. These observations prompted us to examine the relation between GSK3β, APC, and β-catenin in mammalian cells.

B. Rubinfeld, I. Albert, E. Porfiri, S. Munemitsu, P. Polakis, Onyx Pharmaceuticals, 3031 Research Drive, Richmond, CA 94806, USA.
C. Fiol, Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202, USA.

*To whom correspondence should be addressed.

To investigate whether GSK3β and the APC–β-catenin complex interact, we overexpressed Myc-tagged GSK3β in SW 480 colon cancer cells, immunoprecipitated either Myc-GSK3β or β-catenin, and then analyzed the precipitates for the presence of APC, β-catenin, and Myc-GSK3β. Both APC and β-catenin coimmunoprecipitated with antibody specific to the Myc epitope and, conversely, Myc-GSK3β was detected in the β-catenin immunoprecipitates (Fig. 1). These associations were observed with WT but not with kinase-dead Myc-GSK3β (a catalytically inactive mutant of GSK). To examine this association in the absence of ectopic cDNA expression, we performed GSK assays on β-catenin immunoprecipitates from four different cell lines. Specific GSK activity was detected in the β-catenin immunocomplexes from COLO 205 and SW 480 human colon cancer cells, but not from 293 human embryonic kidney cells or AtT20 murine pituitary tumor cells (Fig. 2A). In addition, immunoprecipitates of endogenous GSK3β contained β-catenin and the truncated mutant APC (*10*) endogenous to SW 480 cells (Fig. 2B). Similar results were obtained with COLO 205 cells, also mutant for APC; but WT APC could not be coimmunoprecipitated from either 293 or AtT20 cell lysates by the antibody to GSK3β (*11*). To verify that GSK3β entered into a complex with

β-catenin, we added purified Glu-Glu–tagged GSK3β to cell lysates and analyzed the recovered GSK3β for the presence of β-catenin. β-Catenin was detected in the GSK3β affinity precipitates from COLO 205 and SW 480 cells but not from 293 and AtT20 cells (Fig. 2C).

The COLO 205 and SW 480 cells contain mutant APC and large amounts of cytoplasmic uncomplexed β-catenin, whereas 293 and AtT20 cells express WT APC and small amounts of uncomplexed β-catenin (*5*, *11*). These observations suggest that the accumulation of free β-catenin may be required to promote the association of GSK3β with APC. To test this possibility, we used a mutant β-catenin (ΔN89β-catenin) lacking an NH_2-terminal sequence that is essential to its rapid turnover in vivo (*11*). This mutant β-catenin accumulates in large amounts both as an uncomplexed protein and in a complex with WT APC (*11*). Immunoprecipitates of GSK3β from these cells contained large amounts of APC relative to amounts in controls (Fig. 2D). Moreover, immunoprecipitates of GSK3β from 293 cells transiently expressing the ΔN89β-catenin also contained WT APC (Fig. 2D). APC was not detected in GSK3β immunoprecipitates from control 293 cells, but small amounts were identified in cells transiently overexpressing WT β-catenin. These results suggest that GSK3β binds to APC in a β-catenin–dependent manner.

To examine whether GSK3β was preferentially bound to APC or β-catenin, we measured GSK activity immunoprecipitated by various antibodies to β-catenin relative to that immunoprecipitated by antibodies to APC. The APC3 antibody, which does not recognize mutant APC (*3*), was used as a control. The amounts of GSK activity immunoprecipitated by antibodies to β-catenin and APC were roughly comparable; however, there was far less β-catenin in the APC immunoprecipitate (Fig. 3).

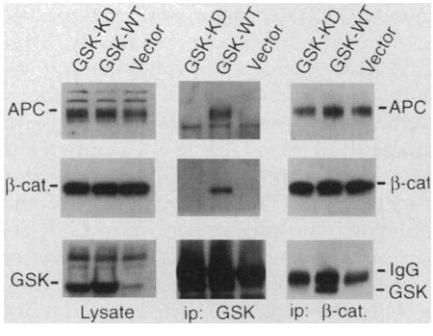

Fig. 1. Association of APC and β-catenin with epitope-tagged GSK3β. Lysates from SW 480 cells transiently transfected with vector or either WT or kinase-dead (KD) GSK3β fused to a Myc epitope were analyzed directly (lysate) or subjected to immunoprecipitation (*16*, *24*) with antibody to the Myc epitope (GSK) or β-catenin (β-cat.). The samples were analyzed by SDS–polyacrylamaide gel electrophoresis (SDS-PAGE) and immunoblotting. The top, middle, and bottom sections of the blots were developed with antibody to APC, β-catenin, and GSK3β, respectively. The APC in these cells is a truncated mutant (~160 kD). IgG indicates the immunoglobin heavy chain.

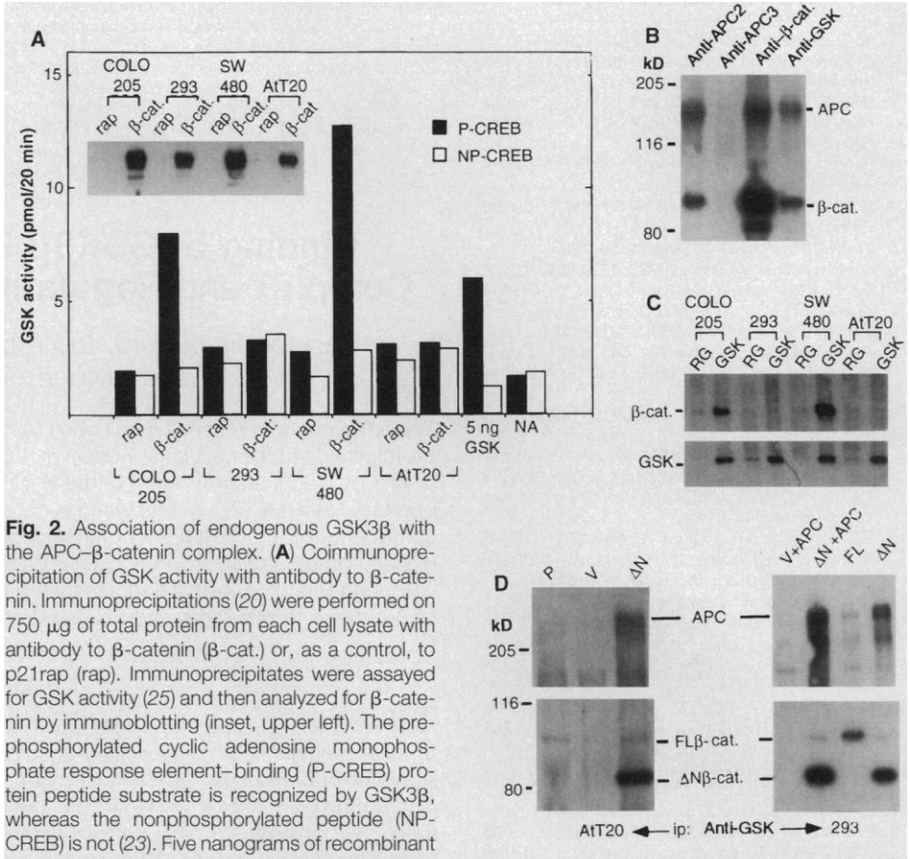

Fig. 2. Association of endogenous GSK3β with the APC–β-catenin complex. (**A**) Coimmunoprecipitation of GSK activity with antibody to β-catenin. Immunoprecipitations (*20*) were performed on 750 μg of total protein from each cell lysate with antibody to β-catenin (β-cat.) or, as a control, to p21rap (rap). Immunoprecipitates were assayed for GSK activity (*25*) and then analyzed for β-catenin by immunoblotting (inset, upper left). The prephosphorylated cyclic adenosine monophosphate response element–binding (P-CREB) protein peptide substrate is recognized by GSK3β, whereas the nonphosphorylated peptide (NP-CREB) is not (*23*). Five nanograms of recombinant purified GSK3β was used as a positive control, and no addition (NA) was used as a negative control. (**B**) Coimmunoprecipitation of APC and β-catenin with endogenous GSK3β. Lysates from SW 480 cells were incubated with antibodies to the central and COOH-terminal regions of APC (anti-APC2 and anti-APC3, respectively), to β-catenin (anti–β-cat.), or to GSK3β (anti-GSK). Antibody to APC3 does not recognize mutant APC protein. Immunoprecipitates (*20*) were analyzed for β-catenin and APC by SDS-PAGE and immunoblotting. (**C**) Affinity precipitation of β-catenin with purified GSK3β protein (*24*). Protein-equivalent lysates from the indicated cells were incubated with either 2 μg of purified Glu-Glu epitope-tagged GSK3β or Glu-Glu rapGAP (RG) protein. The GSK3β and rapGAP proteins were recovered on Glu-Glu antibody coupled to protein G–Sepharose and then analyzed for β-catenin and GSK3β by SDS-PAGE and immunoblotting. (**D**) Conditional association of WT APC with GSK. Left: AtT20 cells stably expressing the ΔN89β-catenin mutant (ΔN) or carrying empty vector (V) or the parental cell (P) were lysed and normalized for protein, and immunoprecipitations were performed with antibody to GSK3β (*20*). Right: 293 cells transiently expressing (*24*) ΔN89β-catenin (ΔN), full-length β-catenin (FL), ΔN89β-catenin and APC (ΔN+APC), or vector control and APC (V+APC) were lysed and subjected to immunoprecipitation with antibody to GSK3β (*20*). Immunoprecipitates were analyzed for APC (top) and β-catenin (bottom) by SDS-PAGE and immunoblotting.

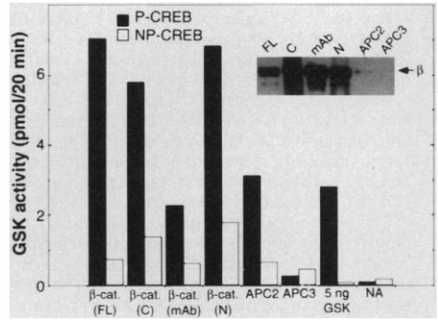

Fig. 3. Comparison of GSK activity in β-catenin (β-cat.) and APC immunoprecipitates. Protein-equivalent lysates from SW 480 cells were subjected to immunoprecipitation with four different antibodies to β-catenin (*20*) or with antibodies to the central and COOH-terminal regions of APC (APC2 and APC3, respectively). Epitopes recognized by APC3 are absent in the SW 480 mutant APC (*3*). The immunocomplexes were assayed for GSK activity (*25*). Recombinant purified GSK3β was used as positive control, and no addition (NA) was used as a negative control. The immunoprecipitates were then analyzed for β-catenin by SDS-PAGE and immunoblotting (inset, upper right).

This indicates that GSK3 activity is not uniformly associated with the total pool of β-catenin but is relatively enriched in the APC–β-catenin complex.

We had previously noted an increase in the electrophoretic mobility of endogenous APC after the down-regulation of β-catenin in SW 480 cells (*5*). Considering this together with the β-catenin–dependent association of GSK3β with APC, we postulated that APC might be a substrate for GSK3β. Phosphorylation experiments with various APC fragments (*11*) revealed that the central region (APC25) was a good substrate for GSK3β (Fig. 4A). APC25 was purified as a phosphoprotein from insect Sf9 cells and was therefore dephosphorylated in vitro before use. The dephosphorylated APC25 was a poor substrate for GSK3β unless it was prephosphorylated by protein kinase A (PKA). Under these conditions, 3 mol of phosphate per mole of APC25 were incorporated after incubation with GSK3β (Fig. 4A). Under these same conditions, we were unable to observe substantial phosphorylation of β-catenin (*11*). The PKA was used as a best-guess priming reagent because some GSK3β substrates require prephosphorylation at the +4 position [SXXXS(P)] relative to the phospho-acceptor residue (*12*). All seven of the repeated 20–amino acid sequences (*1*) contained in APC (Fig. 4, top) contain a conserved SXXXS motif.

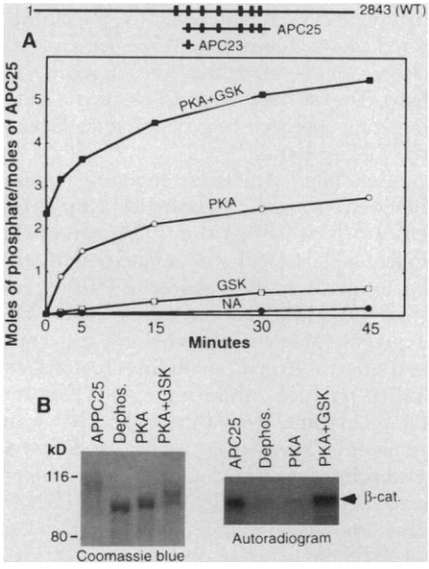

Fig. 4. Phosphorylation of APC by GSK3β and its effect on binding to β-catenin. (**A**) In vitro phosphorylation (*25*). Fragments of APC (APC25 and APC23) and their positions with respect to the intact protein and the locations of the repeated 20–amino acid sequences (solid rectangles) are represented at top. Baculovirus-produced APC25 was first dephosphorylated in vitro and then 2 μg was incubated in the presence of γ-[^{32}P]ATP with PKA, GSK3β (GSK), PKA followed by GSK3β (PKA+GSK), or no kinase (NA). Reactions were terminated by nitrocellulose filtration, and the filters were quantitated by scintillation counting. (**B**) β-catenin binding analysis (*25*). Purified APC25 was dephosphorylated (dephos.) and then rephosphorylated in vitro with PKA alone or PKA followed by GSK3β (PKA+GSK). A Coomassie blue–stained SDS-PAGE gel is shown at left. Two micrograms of each of these proteins was added to wheat germ lysate containing in vitro–translated radiolabeled β-catenin and then recovered on Glu-Glu antibody coupled to protein G–Sepharose. The immunoprecipitates were analyzed for β-catenin by autoradiography of SDS-PAGE gels (autoradiogram at right).

Overexpression of GSK3β in the SW 480 cell resulted in larger amounts of APC coimmunoprecipitated by antibody to β-catenin (Fig. 1). As APC25 contains binding sites for β-catenin (*13*), we tested whether its phosphorylation by GSK3β would facilitate binding. Relative to untreated baculovirus-produced APC25, the dephosphorylated protein exhibited enhanced mobility on SDS-polyacrylamide gels and weak binding to β-catenin (Fig. 4B). Rephosphorylation by PKA produced an upward mobility shift but did not restore β-catenin binding. Finally, subsequent phosphorylation by GSK3β produced an additional mobility shift and restored binding to β-catenin. Similar results were obtained with the smaller APC23 fragment (Fig. 4) (*11*).

Together, the findings that APC regulates β-catenin amounts in vivo (*5*) and that GSK3 regulates ARMADILLO (β-catenin) signaling in *Drosophila* development (*9*) suggest that APC and GSK3β may function in concert to control β-catenin amounts. We demonstrated that GSK3β binds to APC when there is excess uncomplexed β-catenin and that GSK3β can phosphorylate APC in a region of the protein that can down-regulate β-catenin in vivo (*5*). Moreover, this phosphorylation enhances the ability of this region of APC to interact with β-catenin in vitro. One hypothesis is that APC senses excess intracellular β-catenin, which leads to a stronger association with GSK3β. This in turn may result in the phosphorylation of the central region of APC to initiate the turnover of β-catenin. Alternatively, the accumulation of β-catenin may provide a positive signal for the recruitment of GSK3β to APC, allowing it to access preferred kinase substrates. Our data demonstrate a biochemical connection between GSK3β and β-catenin and thereby complement the genetic observations made in *Drosophila* (*9*, *14*).

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20. Polyclonal antibodies to the central (APC2) and COOH-terminal (APC3) regions of APC are described in (*3*). Monoclonal antibodies to the Myc (*15*) and Glu-Glu (*16*) epitopes were partially purified on DE-52 cellulose and used at 2 μg/ml for immunoblotting or were covalently coupled to protein G–Sepharose for immunoprecipitations. β-catenin antibodies referred to as β-cat. (C) and β-cat. (N) are described in (*21*) and (*22*), respectively, and β-cat. (mAb) was from Transduction Laboratories (Lexington, KY). Antibodies to full-length β-catenin and GSK3β were raised in rabbits against their respective purified proteins produced in the baculovirus-Sf9 cell system, and the sera were then affinity-purified against the immobilized proteins. For immunoprecipitations, cells were lysed in Triton X-100 lysis buffer, and 150 μl of lysate (total protein, 5 mg/ml) was subjected to immunoprecipitation as described in (*5*). For immunoblotting, all polyclonal sera were used at 1/1000 dilution and purified antibodies were used at 0.2 μg/ml. Blots were developed with the use of the ECL system (Amersham) or, for β-catenin, with the use of ^{125}I-labeled protein A at 0.5 μCi/ml (Amersham).
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24. All cell lines were from the American Type Culture Collection. The GSK3β cDNA was isolated from a human granulocyte cDNA library (Stratagene) and subcloned into the pCDNA3 expression vector (Invitrogen) containing either the Myc (*15*) or Glu-Glu (*16*) epitope tags. Kinase-dead GSK3β was constructed by site-directed mutagenesis to change lysines 85 and 86 to methionine and isoleucine, respectively. The full-length β-catenin cDNA (*13*) and the ΔN89 mutant (codons 90 to 781) were subcloned into CMV neo Bam vector (*17*) containing the Glu-Glu epitope. Transfection of SW 480 and 293 cells was performed by means of a Lipofectin (BRL) procedure (*5*) and a modified calcium phosphate precipitation method (*18*), respectively. For the derivation of AtT20 lines, cells were seeded at 3.0×10^5 cells per 3.5-cm well and transfected with 2 μg of the indicated β-catenin cDNA plasmid

with the use of 10 μl of Lipofectamine reagent (BRL, Gibco) in Dulbecco's modified Eagle's medium (DMEM). The next day the medium was changed to DMEM with serum, and the cells were cultured for 1 day before selection in G418 (400 μg/ml) for 3 to 4 weeks. Purified GSK3β and APC25 were produced as in (*19*). The affinity precipitation of β-catenin from SW 480 cell lysates by purified GSK3β was performed by addition of 2 μg of GSK3β protein to 150 μl of lysate. After a 2-hour incubation at 40°C, the GSK3β was recovered on Glu-Glu antibody coupled to protein G–Sepharose and then analyzed by immunoblotting (*20*).

25. For GSK assays we followed the procedure in (*23*). Immunoprecipitates were prepared for GSK assay by a final wash in kinase assay buffer [25 mM tris (pH 7.5), 5 mM $MgCl_2$, 1 mM dithiothreitol, and 4% glycerol] and then resuspended in 20 μl of buffer containing 50 μM peptide substrate and 50 μM γ-[^{32}P]adenosine triphosphate (ATP) (10,000 cpm/pmol). Assays were performed for 20 min at 30°C, and 10 μl was analyzed for incorporation of ^{32}P. For the phosphorylation of APC in vitro, Glu-Glu–tagged APC25 was produced with the baculovirus-Sf9 cell system (*13*) and then collected from cell lysates on Glu-Glu antibody coupled to protein G–Sepharose. The immobilized APC25 was then dephosphorylated with 4000 units of Lambda phosphatase (New England Biolabs) at 30°C for 2 hours. The dephosphorylated APC25 was eluted from the washed beads with Glu-Glu peptide and then phosphorylated at 30°C in 50 μl of kinase assay buffer containing APC25 (2 to 5 μg) and PKA (2 μg/ml) (Sigma), GSK3β (0.25 μg/ml), or the two kinases added sequentially. For quantitation (Fig. 4A), 50 μM γ-[^{32}P]ATP (10,000 cpm/pmol) was included in the reactions, and radioactive protein was collected by vacuum filtration over nitrocellulose filters. Phosphorylations in Fig. 4B were performed with unlabeled ATP and the phosphoproteins were repurified before being used for affinity precipitation of in vitro–translated β-catenin (wheat germ TNT system, Promega) (*13*).

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Retinal Degeneration in Mice Lacking the γ Subunit of the Rod cGMP Phosphodiesterase

Stephen H. Tsang, Peter Gouras, Clyde K. Yamashita, Hild Kjeldbye, John Fisher,* Debora B. Farber, Stephen P. Goff†

The retinal cyclic guanosine 3′,5′-monophosphate (cGMP) phosphodiesterase (PDE) is a key regulator of phototransduction in the vertebrate visual system. PDE consists of a catalytic core of α and β subunits associated with two inhibitory γ subunits. A gene-targeting approach was used to disrupt the mouse PDEγ gene. This mutation resulted in a rapid retinal degeneration resembling human retinitis pigmentosa. In homozygous mutant mice, reduced rather than increased PDE activity was apparent; the PDEαβ dimer was formed but lacked hydrolytic activity. Thus, the inhibitory γ subunit appears to be necessary for integrity of the photoreceptors and expression of PDE activity in vivo.

Hereditary photoreceptor cell degenerations called retinitis pigmentosa (RP) define a group of genetic diseases causing blindness that affect 1 in 3000 individuals worldwide (*1*). Autosomal dominant, autosomal recessive, sex-linked, and mitochondrial inheritance patterns have been described for RP. A number of mutations affecting the visual pigment rhodopsin (*2*) as well as peripherin/RDS (*3*), a protein of unknown function and localized in the rims of the outer segment discs, result in different forms of dominantly inherited RP (*1*). Most autosomal recessive forms of the disease result from mutations in genes encoding phototransduction proteins (*4*), but the mechanism whereby these mutated genes produce destruction of photoreceptors is not well understood.

Phototransduction begins with the absorption of light by rhodopsin (*5*–*6*). Photoactivated rhodopsin stimulates the α subunit of a heterotrimeric guanine nucleotide binding protein termed transducin (Tαβγ) to exchange its bound guanosine diphosphate for guanosine triphosphate (GTP) (*7*). The transducin (Tα)-GTP complex dissociates from Tβγ and binds to the inhibitory γ subunits of cyclic guanosine 3′,5′-monophosphate (cGMP) phosphodiesterase ($PDE\alpha\beta\gamma_2$) (*8*). This step removes the constraint that the PDEγ (11 kD) subunit imposes on the catalytic α (88 kD) and β (84 kD) subunits of the heterotetrameric PDE (*7*–*9*) and increases its hydrolytic activity almost 300-fold (*10*). The activated PDE lowers the intracellular concentration of cGMP (*11*), thereby closing cGMP-gated cation channels on the rod plasma membrane and initiating a neural response to light (*12*). Termination of excitation requires quenching of photoexcited rhodopsin and Tα, deactivation of PDE by reassociation of PDEγ to the PDE αβ catalytic subunits, and restoration of the cGMP concentration through activation of guanylate cyclase. The binding of Tα to PDEγ accelerates the intrinsic guanosine triphosphatase activity of Tα, which also quenches the light response (*10*).

Mutations in the PDEβ subunit gene cause retinal degenerations in both *rd1*/*rd1* mice (*13*) and Irish Setter dogs (*14*) and are one of the causes of autosomal recessive RP (*15*). Mutations in the PDEα gene can also produce RP (*16*), but no defects have yet been found in the PDEγ gene (*17*). The absence of PDEγ might be expected at least initially to reduce rod cGMP levels by allowing the constitutively uninhibited PDEαβ to hydrolyze cGMP. Consequently, the cGMP-gated cation channels would be permanently closed, eliminating the rod's response to light. To test these notions, we used a gene-targeting approach to generate mice lacking the PDEγ subunit.

Genomic sequences encoding mouse PDEγ were used to construct a targeting DNA (*18*) in which the third exon of the PDEγ gene (*Pdeg*) was replaced with the bacterial neomycin resistance (Neo) gene (Fig. 1A). The mutant allele could not express sequences encoded by the third and fourth exons, including regions required for PDE inhibition (*19*). CCE and R1 embryonic stem (ES) cells (*18*) were transformed with this vector, and G418-gancyclovir–resistant clones were recovered and screened by Southern (DNA) blot analysis. Six independently targeted CCE ES clones were obtained out of 2500 colonies screened, whereas eight mutated clones were recovered from R1 ES cells out of 400 colonies screened. Targeted ES cells were injected into C57BL/6 or MF1 blastocysts that were reimplanted into the uteri of pseudopregnant foster mothers (*20*). The resulting chimeras were bred to generate offspring carrying the mutant allele in the heterozygous form ($Pdeg^{tm1}/+$) in the germ line; intercrosses generated mutant homozygous animals ($Pdeg^{tm1}/Pdeg^{tm1}$) (Fig. 1, B and C). $Pdeg^{tm1}/Pdeg^{tm1}$ homozygous mice were healthy and fertile; thus, PDEγ is not essential for normal prenatal development. Protein immunoblot analysis of a $Pdeg^{tm1}/Pdeg^{tm1}$ retinal homogenate with antisera to either NH_2- or COOH-terminal portions of PDEγ confirmed that the mutant allele did not encode detectable proteins (Fig. 1D). Chimeras were bred with 129/Sv//Ev animals for generation of an inbred line. The $Pdeg^{tm1}/Pdeg^{tm1}$ mice were crossed with MF1, Swiss Webster, and C57BL/6 mice to investigate

S. H. Tsang, J. Fisher, S. P. Goff, Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics, Columbia University, College of Physicians and Surgeons, New York, NY 10032, USA.
P. Gouras and H. Kjeldbye, Edward Harkness Eye Institute, Department of Ophthalmology, Columbia University, College of Physicians and Surgeons, New York, NY 10032, USA.
C. K. Yamashita and D. B. Farber, Jules Stein Eye Institute, Molecular Biology Institute and Department of Ophthalmology, UCLA School of Medicine, Los Angeles, CA 90095, USA.

*Present address: Regeneron Pharmaceuticals, Tarrytown, NY 10591, USA.
†To whom correspondence should be addressed.