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9. The 41-nt D5, containing seven flanking nucleotides, was synthesized in two pieces on an ABI 392 DNA/RNA synthesizer and worked up according to standard procedures [S. A. Scaringe, C. Francklyn, N. Usman, *Nucleic Acids Res.* **18**, 5433 (1990)]. Oligonucleotides were purified on a Waters high-performance liquid chromatography (HPLC) system with a Machery-Nagel Nucleogen DEAE 60-7 column. Oligonucleotides were then desalted with ABI Oligonucleotide Purification Cartridges following the manufacturer's instructions.
10. $K_m = 1.5 \pm 0.6$ μM, $k_{cat} = 0.02 \pm 0.002$ min^{-1}, and $k_{cat}/K_m = 1.3 \times 10^4$ M^{-1} min^{-1}. Parameters were determined under reaction conditions of 100 mM $MgCl_2$, 500 mM KCl, and 40 mM MOPS (pH 7.5) at 45°C as described (*4*).
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12. In predominantly RNA duplexes, a single deoxyribonucleotide is expected to exist primarily in the C3′-*endo* conformation of its neighbors, resulting in minimal perturbations of the structure. Although twofold effects on binding constants of oligonucleotide strands have been observed (*6*), these would be invisible here because each D5 chimera was preincubated before reaction to ensure full annealing of the strands. Aside from effects on D5 duplex stability, single deoxynucleotides may affect electrostatics and local water structure. However, if water organization is important to catalytic activity and such activity is mediated by a 2′-OH group, we consider such effects to represent important contributions to the catalytic process.
13. Effects smaller than 10-fold were observed for several of the deoxynucleotide-substituted D5 derivatives. Description of these smaller effects is detailed in (*11*).
14. Competitive inhibition was evaluated under k_{cat}/K_m conditions for WT D5 (50 nM D5, 1 nM ^{32}P-exD123), which are the most sensitive for measurement of competitor effects [J. A. Doudna and T. R. Cech, *RNA* **1**, 36 (1995)]. WT D5 was in one piece, whereas competitor chimeric D5 molecules were provided in excess as two-piece annealed strands (3 μM). Competitor D5 derivatives containing both single- and double-deoxynucleotide substitutions were tested, and for substitutions at similar positions the same results were obtained. Kinetics were measured under standard conditions (*10*).
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Uncoupling of GTP Binding from Target Stimulation by a Single Mutation in the Transducin α Subunit

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Glutamic acid–203 of the alpha subunit of transducin (α_T) resides within a domain that undergoes a guanosine triphosphate (GTP)–induced conformational change that is essential for effector recognition. Changing the glutamic acid to an alanine in bovine α_T yielded an alpha subunit (α_TE203A) that was fully dependent on rhodopsin for GTP–guanosine diphosphate (GDP) exchange and showed GTP hydrolytic activity similar to that measured for wild-type α_T. However, unlike the wild-type protein, the GDP-bound form of α_TE203A was constitutively active toward the effector of transducin, the cyclic guanosine monophosphate phosphodiesterase. Thus, the α_TE203A mutant represents a short-circuited protein switch that no longer requires GTP for the activation of the effector target phosphodiesterase.

Heterotrimeric GTP–binding proteins (G proteins) serve as molecular switches in various receptor-coupled signal transduction pathways including those responsible for hormone-regulated adenylyl cyclase activity, receptor-stimulated phosphoinositide lipid turnover, ion channel regulation, olfaction, taste, and vertebrate vision (*1*, *2*). The phototransduction cascade in which rhodopsin activates the G protein transducin has frequently been used as a paradigm for receptor signaling mediated by G proteins. The three-dimensional structures of α_T bound to GDP and α_T bound to guanosine 5′-O-(3-thiotriphosphate) (GTP-γ-S), as well as the aluminum fluoride (AlF_4^-)–activated α_T subunit, have been solved (*3*–*5*). This structural information provides a foundation for understanding the molecular basis by which rhodopsin-stimulated GTP binding to transducin converts this molecule into an activated transducer that directs the regulation of a specific effector protein.

Direct comparisons of the tertiary structures of the GDP-bound and GTP-γ-S–bound α_T subunits indicate that there are three regions that undergo changes in tertiary conformation as an outcome of GTP-GDP exchange (*4*). Two of these regions, designated as switch I (Ser^{173} to Thr^{183} in α_T) and switch II (Phe^{195} to Thr^{215} in α_T), are analogous to conformationally sensitive regions found in the Ras and EF-Tu proteins, whereas a third region, designated switch III (Asp^{227} to Arg^{238} in α_T), may undergo structural changes that are specific to heterotrimeric G proteins (*4*). The switch II region is especially important because it serves as a critical interface, mediating the changes induced within the switch I region by GTP binding into effector recognition. Thus, individual residues within the switch II domain have served as indicators for G protein activation; two examples being Arg^{204} of the α_T subunit, which is no longer sensitive to trypsin when α_T is in the GTP-bound (or AlF_4^--bound) state, and Trp^{207} of α_T, which shows an enhanced fluorescence when activating li-

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gands (GTP, GTP-γ-S, or AlF_4^-) bind to the α_T subunit.

A third amino acid within the switch II region of α_T, Glu^{203}, is highly conserved in G protein α subunits, suggesting that this residue may be essential for some aspect of the activation-deactivation cycle of α_T. It was proposed that Glu^{203} functions in the GTP hydrolytic event by serving as a general base catalyst in the nucleophilic attack on the γ phosphate by water (*3*). However, this is not the case for the α_{i1} subunit (*6*). We also found that this cannot be the case for α_T when we compared the relative amounts of γ-$^{32}P_i$ (P_i is inorganic phosphate) produced through GTP hydrolysis by the *Spodoptera frugiperda*–expressed wild-type α_T subunit and by the α_TE203A mutant (for which Glu^{203} has been changed to an alanine residue). The wild-type α_T subunit and α_TE203A were expressed and purified as glutathione-S-transferase (GST) fusion proteins (*7*). When equivalent amounts of the recombinant α_T subunits and α_T purified from bovine retina were assayed, the α_TE203A mutant had a guanosine triphosphatase (GTPase) activity that was fully stimulated by phosphatidylcholine vesicles containing purified rhodopsin and the purified retinal transducin beta gamma complex ($\beta\gamma_T$) and essentially identical to that measured for both the wild-type protein and the native retinal α_T subunit (Fig. 1A). The E203A mutant also had the same capability for undergoing rhodopsin- and $\beta\gamma_T$-stimulated exchange of [^{35}S]GTP-γ-S for GDP as the native retinal α_T subunit or the wild-type recombinant protein (Fig. 1B).

These findings suggest that the α_TE203A mutant couples properly to both rhodopsin and the $\beta\gamma_T$ complex. This is further shown by the effects of $\beta\gamma_T$ and rhodopsin on the pertussis toxin–catalyzed adenosine diphosphate (ADP) ribosylation of the wild-type α_T and the α_TE203A subunit (Fig. 2). The pertussis toxin–catalyzed ADP ribosylation of α_i and α_T subunits is promoted by $\beta\gamma$ subunit complexes and inhibited by receptor binding [the latter presumably reflects the overlap of the receptor binding domain with the COOH-terminal cysteine residue that serves as the site for ADP ribosylation (*8*)]. The ability of $\beta\gamma_T$ to stimulate ADP ribosylation and the ability of rhodopsin to block this modification were identical for the wild-type α_T subunit and for the α_TE203A mutant (Fig. 2).

We examined whether mutating Glu^{203} to alanine affected the ability of α_T to undergo an activating conformational change. The conformational change occurring in a G protein α subunit as an outcome of GTP-GDP exchange, or after the binding of AlF_4^-, results in enhanced tryptophan fluorescence (*9–12*). The x-ray crystallographic structures for the α_T subunit (*3*, *4*) provide a possible mechanism for the fluorescence enhancement of Trp^{207}. Specifically, Trp^{207}, which lies within the switch II domain, is accessible to solvent in the GDP-bound state but is buried between the side chains of two hydrophobic residues (Leu^{245} and Ile^{249}) when α_T is in the active state. The movement of Trp^{207} and other residues within switch II toward the β4-α3 loop that is immediately NH_2-terminal from switch III constitutes one of the major structural alterations that accompany the binding of activating ligands to the α_T subunit. The ability of Trp^{207} to undergo this movement was not compromised by mutation of Glu^{203}; the AlF_4^--induced fluorescence enhancement for the E203A mutant was indistinguishable from that of the na-

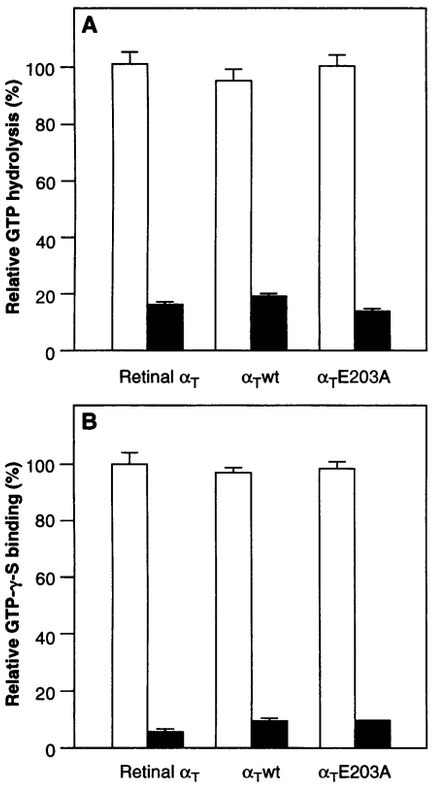

Fig. 1. GTP-binding and GTPase activities of the native retinal α_T subunit, the wild-type α_T, and the α_TE203A mutant. (**A**) Relative GTPase activities of the α_T subunit from bovine retina, insect cell–expressed α_T ($\alpha_T$wt), and insect cell–expressed α_TE203A mutant. Equal amounts (100 nM) of the α_T subunits were mixed with retinal $\beta\gamma_T$ (100 nM) and assayed for [γ^{32}P]GTP hydrolysis (*17*) in the presence (open bars) and absence (solid bars) of rhodopsin (30 nM) that had been inserted into phosphatidylcholine vesicles. The GTPase activities of the recombinant proteins are reported relative to that of the bovine retinal protein (defined as 100%; 150 to 180 pmol [^{32}P]P_i released in 10 min). (**B**) Relative [^{35}S]GTP-γ-S–binding activities of the retinal α_T subunit and wild-type α_T or α_TE203A mutant expressed in insect cells. Equal amounts (100 nM) of the α_T subunits were mixed with retinal $\beta\gamma_T$ (100 nM) and assayed in the presence (open bars) and absence (solid bars) of rhodopsin (30 nM) that had been inserted into phosphatidylcholine vesicles for [^{35}S]GTP-γ-S–binding activity (*19*). The [^{35}S]GTP-γ-S–binding to retinal α_T, in the presence of rhodopsin and $\beta\gamma_T$, is listed as 100%; 10 pmol of [^{35}S]GTP-γ-S–binding to 20 pmol of retinal α_T after 20 min at room temperature.

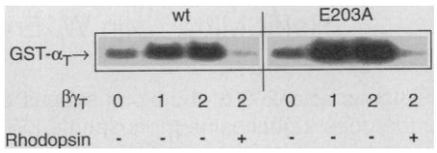

Fig. 2. Pertussis toxin–catalyzed ADP ribosylation of the retinal α_T subunit, the wild-type GST-α_T, and the GST-α_TE203A mutant. Equal amounts of bovine retinal α_T (2 μg), wild-type GST-α_T, or GST-α_TE203A mutant were incubated with the indicated amounts in micrograms of the retinal $\beta\gamma$T subunit complex and subjected to pertussis toxin–catalyzed ADP ribosylation in the presence of [α-^{32}P]NAD (*20*). At the end of the reaction period (1 hour), Laemmli sample buffer was added, and the samples were subjected to SDS-PAGE (13.5% acrylamide).

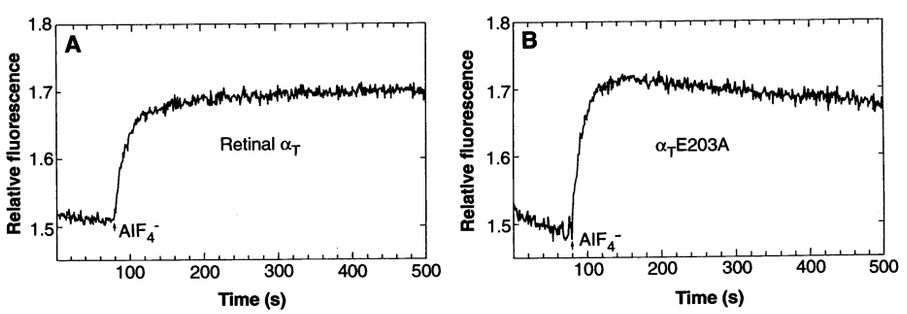

Fig. 3. Aluminum fluoride–induced enhancement of the intrinsic tryptophan fluorescence of the α_T subunit. (**A**) Retinal α_T (250 nM) or (**B**) α_TE203A mutant (250 nM) was equilibrated in a stirred quartz cuvette and the excitation wavelength was fixed at 280 nm. The emission was monitored continuously at 340 nm and a mixture of $AlCl_3$ (30 μM) and NaF (5 mM) was added to form an AlF_4^- complex and activate the α_T subunits.

tive retinal α_T (Fig. 3).

Because the E203A mutant appeared to undergo a normal activating conformational change, on the basis of the above criterion, it was surprising to find that the GDP-bound form of this mutant was able to elicit a significant stimulation of the cyclic guanosine monophosphate (cGMP) phosphodiesterase (PDE). We measured the relative abilities of the native retinal α_T, the wild-type GST-α_T subunit expressed in insect cells, and the GST-α_TE203A mutant to catalyze cGMP PDE activity (Fig. 4). The retinal α_T subunit, when loaded with GTP-γ-S in a rhodopsin- and $\beta\gamma_T$-dependent manner, caused a strong stimulation of PDE activity, whereas the GDP-bound form of retinal α_T showed no stimulation. Essentially identical results were obtained with the *S. frugiperda*–expressed wild-type α_T subunit. However, the α_TE203A mutant stimulated the PDE activity in the absence of rhodopsin- and $\beta\gamma_T$-stimulated GTP-γ-S loading. There was a slight further stimulation when phosphatidylcholine vesicles containing rhodopsin, $\beta\gamma_T$, and GTP-γ-S were added to the assays. The PDE activities stimulated by the α_TE203A mutant, even in the GDP-bound state, were consistently 20 to 40% higher than the PDE activities stimulated by either the native retinal α_T–GTP-γ-S complex or the recombinant, wild-type GTP-γ-S–bound α_T. This reflects the fact that under our assay conditions, typically only 40 to 50% of the total native retinal (or recombinant) α_T subunits can be loaded with GTP-γ-S in a rhodopsin- and $\beta\gamma_T$-dependent manner.

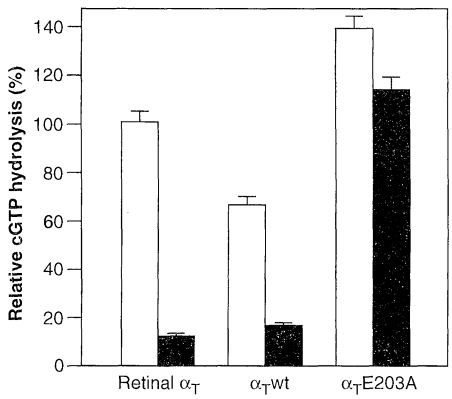

Fig. 4. Relative cGMP phosphodiesterase (PDE) activities of the retinal α_T subunit and wild-type α_T ($\alpha_T$wt) or the α_TE203A mutant expressed in insect cells. The retinal α_T subunits were assayed in the GTP-γ-S–bound state (open bars) or GDP-bound state (solid bars) for their ability to stimulate cGMP hydrolysis by the cGMP PDE (*18, 21*). The rate of cGMP hydrolysis resulting from the activation of the PDE by the recombinant α_T subunits is reported relative to the activity measured for the GTP-γ-S–loaded retinal α_T (100%, 800 to 1000 nmol of cGMP hydrolyzed per second and per nanomole of PDE).

Because the α_TE203A mutant does not need to be bound to GTP-γ-S to stimulate PDE activity, its ability to activate the effector enzyme is not limited by this loading efficiency.

The ability of the α_TE203A mutant to stimulate cGMP hydrolysis by the PDE, in the absence of GTP-γ-S–GDP exchange, cannot be attributed to the possibility that some percentage of the recombinant mutant exists in the GTP-bound state. This possibility was ruled out by the findings that the α_TE203A mutant is fully capable of being ADP-ribosylated in response to pertussis toxin treatment and undergoes an AlF_4^--induced change in tryptophan fluorescence (which requires that the α_T subunit is initially in the GDP-bound state) that is indistinguishable from the fluorescence changes measured with the native retinal subunit. Thus, the data indicate that the α_TE203A mutant is constitutively active in its ability to stimulate the target enzyme.

The x-ray crystallographic studies of α_T reveal that Glu^{203} is solvent-exposed and points away from the β4-α3 loop (residues 241 to 255) in both the α_T-GDP and α_T–GTP-γ-S structures (*3, 4*). In addition, Glu^{203} forms an ion pair with Lys^{206} in both α_T structures (*5*). Thus, we propose that the Glu^{203}-Lys^{206} interaction helps maintain the α2 helical region (that is, switch II; residues 200 to 210) in an inactive conformation when α_T is bound to GDP. Under a normal activation event (that is, upon exchange of GTP for GDP), Gly^{199} becomes hydrogen bonded to the γ-phosphate of GTP, which in turn is thought to convert α2 from a severely distorted 3-10 helix into a more ideal 3-10 helix. This change is accompanied by the movement of the side chains of Arg^{201} and Arg^{204} toward Glu^{241} (with corresponding changes in electrostatic interactions) and the movement of Trp^{207} toward Leu^{245} and Ile^{249}. One possibility that has been raised (*4*) is that as an outcome of these changes, an acidic cluster of amino acids (Asp^{233}, Asp^{234}, and Glu^{235}) within switch III is brought into position to bind to a stretch of basic amino acids within the γ subunit of the target (PDE) molecule (γ_{PDE}). However, we generated a triple mutant in which Asp^{233}, Asp^{234}, and Glu^{235} were all changed to alanine residues and found that this recombinant α_T was still capable of stimulating PDE activity (*13*). These findings indicate that the cluster of acidic amino acids (residues 233 to 235) on α_T are not a critical binding site for the effector γ_{PDE} subunit, and that the movement of the switch II domain may provide γ_{PDE} with access to another site on α_T [perhaps the putative stimulatory contact site, residues 293 to 314 (*14, 15*)].

In summary, we propose that the substitution of an alanine residue for a glutamic acid at position 203 in α_T allows the movement of Arg^{201} or Arg^{204} or both toward Glu^{241} and promotes effector binding, even in the absence of GTP-GDP exchange. Thus, the α_TE203A mutant does not require GTP binding (through an interaction with Gly^{199}) for effector recognition. Apparently, the movement of the NH_2-terminal portion of the switch II domain is sufficient to mediate effector recognition. Trp^{207} within the GDP-bound form of the α_TE203A mutant apparently does not move toward Leu^{245} and Ile^{249}, as the addition of AlF_4^-, which mimics the action of GTP, still induced the necessary movement of Trp^{207} to yield an enhanced fluorescence signal. Thus, the results presented here demonstrate an uncoupling of both receptor-stimulated GTP-GDP exchange and GTPase activity from G protein stimulation of effector activity. In addition, they further pinpoint a region within the switch II domain that is essential for the generation of target recognition and show that target or effector stimulation can occur independently of changes in tryptophan fluorescence, a read-out that up to now has been taken as a direct monitor of G protein activation.

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7. The GST-α_T subunits were expressed with a pGEXKG construct. The construct pGEXKG-α_T was made by digesting the bovine α_T complementary DNA (cDNA) in pUC19 with Nhe I and subjecting it to mung bean nuclease to blunt the Nhe I 5′ overhang, thereby generating a blunt NH_2-terminus, and digesting it further with Xba I. The GST fusion vector pGEXKG was prepared to receive the α_T insert by digestion with Sma I and Xba I. This construct (pGEXKG-α_T) was used for the insect cell expression of GST-α_T. We used the Eco RI site in pGEXKG-α_T directly downstream from the start ATG codon of the GST protein to insert a linker (5′-ATCTAG**ATG**CTAT-3′) containing an additional Xba I site. The 1.8-kb GST-α_T cDNA was thus placed between two Xba I sites in the resultant clone pGEXKG-lin-α_T. This Xba I fragment was ligated into the pVL1393 transfer vector. To generate the α_TE203A mutant, we used the polymerase chain reaction (PCR) to flank a 560–base pair (bp) Sma I–Bgl II fragment of the α_T cDNA by Xba I and Eco RI sites. This fragment, the target for mutagenesis, was ligated into RF-M13mp19. Mutations were obtained with an M13-based in vitro mutagenesis protocol (Amersham) according to manufacturer's instructions. A 470-bp Sma I–Bgl II mutated insert of α_T was ligated into pGEXKG-lin-α_T from which the corresponding 470-bp (wild-type) insert was removed by digestion with Sma I and Bgl II. The 1.8-kb Xba I fragment was ligated into the baculovirus transfer vector pVL1393 to give the construct pVL1393-GST-α_TE203A. Transfection of Sf9 insect cells was done either by cationic liposome-mediated transfection in the presence of linearized AcMNPV DNA (Invitrogen) or with the Baculogold

Transfection kit (Pharmingen). In each case the recombinant extracellular virus (rECV) was purified by a limiting dilution dot-blot hybridization procedure (*16*). The cells were infected at 80% confluence with rECV at a multiplicity of infection of 10 and harvested typically 72 hours after infection. For purification of recombinant GST-α_T, frozen cell pellets were thawed and resuspended in HMDN buffer [20 mM Hepes (pH 7.4), 5 mM $MgCl_2$, 1 mM dithiothreitol (DTT), and 100 mM NaCl] containing Triton X-100 (1%) or cholate (1%) and the protease inhibitors aprotinin and leupeptin. Cells were lysed by sonication on ice, the lysates were centrifuged at 40,000*g*, and sedimented material was discarded. The soluble GST fusion proteins were purified by glutathione-agarose affinity chromatography. Purified proteins were dialysed against HMDN buffer (pH 7.4) before use in assays. The protease thrombin was used (at a fusion protein:protease ratio of 50:1) to separate the GST and α_T proteins by cutting at a thrombin recognition site. Digestion was done in HMDN buffer supplemented with 2.5 mM $CaCl_2$, at 4°C for 3 hours. Liberated GST subunits and undigested GST-α_T were removed by glutathione-agarose affinity chromatography to yield purified wild-type α_T and α_TE203A.

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17. The GTPase activities of retinal α_T and the recombinant α_T subunits were measured as described (*12*). Briefly, the individual proteins (100 nM α_T, 100 nM retinal $\beta\gamma_T$) and phosphatidylcholine vesicles containing rhodopsin (*12*) were assayed for 10 min in a total volume of 200 μl at room temperature. The reactions were done in 20 mM sodium Hepes (pH 7.4), 5 mM $MgCl_2$, 1 mM DTT, and 100 mM NaCl and were initiated by the addition of GTP and [γ-^{32}P]GTP to a final concentration of 1 μM. After 10 min, the reactions were quenched by the addition of 5 ml of 10% ammonium molybdate solution. The [γ-^{32}P]P_i released upon GTP hydrolysis was extracted by the addition of 5 ml of a 1:1 mixture of isobutanol and benzene and quantitated by liquid scintillation counting. Background hydrolysis of GTP was measured by quantitating P_i release by rhodopsin-containing lipid vesicles and $\beta\gamma$T without added α_T-GDP, or by measuring the GTPase activity by α_T-GDP with $\beta\gamma_T$ in the absence of rhodopsin-containing lipid vesicles.

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19. The GTP-γ-S–binding activities of retinal α_T and recombinant α_T subunits were measured in a manner similar to the GTPase assays (*17*). Reactions were initiated by the addition of a mixture of GTP-γ-S and [^{35}S]GTP-γ-S to a final concentration of 1 μM. The reaction mixtures were incubated for 20 min at room temperature, then quenched by the addition of ice-cold buffer containing 20 mM sodium Hepes (pH 7.4), 5 mM $MgCl_2$, and 100 mM NaCl, and then added to 0.45-μm nitrocellulose filters on a suction manifold. The filters were washed twice with 3 ml of buffer and counted in 3 ml of Liquiscient (National Diagnostics). Nonspecific binding was determined by measuring [^{35}S]GTP-γ-S binding to α_T-GDP with $\beta\gamma_T$ in the presence of lipid vesicles that lacked rhodopsin, or by measuring binding to $\beta\gamma_T$- and rhodopsin-containing lipid vesicles in the absence of added α_T-GDP.
20. Pertussis toxin–catalyzed ADP ribosylation was performed by incubating the α_T subunits (2 μg) with lipid vesicles lacking rhodopsin (*12*) and various amounts of the $\beta\gamma_T$ subunit complex (0 to 2 μg) at room temperature in a total volume of 40 μl in the presence of 250 ng of activated pertussis toxin, 1.25 μM nicotinamide adenine dinucleotide (NAD), 0.5 μCi per assay of [α-^{32}P]NAD, 50 mM tris-Cl (pH 8.0), 3 mM $MgCl_2$, 1 mM DTT, 50 μM GTP, and 0.5 mM adenosine triphosphate. The reactions were incubated for 1 hour at room temperature and then Laemmli sample buffer was added and the samples subjected to SDS–polyacrylamide gel electrophoresis (PAGE) and autoradiography.
21. Measurements of cyclic GMP (cGMP) hydrolysis by the retinal cGMP-dependent PDE were done as described (*18*). Briefly, a pH microelectrode was used to measure the decrease in pH that resulted from the production of one proton for each molecule of cGMP hydrolyzed by the PDE. All assays were done at room temperature in a final volume of 200 μl in a buffer containing 5 mM Hepes (pH 8.0), 5 mM $MgCl_2$, and 100 mM NaCl. All PDE assays contained 10 pmol of intact holoPDE and when measuring the GTP-γ-S–bound α_T-stimulated activity, 15 pmol of the α_T–GTP-γ-S complex. Loading of the α_T subunits with GTP-γ-S was done with rhodopsin that had been reconstituted into phosphatidylcholine vesicles and purified retinal $\beta\gamma_T$ (*12*). Equivalent amounts of the different α_T subunits, as quantitated by [^{35}S]GTP-γ-S binding (*12*), were added to the PDE assays. The PDE assays were initiated by the addition of cGMP to a final concentration of 5 mM, and the change in pH (in millivolts) was measured once per second. At the end of the assay period (~200 s), the buffering capacity (millivolts per nanomole) was determined by the addition of 0.1 μmol of KOH to the reaction mixture. The rate of hydrolysis of cGMP (nanomoles per second) was determined from the ratio of the slope of the pH record (millivolts per second) and the buffering capacity of the assay buffer (millivolts per nanomole).

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Rearrangement-Enhancing Element Upstream of the Mouse Immunoglobulin Kappa Chain J Cluster

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Transcriptional regulatory elements have been shown to be necessary but not sufficient for the developmental regulation of immunoglobulin gene rearrangement in mouse precursor B cells. In the chicken λ light chain locus, additional elements in the V-J intervening sequence are involved in negative and positive regulation of rearrangement. Here, mutation of the mouse homolog of a chicken element, located in the V_κ-J_κ intervening sequence upstream of the J_κ cluster, was shown to significantly decrease rearrangement. This cis-acting recombination-enhancing element affects the rearrangement process without being involved in regulating transcription.

During differentiation in the bone marrow, most B cell precursors undergo successive rounds of immunoglobulin (Ig) gene rearrangement: D-J then V-DJ joining for the heavy chain, followed by V-J joining for the light chains (*1*, *2*). Because the same recombinase machinery is involved at each stage, specific elements are thought to account for such a developmental regulation by controlling the accessibility of the loci (*3*). Experiments with transgenic recombination substrates as well as homologous recombination have shown that transcriptional regulatory elements are necessary for these events to occur (*4*–*6*).

Using a chicken λ light chain transgene in its natural germline configuration, we previously showed that, in addition to the enhancer and promoter, other elements located in the V-J intervening sequence regulate rearrangement of this locus in mouse pre-B cells (*6*, *7*). One of these additional elements reduces rearrangement, hence we call it a "silencer." The silencer is flanked by one element (or possibly two) counteracting its effect, hence we call it an "antisilencer" (*6*). We proposed that the silencer and antisilencer are involved in ensuring allelic exclusion at the chicken λ locus (*8*). One of the chicken antisilencer elements is homologous in sequence and location to two palindromic motifs (called KI and KII) located in the mouse V_κ-J_κ intervening sequence upstream of the J_κ1 gene segment and previously described as binding sites for a factor present in mouse pre-B and B cells (*9*).

To investigate the role of the KI and KII sites in regulating mouse κ light chain rearrangement, we generated KI-KII mutant mice using the gene-targeting method in mouse embryonic stem (ES) cells (*10*). The vector used for transfection contained a murine J_κ genomic fragment (*11*) in which the KI and KII sites were altered by site-directed mutagenesis to replace 8 and 9 base pairs (bp) of the KI and KII sites, respectively, in order to modify the overall structure of these sites (Fig. 1A). In addition, we used the Cre-*lox*P system of bacteriophage

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