accord with non-geometric landmarks that could have improved their performance, even though most children turned and inspected the room before searching and used the same landmarks in a different search task. These findings provide evidence for a common shape-based orientation mechanism in humans and other mammals and for informational encapsulation[8] in the child's mechanism.

In contrast to young children and mature rats, human adults conjoined geometric and non-geometric information to reorient themselves. Their performance suggests that some representational systems become more accessible and flexible over development and evolution[13,14]. Studies of the mechanisms underlying the increase in flexibility for reorientation may shed light on uniquely human capacities for problem solving. □

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An eye-specific Gβ subunit essential for termination of the phototransduction cascade

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HETEROTRIMERIC G proteins couple various receptors to intracellular effector molecules. Although the role of the Gα subunit in effector activation, guanine nucleotide exchange and GTP hydrolysis has been well studied[1–4], the cellular functions of the Gβ subunits are less well understood[5,6]. Gβγ dimers bind Gα subunits and anchor them to the membrane for presentation to the receptor[7–9]. In specific systems, the Gβ subunits have also been implicated in direct coupling to ion channels and to effector molecules[10–19]. We have isolated *Drosophila melanogaster* mutants defective in an eye-specific G-protein β-subunit (Gβe), and show here that the β-subunit is essential for G-protein–receptor coupling *in vivo*. Remarkably, Gβ mutants are also severely defective in the deactivation of the light response, demonstrating an essential role for the Gβ subunit in terminating the active state of this signalling cascade.

Genetic screening[20] for mutations in a photoreceptor-cell-specific Gβ subunit (Gβe)[21] isolated two alleles, $G\beta e^1$ and $G\beta e^2$. Both alleles have missense mutations which severely reduce the levels of the Gβ subunit (Fig. 1*a*). $G\beta e^1$ has a tyrosine substituted for a cysteine at amino acid 293 and produces <0.5% of the wild-type levels of the Gβ subunit, whereas the $G\beta e^2$ allele has a glycine substituted for a glutamate at residue 288 and produces ~5% of wild-type protein levels.

When whole-cell patch clamp recordings[22,23] were used to analyse the electrophysiological response of the $G\beta e^1$ and $G\beta e^2$ mutant photoreceptors to light stimuli, both Gβ alleles showed a dramatic loss of light sensitivity, $G\beta e^1$ mutants showing a reduction of nearly two orders of magnitude (Fig. 1*b*). The kinetics of their photoresponse also differed markedly from that of wild

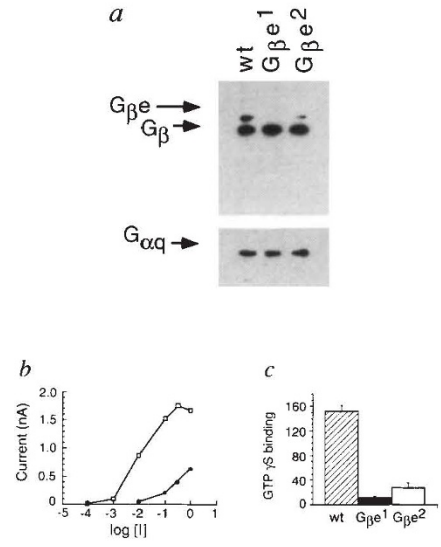

FIG. 1 *a*, Quantification of Gβe protein levels. $G\beta e^1 = 0.4\% \pm 0.2$; $G\beta e^2 = 4.6\% \pm 5.7$ (range 0.8–16%). Similar results were obtained using two different antibodies with two different specificities. Gβ and $G_{\alpha q}$ refer to a brain-specific isoform of Gβ[32] and to the photoreceptor-cell-specific $G_{\alpha q}$[27]. As neither mutation affected the levels of Gβe transcripts (data not shown), the dramatic reduction of Gβe levels in these missense mutants is probably due to the synthesis of misfolded or unstable proteins that are rapidly degraded in the cell. Neither Gβ mutation affected the expression of a photoreceptor-cell-specific Gα subunit, or of several other molecules involved in the phototransduction cascade (data not shown). *b*, Intensity response functions for wild-type (w^{1118}) and mutant photoreceptors. Note that 100-fold stronger light was required to evoke a minimal current from $G\beta e^1$ photoreceptors (closed circles) than from wild-type cells (open squares). Equivalent results were obtained from seven cells from each genotype. Photoreceptors were isolated and patch clamp recordings performed as previously described[22]. *c*, Blue-light-stimulated eye-specific GTP-γS binding. Dark-adapted *Drosophila* head sections were stimulated with blue, 480-nm light (R→M conversion) or red, 610-nm light (M→R conversion). GTP-γS binding to eye tissue was determined by autoradiography. In wild-type controls, GTP-γ[^{35}S] binds to target substrates in the compound eyes following blue light illumination which activates Rh1 rhodopsin. However, no retina-specific binding is observed following stimulation with red light (610 nm). Gβe mutants showed dramatically reduced levels of blue-light-stimulated GTP-γ[^{35}S] eye-specific binding. Values shown indicate per cent of maximal wild-type response at saturating blue light intensity (w^{1118}, $n=9$; $G\beta e^1$, $n=7$; $G\beta e^2$, $n=7$).
METHODS. Cryostat sections (10 μm thick), each containing ~40 fly heads on nitrocellulose filters (Schleicher and Schull BA85, 25-mm circles), were pre-flashed for 10 s with red light (Schott RG-610 filter) in 50 mM 4-morpholine propanesulphonic acid, pH 6.7, 5 mM $MgCl_2$, 2 mM 2-mercaptoethanol, 2 μg ml^{-1} pepstatin, 10 μg ml^{-1} leupeptin, 2 mM benzamidine, 0.2 mM ATP. Sections were then stimulated with red or blue (Schott BG-28) test flashes in the presence of 96 nM GTP-γS and 4 nM GTP-γ[^{35}S]. After the light flashes, the filters were incubated in the dark for 1 min and washed with 10 mM sodium phosphate, pH 7.5, 150 mM NaCl, 0.1% Tween-20, then with 0.1 × SSC. Details of the procedure will be published elsewhere.

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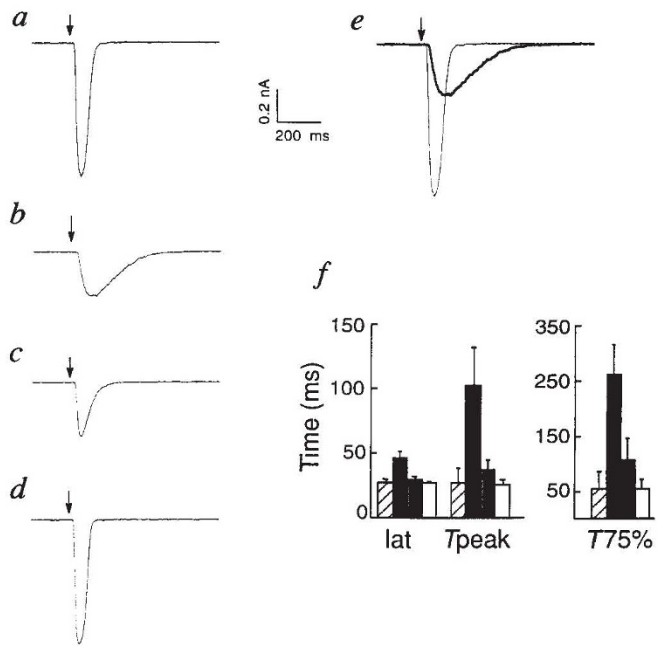
type (Fig. 2). Whereas wild-type photoreceptors responded to intense light flashes with short latencies and short times to peak (27.3 ± 2.42 and 27.6 ± 10.8 ms, respectively[22,24]) (Fig. 2*a*, *f*), in $G\beta e^1$ mutants both parameters were dramatically increased (46.3 ± 5.99 and 103.6 ± 29 ms, respectively; $P < 0.0001$) (Fig. 2*b*, *e*, *f*). These slower response kinetics probably result from reduced levels of Gαβγ holocomplexes available to couple to the downstream effectors. Interestingly, activation kinetics in the less severe $G\beta e^2$ allele were only slightly altered (latency, 30.4 ± 1.67; time to peak response, 37.6 ± 8.17 ms; Fig. 2*f*). This shows that 5% of the wild-type levels of Gβe polypeptide are sufficient for normal activation responses and is consistent with a catalytic role for Gβ in the activation phase of the G-protein cycle. The changes in physiology of $G\beta e$ mutants are due solely to the loss of Gβe, as reintroduction of the wild-type $G\beta e^+$ gene into $G\beta e^1$ mutant hosts by germ-line transformation fully rescued these defects (Fig. 2*d*, *f*).

Phototransduction in *Drosophila* occurs via a phospholipase-C-mediated signalling cascade[25,26]. Light-activated rhodopsin interacts with a photoreceptor-cell-specific $G_{\alpha q}$ (ref. 27) which activates a phospholipase C effector. Biochemical evidence suggests that the reduced sensitivity of $G\beta$ mutants is due to a defect in the activation of $G_{\alpha q}$. An *in-situ* assay of GTP-γS binding to *Drosophila* eyes *in vivo* showed that light-induced GTP binding was dramatically reduced in $G\beta^1$ mutants (Fig. 1*c*). The magnitude of the loss paralleled the loss of sensitivity of the electrical response (Fig. 1*a*, and data not shown). These results suggest that the changes in light sensitivity, latency and time to peak in $G\beta e$ mutants reflect the inability of the G_α subunit to couple efficiently to rhodopsin in the absence of Gβ and demonstrate the essential requirement of Gβ for Gα function *in vivo*.

In addition to the expected defects in the activation phase of the light response (that is, coupling to the receptor molecule), the rate of deactivation (defined as the recovery of the photoresponse) of the phototransduction cascade (Fig. 2*e*, *f*) was also dramatically and unexpectedly decreased in Gβ mutants. To quantify the data, we measured deactivation as the time from peak response to 75% attenuation of the peak current (referred to as *T* 75%). Wild-type photoreceptors had *T* 75% values of 55.3 ± 32.2 ms, compared with 261.6 ± 52.7 ms ($P < 0.0001$) in $G\beta e^1$ mutants. These deactivation defects are indeed due to the loss of Gβe, as wild-type kinetics were restored after germ-line transformation with a cloned copy of the wild-type Gβe gene (Fig. 2*f*). $G\beta e^2$ mutants also showed defective deactivation kinetics (*T* 75% = 106.4 ± 38.84; $P < 0.05$; Fig. 2*f*), demonstrating that 5% of the wild-type levels of the protein are not sufficient for normal deactivation.

When light-activated channels open, extracellular calcium enters the photoreceptor cell, promoting termination of the light response[22,23]. Thus, the altered deactivation kinetics of $G\beta e$ mutants might be due to a decrease in calcium influx resulting from the reduced sensitivity of the $G\beta e$ mutant photoreceptors. We analysed the electrophysiological response of wild-type and $G\beta e$ mutants in the absence of extracellular calcium so as to eliminate the contribution of calcium-mediated deactivation mechanisms. Figure 2*g* shows that even in calcium-free recording medium, $G\beta e^1$ photoreceptor deactivation kinetics are still markedly reduced compared with wild-type cells.

The $G\beta$ deactivation defects were examined further by analysing the kinetics of recovery of the photoresponse following continuous activation of the photoreceptor cell. This prolonged depolarizing afterpotential occurs whenever there is an excess of activated metarhodopsin over free arrestin[20]. A prolonged depolarizing afterpotential can be experimentally terminated by photoconversion of metarhodopsin back to rhodopsin with 580-nm light[28]. Because metarhodopsin in such a case is instanta-



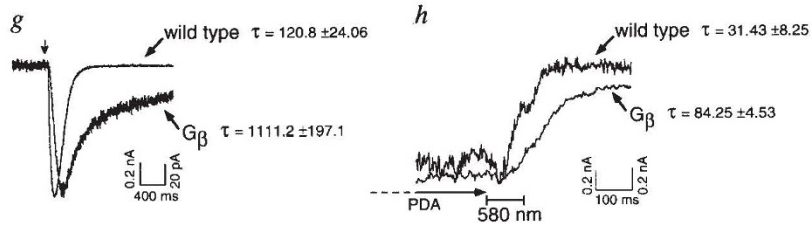


FIG. 2 $G\beta$ mutants have defective activation and deactivation kinetics. Whole-cell voltage clamp recordings[22,23] of light-activated currents from wild type (w^{1118}) (*a*), $G\beta e^1$ (*b*), $G\beta e^2$ (*c*) and $G\beta e^1$; P[w$^+$, $G\beta e^+$] (*d*) transgenic flies. e, A superimposition of the wild-type and $G\beta e^1$ responses on the same current and time scales. Arrows indicate the position of 10-ms light flashes of maximal light intensity (log [*I*] = 0 in Fig. 1*b*). *f*, Histograms of latency, time to peak and time to 75% deactivation (mean ±s.d.; note the different time scales). Each data point represents 4–6 independent cells, each with a minimum of three individual electrophysiological recordings. Hatched bars, wild-type; solid bars, $G\beta e^1$; shaded bars, $G\beta e^2$; open bars, $G\beta e^1$; P[w$^+$, $G\beta e^+$]. lat, latency (time from stimulus onset to initiation of inward current); *T*peak, time required to reach peak current from onset of stimulus; *T* 75%, time from peak response to 75% attenuation of the peak current. *g*, Wild-type and $G\beta e^1$ responses to 10-ms flashes in nominal calcium-free bath solution. For quantitative evaluation of the data, the tail of the deactivation phase of the light-activated current was fitted to a single exponential function and the time constant (τ) was measured (this is necessary owing to the extremely long deactivation times of $G\beta e^1$ mutants in the absence of extracellular calcium). Average τ values (mean ±s.d.) are shown for wild type ($n = 7$) and $G\beta e^1$ ($n = 4$). *h*, A prolonged depolarizing afterpotential (PDA) was induced in $G\beta e^1$ and wild-type photoreceptors with a 100-ms pulse of 480-nm light (R→M conversion). The afterpotential was terminated 1.3 s later with a 100-ms pulse of 580-nm light (M→R conversion). The traces show the last 100 ms of the afterpotential and the deactivation phases following the 580-nm light pulse. Average τ values (mean ±s.d.) for wild type ($n = 4$) and $G\beta e^1$ ($n = 3$) are indicated.

neously inactivated, it is possible to determine whether defects in receptor coupling or inactivation produce the slow kinetics of deactivation seen in the *Gβe* mutants. Upon light-induced inactivation of metarhodopsin, *Gβe* mutants still showed very slow deactivation kinetics (Fig. 2*h*), indicating that the process responsible for the deactivation defect in *Gβe* mutants lies downstream of receptor activation and inactivation.

Although much is known about G-protein function in reconstituted systems, less is known about the function of Gβ and Gγ *in vivo*. The demonstration that 5% of the wild-type levels of Gβ[2] in the weaker mutant 'rescues' the activation defects but does not rescue the deactivation phase of the response highlights the two aspects of Gβ requirements *in vivo*: first, there is a catalytic requirement in coupling, where Gβ probably functions by shuttling many Gαs to the receptors, and second, there is a stoichiometric requirement where Gβ may need to complex with an activated component to shut off the response.

Termination of this signalling cascade may be modulated by Gβ at several steps. Gβ may act at the level of Gα or the Gα : effector complexes[29]. It may also be required at a different point in the signalling pathway. Pitcher *et al.*[30] showed that Gβγ is necessary for phosphorylation of the β-adrenergic receptor by recruiting the β-adrenergic recelptor kinase to the membrane. However, as the deactivation defect of *Gβe* mutants cannot be rescued following photochemical inactivation of rhodopsin (Fig. 2*h*), Gβ is not required at the level of the receptor molecule. Ron *et al.*[31] showed that homologues of Gβ subunits may function as binding sites for protein kinase C. Interestingly, *Drosophila* mutants defective in an eye-specific isoform of protein kinase Cα have deactivation defects similar to *Gβe* mutants[22,24]. The strict requirement for Gβ in modulating the shut-off of the response may be particularly important in signalling cascades like phototransduction, where Gβγ molecules are very abundant and fast deactivation kinetics are fundamental for proper temporal resolution of closely spaced stimuli. □

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A matrix metalloproteinase expressed on the surface of invasive tumour cells

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GELATINASE A (type-IV collagenase; M_r 72,000) is produced by tumour stroma cells and is believed to be crucial for their invasion and metastasis, acting by degrading extracellular matrix macromolecules such as type IV collagen[1–3]. An inactive precursor of gelatinase A (pro-gelatinase A) is secreted and activated in invasive tumour tissue[4–7] as a result of proteolysis which is mediated by a fraction of tumour cell membrane that is sensitive to metalloproteinase inhibitors[4,5]. Here we report the cloning of the complementary DNA encoding a new matrix metalloproteinase with a potential transmembrane domain. Expression of the gene product on the cell surface induces specific activation of pro-gelatinase A *in vitro* and enhances cellular invasion of the reconstituted basement membrane. Tumour cells of invasive lung carcinomas, which contain activated forms of gelatinase A, were found to express the transcript and the gene product. The new metalloproteinase may thus trigger invasion by tumour cells by activating pro-gelatinase A on the tumour cell surface.

We screened cDNAs with homology to conserved regions in matrix metalloproteinase (MMP) genes (Fig. 1 legend) and isolated a unique 3.4-kilobase (kb) cDNA fragment (MMP-X1) from a human placenta cDNA library. This cDNA encodes a unique protein of 582 amino acids (M_r 66K) which can be closely aligned with known MMP family members[8,9] (Fig. 1*a*, *b*). The MMP-X1 protein contains a potential transmembrane domain at the C terminus (24 hydrophobic amino acids; black overbar in Fig. 1*a*), which does not exist in other MMPs. We will call the product MT-MMP, for membrane-type matrix metalloproteinase.

To identify the gene product, we transfected COS-1 cells with MT-MMP plasmid and immunoprecipitated cell lysates labelled with ^{35}S-methionine with monoclonal antibodies (antibodies 113-5B7, 114-1F2 and 118-3B1) raised against synthetic MT-MMP peptides. These antibodies recognized different epitopes but immunoprecipitated a common 63K protein only from the transfected cells (Fig. 2*a*). We could detect no MT-MMP released into the medium, whereas a tissue inhibitor of metalloproteinases type-1 (TIMP-1), which was co-expressed as a secretory protein, was found in both the cell lysate and culture medium (Fig. 2*b*). MT-MMP was also detected in the plasma membrane fraction of the transfected cells (Fig. 2*c*) but TIMP-1 was not (data not shown). Immunohistochemical staining of COS-1 cells transfected with MT-MMP plasmid localized the product to the cell surface (Fig. 2*d*).

The expression of MT-MMP on the cell surface fits the requirements of an activator for pro-gelatinase A[2–7]. We tested this idea by transfecting MT-MMP plasmid into human fibrosarcoma HT1080 and mouse fibroblast NIH3T3 cell lines which secrete pro-gelatinase A and pro-gelatinase B (92K type-IV procollagenase) into the culture supernatant[10,11], as detected by gelatin zymography (66K and 90K bands, respectively, in Fig. 3*a*).

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