

Slowed recovery of rod photoresponse in mice lacking the GTPase accelerating protein RGS9-1

Ching-Kang Chen*, Marie E. Burns†, Wei He‡, Theodore G. Wensel‡, Denis A. Baylor† & Melvin I. Simon*§

* Division of Biology, 147-75, California Institute of Technology, Pasadena, California 91125, USA

† Department of Neurobiology, Stanford University Medical Center, Stanford, California 94305, USA

‡ Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030, USA

Timely deactivation of the α -subunit of the rod G-protein transducin ($G\alpha t$) is essential for the temporal resolution of rod vision¹. Regulators of G-protein signalling (RGS) proteins accelerate hydrolysis of GTP by the α -subunits of heterotrimeric G proteins²⁻⁴ *in vitro*. Several retinal RGS proteins can act *in vitro*

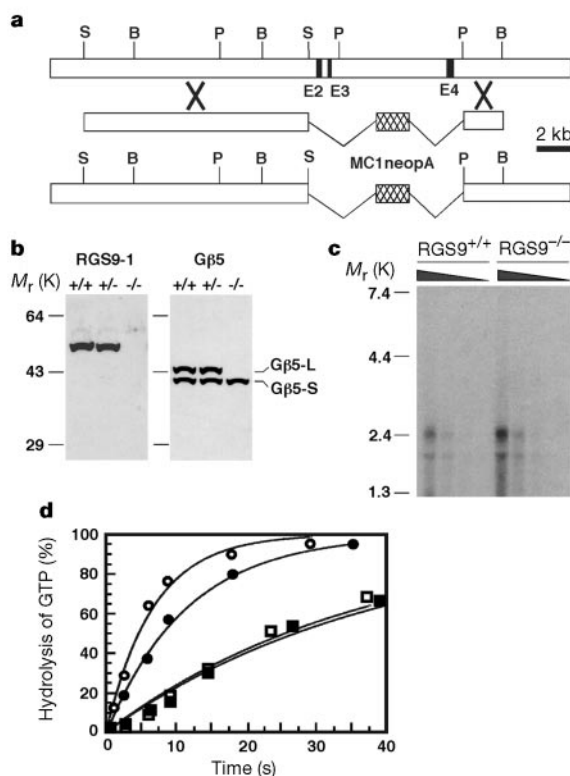


Figure 1 Inactivation of the RGS9 gene results in the loss of RGS9-1 and G β 5-L in the retina and a reduced rate of hydrolysis of GTP by transducin. **a**, Inactivation of the RGS9 gene. Top, Partial genomic structure of the RGS9 gene; middle, targeting construct; bottom, altered RGS9 locus. The targeting construct contained 9 kb (long arm) of the 3' downstream and 1.3 kb (short arm) of the 5' upstream homologous sequence. The MC1neopA cassette (hatched box) replaced a 5.5-kb fragment that contained three exons corresponding to amino-acid residues 19–105. Restriction sites: B, *Bam*HI; P, *Pst*I, S, *Spe*I. **b**, Immunoblot analysis of RGS9-1 and G β 5 protein levels in retinal homogenates derived from mice at 1 month old. The levels of RGS9-1 and G β 5 were similar in RGS9^{+/+} and RGS9^{+/-} retinas. **c**, Northern blot analysis of G β 5-L mRNA. From left to right, 4, 2, 1 and 0.5 μ g of total retinal RNA derived from mice at 1 month of age were loaded per lane. The retina-specific exon of the G β 5 gene¹¹ was used as a probe. **d**, Single-turnover GTP hydrolysis by G αt in ROS from RGS9^{+/+} (circles) and RGS9^{-/-} (squares) mice with (open) or without (filled) exogenous PDE- γ (1.33 μ M). Rate constants were determined by fitting the results with a single exponential function and are given in the text.

as GTPase accelerating proteins (GAP) for G αt ⁵⁻⁸. Recent reconstitution experiments indicate that one of these, RGS9-1, may account for much of the G αt GAP activity in rod outer segments (ROS)^{8,9}. Here we report that ROS membranes from mice lacking RGS9-1 hydrolyse GTP more slowly than ROS membranes from control mice. The G β 5-L protein that forms a complex with RGS9-1 (ref. 10) was absent from RGS9^{-/-} retinas, although G β 5-L messenger RNA was still present. The flash responses of RGS9^{-/-} rods rose normally, but recovered much more slowly than normal. We conclude that RGS9-1, probably in a complex with G β 5-L, is essential for acceleration of hydrolysis of GTP by G αt and for normal recovery of the photoresponse.

To investigate the functional role of RGS-9 in retinal photoreceptors, we inactivated both alleles of the RGS-9 gene by replacing a 5.5-kilobase (kb) genomic fragment containing exons 2 to 4 with a neomycin resistance marker, MC1neopA (Fig. 1a; see Methods). Heterozygous and homozygous knockout mice had normal retinal morphologies up to eight months of age (data not shown). Western blot analysis showed that the retinas of RGS9^{+/+} and RGS9^{+/-} mice contained similar amounts of RGS9-1, whereas no RGS9-1 was detectable in RGS9^{-/-} retinas (Fig. 1b). RGS9^{-/-} retinas contained normal amounts of other photoreceptor proteins including recoverin, transducin, rhodopsin kinase, guanylyl cyclase E and guanylyl cyclase F (data not shown). However, the long form of the fifth member of the G-protein β -subunit (G β 5-L)^{11,12} was absent from RGS9^{-/-} retinas (Fig. 1b), although normal amounts of G β 5-L mRNA were still present (Fig. 1c). G β 5 isoforms form complexes

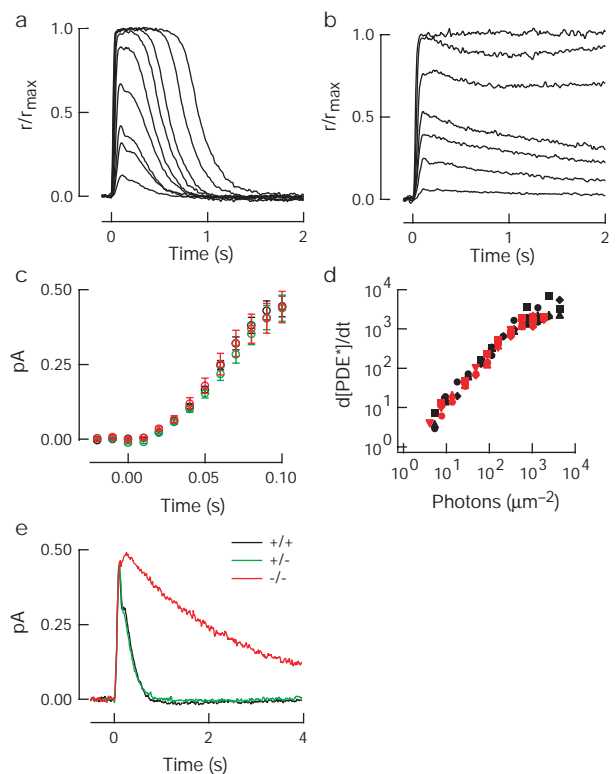


Figure 2 Slow recovery of the photoresponse in the absence of RGS9-1 and G β 5-L. Representative families of responses from a control rod (**a**) and an RGS9^{-/-} rod (**b**) to flashes of 9.6–1350 photons μ m⁻² (**a**) or 4.0–572 photons μ m⁻² (**b**). The dark currents were 14.7 pA (**a**) and 7.7 pA (**b**). Each trace is the average of 2–31 responses. **c**, Rising phases of the population average single photon response of RGS9^{-/-} (red, $n = 16$), RGS9^{+/-} (green, $n = 29$) and control rods (black, $n = 12$). Error bars indicate s.e.m. **d**, Initial rate of change of the light-activated PDE activity ($d[PDE^*]/dt$) in five control rods (black) and five RGS9^{-/-} rods (red) as described^{18,20,21}. **e**, Average single photon responses from **c** shown on a longer time scale. In each panel the flash was delivered at $t = 0$.

with several RGS proteins including RGS6, RGS7, RGS9 and RGS11 (refs 10, 13–15), which interact with Gβ5 through their G-protein γ-like (GGL) domains^{14–15}. The absence of Gβ5-L in RGS9^{-/-} retinas indicates that RGS9-1 may be required for the translation or the stability of Gβ5-L in photoreceptors.

In the absence of RGS9-1, hydrolysis of GTP was significantly slowed (Fig. 1d). In RGS9^{+/+} ROS, the rate constant of GTP hydrolysis (k_{inact}) by Gαt was $0.087 \pm 0.0021 \text{ s}^{-1}$ (mean \pm s.e.m.); in RGS9^{-/-} ROS it was $0.026 \pm 0.0021 \text{ s}^{-1}$. The k_{inact} in RGS9^{-/-} ROS was comparable to that for isolated Gαt, indicating that RGS9-1 is indeed essential for the acceleration of hydrolysis of GTP by Gαt. Although k_{inact} for both RGS9^{+/+} and RGS9^{-/-} ROS was considerably slower than the rate of recovery of the flash responses (see below), we attribute the slow *in vitro* rate to the more than 50-fold dilution of cellular contents in the assay.

RGS9-deficient rods provided a unique opportunity to investigate the contribution of the effector γ-subunit of cyclic GMP phosphodiesterase (PDEγ) to Gαt deactivation (Fig. 1d). When the diluted ROS used in our assays were supplemented with PDEγ, the rate constant of hydrolysis of GTP in RGS9^{+/+} ROS increased to $0.148 \pm 0.011 \text{ s}^{-1}$, consistent with previous reports that PDEγ can speed up hydrolysis of GTP by Gαt^{16,17}. In contrast, exogenous PDEγ had no effect on hydrolysis of GTP in RGS9^{-/-} ROS ($k_{\text{inact}} = 0.027 \pm 0.002 \text{ s}^{-1}$), indicating that PDEγ by itself has no GTPase accelerating activity. The effect of exogenous PDEγ in control ROS membranes therefore depends on RGS9-1, consistent with previous reports that the G-protein–effector complex is the preferred target for RGS proteins^{17,18}.

To investigate the function of RGS9-1 in phototransduction, we recorded from individual rod cells using suction electrodes¹⁹. Responses from RGS9^{+/+} (Fig. 2a) and RGS9^{-/-} (Fig. 2b) rods revealed a specific defect in the recovery phase of the RGS9^{-/-} responses over a wide range of flash strengths (Fig. 2b, d). Single exponential fits to the recovery phase of the dim flash response gave time constants of about 0.2 s (+/+) and 2.5 s (-/-), and the integration time of the dim flash response was prolonged sevenfold in the knockout rods (Table 1).

The deletion of RGS9 had no effect on the amplitude of flash responses, as indicated by the values of flash sensitivity, single photon response amplitude and half-saturating flash strength listed in Table 2. The rising phases of the average single photon responses were virtually identical in +/+, +/- and -/- rods (Fig. 2c), and the initial rate of change of the light-dependent PDE activity^{18,20,21} was indistinguishable in control and knockout rods over a wide range of flash strengths (Fig. 2d). This indicates that

interactions between rhodopsin and transducin, as well as transducin and PDE, proceeded normally in RGS9^{-/-} rods.

Although the rising phases of RGS9^{-/-} and RGS9^{+/+} responses coincided, RGS9^{-/-} dim flash responses continued to rise for a longer time, so that they reached peak amplitude slightly later, on average, than control responses (Table 1), consistent with a prolonged lifetime of activated Gαt. The rate of activation in RGS9^{-/-} responses slowed about 170 ms after the flash, so that the response amplitude was not significantly larger than that of controls (Table 2). This slowing may result from a decrease in the catalytic activity of rhodopsin, as rhodopsin kinase and arrestin begin to deactivate rhodopsin by this time^{20,22}.

The effect of the RGS9 deletion resembles that of dialysing GTPγS into truncated salamander rods²³ and is consistent with the sole deficit in RGS9^{-/-} rods being a prolongation of the lifetime of activated Gαt. The RGS9^{-/-} phenotype indicates that RGS9 is essential for the high rate of GTP hydrolysis in normal rods.

In RGS9^{-/-} rods, unlike control rods, the rate of recovery of the

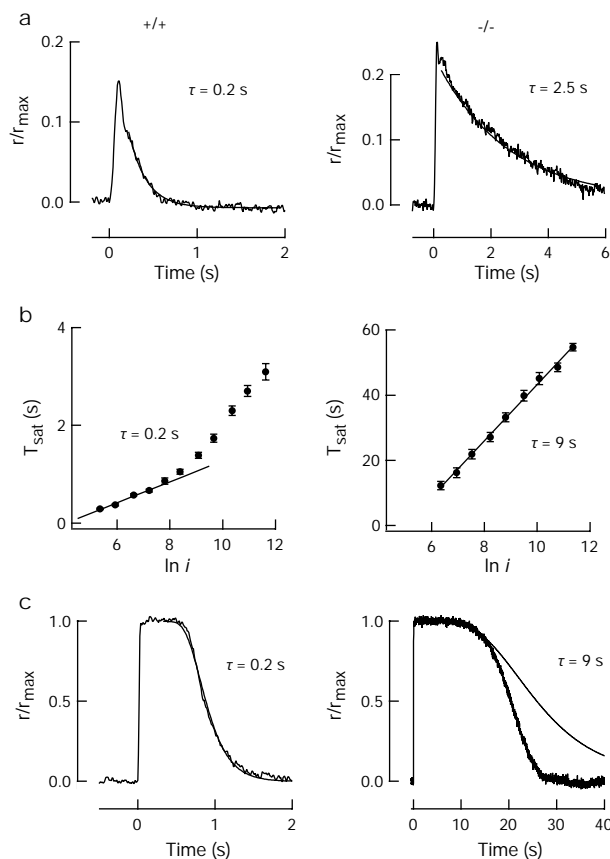


Figure 3 Dependence of recovery on flash strength. **a**, Representative mean dim flash responses from a control rod (left) and an RGS9^{-/-} rod (right). The falling phases were fitted by single exponential functions. **b**, Rate-limiting time constant of recovery from saturating flashes was greatly slowed in RGS9^{-/-} rods. In control rods, the rate-limiting time constant of recovery from saturating flashes was 0.2 s (left, collected results from 13 cells, 6–13 measurements used to determine each mean value). In RGS9^{-/-} rods (right), the recovery time constant was 8.6 s (collected results from 8 cells, 4–8 measurements used to determine each mean value). Error bars indicate s.e.m. **c**, In a control rod, the entire time course of recovery of the saturating response (left; same rod as in **a**) was fitted by a single exponential function with a 0.2-s time constant (thick line). However, in the saturating response of a RGS9^{-/-} rod (right; same rod as in **a**), a single exponential function with a 9-s time constant did not fit the entire recovery phase. Instead, the deactivation speeded as the response recovered. Flash strengths for the control responses were 9.55 (**a**) and 1,350 (**c**) photons μm^{-2} , those for RGS9^{-/-} responses were 13.6 (**a**) and 572 (**c**) photons μm^{-2} . Dark currents for the control and RGS9^{-/-} rods were 10.8 and 7.7 pA, respectively.

Table 1 Kinetic characteristics of control and RGS9^{-/-} flash responses

Strains	Integration time (ms)	Time to peak (ms)	Dim flash τ_{rec} (s)	Saturating flash τ_{rec} (s)
RGS9 ^{+/+}	267 \pm 26 (12)	104 \pm 3 (12)	0.18 \pm 0.01 (12)	0.27 \pm 0.02 (13)
RGS9 ^{+/-}	250 \pm 26 (29)	123 \pm 8 (30)	0.18 \pm 0.02 (29)	0.22 \pm 0.01 (24)
RGS9 ^{-/-}	1,881 \pm 129 (15)	283 \pm 39 (18)	2.60 \pm 0.32 (18)	8.99 \pm 0.22 (8)

All values are mean \pm s.e.m. The number of rods used to determine each parameter is given in parentheses. The dim flash τ_{rec} was measured by fitting a single exponential function to the falling phase of a dim flash response. Saturating flash τ_{rec} was determined as the slope of the linear relation between the time spent in saturation and the natural log of the flash strength^{24,25}.

Table 2 Sensitivity characteristics of control and RGS9^{-/-} flash responses

Strains	i_0^* (photons μm^{-2})	Single photon response amplitude (pA)	Flash sensitivity (pA per photons per μm^{-2})	$I_{1/2}^\dagger$ (pA)
RGS9 ^{+/+}	66.7 \pm 6.4 (12)	0.46 \pm 0.04 (12)	0.16 \pm 0.03 (12)	12.3 \pm 0.8 (13)
RGS9 ^{+/-}	77.0 \pm 3.5 (32)	0.47 \pm 0.05 (29)	0.11 \pm 0.02 (20)	10.5 \pm 0.6 (32)
RGS9 ^{-/-}	65.0 \pm 6.9 (18)	0.55 \pm 0.08 (16)	0.11 \pm 0.01 (16)	8.8 \pm 0.5 (23)

* Flash strength that elicited a half-maximal response.

† Inward current in darkness. $I_{1/2}$ was somewhat smaller in -/- rods than in +/+, which might be explained by a small reduction in the length of the outer segment. Such an effect has been observed in other rods with impairments in deactivation of the light response^{20,22,30}.

flash responses slowed progressively as the flash strength was raised. Dim flash responses of RGS9^{-/-} rods recovered exponentially with a 2.5-s time constant (Fig. 3a, right; Table 1). However, the time constant of the rate-limiting deactivation step in saturated responses, given by the slope of the relationship between the time that the response remained saturated (T_{sat}) and the log of the flash strength^{24,25}, was 9 s (Fig. 3b, right). Although saturating responses of RGS9^{-/-} rods began to decline with a time constant of 9 s, the deactivation speeded as the response recovered (Fig. 3c, right). In sharp contrast, both dim and moderately strong saturating responses of +/+ rods recovered exponentially with a time constant of 0.2 s (Fig. 3a, c, left; Table 1) and the time constant of the rate-limiting deactivation step in saturated responses was also 0.2 s (straight line in Fig. 3b, left, up to $\ln i = 8.5$). At flash strengths exceeding $\ln i = 8.5$ in control rods, the slope steepened to 0.7 s. This effect is attributable to slowed deactivation of the cascade when more than one photoisomerization occurs per disc face. Multiple photoisomerizations per disc face would cause the concentration of activated G α t to exceed that of PDE γ and thereby decrease the rate at which G α t deactivates²⁵.

A striking feature of the RGS9^{-/-} phenotype was the dependence of the recovery rate on flash strength at strengths below those that would cause multiple photoisomerizations per disc face. Indeed, in all 15 cells examined, the recovery rate appeared to vary continuously with the response amplitude, accelerating as the response fell (for example, Fig. 3c, right). In control rods at similar flash strengths recovery was faster and governed by a single time constant. By assuming that the rate of G α t deactivation varies linearly with the inward current in RGS9^{-/-} rods, we could simulate their responses over a wide range of flash strengths (Fig. 4, right), whereas the

responses of control rods were well fitted by assuming that transducin deactivated with a single time constant of 0.2 s (Fig. 4, left). Our interpretation is that RGS9^{-/-} rods may contain a relatively weak GTPase accelerating factor whose activity varies with membrane current because it depends on the concentration of calcium or cyclic GMP, both of which vary with the membrane current. The fact that the dim flash response in RGS9^{-/-} rods fell with a time constant 10-fold longer than that in control rods indicates that the residual accelerating factor might contribute little to the GTPase activity of normal rods.

We conclude that RGS9-1 is required for normal deactivation of transducin and response recovery in retinal rods. In the absence of RGS9-1, PDE γ has no GTPase accelerating activity. Thus, RGS9-1 is the essential GTPase accelerating factor in rods and the G α t-PDE γ complex is its preferred target. In addition, our results indicate that RGS9-1 is required for stable expression of G β 5-L. This is the first demonstration, to our knowledge, that an RGS protein is required for the proper function of a G-protein cascade in mammals. □

Methods

Transgenic mice

RGS9^{-/-} mice were generated by standard procedures²⁶. Transfected embryonic stem cell clones derived from 129SvJ mice were screened for homologous recombination by polymerase chain reaction (PCR) using a primer inside the MCI neopA cassette (neo-3, 5'-TCGCCGCTCCCGATTCCGAGCGCA-3') and a primer outside the 1.3-kb short arm (RGS9-ko1, 5'-GAGAAAAGGATCCAGGAACCTGTAG-3'). Positive clones were micro-injected into blastocysts derived from C57/B6 to generate chimaeric mice, which were then mated with either C57/B6 or 129SvJ to produce hemizygous knockouts (RGS9^{-/-}). RGS9^{-/-} mice were produced by inbreeding of RGS9^{+/-} mice. All experimental procedures complied with NIH guidelines as approved by the Institutional Animal Care and Use Committee of the California Institute of Technology.

Immunoblots

Proteins were detected by western blotting of 15 μ g total retinal proteins. The polyclonal antibodies used for detecting photoreceptor-specific proteins were: CT-215 (anti-G β 5, used at 1:2,500 dilution); CT-317 (anti-RGS9-1, 1:1,000); T α -1A (anti-G α t, 1:2,000); 8585 (anti-rhodopsin kinase, 1:3,000); 8698 (anti-recoverin, 1:5,000); α -GC-E (anti-guanylyl cyclase E, 1:10,000); and α -GC-F (anti-guanylyl cyclase F, 1:10,000).

Single-turnover GTPase assay

Retinas were isolated from dark-adapted mice under dim red light, and ROS were isolated from 15 retinas using standard discontinuous sucrose gradient centrifugation procedures. ROS were hypotonically shocked in 90 μ l H₂O for 10 s by vortexing and then resuspended in 100 μ l GAPN buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 2 mM MgCl₂, solid PMSF and 1 mM DTT). Single turnover G α t GTPase assays were carried out in triplicate as described²⁷ with or without exogenous PDE γ . Final concentrations were: Rhodopsin, 20 μ M; recombinant (His)₆-PDE γ (ref. 28), 0 or 1.33 μ M; AMP-PNP, 50 μ M; in GAPN buffer.

Electrophysiology

All mice used in physiological experiments were housed in 12-h cyclic light and dark-adapted overnight before we isolated the retinas and made recordings. Small pieces of retina were perfused with bicarbonate buffered Locke's solution (112.5 mM NaCl, 3.6 mM KCl, 2.4 mM MgCl₂, 1.2 mM CaCl₂, 10 mM HEPES (pH 7.4), 0.02 mM EDTA, 20 mM NaHCO₃, 3 mM Na₂-succinate, 0.5 mM Na-glutamate, 10 mM glucose and 0.1% vitamin and amino acids supplement solution (Sigma)) warmed to 34–37 °C and bubbled with 95% O₂/5% CO₂. We drew outer segments of single rods into a suction electrode that contained 140 mM NaCl, 3.6 mM KCl, 2.4 mM MgCl₂, 1.2 mM CaCl₂, 3 mM HEPES (pH 7.4), 0.02 mM EDTA and 10 mM glucose. Brief flashes (10 ms) of 500-nm light of calibrated intensity were used to stimulate the rods. Single-cell recording procedures were done as described¹⁸.

The time spent in saturation was taken as the interval between the midpoint of the flash and the point at which the response recovered to 10% of its initial value. We obtained the form and amplitude of the single photon response by comparing the squared mean and the time-dependent ensemble variance of a series of responses to dim flashes of fixed strength¹⁹. The mean linear response to at least 25 dim flashes was squared and scaled so that its rising phase coincided with that of the time-dependent variance. The scaling factor for the squared mean response is $1/n$ where n is the mean number of photoisomerizations per trial¹⁹. The mean response, divided by n , gave the estimated form and amplitude of the single photon response.

The time course of the light-activated PDE activity, PDE^{*}(t), was calculated as described^{18,20,21}. The initial rate of change of PDE activation, $d[\text{PDE}^*(t)]/dt$, was determined by measuring the slope of a straight line fitted to PDE^{*}(t) between the time of the flash and the time at which the PDE activity reached its maximum value, a time window which varied from 100 ms for dim flashes to 30 ms for saturating flashes.

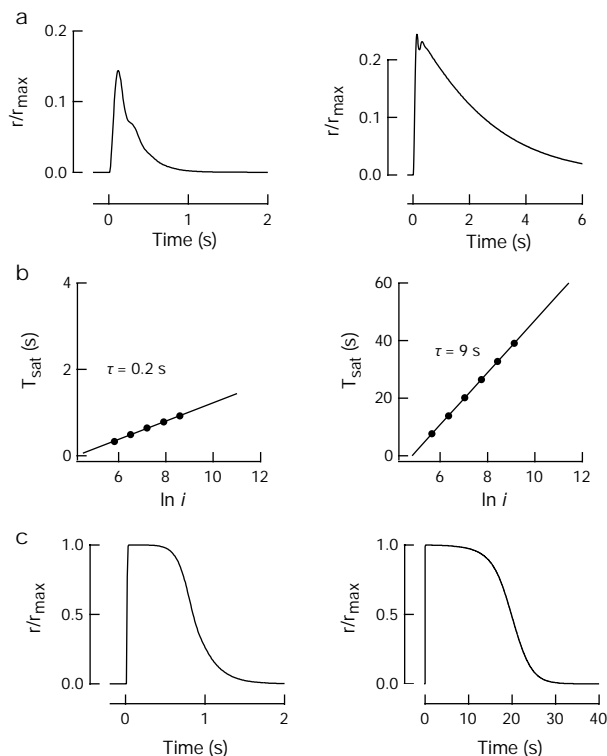


Figure 4 Simulated flash responses of rods. Left, control; right, RGS9^{-/-} rod. **a**, Dim and **c**, saturating flashes. For +/+ responses, G α t was assumed to deactivate with a single time constant of 0.2 s. For RGS9^{-/-} responses, G α t was assumed to deactivate with a time constant that increased linearly with the inward current. In all other respects, control and RGS9^{-/-} rods were assumed to be identical (see Methods). **b**, Dependence of the time in saturation (T_{sat}) on flash strength calculated for control and RGS9^{-/-} simulated responses.

Mathematical simulations

An Igor-based modelling program (written by F. Rieke, University of Washington) was adapted to compute the theoretical flash responses for control and RGS9^{-/-} responses. In this simulation, diffusion was ignored and the interior of the outer segment was assumed to be well-stirred. Theoretical flash responses were calculated using equations 4, 5, 6 and 7 in ref. 29. Rhodopsin activity was assumed to undergo first-order decay, and channel activation was assumed to vary with the cube of the cGMP concentration. For control responses, the time constant of PDE (or, equivalently G α t) deactivation was 0.2 s, whereas in RGS9^{-/-} rods the time constant was assumed to vary linearly with the inward current, being 9 s when the current was zero and 2.5 s when the current was at its dark level. All other parameters used to fit +/+ and RGS9^{-/-} were identical and were: rhodopsin lifetime: 0.09 s; basal PDE activity: 9 s⁻¹; dark [Ca²⁺]: 400 nM; minimum [Ca²⁺] in saturating light: 50 nM; Hill coefficient of guanylate cyclase for Ca²⁺: 2.3; half-maximal activation of guanylate cyclase by Ca²⁺: 400 nM; Hill coefficient of channels for cGMP: 3; time constant of Na⁺/Ca²⁺, K⁺ exchange: 40 ms. To simulate the responses of the cells in Fig. 3, the dark currents were taken as 10.8 pA (+/+) and 7.7 pA (RGS9^{-/-}).

Received 5 October; accepted 24 November 1999.

- Arshavsky, V. Y. & Pugh, E. N. Lifetime regulation of G protein-effector complex: emerging importance of RGS proteins. *Neuron* **20**, 11–14 (1998).
- Berman, D. M. & Gilman, A. G. Mammalian RGS proteins: barbarians at the gate. *J. Biol. Chem.* **273**, 1269–1272 (1998).
- Wieland, T. & Chen, C.-K. Regulator of G-protein signaling: a novel protein family involved in timely deactivation and desensitization of signaling via heterotrimeric G-proteins. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **360**, 14–20 (1999).
- De Vries, L. & Farquhar, M. G. RGS proteins: more than just GAPs for heterotrimeric G proteins. *Trends Cell Biol.* **9**, 138–144 (1999).
- Nekrasova, E. R. et al. Activation of transducin guanosine triphosphatase by two proteins of the RGS family. *Biochemistry* **36**, 7638–7643 (1997).
- Chen, C.-K., Wieland, T. & Simon, M. I. RGS-r, a retinal specific RGS protein, binds an intermediate conformation of transducin and enhances recycling. *Proc. Natl Acad. Sci. USA* **93**, 12885–12889 (1996).
- Faurobert, E. & Hurley, J. B. The core domain of a new retina specific RGS protein stimulates the GTPase activity of transducin in vitro. *Proc. Natl Acad. Sci. USA* **94**, 2945–2950 (1997).
- He, W., Cowan, C. W. & Wensel, T. G. RGS9, a GTPase accelerator for phototransduction. *Neuron* **20**, 95–102 (1998).
- Cowan, C. W., Fariss, R. N., Sokal, I., Palczewski, K. & Wensel, T. G. High expression levels in cones of RGS9, the predominant GTPase accelerating protein of rods. *Proc. Natl Acad. Sci. USA* **95**, 5351–5356 (1998).
- Makino, E. R., Handy, J. W., Li, T. & Arshavsky, V. Y. The GTPase activating factor for transducin in rod photoreceptors is the complex between RGS9 and type 5 G protein β subunit. *Proc. Natl Acad. Sci. USA* **96**, 1947–1952 (1999).
- Watson, A. J., Katz, A. & Simon, M. I. A fifth member of the mammalian G-protein β -subunit family. Expression in brain and activation of the β 2 isotype of phospholipase C. *J. Biol. Chem.* **269**, 22150–22156 (1994).
- Watson, A. J., Aragay, A. M., Slepak, V. Z. & Simon, M. I. A novel form of the G protein β subunit G β 5 is specifically expressed in the vertebrate retina. *J. Biol. Chem.* **271**, 28154–28160 (1996).
- Cabrera, J. L., de Freitas, F., Satpaev, D. K. & Slepak, V. Z. Identification of the G β 5-RGS7 protein complex in the retina. *Biochem. Biophys. Res. Commun.* **249**, 898–902 (1998).
- Snow, B. E. et al. A G protein γ subunit-like domain shared between RGS11 and other RGS proteins specifies binding to G β 5 subunits. *Proc. Natl Acad. Sci. USA* **95**, 13307–13312 (1998).
- Levay, K., Cabrera, J. L., Satpaev, D. K. & Slepak, V. Z. G β 5 prevents the RGS7-G α o interaction through binding to a distinct G γ -like domain found in RGS7 and other RGS proteins. *Proc. Natl Acad. Sci. USA* **96**, 2503–2507 (1999).
- Arshavsky, V. Y. & Bownds, M. D. Regulation of deactivation of photoreceptor G protein by its target enzyme and cGMP. *Nature* **357**, 416–417 (1992).
- Pages, F., Deterre, P. & Pfister, C. Enhanced GTPase activity of transducing when bound to cGMP phosphodiesterase in bovine retinal rods. *J. Biol. Chem.* **267**, 22018–22021 (1992).
- Tsang, S. H. et al. Role for the target enzyme is deactivation of photoreceptor G protein in vivo. *Science* **282**, 117–121 (1998).
- Baylor, D. A., Lamb, T. D. & Yau, K. W. Responses of retinal rods to single photons. *J. Physiol. (Lond.)* **288**, 613–634 (1979).
- Chen, C.-K. et al. Abnormal photoresponses and light-induced apoptosis in rods lacking rhodopsin kinase. *Proc. Natl Acad. Sci. USA* **96**, 3718–3722 (1999).
- Lamb, T. D. & Pugh, E. N. A quantitative account of the activation steps involved in phototransduction in amphibian photoreceptors. *J. Physiol.* **449**, 719–758 (1992).
- Xu, J. et al. Prolonged photoresponses in transgenic mouse rods lacking arrestin. *Nature* **389**, 505–509 (1997).
- Sagoo, M. S. & Lagnado, L. G-protein deactivation is rate-limiting for shut-off of the phototransduction cascade. *Nature* **389**, 392–395 (1997).
- Pepperberg, D. R. et al. Light-dependent delay in the falling phase of the retinal rod photoresponse. *Vis. Neurosci.* **8**, 9–18 (1992).
- Lyubarsky, A. L. & Pugh, E. N. Recovery phase of the murine rod photoresponse reconstructed from electroretinographic recordings. *J. Neurosci.* **16**, 563–571 (1996).
- Ramirez-Solis, R., Davis, A. C. & Bradley, A. in *Methods in Enzymology: Guide to Techniques in Mouse Development* (eds Wassarman, P. M. & DePamphilis, M. L.) 855–878 (Academic, San Diego, 1993).
- Cowan, C. W., Wensel, T. G. & Arshavsky, V. Y. in *Methods in Enzymology: Vertebrate Phototransduction and the Visual Cycle* (ed. Palczewski, K.) 524–538 (Academic, San Diego, 2000).
- Wieland, T., Chen, C.-K. & Simon, M. I. The retinal specific protein RGS-r competes with the γ subunit of cGMP phosphodiesterase for the α subunit of transducin and facilitates signal termination. *J. Biol. Chem.* **272**, 8853–8856 (1997).
- Rieke, F. & Baylor, D. A. Single-photon detection by rod cells of the retina. *Rev. Mod. Phys.* **70**, 1027–1036 (1998).

30. Makino, C. L., Flannery, J. G., Chen, J. & Dodd, R. L. in *Photostasis and Related Phenomenon* (eds Williams, T. P. & Thistle, A. B.) 129–151 (Plenum, New York, 1998).

Acknowledgements

We thank members of the Caltech Transgenic Core Facility for their technical support, and R. Lefkowitz, J. Hurley and H. Jurgen-Fuller for antibodies. This work was supported by grants from the NIH.

Correspondence and requests for materials should be addressed to M.I.S. (e-mail: simonm@starbase1.caltech.edu).

Food and metabolic signalling defects in a *Caenorhabditis elegans* serotonin-synthesis mutant

Ji Ying Sze^{*†}, Martin Victor[‡], Curtis Loer[§], Yang Shi[‡] & Gary Ruvkun^{*}

^{*} Department of Molecular Biology, Massachusetts General Hospital, Department of Genetics, Harvard Medical School, Boston, Massachusetts 02114, USA

[‡] Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115, USA

[§] Department of Biology, University of San Diego, San Diego, California 92110, USA

The functions of serotonin have been assigned through serotonin-receptor-specific drugs and mutants^{1,2}; however, because a constellation of receptors remains when a single receptor subtype is inhibited, the coordinate responses to modulation of serotonin levels may be missed. Here we report the analysis of behavioural and neuroendocrine defects caused by a complete lack of serotonin signalling. Analysis of the *C. elegans* genome sequence showed that there is a single tryptophan hydroxylase gene (*tph-1*)—the key enzyme for serotonin biosynthesis. Animals bearing a *tph-1* deletion mutation do not synthesize serotonin but are fully viable. The *tph-1* mutant shows abnormalities in behaviour and metabolism that are normally coupled with the sensation and ingestion of food: rates of feeding and egg laying are decreased; large amounts of fat are stored; reproductive lifespan is increased; and some animals arrest at the metabolically inactive dauer stage. This metabolic dysregulation is, in part, due to downregulation of transforming growth factor- β and insulin-like neuroendocrine signals. The action of the *C. elegans* serotonergic system in metabolic control is similar to mammalian serotonergic input to metabolism and obesity².

Serotonin is synthesized from tryptophan by an enzymatic pathway: tryptophan hydroxylase (TPH) catalyses the rate-limiting first step, and the dual functional 5-hydroxytryptophan (5HTP)/L-dopa decarboxylase then matures 5HTP to serotonin (Fig. 1a)¹. There is one probable orthologue of mammalian tryptophan hydroxylase in the *C. elegans* genome, ZK1290.2, which we call *tph-1* (Fig. 1b). Two other *C. elegans* aromatic amino-acid hydroxylase (AAAH) gene family members are B0432.5, a probable tyrosine hydroxylase that catalyses dopamine synthesis and is expressed in dopaminergic neurons (R. Lints and S. Emmons, personal communication), and K08F8.4, a probable phenylalanine hydroxylase that is not expressed in neurons³. *tph-1* shares higher amino-acid identity in the 330-amino-acid catalytic domain with mammalian tryptophan hydroxylase (61%) than with mammalian phenylalanine (53%) or

[†] Current address: Department of Anatomy and Neurobiology, College of Medicine, University of California, Irvine, California 92697-1280, USA.