pretation of the pseudo-twofold symmetry to be different. With reference to Fig. 2 of this report and figure 3 in the article of Ikura *et al.* (*27*), helices II and VI are almost antiparallel in the crystal structure and at an angle in the NMR structure where the peptide Trp residue is also in a different conformation. The NMR structure describes a disordered region comprising residues 74 to 82. On the other hand, the crystal structure shows an extended peptide segment from residues 73 to 77, with well-defined helices preceeding (IV) and following (V) the segment. Thus we have obtained the ϕ and ψ torsion angles of the segment and have some understanding of the geometrical transformation in going to the CaM bound form from the unbound form. The origin of the differences between the NMR and x-ray structures is under investigation by both laboratories.

27. M. Ikura *et al.*, *Science* **256**, 632 (1992).
28. W. Steigemann, thesis, Technische Universität, München (1974).
29. J. S. Sack, *J. Mol. Graphics* **6**, 244 (1988).
30. M. Carson, *ibid.* **5**, 103 (1987).
31. We thank B. E. Kemp for the peptide sample and our colleagues in both laboratories for helpful discussions and assistance. Supported by the Howard Hughes Medical Institute and W. M. Keck Foundation (F.A.Q.), an NIH grant (A.R.M.), and a Cardiovascular Discovery Award from Glaxo (A.R.M. and F.A.Q.).

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Deactivation of Visual Transduction Without Guanosine Triphosphate Hydrolysis by G Protein

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G proteins couple receptors to their target enzymes in many signal transduction cascades. It has generally been thought that deactivation of such cascades cannot occur without the hydrolysis of guanosine triphosphate (GTP) by G protein. This requirement has now been reexamined in both vertebrate and invertebrate phototransduction. Results indicate that GTP hydrolysis is not required for deactivation. Evidence is presented for an alternative model in which the target enzyme is deactivated by an inhibitory factor that is available even when GTP hydrolysis is blocked.

G protein–coupled receptors initiate signal transduction by catalyzing the exchange of GTP for guanosine diphosphate (GDP) on G proteins (*1*). The G protein with GTP bound to it can then activate its target enzyme. All steps in the cascade must deactivate when the signal is removed. According to what has become the standard model for G protein–mediated transduction, deactivation of the target enzyme depends directly on the hydrolysis of the bound GTP by the intrinsic GTPase of the G protein.

We questioned the standard model because it seems unable to account for the effect of nonhydrolyzable GTP analogs such as guanosine-5′-*O*-3-thiotriphosphate (GTPγS) (*2*) on G protein–mediated phototransduction in *Limulus*. If target enzyme deactivation cannot occur without GTP hydrolysis, each G protein that binds a nonhydrolyzable analog should generate a maintained increase in target enzyme activity and a maintained increase in the output of the transduction cascade. Thus, the G proteins that bind analogs during a stimulus should generate a maintained aftereffect, an output that persists even after the stimulus is removed (the response would normally return to base line). An aftereffect has been observed in *Limulus* photoreceptors (*3*, *4*) but surprisingly appears to be pulsatile waves (Fig. 1A, middle trace) rather than a maintained output. These spontaneous waves initiated at the G protein level are similar kinetically to the single photon response (*4*), the elementary unit of the light response, but have a smaller average size. We found no evidence of a concurrent maintained output because the average shift in resting potential was a negligible 0.46 ± 0.47 mV (SEM; $n = 4$). This complete return to base line after each wave indicates that the aftereffect is entirely pulsatile.

When further binding of GTPγS was induced by additional light, the pulsatile events became so frequent that they superposed to form a maintained, noisy aftereffect (Fig. 1A, bottom trace). The detection of the pulsatile nature of the aftereffect in *Limulus* photoreceptors is possible because the high transduction gain makes it possible to detect the events produced by a single G protein (*4*). In systems with less gain, only the larger maintained aftereffect resulting from the superposition of waves might be detected. Difficulty in explaining the pulsatile nature of the aftereffect in terms of the standard model led us to devise more rigorous tests of this theory.

According to the standard model, blocking hydrolysis should prevent any deactivation of the response at the offset of a long-duration light (*5*). Testing this prediction in living cells by injecting them with GTPγS is complicated by the fact that some deactivation will occur when G proteins bind endogenous, hydrolyzable GTP instead of the injected GTPγS. To estimate the relative binding of GTP and of the

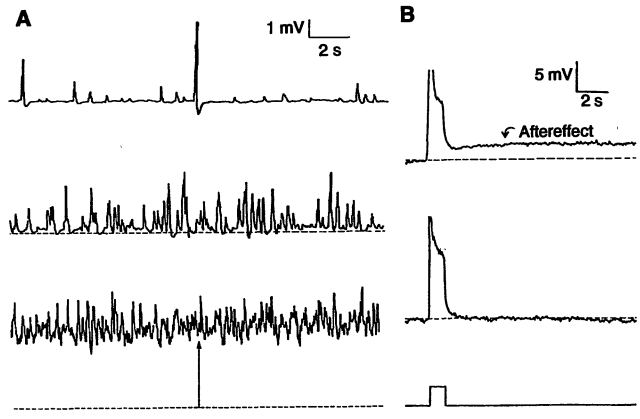

Fig. 1. Deactivation in *Limulus* ventral photoreceptors injected with GTPγS. (**A**) Pulsatile aftereffect of light in low intracellular concentration of GTPγS (≈20 μM). Top trace: before injection, low rate of spontaneous waves typical of these photoreceptors due to spontaneous activation of early stages of transduction (*23*). GTPγS was then pressure-injected, and light [50 flashes, 30-ms duration at 4×10^5 light-activated rhodopsin isomerizations (R*)/s] induced binding of GTPγS to G protein. The cell was then allowed to dark adapt. Middle trace: aftereffect of first light. The rate of waves in the dark was elevated but the base line was negligibly different from preinjection level (dashed line). Bottom trace: aftereffect of second identical light. Waves became so frequent that they superposed to form a maintained, noisy depolarization from the base line (arrow). Electrode contained 0.5 mM GTPγS, 10 mM Hepes, and 300 mM potassium aspartate, pH ≈ 7.0. (**B**) Deactivation of light response in high concentration of GTPγS. Top trace: receptor potential following injection of the GTPγS to 2.3 mM. At the termination of the long duration light (1 s, 5×10^5 R*/s) the voltage rapidly deactivated 80% from the plateau response, leaving only a small maintained aftereffect similar to that shown in Fig. 1A. For controls see (*24*). Middle trace: receptor potential following injection of GTP to 2.2 mM in a different cell. No aftereffect was elicited by light (1 s, 1×10^4 R*/s). Bottom trace: light stimulus. Electrode solutions: 100 mM GTPγS (or GTP, bottom trace), 4.5 mM Fast Green FCP, 300 mM potassium aspartate, 100 mM Hepes, and 5 mM KCl.

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analog, we determined the concentration of injected analog by coinjecting a dye and measuring the change in cellular absorbance (6). For an intracellular concentration of 2.3 mM GTPγS (Fig. 1B, top trace) we calculate as a worst case that no more than 25% of the light-induced nucleotide exchange will result in the binding of GTP rather than GTPγS (7). Although the standard model predicts that only this fraction of the light response should deactivate, we observed greater than 80% deactivation at the offset of the light (n = 6). These results suggest that there is a large component of deactivation that does not require hydrolysis.

To investigate the role of GTP hydrolysis further we turned to the vertebrate rod photoreceptor in which G protein activates the target enzyme, guanosine-3′5′-monophosphate phosphodiesterase (cGMP PDE) (8). An important technical advantage of this system is that the activity of the target enzyme can be measured in real time in vitro (9), which allows nucleotide concentrations to be manipulated and verified. The light-stimulated PDE of homogenates of *Bufo marinus* rod outer segments (ROSs) was measured (10) with a standard pH assay. Under conditions where GTPγS was the only added guanine trinucleotide (Fig. 2A), responses to light deactivated by over 66% (average 54 ± 2% SEM; n = 29). Similar deactivation in GTPγS has recently been observed in bovine rod preparations (11). To determine whether this deactivation was due to GTP (endogenous, contaminant, or generated by transphosphorylation) we analyzed the nucleotide content of ROS homogenates using high-pressure liquid chromatography (HPLC) analysis in parallel with PDE assays in two experiments (12). GTP was present at a concentration <3% of GTPγS at the time corresponding to the light flash in preparations that exhibited deactivation of >55%. The observed deactivation cannot be attributed to G proteins that bound GTP. Furthermore, control experiments (13) indicated that deactivation could not be attributed to the added adenosine triphosphate (ATP), to a decrease in cGMP concentration, to end-product inhibition of PDE by GMP (14), to calcium-dependent modulation of PDE (15), to nonlinearity of the assay, or to inhomogeneity of the membrane preparation. Thus in vertebrate rods, as in *Limulus* photoreceptors, deactivation of transduction can occur without GTP hydrolysis.

Rod photoreceptor PDE is held inactive by inhibitory γ subunits and becomes activated when G protein binds γ (16). According to the standard model, G protein must hydrolyze GTP before deactivation can occur (9, 17), and more specifically hydrolysis is required for γ to be released and again inhibit PDE. Hydrolysis-independent deactivation cannot be accounted for by this model but can be explained by a simple modification in which the enzyme is deactivated by an inhibitory factor (Fig. 3) that is available even when GTP hydrolysis is blocked. To test for such a factor we added trypsin-activated PDE (tPDE*) to extracts of ROS homogenates [trypsin selectively degrades γ (8, 18)]. As shown in Fig. 2B, the tPDE* activity completely deactivated. This deactivation was not seen when tPDE* was added to buffer (Fig. 2C) or to extracts of muscle. These results indicate that rods contain an inhibitory factor

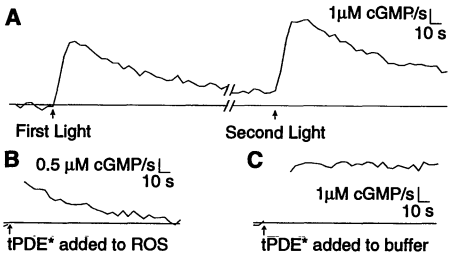

Fig. 2. Deactivation of light-stimulated activity of vertebrate rod PDE in GTPγS and of added trypsin-activated PDE as evidence for endogenous inhibitory factor. (**A**) Substantial deactivation of PDE after light flashes in the presence of GTPγS. Flash of dim light (0.0002% R* was activated) resulted in light-stimulated activity that deactivated 67% after the first light and 66% after the second light. Initial dark rate (horizontal line), 1.0 μM cGMP/s. Rhodopsin concentration, 5 μM. Added to ROS homogenate: 0.1 mM ATP, 1.25 mM GTPγS, 10 mM cGMP. (**B**) Trypsin-activated PDE (tPDE*), where the asterisk indicates an active state, completely deactivated when added to dark-adapted ROS homogenates to 1.15 nM (n = 3). Addition of solutions to the vials caused motion-induced artifacts that have been omitted. Trace shown is an average of two experiments from the same preparation. Dark rate before addition of tPDE*, 1.1 μM cGMP/s. Rhodopsin concentration, 14 μM. The only added nucleotide was 10 mM cGMP. (**C**) Addition of tPDE* to buffer solution without ROS to 1.15 nM resulted in steady activity. The higher level of maximal activity in (C) than in (B) indicates a rapid, unresolved deactivation upon addition of the tPDE* to ROS homogenates. The only added nucleotide was 10 mM cGMP.

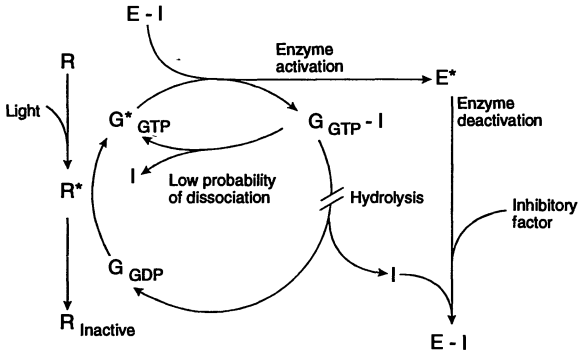

Fig. 3. Model for deactivation of target enzyme based on vertebrate rod transduction. Light-stimulated rhodopsin (R*) activates G protein (G) by catalyzing exchange of the bound guanine nucleotide. The target enzyme (E) is initially inactive because of the bound inhibitory subunit (I) but becomes active when G protein binds I. Enzyme deactivation is not directly linked to GTP hydrolysis because the inhibitory factor can turn off E* in the absence of GTP hydrolysis (indicated by the break). The inhibitory factor may be the same as the inhibitor, but other possibilities exist (19). Without the inhibitory factor, enzyme deactivation depends directly on GTP hydrolysis to make the inhibitor available, which is the standard model. For simplicity, target enzyme regulation is depicted by one inhibitor rather than two (22), but this does not change our argument.

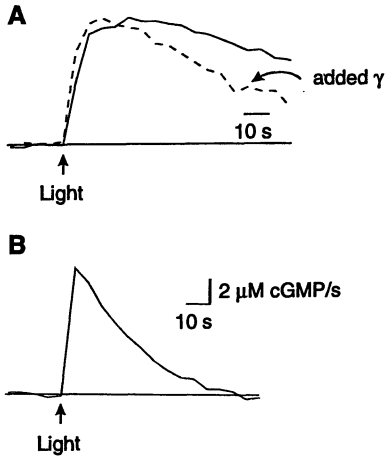

Fig. 4. Acceleration of deactivation by addition of exogenous inhibitory subunit γ, a fusion protein expressed in *Escherichia coli* (25). (**A**) With no added γ, PDE deactivated in GTPγS (solid line) after a light flash (0.0013% R* was activated) to 44% by the end of the trace, with a final deactivation of 82%. With 50 nM exogenous γ, PDE deactivated more rapidly (dashed line) to 76% by the end of the trace, and the final deactivation was complete. Concentration of exogenous γ is comparable to 0.5 × [PDE] in the ROS homogenate (26). Traces were normalized to facilitate comparison. Control: dark rate, 1.6 μM cGMP/s, and maximal light-activated rate, 7.5 μM cGMP/s. With exogenous γ: dark rate, 1.2 μM, cGMP/s, and maximal light-activated rate, 4.6 μM cGMP/s. (**B**) PDE deactivation following a light flash (0.013% R* was activated) in the presence of GTP. Dark rate, 1.6 μM cGMP/s. All data (A and B) were from the same ROS preparation. Rhodopsin concentration, 9.3 μM. Added to ROS homogenates: 10 mM cGMP, 50 mM hydroxylamine, and 1.25 mM GTPγS in (A) or 1.25 mM GTP in (B).

capable of deactivating tPDE*.

A further prediction of the model in Fig. 3 is that enhancement of the concentration of the inhibitory factor should speed deactivation. Although the identity of the endogenous inhibitory factor is not known (*19*), one of the possibilities is γ itself. Therefore, we supplemented ROS homogenates with exogenous γ and found that it enhanced the rate and extent of deactivation (Fig. 4A) (n = 16). The response in GTP without added γ is included for reference (Fig. 4B). The faster recovery time in GTP compared to that in GTPγS is consistent with the model in Fig. 3 because there are two pathways available for deactivation when hydrolysis is possible but only one when hydrolysis is blocked. The relative contribution of these reactions to deactivation in vivo cannot be inferred from in vitro measurements because of the unavoidable dilution of inhibitory factors relative to their concentrations in the intact cell.

The published physiological experiments on vertebrate rod photoreceptors are consistent with our proposal for hydrolysis-independent deactivation. After introduction of nonhydrolyzable GTP analogs into intact vertebrate rods, the response to nonsaturating lights turned off relatively normally (*20*). These results are similar to those in Fig. 2A but cannot be taken as definitive evidence for hydrolysis-independent deactivation because the amount of deactivation due to the binding of endogenous GTP was not determined. More direct evidence for the presence of inhibitory factors comes from experiments showing that the injection of activated PDE into intact rods produced a response that then completely deactivated (*21*).

The model in Fig. 3 suggests an explanation for why an aftereffect occurs when hydrolysis is blocked. G protein undergoes functional deactivation when it forms the G-I complex because it cannot activate additional enzyme molecules. However, this deactivation is reversible because there is some probability that the inhibitor will dissociate (*22*), leaving the G protein (with GTPγS bound) free to activate another enzyme. If the concentration of this complex becomes high enough to result in frequent dissociations and activation of target enzyme molecules, an aftereffect will be generated. As shown in Fig. 1A, the aftereffect in *Limulus* is pulsatile. If the *Limulus* enzyme is regulated by an inhibitory subunit as in Fig. 3, pulses will occur because each enzyme that becomes active will be turned off by the inhibitory factor with kinetics like those of single photon responses. According to this view, GTP hydrolysis provides a second and irreversible step in G protein deactivation, preventing the accumulation of the G-I complex.

Deactivation of G protein–mediated transduction can occur by a pathway that is independent of GTP hydrolysis. The role of hydrolysis-independent deactivation in other transducing systems remains to be determined. Control of the inhibitory factors involved in hydrolysis-independent deactivation could affect the duration of enzyme activation, thereby providing a mechanism for controlling the amplification of G protein–mediated transduction.

REFERENCES AND NOTES

1. L. Stryer and H. R. Bourne, *Annu. Rev. Cell Biol.* **2**, 391 (1986); A. G. Gilman, *Annu. Rev. Biochem.* **56**, 615 (1987).
2. G. Yamanaka, F. Eckstein, L. Stryer, *Biochemistry* **24**, 8094 (1985).
3. S. R. Bolsover and J. E. Brown, *J. Physiol. (London)* **332**, 325 (1982).
4. A. Kirkwood, D. Weiner, J. E. Lisman, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3872 (1989).
5. Long-duration light was used so that the response would have reached steady state before stimulus offset. The return toward base line at offset therefore cannot be attributed to a compensatory enzyme that responds to changes produced by the target enzyme activity.
6. Intracellular concentration of the coinjected dye, Fast Green FCP, was measured by the decrease in light (610 nm) transmitted through the cell as measured by an EG & G (Wellesley, MA) Model 1192 photomultiplier.
7. The fraction of the total light-induced nucleotide exchange leading to the binding of GTPγS is given by a simple formula, provided that the nucleotide concentration is high relative to the binding constants:

$$1/(1 + k\,[\mathrm{GTP}]/[\mathrm{GTP}\gamma\mathrm{S}])$$

where k is the ratio of the apparent Michaelis constant, K_m, for GTPγS to the apparent K_m for GTP. For squid G protein, k is 0.1 (P. Robinson, unpublished data), but we make the worst case assumption that the value is 0.24, the highest measured for any G protein [G. Yamanaka, F. Eckstein, L. Stryer, *Biochemistry* **25**, 6149 (1986)]. The concentration of GTP in squid retina is 0.5 mM [M. Fathi, M. Tsacopoulos, V. Raverdino, M. Porthault, *J. Chromatog.* **563**, 356 (1991)], but as an upper limit we assume 2 mM, the highest concentration measured in any cells [M. S. Biernbaum and M. D. Bownds, *J. Gen. Physiol.* **85**, 83 (1985)]. The GTPγS solution (Boehringer Mannheim, Indianapolis, IN) was 66% GTPγS and 33% GDP as analyzed with HPLC. We make the worst case assumption that all the contaminating GDP injected was converted to GTP by transphosphorylation.
8. L. Stryer, J. B. Hurley, B. K. Fung, *Trends Biochem. Sci.* **6**, 245 (1981); J. B. Hurley and L. Stryer, *J. Biol. Chem.* **257**, 8188 (1982).
9. R. Yee and P. A. Liebman, *J. Biol. Chem.* **253**, 8902 (1978).
10. We obtained ROSs by shaking dark-adapted retinas of *Rana catesbiana* or *Bufo marinus* in a buffer solution (65 mM KCl, 35 mM NaCl, 2 mM $MgCl_2$, 10 mM Hepes, 1 mM dithiothreitol, 0.1 mM phenylmethane sulfonyl fluoride, aprotinin 500 KIU/ml; pH 7.8) and then shearing them. We assayed the PDE activity by monitoring the protons (produced when cGMP was hydrolyzed) with a pH microelectrode [MI410 combination or MI405 with an MI402 reference electrode (Microelectrodes Inc., Londonderry, NH)] in a pH range of 7.75 to 7.60. PDE activity was calculated as the rate of change of pH. Nucleotides were added to the following concentrations unless indicated otherwise: 0.1 mM ATP, 1.25 mM GTP or 1.25 mM GTPγS, and 10 mM cGMP. In some preparations 0.1 mM GDP was added to keep the dark rate low. Preparation and manipulation of ROS were performed under infrared illumination.
11. J. Hurley, unpublished data.
12. To measure maximal GTP levels, we used ROS preparations with 0.1 mM added GDP. These preparations were not exposed to light. Nucleotides were added at the times and concentrations used in the pH assay, although cGMP was omitted to avoid overloading the column (analyzed cGMP had no contaminating GDP or GTP). Portions of the ROS suspension were withdrawn and immediately quenched with equal volumes of 2% perchloric acid and then neutralized and centrifuged for 10 min at 4°C. We used a Hewlett-Packard 1090 with a reversed-phase ion-exchange 3-μm bead, pecosphere 3CR C_{18} column (Perkin-Elmer, Norwalk, CT), monitoring absorbance at 254 nm and using a flow rate of 0.5 ml/min. The mobile phase was buffer A [100 mM KH_2PO_4 and 5.0 mM TBAHS (tetrabutylammonium hydrogen sulfate) at pH 6.5] and buffer B (100 mM KH_2PO_4, 5.0 mM TBAHS, and 4% methanol at pH 6.5). The elution procedure first used 100% buffer A, then 80% buffer A at 8 min, 40% buffer A at 18 min, and 100% buffer B at 25 min for the rest of the run.
13. Deactivation in GTPγS was not due to the cGMP concentration dropping below a level of saturation for PDE because the restoration of cGMP to its starting concentration after a light flash did not increase PDE activity. We evaluated end-product inhibition of PDE by adding GMP to ROS homogenates before a light flash in the presence of GTPγS. The light-stimulated PDE activity was only inhibited 18.8 ± 3.0% (SEM; n = 3) by addition of 2 mM GMP, a concentration several times higher than the amount of GMP produced by the time deactivation occurred. Deactivation of PDE was comparable to that measured in ATP when hydroxylamine (10 to 50 mM) [K. P. Hofmann, D. Emeis, P. P. M. Schnetkamp, *Biochim. Biophys. Acta* **725**, 60 (1983)] was substituted for ATP [P. A. Liebman and E. N. Pugh, Jr., *Nature* **287**, 734 (1980); A. Sitaramayya and P. A. Liebman, *J. Biol. Chem.* **258**, 1205 (1983)] to deactivate light-stimulated rhodopsin (R*) (Fig. 4A). The extent and the time course of deactivation were unaffected by inclusion of 1 mM dibromo BAPTA, a Ca^{2+} buffer (n = 3). The linearity of the pH assay was ≈90% across the pH range used, as verified by using tPDE* (Fig. 2C). Sonication did not alter the time course of deactivation.
14. N. Bennett and A. Clerc, *Biochemistry* **28**, 7418 (1989).
15. P. Robinson, S. Kawamura, B. Abramson, M. Bounds, *J. Gen. Physiol.* **76**, 631 (1980); S. Kawamura and M. D. Bownds, *ibid.* **77**, 571 (1981); A. E. Barkdoll III, E. N. Pugh, Jr., A. Sitaramayya, *ibid.* **93**, 1091 (1989); S. Kawamura and M. Murakami, *Nature* **349**, 420 (1991).
16. A. Yamazaki, P. J. Stein, N. Chernoff, M. W. Bitensky, *J. Biol. Chem.* **258**, 8188 (1983); P. Deterre *et al.*, *Proteins Struct. Funct. Genet.* **1**, 188 (1986); T. G. Wensel and L. Stryer, *ibid.*, p. 90; A. Sitaramayya, J. Harkness, J. H. Parkes, C. Gonzalez-Oliva, P. A. Liebman, *Biochemistry* **25**, 651 (1986).
17. G. L. Wheeler and M. W. Bitensky, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4238 (1977).
18. N. Miki, J. M. Baraban, J. J. Keirns, J. J. Boyce, M. W. Bitensky, *J. Biol. Chem.* **250**, 6320 (1975).
19. If γ is the inhibitory factor that we observed, it may exist either free or bound. One bound source could be PDE itself. If one γ is sufficient to inactivate PDE significantly (*14*) then PDE with two γ's may donate one to inactivate light-activated PDE without itself becoming significantly active (*22*). Alternatively, PDE might be inactivated by inhibitory subunits from a soluble form of PDE [P. G. Gillespie, R. K. Prusti, E. D. Apel, J. A. Beavo, *J. Biol. Chem.* **264**, 12187 (1989)] or by the GDP-bound form of the alpha subunit of transducin [S. Kroll, W. J. Phillips, R. A. Cerione, *ibid.*, p. 4490].
20. T. D. Lamb and H. R. Matthews, *J. Physiol. (London)* **407**, 463 (1988); H. Kondo and W. H.

Miller, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1322 (1988).
21. J. W. Clack, B. Oakley II, P. J. Stein, *Nature* **305**, 50 (1983).
22. P. Deterre, J. Bigay, F. Forquet, M. Robert, M. Chabre, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2424 (1988).
23. A. R. Adolph, *J. Gen. Physiol.* **48**, 297 (1964); J. Lisman, *ibid.* **85**, 171 (1985).
24. No appreciable metabolism of the injected GTPγS occurred in the dark because illuminating the photoreceptor either as soon as possible after injection (3.5 min) or long after injection (50 min) gave similar results. Also, light did not significantly induce hydrolysis of GTPγS because a second light given to a photoreceptor long after the first also induced an aftereffect.
25. R. L. Brown and L. Stryer, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 4922 (1989).
26. W. Baehr, M. J. Devlin, M. L. Applebury, *J. Biol. Chem.* **254**, 11669 (1979).
27. We thank T. Wensel for valuable discussions and providing the trypsin-activated PDE; L. Brown and L. Stryer for providing the γ; R. Cote for performing the initial HPLC analysis and for sharing his HPLC equipment and expertise; Y. K. Ho for suggesting the trypsin-activated PDE experiment; and I. Levitan, D. Oprian, and E. Richard for comments on this manuscript. Supported by NIH grant EY 01496 (to J.L.).

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The *met* Proto-Oncogene Receptor and Lumen Formation

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The *met* proto-oncogene product (Met) and its ligand, hepatocyte growth factor/scatter factor (HGF/SF), have been implicated in cell mitogenic response, cell motility, and the promotion of the ordered spatial arrangement of tissue. By means of confocal laser-scanning microscopy, it was shown that Met is expressed in cells bordering lumen-like structures that resemble ducts in the human mammary cell line T47D. In human breast tissue biopsies, Met staining was intense in normal cells bordering mammary ducts but was reduced in adjacent tumor tissue. Met staining in lumen-forming organs colocalizes with staining of antibody to phosphotyrosine, which suggests that the Met receptor and its substrates may be activated in lumen structures or ducts. HGF/SF treatment of human epithelial carcinoma cell lines resulted in the formation of lumen-like structures in vitro. Reduced expression of Met could be related to the extent of tumor cell differentiation.

The human *met* proto-oncogene product (Met), a member of the family of tyrosine kinase growth factor receptors, was identified by means of the activated oncogene *tpr-met* (*1*, *2*). Met is synthesized as a glycosylated 170-kD precursor and cleaved in the external (ligand-binding) domain to yield a mature disulfide-linked α (50-kD), β (140-kD) heterodimer (*1*, *3*, *4*). The Met receptor is expressed in a variety of tissue and cell types, but the highest concentrations are found in epithelial cells (*5*, *6*). Hepatocyte growth factor (HGF) has been shown to be the ligand for the Met receptor. Under physiological conditions, the kinase activity of Met is dependent on the binding of the mature heterodimeric receptor to its ligand (*7*). HGF is a mediator of liver regeneration both in humans and in rodents (*8*) and is a powerful mitogen for hepatocytes and epithelial cells (*9*). Scatter factor (SF) is identical to HGF (*10*, *11*) and was independently shown both to promote epithelial cell motility (scattering) and to cause certain epithelial cell lines to become invasive when assayed in vitro (*11*, *12*). HGF/SF has been shown to be involved in the differentiation that causes Madin Darby canine kidney (MDCK) epithelial cells to morphologically change into branching tubules (*13*).

The expression of Met in cells and tissues of both human and mouse origin was determined. Immunoprecipitation and protein-immunoblot analyses showed that Met is expressed in the human breast carcinoma cell line T47D (*14*, *15*). The distribution of Met in T47D cells was examined by immunofluorescence and confocal laser-scanning microscopy (CLSM) with C28 (*16*) or C200 (*17*) rabbit antibodies to a COOH-terminal or an NH_2-terminal peptide of Met, respectively (Figs. 1 through 3). Controls were prepared with C28 antiserum in the presence of competing peptide or without C28 antiserum.

T47D cells in suspension form lumen-like structures resembling mammary ducts (*14*). Analyses of T47D cells in paraffin sections stained with C28 antibody (*16*) showed intense fluorescent staining in cells bordering lumen-like structures (Fig. 1). A marked decrease in intensity was observed when competing C28 peptide was added to the primary antibody or when the primary antibody was omitted (*18*, *19*). Moreover, T47D cells stained with the C200 antibody, directed against the Met extracellular domain, also showed intense fluorescent staining in cells bordering the lumen-like structures (Fig. 1). We observed an 80-fold (± 5-fold) greater fluorescence by CLSM in cells that form the lumen border than in adjacent cells (Fig. 2). The subcellular localization of Met in the T47D cells was also investigated by electron microscopy with indirect immunogold labeling and the C28 Met antibody (*17*). These analyses show that Met was localized to microvilli that protrude into the lumen (Fig. 3).

The intense Met-specific staining of T47D cells bordering the lumen prompted us to examine Met expression in normal and abnormal human breast tissue. Fifty human breast carcinoma biopsies were examined by CLSM with C28 Met antibody (*17*). Normal and tumor tissue from a representative breast biopsy is shown (Fig. 4). We found intense Met fluorescence in cells that form a normal mammary duct (Fig. 4A). Thus, the intensity of staining was always greater (~80-fold) in the duct-forming cells than in the adjacent nonductal cells (*18*, *20*). The pattern of Met staining in the mammary duct was similar to but higher than that observed in the T47D lumen-like structures (Figs. 1 through 3). By contrast, Met staining in adjacent breast tumor tissue was always reduced, but even in the disrupted architecture of the tumor, Met fluorescence was evident in lumen- or duct-like structures (Fig. 4C).

Met is rapidly phosphorylated on tyrosine residues after HGF/SF activation (*7*), and labeling with a monoclonal antibody to phosphotyrosine (anti–P-Tyr) colocalizes with Met staining in cells activated with the Met ligand HGF/SF (*15*). We co-stained breast tissue samples with anti–P-Tyr and found intense anti–P-Tyr fluorescence that colocalized with Met staining in the cells forming the normal breast duct (Fig. 4B). These results suggest that Met and its substrates may be activated in these cells. However, we cannot exclude that other tyrosine kinase receptors and other substrates are activated. As with the lower Met staining of tumor tissue (Fig. 4C), we also observed reduced amounts of anti–P-Tyr staining in the tumor cells (Fig. 4D); however, even the lower amounts of anti–P-Tyr fluorescence colocalized with Met staining. It has been proposed that HGF may be a tumor suppressor gene (*21*), and this has also been suggested for *met* (*22*); both of these genes are localized on the long arm of chromosome 7. Monosomy 7 or 7q

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