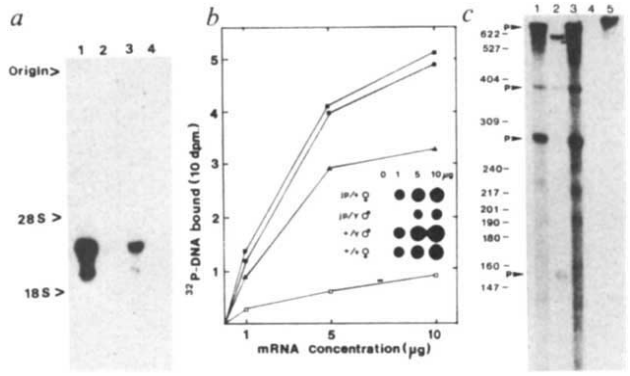


Fig. 4 *a*, Northern blot analysis of RNA from the brains of control (lane 1), hemizygous *jp*/Y (lane 2) and heterozygous *jp*/+ mice (lane 3). Lane 4, purified ribosomal RNA (20 μg). The 5.1-kb and 2.0-kb positions indicate 28S and 18S mouse ribosomal RNAs, respectively. *b*, Dot hybridization analysis of PLP-specific mRNA from mouse brains. ■, Male +/Y control; ●, female +/+ control mice; ▲, heterozygous *jp*/+ female; □, hemizygous *jp*/Y male. The inset shows the autoradiographic spots (2 days' exposure) used for this analysis. Control autoradiographic spots obtained using equal quantitites of *Escherichia coli* transfer RNA gave no signal. *c*, S_1 protection experiment using poly(A)$^+$ RNA from *jimpy* mice. The 795-nucleotide *Pst*I–*Pst*I fragment[6] was subcloned into vector M13mp9. Radiolabelled single-stranded DNA was prepared according to Burke[16]. Aliquots of 60,000 c.p.m. per probe were hybridized with 0.12 μg of poly(A)$^+$ RNA from male +/Y mice (lane 1), 1.4 μg of poly(A)$^+$ RNA from hemizygous *jimpy* male *jp*/Y (lane 2) or 0.5 μg of poly(A)$^+$ RNA from female heterozygous *jp*/+ mice (lane 3), plus 10 μg of carrier yeast tRNA. Lane 4, 5,000 c.p.m. of unhybridized probe which had been digested with S_1. Lane 5, 5,000 c.p.m. of unhybridized probe that had not been S_1 digested. After digestion with S_1 nuclease, the resulting mixture was electrophoresed through 5% acrylamide/8 M urea gel. Autoradiogram exposure was at −70 °C plus intensifying screen for 8 h. Size markers are pBR322 digested by *Hpa*II. P, protected; ND, not digested.

Methods. *a*, Total cellular RNA from 3-week-old mice was prepared, electrophoresed and transferred to nitrocellulose as described elsewhere[17,18]. Blots were prehybridized at 42 °C in 50% formamide, 5 × SSC, 5 × Denhardt's[19], 20 mM sodium phosphate *p*H 6.8, 250 μg ml^{-1} salmon sperm DNA, 0.1% SDS, then hybridized with nick-translated ^{32}P-labelled probe[20] in the same solution. Blots were washed four times in 2 × SSC/0.1% SDS/0.1% pyrophosphate at room temperature and twice in 0.1 × SSC/0.1% SDS/0.1% pyrophosphate for 15 min each at 50 °C, then exposed to Kodak X-Omat AR film at −70 °C for 1 day with Cronex Lightning Plus intensifying screen. *b*, Samples of poly(A)$^+$ RNA (1–10 μg) were treated with formaldehyde and spotted onto sheets of nitrocellulose according to the method of White and Bancroft[21], prehybridized and hybridized as for the Northern analysis. Individuals blots were cut out and counted in scintillation fluid and standard curves generated by plotting counts per min of probe bound per dot against μg of RNA immobilized per dot.

observed using the 5′ rat cDNA probe and wild-type mouse brain mRNA are identical to those seen with rat brain mRNA (data not shown). It remains possible that this pattern is related to the presence of a mRNA coding for the DM-20 molecule, which differs from PLP by the deletion of an internal fragment of about 40 amino acids[11], and further experiments are in progress to test this hypothesis and to define the mutational site further. With this finding, mutations in the structural genes coding for at least two different myelin proteins have been shown to lead to myelin deficiency and severe neurological disorders[4]. In both cases, the structural gene mutation leads to lowered steady-state mRNA levels, although it is not known whether this is due to a transcriptional or post-transcriptional event. The correlation reported here between the *jimpy* mutation and the PLP structural gene extends previous findings suggesting that *plp* is on the X chromosome[12,13] and underlines the interest in myelin as a model system for study of gene–gene product interactions.

Received 6 January; accepted 3 April 1986.

1. Hogan, E. L. & Greefield, S. in *Myelin* (ed. Morell, P.) 489-514 (Plenum, New York, 1984).
2. Lees, M. B. & Brostoff, S. W. in *Myelin* (ed. Morell, P.) 197-224 (Plenum, New York, 1984).
3. Roach, A., Boylan, K., Horvath, S., Prusiner, S. B. & Hood, L. E. *Cell* **34**, 799-806 (1983).
4. Roach, A., Takahashi, N., Pravtcheva, D., Ruddle, F. & Hood, L. E. *Cell* **42**, 149-155 (1985).
5. Ford, F. R. *Diseases of the Nervous System in Infancy Childhood and Adolescence* (ed. Thomas, C.) 831-833 (Springfield, Illinois, 1960).
6. Dautigny, A. *et al. FEBS Lett.* **188**, 33-36 (1985).
7. Amar, L. C., Arnaud, S., Cambrou, J., Guenet, J.-L. & Avner, P. R. *EMBO J.* (in the press).
8. Davisson, M. T. & Roderick, T. H. *Mouse News Lett.* **83**, 7-8 (1985).
9. Milner, R. J. *et al. Cell* **42**, 931-939 (1985).
10. Alliel, P. M. *et al. Hoppe-Seyler's Z. physiol. Chem.* **366**, 762 (abstr.) (1985).
11. Trifilieff, E. *et al. C.r. hebd. Séanc. Acad. Sci., Paris* **300**, Sér. III, 241-247 (1985).
12. Willard, F. & Riordan, J. R. *Science* **230**, 940-942 (1985).
13. Dautigny, A. *et al. 616th Meet. biochem. Soc.*, London, Abstr. 10 (1985).
14. Camargo, M. & Cervenka, J. *J. hum. Genet.* **34**, 757-780 (1982).
15. Nesbitt, M. N. & Francke, V. in *Genetic Variants and Strains of the Laboratory Mouse* (ed. Green, M. C.) 316-324 (Fischer, Stuttgart, 1981).
16. Burke, J. F. *Gene* **30**, 63-68 (1984).
17. Kaplan, B. B., Bernstein, S. L. & Gioio, A. *Biochem. J.* **183**, 181-184 (1979).
18. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5201-5215 (1980).
19. Denhardt, D. T. *Biochem. biophys. Res. Commun.* **23**, 641-646 (1966).
20. Rigby, P. W. J., Dieckman, M., Rhodes, C. & Berg, P. *J. molec. Biol.* **113**, 237-251 (1977).
21. White, B. A. & Bancroft, F. C. *J. biol. Chem.* **257**, 8569-8572 (1982).

Light-dependent phosphorylation of rhodopsin by β-adrenergic receptor kinase

Jeffrey L. Benovic*, Federico Mayor Jr*, Robert L. Somers†, Marc G. Caron* & Robert J. Lefkowitz*

* Howard Hughes Medical Institute, Departments of Medicine, Biochemistry and Physiology, Duke University Medical Center, Durham, North Carolina 27710, USA
† National Eye Institute, National Institutes of Health, Bethesda, Maryland 20892, USA

The structural components involved in transduction of extracellular signals as diverse as a photon of light impinging on the retina or a hormone molecule impinging on a cell have been highly conserved. These components include a recognition unit or receptor (for example, the β-adrenergic receptor (βAR) for catecholamines or the 'light receptor' rhodopsin), a guanine nucleotide regulatory or transducing protein, and an effector enzyme (for example, adenylate cyclase or cyclic GMP phosphodiesterase)[1,2]. Molecular cloning has revealed that the βAR shares significant sequence and three-dimensional homology with rhodopsin[3]. The function of the βAR is diminished by exposure to stimulatory agonists, leading to desensitization[4]. Similarly, 'light adaptation' involves decreased coupling of photoactivated rhodopsin to cGMP phosphodiesterase activation[5–7]. Both forms of desensitization involve receptor phosphorylation. The latter is mediated by a unique protein kinase, rhodopsin kinase, which phosphorylates only the light-bleached form of rhodopsin[8–10]. An analogous enzyme (termed βAR kinase or βARK) phosphorylates only the agonist-occupied βAR[11]. We report here that βARK is also capable of phosphorylating rhodopsin in a totally light-dependent fashion. Moreover, rhodopsin kinase can phosphorylate the agonist-occupied βAR. Thus the mechanisms which regulate the function of these disparate signalling systems also appear to be similar.

Phosphorylation of the β-adrenergic receptor by βARK is almost totally dependent on agonist occupancy of the receptor, as demonstrated in Fig. 1 (compare lanes 1 and 2). βARK is also able to phosphorylate rhodopsin in an almost totally light-dependent manner (Fig. 1, lanes 4, 5). At comparable levels

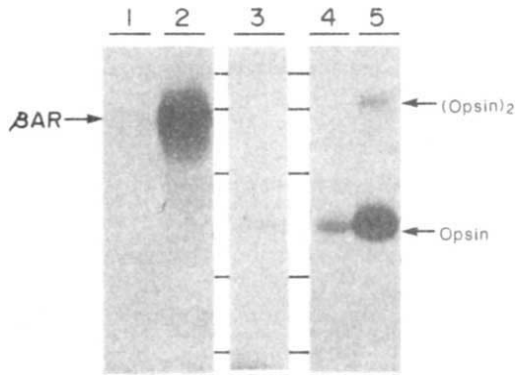


Fig. 1 Phosphorylation of the β-adrenergic receptor and rhodopsin by the β-adrenergic receptor kinase. Lane 1 contains 0.6 pmol βAR; lane 2, 0.6 pmol βAR and 20 μM (−)isoprenaline; lane 3, 0.6 pmol rhodopsin in the light; lane 4, 250 pmol rhodopsin in the dark; lane 5, 250 pmol rhodopsin in the light. The results shown are representative of three experiments. Relative molecular mass (M_r) standards (94,000 (94K), 67K, 45K, 30K and 20.1K) are indicated by marks between lanes 2 and 3 and between 3 and 4.
Methods. Receptors were incubated for 60 min at 30 °C with 20 mM Tris-HCl *p*H 7.5, 20 mM NaCl, 5 mM sodium phosphate, 2 mM EDTA, 5 mM $MgCl_2$, 5 mM NaF, 0.05 mM [γ-^{32}P]ATP, (2,000 c.p.m. per pmol) and 0.3 μg βARK in a total volume of 50 μl. Reactions containing βAR were stopped by adding 1 ml of 100 mM NaCl, 10 mM Tris-HCl *p*H 7.2, 1% digitonin. After incubation for 30 min the samples were centrifuged and the supernatants desalted on Sephadex G-50 using 100 mM NaCl, 10 mM Tris-HCl *p*H 7.2, 0.05% digitonin. βAR was then repurified using an alprenolol affinity resin as described elsewhere[11]. After lyophilization, 100 μl of SDS buffer (8% SDS, 10% glycerol, 5% β-mercaptoethanol, 25 mM Tris-HCl *p*H 6.5) was added to each sample. Reactions containing rhodopsin were stopped by adding 50 μl of SDS buffer. Samples were then electrophoresed on 10% homogeneous SDS-polyacrylamide gels[17]. Gels were dried before autoradiography for 8 h (lanes 1-3) or 15 min (lanes 4, 5). βARK was partially purified from bovine brain by $(NH_4)_2SO_4$ precipitation (13-26%) of a high-speed supernatant fraction. The precipitate was then chromatographed on Ultrogel AcA34, DEAE Sephacel and CM Fractogel. Kinase activity was eluted with 20 mM Tris-HCl *p*H 7.5, 1 mM EDTA, 0.1 mM phenylmethylsulphonyl fluoride, 10 μg ml^{-1} leupeptin, 5 μg ml^{-1} pepstatin, 10 μg ml^{-1} benzamidine (J.L.B., F.M. Jr, R.J.L. and M.G.C., manuscript in preparation). The preparation used was ~20% pure and had a specific activity of ~35 pmol P_i per min per mg using 0.02 μM βAR as substrate. The preparation did not contain any contaminating protein kinase C or cyclic AMP-dependent protein kinase[11]. βAR was purified from hamster lung to >95% homogeneity by affinity chromatography and molecular-sieve HPLC as described elsewhere[18]. Purified βAR was reconstituted into phosphatidylcholine vesicles[11,19] before phosphorylation. Rod outer segments (ROS) were prepared from bovine retinas by stepwise sucrose gradient centrifugation[13]. Rhodopsin kinase-free ROS were prepared by treatment with 5 M urea[10] and consisted of ~95% rhodopsin, as assessed by Coomassie blue staining of SDS-polyacrylamide gels. The urea treatment effectively eliminates the endogenous rhodopsin kinase activity as rhodopsin phosphorylation in our ROS preparations was observed only in the presence of added kinase.

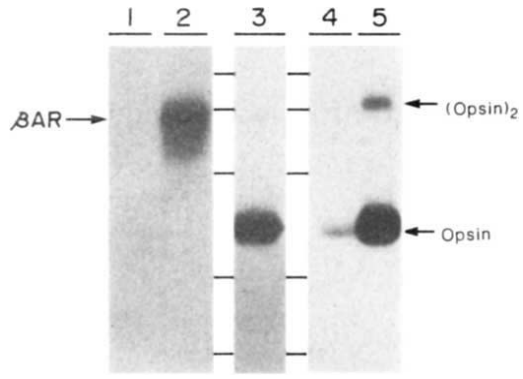


Fig. 2 Phosphorylation of the β-adrenergic receptor and rhodopsin by rhodopsin kinase. Lane 1 contains 0.6 pmol βAR; lane 2, 0.6 pmol βAR and 20 μM (−)isoprenaline; lane 3, 0.6 pmol rhodopsin in the light; lane 4, 250 pmol rhodopsin in the dark; lane 5, 250 pmol rhodopsin in the light. Incubations were identical to those described for Fig. 1, except that they contained 3 μg of rhodopsin kinase instead of βARK. βAR was repurified as described in Fig. 1 legend to remove the rhodopsin kinase autophosphorylation band which runs at M_r ~64K. Reactions containing rhodopsin were stopped by adding 50 μl of SDS buffer before electrophoresis on 10% polyacrylamide gels. Autoradiography of the dried gel was for 24 h (lanes 1-3) or 8 min (lanes 4, 5). The results shown are representative of three experiments. M_r standards are the same as in Fig. 1.
Methods. Rhodopsin kinase was partially purified from bovine ROS extracted with 10 mM potassium phosphate, 3 mM EDTA, 2 mM EGTA *p*H 6.8. The high-speed supernatant from this extract was dialysed against 20 mM potassium phosphate, 0.5 mM EDTA, 0.5 mM EGTA, 20% glycerol *p*H 6.8 before chromatography on a Blue Sepharose column. Rhodopsin kinase activity was eluted with a KCl gradient before dialysis against 0.1 M potassium phosphate, 0.1 mM EDTA, 0.1 mM EGTA, 50% glycerol *p*H 6.8 (R.L.S., manuscript in preparation). The preparation used was ~10% pure and had a specific activity of ~13 nmol P_i per min per mg using 10 μM rhodopsin as substrate. The preparation did not contain any contaminating protein kinase C or cAMP-dependent protein kinase. βAR was purified and reconstituted as described in Fig. 1 legend. Urea-treated ROS were prepared as described in Fig. 1 legend.

(0.6 pmol), βAR is a significantly better substrate for βARK than is rhodopsin (Fig. 1, lanes 2, 3). Figure 2 shows that analogous results are achieved using rhodopsin kinase. Thus, phosphorylation of rhodopsin is almost totally light-dependent (Fig. 2, compare lanes 4 and 5) whereas phosphorylation of βAR is almost totally agonist-dependent (lanes 1, 2). In addition, while βARK preferentially phosphorylates βAR, rhodopsin kinase phosphorylates rhodopsin better than βAR (Fig. 2, lanes 2, 3). The large apparent difference in kinase specificity, with βARK being much better at phosphorylating βAR compared with rhodopsin and rhodopsin kinase being only slightly better at phosphorylating rhodopsin, may reflect the fact that only the bleaching intermediates metarhodopsins II and III can serve as effective substrates for rhodopsin kinase[10,12]. Thus, the actual substrate concentration of rhodopsin in our experiments may in fact be lower than that of βAR. However, this does not alter the fact that βAR is the preferred substrate for βARK while rhodopsin is preferred by rhodopsin kinase.

The striking light dependence of rhodopsin phosphorylation by βARK is demonstrated further in Fig. 3. At the 2-h time point a 60-fold increase in phosphorylation was observed in the presence of light. The low stoichiometry (~0.07 mol per mol) probably reflects the relatively low amount of βARK used in these experiments. To compare the sites phosphorylated by rhodopsin kinase and βARK, HPLC tryptic peptide mapping of phosphorylated rhodopsin was performed. The peptide maps appeared virtually identical, with only a single major peptide. Phosphoamino-acid analysis of this peptide revealed predominantly phosphoserine together with a small amount of phosphothreonine, for both kinases (data not shown).

Rhodopsin kinase phosphorylates rhodopsin at as many as nine different sites[13], with seven of these residues (serines and threonines) being clustered in the carboxy-terminal 15 amino acids[14]. The sequence of the β-adrenergic receptor, deduced recently from the cloned gene, indicates that a similar serine- and threonine-rich region exists in its carboxy terminus[3]. The actual phosphorylation sites on the βAR have yet to be

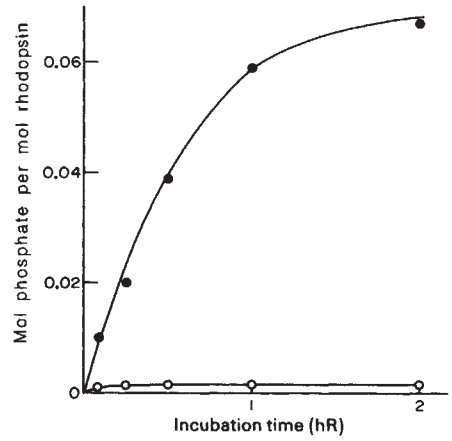


Fig. 3 Time course of rhodopsin phosphorylation by the β-adrenergic receptor kinase. Urea-treated ROS (250 pmol of rhodopsin) were incubated at 30 °C with CM Fractogel-purified βARK (0.3 μg protein) in the dark (○) or under continuous illumination with white light (●) for the times indicated. The incubations also contained 20 mM Tris-HCl *p*H 7.5, 20 mM NaCl, 2 mM EDTA, 5 mM $MgCl_2$ and 0.05 mM [γ^{32}P]ATP (1,600 c.p.m. per pmol). Reactions were stopped by adding 50 μl of SDS buffer before electrophoresis on a 10% polyacrylamide gel. After autoradiography the rhodopsin bands were excised and counted in a Packard scintillation counter. The results shown are representative of three experiments.

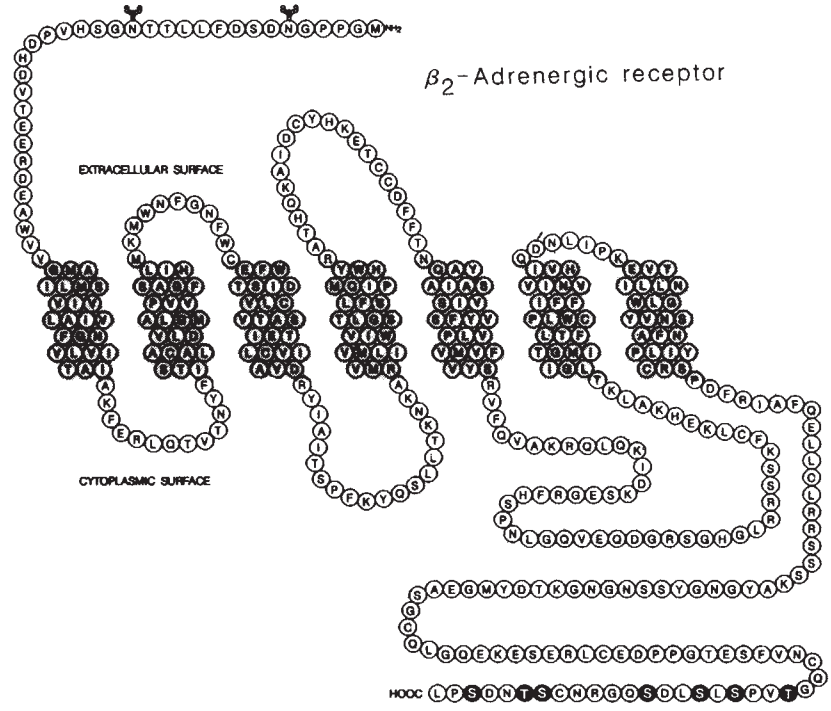
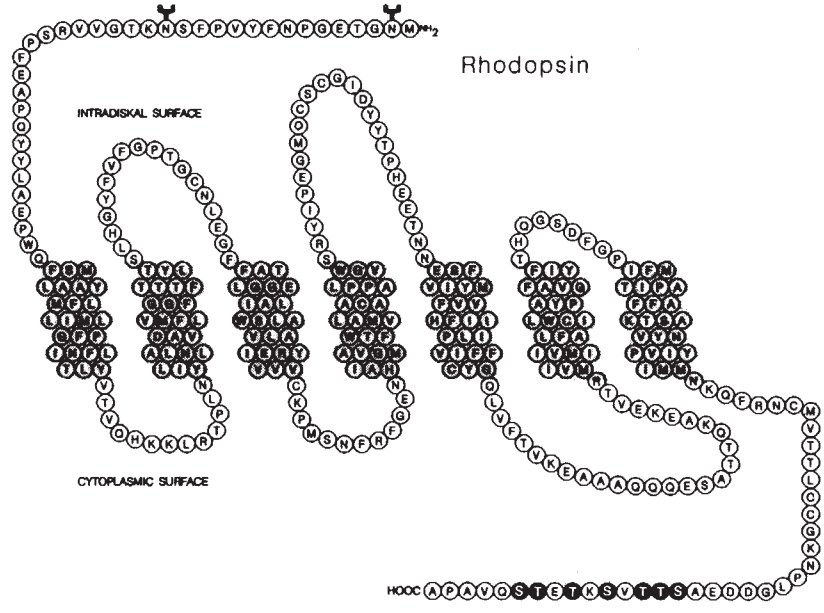


Fig. 4 Structure of the β-adrenergic receptor and rhodopsin polypeptide chains as they may be organized within the membrane. The seven known rhodopsin kinase phosphorylation sites on rhodopsin are represented by solid circles, as is a similar serine- and threonine-rich region near the carboxy terminus of βAR. Amino-acid sequences of the two proteins are taken from refs 3 and 20.



determined, however, the carboxy-terminal domain seems a likely region. Figure 4 compares the structures of the βAR and rhodopsin polypeptide chains as they may be organized within the membrane. The seven known phosphorylation sites on rhodopsin and seven possible sites of phosphorylation on the βAR are shown as solid circles.

We have found that βARK, an enzyme which specifically phosphorylates only the agonist-occupied form of the β-adrenergic receptor, can also phosphorylate the same region on rhodopsin as does rhodopsin kinase, in a light-dependent manner. This result suggests that in addition to the striking homologies in the structure and function of signal transduction components which exist between the two systems, their biochemical mechanisms of regulation are also analogous. Phosphorylation of bleached rhodopsin by rhodopsin kinase appears to lead to the attenuation of its interaction with transducin[15]. Phosphorylation of the β-adrenergic receptor by βARK in response to agonist occupancy of the receptor also appears to lead to an uncoupling of the receptor from the stimulatory guanine nucleotide regulatory protein, and this in turn is associated with attenuation of adenylate cyclase responsiveness (J.L.B., F.M. Jr, M.G.C. and R.J.L., manuscript in preparation).

Because phosphorylation of the receptor appears to be important for physiological adaptation (desensitization) of the responses associated with both the β-adrenergic receptor and the light receptor rhodopsin, phosphorylation may also represent an important control mechanism for other hormone-receptor systems. Recently, we have shown that desensitization of the α_1-adrenergic receptor, a receptor coupled to the phosphatidylinositol-Ca^{2+} signal transduction pathway, is also associated with its phosphorylation[16]. Moreover, desensitization or tachyphylaxis is a phenomenon which is common to many physiologically responsive systems. Thus, phosphorylation of the receptor component of hormone- and drug-responsive systems by a family of specific receptor kinases may represent a major mechanism by which cellular responsiveness to a variety of signals is regulated.

We acknowledge the assistance of Lynn Tilley in the preparation of this manuscript. This work was supported in part by NIH grant HL-16037. F.M. is a recipient of a Fulbright-Spain Ministry of Science postdoctoral fellowship.

Received 10 April; accepted 6 May 1986.

1. Fung, B. K.-K. & Stryer, L. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2500-2504 (1980).
2. Lefkowitz, R. J., Stadel, J. M. & Caron, M. G. *A. Rev. Biochem.* **52**, 159-186 (1983).
3. Dixon, R. A. F. *et al. Nature* **321**, 75-79 (1986).
4. Sibley, D. R. & Lefkowitz, R. J. *Nature* **317**, 124-129 (1985).
5. Liebman, P. A. & Pugh, E. N. *Nature* **287**, 734-736 (1980).
6. Sitaramayya, A. & Liebman, P. A. *J. biol. Chem.* **258**, 1205-1209 (1983).
7. Wilden, U., Hall, S. W. & Kuhn, H. *Proc. natn. Acad. Sci. U.S.A.* **83**, 1174-1178 (1986).
8. Kuhn, H. & Dreyer, W. J. *FEBS Lett.* **20**, 1-6 (1972).
9. Bownds, D., Dawes, J., Miller, J. & Stahlman, M. *Nature new Biol.* **237**, 125-127 (1972).
10. Shichi, H. & Somers, R. L. *J. biol. Chem.* **253**, 7040-7046 (1978).
11. Benovic, J. L., Strasser, R. H., Caron, M. G. & Lefkowitz, R. J. *Proc. natn. Acad. Sci. U.S.A.* **83**, 2797-2081 (1986).
12. Paulsen, R., Miller, J. A., Brodie, A. E. & Bownds, M. D. *Vision Res.* **15**, 1325-1332 (1975).
13. Wilden, U. & Kuhn, H. *Biochemistry* **21**, 3014-3022 (1982).
14. Hargrave, P. A. *et al. Neurochem. Int.* **1**, 231-244 (1980).
15. Shichi, H., Yamamoto, K. & Somers, R. L. *Vision Res.* **24**, 1523-1531 (1984).
16. Leeb-Lundberg, L. M. F. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 5651-5655 (1985).
17. Laemmli, U. K. *Nature* **227**, 680-685 (1970).
18. Benovic, J. L., Shorr, R. G. L., Caron, M. G. & Lefkowitz, R. J. *Biochemistry* **23**, 4510-4518 (1984).
19. Cerione, R. A., Strulovici, B., Benovic, J. L., Lefkowitz, R. J. & Caron, M. G. *Nature* **306**, 562-566 (1983).
20. Ovchinnikov, Y. A. *FEBS Lett.* **148**, 179-191 (1982).

Stimulation of haematopoiesis in primates by continuous infusion of recombinant human GM-CSF

Robert E. Donahue*, Elizabeth A. Wang*, David K. Stone*, Robert Kamen*, Gordon G. Wong*, Prabhat K. Sehgal†, David G. Nathan‡ & Steven C. Clark*

* Genetics Institute, Inc., Cambridge, Massachusetts 02140, USA
† New England Regional Primate Research Center, Southborough, Massachusetts 01722, USA
‡ Division of Hematology/Oncology, Dana Farber Cancer Institute and the Children's Hospital, Boston, Massachusetts 02115, USA

Certain proteins are known to play an important part in the proliferation, differentiation and functional activation of haematopoietic progenitor cells *in vitro*[1,2]. These proteins include erythropoietin and various colony-stimulating factors (CSFs), one of which is granulocyte–macrophage colony-stimulating factor (GM-CSF). Recently, both murine[3,4] and human GM-CSF[5–7] have been purified to homogeneity and complementary DNAs encoding them have been cloned. Although the *in vitro* activity of recombinant human GM-CSF has been investigated intensively (refs 5–15; see ref. 8 for a review), little is known about the functional activity of this protein *in vivo*. There is strong evidence that colony-stimulating activities produced by various human and murine tumour tissues and cell lines can stimulate granulopoiesis in mice[16–26], as can human urinary extracts[27,28]. A partially purified preparation of human urinary colony-stimulating factor, however, proved only marginally effective in stimulating granulopoiesis in humans[29]. All these studies suffer from the lack of a homogeneous preparation of colony-stimulating factor. It has recently been shown that recombinant murine multi-CSF or interleukin-3 can stimulate haematopoiesis in mice *in vivo*[30,31]. Large-scale production of recombinant human GM-CSF now permits us to examine its effects *in vivo* using a primate model. We find that the continuous infusion of GM-CSF in healthy monkeys rapidly elicits a dramatic leukocytosis and a substantial reticulocytosis. A similar effect has been observed in one pancytopenic, immunodeficient rhesus macaque. These results suggest that GM-CSF could prove useful in several clinical situations.

To analyse the biological activities of recombinant human GM-CSF on simian progenitor cells, adherence-depleted simian bone marrow mononuclear cells were plated in 0.9% methylcellulose cultures at a cell concentration of 2.5×10^4 cells per ml. In this culture system, picomolar concentrations of purified human recombinant GM-CSF stimulated the terminal differentiation of simian granulocyte/macrophage progenitors (CFU-GM). Eosinophil colonies were not specifically counted (Fig. 1). These results support the earlier observation that conditioned media from the hairy-cell leukaemia cell line Mo can stimulate monkey granulocyte–monocyte colony formation[32]. In the presence of erythropoietin, the hormone also induced the proliferation and differentiation of early erythroid (BFU-E) and multipotential progenitors (CFU-GEM) (Fig. 1). These data confirm previous results obtained with human progenitors which show that GM-CSF is a multi-lineage haematopoietin[11–15]. GM-CSF derived from *Escherichia coli* was as effective in stimulating colony formation as was the glycosylated protein derived from mammalian cells, indicating that carbohydrate modification has little effect on the *in vitro* activity of the haematopoietin (results not shown).

We next analysed the ability of GM-CSF to stimulate the proliferation of enriched populations of simian progenitor cells when plated at low cell densities (data not shown). To determine whether recombinant human GM-CSF acts directly on simian haematopoietic progenitor cells or indirectly through accessory cells, an additional panning step was performed on the adherence-depleted simian bone marrow mononuclear cells so as to remove T cells, B cells, monocytes, natural killer cells and granulocytes. This depletion did not affect the ability of GM-