Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 11067–11070, December 1991
Biochemistry

Isolation of *Drosophila* genes encoding G protein-coupled receptor kinases

(guanine nucleotide-binding proteins/receptor desensitization/regulation/phosphorylation)

J. AARON CASSILL, MICHAEL WHITNEY, CLÁUDIO A. P. JOAZEIRO, ANN BECKER, AND CHARLES S. ZUKER

Howard Hughes Medical Institute and Departments of Biology and Neuroscience, University of California, San Diego, La Jolla, CA 92093

Communicated by Dan L. Lindsley, September 20, 1991

ABSTRACT **G protein-coupled receptors are regulated via phosphorylation by a variety of protein kinases. Recently, termination of the active state of two such receptors, the β-adrenergic receptor and rhodopsin, has been shown to be mediated by agonist- or light-dependent phosphorylation of the receptor by members of a family of protein-serine/threonine kinases (here referred to as G protein-coupled receptor kinases). We now report the isolation of a family of genes encoding a set of *Drosophila* protein kinases that appear to code for G protein-coupled receptor kinases. These proteins share a high degree of sequence homology with the bovine β-adrenergic receptor kinase. The presence of a conserved family of G protein-coupled receptor kinases in vertebrates and invertebrates points to the central role of these kinases in signal transduction cascades.**

G protein-coupled receptors mediate responses to a wide range of extracellular stimuli, including hormones, neurotransmitters, peptides, odorants, and light (reviewed in refs. 1–3). These receptors share several structural features including seven putative transmembrane domains, the presence of clustered serine and threonine residues in the cytoplasmic C-terminal tail of the molecule, and generally a disulfide bond between the second and third cytoplasmic loops (1, 4, 5). Nearly 100 members of this superfamily of membrane receptors have been identified and many of the heterotrimeric GTP-binding proteins that couple their response to intracellular effectors have been characterized (1–3).

The β-adrenergic receptor and the light receptor rhodopsin are two of the best characterized G protein-coupled receptors. Studies of the activation and inactivation properties of these two receptors showed that, *in vitro*, rapid quenching of the receptor requires ATP (6–9). Indeed, ligand-dependent (or light-dependent) activation of the receptor results in the activation of a specific protein kinase that phosphorylates numerous serine and threonine residues clustered on the C-terminus of the receptor (10–14). Phosphorylation alone results in only a slight decrease in receptor activity, but causes the receptors to become high-affinity substrates for a protein known as arrestin (15–17). Arrestin binding is then thought to "turn off" the active state of the receptor by preventing the receptor from coupling to the G protein.

The kinases responsible for phosphorylating the activated forms of the β-adrenergic receptor and rhodopsin are the β-adrenergic receptor kinase (BARK) (18) and rhodopsin kinase (ROK) (16, 19), respectively. Both of these proteins have been purified to homogeneity and their specificities and activities have been assayed *in vitro* in reconstituted systems (18–22). Interestingly, rhodopsin and the β-adrenergic receptor can be properly phosphorylated by each other's kinase, albeit at lower affinity, and only in their agonist (or light)-activated state (23). The presence of potential phosphorylation sites in the C-terminal domain of most G protein-coupled receptors (1), together with the established involvement of receptor phosphorylation in desensitization mechanisms (reviewed in refs. 1 and 3), suggests that G protein-coupled receptor kinases (GPRKs) define a family of protein-serine/threonine kinases.

Although many of the intracellular components involved in G-protein signaling have been identified at the molecular level in both vertebrates and invertebrates (reviewed in refs. 1–4), little is known about the regulation of these processes *in vivo*. In an effort to carry out a molecular genetic dissection of the role of receptor phosphorylation, we undertook a molecular screen designed to isolate GPRKs in *Drosophila*. Benovic *et al.* (24) have isolated and characterized a cDNA encoding bovine BARK. Because of the functional similarity between BARK and ROK (23), we reasoned that members of the GPRK family might share a high degree of structural similarity. Our strategy relied on screening for kinases similar to BARK. We now report that *Drosophila* contains a family of genes encoding proteins with a high degree of sequence similarity to the bovine BARK, and we describe the detailed molecular characterization of two members of that family.* The identification of these proteins in *Drosophila*, a system particularly suited for molecular genetic analysis, may provide significant insight into their *in vivo* role.

MATERIALS AND METHODS

PCR. Reaction mixtures contained 10 μg of *Drosophila* retinal cDNA, 2.5 μg of each oligonucleotide primer, and 5 units of *Taq* polymerase (AmpliTaq, Cetus) in a final volume of 25 μl. Reactions were carried out in buffer recommended by the manufacturer (Cetus), supplemented with 0.2 mM dNTPs. Amplification was carried out in four cycles of 1 min at 94°C, 3 min of ramped cooling to 58°C, 1 min at 58°C, 1 min of ramped warming to 72°C, and 2 min at 72°C, followed by 30 cycles of 1 min at 94°C, 1 min at 65°C, and 3 min at 72°C.

DNA Sequencing. DNA sequence was determined according to the chain-termination procedure (25) using T7 polymerase sequencing kits (Pharmacia). Templates were generated by limited exonuclease III digestions (26). Sequence alignments were generated using the University of Wisconsin Genetics Computer Group programs.

DNA Hybridizations. DNA probes were labeled by random oligonucleotide priming (Amersham multiprime system). Hybridizations were carried out at 65°C in 5× SSC (1× SSC is 150 mM NaCl/15 mM sodium citrate)/100 mM NaH_2PO_4, pH 6.8/0.04% bovine serum albumin/0.04% polyvinylpyrrolidone 40/0.04% Ficoll/0.5% SDS. Filters were washed in

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ROK, rhodopsin kinase; BARK, β-adrenergic receptor kinase; GPRK, G protein-coupled receptor kinase.

*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M80493 and M80494).

0.2× SSC/0.5% SDS at 65°C. Low-stringency hybridizations were carried out at 42°C in 7× SSC/140 mM NaH_2PO_4, pH 6.8/20% formamide/0.04% bovine serum albumin/0.04% polyvinylpyrrolidone 40/0.04% Ficoll/0.5% SDS and washed at 50°C in 2× SSC.

***In Situ* Hybridization to Polytene Chromosomes and Tissue Sections.** Polytene chromosome squashes (Canton-S strain) were prepared as described (27). Hybridizations with biotinylated DNA probes were carried out according to Langer-Sofer *et al.* (28) with the following modifications: DNA was nick-translated using biotin-labeled dUTP (Bio-16-dUTP; Enzo Biochem) and hybrids were detected with the Detek-I-HRP detection kit (Enzo Biochem). Preparation of 8-μm frozen sections and *in situ* hybridization were as described by Hafen *et al.* (29), except that the Pronase treatment was omitted in the pretreatment of the sections and probes labeled with digoxigenin-dUTP (Boehringer Mannheim Genius kit) were used.

Generation of Antibodies Against GPRK-1 and -2 and Western Blots. Antibodies were generated against fusion proteins expressed in bacteria using the expression vector pGEMEX-1 (Promega). The GPRK-1 antibodies were generated against a region encoding amino acid residues 394–701, and the GPRK-2 antibodies against a fragment encoding residues 142–433. Fusion proteins were induced and purified as described by Studier *et al.* (30). Antibodies were raised in rats as described (31). Sera from positive animals were affinity-purified over a Bio-Rad Affi-Gel affinity column coupled with 500 μg of fusion protein. Western blot analysis was carried out exactly as described (31). Either 20 retinas, 10 heads, or one body of w^{1118} flies or 10 heads from *eya* (eyes absent) mutant flies (32) were added to 10 μl of sample buffer, sonicated for 20 sec, and boiled for 5 min before the proteins were separated by electrophoresis in a 10% polyacrylamide gel.

RESULTS AND DISCUSSION

Isolation and Sequence Analysis of GPRK-1 and GPRK-2 cDNA. To isolate sequences encoding potential GPRKs, we designed degenerate oligonucleotides corresponding to regions conserved in BARK and known *Drosophila* serine/threonine kinases (24, 33–35). These oligonucleotides were used as amplification primers in PCRs templated with a pooled collection of *Drosophila* retinal cDNAs. Oligonucleotide 1 encoded the sequence (Ile/Val)-(Ile/Val)-Tyr-Arg-Asp-Leu-Lys, which is within the conserved catalytic domain of serine/threonine kinases (ATP-binding site) (35, 36). The reverse complement of oligonucleotide 2 encoded the sequence Tyr-Met-Ala-Pro-Glu-(Ile/Val)-(Ile/Val), which is also conserved in serine/threonine kinases, and is generally found 135–180 nucleotides downstream from the oligonucleotide 1 sequence (35, 36). These two oligonucleotides flank domain VII [as defined by Hanks *et al.* (35)], which contains the amino acid sequence Asp-Phe-Gly, the most highly conserved short motif found in serine/threonine kinases (35).

The PCR products were directionally cloned into the vector pBluescript KS(−) (Stratagene) and the individual inserts were analyzed for size. Inserts greater than 70 base pairs (the relative position of the Asp-Phe-Gly motif in this domain) were characterized further. Of 221 cloned PCR products for which the DNA sequence was determined, 10 defined *Drosophila* kinase sequences not found in current nucleic acid/protein data bases (GenBank, on-line July 1, 1991). Of these, 4 showed a high level of similarity to BARK (Fig. 1). Three of these contained the variant Asp-Leu-Gly instead of the conserved Asp-Phe-Gly motif; this variant sequence has been previously found only in bovine BARK and one other kinase (*daf*-1, refs. 36 and 37). We named these four clones Gprk-1 through -4, in decreasing order of similarity to BARK. The two cloned PCR fragments which showed the highest level of amino acid sequence similarity to BARK, Gprk-1 and Gprk-2, were further characterized. We used the cloned PCR fragments as probes to screen *Drosophila* genomic and cDNA libraries and obtained several cDNA and genomic clones for each. The nucleotide sequences of the largest cDNAs corresponding to Gprk-1 (2.3 kilobases) and Gprk-2 (2.8 kilobases) were determined; both cDNA classes were found to contain a single large open reading frame.

Gprk-1 encodes an 80-kDa polypeptide displaying 79% amino acid similarity (and 65% amino acid identity) to bovine BARK (Fig. 2*A*). The sequence similarity extends throughout the entire length of the molecule with the catalytic domain displaying the highest degree of homology (Fig. 2*B*). This high degree of sequence conservation between species that diverged hundreds of millions of years ago suggests that GPRK-1 may be the functional homolog of BARK. Interestingly, no adrenergic signaling system has been identified in *Drosophila*. In this regard, BARK has been shown to also phosphorylate agonist-activated α_{2a}-adrenergic receptors (38) and M2 muscarinic cholinergic receptors (39). Thus, it is quite likely that BARK represents a kinase with a broader range of substrate specificities than originally suggested (see *Concluding Remarks*).

Gprk-2 encodes a 50-kDa polypeptide displaying 60% amino acid similarity and 39% identity to BARK (Fig. 2*A*), and 60% amino acid similarity with GPRK-1. The most conserved region of GPRK-2 is the catalytic domain, which displays 68% similarity to the corresponding domain of BARK (Fig. 2*B*). Although GPRK-2 is significantly smaller than BARK (or GPRK-1; see Figs. 2*A* and 3), it contains all of the conserved domains found in protein-serine/threonine kinases. Indeed, homology region 1 of the catalytic domain (35, 36) begins at residue 29. The equivalent domains of GPRK-1 and BARK begin at residues 199 and 198, respectively. Since the N-terminal domains of many types of serine/threonine kinases contain sequences involved in autoregulation of the enzyme (35, 40), it is possible that GPRK-2 represents a variant that may require an additional regulatory subunit.

Distribution of GPRK-1 and GPRK-2 Proteins. Since G protein-coupled receptors are involved in a wide range of signaling processes, it is difficult to assign a role to GPRK-1 and -2 based on sequence similarity alone. *In situ* hybridization to polytene chromosomes showed that Gprk-1 localizes to the second chromosome at position 41B3-C2. Gprk-2 maps to the third chromosome at position 100C3-5. No mutations

```
          313                                                    361
BARK      VVYRDLKPaN ILLDEhGHVR ISDLGLAcDF SkKkphas        VGThGY MAPEV
GPRK-1    VVYRDLKPaN ILLDENGHIR ISDLGLAcDF SkKkphas.. ....VGThGY MAPEI
GPRK-2    VVYRDFKPeN ILLDDhGHVR ISDLGLAvEi pegemvrg.. ...RVGTvGY MAPEV
GPRK-3    VVYRDLKskN ILvksNlsca IgDLGLAvrh veKndsvdip sthRVGTkrY MAPEI
GPRK-4    VVYRDLKPkN LLLDhNmHak IaDFGLs... ...nmmldge flrtsaspnY MAPEV
```

FIG. 1. Isolation of GPRKs. Shown is an amino acid alignment of four *Drosophila* sequences (GPRK-1 to -4) displaying a high degree of similarity to bovine BARK (24). Horizontal lines above the sequences indicate the residues encoded by the PCR primers used in the amplification reactions. Amino acids are indicated by their single-letter code. Identical and conservative amino acids are shown by uppercase letters. Shading indicates amino acid identities.

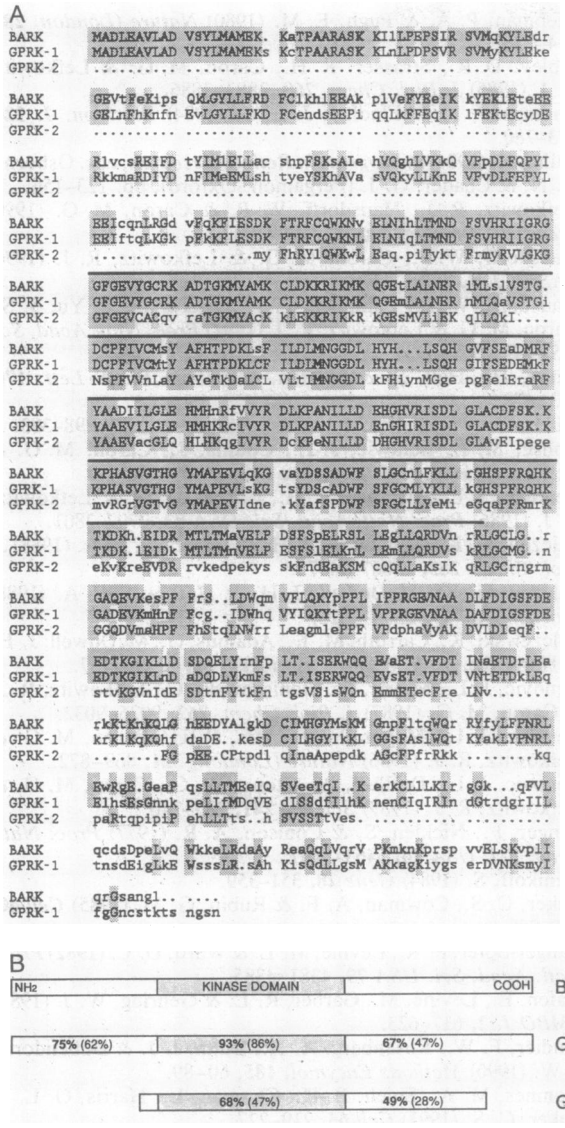

FIG. 2. Sequence alignment of GPRK-1, GPRK-2, and BARK. (*A*) Shown is an alignment of the deduced amino acid sequence of GPRK-1 and GPRK-2 with bovine BARK (24). The alignment has been optimized for the largest number of matches with the minimum number of gaps. Identical and conservative amino acids are shown by uppercase letters. Shaded residues indicate amino acid identity between at least two of the proteins. The catalytic domain is indicated by an overline. (*B*) Amino acid similarity between the various domains of the *Drosophila* GPRKs and BARK. The level of identity for these domains is in parentheses. Overall, GPRK-1 and BARK display 79% amino acid similarity, while GPRK-2 and BARK display 60% sequence similarity.

affecting development or adult functions have been isolated at or near either of these cytogenetic locations. To determine in which cells and tissues these proteins are expressed, we raised antibodies against each polypeptide and examined their sites of accumulation by Western blot analysis (Fig. 3) and immunohistochemical staining of frozen tissue sections of wild-type flies. In particular, we wanted to determine whether either of these two kinases might be enriched in the visual system and thus be a likely candidate for ROK. Therefore, we also assayed the presence of these proteins in *Drosophila* mutants lacking the adult compound eyes [eyes absent, *eya* (32)]. Both GPRK-1 and GPRK-2 were found in all of the samples examined (Fig. 3). These results were confirmed by immunohistochemical stainings, which showed that both proteins are found ubiquitously in the adult animal,

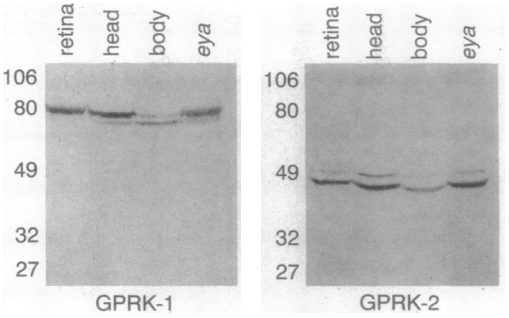

FIG. 3. Expression pattern of GPRK-1 and GPRK-2. Western blots of protein extracts prepared from retinas, heads, and bodies of wild-type (w^{1118}) flies and from heads of mutant *eya* (eyes absent) flies (32) were probed with antibodies against GPRK-1 or GPRK-2. The positions of molecular size markers are indicated (kDa). Anti-GPRK-1 recognized a protein of ≈80 kDa. Anti-GPRK-2 recognized a protein of ≈50 kDa. The additional band recognized by each antibody most likely resulted from degradation of the proteins during sample preparation, since their presence varied in different preparations of the same tissues (data not shown). Both proteins were present in all samples tested, demonstrating that their expression is not limited to the visual system or the brain.

with low levels of expression found in all tissues examined (data not shown). We also examined the developmental expression of both proteins by Western blot analysis of protein from embryos, first-, second-, and third-instar larvae, and early and late pupae. Both GPRK-1 and GPRK-2 were present at all stages of development (data not shown).

***Drosophila* Contains a Family of Gprk-Like Genes.** To determine whether additional Gprk-like sequences are present in the *Drosophila* genome, we carried out low-stringency Southern blot hybridizations to genomic DNA using radiolabeled Gprk-1 as a probe. A 150-base-pair fragment of Gprk-1 containing kinase domains VI–VIII (35, 36) was hybridized to genomic DNA digested with *Bam*HI, *Bgl* II, *Eco*RI, or *Pst* I. This fragment is colinear with the corresponding genomic sequence and is not digested by any of these restriction endonucleases, yet it hybridized to multiple bands at low stringency (Fig. 4). These signals do not correspond to those resulting from hybridization with either Gprk-3 or Gprk-4, and they remain even after high-stringency washes (data not shown; see *Materials and Methods*). Based on the number of expected fragments hybridizing to Gprk-1 and Gprk-2 genomic sequences, we estimate the presence of at least two additional members of this gene family.

Concluding Remarks. G protein-coupled receptors mediate cellular responses to a diverse range of extracellular stimuli. Receptor phosphorylation appears to be a widely utilized strategy for modulating receptor function. Although protein kinase A (1, 12, 13) and protein kinase C (42) appear to be involved in the regulation of a number of G protein-coupled receptors, there appears to be a functionally conserved family of protein kinases that mediate receptor desensitization at high agonist concentrations; BARK and ROK represent two members of this family (1, 12, 16, 19). In contrast to protein kinase C and protein kinase A, BARK- and ROK-mediated phosphorylation does not desensitize the receptor (1, 12, 13, 16). However, the phosphorylation of the receptor by one of these kinases is a prerequisite for arrestin binding, which results in uncoupling of the receptor from G proteins (15–17).

Although there are hundreds of G protein-coupled receptors, a search for BARK homologs has produced only a few related protein kinases (ref. 43 and this study). However, since most G protein-coupled receptors couple to a small number of G proteins (e.g., a few each of G_s, G_i, G_o, G_q) and intracellular effectors (e.g., adenylate cyclase, phospholi-

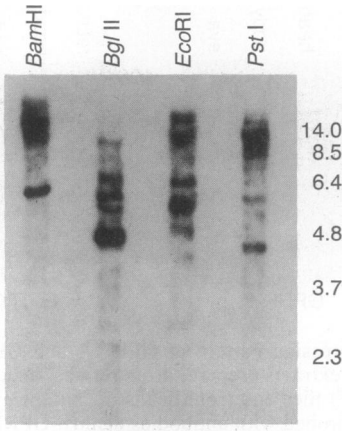

FIG. 4. Gprk-1-related sequences in the *Drosophila* genome. Genomic *Drosophila* DNA was digested with *Bam*HI, *Bgl* II, *Eco*RI, or *Pst* I, and the fragments were separated in a 0.8% agarose gel with Tris/borate EDTA buffer, denatured, and transferred to nitrocellulose (41). The DNA blot was hybridized at low stringency with a 150-base-pair fragment from Gprk-1 that is colinear with the corresponding genomic DNA sequence and is not cleaved by any of the restriction endonucleases used. The probe hybridized to multiple restriction fragments, indicating the presence of related sequences. The multiple hybridization signals persisted even after washing at high stringency (0.2× SSC, 65°C; data not shown), indicating a high degree of sequence similarity. Other 150-base-pair fragments encoding the corresponding catalytic domains from four other kinases produced single hybridizing fragments on the same filter (data not shown). The positions of DNA markers are indicated (kilobases).

pase C, cGMP phosphodiesterase; refs. 1–3), it may not be unexpected to find that only a small number of GPRKs modulate a wide range of receptors. In this regard, it would be of interest to determine whether the β-adrenergic receptor indeed represents the primary target of BARK. The isolation of *Drosophila* mutants lacking GPRK-1 and GPRK-2 may provide significant insight into the nature of the *in vivo* substrates of these kinases and the biology of these proteins in general.

We gratefully acknowledge Sam Lehman for his efforts in the early stages of this project. We thank Mike Socolich for his help with chromosome *in situ* hybridizations and Lorraine Chuman for assistance in manuscript preparation. We thank members of the Zuker lab for valuable comments on the manuscript. This work was supported by the Howard Hughes Medical Institute. J.A.C. is supported by a postdoctoral grant from the National Eye Institute. C.S.Z. is a Pew Scholar, a McKnight Fellow, a March of Dimes Basill O'Connor Fellow, and an Investigator of the Howard Hughes Medical Institute.

1. Dohlman, H. G., Thorner, J., Caron, M. G. & Lefkowitz, R. J. (1991) *Annu. Rev. Biochem.* **60**, 653–688.
2. Simon, M. I., Strathmann, M. P. & Gautam, N. (1991) *Science* **252**, 802–808.
3. Stryer, L. & Bourne, H. R. (1986) *Annu. Rev. Cell Biol.* **2**, 391–420.
4. O'Dowd, B. F., Lefkowitz, R. J. & Caron, M. G. (1989) *Annu. Rev. Neurol.* **12**, 67–83.
5. Strader, C. D., Sigal, I. S. & Dixon, R. A. F. (1989) *FASEB J.* **3**, 1825–1832.
6. Kühn, H. & Dreyer, W. J. (1972) *FEBS Lett.* **20**, 1–6.
7. Kühn, H., Cook, J. H. & Dreyer, W. J. (1973) *Biochemistry* **12**, 2495–2502.
8. Liebman, P. A. & Pugh, E. M. (1980) *Nature (London)* **287**, 734–736.
9. Sibley, D. R., Strasser, R. H., Caron, M. G. & Lefkowitz, R. J. (1985) *J. Biol. Chem.* **260**, 3883–3886.
10. Thompson, P. & Findlay, J. B. C. (1984) *Biochem. J.* **220**, 773–780.
11. Kühn, H. (1984) in *Progress in Retinal Research*, eds. Osborne, N. N. & Chader, G. J. (Pergamon, Oxford), pp. 123–156.
12. Lefkowitz, R. J., Hausdorff, W. P. & Caron, M. G. (1990) *Trends Pharmacol. Sci.* **11**, 190–194.
13. Hausdorff, W. P., Caron, M. G. & Lefkowitz, R. J. (1990) *FASEB J.* **4**, 2881–2889.
14. Hausdorff, W. P., Campbell, P. T., Ostrowski, J., Yu, S. S., Caron, M. G. & Lefkowitz, R. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2979–2983.
15. Kühn, H., Hall, S. & Wilden, U. (1984) *FEBS Lett.* **176**, 473–478.
16. Kühn, H. & Wilden, U. (1987) *J. Recept. Res.* **7**, 298–383.
17. Lohse, M. J., Benovic, J. L., Codina, J., Caron, M. G. & Lefkowitz, R. J. (1990) *Science* **248**, 1547–1550.
18. Benovic, J. L., Strasser, R. H., Caron, M. G. & Lefkowitz, R. J. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2797–2801.
19. Palczewski, K., McDowell, J. H. & Hargrave, P. A. (1988) *J. Biol. Chem.* **263**, 14067–14073.
20. Palczewski, K., McDowell, J. H. & Hargrave, P. A. (1988) *Biochemistry* **27**, 2306–2313.
21. Palczewski, K., Carruth, M. E., Adamus, G., McDowell, J. H. & Hargrave, P. A. (1990) *Vision Res.* **30**, 1129–1137.
22. Benovic, J. L., Mayor, F., Staniszewski, C., Lefkowitz, R. J. & Caron, M. G. (1987) *J. Biol. Chem.* **262**, 9026–9032.
23. Benovic, J. L., Mayor, F., Somers, R. L., Caron, M. G. & Lefkowitz, R. J. (1986) *Nature (London)* **321**, 869–872.
24. Benovic, J. L., DeBlasi, A., Stone, W. C., Caron, M. G. & Lefkowitz, R. J. (1989) *Science* **241**, 235–240.
25. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
26. Henikoff, S. (1984) *Gene* **28**, 351–359.
27. Zuker, C. S., Cowman, A. F. & Rubin, G. M. (1985) *Cell* **40**, 851–858.
28. Langer-Sofer, P. R., Levine, M. L. & Ward, D. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4381–4385.
29. Hafen, E., Levine, M., Garber, R. L. & Gehring, W. J. (1983) *EMBO J.* **2**, 617–623.
30. Studier, F. W., Rosenberg, A. H., Dunn, J. J. & Dubendorff, J. W. (1990) *Methods Enzymol.* **185**, 60–89.
31. Stamnes, M. A., Shieh, B.-H., Chuman, L., Harris, G. L. & Zuker, C. S. (1991) *Cell* **65**, 219–227.
32. Sved, J. (1986) *Drosophila Inf. Serv.* **73**, 169.
33. Kalderon, D. & Rubin, G. M. (1988) *Genes Dev.* **2**, 1539–1556.
34. Schaeffer, E., Smith, D., Mardon, G., Quinn, W. & Zuker, C. S. (1989) *Cell* **57**, 403–412.
35. Hanks, S. K., Quinn, A. N. & Hunter, T. (1988) *Science* **241**, 42–52.
36. Hanks, S. K. (1991) *Curr. Opinion Struct. Biol.* **1**, 369–383.
37. Georgi, L. L., Albert, P. S. & Riddle, D. L. (1990) *Cell* **61**, 635–645.
38. Benovic, J. L., Regan, J. W., Matsui, H., Mayor, F., Cotecchia, S., Leeb-Lundberg, L. M. F., Caron, M. G. & Lefkowitz, R. J. (1987) *J. Biol. Chem.* **262**, 17251–17253.
39. Kwatra, M. M., Benovic, J. L., Caron, M. G., Lefkowitz, R. J. & Hosey, M. M. (1989) *Biochemistry* **28**, 4543–4547.
40. Hardie, G. (1988) *Nature (London)* **335**, 592–593.
41. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
42. Bouvier, M., Guilbault, N. & Bonin, H. (1991) *FEBS Lett.* **279**, 243–248.
43. Benovic, J. L., Onorato, J. J., Arriza, J. L., Stone, W. C., Lohse, M., Jenkins, N. A., Gilbert, D. J., Copeland, N. G., Caron, M. G. & Lefkowitz, R. J. (1991) *J. Biol. Chem.* **266**, 14939–14946.