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Biophysical Chemistry 56 (1995) 183–188

Biophysical Chemistry

Mechanism of rhodopsin phosphorylation

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Abstract

A key reaction in the inactivation of rhodopsin is its phosphorylation by rhodopsin kinase. In recent years, extensive studies related to rhodopsin kinase function and enzymatic properties were carried out. Rhodopsin kinase is a Ser/Thr protein kinase and a member of the G protein-coupled receptor kinases sub-family (GRKs) which consists of six recently identified members. Photolyzed rhodopsin is phosphorylated by rhodopsin kinase sequentially, with the first phosphate transferred preferentially to Ser-338, and subsequent phosphates transferred to Ser-343 and Thr-336. The binding of arrestin to the receptor, and reduction of the photolyzed chromophore all-*trans*-retinal to all-*trans*-retinol limits physiologically significant phosphorylation at no more than three sites (H. Ohguro, R.S. Johnson, L.H. Ericsson, K.A. Walsh and K. Palczewski, Biochemistry, 33 (1994) 1023). A similar phosphorylation reaction is implicated in most, if not all, G protein-coupled receptors during their desensitization.

Keywords: Rhodopsin; Rhodopsin kinase; Phototransduction; Rod outer segment

1. Introduction

Light-dependent phosphorylation of rhodopsin was first detected in rod outer segment (ROS) homogenates [1–3], and it was also observed in vivo during quenching mechanisms of phototransduction [4–7]. Rhodopsin kinase phosphorylates photolyzed rhodopsin, and prevents continuous activation of the rod-specific G_T protein, transducin (Fig. 1, see also Section 4 'Functions'). Rhodopsin kinase (RK) was purified [8–11], and subsequently the amino acid sequence was elucidated [12]. The enzyme is classified as a Ser/Thr protein kinase and a member of G protein-coupled receptor kinases sub-family (GRKs) [13,14].

2. Biochemical properties

Rhodopsin kinase (MW 62,934 Da) is a soluble protein kinase that is expressed exclusively in retinal photoreceptor cells and in the pineal gland [12,14–16]. Rhodopsin kinase catalyses phosphorylation of photolyzed rhodopsin at several Ser and Thr residues [2,17]. A unique proteolysis of rhodopsin by endoproteinase Asp-N removes all phosphorylation sites, suggesting that they are located at the C-terminus [18]. Further sub-digestion of the C-terminal peptide (Fig. 2) and mass spectroscopy allowed us to identify the phosphorylation sites on the rhodopsin molecules (Fig. 2) [19–21]. The phosphorylation reaction is

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0301-4622/95/$09.50
SSDI 0301-4622(95)00031-3

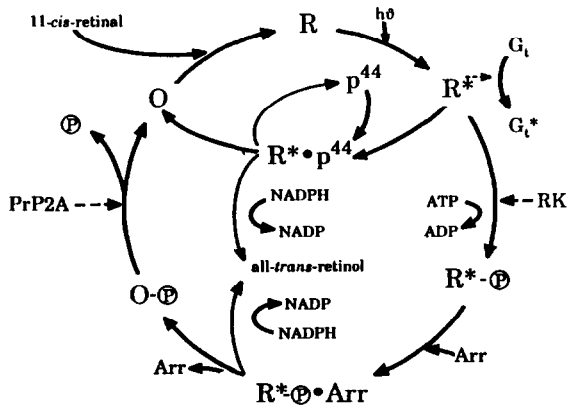

Fig. 1. Flux diagram of rhodopsin deactivation and regeneration. Phototransduction begins with photo-isomerization of rhodopsin's chromophore, 11-*cis*-retinal to all-*trans*-retinal, resulting in formation of *meta*-rhodopsin II (R*), that initiates the excitation cascade of reactions by the activation of several thousand G_T-proteins [52,53]. To terminate the light signal, *meta*-rhodopsin II is deactivated by phosphorylation at multiple sites and binding of a regulatory protein, arrestin. Further decay of *meta*-rhodopsin II results from the reduction of the photolyzed free all-*trans*-retinal into all-*trans*-retinol by disc membrane associated retinol dehydrogenase [54]. Once this reduction has occurred, arrestin dissociates from now inactivated opsin (O). The chromophore is regenerated and phosphates are removed by protein phosphatase 2A (PrP 2A, [55]). A short-cut in the regeneration can occur with the help of membrane associated p^{44}, which may regulate the availability of R* for the interaction with G_T. The complex between p^{44}/arrestin and activated rhodopsins fall apart, by reduction of all-*trans*-retinal to all-*trans*-retinol. In vivo, p^{44} interaction with R* could be the only physiologically relevant mechanism of quenching photolyzed rhodopsin.

sequential with the first phosphate transferred preferentially to Ser-338 of rhodopsin, and subsequent phosphorylations are at Ser-343 and Thr-336 [19]. Rhodopsin kinase is a highly specific enzyme that phosphorylates: (i) photoactivated rhodopsin [1–3]; (ii) topologically related receptors such as photolyzed iodopsin (chicken red cone pigment) [22] and agonist-occupied $ß_2$-adrenergic receptor [23]. Rhodopsin kinase phosphorylates peptide substrates only marginally and presumably by different mechanisms than that of receptors, as reflected by (i) large differences in V_{max}/K_M [9,24–28], (ii) a different mechanism of inhibition by the antibody directed against the N-terminal portion of rhodopsin kinase [16], and (iii) lack of peptide phosphorylation by a mutant of rhodopsin kinase K491A, but high activity of this mutant against photolyzed rhodopsin (K. Palczewski, H. Ohguro, R. Premont and J. Inglese, in press). As a donor of phosphate, Mg–ATP is a better substrate than Mg–GTP, and the kinase requires an extra Mg^{2+} for maximum activity [11,24]. Inhibitors of rhodopsin kinase are: polyanions, peptides derived from the cytosolic surface of rhodopsin, and nucleoside sangivamycin [24,29,30].

3. Structure

Rhodopsin kinase is a monomeric protein with an overall structure related to other protein kinases, and in particular to the G protein-coupled receptor kinases (Fig. 3) [12,13]. The catalytic domain is located in the mid-region of the rhodopsin kinase sequence (position 185–455) and the N-terminal region was implicated in the recognition of photoactivated rhodopsin. The catalytic domain of rhodopsin kinase contains essential motifs of protein kinases: G–x–G–x–x–G implicated in a fold that is responsible for ATP binding at position 193; invariable Asp-332 and Lys-216 involved in catalysis. Ten Cys residues identified in the sequences, from which an unknown number of disulfide bridges may be formed. Rhodopsin kinase is post-translationally modified:

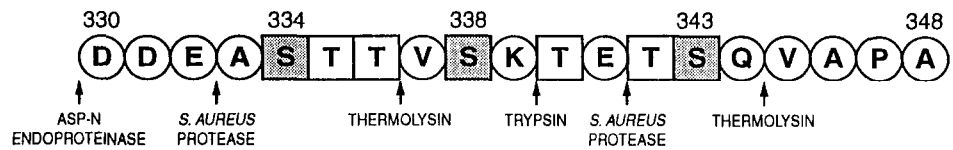

Fig. 2. Model of the C-terminal 19-residue region of rhodopsin used to identify the sites of phosphorylation. This peptide, obtained by cleavage of rod outer segment membranes with endoproteinase Asp-N, contains three Ser (shaded squares) and four Thr residues (open squares), which are potential sites of phosphorylation by rhodopsin kinase. Cleavage sites by thermolysin, trypsin, and *S. aureus protease* V8 are shown by arrows. Further analysis by mass spectrometry and Edman degradation allowed us to identify the sites of phosphorylation [19,51].

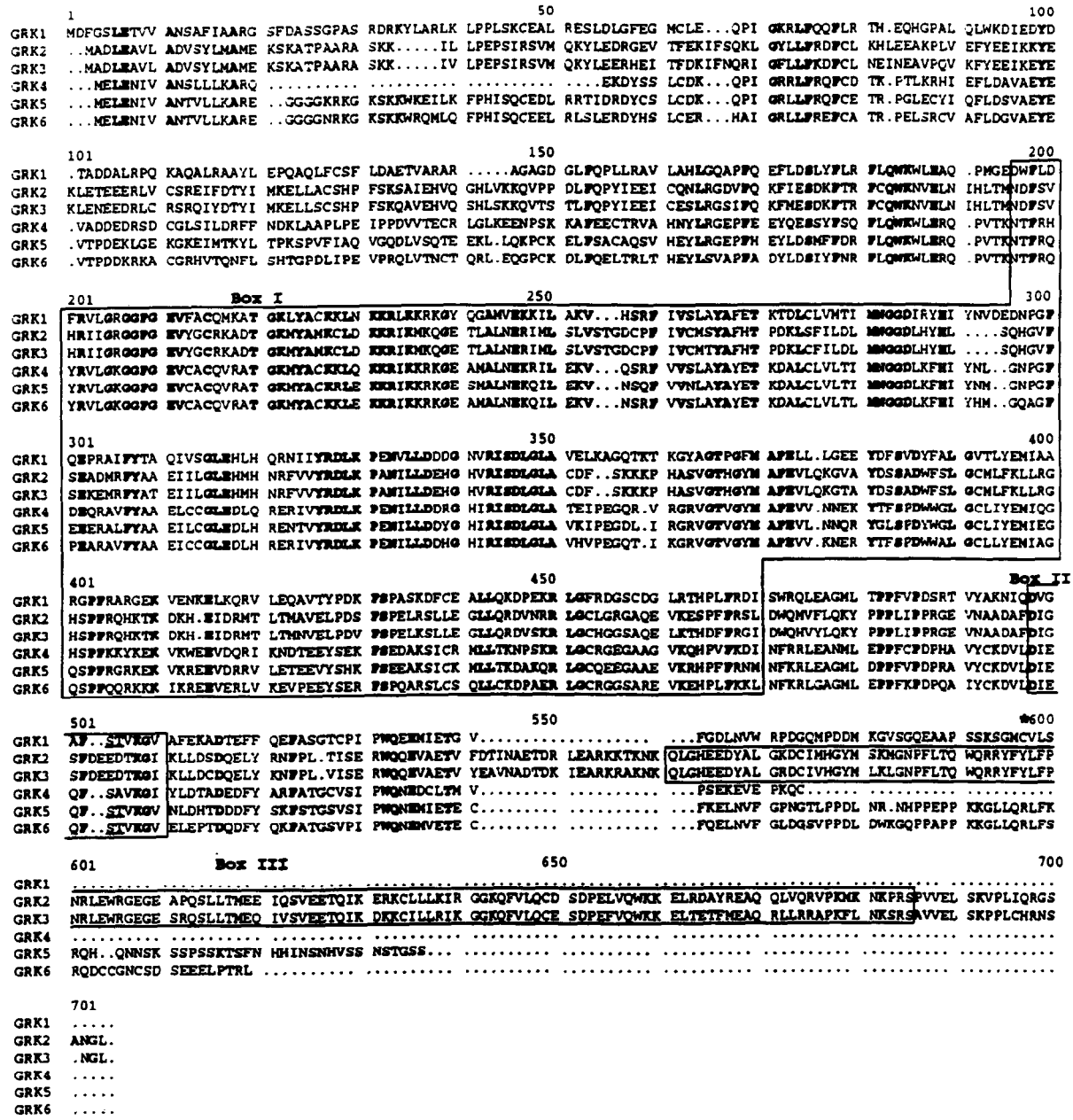

Fig. 3. The amino acid sequence alignment of G protein-coupled receptor kinases (GRKs). GRK1, bovine rhodopsin kinase [12]; GRK2, bovine ß-adrenergic receptor kinase 1 [56]; GRK3, bovine ß-adrenergic receptor kinase 2 [57]; GRK4, human IT11-A protein kinase [58]; GRK5, human GRK5 [59]; GRK6, human GRK6 [60] are aligned. Box I represents catalytic domain, box II is autophosphorylation region, and box III is the Gßγ-binding domain (reviewed by Inglese et al. [11]). Autophosphorylated residues in GRK 1,5,6 are underlined and the isoprenylation site in GRK1 is indicated by asterisk. The sequence similarity between: (i) rhodopsin kinase and other GRKs is 68 to 78%; (ii) ßARKs is 96%; and (iii) GRK5 and 6 is 90%.

(1) at the C-terminus by farnesylation, proteolysis, and α-carboxylmethylation [31,32]; (2) by autophosphorylation at residues Ser-21, Ser-488, and Thr-489 [33,34]; and (3) at the N-terminus which is blocked by an unidentified modification.

Double mutations at residues Ser-488 and Thr-489 in rhodopsin kinase to neutral Ala or acidic residue(s) Asp decrease autophosphorylation to sub-stoichiometrical levels, while single mutations at either residue reduce autophosphorylation by half. Mutations in the autophosphorylation region affected the K_M for ATP, increase 'dark activity' toward rhodopsin, and change the initial sites of phosphorylation, implying that this region is involved in the ATP binding and may regulate selectivity of the site of phosphorylation on photolyzed rhodopsin (K. Palczewski, H. Ohguro, R. Premont and J. Inglese, in press).

Rhodopsin kinase is activated by photolyzed rhodopsin upon binding to the cytoplasmic loops of photolyzed rhodopsin, presumably by the V–VI loop, while the C-terminal region of rhodopsin does not appear to be critical in anchoring rhodopsin kinase to disk membranes [18,27,28]. The site on rhodopsin kinase where it binds to photolyzed rhodopsin (the activation site, presumably the N-terminus)[16] is separated from the site of phosphorylation (the catalytic site, mid-portion of rhodopsin kinase) [12]. The activation mechanisms involve the conformational change that brings the ATP binding site(s) and the peptide binding site into closer proximity, resulting in more efficient phospho-transfer catalysis. Binder et al. [35], reported that at low levels of bleaching, even non-bleached rhodopsin is phosphorylated, suggesting that when kinase is activated by photolyzed rhodopsin, it might phosphorylate the C-terminal regions of non-photolyzed rhodopsin located nearby. In general, this mechanism distinguishes rhodopsin kinase from other non-receptor protein kinases.

Interestingly, rhodopsin kinase is capable of phosphorylating opsin molecule, but only in the presence of photolyzed chromophore, all-*trans*-retinal [36], or several retinal analogs, including retinoids lacking the 13 methyl or the two terminal carbons of the polyene chain (K. Palczewski, S. Jager, J. Buczylko, R.K. Crouch, L.D. Bredberg, K.P. Hofmann, M.-A. Asson-Batres and J.C. Saari, Biochemistry, 33, 13741–13750). Since all-*trans*-retinal could accumulate due to low and reversible retinol dehydrogenase activity in outer ROS in physiological condition this reverse reaction could impede the recycling of rhodopsin at high bleach levels (K. Palczewski, S. Jager, J. Buczylko, R.K. Crouch, L.D. Bredberg, K.P. Hofmann, M.-A. Asson-Batres and J.C. Saari, Biochemistry, 33, 13741–13750). This property could be utilized in designing specific inhibitors of the constitutively active opsin [37] in patients with retinitis pigmentosa who are affected by mutation in the chromophore attachment site at residues Lys-296 [38].

4. Functions

In retinal photoreceptor cells, during transition of photolyzed rhodopsin to inactivated opsin, the receptor assumes three relatively stable conformations *meta*-rhodopsin: I, II, and III [39,40]. *meta*-Rhodopsin II [1] binds to and activates a photoreceptor-specific G protein, and initiates the signal-amplifying cascade of reactions (Fig. 1) [41,42]. This activated state of rhodopsin, along with *meta*-rhodopsin I [44], is phosphorylated by rhodopsin kinase [45,46], a major protein kinase in rod outer segments with an estimated concentration of 10 to 50 μM [11]. The dissociation of the kinase is followed by the binding of arrestin, thereby preventing continuous G protein activation [36,47,48]. It is conceivable that alternative mechanisms that do not include rhodopsin phosphorylation are also taking place and involve a splice variant of arrestin [49,50], as depicted in Fig. 1.

Acknowledgements

Supported by NIH Grants EY08061 and EY01730, pre-doctoral fellowship from Merck Foundation, and by an award from Research to Prevent Blindness to the Department of Ophthalmology at the University of Washington; and by the Human Frontier Science

[1] meta-Rhodopsin II (refereed also as R*) is not a single entity, but a mixture of several forms (at least two, see [43]).

Program. K.P. is a Jules and Doris Stein Professor of Research to Prevent Blindness.

References

[1] D. Bownds, J. Dawes, J. Miller and M. Stahlman, Nature New Biol., 237 (1972) 125.
[2] H. Kühn and W.J. Dreyer, FEBS Lett., 20 (1972) 1.
[3] R.N. Frank, H.D. Cavangh and K.R. Kenyon, J. Biol. Chem., 248 (1973) 596.
[4] H. Kühn, Nature, 250 (1974) 588.
[5] H. Kühn, in N. Osborne and G.J. Chader (Editors), Progress in Retinal Research, Vol. 3, Pergamon Press, 1984, p. 123.
[6] M.S. Birnbaum, B.M. Bider and M.D. Bownds, Vis. Neurosci., 7 (1991) 499.
[7] K. Palczewski and J.L. Benovic, Trends Biol. Sci., 16 (1991) 387.
[8] K. Palczewski, J.H. McDowell and P.A. Hargrave, J. Biol. Chem., 263 (1988) 14067.
[9] D.J. Kelleher and G.L. Johnson, J. Biol. Chem., 265 (1990) 2632.
[10] J. Buczylko, C. Gutmann and K. Palczewski, Proc. Natl. Acad. Sci. USA, 88 (1991) 2568.
[11] K. Palczewski, in P.A. Hargrave (Editor), Method in Neuroscience, Vol. 15, Academic Press, 1993, p. 217.
[12] W. Lorenz, J. Inglese, K. Palczewski, J.J. Onorato, M.G. Caron and R.J. Lefkowitz, Proc. Natl. Acad. Sci. USA, 88 (1991) 8715.
[13] J. Inglese, N.J. Freedman, W.J. Koch and R.J. Lefkowitz, J. Biol. Chem., 268 (1993) 23735.
[14] R.L. Somers and D.C. Klein, Science, 226 (1984) 182.
[15] K. Palczewski, M.E. Carruth, G. Adamus, J.H. McDowell and P.A. Hargrave, Vision Res., 30 (1990) 1129.
[16] K. Palczewski, J. Buczylko, L. Lebioda, J.W. Crabb and A.S. Polans, J. Biol. Chem., 268 (1993) 6004.
[17] P. Thompson and J.B.C. Findlay, Biochem. J., 220 (1984) 773.
[18] K. Palczewski, J. Buczylko, M.W. Kaplan, A.S. Polans and J. W Crabb, J. Biol Chem., 266 (1991) 12949.
[19] H. Ohguro, K. Palczewski, K.A. Walsh and R.S. Johnson, Biochemistry, 32 (1993) 5718.
[20] J.H. McDowell, J.P. Nawrocki and P.A. Hargrave, Biochemistry, 32 (1993) 4968.
[21] D.I. Papac, J.E. Oatis, R.K. Crouch and D.R. Knapp, Biochemistry, 32 (1993) 5930.
[22] Y. Fukada, K. Kokame, T. Okano, Y. Shichida, T. Yoshizawa, J.H. McDowell, P.A. Hargrave and K. Palczewski, Biochemistry, 29 (1990) 10102.
[23] J.L. Benovic, F. Mayer, Jr, R.L. Somers, M.G. Caron and R.J. Lefkowitz, Nature, 321 (1986) 869.
[24] K. Palczewski, J.H. McDowell and P.A. Hargrave, Biochemistry, 27 (1988) 2306.
[25] K. Palczewski, A. Arendt, J.H. McDowell and P.A. Hargrave, Biochemistry, 28 (1989) 8764.
[26] J.J. Onorato, K. Palczewski, J.W. Regan, M.J. Caron, R.J. Lefkowitz and J.L. Benovic, Biochemistry, 30 (1991) 5118.
[27] C. Fowles, R. Sharma and M. Akhtar, FEBS Lett., 238 (1988) 56.
[28] N.G. Brown, C. Fowles, R. Sharma and M. Akhtar, Eur. J. Biochem., 208 (1992) 659.
[29] K. Palczewski, N. Kahn and P.A. Hargrave, Biochemistry, 29 (1990) 6267.
[30] K. Palczewski, G. Rispoli and P.B. Detwiler, Neuron, 8 (1992) 1.
[31] J. Inglese, J.F. Glickman, W. Lorenz, M.G. Caron and R.J. Lefkowitz, J. Biol. Chem., 267 (1992) 1422.
[32] J.S. Anant and B.K.-K. Fung, Biochem. Biophys. Res. Commun., 183 (1992) 468.
[33] K. Palczewski, J. Buczylko, P. Van Hooser, S.A. Carr, M.J. Huddleston and J.W. Crabb, J. Biol. Chem., 267 (1992) 18991.
[34] J.A. Crabb, C. Johnson, K. West, J. Buczylko, K. Palczewski, J. Hou, K. McKeehan, M. Kan, W.L. McKeehan, M.J. Huddleston and S.A. Carr, in R.H. Angeletti (Editor), Techniques in Protein Chemistry, Academic Press, 1993, p. 171.
[35] B.M. Binder, M.S. Biernbaum and M.D. Bownds, J. Biol. Chem., 265 (1990) 15333.
[36] K.P. Hofmann, A. Pulvermüller, J. Buczylko, P. Van Hooser and K. Palczewski, J. Biol. Chem., 267 (1992) 15701.
[37] P.R. Robinson, G.B. Cohen, E.A. Zhukovsky and D.D. Oprian, Neuron, 9 (1992) 719.
[38] P.R. Robinson, J. Buczylko, H. Ohguro and K. Palczewski, Proc. Natl. Acad. Sci. USA 91, 5411–5415.
[39] K.P. Hofmann, Photobiochem. Photobiophys., 13 (1986) 309.
[40] T. Yoshizawa and H. Kandori, in N. Osborne and G.J. Chader (Editors), Progress in Retinal Research, Vol. 11, Pergamon Press, 1991, p. 33.
[41] M. Chabre and P. Deteree, Eur. J. Biochem., 179 (1989) 255.
[42] L. Lagnado and D. Baylor, Neuron, 8 (1992) 995.
[43] S. Arnis and K.P. Hofmann, Proc. Natl. Acad. Sci. USA, 90 (1993) 7849.
[44] A. Pulvermuller, K. Palczewski and K.P. Hofmann, Biochemistry, 32 (1993) 14082.
[45] A. Sitaramayya and P.A. Liebman, J. Biol. Chem., 258 (1983) 1205.
[46] A. Sitaramayya and P.A. Liebman, J. Biol. Chem., 258 (1983) 12106.
[47] U. Wilden and H. Kühn, Biochemistry, 21 (1982) 3014.
[48] U. Wilden, S. Hall and H. Kühn, Proc. Natl. Acad. Sci. USA, 83 (1986) 1174.
[49] K. Palczewski, J. Buczylko, H. Ohguro, R.S. Annan, S.A. Carr, J.W. Crabb, M.W. Kaplan, R.S. Johnson and K.A. Walsh, Protein Sci., 3 (1994) 314.
[50] W.C. Smith, A.H. Milam, D. Dugger, A. Arendt, P.A. Hargrave and K. Palczewski, J. Biol. Chem., 269, 15407.
[51] H. Ohguro, R.S. Johnson, L.H. Ericsson, K.A. Walsh and K. Palczewski, Biochemistry, 33 (1994) 1023.
[52] M.P. Gray-Keller, M.S. Biernbaum and M.D. Bownds, J. Biol. Chem., 265 (1990) 15323.

[53] M. Kahlert, D.R. Pepperberg and K.P. Hofmann, Nature, 345 (1990) 537.
[54] J.C. Saari, in N. Osborne and G.J. Chader (Editors), Progress in Retinal Research, Vol. 9, Pergamon Press, 1990, p. 363.
[55] K. Palczewski, P.A. Hargrave, J.H. McDowell and T.S. Ingebritsen, Biochemistry, 27 (1989) 415.
[56] J.L. Benovic, A. DeBlasi, W.C. Stone, M.G. Caron and R.J. Lefkowitz, Science, 246 (1989) 235.
[57] J.L. Benovic, J.J. Onorato, J.L. Arriza, W.C. Stone, M. Lohse, N.A. Jenkins, D.J. Gilbert, N.G. Copeland, M.G. Caron and R.J. Lefkowitz, J. Biol. Chem., 266 (1991) 14939.
[58] C. Ambrose, M. James, G. Barnes, C. Lin, G. Bates, M. Altherr, M. Duyao, N. Groot, D. Church, J.J. Wasmuth, H. Lehrach, D. Housman, A. Bucker, J.F. Gusella and M.E. MacDonald, Hum. Mol. Genet., 1 (1992) 697.
[59] P. Kunapuli and J.L. Benovic, Proc. Natl. Acad. Sci. USA, 90 (1993) 5588.
[60] J.L. Benovic and J. Gomez, J. Biol. Chem., 268 (1993) 19521.