ANTHONY ARTALE
MED LIB NATHAN CUMMINGS CTR (S-46)
MEMORIAL SLOAN KETTERING CANCER CTR
1275 YORK AVENUE
NEW YORK, NY 10021
UNITED STATES

Client Number: DDS36478
Title: **TRENDS IN PHARMACOLOGICAL SCIENCES**
DB Ref. No.: IRN10366349
ISSN: ISSN01677691
Vol./Issue: 15
Date: 1994
Pages: 170-172
Article Title: A COMMON STEP FOR SIGNAL TRANSDUCTION IN
Article Author: OLIVEIRA L
Report Number: IRN10366349
Publisher: ELSEVIER/NORTH HOLLAND,

Estimated cost for this 3 page document: $10.2 document supply fee + $30 copyright = $40.2

6 Alonso-Llamazares, A., Zamanillo, D., Fernandez, A., Chinchetru, M. A. and Calvo, P. (1993) *NeuroReport* 4, 1266–1268
7 Battaglia, G., Shannon, M., Borgundvaag, B. and Titeler, M. (1983) *J. Neurochem.* 41, 538–544
8 Morrow, A. L. and Creese, I. (1986) *Mol. Pharmacol.* 29, 321–330
9 Johnson, R. D. and Minneman, K. P. (1987) *Mol. Pharmacol.* 31, 239–246
10 Han, C., Abel, P. W. and Minneman, K. P. (1987) *Mol. Pharmacol.* 32, 505–510
11 Hanft, G. and Groß, G. (1989) *Br. J. Pharmacol.* 97, 691–700
12 Boer, R., Grassegger, A., Schudt, C. H. and Glossmann, H. (1989) *Eur. J. Pharmacol.* 172, 131–138
13 Michel, A. D., Loury, D. N. and Whiting, R. L. (1989) *Br. J. Pharmacol.* 98, 883–889
14 Sleight, A. J., Koek, W. and Bigg, D. C. H. (1993) *Eur. J. Pharmacol.* 238, 407–410
15 Han, C. and Minneman, K. P. (1991) *Mol. Pharmacol.* 40, 531–538
16 Hiramatsu, Y., Muraoka, R., Kigoshi, S. and Muramatsu, I. (1992) *Br. J. Pharmacol.* 105, 1071–1076
17 Kenakin, T. P. (1993) *Trends Pharmacol. Sci.* 14, 431–432
18 Hall, J. M., Caulfield, M. P., Watson, S. P. and Guard, S. (1993) *Trends Pharmacol. Sci.* 14, 47–52
19 Ohmura, T., Oshita, M., Kigoshi, S. and Muramatsu, I. (1992) *Br. J. Pharmacol.* 107, 697–704
20 Blue, D. R. and Clarke, D. E. (1990) *Br. J. Pharmacol.* 99, 204P
21 Clarke, D. E., Whiting, R. L., Pfister, J. and Blue, D. R. (1991) *Br. J. Pharmacol.* 102, 196P
22 Williams, T. J. and Clarke, D. E. (1994) *Br. J. Pharmacol.* 112, 207P
23 Cotecchia, S. *et al.* (1988) *Proc. Natl Acad. Sci. USA* 85, 7159–7163
24 Pimoule, C., Graham, D. and Langer, S. Z. (1992) *Br. J. Pharmacol.* 105, 233P
25 Schwinn, D. A. *et al.* (1990) *J. Biol. Chem.* 265, 8183–8189
26 Lomasney, J. W. *et al.*, (1991) *J. Biol. Chem.* 266, 6365–6369
27 Perez, D. M., Piascik, M. T. and Graham, R. M. (1991) *Mol. Pharmacol.* 40, 876–883
28 Forray, C. *et al.* (1994) *Mol. Pharmacol.* 45, 703–708
29 Michel, M. C., Kerker, J., Branchek, T. A. and Forray, C. (1993) *Mol. Pharmacol.* 44, 1165–1170
30 Rokosh, D. G. *et al.* (1994) *Biochem. Biophys. Res. Commun.* 200, 1177–1184
31 Oshita, M., Kigoshi, S. and Muramatsu, I. (1993) *Br. J. Pharmacol.* 108, 6–7
32 Kenny, B. A. *et al.* (1994) *Br. J. Pharmacol.* 111, 1003–1008
33 Goetz, A., Lutz, M., Carpi, E., Rimele, T. and Saussy, D. (1993) *FASEB J.* 7, A696
34 Michel, M. C. and Insel, P. A. (1994) *Br. J. Pharmacol.* 112, 59P
35 Clarke, D. E. *et al. Proceedings of the IUPHAR Satellite 'Pharmacology of Adrenoceptors'* (in press)
36 Graziadei, I., Zernig, G., Boer, R. and Glossman, H. (1989) *Eur. J. Pharmacol.* 172, 329–337

HEAT: 2-β-4-hydroxy-3-iodophenylethyl-aminomethyltetralone
WB4101: 2-(2,6-dimethoxyphenoxyethyl)-aminoethyl-1,4-benzodioxane

A common step for signal transduction in G protein-coupled receptors

G protein-coupled receptors form a large superfamily of proteins that transduce signals across the cell membrane. At the external side they receive a ligand (or a photon in the case of opsins), and at the cytosolic side they activate a G protein. G protein-coupled receptors can be divided into three main families: rhodopsin-like, secretin-like and metabotropic glutamate-like receptors[1]. All these receptors consist of a single protein chain that crosses the membrane seven times, similar to the seven-transmembrane-helix bundle of bacteriorhodopsin[2]. Most ligands bind between the membrane helices, but the periplasmic loops are sometimes also involved in ligand recognition. The second and third cytosolic loop and part of the (cytosolic) C-terminus of the receptors are involved in G protein recognition and binding.

Hundreds of G protein-coupled receptors have been identified, and they can be activated by a multitude of agonists. Five steps are involved in the process of G protein-coupled-receptor activation[3]: (1) creation of the signal by absorption of a photon or by ligand binding; (2) transduction of the signal through the membrane; (3) interaction with the G protein; (4) activation of the G protein; and (5) activation of the second messenger. Evidence presented below indicates that an early step in G protein activation may follow a common scheme in all rhodopsin-like G protein-coupled receptors.

Modelling studies

Most modelling studies are based on the assumption that G protein-coupled receptors are structurally homologous to bacteriorhodopsin. The merits of the different modelling procedures have recently been reviewed[4,5]. We have constructed explicit atomic models of the transmembrane region of many G protein-coupled receptors based on the structural framework of bacteriorhodopsin and the alignment shown in Fig. 1. As there is no structural information for the loops in bacteriorhodopsin, G protein-coupled receptor loops cannot be modelled by homology.

Although the mutual alignment of G protein-coupled receptors is straightforward[1,6], the alignment of G protein-coupled receptors with bacteriorhodopsin is problematic. Consequently, many alignments have been suggested that differ primarily by one-turn helical shifts[1,6–8]. Despite these differences, models based on these alignments have common features: residues involved in ligand interaction cluster in the same spatial region and conserved polar residues form a pocket between the intracellular halves of the helices I, III, VI and VII, in particular residues Asn130, Asp224 and Asn729 (Figs 1 and 2). This region corresponds to the proton channel locus in bacteriorhodopsin[2] and to the allosteric site described for some G protein-coupled receptors[9,10].

The polar allosteric pocket

In the signal transduction pathway of G protein-coupled receptors, allosteric effects regulate ligand binding, G protein coupling, and binding of GDP and GTP to the G protein[11,12]. Mutation experiments have provided evidence that the polar allosteric pocket has a central role in these processes. For example, mutations in this pocket can affect the cation dependent regulation of ligand binding[9,10] and/or G protein coupling. Mutation of Asp224 to Asn abolished the cation effect on the allosteric site[10] and weakened the agonist binding to G protein-coupled receptors without affecting antagonist binding[13,14]. The most striking effect involving the polar pocket resulted from mutating Asp339 in the α- and β-adrenoceptors, muscarinic acetylcholine M_1 receptors and mammalian opsins: receptors had increased

affinity for their ligands but reduced or even absent G protein coupling[15–17].

Recent experiments on the receptor for gonadotropin-releasing hormone provide additional evidence for the importance of the polar character of the pocket. Zhou *et al.*[18] showed that the residue pair Asn224 and Asp729 can be inverted to Asp224 and Asn729 without loss of function, whereas the Asp224 and Asp729 pair was not functional. These results suggest either a direct contact between these residues[18], or a requirement for charge conservation in the polar pocket.

G protein binding

The Asp-Arg-Tyr (339–341) motif at the cytosolic end of helix III and the loops between the helices III–IV and V–VI as well as the C-terminal domain are important for G protein coupling and activation. Flash photolysis experiments on mutated rhodopsins were used to determine the roles of these cytosolic regions[19]. The Glu-Arg-Tyr motif (Asp-Arg-Tyr in most other receptors) was shown to be essential for transducin coupling, whereas the other regions appeared to be more important for activation of this G protein. The key role of the Asp-Arg-Tyr sequence was also shown for angiotensin II receptors, where mutating Asp-Arg-Tyr into Gly-Gly-Ala abolished G protein coupling[20].

The arginine switch

Highly conserved residues normally have a functional role in proteins, and the function that is common to all G protein-coupled receptors is that the signal has to be transduced to the G protein. The Arg340 of the Asp-Arg-Tyr sequence is the only residue that is fully conserved in all rhodopsin-like G protein-coupled receptors and it is therefore reasonable to suggest that this residue plays a pivotal role in the signal transduction process. As Arg340 is located both near the polar pocket and the cytosol, it may have a 'switching' role, which is expressed through alternative conformations of its side chain (Fig. 2). The arginine switch is: (1) off when the Arg340 side chain is in the polar pocket; and (2) on when the Arg340 side chain is shifted toward the cytosol where it can bind the G protein. The shift is caused by a rearrangement of the polar pocket as a result of either ligand binding or increased concentration of G protein in the cytosol.

This signalling process can explain the allosteric effect observed for several receptors: agonist binding triggers a small conformational change, such as the rotamer shift of one or more residues, that disturbs the polar pocket, shifts the Arg340 side chain and favours G protein binding. This mechanism can also work in the opposite direction: a G protein can cause the Arg340 side chain to move out of the polar pocket, which triggers a reverse

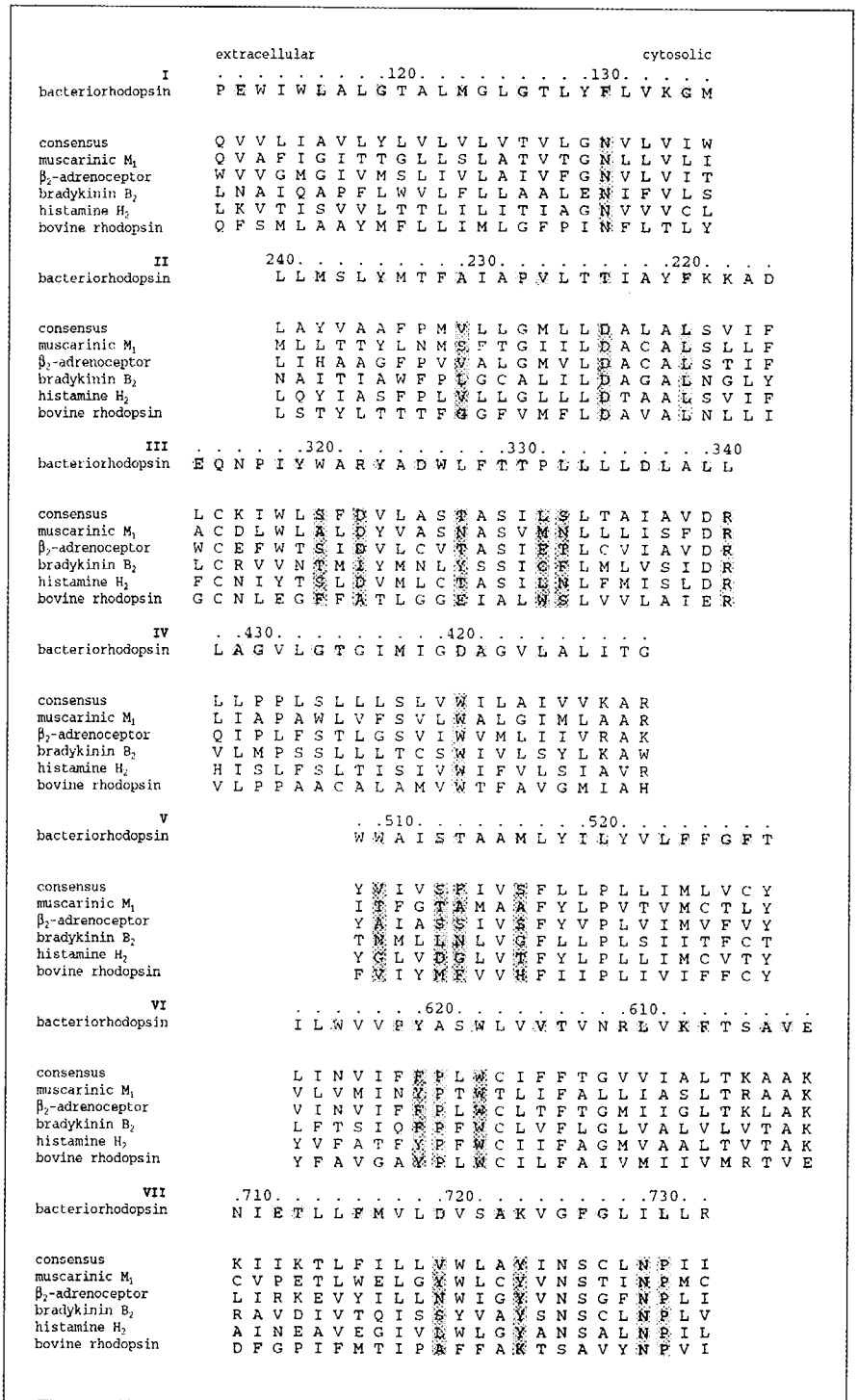

Fig. 1. Aligned sequences of the transmembrane helices bacteriorhodopsin and representative G protein-coupled receptors. The **shaded** *residues point into the central cavity of bacteriorhodopsin. Residues that align vertically in this figure are equally deep in the membrane.* **Pink** *residues are involved in ligand binding and* **blue** *residues are conserved. The residues are numbered such that the 100s digit indicates the helix number.*

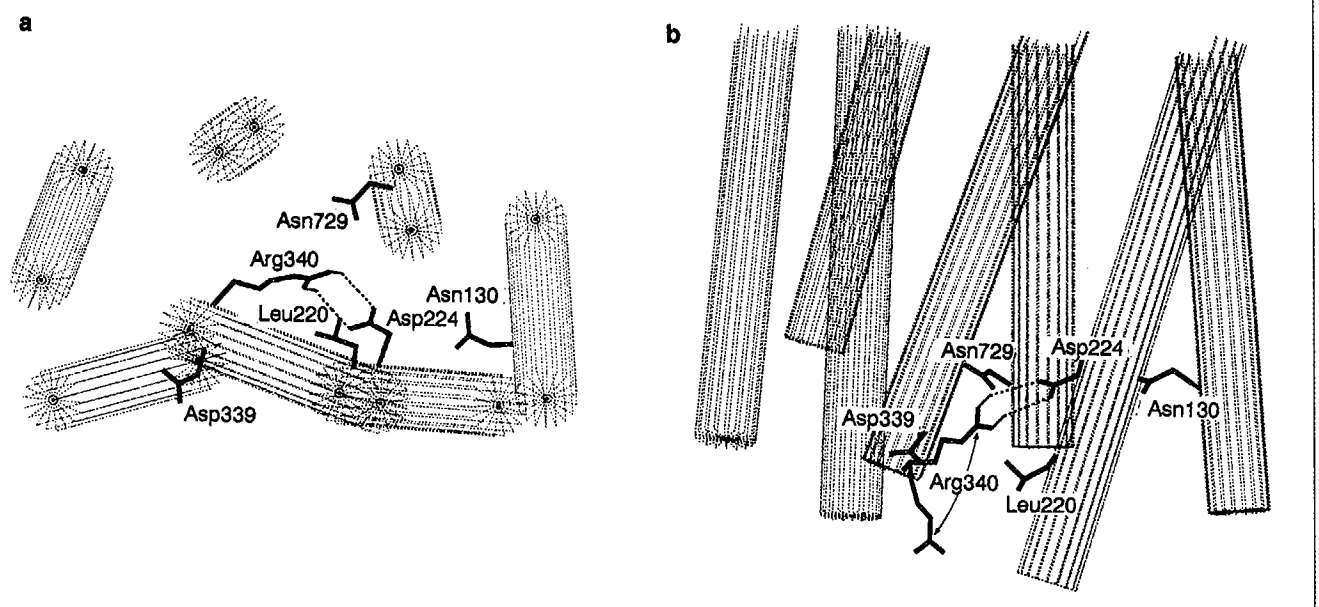

Fig. 2. Structural arrangement of the arginine switch and residues in the polar pocket. Transmembrane helices are shown as **cylinders** *and hydrogen bonds are indicated by* **dashed lines**. **a:** *View perpendicular to the membrane.* **b:** *View in the plane of the membrane. The two alternative switch positions of* **Arg340** *are connected by an* **arrow**.

chain of events leading to modified affinity for the ligand.

□ □ □

Mechanistic models at the single residue level, such as that of the arginine switch, are most useful in focusing further experimental effort. Several conserved residues near Arg340 and the polar pocket appear to be ideal candidates for analysis in this context. In our model, the highly conserved Leu220 is positioned to orient the Arg340 side chain through hydrophobic interaction. Additional position-specific interactions may involve the predominantly polar residues 725 and 726 and the conserved Tyr734 (which is required for agonist-mediated β_2-adrenoceptor sequestration[21]). It will be interesting to see which, if any, role these residues have in signal transduction. Although the arginine-switch hypothesis may aid in the explanation of the allosteric coupling of ligand binding to G protein binding, a comprehensive view of the exquisite chain of molecular events in the action of G protein-coupled receptors may have to await the solution of a first crystal structure.

L. OLIVEIRA, A. C. M. PAIVA, C. SANDER* AND G. VRIEND*

*Escola Paulista de Medicina, Sao Paulo, Brazil and *BIOcomputing, EMBL, Heidelberg, Germany.*

A multiple sequence alignment of all known G protein-coupled receptors is available from the TM7 file server at the EMBL. This server also holds three-dimensional models for the transmembrane regions of more than 200 G protein-coupled receptors. Send a message containing the word 'HELP' to TM7@EMBL-Heidelberg.DE over Internet to get a complete list of G protein-coupled receptor-related services.

Acknowledgements

The authors thank Thue Schwartz for stimulating discussions and for critically reading the manuscript.

References

1 Oliveira, L., Paiva, A. C. M. and Vriend, G. (1993) *J. Comput. Aided Mol. Design* 7, 649–658
2 Henderson, R. *et al.* (1990) *J. Mol. Biol.* 212, 899–929
3 Kaziro, Y. *et al.* (1991) *Annu. Rev. Biochem.* 60, 349–400
4 Westkaemper, R. B. and Glennon, R. A. (1993) *Med. Chem. Res.* 3, 317–334
5 Hoflack, J., Trumpp-Kallmeyer, S. and Hibert, M. (1994) *Trends Pharmacol. Sci.* 15, 7–9
6 Baldwin, J. M. (1993) *EMBO J.* 12, 1693–1703
7 Cronet, P., Sander, C. and Vriend, G. (1993) *Prot. Eng.* 6, 59–64
8 Hibert, M., Trumpp-Kallmeyer, S., Bruinvels, A. and Hoflack, J. (1991) *Mol. Pharmacol.* 40, 8–15
9 Nunnari, J. M., Repaske, M. G., Brandon, S., Cragoe, E. J. and Limbird, L. E. (1987) *J. Biol. Chem.* 262, 12387–12392
10 Neve, K. A. (1991) *Mol. Pharmacol.* 39, 570–578
11 Hepler, J. R. and Gilman, A. G. (1992) *Trends Pharmacol. Sci.* 17, 383–387
12 Gilman, A. G. (1987) *Annu. Rev. Biochem.* 56, 615–649
13 Chung, F-Z., Wang, C-D., Potter, P. C., Venter, J. C. and Fraser, C. M. (1988) *J. Biol. Chem.* 263, 4052–4055
14 Strader, C. D., Sigal, I. S., Register, R. B., Candelore, E. R. and Dixon, R. A. F. (1987) *Proc. Natl Acad. Sci. USA* 84, 4384–4388
15 Fraser, C. M., Chung, F-Z., Wang, C-D. and Venter, J. C. (1988) *Proc. Natl Acad. Sci. USA* 85, 5478–5482
16 Fraser, C. M. (1989) *J. Biol. Chem.* 264 9266–9270
17 Wang, C-D. *et al.* (1991) *Mol. Pharmacol* 40, 168–179
18 Zhou, W. *et al.* (1994) *Mol. Pharmacol.* 45, 165–170
19 Franke, R. R., König, B., Sakmar, T. P., Khorana, H. G. and Hofmann, K. P. (1990) *Science* 250, 123–125
20 Ohyama, K., Yamano, Y., Chaki, S., Kondo, T. and Inagami, T. (1992) *Biochem. Biophys. Res. Commun.* 189, 677–683
21 Barak, L. S. *et al.* (1994) *J. Biol. Chem.* 269, 2790–2795