FEBS 20261 FEBS Letters 430 (1998) 37–40

Minireview

G protein-coupled receptor kinase 2 (GRK2): mechanisms of regulation and physiological functions

Anna M. Aragay, Ana Ruiz-Gómez, Petronila Penela, Susana Sarnago, Ana Elorza, M. Carmen Jiménez-Sainz, Federico Mayor, Jr.*

Departamento de Biología Molecular, Centro de Biología Molecular "Severo Ochoa" (Consejo Superior de Investigaciones Científicas - Universidad Autónoma de Madrid), 28049 Madrid, Spain

Received 22 April 1998

Abstract G protein-coupled receptor kinase 2 (GRK2) plays a key role in determining the rate and extent of G protein-coupled receptor (GPCR) desensitization and resensitization. Recent data indicate that GRK2 activity, subcellular distribution and expression are tightly regulated. The important physiological function of GRK2 as a modulator of the efficacy of GPCR signal transduction systems is exemplified by its relevance in cardiovascular physiopathology as well as by its emerging role in the regulation of chemokine receptors.

Key words: G protein-coupled receptor kinase; Desensitization; Cardiovascular; Chemokine receptor

The cellular responses to agonists acting through G protein-coupled receptors (GPCRs) are usually rapidly attenuated, a process that is critical to prevent the uncontrolled stimulation of cells. Mechanisms of attenuation include removal of agonist from the extracellular fluid and receptor desensitization, endocytosis and down-regulation. Receptor phosphorylation by specific G protein-coupled receptor kinases (GRKs) plays a key role in triggering rapid desensitization [1–5]. The GRK-mediated phosphorylation of the agonist-occupied receptor promotes the binding of a member of the family of uncoupling proteins termed β-arrestins, resulting in the uncoupling of the receptor from G proteins.

There are six known members of the GRK family, GRK1 to GRK6, which share a number of structural and functional similarities [2,4,6,7] and differ in both an N-terminal domain of largely unknown function, and a C-terminal domain of variable length, that contains specific determinants for membrane attachment. GRK2 is a ubiquitous member of the GRK family which has been shown to phosphorylate a variety of GPCRs (see [3] for a recent review). Recent evidence indicates that, besides promoting receptor uncoupling, GRK2 would also directly participate in GPCR sequestration, thus triggering receptor dephosphorylation and recycling [5,8–10]. Moreover, GRK2 and β-arrestin have been suggested to participate in the regulation of the mitogen-activated protein kinase cascade by GPCR [11]. This fact, together with the recently reported phosphorylation of tubulin by GRK2 [12], further stresses the relevance of this kinase in GPCR signaling. This review focuses on the mechanisms of regulation of GRK2 activity and subcellular distribution, as well as on some physiological processes in which an important participation of this kinase has recently been unveiled.

1. Modulation of GRK2 subcellular distribution and activity

GRK2 activity and subcellular localization appear to be subject to complex regulatory processes. Although GRK2 was initially described as a soluble, cytosolic enzyme that transiently translocates to the plasma membrane upon receptor activation, recent data indicate that several GRK2 pools exist inside the cells: cytosolic, plasma membrane-bound and microsomal membrane-bound [11,13–15]. Interestingly, GRK2 activity is regulated upon interaction with several proteins and lipids in the three cellular pools.

GRK2 translocation to the plasma membrane seems to be facilitated by its interaction with free Gβγ subunits released upon receptor activation, as well as with the agonist-occupied form of the receptor itself [2,6]. A recent report has shown that these mechanisms occur in intact cells, and that there is a selective interaction of specific pools of Gβγ subunits with receptors and GRKs [16]. The interaction with Gβγ subunits does not only facilitate the translocation of the kinase to the plasma membrane, but synergistically enhances the agonist-dependent phosphorylation of the receptor [17,18]. The Gβγ binding domain is located in the C-terminal portion of the kinase (residues 546–670), and partially overlaps with a pleckstrin homology (PH) domain (residues 553–651) which N-terminal portion has been suggested to mediate the interaction of GRK2 with phosphatidyl inositol 4,5-bisphosphate (PIP_2) and other phospholipids. The binding of GRK2 to both Gβγ subunits and PIP_2 would be needed for full kinase activation [17,18]. However, the exact mechanism by which GRK2 translocates to the plasma membrane is not fully understood and deserves further investigation.

On the other hand, a significant amount of GRK2 is associated to internal microsomal membranes by means of electrostatic interactions between an N-terminal region of the kinase (residues 88–145) and an unknown protein which is an integral component of the microsomal membrane [13,14]. Most interesting is the fact that the interaction of GRK2 with the unidentified anchoring protein leads to inactivation of the bound kinase. Although Gβγ subunits do not play a preferential role in determining GRK2 subcellular distribution under basal conditions [15], microsome-bound GRK2 activity can be enhanced by stimulation of endogenous heterotrimeric G proteins [14]. Nevertheless, the role of the GRK2 pool in internal membranes still remains unclear. An interesting hy-

*Corresponding author. Fax: +34 (91) 397 47 99.
E-mail: fmayor@cbm.uam.es

PII: S0014-5793(98)00495-5

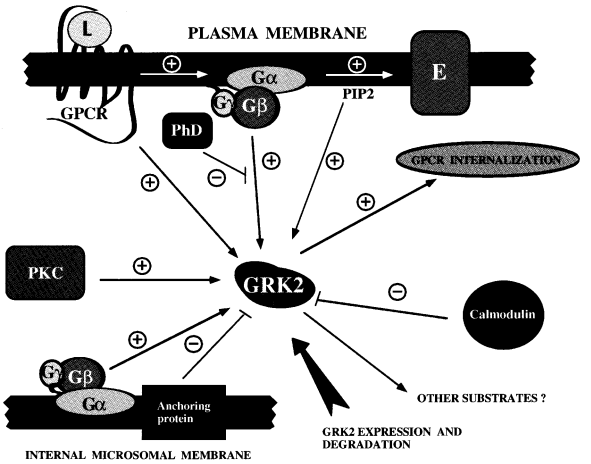

Fig. 1. Modulation of GRK2 activity and subcellular distribution. Upon G protein-coupled receptor (GPCR) activation by ligands (L), GRK2 translocates to the plasma membrane, where its activity is regulated by interaction with the activated GPCR, Gβγ subunits of G proteins and inositol phospholipids (PIP2). Phosducin (PhD) can inhibit Gβγ-mediated modulation. GRK2 participates in GPCR internalization and may phosphorylate other substrates different from activated GPCR. Calmodulin, PKC phosphorylation and the interaction of GRK2 with a microsomal anchoring protein participate in the modulation of GRK2 activity and subcellular localization. See text for details.

pothesis is that this pool acts as a reservoir for its translocation to the plasma membrane upon receptor activation. Alternatively, or in addition, it may also reflect other cellular functions of GRK2. In this context, recent data have shown that GRK2 colocalizes in endosomes with β-adrenergic receptors during agonist-induced receptor internalization [10], and that the kinase cellular complement determines the rate and extent of GPCR desensitization and resensitization [5,8,19]. Furthermore, GRK2 is also associated with other cellular structures, such as microtubules purified from bovine brain, and is able to phosphorylate tubulin ([12] and C. Murga and F. Mayor, Jr., unpublished observations). A better understanding of the distribution and traffic of GRK2 between these different subcellular locations would help to ascertain its different cellular roles.

Other mechanisms appear to contribute to the regulation of GRK2 activity. Calmodulin has been reported to inhibit agonist-dependent phosphorylation of muscarinic receptors or rhodopsin by GRK2 in a Ca^{2+}-dependent manner [20,21]. Recent studies suggest that calmodulin may also inhibit GRK activity in a Ca^{2+}-independent manner [22]. In addition, the cytosolic phosphoproteins phosducin and phosducin-like proteins inhibit GRK2 activity towards GPCR, probably by competing with GRK2 for free Gβγ subunits [23]. Finally, it has recently been shown that GRK2 and GRK5 activities are regulated by PKC phosphorylation [24–26]. Phosphorylation of GRK2 by PKC leads to an activation of the kinase, probably by increasing GRK2 ability to bind to the plasma membrane. It is tempting to suggest that phosphorylation by other unidentified kinases or interaction with additional proteins may also alter GRK2 functions. In fact, our laboratory has recently identified a cytosolic factor able to modulate GRK2 activity (A. Ruiz-Gomez, A. Elorza and F. Mayor, Jr., in preparation). In summary, regulation of GRK2 activity and subcellular distribution is a complex process that involves the interaction of the kinase with anchoring proteins in several cellular membranes, as well as the modulation of its activity by targeting, phosphorylation or binding to multiple proteins in different cellular pools (see Fig. 1). The occurrence of such complex regulatory mechanisms is consistent with a key role of GRK2 in GPCR signaling processes.

2. Physiological functions

Recent reports suggest that the extent of agonist-mediated desensitization and sequestration of GPCR depends on the cellular content of GRKs and β-arrestins [19,27]. Therefore, in a given tissue, different GPCRs would desensitize/resensitize at different rates, depending on the particular receptor/GRK/arrestin expression pattern. GRK2 is ubiquitously expressed, with higher levels found in brain, leukocytes, heart and spleen, followed by lung and kidney [7]. Such expression pattern suggests a particularly important role of GRK2 in neurotransmission, cardiovascular function and immune and inflammatory responses.

2.1. GRK2 and cardiovascular function

GPCRs mediate the actions of messengers such as catecholamines, endothelins or angiotensin, which are key regulators of cardiovascular functions and are involved in cardiovascular development and in the control of growth and remodeling of cardiovascular cells. GRK2 has been shown to modulate several types of adrenergic, angiotensin and endothelin receptors [3,5]. Such receptor systems are also important pharmacological targets in the management of chronic heart failure, angina pectoris or hypertension, and desensitization of GPCR is clinically relevant in several cardiovascular diseases [28–30].

An important feature of heart failure is reduced β-adrenergic-stimulated cyclase activity, due to both diminished β1 receptor number (down-regulation) and impaired receptor function (uncoupling) [31]. Interestingly, mRNA levels for GRK2 as well as kinase activity are increased almost three-fold in the ventricles of congestive heart failure patients [5,32]. GRK2 levels are also altered in mouse models of cardiac hypertrophy [33], suggesting that GRK2 is an important factor underlying alterations of adrenergic signaling in such situation [31,34]. An increased GRK2 activity has also been found in hypertensive patients which show reduced β2AR responsiveness [35]. Consistent with a key role for GRK2 in cardiac function, cardiac contractility can be decreased or enhanced in transgenic mice overexpressing GRK2 or GRK2 inhibitor constructs, respectively [36]. Increased GRK activity seems to be an early step that preceded changes in the expression of adenylyl cyclase isoforms also taking place in heart failure [37]. Moreover, the disruption of the GRK2 gene results in marked myocardial hypoplasia and embryonic death in mice, indicating a crucial role for this kinase in cardiac cell growth and differentiation [38]. It is important to note that although GRK2 and GRK3 share a high degree of homology and overlapping patterns of substrate specificity 'in vitro', the disruption of the GRK3 gene allows for normal embryonic and postnatal development and only leads to the loss of odorant receptor desensitization [39]. To ascertain the mechanisms and signals governing the expression and activity levels of GRKs and arrestins in cardiovascular cells may prove important for understanding some key aspects of cardiovascular physiopathology.

2.2. GRKs and desensitization of chemokine receptors

Chemokine receptors cause chemotactic and pro-adhesion effects in leukocytes in response to locally produced chemoattractants. The responsiveness of the cells to chemoattractants is transient and results in rapid receptor desensitization. The rapid modulation of signaling is likely to be important in maintaining the ability of leukocytes to sense the gradient of chemoattractant and in the regulation of the inflammatory response [40,41]. Therefore, the biochemical relationship between receptor-mediated activation, desensitization and resensitization appears to be important in the response to injury and infection.

Chemokines promote cellular signaling by interaction with different GPCRs, several of which have been recently identified as cofactors in the internalization of the human immunodeficiency virus [42]. Molecular cloning studies have identified several groups of chemokines. The best known are the C-X-C and CC-chemokines, according to the position of the conserved cysteine residues. Among the C-X-C chemoattractants are the peptides IL-8, complement content C5a and *N*-formylated peptides (e.g. fMLP), which are predominantly chemotactic for neutrophils. The C-C family, which includes macrophage inflammatory protein 1a and 1b (MIP-1a and -1b), monocyte chemotactic protein 1 (MCP-1) and RANTES (regulated upon activation, normal T expressed and secreted), is chemotactic predominantly for monocytes and lymphocyte subsets [43].

The functional importance of phosphorylation by GRKs in chemokine receptor desensitization has been recently reported. In the case of IL-8 receptors, it has been shown that ligand binding to both IL-8RA and IL-8RB results in rapid receptor phosphorylation in its C-terminal domain, desensitization and sequestration [44–46]. The results are consistent with the agonist-dependent and agonist-independent phosphorylation of the receptor by GRK proteins and protein kinase C, respectively. The receptors for fMLP and C5a have also been demonstrated to become rapidly phosphorylated in a similar process [47,48]. In the case of the formyl peptide receptor, Prossnitz et al. [49] showed that GRK2 was able to phosphorylate its C-terminal cytoplasmic tail, whereas GRK3 had 50% of the activity of GRK2, and GRK5 and GRK6 had no detectable effect. Recently, the signaling and internalization of the CXCR4 receptor was demonstrated to be regulated by both receptor phosphorylation-dependent and -independent mechanisms [50].

A more direct involvement of GRK proteins has been demonstrated for CC-chemokine receptor desensitization. The CCR-2B receptor is rapidly phosphorylated and internalized upon MCP-1 induction in monocytes and transfected HEK293 cells. Co-expression of CCR-2B in *Xenopus* oocytes with GRK3, but not GRK2, specifically blocked receptor activation by MCP-1 [51]. More interestingly, a direct interaction between GRKs and this chemokine receptor has recently been reported under physiological circumstances, by showing co-immunoprecipitation of the ligand-activated CCR-2 receptor and GRK2 in monocytes [52]. In these cells, GRK2 translocates to membranes upon MCP-1 stimulation, leading to CCR-2 receptor phosphorylation. One important finding was the fact that GRK2 forms a multimolecular complex with the activated receptor and β-arrestin [52]. The CCR-2B receptor expressed in HEK293 cells can also be phosphorylated and desensitized by the GRK3 besides GRK2. Unlike the CCR-2 receptor, CCR-5 was desensitized and phosphorylated only following overexpression of GRKs in HEK293 cells [53]. CCR-5 could be phosphorylated by overexpression of GRK-2, -3, -5 or -6 in this cell type, but it is still not known which GRK regulates the CCR-5 in lymphocytes under physiological conditions. In addition, CCR-5 internalization in HEK293 cells was only observed upon overexpression of β-arrestin 1 or 2. This constitutes another example about how the biochemical complements of signaling molecules in given cell lines might determine the selectivity of the signal transduction pathways. The fact that other chemokine receptors, like the CCR-2, are desensitized in HEK293 cells by endogenous GRKs may be explained by a lower affinity of the CCR-5 receptor for these kinases. Interestingly, the extent of desensitization of the CCR-2 receptor is nevertheless diminished in HEK293 cells with respect to monocytes, which contain higher levels of GRK2 [52]. These observations raise the possibility that the CC-chemokine receptors require high levels of expression of GRKs and β-arrestins to be effectively desensitized and internalized, consistent with the pattern of expression of these proteins in leukocytes. They also indicate that variation in the complement of the GRKs may be an important physiological mechanism in determining the specificity of regulation of certain GPCRs [53].

Some CC-chemokine receptors, like the CCR-5, act as a co-receptor required for HIV-1 internalization [42]. Although some reports have shown that this process can occur in absence of desensitization and internalization of the chemokine receptor [54,55], more experiments are needed to assess the role of GPCR signalling, modulation and down-regulation in the infection process. On the other hand, it has recently been shown that neither phosphorylation of C-tail serines or threonines nor internalization of chemokine receptors affects chemotaxis measured in an unidirectional assay [56,57]. Therefore, the cells seem to migrate in a process that does not require continuous modulation at the level of receptor. However, whether receptor internalization/desensitization are important for a migrating cell to change direction deserves further research.

In summary, GRK2 appears to have a key role in the desensitization of a variety of chemokine receptors. Although it will be necessary to perform more in vivo experiments to ascertain which GRKs are responsible for the desensitization of each particular chemokine receptor in leukocytes, modulation of GRK2 activity and expression emerges as a new target to regulate cell responses to chemokines.

Acknowledgements: The authors thank A. Morales for editorial assistance. The work in our laboratory is supported by grants from Ministerio de Educación y Cultura (DGICYT/PM95-0033) and the European Union. The Centro de Biología Molecular receives an institutional grant from Fundación Ramón Areces.

References

[1] Chuang, T.T., Iacovelli, L., Sallese, M. and De Blasi, A. (1996) Trends Pharmacol. Sci. 17, 416–421.
[2] Lohse, M.J., Krasel, C., Winstel, R. and Mayor Jr., F. (1996) Kidney Int. 49, 1047–1052.
[3] Palczewski, K. (1997) Eur. J. Biochem. 248, 261–269.
[4] Bohm, S.K., Grady, E.F. and Bunnett, N.W. (1997) Biochem. J. 322, 1–18.
[5] Mayor, Jr., F., Penela, P. and Ruiz-Gómez, A. (1998) Trends Cardiovasc. Med., 8, 235–241.

[6] Premont, R.T., Inglese, J. and Lefkowitz, R.J. (1995) FASEB J. 9, 175–182.
[7] Sterne-Marr, R. and Benovic, J.L. (1995) Vitam. Horm. 51, 193–234.
[8] Ferguson, S.S., Menard, L., Barak, L.S., Koch, W.J., Colaprieto, A.M. and Caron, M.G. (1995) J. Biol. Chem. 270, 24782–24789.
[9] Ferguson, S.S., Dowry, W.E., Colaprieto, A.M., Barak, L.S., Menard, L. and Caron, M.G. (1996) Science 271, 363–366.
[10] Ruiz-Gómez, A. and Mayor Jr., F. (1997) J. Biol. Chem. 272, 9601–9604.
[11] Daaka, Y., Luttrell, L.M., Ahn, S., Della Roca, G.J., Ferguson, S.S.G., Caron, M.G. and Lefkowitz, R.J. (1998) J. Biol. Chem. 273, 685–688.
[12] Pitcher, J.A., Hall, R.A., Daaka, Y., Zhang, J., Ferguson, S.S.G., Hester, S., Miller, S., Caron, M.G., Lefkowitz, R.J. and Barak, L.S. (1998) J. Biol. Chem., in press.
[13] Garcia Higuera, I., Penela, P., Murga, C., Egea, G., Bonay, P., Benovic, J.L. and Mayor Jr., F. (1994) J. Biol. Chem. 269, 1348–1355.
[14] Murga, C., Ruiz Gómez, A., Garcia Higuera, I., Kim, C.M., Benovic, J.L. and Mayor Jr., F. (1996) J. Biol. Chem. 271, 985–994.
[15] Murga, C., Esteban, N., Ruiz Gómez, A. and Mayor Jr., F. (1997) FEBS Lett. 409, 24–28.
[16] Daaka, Y., Pitcher, J.A., Richardson, M., Stoffel, R.H., Robishaw, J.D. and Lefkowitz, R.J. (1997) Proc. Natl. Acad. Sci. USA 94, 2180–2185.
[17] Pitcher, J.A., Fredericks, Z.L., Stone, W.C., Premont, R.T., Stoffel, R.H., Koch, W.J. and Lefkowitz, R.J. (1996) J. Biol. Chem. 271, 24907–24913.
[18] DebBurman, S.K., Ptasienski, J., Benovic, J.L. and Hosey, M.M. (1996) J. Biol. Chem. 271, 22552–22562.
[19] Menard, L., Ferguson, S.S., Zhang, J., Lin, F.T., Lefkowitz, R.J., Caron, M.G. and Barak, L.S. (1997) Mol. Pharmacol. 51, 800–808.
[20] Chuang, T.T., Paolucci, L. and De Blasi, A. (1996) J. Biol. Chem. 271, 28691–28696.
[21] Haga, K., Tsuga, H. and Haga, T. (1997) Biochemistry 36, 1315–1321.
[22] Pronin, A.N., Satpaev, D.K., Slepak, V.Z. and Benovic, J.L. (1997) J. Biol. Chem. 272, 18273–18280.
[23] Hekman, M., Bauer, P.H., Sohlemann, P. and Lohse, M.J. (1994) FEBS Lett. 343, 120–124.
[24] Chuang, T.T., LeVine, H. and De Blasi, A. (1995) J. Biol. Chem. 270, 18660–18665.
[25] Winstel, R., Freund, S., Krasel, C., Hoppe, E. and Lohse, M.J. (1996) Proc. Natl. Acad. Sci. USA 93, 2105–2109.
[26] Pronin, A.N. and Benovic, J.L. (1997) J. Biol. Chem. 272, 3806–3812.
[27] Schlador, M.L. and Nathanson, N.M. (1997) J. Biol. Chem. 272, 18882–18890.
[28] Bristow, M.R. (1993) J. Am. Coll. Cardiol. 22, 61–71.
[29] Brodde, O.E. and Michel, M.C. (1992) J. Hypertens. 10, 715–722.
[30] Castellano, M. and Bohm, M. (1997) Hypertension 29, 715–722.
[31] Hammond, H.K. (1993) Circulation 87, 652–654.
[32] Ungerer, M., Bohm, M., Elce, J.S., Erdmann, E. and Lohse, M.J. (1993) Circulation 87, 454–463.
[33] Choi, D.J., Koch, W.J., Hunter, J.J. and Rockman, H.A. (1997) J. Biol. Chem. 272, 17223–17229.
[34] Shing, K., Ballingand, K.L., Fisher, T.A., Smith, T.W. and Kelly, R.A. (1996) J. Biol. Chem. 271, 1111–1117.
[35] Gros, R., Benovic, J.L., Tan, C.M. and Feldman, R.D. (1997) J. Clin. Invest. 99, 2087–2093.
[36] Koch, W.J., Rockman, H.A., Samama, P., Hamilton, R.A., Bond, R.A., Milano, C.A. and Lefkowitz, R.J. (1995) Science 268, 1350–1353.
[37] Ping, P., Anzai, T., Gao, M. and Hammond, H.K. (1997) Am. J. Physiol. 273, H707–717.
[38] Jaber, M., Koch, W.J., Rockman, H., Smith, B., Bond, R.A., Sulik, K.K., Ross Jr., J., Lefkowitz, R.J., Caron, M.G. and Giros, B. (1996) Proc. Natl. Acad. Sci. USA 93, 12974–12979.
[39] Peppel, K., Boekhoff, I., MacDonald, P., Breer, H., Caron, M.G. and Lefkowitz, R.J. (1997) J. Biol. Chem. 272, 25425–25428.
[40] Murphy, P.M. (1994) Annu. Rev. Immunol. 12, 593–633.
[41] Schall, T.J. and Bacon, K.B. (1994) Curr. Opin. Immunol. 6, 865–873.
[42] Feng, Y., Broder, C.C., Keurredy, P.E. and Berger, E.A. (1996) Science 272, 872–877.
[43] Baggiolini, M. (1998) Nature 392, 565–568.
[44] Mueller, S.G., Schraw, W.P. and Richmond, A. (1995) J. Biol. Chem. 270, 10439–10448.
[45] Mueller, S.G., White, J.R., Schraw, W.P., Lam, V. and Richmond, A. (1997) J. Biol. Chem. 272, 8207–8214.
[46] Richardson, R.M., Dubose, R.A., Ali, H., Tomhave, E.D., Haribabu, B. and Snyderman, R. (1995) Biochemistry 34, 14193–14201.
[47] Ali, H., Richardson, R.M., Tomhave, E.D., Didsbury, J.R. and Snyderman, R. (1993) J. Biol. Chem. 268, 24247–24254.
[48] Giannini, E. and Boulay, F. (1995) J. Immunol. 154, 4055–4064.
[49] Prossnitz, E.R., Kim, C.M., Benovic, J.L. and Ye, R.D. (1995) J. Biol. Chem. 270, 1130–1137.
[50] Haribabu, B., Richardson, R.M., Fisher, I., Sozzani, S., Peiper, S.C., Horuk, R., Ali, H. and Snyderman, R. (1997) J. Biol. Chem. 272, 28726–28731.
[51] Franci, C., Gosling, J., Tsou, C.-L., Coughlin, S.R. and Charo, I.F. (1996) J. Immunol. 157, 5606–5612.
[52] Aragay, A.M., Mellado, M., Frade, J.M.R., Martin, A.M., Jimenez-Sainz, M.C., Martinez-A., C. and Mayor Jr., F. (1998) Proc. Natl. Acad. Sci. USA 95, 2985–2990.
[53] Aramori, I., Zhang, J., Ferguson, S.S.G., Bieniasz, P.D., Cullen, B.R. and Caron, M. (1997) EMBO J. 16, 4606–4616.
[54] Gosling, J., Monteclaro, F.S., Atchinson, R.E., Arai, H., Tsou, C.-L., Goldsmith, M.A. and Charo, I.F. (1997) Proc. Natl. Acad. Sci. USA 94, 5061–5066.
[55] Alkatib, G., Locati, M., Kennedy, P.E., Murphy, P.M. and Berger, E.A. (1997) Virology 234, 340–348.
[56] Arai, H., Monteclaro, F.S., Tsou, C-L., Franci, C. and Charo, I.F. (1997) J. Biol. Chem. 272, 25037–25042.
[57] Hsu, M.H., Chiang, S.C., Ye, R.Y. and Prossnitz, E.R. (1997) J. Biol. Chem. 272, 29426–29429.