β-Arrestin2 Is Critically Involved in CXCR4-mediated Chemotaxis, and This Is Mediated by Its Enhancement of p38 MAPK Activation*

Yue Sun‡, Zhijie Cheng‡, Lan Ma§, and Gang Pei‡

From the Laboratory of Molecular Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yue Yang Road, Shanghai 200031, People’s Republic of China, and National Laboratory of Medical Neurobiology, Fudan University Medical Center, Shanghai 200040, People’s Republic of China.

Received for publication, July 19, 2002, and in revised form, September 16, 2002 Published, JBC Papers in Press, October 4, 2002, DOI 10.1074/jbc.M207294200

Chemotaxis mediated by chemokine receptors such as CXCR4 plays a key role in lymphocyte homing and hematopoiesis as well as in breast cancer metastasis. We have demonstrated previously that β-arrestin2 functions to attenuate CXCR4-mediated G protein activation and to enhance CXCR4 internalization. Here we show further that the expression of β-arrestin2 in both HeLa and human embryonic kidney 293 cells significantly enhances the chemotactic efficacy of stromal cell-derived factor 1α, the specific agonist of CXCR4, whereas the suppression of β-arrestin2 endogenous expression by antisense or RNA-mediated interference technology considerably attenuates stromal cell-derived factor 1α-induced cell migration. Expression of β-arrestin2 also augmented chemokine receptor CCR5-mediated but not epidermal growth factor receptor-mediated chemotaxis, indicating the specific effect of β-arrestin2. Further analysis reveals that expression of β-arrestin2 strengthened CXCR4-mediated activation of both p38 MAPK and ERK, and the suppression of β-arrestin2 expression blocked the activation of two kinases. Interestingly, inhibition of p38 MAPK activation (but not ERK activation) by its inhibitors or by expression of a dominant-negative mutant of p38 MAPK effectively blocked the chemotactic activity of β-arrestin2. Expression of a dominant-negative mutant of ASK1 also exerted the similar blocking effect. The results of our study suggest that β-arrestin2 can function not only as a regulator of CXCR4 signaling but also as a mediator of stromal cell-derived factor 1α-induced chemotaxis and that this activity probably occurs via the ASK1/p38 MAPK pathway.

Chemokines are a family of soluble peptides that promote the recruitment of various types of leukocytes to sites of inflammation and to secondary lymphoid organs (1, 2). On the basis of the first two of four conserved Cys residues, chemokines are divided into four subfamilies: α (C-X-C), β (C-C), γ (C), and δ (C-X-X-C) (3–5). SDF-11 is an 8-kDa CXC chemokine originally isolated from a bone marrow stromal cell line (6). CXCR4, a well known chemokine receptor that functions as a CD4-associated human immunodeficiency virus type 1 (HIV-1) coreceptor (7–9), is the only receptor for SDF-1α, whereas SDF-1β is the sole chemokine for CXCR4 (10, 11). The constitutive expression of SDF-1 and CXCR4 in a large number of tissues (12–15) suggests the broad spectrum of their biological functions. SDF-1/CXCR4 plays an important role in lymphocyte homing and hematopoiesis and is mandatory for various development processes, in particular the development of the brain, heart, and blood vessels (16–19). Knock-out mice that lack CXCR4 or SDF-1 are embryologically lethal (17–19). It has recently been demonstrated that chemotaxis induced by CXCR4 is involved in breast cancer metastasis (20).

As a G protein-coupled receptor (GPCR), CXCR4 can be regulated by β-arrestin, which is a well known regulator of GPCRs. Our previous study demonstrates that β-arrestin can functionally interact with CXCR4 on SDF-1 stimulation and thus significantly attenuate CXCR4-mediated G-protein activation and promote CXCR4 internalization (21). Recent discoveries indicate that β-arrestin also plays an important role as a scaffold that links GPCRs to mitogen-activated protein kinase (MAPK) cascades such as Raf, ERK, ASK1, and c-Jun NH2-terminal kinase 3 (22). Furthermore, formation of β-arrestin complexes with Hck or c-Fgr after stimulation of CXCR1 by interleukin 8 leads to Hck or c-Fgr activation that regulates CXCR1-mediated granule exocytosis (23). A very recent report shows that lymphocytes from β-arrestin2-deficient mice are strikingly impaired in their ability to respond to CXCL12 (SDF-1) in migration assays (24), but the underlying mechanism is unclear.

Accumulating evidence demonstrates that p38 MAPK is functionally involved in cell migration and chemotaxis. Oddly enough, however, both p38 MAPK and ERK can respond to chemotactic stimulation. In human endothelial cells, inhibition of p38 MAPK activity by its specific inhibitor leads to blockage of EGFr-induced cell migration, and this is correlated to its attenuation of heat shock protein 27 phosphorylation and actin reorganization (25). It is also reported that inhibition of p38 MAPK abrogates methyl-accepting chemotaxis protein-1-induced chemotaxis (26). However, the potential role of p38 MAPK in CXCR4-mediated chemotaxis is not known yet.

EXPERIMENTAL PROCEDURES

Materials—SDF-1α was purchased from Leinco Technologies, Inc. (St. Louis, MO), PD98059, U0126, SB203580, SKF86024, and protein-coupled receptor; HEK, human embryonic kidney; CMV, cytomegalovirus; iRNA, RNA-mediated interference; RANTES, regulated on activation normal T cell expressed and secreted.

© 2002 by The American Society for Biochemistry and Molecular Biology, Inc.
SB202474 were from Calbiochem. The anti-ASK1 polyclonal antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-β-arrestin polyclonal antibody was produced in our laboratory as described previously (30, 31). Briefly, ASK1 (K709R) was subcloned by PCR from ASK1 plasmid that was a generous gift of Dr. Yang Shi (Dept. of Pathology, Harvard Medical School, Boston, MA). A 22-nucleotide oligonucleotide (oligonucleotide 1) that corresponded to nucleotides 216–237 of the β-arrestin2 coding region was first inserted into the BS/U6 vector with ApaI (blunted) and XhoI. The inverted motif that contains the six-nucleotide spacer and five Ts (oligonucleotide 2) was then subcloned into the XhoI and EcoRI sites of the intermediate plasmid to generate BS/U6/β-arrestin2. Oligonucleotide 1 is 5'-GGCTTGTCCTTCCGAAAGACA-3' (forward) and 5'-AGCTTGCTTTGCGGAAGGACAAGCC-3' (reverse). Oligonucleotide 2 is 5'-AGCTTGCTTTGCGGAAGGACAAGCC-3' (forward) and 5'-AATTCGAAAAAGCTTTGCTTCCCGCAAGA-3' (reverse). The sequences for the body of the small interfering RNA for β-arrestin2 were taken from GenBank™ accession no. NM_004313 (nucleotides 270–290).

Cell Culture and Transfection—Human embryonic kidney (HEK) 293 cells were cultured in minimum Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum. HeLa cells were cultured in 1640 supplemented with 10% fetal bovine serum. For stable transfection, HEK293 cells were transfected with 3 µg of DNA that contained a neomycin-selectable marker by using LipofectAMINE (Invitrogen) according to the manufacturer’s instructions. Stable clones were selected and maintained in G418 (800 µg/ml). For transient transfection, HEK293 cells were transfected by using the calcium phosphate-DNA co-precipitation method as described previously (28).

Adenovirus Construction—Recombinant adenoviruses that encode β-galactosidase or β-arrestin2 or β-arrestin2 antisense were prepared as described previously (30, 31). Briefly, β-galactosidase, β-arrestin2, or β-arrestin2 antisense was cloned into shuttle vector pAdTrack-CMV by using standard cloning protocols, and the shuttle vectors were recombined with pAdEasy-1, which harbors a CMV-driven green fluorescent protein, to form the viral constructs. The adenoviral plasmids were transfected into the HEK293 cells to generate recombinant adenovirus.

Chemotaxis Assay—The assays were performed in blind well chambers (Neuroprobe, Gaithersburg, MD) as described (32). For each agonist concentration tested, cells migrated through to the underside of the membrane were counted in five high power fields, in a blinded fashion. The migration index for each experiment was calculated as the mean number of cells that migrated toward medium-containing agonist divided by the mean number of cells that migrated toward medium-containing bovine serum albumin only.

MAPK Phosphorylation and Western Blotting—After serum starvation for 12 h in 0.1% bovine serum albumin-minimum Eagle’s medium, the cells were stimulated with SDF-1α and lysed in 10 mM Tris-HCl, pH 7.4, 5 mM EDTA, 2% SDS, and 1% 2-mercaptoethanol. Aliquots of the whole cell extracts prepared were subjected to 10% SDS-polyacrylamide gel electrophoresis and then electroblotted onto nitrocellulose membranes. The total amounts of ERK and phosphorylated ERK were detected by anti-total-ERK antibodies and anti-phospho-ERK antibodies (New England Biolabs Inc., respectively). The total amounts of p38 MAPK and phosphorylated p38 MAPK were detected by anti-total-p38 antibodies and anti-phospho-p38 antibodies (New England Biolabs Inc., respectively).

Kinesin Assay—After being stimulated with SDF-1α in serum-free medium, the cells were lysed in ice-cold lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na3VO4, 1 mg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride). Cell extracts were clarified by centrifugation at 15,000 × g for 10 min. Next the supernatants were subjected to immunoprecipitation to detect p38 MAPK kinase activity by using the p38 MAPK kinase assay kit (New England Biolabs Inc.) according to the manufacturer’s instructions.

Statistical Analysis—Data were analyzed by Student’s t test to compare independent means with pooled estimates of common variations.
RESULTS

Change in β-Arrestin2 Expression Altered CXCR4-mediated Chemotaxis in HeLa Cells—HeLa cells express endogenous CXCR4 receptor (33), and under the conditions of this study SDF-1α could induce HeLa cell chemotaxis in a dose-dependent manner (data not shown). When the cells were pretreated with CXCR4-specific antagonist T140 (300 nM), SDF-1α-induced chemotaxis was totally inhibited (Fig. 1A), whereas as a control EGF-induced chemotaxis was not affected by T140 (Fig. 1A). Thus, HeLa cells were used as a model system to explore the potential effect of β-arrestin2 on CXCR4-mediated chemotaxis.

The recombinant adenoviruses that encode β-arrestin2 or β-arrestin2 antisense were constructed as described under “Experimental Procedures.” The expression of β-arrestin2 in HeLa cells was detected in Western blotting (Fig. 1B) 80 h after adenovirus infection. The expression of endogenous β-arrestin2 was reduced to approximately 40% by the β-arrestin2 antisense adenovirus infection and was enhanced approximately 5-fold by the β-arrestin2 adenovirus infection as compared with the control of β-galactosidase adenovirus infection. However, the expression of actin was not affected under the same conditions (Fig. 1B). In the chemotaxis assay, when the expression of β-arrestin2 was reduced in HeLa cells, SDF-1α-induced chemotaxis was severely decreased when the maximum chemotactic index was decreased from 3.8 to 2.5 (Fig. 1C). In contrast, when β-arrestin2 was overexpressed in HeLa cells the sensitivity of the cells to SDF-1α was greatly increased; the concentration needed to induce maximum chemotaxis was reduced from 30 to 3 nM (Fig. 1C).

To further test the specificity of β-arrestin2 effect, EGF-induced chemotaxis in the infected HeLa cells was measured under the same conditions. The results revealed that infection by neither β-arrestin2 adenovirus nor β-arrestin2 antisense adenovirus could alter EGF-mediated chemotaxis (Fig. 1D). This indicates that the potential for chemotaxis in these cells was not changed, and that the effect of β-arrestin2 on chemotaxis was receptor-specific.

Change in β-Arrestin2 Expression Altered CXCR4-mediated Chemotaxis in HEK293 Cells—To confirm the above phenomenon, β-arrestin2 or β-galactosidase was transfected to the CXCR4-expressed stable cell line of HEK293 cells (CXCR4-HEK293 cells), and the chemotaxis assay was performed 48 h after transfection. The expression of transfected β-arrestin2 was approximately 7-fold higher than the endogenous level (Fig. 2A). To make sure that only the transfected cells were counted in the assay, green fluorescent protein was co-transfected with β-arrestin2 or β-galactosidase, and the chemotactic cells with green fluorescence were counted. As in the HeLa cells, the concentration of SDF-1α to induce the maximum chemotaxis was reduced from 3 to 0.3 nM after HEK293 cells were transfected with β-arrestin2 as compared with those with β-galactosidase (Fig. 2B). In contrast, the EGF-induced chemotaxis in the β-arrestin2-transfected HEK293 cells was not changed (Fig. 2C).
To explore the potential function of β-arrestin2 on chemotaxis mediated by other chemokine receptors, β-arrestin2 or β-galactosidase was transfected to the CCR5-expressed stable cell line of HEK293 cells. The results showed that the chemotactic sensitivity of the β-arrestin2-transfected HEK293 cells to RANTES stimulation was also greatly increased as compared with the control cells (Fig. 2D).

To explore the potential function of β-arrestin2 on chemotaxis mediated by other chemokine receptors, β-arrestin2 or β-galactosidase was transfected to the CCR5-expressed stable cell line of HEK293 cells. The results showed that the chemotactic sensitivity of the β-arrestin2-transfected HEK293 cells to RANTES stimulation was also greatly increased as compared with the control cells (Fig. 2D).

Inhibition of β-Arrestin2 Expression by RNAi Reduced SDF-1α but Not EGF-induced Chemotaxis—Double-stranded RNA-mediated interference (RNAi) has recently emerged as a powerful reverse genetic tool to silence gene expression in multiple organisms (34–36). Using this technology, we transfected RNAi plasmid of β-arrestin2 or the control vector into the CXCR4-HEK293 cells, and the expression of endogenous β-arrestin2 was remarkably reduced to approximately 20% of the control level as detected by Western blotting (Fig. 3A). However, the expression of actin and β-arrestin1 was not affected under the same conditions. Further experiments showed that when β-arrestin2 expression was reduced by RNAi, the chemotaxis mediated by CXCR4 was also greatly attenuated (Fig. 3B), but the chemotaxis mediated by EGFR was not changed under the same conditions (Fig. 3C).

β-Arrestin2 Functionally Mediates the SDF-1α-stimulated Activation of ERK and p38 MAPK—To test the effect of β-arrestin2 on the CXCR4-mediated activation of ERK1/2 and p38 MAPK, the widely used phospho-specific antibodies against two kinases were applied in the Western blotting to detect their activation. When treated with SDF-1α in CXCR4-HEK293 cells, ERK and p38 MAPK were activated in a dose-dependent manner (Fig. 4). The activation of ERK was significantly enhanced by co-transfection with β-arrestin2 at 0.3 nM SDF-1α, whereas the activation of p38 MAPK was significantly enhanced at the basal and the two SDF-1α concentrations tested (Fig. 4). This result indicates that...
β-arrestin2 can functionally mediate the activation of both kinases. In contrast, when β-arrestin2 expression in CXCR4-HEK293 cells was reduced by its RNAi, the activation of both p38 MAPK and ERK was significantly attenuated at 3.0 nM SDF-1α as shown in Fig 5.

**EGF-stimulated Activation of p38 MAPK Is Not Altered by Expression of β-Arrestin2**—To further test the specificity of β-arrestin2 effect on p38 MAPK activation, EGF-stimulated activation of p38 MAPK in the same CXCR4-HEK293 cells was detected under the same conditions. The results revealed that activation of p38 MAPK stimulated by EGF could not be altered by co-transfection with β-arrestin2 at the two EGF concentrations tested (Fig. 6, A and B). To directly measure the change in kinase activity in p38 MAPK, ATF2 was used as the substrate in the kinase assay (37). The results showed that the ATF2 phosphorylation by p38 MAPK after EGF stimulation did not change by co-transfection with β-arrestin2 as compared with those with β-galactosidase (Fig. 6C), whereas it was remarkably enhanced after SDF-1α stimulation under the same conditions (Fig. 6D). This finding not only confirms the effect of β-arrestin2 on p38 MAPK activation but also indicates that the effect was receptor-specific, a result that agrees with the data from the chemotaxis assay (Figs. 1, 2, and 3).

**Inhibition of p38 MAPK Activation (but Not ERK Activation) by Its Inhibitors or by Expression of a Dominant-negative Mutant of p38 MAPK Blocked the Chemotactic Effect of β-Arrestin2**—Because β-arrestin2 expression enhanced both ERK and p38 MAPK activation, the specific inhibitors of these two kinases were used to test their role in the chemotactic effect of β-arrestin2. Pretreated with 2 or 6 μM SB203580 (a specific inhibitor of p38 MAPK), the chemotaxis index of CXCR4-HEK293 cells that transfected with β-arrestin2 was decreased from 3.5 to 2.1 or 1.4, respectively (Fig. 7A). The latter was comparable to the level of inhibition of chemotaxis index (1.5) by the transfection of RNAi plasmid of β-arrestin2 (Fig. 7A). Application of another p38 MAPK inhibitor, SKF86002, gave similar results, whereas pretreatment with SB202474 (a negative control of SB203580) or ERK pathway inhibitors PD98059 or U0126 did not have any effect on chemotaxis (Fig. 7A). This result indicates that the activation of p38 MAPK but not that of ERK is likely involved in the chemotactic effect of β-arrestin2.

To further independently test the role of p38 MAPK in the chemotactic effect of β-arrestin2, a dominant-negative p38 MAPK (p38AF) was co-transfected with β-arrestin2 to the CXCR4-HEK293 cells. As shown in Fig. 7B, both the maximal chemotactic response and the efficacy of the response to SDF-1α were greatly decreased by the co-transfection of p38AF, consistent with the results from the above study with use of the p38 inhibitors.

**Expression of the Dominant-negative Mutant of ASK1 (K709R) Blocks the Effect of β-Arrestin2 on Chemotaxis**—ASK1 is one of the upstream MAPKKKs of p38 MAPK and c-Jun NH2-terminal kinase. SDF-1α-induced p38 MAPK activation was attenuated after the catalytically inactive mutant ASK1 (K709R) and β-arrestin2 were co-transfected in the CXCR4-HEK293 cells as compared with those of the control (co-transfection with β-galactosidase and β-arrestin2) (Fig. 7C). Consistently, SDF-1α-induced chemotaxis was also remarkably reduced, because the concentration of SDF-1α needed for the maximum response was increased from 0.3 to 3 nM (Fig. 7D) by the co-transfection of ASK1 (K709R). These data further support the notion that the ASK1/p38 MAPK pathway may mediate the chemotactic effect of β-arrestin2.

When the expression of β-arrestin2 was severely reduced by RNAi, the remainder of the SDF-1α-induced chemotaxis would
be mediated largely in a β-arrestin2-independent manner. This was indeed the case as demonstrated by results from the co-expression of p38 (AF) or ASK1 (K709R) with β-arrestin2 RNAi (Fig. 7E), which only slightly reduced the SDF-1α-induced chemotaxis. These results further indicate the function of the β-arrestin2/ASK1/p38 MAPK pathway in CXCR4-mediated chemotaxis.

**DISCUSSION**

As a key regulator of GPCR signaling, β-arrestins carry out many important roles in GPCR-mediated biological functions. For example, both the duration of action and the analgesic potency of single doses of morphine are markedly enhanced in β-arrestin2 knock-out mice as compared with wild-type littermates, and β-arrestin2 knock-out mice fail to develop tolerance to the repeated administration of morphine (38). Another example is that β-arrestin regulates interleukin 8-induced granule release of the neutrophil leukocytes (23). By positively or negatively manipulating the expression level of β-arrestin2 in two different kinds of cell lines, this study further demonstrates that β-arrestin2 is critically involved in the chemokine-mediated chemotaxis. The changes in CXCR4-mediated chemotaxis are nicely correlated with the change of β-arrestin2 expression levels in the two systems. This correlation coincides with the findings of a recent report that used lymphocytes from β-arrestin2-deficient mice (24). Altogether, all of the results provide at least one physiological explanation for the wide and rich expression of β-arrestins in the blood system and further imply a potential role for β-arrestins in the immunological functions via their regulation of chemokine-induced chemotaxis.

β-Arrestins have been shown to possess dual functions in regulating the signals of GPCRs. β-Arrestins quench some signals from the activated receptor such as in cAMP-dependent protein kinase pathways, and they mediate other signals such as those involved in the MAPK cascades. It has been reported that β-arrestins play a crucial role in β2-adrenergic receptor-mediated ERK activation (39), and our previous work also demonstrates that β-arrestin2 can enhance the CXCR4-mediated activation of ERK (21). Our current study further reveals that β-arrestin2 is crucially involved in the chemokine-induced activation of p38 MAPK. More interestingly, activation of p38 MAPK by SDF-1α induces cell chemotaxis significantly. The molecular mechanism of β-arrestin2 enhancement of chemokine-induced chemotaxis is currently unclear, but it seems to be mediated by ASK1, the upstream kinase of p38 MAPK. However, more study is required to reveal the detailed pathway from β-arrestin2 to p38 MAPK.

As a good control, the current investigation shows that the changes in the level of β-arrestin2 expression does not alter EGFR-mediated chemotaxis or EGF-induced activation of p38 MAPK. This indicates that the effect of β-arrestin2 is not on the general apparatus of cell chemotaxis and migration; rather, it works through its specific signal pathways and under the control of GPCRs. It may also be worthwhile to point out that the current study successfully used DNA vector-based RNAi technology to significantly inhibit the endogenous expression of β-arrestin2 in a mammalian cell line, and the inhibition of β-arrestin2 expression is well correlated to its effect on chemotaxis. Another construction of RNAi plasmid of β-arrestin2 was also used, and it showed a similar ability to block β-arrestin2 expression (data not shown).
has been difficult in our laboratory to block β-arrestin2 expression in HEK293 cells by using oligo-based or virus-based antisense technology, probably because of the high expression level and/or the slow degradation of β-arrestin2. DNA vector-based RNAi technology to block β-arrestin2 apparently provides a useful tool for future study of the various functions of β-arrestin2.

Acknowledgments—We thank Dr. Jiahuai Han for ASK1 construct, Dr. Shi Yang for the gift of BSU6 vector, and Dr. Stephen C. Peiper for T140. We also thank Linhua Qin, Yaya Wang, Hua Gao, Yongxin Yu, Bin Qu, and Shunmei Xin for their technical assistance and Kun Ling, Zhu Wang, Lili Ji, and Peihua Wu for kind help. We also thank Ping Wang, Wenbo Zhang, Nanjie Xu, and Yanxiang Ni for helpful discussion.

Fig. 7. Effects of p38 MAPK or ERK on the chemotaxis effect of β-arrestin2. A, CXCR4-HEK293 cells transfected with β-arrestin2 were pretreated with p38 MAPK-specific inhibitors (SB203580 or SKF86002) or negative control of SB203580 (SB202474) or ERK pathway-specific inhibitors (PD98059 or U0126) for 1 h, and then the cells were subjected to chemotaxis assay on SDF-1α (0.3 nM). *, p < 0.05 compared with SDF-1α alone. B, CXCR4-HEK293 cells were co-transfected with β-arrestin2 and control plasmid (β-galactosidase) or p38AF, respectively, and then the cells were subjected to chemotaxis assay on SDF-1α at the concentrations indicated. C, CXCR4-HEK293 cells were co-transfected with β-arrestin2 and control plasmid (β-galactosidase) or ASK1 (K709R), respectively. The cells were incubated without (basal) or with 3.0 nM SDF-1α for 5 min, and the cells were solubilized and extracts were subjected to immunoblotting by using anti-phospho-p38 MAPK antibodies. The same blots were reprobed with anti-p38 MAPK antibodies to check for total protein content after stripping and blocking. D, CXCR4-HEK293 cells were co-transfected with β-arrestin2 and control plasmid (β-galactosidase) or ASK1 (K709R), respectively, and the cells were subjected to chemotaxis assay on SDF-1α at the concentrations indicated. E, CXCR4-HEK293 cells were co-transfected with β-arrestin2 RNAi and control plasmid (β-galactosidase) or ASK1 (K709R), respectively, and the cells were subjected to chemotaxis assay on SDF-1α at the concentrations indicated. Data are means ± S.E. of at least three independent experiments.

REFERENCES
1. Baggiolini M. (1998) Nature 392, 565–568
2. Wells, T. N., Power, C. A., Lusti-Narasimhan, M., Hoogewerf, A. J., Cooke, R. M., Chung, C. W., Peitsch, M. C., and Proudfoot, A. E. (1996) J. Leukocyte Biol. 59, 53–60
3. Rollins, B. J. (1997) Blood 90, 909–928
4. Premack, B. A., and Schall, T. J. (1996) Nat. Med. 2, 1174–1178
5. Luster, A. D. (1998) N. Engl. J. Med. 338, 436–445
6. Tashiro, K., Tada, H., Heilker, R., Shirouzu, M., Nakano, T., and Honjo, T. (1993) Science 261, 600–603
7. Berger, E. A., Murphy, P. M., and Farber, J. M. (1999) Annu. Rev. Immunol. 17, 657–700
8. Littman, D. R. (1998) Cell 93, 677–680
β-Arrestin2 Enhances Chemokine-induced Chemotaxis

9. Hoffman, T. L., and Doms, R. W. (1998) AIDS 12 (suppl. A), S17–S26
10. Bleul, C. C., Farzan, M., Choe, H., Parolin, C., Clark-Lewis, I., Sodroski, J., and Springer, T. A. (1996) Nature 382, 829–833
11. Oberlin, E., Amara, A., Bachelet, F., Bessia, C., Virelizier, J. L., Arenzana-Seisdedos, F., Schwartz, O., Heard, J. M., Clark-Lewis, I., Legler, D. F., Losetscher, M., Baggioni, M., and Moser, B. (1996) Nature 382, 833–835
12. Shirozu, M., Nakano, T., Inazawa, J., Tashiro, K., Tada, H., Shinozawa, T., and Henjo, T. (1995) Genomics 28, 495–500
13. McGrath, K. E., Konisky, A. D., Maltby, K. M., McGann, J. K., and Palis, J. (1999) Dev. Biol. 213, 442–456
14. Loetscher, M., Geiser, T., O’Reilly, T., Zwahlen, R., Baggioni, M., and Moser, B. (1994) J. Biol. Chem. 269, 232–237
15. Zhang, L., He, T., Talal, A., Wang, G., Frankel, S. S., and Ho, D. D. (1998) J. Virol. 72, 5035–5045
16. Bleul, C. C., Puhlbrugge, R. C., Casasnovas, J. M., Aiuti, A., and Springer, T. A. (1996) J. Exp. Med. 184, 1101–1109
17. Nagasawa, T., Hirota, S., Tachibana, K., Takakura, N., Nishikawa, S., Kitamura, Y., Yoshida, N., Rikutani, H., and Kishimoto, T. (1996) Nature 382, 635–638
18. Zou, T. R., Kottmann, A. H., Kuroda, M., Tanisuchi, I., and Littman, D. R. (1998) Nature 393, 595–599
19. Tachibana, K., Hirota, S., Izasa, H., Yoshida, H., Kawabata, K., Kataoka, Y., Kitamura, Y., Matsushima, K., Yoshida, N., Nishikawa, S., Kishimoto, T., and Nagasawa, T. (1998) Nature 393, 591–594
20. Muller, A., Homey, B., and Zlotnik, A. (2001) Nature 410, 50–56
21. Cheng, Z. J., Zhao, J., Sun, Y., Hu, W., Wu, Y. L., Cen, B., Wu, G. X., and Pei, G. (2000) J. Biol. Chem. 275, 2479–2485
22. McDonald, P. H. et al. (2000) Science 290, 1574–1577
23. Barlic, J., Andrews, J. D., Kelvin, A. A., Bosinger, S. E., DeVries, M. E., Xu, L., Dobransky, T., Feldman, R. D., Ferguson, S. S., and Kelvin, D. J. (2000) Nat. Immunol. 1, 227–233
24. Fong, A. M., Premont, R. T., Richardson, R. M., Yu, Y. R., Leffkowitz, R. J., and Patel, D. D. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 7478–7483
25. Rousseau, S., Houle, F., Landry, J., and Hsu, J. (1997) Oncogene 15, 2169–2177
26. Ashida, N., Arai, H., Yamasaki, M., and Kita, T. (2001) J. Biol. Chem. 276, 16555–16560
27. Yu, Q. M., Cheng, Z. J., Zhou, T. H., Cen, B., Guo, S. Q., and Pei, G. (1999) Sheng Wu Huaxue Xue Bao Wu Li Xue Boxue 31, 344–346
28. Ling, K., Wang, P., Zhao, J., Wu, Y. L., Cheng, Z. J., Wu, G. X., Hu, W., Ma, L., and Pei, G. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 7922–7927
29. Zou, Y. R., Stock, C., Affar, B., Goy, F., Shi, Y., Forrester, W. C., and Shi, Y. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 5615–5620
30. Sundell, S. J., Louden, B. P., and Renovic, J. L. (1999) Biochemistry 38, 8723–8732
31. He, T. C., Zhou, S., da Costa, L. T., Yu, J., Kinzler, K. W., and Vogelstein, B. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2569–2574
32. Neutype, E. R., and Bourne, H. R. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 14489–14494
33. de Parseval, A., and Elder, J. H. (2001) J. Virol. 75, 4528–4539
34. Chuang, C. F., and Meyerowitz, E. M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 4985–4990
35. Misquitta, L., and Paterson, B. M. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1451–1456
36. Tsuschl, T., Zamore, P. D., Lehmann, R., Bartel, D. P., and Sharp, P. A. (1999) Genes Dev. 13, 3191–3197
37. Jing, Q., Xin, S. M., Cheng, Z. J., Zhang, W. B., Zhang, R., Qin, Y. W., and Pei, G. (1999) Circ. Res. 84, 831–839
38. Bohn, L. M., Leffkowitz, R. J., Gaintinolin, R. R., Peppel, K., Caren, M. G., and Lin, P. F. (1999) Science 286, 2495–2498
39. Luttrell, L. M., Ferguson, S. S., Daaka, Y., Miller, W. E., Maudsley, S., Della Rocca, G. J., Lin, F., Kawakatsu, H., Owada, K., Luttrell, D. K., Caron, M. G., and Leffkowitz, R. J. (1999) Science 283, 655–661
40. Ayala, J. M., Goyal, S., Liverton, N. J., Claremon, D. A., O’Keefe, S. J., and Hanlon, W. A. (2000) J. Leukocyte Biol. 67, 869–875
41. Jing, Q., Xin, S. M., Zhang, W. B., Wang, P., Qin, Y. W., and Pei, G. (2000) Circ. Res. 87, 52–59