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Endocytic trafficking of chemokine receptors

Adriano Marchese

Department of Pharmacology, Health Sciences Division, Loyola University Chicago, Maywood, IL 60153

Abstract

Chemokine receptors belong to the super family of G protein-coupled receptors (GPCRs). The cognate ligands for chemokine receptors are small circulating proteins known as chemokines. Upon binding to their cognate chemokines, receptors are rapidly desensitized, internalized onto early endosomes and sorted either into a recycling pathway or degradative pathway. Chemokine receptor trafficking is essential because it limits the magnitude and duration of signaling by removing receptors from the cell surface thereby limiting access to their ligands, but it also delivers bound chemokines to lysosomes for degradation. Once on endosomes receptors are sorted into a recycling pathway contributing to resensitization of receptor signaling or they are sorted into the degradative pathway leading to long-term attenuation of signaling. Recent studies have revealed some key information regarding the molecular determinants mediating chemokine receptor internalization and have shed light on the mechanisms dictating sorting into either the recycling or degradative pathways. Here I discuss our current understanding of the mechanisms mediating chemokine receptor trafficking with a focus primarily on recent findings for the chemokine receptor CXCR4.

Introduction

There are more than 50 chemokines and at least 19 defined receptors [1]. Chemokines and their receptors are mostly known for their roles in chemotaxis of multiple blood cell types [1,2], however several receptors are also expressed in other tissues where they have other roles [3]. Chemokine receptors are also involved in several pathologies including cancer, HIV and inflammation [1,4–6]. Remarkably, defects in endocytic trafficking of chemokine receptors may be a contributing factor to cancer progression. For example, the chemokine receptor CXCR4 is over-expressed in many types of cancers [4,7], and in a subset of breast cancers its expression may in part be due to a defect in its ubiquitination and lysosomal trafficking and degradation contributing to increased CXCR4 levels on the cell surface and greater metastatic potential of cancer cells [8]. This highlights the fact that it is essential that the responsiveness of chemokine receptors to activation by their cognate ligands is tightly

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Address correspondence to: Adriano Marchese, Department of Pharmacology Health Sciences Division Loyola University Chicago 2160 S. 1st Ave. Maywood, IL 60153, Tel: 708-216-3456, amarchese@lumc.edu.

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controlled and that endocytic trafficking plays an important role in this regulation. Despite this the molecular mechanisms mediating endocytic trafficking of chemokine receptors remain poorly understood.

Chemokine receptors undergo rapid agonist-induced internalization via a mechanism that involves G protein-coupled receptor kinase (GRK) mediated phosphorylation and β -arrestin binding leading to G protein uncoupling and receptor internalization via clathrin-coated pits [9]. Although β -arrestins mediate agonist-dependent internalization of chemokine receptors, a direct involvement of AP2 may also be required for a subset of chemokine receptors. AP2, a core component of clathrin-coated pits at the plasma membrane, is a heterotetrameric protein complex comprised of β_2 , α , σ and μ_2 subunits [10]. The β_2 -adaplin subunit interacts with β -arrestins and is required for β -arrestin-dependent internalization of GPCRs via clathrin-coated pits [11-13]. The μ_2 subunit interacts with dileucine motifs ([DE]XXX[LI]) in the carboxyl terminal tails of membrane spanning proteins found near acid residues thereby promoting their internalization via clathrin-coated pits [10]. Two putative dileucine motifs (FRHGILKLL) are present within the carboxyl terminal tail of the chemokine receptor CXCR2, although not in the context of acidic residues [14] (Fig. 1). When the Ile/Leu pair and/or Leu pair are changed to alanine residues, internalization of the mutant receptors is attenuated. The mutant receptors show normal agonist-induced phosphorylation and binding to β -arrestins as assessed by co-immunoprecipitation, suggesting that AP2 acts independent of β -arrestins to promote CXCR2 internalization. However, β -arrestins are also likely involved in CXCR2 internalization [15]. CXCR4 also has a putative dileucine motif sequence within its carboxyl-terminal tail (GSSLKIL) (Fig. 1). Mutation of the Ile/Leu pair within this motif to alanine residues attenuates agonist-induced internalization of the receptor [16], although not in all cell types [17]. Direct support for a role for AP2 in CXCR4 internalization was provided in a recent study that showed that siRNA mediated depletion of the μ_2 subunit attenuates agonist-induced internalization of CXCR4 in HeLa cells [18]. It remains to be determined whether AP2 interacts directly with CXCR4. Scanning the amino acid sequence of C-X-C chemokine receptors reveals that dileucine-like motif elements are present in several receptors (Fig. 1), suggesting that dileucine motifs may have a broad role in chemokine receptor internalization. As AP2 is also known to interact with β -arrestin to mediate GPCR endocytosis, therefore, for at least CXCR2 and CXCR4, a complex interplay between receptor/ β -arrestin/AP2 is required for internalization, although mechanistic insight is lacking.

Chemokine receptor internalization leads to ligand degradation

Once internalized onto early endosomes GPCRs are either sorted into the recycling pathway or the degradative pathway [19] (Fig. 2). Many chemokine receptors readily enter the recycling pathway upon internalization and rapidly return to the cell surface where they contribute to resensitization of receptor signaling. For example, chemokine receptor CCR5 is relatively resistant to agonist-induced degradation, even upon chronic treatment with agonist most CCR5 recycles back to the plasma membrane [20,21**]. CCR7 also internalizes and it too is resistant to degradation upon binding to either of its cognate ligands CCL19 or CCL21 as it readily enters the recycling pathway [22]. One function of chemokine receptor internalization may be to deliver bound chemokines to lysosomes for

degradation. Upon binding to their cognate chemokines, receptor/chemokine complexes co-internalize onto endosomes where they disassociate owing to the acidic environment of the endocytic compartment and while the receptors enter the recycling pathway, chemokines are delivered to lysosomes and are degraded. The chemokines bound to CCR5 and CCR7 are efficiently degraded in lysosomes [22,23]. This may be important because sequestration and removal of circulating chemokines, especially inflammatory chemokines, may limit uncontrolled and potentially harmful recruitment of leukocytes [24].

Mechanisms mediating post endocytic sorting of chemokine receptors

Chemokine receptor recycling may be dependent upon a type-I PDZ-domain binding ligands present at the end of the carboxy-terminus of many chemokine receptors [25,26] (Fig. 1). Deletion or mutation of this motif in CCR5 [25] and CXCR2 [26] redirects receptors towards lysosomes where they are degraded, indicating that receptors are actively recycled possibly by interacting with an unidentified PDZ-domain containing protein. Interestingly, upon prolonged agonist exposure CXCR2 traffics to lysosomes, suggesting that disruption of this interaction may enhance lysosomal sorting [26]. Upon agonist-induced internalization of the β_2 -adrenergic receptor (β_2 AR), it too undergoes very efficient recycling, which is also mediated by a PDZ-ligand located at the carboxy-terminus of the receptor [27,28]. The PDZ-ligand interacts with the PDZ-domain containing protein SNX27, which links the receptor via the WASH (Wiskott-Aldrich syndrome protein and SCAR homologue) actin nucleation complex to the retromer complex in tubule extensions of early endosomes for efficient recycling [28-30]. Whether these factors mediate chemokine receptor recycling remains unknown. However, the carboxyl-terminal tail of CXCR2 interacts with G protein-coupled receptor associated sorting protein 1 (GASP1) [31]. GASP1 has been shown to mediate lysosomal targeting and degradation of a subset of GPCRs through an interaction with their carboxyl-terminal tail [32]. A recent study revealed that GASP1 functions in GPCR sorting to lysosomes by interacting with a newly identified protein called Beclin-2 [33]. So it is possible that CXCR2, and possibly other chemokine receptors, is sorted to lysosomes through a GASP1-Beclin2-dependent process, but this remains to be determined.

Regulation of chemokine receptor trafficking by ubiquitin

In contrast to other chemokine receptors, upon internalization CXCR4 is primarily sorted into the degradative pathway and is efficiently targeted for lysosomal degradation (Fig. 2). This requires agonist induced ubiquitination of carboxyl-terminal tail lysine residues [21]** (Fig. 1). Ubiquitination is preceded by agonist-induced phosphorylation of C-tail serine residues 324/5 at the plasma membrane [34] (Fig. 1). Phosphorylation of these residues leads to recruitment of the E3 ubiquitin ligase AIP4 to the receptor at the plasma membrane thereby enabling ubiquitination of the receptor on nearby lysine residues [21**,35**]. An adaptor protein is not required because AIP4 interacts directly with the receptor on phosphorylated Ser324/5 [34]. Interestingly, the Ser324/5 pair is phosphorylated by PKC and GRK6 [36], suggesting that these kinases drive receptor ubiquitination and degradation, but this remains to be determined. In contrast, while ubiquitination of β_2 AR is also preceded by phosphorylation, it requires β -arrestin-2 to serve as an adaptor to link Nedd4, an E3

ubiquitin ligase related to AIP4, to the receptor [37]. This highlights the fact that GPCRs are ubiquitinated by diverse mechanisms.

Regulation of chemokine receptor sorting by ubiquitination of the ESCRT machinery

The ubiquitin moieties attached to CXCR4 tag the receptor for lysosomal degradation because they mediate entry into the endosomal sorting complex required for transport (ESCRT) pathway [35^{**},38^{*}]. The ESCRT pathway consists of five discrete multi-protein complexes known as ESCRT 0-III and the AAA ATPase Vps4 complex [39]. These complexes act in a sequential and coordinated manner to target ubiquitinated cargo into intraluminal vesicles of multivesicular bodies, which then fuse with lysosomes where degradation occurs. Entry into the ESCRT pathway occurs when the ubiquitin binding domains (UBDs) found in ESCRT-0 bind to ubiquitin moieties on ubiquitinated receptors, including CXCR4 [40,41]. Although ubiquitin modification is necessary for GPCR sorting into the ESCRT pathway, regulation of the sorting machinery by ubiquitin modification also plays an important role in this process [35^{**},38^{*},42,43]. ESCRT-0, and hence CXCR4 sorting into the degradative pathway, is regulated by a complex process that involves endosomal localized AIP4 and β -arrestin-1, revealing a novel role for β -arrestin in endosomal sorting of GPCRs [44]^{*}. ESCRT-0 is comprised of two protein subunits: HRS (hepatocyte growth factor-regulated tyrosine kinase substrate) and STAM (signal transduction adaptor molecule). These subunits have opposing roles in sorting CXCR4 to lysosomes as siRNA mediated depletion of HRS attenuates CXCR4 degradation [35]^{**}, while siRNA mediated depletion of STAM-1 accelerates it [38]^{*}. This may be explained by the fact that β -arrestin-1 interacts directly with STAM-1 and AIP4 to regulate the ubiquitination status of HRS [38]. The ubiquitin moieties on HRS may interact with its own UBD rendering HRS in an auto-inhibitory conformation such that it is unable to perform its sorting function efficiently [45]. Because CXCR4 activation induces HRS ubiquitination, it represents a way in which CXCR4 can regulate its own sorting for degradation [38]^{*}. Therefore ubiquitin acts in *cis* and in *trans* to regulate chemokine receptor endosomal sorting, underscoring the fact that endosomal sorting is complex.

Interestingly, CXCR7 is also regulated by ubiquitination, although in contrast to CXCR4, CXCR7 is constitutively ubiquitinated and agonist-activation induces its deubiquitination [46]. CCR7 is also constitutively ubiquitinated, however, in contrast to CXCR7, agonist has no effect on its ubiquitination status [47]. Constitutive ubiquitination of CXCR7 and CCR7 may serve to regulate cell surface expression of the receptors from an intracellular pool. Identifying the E3 ubiquitin ligase and the deubiquitinating enzymes that regulate CCR7 and CXCR7 ubiquitination will no doubt lead to greater mechanistic insight into this process.

CXCR7 is an interesting receptor because it binds to the chemokines CXCL11 and CXCL12 with high affinity, however, binding to these chemokines does not illicit G protein-coupled second messenger responses [48-50]. Therefore CXCR7 has been considered to be a non-signaling GPCR, although it couples to β -arrestin dependent signaling [48]. Upon agonist binding CXCR7 is rapidly internalized via a β -arrestin-dependent pathway and recycled to the cell surface [46,51]. CXCR7 itself is not degraded, but rather it delivers bound

chemokine to lysosomes for degradation [51,52] (Fig. 2). CXCL12 is also the cognate ligand for CXCR4, which couples very strongly with G protein-dependent signaling [53]. CXCR7 has been shown to function physiologically by interacting with and internalizing CXCL12 and sequestering it from the extracellular milieu, thereby titrating the amount of CXCL12 that is available to act on CXCR4 [54]**. A recent study revealed that this is important during development of the brain [55]**. In cortical interneurons, CXCR7 bound to CXCL12 is internalized and CXCL12 is delivered to lysosomes for degradation, while CXCR7 is recycled to the plasma membrane to initiate another round of binding, internalization, chemokine release and recycling; however, internalized CXCR4 is targeted for lysosomal degradation. In this way, CXCL12 is titrated to a point at which it can then direct the migration of interneurons expressing CXCR4 in a sustained manner to their final destination during development of the neocortex [55]**. Understanding how CXCR7 and CXCR4 are sorted on endosomes may provide additional insights into how they cooperate during development and in diseases in which they are involved.

Conclusion

Chemokine receptor trafficking is important for regulating signaling and ultimately mediating cell migration [8]. Despite this the mechanisms mediating chemokine receptor trafficking remain poorly defined. Identifying the sorting machinery, such as the E3 ubiquitin ligase and deubiquitinating enzymes plus other factors that contribute to either recycling and/or lysosomal sorting will be important in understanding how chemokine receptor trafficking is precisely regulated. This may have significant clinical implications because it may enable modulating chemokine receptor trafficking as a therapeutic strategy to treat cancer and possibly other diseases.

References

1. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med*. 2006; 354:610–621. [PubMed: 16467548]
2. Thelen M, Stein JV. How chemokines invite leukocytes to dance. *Nat Immunol*. 2008; 9:953–959. [PubMed: 18711432]
3. Cardona AE, Li M, Liu L, Savarin C, Ransohoff RM. Chemokines in and out of the central nervous system: much more than chemotaxis and inflammation. *J Leukoc Biol*. 2008; 84:587–594. [PubMed: 18467654]
4. Balkwill F. Cancer and the chemokine network. *Nat Rev Cancer*. 2004; 4:540–550. [PubMed: 15229479]
5. Dorsam RT, Gutkind JS. G-protein-coupled receptors and cancer. *Nat Rev Cancer*. 2007; 7:79–94. [PubMed: 17251915]
6. Garzino-Demo A, DeVico AL, Conant KE, Gallo RC. The role of chemokines in human immunodeficiency virus infection. *Immunol Rev*. 2000; 177:79–87. [PubMed: 11138787]
7. Balkwill F. The significance of cancer cell expression of the chemokine receptor CXCR4. *Semin Cancer Biol*. 2004; 14:171–179. [PubMed: 15246052]
8. Li YM, Pan Y, Wei Y, Cheng X, Zhou BP, Tan M, Zhou X, Xia W, Hortobagyi GN, Yu D, et al. Upregulation of CXCR4 is essential for HER2-mediated tumor metastasis. *Cancer Cell*. 2004; 6:459–469. [PubMed: 15542430]
9. Neel NF, Schutyser E, Sai J, Fan GH, Richmond A. Chemokine receptor internalization and intracellular trafficking. *Cytokine Growth Factor Rev*. 2005; 16:637–658. [PubMed: 15998596]

10. Bonifacino JS, Traub LM. Signals for sorting of transmembrane proteins to endosomes and lysosomes. *Annu Rev Biochem.* 2003; 72:395–447. [PubMed: 12651740]
11. Laporte SA, Oakley RH, Zhang J, Holt JA, Ferguson SS, Caron MG, Barak LS. The beta2-adrenergic receptor/betaarrestin complex recruits the clathrin adaptor AP-2 during endocytosis. *Proc Natl Acad Sci U S A.* 1999; 96:3712–3717. [PubMed: 10097102]
12. Laporte SA, Oakley RH, Holt JA, Barak LS, Caron MG. The interaction of beta-arrestin with the AP-2 adaptor is required for the clustering of beta 2-adrenergic receptor into clathrin-coated pits. *J Biol Chem.* 2000; 275:23120–23126. [PubMed: 10770944]
13. Kim YM, Benovic JL. Differential roles of arrestin-2 interaction with clathrin and adaptor protein 2 in G protein-coupled receptor trafficking. *J Biol Chem.* 2002; 277:30760–30768. [PubMed: 12070169]
14. Fan GH, Yang W, Wang XJ, Qian Q, Richmond A. Identification of a motif in the carboxyl terminus of CXCR2 that is involved in adaptin 2 binding and receptor internalization. *Biochemistry.* 2001; 40:791–800. [PubMed: 11170396]
15. Su Y, Raghuwanshi SK, Yu Y, Nanney LB, Richardson RM, Richmond A. Altered CXCR2 signaling in beta-arrestin-2-deficient mouse models. *J Immunol.* 2005; 175:5396–5402. [PubMed: 16210646]
16. Orsini MJ, Parent JL, Mundell SJ, Marchese A, Benovic JL. Trafficking of the HIV coreceptor CXCR4. Role of arrestins and identification of residues in the c-terminal tail that mediate receptor internalization. *The Journal of biological chemistry.* 1999; 274:31076–31086. [PubMed: 10521508]
17. Signoret N, Rosenkilde MM, Klasse PJ, Schwartz TW, Malim MH, Hoxie JA, Marsh M. Differential regulation of CXCR4 and CCR5 endocytosis. *J Cell Sci.* 1998; 111(Pt 18):2819–2830. [PubMed: 9718374]
18. Malik R, Soh UJ, Trejo J, Marchese A. Novel roles for the E3 ubiquitin ligase atrophin-interacting protein 4 and signal transduction adaptor molecule 1 in G protein-coupled receptor signaling. *J Biol Chem.* 2012; 287:9013–9027. [PubMed: 22275353]
19. Marchese A, Chen C, Kim YM, Benovic JL. The ins and outs of G protein-coupled receptor trafficking. *Trends Biochem Sci.* 2003; 28:369–376. [PubMed: 12878004]
20. Mueller A, Kelly E, Strange PG. Pathways for internalization and recycling of the chemokine receptor CCR5. *Blood.* 2002; 99:785–791. [PubMed: 11806977]
- 21**. Marchese A, Benovic JL. Agonist-promoted ubiquitination of the G protein-coupled receptor CXCR4 mediates lysosomal sorting. *J Biol Chem.* 2001; 276:45509–45512. This was one of the first reports to show that agonist-induced ubiquitination serves as an endosomal sorting signal and targets a mammalian G protein-coupled receptor for lysosomal degradation. [PubMed: 11641392]
22. Otero C, Groettrup M, Legler DF. Opposite fate of endocytosed CCR7 and its ligands: recycling versus degradation. *J Immunol.* 2006; 177:2314–2323. [PubMed: 16887992]
23. Wang JM, Hishinuma A, Oppenheim JJ, Matsushima K. Studies of binding and internalization of human recombinant monocyte chemotactic and activating factor (MCAF) by monocytic cells. *Cytokine.* 1993; 5:264–275. [PubMed: 8218939]
24. Graham GJ. D6 and the atypical chemokine receptor family: novel regulators of immune and inflammatory processes. *Eur J Immunol.* 2009; 39:342–351. [PubMed: 19130487]
25. Delhay M, Gravot A, Ayinde D, Niedergang F, Alizon M, Brelot A. Identification of a postendocytic sorting sequence in CCR5. *Mol Pharmacol.* 2007; 72:1497–1507. [PubMed: 17855654]
26. Baugher PJ, Richmond A. The carboxyl-terminal PDZ ligand motif of chemokine receptor CXCR2 modulates post-endocytic sorting and cellular chemotaxis. *J Biol Chem.* 2008; 283:30868–30878. [PubMed: 18755694]
27. Cao TT, Deacon HW, Reczek D, Bretscher A, von Zastrow M. A kinase-regulated PDZ-domain interaction controls endocytic sorting of the beta2-adrenergic receptor. *Nature.* 1999; 401:286–290. [PubMed: 10499588]

28. Lauffer BE, Melero C, Temkin P, Lei C, Hong W, Kortemme T, von Zastrow M. SNX27 mediates PDZ-directed sorting from endosomes to the plasma membrane. *J Cell Biol.* 2010; 190:565–574. [PubMed: 20733053]
29. Temkin P, Lauffer B, Jager S, Cimerancic P, Krogan NJ, von Zastrow M. SNX27 mediates retromer tubule entry and endosome-to-plasma membrane trafficking of signalling receptors. *Nat Cell Biol.* 2011; 13:715–721. [PubMed: 21602791]
30. Steinberg F, Gallon M, Winfield M, Thomas EC, Bell AJ, Heesom KJ, Tavaré JM, Cullen PJ. A global analysis of SNX27-retromer assembly and cargo specificity reveals a function in glucose and metal ion transport. *Nat Cell Biol.* 2013; 15:461–471. [PubMed: 23563491]
31. Heydorn A, Sondergaard BP, Ersboll B, Holst B, Nielsen FC, Haft CR, Whistler J, Schwartz TW. A library of 7TM receptor C-terminal tails. Interactions with the proposed post-endocytic sorting proteins ERM-binding phosphoprotein 50 (EBP50), N-ethylmaleimide-sensitive factor (NSF), sorting nexin 1 (SNX1), and G protein-coupled receptor-associated sorting protein (GASP). *J Biol Chem.* 2004; 279:54291–54303. [PubMed: 15452121]
32. Zwaenepoel O, Tzenaki N, Vergetaki A, Makrigiannakis A, Vanhaesebroeck B, Papakonstanti EA. Functional CSF-1 receptors are located at the nuclear envelope and activated via the p110delta isoform of PI 3-kinase. *FASEB J.* 2012; 26:691–706. [PubMed: 22084313]
33. He C, Wei Y, Sun K, Li B, Dong X, Zou Z, Liu Y, Kinch LN, Khan S, Sinha S, et al. Beclin 2 functions in autophagy, degradation of G protein-coupled receptors, and metabolism. *Cell.* 2013; 154:1085–1099. [PubMed: 23954414]
34. Bhandari D, Robia SL, Marchese A. The E3 ubiquitin ligase atrophin interacting protein 4 binds directly to the chemokine receptor CXCR4 via a novel WW domain-mediated interaction. *Mol Biol Cell.* 2009; 20:1324–1339. [PubMed: 19116316]
- 35*. Marchese A, Raiborg C, Santini F, Keen JH, Stenmark H, Benovic JL. The E3 ubiquitin ligase AIP4 mediates ubiquitination and sorting of the G protein-coupled receptor CXCR4. *Dev Cell.* 2003; 5:709–722. This was the first report to assign an E3 ubiquitin ligase (AIP4) to agonist induced ubiquitination of a mammalian G protein-coupled receptor. It was also the first report to show that the ESCRT machinery was ubiquitinated by GPCR activation and was important for mediating lysosomal sorting of a mammalian GPCR. [PubMed: 14602072]
36. Busillo JM, Armando S, Sengupta R, Meucci O, Bouvier M, Benovic JL. Site-specific phosphorylation of CXCR4 is dynamically regulated by multiple kinases and results in differential modulation of CXCR4 signaling. *J Biol Chem.* 2010; 285:7805–7817. [PubMed: 20048153]
37. Shenoy SK, Xiao K, Venkataramanan V, Snyder PM, Freedman NJ, Weissman AM. Nedd4 mediates agonist-dependent ubiquitination, lysosomal targeting, and degradation of the beta2-adrenergic receptor. *J Biol Chem.* 2008; 283:22166–22176. [PubMed: 18544533]
- 38*. Malik R, Marchese A. Arrestin-2 interacts with the endosomal sorting complex required for transport machinery to modulate endosomal sorting of CXCR4. *Mol Biol Cell.* 2010; 21:2529–2541. This was one of the first reports to describe a mechanism by which β -arrestins regulate endosomal sorting of a mammalian G protein-coupled receptor. This report showed that β -arrestins modulate the ubiquitination status of components of the ESCRT machinery. [PubMed: 20505072]
39. Henne WM, Stenmark H, Emr SD. Molecular Mechanisms of the Membrane Sculpting ESCRT Pathway. *Cold Spring Harb Perspect Biol.* 2013; 5
40. Raiborg C, Stenmark H. The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. *Nature.* 2009; 458:445–452. [PubMed: 19325624]
41. Shields SB, Oestreich AJ, Winistorfer S, Nguyen D, Payne JA, Katzmann DJ, Piper R. ESCRT ubiquitin-binding domains function cooperatively during MVB cargo sorting. *J Cell Biol.* 2009; 185:213–224. [PubMed: 19380877]
42. Berlin I, Higginbotham KM, Dise RS, Sierra MI, Nash PD. The deubiquitinating enzyme USP8 promotes trafficking and degradation of the chemokine receptor 4 at the sorting endosome. *J Biol Chem.* 2010; 285:37895–37908. [PubMed: 20876529]
43. Sierra MI, Wright MH, Nash PD. AMSH interacts with ESCRT-0 to regulate the stability and trafficking of CXCR4. *J Biol Chem.* 2010; 285:13990–14004. [PubMed: 20159979]

- 44*. Bhandari D, Trejo J, Benovic JL, Marchese A. Arrestin-2 interacts with the ubiquitin-protein isopeptide ligase atrophin-interacting protein 4 and mediates endosomal sorting of the chemokine receptor CXCR4. *J Biol Chem*. 2007; 282:36971–36979. This was the first report to establish a direct role for β -arrestins in endosomal sorting and lysosomal targeting of a G protein-coupled receptor, a function distinct from their roles in G protein-coupled receptor desensitization and internalization. [PubMed: 17947233]
45. Hoeller D, Crosetto N, Blagoev B, Raiborg C, Tikkanen R, Wagner S, Kowanetz K, Breitling R, Mann M, Stenmark H, et al. Regulation of ubiquitin-binding proteins by monoubiquitination. *Nat Cell Biol*. 2006; 8:163–169. [PubMed: 16429130]
46. Canals M, Scholten DJ, de Munnik S, Han MK, Smit MJ, Leurs R. Ubiquitination of CXCR7 controls receptor trafficking. *PLoS One*. 2012; 7:e34192. [PubMed: 22457824]
47. Schaeuble K, Hauser MA, Rippl AV, Bruderer R, Otero C, Groettrup M, Legler DF. Ubiquitylation of the chemokine receptor CCR7 enables efficient receptor recycling and cell migration. *J Cell Sci*. 2012; 125:4463–4474. [PubMed: 22797918]
48. Rajagopal S, Kim J, Ahn S, Craig S, Lam CM, Gerard NP, Gerard C, Lefkowitz RJ. Beta-arrestin-but not G protein-mediated signaling by the “decoy” receptor CXCR7. *Proc Natl Acad Sci U S A*. 2010; 107:628–632. [PubMed: 20018651]
49. Burns JM, Summers BC, Wang Y, Melikian A, Berahovich R, Miao Z, Penfold ME, Sunshine MJ, Littman DR, Kuo CJ, et al. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. *J Exp Med*. 2006; 203:2201–2213. [PubMed: 16940167]
50. Levoe A, Balabanian K, Baleux F, Bachelier F, Lagane B. CXCR7 heterodimerizes with CXCR4 and regulates CXCL12-mediated G protein signaling. *Blood*. 2009; 113:6085–6093. [PubMed: 19380869]
51. Luker KE, Steele JM, Mihalko LA, Ray P, Luker GD. Constitutive and chemokine-dependent internalization and recycling of CXCR7 in breast cancer cells to degrade chemokine ligands. *Oncogene*. 2010; 29:4599–4610. [PubMed: 20531309]
52. Naumann U, Cameron E, Pruenster M, Mahabaleswar H, Raz E, Zerwes HG, Rot A, Thelen M. CXCR7 functions as a scavenger for CXCL12 and CXCL11. *PLoS One*. 2010; 5:e9175. [PubMed: 20161793]
53. Busillo JM, Benovic JL. Regulation of CXCR4 signaling. *Biochim Biophys Acta*. 2007; 1768:952–963. [PubMed: 17169327]
- 54*. Boldajipour B, Mahabaleswar H, Kardash E, Reichman-Fried M, Blaser H, Minina S, Wilson D, Xu Q, Raz E. Control of chemokine-guided cell migration by ligand sequestration. *Cell*. 2008; 132:463–473. This study revealed that CXCR7 acts as a non-signaling receptor *in vivo* by binding to and internalizing CXCL12 thereby creating an extracellular concentration gradient of ligand enabling proper directed migration of CXCR4 expressing cells. [PubMed: 18267076]
- 55*. Sanchez-Alcaniz JA, Haege S, Mueller W, Pla R, Mackay F, Schulz S, Lopez-Bendito G, Stumm R, Marin O. Cxcr7 controls neuronal migration by regulating chemokine responsiveness. *Neuron*. 2011; 69:77–90. This study revealed the intricate cooperativity between CXCR4 and CXCR7 during development of the brain, showing that CXCR7 acts a scavenging receptor *in vivo* by internalizing and degrading CXCL12 in order to titrate the concentration of extracellular CXCL12 and to sustain signaling mediated by the CXCL12 signaling receptor CXCR4. [PubMed: 21220100]
56. Meiser A, Mueller A, Wise EL, McDonagh EM, Petit SJ, Saran N, Clark PC, Williams TJ, Pease JE. The chemokine receptor CXCR3 is degraded following internalization and is replenished at the cell surface by de novo synthesis of receptor. *J Immunol*. 2008; 180:6713–6724. [PubMed: 18453591]
57. Mahabaleswar H, Tarbashevich K, Nowak M, Brand M, Raz E. beta-arrestin control of late endosomal sorting facilitates decoy receptor function and chemokine gradient formation. *Development*. 2012; 139:2897–2902. [PubMed: 22791893]
58. Slagsvold T, Marchese A, Brech A, Stenmark H. CISK attenuates degradation of the chemokine receptor CXCR4 via the ubiquitin ligase AIP4. *Embo J*. 2006; 25:3738–3749. [PubMed: 16888620]

Highlights

- Chemokine receptor internalization is important for regulating signaling and function
- Chemokine receptor trafficking is important for delivering chemokines to lysosomes
- Ubiquitination has diverse roles in chemokine receptor trafficking
- Cooperation between CXCR4 and CXCR7 trafficking controls physiological signaling

Figure 1. Amino acid alignment of the carboxy-terminal tail of C-X-C chemokine receptors

Lysine residues highlighted in blue in the amino acid sequence of CXCR4 have been shown to be involved in ubiquitination and degradation of CXCR4 [21], whereas the lysine residues highlighted in CXCR2 [26] and CXCR3 [56] have been shown to not be involved in ubiquitination and degradation. The lysine residues highlighted in CXCR7 have been shown to be involved in ubiquitination and plasma membrane localization [46]. The serine residues highlighted in red in CXCR4 have been shown to be important for E3 ubiquitin ligase AIP4 binding and ubiquitination and degradation of CXCR4 [21,34]. The dileucine motif-like sequences highlighted in yellow have been shown to be important for agonist-induced internalization of CXCR2 [14] and CXCR4 [21]. The PDZ-like ligand in CXCR2 has been shown to be important for directing the receptor away from the degradative pathway [26]. Transmembrane domain 7 is indicated and the single letter amino acid code is used. The sequence for each receptor was obtained from GenBank under the following accession numbers: U11870 (CXCR1); U11869 (CXCR2); U32674 (CXCR3); AF005058 (CXCR4); X68829 (CXCR5); AF007545 (CXCR6); and BC008459 (CXCR7).

Figure 2. Trafficking patterns of the CXCL12 chemokine receptors CXCR4 and CXCR7

Upon binding to CXCL12, CXCR4 is ubiquitinated by the E3 ubiquitin ligase AIP4 at the plasma membrane. CXCR4 then internalizes to early endosomes and is sorted to lysosomes where it is degraded. Endosomal sorting of ubiquitinated CXCR4 is mediated by a complex and carefully regulated process that involves AIP4, β -arrestin-1, the ESCRT machinery and the AAA ATPase Vps4. In contrast, CXCR7 is constitutively ubiquitinated and upon binding CXCL12, is deubiquitinated and CXCL12 and CXCR7 co-internalize to early endosomes via a β -arrestin-1-dependent pathway. In the acidic environment of the endosome, CXCL12 dissociates from CXCR7 and while CXCR7 enters a Rab11-positive recycling compartment [57], CXCL12 is delivered to lysosomes for degradation. The ubiquitin moieties on CXCR4 target it into invaginating domains of early endosomes or multivesicular bodies, which pinch off and form the intraluminal vesicles. CXCR4 is found on the limiting membrane of multivesicular bodies and on the membranes of the intraluminal vesicles [58]. The multivesicular body fuses with lysosomes where degradation occurs.