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# Endocytic trafficking of chemokine receptors

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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. Receptor sorting into the recycling pathway contributes to resensitization of receptor signaling, whereas sorting into the degradative pathway leads 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.

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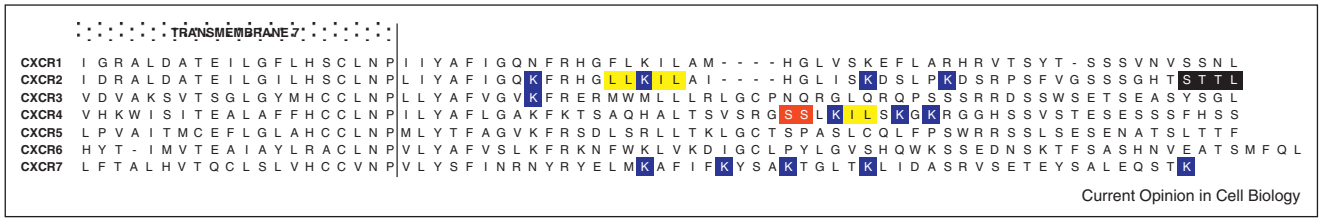
## Introduction

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 overexpressed 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 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 hetero-tetrameric protein complex that is comprised of $\beta_2$, α, σ and $\mu_2$ subunits [10]. The $\beta_2$-adaptin subunit interacts with β-arrestins and is required for β-arrestin-dependent internalization of GPCRs via clathrin-coated pits [11–13]. The $\mu_2$ 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 (FRHG**IL**K**LL**) are present within the carboxyl terminal tail of the chemokine receptor CXCR2, although not in the context of acidic residues [14] (Figure 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 coimmunoprecipitation, 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 (GSSLK**IL**) (Figure 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 $\mu_2$ 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

**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).

reveals that dileucine-like motif elements are present in several receptors (Figure 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] (Figure 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 cointernalize 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 postendocytic sorting of chemokine receptors

Chemokine receptor recycling may be dependent on a type-I PDZ-domain binding ligands present at the end of the carboxy-terminus of many chemokine receptors [25,26] (Figure 1). Deletion or mutation of this motif in CCR5 [25] and CXCR2 [26] redirects receptors toward 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 $\beta_2$-adrenergic receptor ($\beta_2$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.