The chemokine receptor CXCR4 plays critical roles in development, immune function, and human immunodeficiency virus type 1 (HIV-1) entry. Here we demonstrate that, like the CC-chemokine receptors CCR5 and CCR2b, CXCR4 is posttranslationally modified by sulfation of its amino-terminal tyrosines. The sulfate group at tyrosine 21 contributes substantially to the ability of CXCR4 to bind its ligand, stromal derived factor 1α. Tyrosine sulfation plays a less significant role in CXCR4-dependent HIV-1 entry than in CCR5-dependent HIV-1 entry. In some cell lines, CXCR4 is efficiently modified by a chondroitin sulfate chain at serine 18, but neither HIV-1 entry nor stromal derived factor 1α binding was affected by loss of this glycosaminoglycan. These data demonstrate a functional role for tyrosine sulfates in the CXCR4 chemokine receptor family and underscore a general difference in HIV-1 utilization of CCR5 and CXCR4.

CXCR4 is a member of the G-protein-coupled receptor family that serves as a receptor for the chemokine stromal derived factor (SDF)1α (1, 2). CXCR4 is expressed in a wide range of tissues and cell lines. Disruption of either the sdf1-1α or CXCR4 gene resulted in similar embryonic lethal mutations, underscoring a role for this receptor/ligand pair in hematopoiesis, vascularization, and neuronal development (3–5). CXCR4 has also been implicated in breast cancer and melanoma metastasis (6).

In addition to roles in development and cancer, CXCR4 serves as a coreceptor, together with the cellular receptor CD4, for the entry of a subset of isolates of human immunodeficiency virus type 1 (HIV-1) (7). Entry is initiated when the HIV-1 envelope glycoprotein binds CD4 (8). Association with CD4 induces a conformational change in the surface envelope glycoprotein gp120 that allows this glycoprotein to then associate with its natural chemokine ligands (18, 19). Most or all chemokine receptors and all HIV-1 coreceptors have amino-terminal domains that suggest the presence of tyrosine sulfate moieties (18). Tyrosine sulfation plays an important role in HIV-1 entry (9, 10). Association with the chemokine receptor is thought to induce a more dramatic rearrangement of the transmembrane envelope glycoprotein gp41. This latter rearrangement results in the mixing of viral and cellular lipids and ultimately in the entry of the viral capsid into the target cell (11).

Most virions that are initially transmitted to a new host and those that predominate during most of infection utilize the CC-chemokine receptor CCR5 as a principal coreceptor. Coincident with a decline in immune function, HIV-1 isolates emerge that utilize CXCR4 (12). Viruses that utilize CXCR4 can infect a larger pool of mostly naive CD4+ T lymphocytes that express CXCR4 but not CCR5 (13). Most laboratory-adapted isolates of HIV-1 utilize CXCR4 exclusively because cell lines used in tissue culture for viral replication typically express CXCR4 but not CCR5 (14). In addition to these receptors, a number of G-protein-coupled receptors have been identified that serve as coreceptors in tissue culture systems of HIV-1 infection. The role of these minor coreceptors remains unclear, but they have been useful in identifying properties common to HIV-1 coreceptors (15, 16).

The most prominent of these commonalities is the presence of a tyrosine-rich and acidic region in their amino termini (16). This region of CCR5 plays a critical or necessary role in the entry of HIV-1 isolates that utilize this coreceptor (17). The tyrosines of this region of CCR5 (and those of analogous regions of CCR2b and the C5a receptor) are modified by the addition of sulfates (18). In the case of CCR5, these sulfate moieties are important both for HIV-1 entry and for the association of CCR5 with its natural chemokine ligands (18, 19). Most or all chemokine receptors and all HIV-1 coreceptors have amino-terminal domains that suggest the presence of tyrosine sulfates (18). To date, functions for these sulfate groups have been demonstrated for the CC-chemokine receptors CCR2b and CCR5 and for the related receptor of the C5a anaphylatoxin (18, 20, 21).

Here we document the presence of two distinct forms of sulfation on CXCR4. Tyrosine sulfate is present at the amino terminus of CXCR4 and makes a substantial contribution to its association with SDF-1α. In contrast to CCR5, the tyrosine sulfate moieties of CXCR4 did not play a major role in the entry of the five HIV-1 isolates that were assayed. Finally, we observe an additional source of CXCR4 sulfate present at serine 18, a chondroitin sulfate chain, in some but not all cell lines. These data extend the role of amino-terminal tyrosine sulfates to the CXCR4 family and underscore a key difference in HIV-1 utilization of CCR5 and CXCR4.

**EXPERIMENTAL PROCEDURES**

Cells, Plasmids, and Antibodies—The human embryonic kidney cell line HEK293T, the canine thymic stromal cell line CF2Th, the human cervical cancer cell line HeLa, the human myeloid cell line U937, and the human T-lymphocyte cell line Jurkat were obtained from American
Type Culture Collection (ATCC CRL11554, ATCC CRL1430, ATCC CCL2, ATCC CRL1593, and ATCC TIB152, respectively). Codon-optimized and myc- and C9-tagged CXCR4 has been described previously (22). All the CXCR4 variants were made on codon-optimized CXCR4 by the PCR-based QuickChange mutagenesis method (Stratagene) and confirmed by sequencing the entire reading frame. The antibody ID4, which recognizes the C9 tag, and the anti-myc tag antibody, 9E10, were provided by the National Cell Culture Center (Minneapolis, MN). Anti-CXCR4 antibody 12G5, conjugated to phycoerythrin, was obtained from Pharmingen.

Labeling and Immunoprecipitation—Cells were transfected with plasmids expressing CXCR4 or CXCR4 variants by the calcium phosphate method; 1 day later, cells were washed twice in phosphate-buffered saline (PBS), split at a 1:3 ratio, and labeled with [35S]cysteine and [35S]methionine or [35S]sulfate for 1 day. Cysteine and methionine labeling media contained 50 μCi each of [35S]cysteine and [35S]methionine per milliliter of Dulbecco's modified Eagle's medium lacking both amino acids. Sulfate labeling media contains 125 μCi [35S]sulfate/ml sulfate-free media (ICN). In some cases, cells were treated with 3 μg/ml N-glycosylation inhibitor tunicamycin added to cell culture media 5 h before labeling and also added to the labeling media. Labeled cells were lysed in 1% N-dodecyl-β-D-maltoside (Anatrace) in PBS containing protease inhibitor mixtures for mammalian cells (Sigma and Roche Biochemicals). After cell debris was removed by centrifugation at 14,000 rpm for 10 min at 4°C, supernatant was immunoprecipitated by the ID4 antibody. Immunoprecipitates were washed twice with 1% N-dodecyl-β-D-maltoside/PBS containing 0.5% SDS and once with PBS. Reducing Laemmli sample buffer was added, and samples were treated at 50°C for 10–15 min before analyzed by SDS-PAGE. Typically, a 9-fold greater volume of [35S]sulfate-labeled samples was analyzed relative to samples radiolabeled with cysteine and methionine. Endogenous CXCR4 was immunoprecipitated using the anti-CXCR4 antibody 12G5 from HeLa cells lysed with 1% CHAPS (Anatrace) in PBS.

HIV-1 Entry Assays—HIV-1 entry into C2TH cells expressing CD4 and wild-type or variant coreceptors has been described previously (14). Briefly, HIV-1 proviral DNA lacking a functional envelope gene and encoding choloramphenicol acetyltransferase as a reporter was cotransfected into HEK293T cells with plasmids encoding the envelope glycoproteins of the CCRX isolates 89.6 or MCGP1 or of the X4 isolates CF402.1, SG3, or HXBc2. Viruses harvested from the cell culture supernatant corresponding to 10,000–20,000 cpm reverse transcriptase activity were incubated with target cells overnight. C2TH cells were transfected with 3.0 μg of pcDNA3.1-C4D plasmid and a varying amount (0–1.0 μg) of wild-type or variant CXCR4-expressing plasmids in 6-well plates. The following day, cells were replated onto 6-well plates for FACS analysis and infection. Approximately 48 h after transfection, one set of cells was used to measure the receptor expression level, and the other sets were infected with reporter viruses. Expression of wild-type CXCR4 and the variants was measured by the 12G5 antibody (Pharmingen) that recognizes domains other than the CXCR4 amino terminus (23). Infected cells were washed with PBS after overnight incubation with viruses, and choloramphenicol acetyltransferase activity was measured in the lysed cells ~60 h after infection.

Enzymatic Digestion of CXCR4 Carbohydrates—Cells transfected and labeled with [35S]cysteine and [35S]methionine or [35S]sulfate were lysed and immunoprecipitated as described above and washed in 1% N-dodecyl-β-D-maltoside/PBS and in PBS. Typically, immunoprecipitates from ~1–5×106 cells were digested in a total volume of 150 μl 1.5 h at 37°C with enzyme. To digest the high molecular weight smear coprecipitated with CXCR4, 10 million chondroitin ABC lyase (EC 4.2.2.4; Sigma) or 10 milliunits of heparinase (heparin lyase; Calbiochem), heparitinase I (heparin lyase I; Calbiochem), heparitinase II (heparin lyase II; Calbiochem), or a mixture of all three heparinases were used. Chondroitinase buffer contains 150 mM NaCl, 1 mM CaCl2, and 1 mM MgCl2 in 20 mM Tris, pH 8. Heparinase buffer contains 500 mM NaCl, 5 mM calcium acetate, and 1 mM MgCl2 in 50 mM Heps, pH 7.4. Digests were washed once in PBS and analyzed by SDS-PAGE. Detection of O-glycans was performed using the glycoprotein deglycosylation kit (Calbiochem) or O-glycanase (Glyko). In the former case, 1 milliunit of N-acetylgalactosaminidase, 1.5 milliunits of 1,4-galactosaminidase, 40 milliunits of N-acetylgalactosaminidase, and 2 milliunits of neuraminidase were used together. Alternatively, 1 milliunit of O-glycanase (Glyko) and 5 milliunits of neuraminidase (Roche Biochemicals) were used together. In each case, digestion of O-glycan moieties of CCR5 was used to control for enzyme efficiency. Washed immunoprecipitates were digested with enzymes in a total volume of 50 μl for 2 h at 37°C. After digestion, samples were analyzed by SDS-PAGE. Chondroitinase treatment of HeLa cells was performed by incubating 1×106 cells with 500 milliunits of chondroitin ABC lyase in 0.5 ml of medium.

SDF-1α Binding to Cells Expressing CXCR4 Variants—Binding experiments were performed with HEK293T transfected with wild-type CXCR4 or the variants or HeLa cells. Two days after transfection, cells were washed in PBS and incubated at 2×106 cells/ml in binding buffer (50 mM Hepes, pH 7.4, 1 mM CaCl2, 1 mM MgCl2, 0.2% bovine serum albumin, and 0.1% NaN3) containing 5 μM diazyl fetal bovine serum (Invitrogen) or 1:500 diluted Mega Blox3 (CEL Associates, Inc.) for 1 h at room temperature before being used for the binding experiment. Fifty μl of cells were incubated with 0.2 μM [35S]SDF-1α (PerkinElmer Life Sciences) and 0–300 nM unlabeled SDF-1α (Peprotech) in 50 μl of binding buffer for 30 min at 37°C. Cells were washed once in binding buffer and once in binding buffer containing 0.5 mM NaCl. In parallel, FACS analysis was performed on 2×105 of the same cells stained with 12G5-phycoerythrin or the anti-myc antibody 9E10.

RESULTS

Tyrosine Sulfation of CXCR4—The amino terminus of CCR5 contains at least three sulfated tyrosines that play a major role in the entry of HIV-1 viruses that utilize CCR5 as a coreceptor as well as in the association of CCR5 with its natural chemokine ligands (18, 24). Amino-terminal tyrosine sulfation also participates in the ligand association of two additional chemotactic G-protein-coupled receptors: CCR2b and the C5a receptor (20, 21). We therefore sought to identify and determine a role of tyrosine sulfate of CXCR4, the other principal coreceptor of HIV-1.

Fig. 1 demonstrates that CXCR4 efficiently incorporates sulfate. HEK293T cells were transfected with plasmids encoding carboxyl-terminally tagged wild-type CXCR4 or a CXCR4 variant (FFF; see Table I) in which the three amino-terminal tyrosines had been altered to phenylalanine. Cells were divided and radiolabeled with either [35S]cysteine and [35S]methionine or [35S]sulfate, and CXCR4 and FFF were immunoprecipitated with the antibody ID4. As in the case of the C5a receptor (20), three forms of CXCR4 could be observed: (a) a predominant glycosylated form (indicated by the top arrowhead in Fig. 1); (b) a smaller, less glycosylated form; and (c) a fragment smaller than the expected size of unmodified CXCR4 (Fig. 1, lane 1). Treatment with the N-glycosylation inhibitor tunicamycin reduced the mobility of the predominant form (lane 2), consistent with the previous observation of CXCR4 N-glycosylation (25). An identical pattern was also observed with the FFF variant (lanes 3 and 4). Only the predominant form of FFF CXCR4 was observable in cells labeled with [35S]sulfate. Sulfate incorporation was markedly lower but still observable in the FFF variant as compared with wild-type CXCR4 (compare lanes 5 and 6 to lanes 7 and 8 of Fig. 1). We conclude that one or more of the amino-terminal tyrosines of CXCR4 are sulfated and that additional sulfate moieties are present on the receptor.

CXCR4 Amino-terminal Tyrosines and HIV-1 Entry—We then investigated the role of this sulfation in HIV-1 entry and

![Fig. 1. CXCR4 amino-terminal tyrosines are sulfated.](image-url)
in SDF-1α association. Fig. 2 demonstrates that HIV-1 pseudotyped with the envelope glycoproteins of various HIV-1 isolates that utilize CXCR4 is variably sensitive to alteration of the amino-terminal tyrosines of CXCR4. Little or no difference in the entry of viruses pseudotyped with the envelope glycoproteins of the ELI, SG3, and HXB2 isolates was observed in cells expressing comparable levels of CXCR4 or the FFF variant (Fig. 2A). Entry of the 89.6 and CF402.1 into cells expressing FFF was approximately 60% as efficient as entry into cells expressing CXCR4 (Fig. 2B). In contrast to the still efficient entry of 89.6 with the FFF CXCR4 variant, a CCR5 variant whose amino-terminal tyrosines have been altered to phenylalanine does not support any detectable entry by the 89.6 isolate (18). These data show that the role of tyrosine sulfate at the amino terminus of CXCR4 for HIV-1 entry varies among isolates and is less pronounced than what we have previously observed in the case of CCR5.

**SDF-1α Binding to the FFF Variant**—In contrast to HIV-1 entry, alteration of CXCR4 amino-terminal tyrosines had a substantial effect on the binding of SDF-1α. Fig. 3 shows that HEK293T cells transfected with wild-type CXCR4 bound SDF-1α much more efficiently than cells expressing comparable levels of the FFF variant (higher expression than wild-type CXCR4 in Fig. 3A, lower expression than wild-type CXCR4 in Fig. 3B), as determined by the amino-terminal tag antibody 9E10. The affinity of SDF-1α for the FFF variant was determined to be 4- to 5-fold lower than that of wild-type CXCR4 (Fig. 3A), and cells expressing the FFF variant bound less than half the [35S]SDF-1α as cells expressing comparable levels of wild-type CXCR4 when incubated with 0.2 nM radiolabeled SDF-1α (Fig. 3, A and B). These data imply that sulfate groups on one or more of the amino-terminal tyrosines of CXCR4 contribute substantially to its association with SDF-1α.

**Residual Sulfation at the Amino Terminus of CXCR4**—We also investigated the source of the residual sulfate observed on the FFF variant (Fig. 1, lane 8). Fig. 4A demonstrates that this residual sulfate is not associated with extracellular tyrosines other than the three in the amino terminus. A CXCR4 variant in which every extracellular tyrosine has been altered to phenylalanine (PFFPF; see Table I) and treated with the N-glycosylation inhibitor tunicamycin still incorporates [35S]Sulfate (Fig. 4A, lanes 2 and 5) with an efficiency similar to that of the FFF variant. Also, a panel of O-glycosidases that altered the mobility of CCR5 had no effect on the mobility or sulfate incorporation of CXCR4 or the FFF variant (data not shown). In contrast, a CXCR4 variant lacking residues 2–25 of its amino terminus (ΔNX4; see Table I) does not incorporate radiolabeled sulfate (Fig. 4A, lanes 3 and 7).

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### TABLE I

**CXCR4 variants used in this study**

| Receptor | Tyrosine | Chondroitin Sulfate | Sulfate |
|----------|----------|---------------------|---------|
| CXCR4    | MEG15I7SYTNTENQQGDDGSMKPC | +       | +       |
| ΔNX4     | MEG15I7SYTNTENQQGDDGSMKPC | +       | +       |
| FFF      | MEG15I7SYTNTENQQGDDGSMKPC | +       | +       |
| FFF-TST/AAA | MEG15I7D4IAENQGDDGSMKPC | +       | +       |
| FFAFAA   | MEG15I7SYTNTENQQGDDGSMKPC | +       | +       |
| TTAATA   | MEG15I7SYTNTENQQGDDGSMKPC | +       | +       |
| TTAAT   | MEG15I7SYTNTENQQGDDGSMKPC | +       | +       |
| TTAFFS   | MEG15I7SYTNTENQQGDDGSMKPC | +       | +       |

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**Fig. 2** HIV-1 entry on cells expressing the FFF CXCR4 variant. C82Th cells were cotransfected with plasmids encoding CD4 and either wild-type CXCR4 (○) or the FFF variant (□), and assayed for their ability to support infection by HIV-1 pseudotyped with the envelope glycoproteins of the indicated isolates. In parallel, an aliquot of transfected cells was analyzed by FACs using the anti-CXCR4 antibody 12G5. The vertical axis indicates chloramphenicol acetyltansferase activity, a measure of infection, and the horizontal axis indicates CXCR4 or variant receptor expression, reported as the mean fluorescence obtained from FACs analysis. A and B show two representative experiments of two or more experiments performed for each isolate assayed.

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**Fig. 5** Role of CXCR4 Modifications

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**Non-tyrosine Sulfate Signal Associated with Amino-terminal Serines**—Fig. 4B localizes this residual sulfation to one of two amino-terminal serines. Alteration to alanine of two threonines and one serine (residues 8, 9, and 13) at the very aminoterminal end of CXCR4 (variant FFF-TST/AAA; see Table I) did not alter the ability of the FFF variant to incorporate sulfate in transfected HEK293T cells (Fig. 4B, lanes 4 and 5). However, when serine 18 and serine 23 of the FFF variant were altered to alanine (FFFAFA), no sulfate incorporation was detectable (lane 6). Note that this variant has also lost the diffuse high molecular weight sulfated smear present in all CXCR4 variants containing these serines. Sulfate incorporation was also not observed with an FFF variant in which serine 18 alone was altered to alanine (FFAFAS; shown in Fig. 7A). We conclude that serine 18 is associated with two forms of sulfated molecules. First, serine 18 incorporates sulfate either directly or, more likely, in a small carbohydrate form that is not digested by standard O-glycosidases and that does not alter the mobility of CXCR4 on SDS-PAGE. Second, the diffuse high molecular weight sulfate signal associated with CXCR4 is also dependent on serine 18.

**CXCR4 Is Modified by Chondroitin Sulfate at Serine 18**—Fig. 5 clarifies the nature of this diffuse high molecular weight sulfate signal. As shown in Fig. 5A, the diffuse sulfate signal is much more pronounced in HeLa cells transfected with CXCR4
and labeled with $^{35}$S sulfate than in HEK293T cells (compare lanes 1 and 4). This signal disappears if either serine 18 and 23 (YYAYA) or serine 18 alone (YYAYS) is altered to alanine (Fig. 5A). When immunoprecipitates of $^{35}$S sulfate-labeled HeLa cells transfected with wild-type CXCR4 were treated with chondroitin ABC lyase, the high molecular weight sulfate signal disappeared, and the signal associated with CXCR4 increased (Fig 5B; lane 2). No effect was observed with cells incubated with chondroitinase buffer alone (lane 1) or with a mixture of heparinase, heparitinase I, and heparitinase II (lane 3). We conclude that the high mobility sulfate signal is the result of a chondroitin chain attached at serine 18 of CXCR4. We further conclude that CXCR4 modified by chondroitin is the major form of CXCR4 in HeLa cells.

Fig. 5C further documents cell type variation associated with this glycosaminoglycan attachment. Sulfate signal associated
with chondroitin chain modification could be observed in HeLa cells, to a lesser extent in Cf2Th canine thymocytes, and to an even lesser extent in HEK293T cells. No observable chondroitin modification was detected in U937 or Jurkat cell lines (Fig. 5C) or in primary lymphocytes (data not shown).

Investigation of any potential role for this chondroitin sulfate chain in enhancing or inhibiting HIV-1 entry or SDF-1α binding did not yield positive results. Cf2Th cells expressing the YYAYS variant could be as efficiently infected by HIV-1 pseudotyped with the envelope glycoproteins of the HXBc2, YYAFS or YYAFS variants in which this tyrosine is present (YYAYS and FFAYS). We conclude that a CXCR4 variant in which three amino-terminal tyrosines, binds SDF-1α substantially less efficiently than wild-type YYAYS but more efficiently than FFAFS or YYAFS. We conclude that the sulfate group at tyrosine 21 makes a substantial contribution to the efficiency of the SDF-1α binding to wild-type CXCR4 plotted against receptor expression.

We then assessed the relative contribution of the sulfate group at tyrosine 21. As expected, a CXCR4 variant in which serine 18 is altered to alanine (YYAYS) efficiently incorporates sulfate, whereas a CXCR4 variant in which three amino-terminal tyrosines and serine 18 are altered (FFAFS) incorporates no [35S]sulfate (lanes 3 and 4). If serine 18 and tyrosine 21 are altered (YYAFS), sulfate incorporation is substantially attenuated (lanes 5 and 6). If tyrosines 7 and 12 are altered to phenylalanine, but tyrosine 21 is left unaltered (FFAFS), only a modest reduction in sulfate incorporation is observed. These data indicate that the tyrosine 21 is efficiently sulfated and that one or both of tyrosines 7 and 12 are also less efficiently sulfated.

Posttranslational modifications of the amino termini of CC-chemokine receptors, in particular tyrosine sulfation and O-glycosylation, play a critical role in the ability of these receptors to associate with their natural ligands (18, 19). In particular, tyrosine sulfation of CCR5 and CCR2b and O-glycosylation of CCR5 contribute to binding to their respective chemokine ligands (18, 19, 21). Similarly, we have shown that tyrosine sulfate moieties on the chemotactic receptor for C5a...
contribute to association and signaling through the C5a anaphylatoxin (20). Most or all chemokine receptors have a motif that is suggestive of tyrosine sulfation, and we have hypothesized that these sulfate moieties are critical to the function of chemokine receptors and related molecules (18). Here we extend these observations to the first CXC-chemokine receptor, CXCR4. In particular, the sulfated group at tyrosine 21 clearly participates in SDF-1α association with CXCR4. We have also hypothesized that most or all HIV-1 coreceptors are tyrosine-sulfated. In the case of CCR5, the sulfated groups play an important role in the ability of CCR5 to support HIV-1 entry (18). However, we observe here that the tyrosine sulfated groups at the amino terminus of CXCR4 play a much less important role in entry of CXCR4-utilizing isolates than do tyrosine sulfated groups of CCR5 in the entry of all CCR5-dependent HIV-1 isolates assayed previously (including 89.6, JR-FL, YU2, and ADA). This is best observed in the case of the dual-tropic isolate 89.6, which can utilize both CCR5 and CXCR4. No detectable entry of the 89.6 virus was observed on cells expressing a CCR5 variant whose four amino-terminal tyrosines were altered to phenylalanine under conditions that carefully control for receptor expression (18). In contrast, we show here that the analogous CXCR4 variant (denoted FFF here) allowed for efficient entry of the same isolate. The relative lack of sensitivity of the five HIV-1 isolates assayed here to alteration in the amino-terminal tyrosines of CXCR4 is consistent with their generally lower reliance on the amino terminus of CXCR4 than on the amino terminus of CCR5. We and others have found, for instance, that deletion of the CCR5 amino terminus ablates entry of all CCR5-dependent isolates assayed, whereas attenuated but still efficient entry of CXCR4-dependent isolates can be observed if the CXCR4 amino terminus is deleted (25). The underlying cause of this very consistent difference in the way viruses utilize CCR5 and CXCR4 remains unclear.

In the process of describing the sulfate groups on CXCR4, we were able to identify two additional forms of sulfate, both associated with CXCR4 serine 18. Most cells appear to modify serine 18 with a sulfated group that appears not to significantly associate with CXCR4 serine 18. Most cells appear to modify the 18. Most cells appear to modify small sulfated group or the chondroitin chain on serine 18, whereas it was observed in cells derived from other tissues. Further investigations into the role and distribution of this glycosaminoglycan modification are under way. Nonetheless, to our knowledge, CXCR4 is the first reported instance of a G-protein-coupled receptor that is modified by a glycosaminoglycan.

CXCR4 is not likely to be the only CXC-chemokine receptor for which tyrosine sulfation plays an important role. Clear motifs for tyrosine sulfation are present at the amino terminus of CXCR1–CXCR6 as well as that of most CC-chemokine receptors. The general importance of tyrosine sulfation to the function of chemokine and chemotactic receptors suggests that regulation of this modification could be useful in the modulation of immune function or in disease states in which chemokine receptors participate.

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