proves representative, the proposed approach may efficiently target secondary PP1 partners with high-affinity RVxF instances. Interestingly, given that the number of primary PP1 partners has stagnated in recent years, secondary interactors show the highest potential for growth via novel discovery approaches like the one proposed by Meiselbach and coworkers. Other recently described PP1 binding motifs [12–14] may inspire additional strategies.

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Specific Probes for Chemokine Receptors

Chemokine receptors have attracted a good deal of public attention as important therapeutic targets for many diseases and disorders. In this issue of *Chemistry & Biology*, Kumar and colleagues propose a new concept of synthetic modular modifications to generate unnatural chemokines, which exhibit high receptor selectivity [1].

In a postgenome and proteome era, selective agonists and antagonists can be highly useful for studies of receptor biology and for clinical applications. Chemokines belong to a chemotactic cytokine family that attracts and induces migration of leukocytes. Chemokines and their receptors play fundamental roles in physiological phenomena. Since these actions are relevant to many pathological disorders such as cancer and AIDS, chemokine receptors are thought to be critical drug targets.

Chemokine receptors are members of the seven-transmembrane G protein-coupled receptor (GPCR) family, which transduce signals of corresponding chemokines. The relationships between chemokines and their receptors are highly interconnected and complicated: a single chemokine recognizes a plurality of receptors, while one chemokine receptor recognizes several chemokines. Numerous chemokines lack receptor selectivity. Unnatural chemokines that have high receptor selectivity would be practically useful, not only as specific molecular probes for biological studies, but also as drug leads for clinical application. Furthermore, the development of systematic strategies to synthesize such unnatural ligands would be desirable.

In this issue of *Chemistry & Biology*, Kumar and colleagues report unnatural synthetic molecules as chemical probes of chemokine receptors. They present the concept of modular modifications to generate unnatural chemokines that possess receptor selectivity [1]. They created synthetically and modularly modified (SMM)-chemokines based on a combination of total chemical synthesis and modular modification. They chose CXCR4 [2] and CCR5 [3–7] as target receptors with the aim of potentially developing anti-AIDS drugs, since these are the two principal coreceptors that are required for HIV-1 entry. In addition to HIV infection/AIDS, CXCR4 has also been shown to be involved in several problematic diseases, such as cancer metastasis [8, 9], leukemia [10, 11], and rheumatoid arthritis [12, 13]. As such, CXCR4 represents one of the greatest therapeutic targets for the above diseases. Although natural chemokines for CXCR4 and CCR5 can inhibit HIV infection by blocking gp120 binding regions on CXCR4 and CCR5, respectively, serious problems remain concerning selectivity, side effects, and toxicity profiles. vMIP-II, which recognizes various chemokine receptors, was chosen among chemokine ligands as the parent molecule for modification [14]. For a detailed discussion of CXCR4/CCR5 antagonists, readers are referred to recent reviews [15, 16].

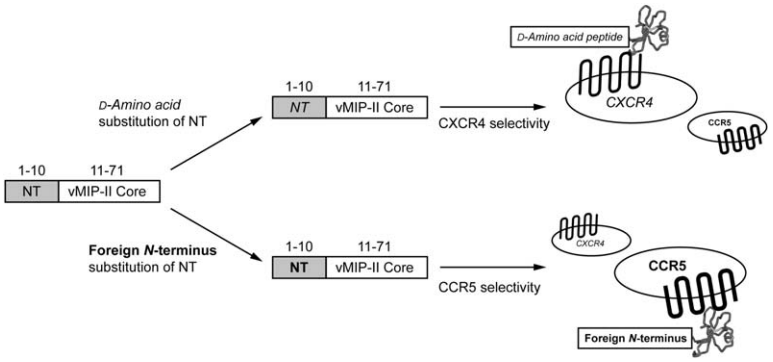

Figure 1. Development of Selective Ligands for CXCR4 or CCR5 through Synthetic Modular Modifications Starting from a Native Chemokine, vMIP-II

Addition of the D-amino acid sequence derived from the N-terminal region (1–10) of vMIP-II to the N terminus of vMIP-II (11–71) led to the development of a new molecule with increased selectivity for CXCR4. Alternatively, addition of the N-terminal (1–10) sequence of MIP-Iβ, which is a foreign chemokine specific for CCR5, to the N-terminus of vMIP-II (11–71) resulted in the development of a CCR5-selective ligand.

Since knockout mice lacking CXCR4 are not viable, the development of new modified ligands that interfere only with HIV-1, but not with SDF-1α (the endogenous ligand) binding on CXCR4, would be ideal. The authors and others previously reported that the N terminus of SDF-1α and vMIP-II is important for CXCR4 binding [17, 18] and that D-amino acid peptides derived from the N-terminal (1–10) sequence of vMIP-II show high binding selectivity for CXCR4 over CCR5 [19]. Thus, the D-amino acid sequence was added to the N terminus of vMIP-II (11–71) to develop RCP168, a new analog with increased selectivity for CXCR4 (Figure 1). RCP168 does not show agonistic activity or interfere with the physiological action induced by SDF-1α (Ca^{2+} mobilization) [1]. However, RCP168 shows potent anti-HIV activity based on inhibition of HIV-1 entry through CXCR4. The RCP168 binding region on CXCR4 overlaps that of HIV-1 gp120, but not that of SDF-1α.

The authors also designed CCR5-specific SMM-chemokines [1]. In this case, they utilized a "foreign N terminus" in order to introduce differential receptor binding (Figure 1). In essence, the N-terminal (1–10) sequence of MIP-Iβ, which is a CCR5-specific chemokine, was adopted. The resulting molecule, which is designated as RCP188, proved to be selective for CCR5. However, RCP188 shows agonistic activity and interferes with Ca^{2+} mobilization induced by MIP-1β, which is in a sharp contrast to the CXCR4-specific SMM-chemokine, RCP168.

Since GPCRs, such as chemokine receptors, are great targets for drug discovery and chemical biology, specific molecular probes are highly useful for characterizing biological functions of receptors. In this regard, the authors' objective to produce specific ligands is to be commended. The present results provide highly useful insights for the future design of synthetic chemokine ligands. These selective chemokines could become in great demand by receptor biologists and pharmacologists. Certainly, RCP168 has promise as a lead for anti-AIDS/HIV drugs. Although Huang and coworkers developed SMM-chemokines, which are specific ligands for CXCR4 or CCR5, it is unclear as to the generality of their strategy. It is ambiguous how one designs ligands that are specific for target receptors, or ligands that do or do not interfere with the physiological actions induced by endogenous ligands. In practice, one might design ligand molecules on a case-by-case basis through trial and error to develop selective ligands. However, it may be challenging to establish a more general concept for molecular design. In the era of chemical biology, there is a great need for targeted molecules, including biological probes to elucidate complicated proteome networks. As a consequence, this field of research will be dramatically developed in future.

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