

## 選殖及分析 CXCR4 and CCR5 的啟動子

**Identification of the Cis-acting Elements on the Promoters of the CXCR4 and CCR5 during the Monocytic Cells Differentiated by Retinoic Acid**

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## 一、中文摘要

據估計，至公元2000年時，全世界將有3-4千萬人被人類免疫不全病毒（愛滋病毒；HIV-1）感染，而其中之一半是發生在亞洲。目前國內愛滋病毒帶原者雖不多，但由於社會價值觀念改變，社交及旅遊活動增加，因此，臺灣未來可能爆發愛滋病毒的嚴重流行。

HIV-1 要進入 CD4 細胞必須透過與細胞表面之主要受體（CD4）及其中一個副受體（CXCR4 或 CCR5）的結合之機轉。已知 CXCR4 蛋白質是做為嗜 T 淋巴球 HIV-1 株的副受體，而 CCR5 是嗜巨噬細胞 HIV-1 株的副受體。在臨床上，嗜巨噬細胞 HIV-1 株是早期病毒感染（尤其是經由異性性交）的主力，到了後期（愛滋病階段），絕大部份的病毒變為嗜 T 淋巴球 HIV-1 株並使用 CXCR4 為副受體。由於 HIV-1 使用不同蛋白質，在不同的疾病階段，做為感染不同細胞的副受體，所以許多研究就針對這兩個蛋白質的表達做深入之探討。最近之實驗發現 Monocyte（嗜巨噬細胞的母細胞）其實也會表達 CXCR4，並且可以輕易的被嗜 T 淋巴球 HIV-1 株所感染，但是一但分化成為嗜巨噬細胞（受 Retinoic Acid 刺激），則變為可被巨噬細胞 HIV-1 株感染但抗拒嗜 T 淋巴球 HIV-1 株，這樣之變化導源於 CXCR4 及 CCR5 在分化時表達的消長。本計畫擬分離並選殖 CXCR4 及 CCR5 基因的啟動子，進而研究此兩啟動子在分化時調控傳譯的差異性。

關鍵詞：關鍵詞：人類免疫不全病毒第一型

(愛滋病毒)；愛滋病毒的受體及副受體；愛滋病毒的嗜性

**Abstract**

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of AIDS (acquired immunodeficiency syndrome) that causes the worst global epidemic known to human history. Cellular entry of HIV-1 requires binding to both CD4 (the primary receptor) and one of the seven transmembrane G-protein-coupled chemokine receptors, CXCR4 or CCR5, as the major co-receptors. CXCR4 has been shown to mediate entry of T-cell line adapted (T-tropic) HIV-1 strains into peripheral blood lymphocytes (PBL) and T-cell, whereas CCR5 on the surface of PBL and monocytes/macrophages mediates the infection of macrophage-tropic (M-tropic) viral strains. The importance of chemokine receptors to the HIV-1 entry and AIDS pathogenesis has therefore promoted intensive investigation on the co-receptor usage during the viral transmission. Recent study has demonstrated that monocytic cell line U937 does expressed CXCR4 on the cell surface that is more susceptible for T-tropic HIV-1 infection. However, upon differentiation to macrophage or more mature phenotype by retinoic acid, certain U937 clones become highly susceptible to M-tropic but resist to T-tropic HIV-1 infections. This dichotomous effects in susceptibility of viral transmission is governed by the differential expression of CXCR4 and CCR5. Other

studies have also confirmed that the expression of different co-receptors on the cell surface controls the HIV-1 infection in particular cell types as well as the disease progression to AIDS. Therefore, it is rationalized to clone the promoters of both the CXCR4 and CCR5 gene for further investigating the gene regulation of these two proteins, using U937 cell line as the model system.

**Keywords:** Human immunodeficiency virus type 1, CXCR4, CCR5, Retinoic Acid

## 二、緣由與目的

It is estimated that 40 millions people will be infected with Human immunodeficiency virus type 1 (HIV-1) in year 2000 worldwide. Epidemic of HIV-1 is no doubt the worst catastrophe in human history. HIV-1, a lentivirus, belongs to a genus of the Retroviridae family, and causes acquired immunodeficiency syndrome (AIDS) in human (1). Depending on the physical conditions of the host, HIV-1 can establish a clinical latent stage in the infected hosts in excess of 15 years (2). One of the mechanisms whereby HIV-1 escapes host immune surveillance, leading thereby to the virus' latent period, is the constant altering of its immunogenic surface antigens.

Human immunodeficiency virus uses CD4<sup>+</sup> as the primary receptor and chemokine co-receptors to enter target cells (3). Chemokine receptors belong to the superfamily of G protein-coupled receptors that have seven transmembrane domains. They can be divided in two groups: the  $\alpha$  (CXC) and the  $\beta$  (CC) subfamilies, which have or does not have a single amino acid, respectively, inserted between the first and the second cysteine residues of the proteins. The binding of chemokines to their receptors induces a rapid calcium influx and inflammatory responses in the receptor-bearing cells (4). However, recent studies have demonstrated that CCR5 and CXCR4 mutants defective in G-protein signaling are

still active in mediating HIV-1 infection (5,6).

The chemokine receptors CXCR4 and CCR5 are the major co-receptors for HIV-1 entry into CD4<sup>+</sup> cells. The importance of chemokine receptors for HIV-1 entry and AIDS pathogenesis has promoted intensive investigations (7,8). It is found that during primary infection, most HIV-1 isolates are macrophage-tropic (M-tropic) and the viral isolates tend to use CCR5 for cell entry (9). While at the later stage, M-tropic viruses tend to become less prominent and are generally replaced by T- cell tropic (T-tropic) viruses (10,11), which use CXCR4 for viral entry. This correlation of the disease progression and HIV-1 tropism is believed to be the result of altering co-receptor usage driven by the selection of new target cells for infection (12). For studying the infection of M-tropic HIV-1, promonocytic cell line, such as U937, has been frequently used as a cell line model (13). However, recent studies demonstrated that certain U937 cell clones are relatively resistant to the infection of M-tropic HIV-1 isolates, but susceptible to T-tropic viruses (14). Upon differentiation of these U937 clones to macrophage or mature phenotype by retinoic acid, U937 cells become highly susceptible to M-tropic but resist to T-cell tropic HIV-1 infections. The change in susceptibility of viral transmission is governed by the differential expression of CXCR4 and CCR5 (15). Since the differential expression of CCR5 or CXCR4 in cells surface is likely to control the HIV-1 infection and the disease development (16,17), it is critical to clone the promoters for both the CXCR4 and CCR5 genes before any investigation on dichotomous effects of the co-receptor expression can be performed. Therefore, molecular cloning techniques are proposed to used to clone these two promoters in this project. To do this, the human genomic DNA will be isolated and followed by polymerase chain reaction (PCR) with primers specific for the promoter sequences. The PCR products will then be cloned into pPCR-Script AMP (SK<sup>+</sup>) vector

and confirmed by automated sequencing. The obtained promoters will then be detailed characterized for the transcriptional regulatory activities mediated by retinoic acid in U937 cells.

### 三、結果與討論

In last year, We have done many important researches. We have isolated human genomic DNA from white blood cells and polymerase chain reaction (PCR) amplification of the CCR5 promoter sequence with *pfu* polymerase. The PCR product was cloned with Stratagene PCR-Script Amp cloning kit, the correct clone has been confirmed by the nucleotide sequence analysis with automated sequencer (ABI Prism 377)( Fig. 1). We have cloned the pCCR5-59029A promoter, when the promoter sequence was compared with the GenBank database, there has several sequences similar to consensus sequences for the transcription factors such as; activating protein-1 (AP-1), CCAAT-binding transcription factor, and NF- $\kappa$ B et al.

For Gel retardation analysis, we have prepared the nuclear extracts from U937 monocytic or Jurkat T cell line with or without retinoic acid or PMA/PHA treatment, respectively. From the induction of cellular transcription factors in these two cell lines, We expected to detect different binding patterns in the CCR5 promoter (Fig. 2).  
**Fig.3** Semi-quantification of CCR5 mRNA from Jurkat cells with or without stimulation PMA+PHA. lane 1、3、5; + PMA+PHA lane 2、4、6; - PMA+PHA. The ratio of CCR5 mRNA from Jurkat cells with PMA+PHA or without stimulation is 4:1. For the footprinting analysis, we have first titrated the DNase 1 concentration, Using 14 ng P<sup>32</sup>- labeled PCR fragment (labeled with one of other primers P029R or PSFCCR5) with the concentrations of DNase 1 from 0.25 to 1.5 unit (Fig. 4A). **Fig. 4B** and **4C**; the Footprinting analysis with 1 unit of DNase 1,

and 0 to 8 ug nuclear extract isolated from Jurkat T cell with or without PMA/PHA stimulation respectively.

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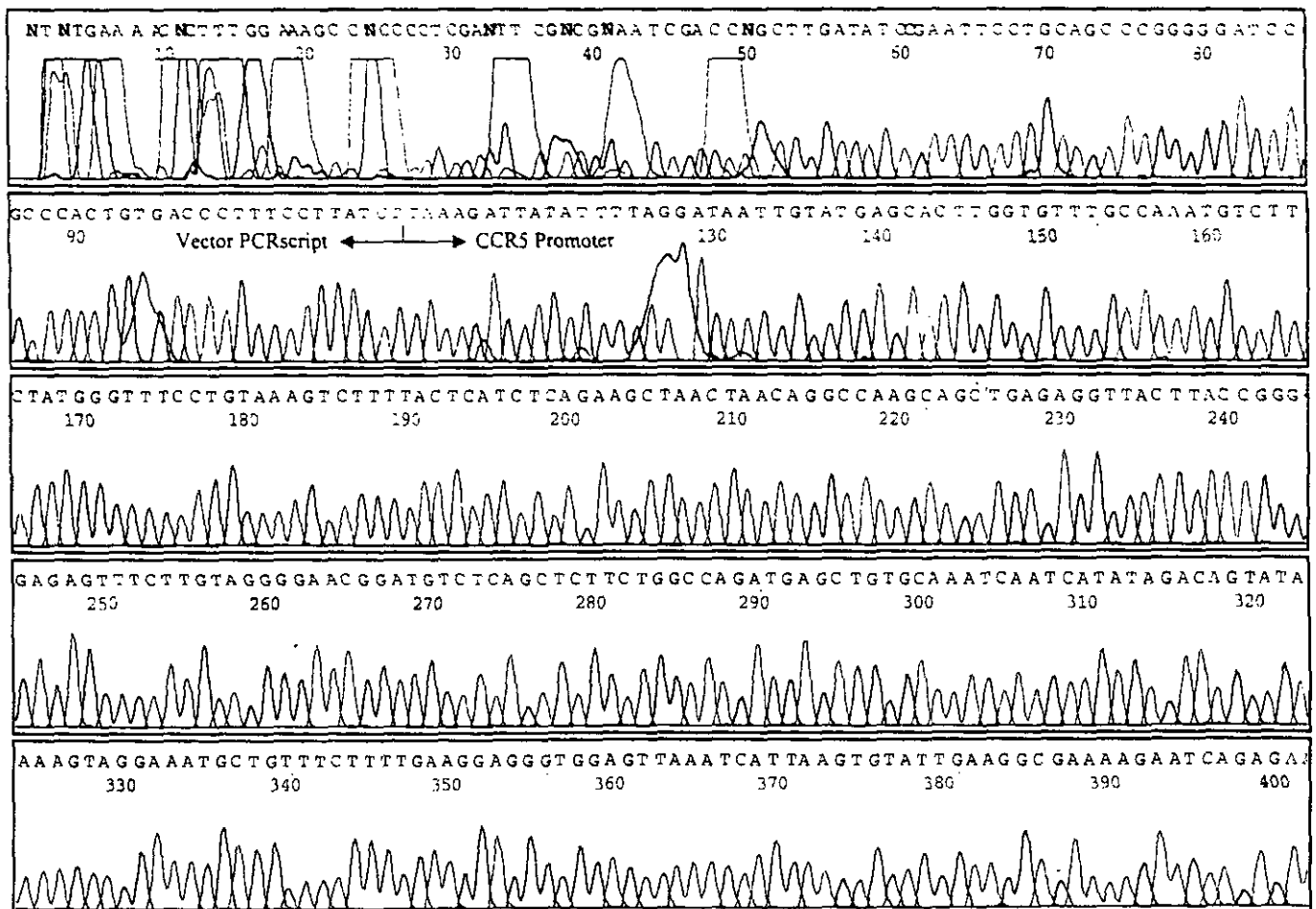
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**Figure 1:** DNA sequence of the promoter CCR5-59029A

CCR5-forward primer: (-986) 5'-AAGCCCAGAGGGGCATCTTGT-3' (-966)

CCR5-reverse primer: (194) 5'-ACTGTGACCCTTTCCTTATCT-3' (173)

The PCR product was cloned with Stratagene PCR-Script Amp cloning vector, the correct clone was confirmed by automated sequencer (ABI Prism 377)



**Figure 2:** Gel retardation , The ANN (AP-1, NF-kB, NFAT) probe binds to nuclear extract from Jurkat or U937 cells with PMA/PHA or retinoic acid stimulation respectively.

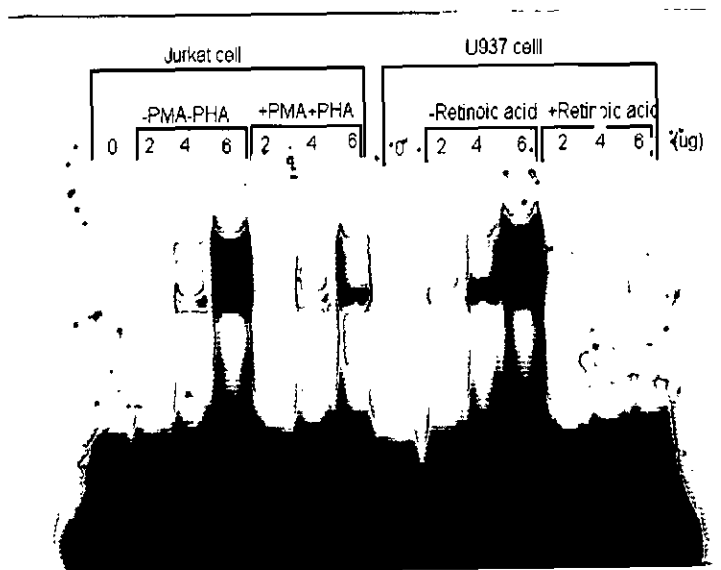


Figure (3A): Three pair of CCR5 primers in CCR5 gene

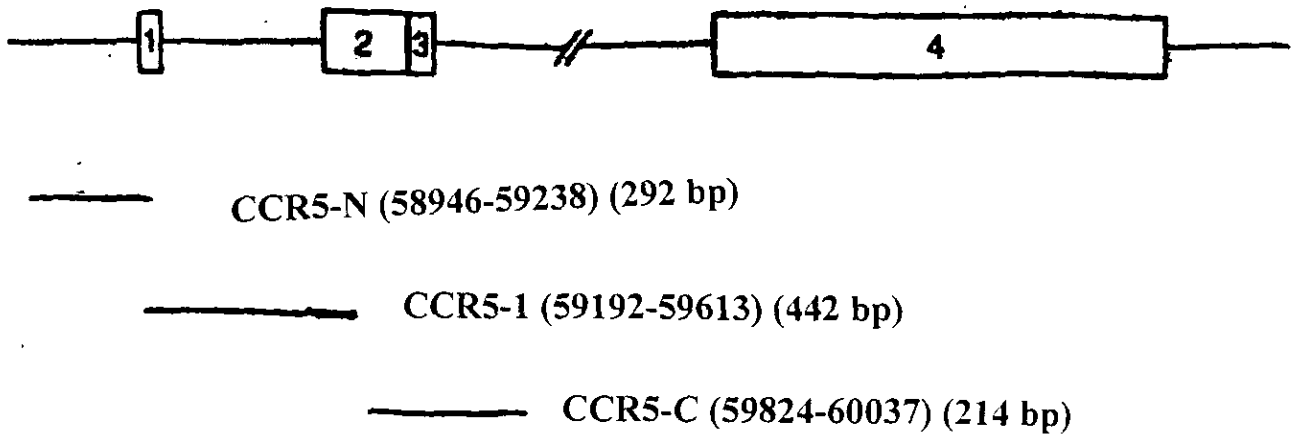
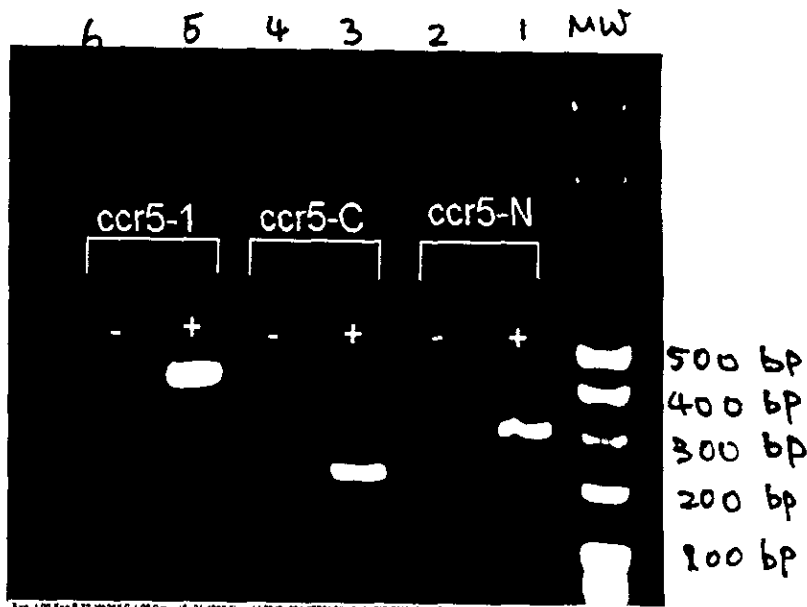


Figure 3 (B): Semi-quantification of CCR5 mRNA from Jurkat cells with or without stimulation PMA/PHA



Lane 1, 3, 5 ; + PMA/PHA  
Lane 2, 4, 6 ; - PMA - PHA

(A)

P029R

PSFCCR5

0.25 0.5 1 2 4 8 16 32 64 128 256 512 1024 2048 4096 8192 16384 32768 65536 131072 262144 524288 1048576 2097152 4194304 8388608 16777216 33554432 67108864 134217728 268435456 536870912 1073741824 2147483648 4294967296 8589934592 17179869184 34359738368 68719476736 137438953472 274877906944 549755813888 1099511627776 2199023255552 4398046511104 8796093022208 17592186044416 35184372088832 70368744177664 140737488355328 281474976710656 562949953421312 1125899906842624 2251799813685248 4503599627370496 9007199254740992 18014398509481984 36028797018963968 72057594037927936 144115188075855872 288230376151711744 576460752303423488 1152921504606846976 2305843009213693952 4611686018427387904 9223372036854775808 18446744073709551616 36893488147419103232 73786976294838206464 147573952589676412928 295147905179352825856 590295810358705651712 1180591620717411303424 2361183241434822606848 4722366482869645213696 9444732965739290427392 18889465931478580854784 37778931862957161709568 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