5.7 million (3.4–9.4 million) people infected. HIV-1 CRF01_AE of central African ancestry is widely circulating in Southeast Asia (Ou *et al.*, 1992, 1993; Weniger and Brown, 1996). CRF01_AE epidemic broke out in the late 1980s among female commercial workers and their clients in Thailand and became prevalent among injecting drug users (IDUs) in this country (Ou *et al.*, 1993) and disseminated to neighboring countries in Asia, including Cambodia, Vietnam, Malyasia, China, Taiwan, Korea, and Japan (Weniger and Brown, 1996; Weniger *et al.*, 1994). Subtype B′ (Thailand variant of subtype B; also referred to as Thai-B virus) (Kalish *et al.*, 1995; Ou *et al.*, 1992, 1993) is a unique subtype B variant that spreads primarily through IDU networks in Southeast Asia (Motomura *et al.*, 2003; Weniger *et al.*, 1994). Two closely related CRFs, CRF07_BC and CRF08_BC, are disseminating rapidly among IDUs in northwestern (Xinjiang Province) (Su *et al.*, 2000) and southeastern (Guangxi Province) China (Piyasirisilp *et al.*, 2000), respectively. A variety of novel CRFs composed of CRF01_AE and subtype B (B′) in Thailand [CRF15_01B (Tovanabutra *et al.*, 2003) and CRF34_01B] and in Malaysia (CRF33_01B) have been identified (Tee *et al.*, 2006).

C. Other HIV-1 Variants of Geographical Relevance

Less prevalent HIV-1 subtypes, but common on a localized scale, are observed in various geographic areas: subtype D, distributed mainly in East Africa (Uganda, Tanzania, and Kenya); subtype F (subsubtype F1) predominant in Romania (mostly children infected through contaminated blood products and unsterilized needle and syringes), and found in a minority of HIV-1-infected people in Brazil; subtype G circulating in West and central Africa, with the highest prevalence in Nigeria as well as in Portugal and northern Spain. A variety of novel CRFs were identified in South America: CRF12_BF (Thomson *et al.*, 2000) and CRF17_BF in Argentina; CRF28_BF, CRF29_BF, and CRF31_BC in Brazil. These new recombinant strains account for ~12% of HIV-1 infections in Latin America (Fig. 4). In Europe, CRF14_BG and its related recombinants are circulating locally in northwestern Spain (Delgado *et al.*, 2002; Thomson *et al.*, 2001) and Portugal (Esteves *et al.*, 2002). The widest range of novel CRFs, including CRF18_cpx (Thomson *et al.*, 2005), CRF19_cpx (Casado *et al.*, 2005), CRF20_BG, CRF22_01A1, CRF23_BG, and CRF24_BG (http://hiv-web.lanl.gov/CRFs/), have been reported in Cuba, where those recombinants account for ~20% of HIV-1 infections (http://www.hiv.lanl.gov/components/hiv-web/new_geography/). Injecting drug use triggered a new HIV-1 epidemic in Eastern Europe: CRF03_AB was originally identified among IDUs in the Russian city of Kaliningrad (Liitsola *et al.*, 1998), and later detected in several cities in Ukraine and Belarus (St. Petersburg, Smolensk, and Perm) (Figs. 3 and 4). Other minor nonrecombinant subtypes (A2, F2, H, J, and K) were detected in central Africa. Most of the remaining CRFs have lesser relevance in epidemic on a global scale.

D. Emergence of HIV-1 Recombinants Worldwide

Although the exact prevalence of recombinant strains is not well known, the preliminary data show that the proportions of discordant *gag/env* samples varied from less than 10% to up to 40% in Africa (McCutchan *et al.*, 1999; Vidal *et al.*, 2000) and 10–30% in some areas in Asia, including central Myanmar (Kusagawa *et al.*, 1998; Motomura *et al.*, 2000, 2003; Takebe *et al.*, 2003) and more than 60% in western part of Yunnan Province (southwestern China) (Yang *et al.*, 2002).

Recombinant viruses have already contributed substantially to the global pandemic, and the likelihood of generating recombinant viruses will continue to increase as the different HIV-1 subtypes spread worldwide (Peeters, 2000). Mixing of different lineages of HIV-1 strains could quickly lead to the evolution of new recombinant strains. Even recombinant viruses will recombine, leading to the evolution of second-generation recombinants, inter-CRF recombinants (ICRs): ICR01_0708 identified among IDUs in Yunnan Province of China is the first example of this category, which is composed of two closely related CRFs in China, CRF07_BC and CRF08_BC (Yang *et al.*, 2003) (Fig. 3).

V. Methods for Identifying HIV Genetic Forms

A. Phylogenetic Sequence Analysis

For subtype classification, phylogenetic sequence analysis is the most reliable method. Various software programs for phylogenetic analysis (i.e., molecular evolutionary genetic analysis, MEGA) are freely available (http://hiv-web.lanl.gov/). Due to the high frequency of recombination in HIV, it is necessary to equip software programs designed for identifying recombination. This is particularly the case for the molecular epidemiological investigation in the areas where different lineages of HIV-1 strains are cocirculating. Softwares designed for detecting recombination, including Simplot (Ray, 2002) and Recombination Identification Program (RIP) (http://hiv-web.lanl.gov/) are useful for this purpose. Figure 5 illustrates an example of phylogenetic tree analysis and recombination breakpoint analyses (bootscanning plot and subregion tree analyses) for identification and characterization of novel recombinant strains in Myanmar (Takebe *et al.*, 2003).

B. Alternative Methods (Heteroduplex Mobility Assay and Serotyping)

Other methods, less expensive and requiring less sophisticated equipments, can be useful as the alternatives for sequencing. This includes serotyping and heteroduplex mobility assay (HMA) (Delwart *et al.*, 1993).

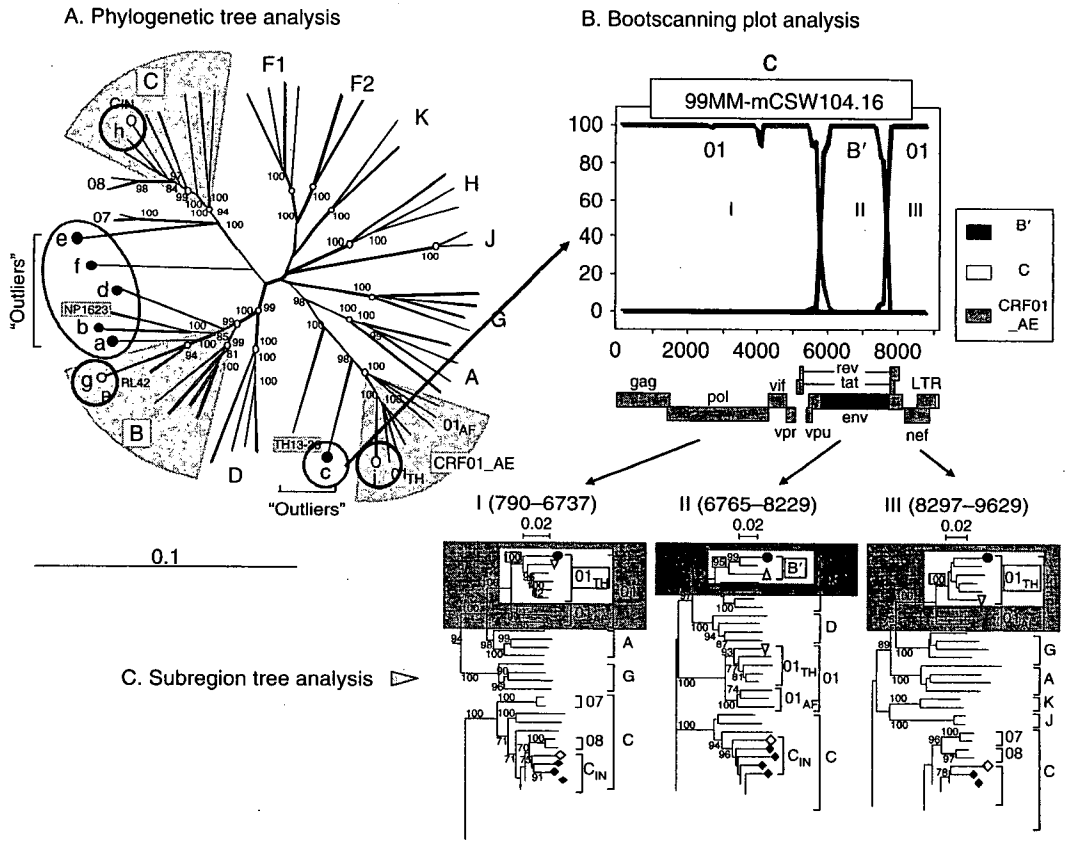

FIGURE 5 Phylogenetic tree analysis (A) and recombination breakpoint analysis for identification novel HIV-1 recombinant strains. Neighbor-joining tree analysis of Myanmar HIV-1 isolates based on near full-length sequences. Strains (g–i) belong to nonrecombinant forms of HIV-1 subtypes B′

Serotyping is a method based on the binding of antibodies present in the patient's sera to the peptides representing a segment of envelop V3 loop of different subtypes (Pau *et al.*, 1993, 1994). Serotyping is particularly useful for analyzing large numbers of specimens for epidemiological studies. However, this assay cannot reliably distinguish between subtypes A and C and cannot detect recombinants. HMA is the method based on electrophoretic mobility of DNA duplexes formed by hybridization of the polymerase chain reaction (PCR)-amplified sequences with reference sequences of different subtypes. However, both methods are less reliable in the areas with high HIV-1 genetic heterogeneity, such as central Africa and some regions in Asia, because of the high frequency of intermediate or incorrect results. These two methods can be useful in the areas, where one or two relatively homogeneous subtypes are prevalent. For instance, serotyping method was successfully applied for the distinction of subtype B and CRF01_AE infections for the study in Thailand (Pau *et al.*, 1993).

VI. Origin of HIVs and Genesis of HIV-1 Pandemic

A. HIV/AIDS as a "Zoonosis"

Current evidence indicates that HIV-1 and HIV-2 entered into human population through multiple zoonotic infections from SIVs-infected nonhuman primates (Hahn *et al.*, 2000). It has been known that HIV-2 and SIV sm have a high degree of genetic and phenotypic homology (Gao *et al.*, 1992), sharing unique open-reading frame, called vpx, in their genomes. Moreover, the habitat of the sooty mangabey closely matches HIV-2 endemicity in West Africa. These close relationships between HIV-2 and SIV-sm led to the hypothesis that HIV-2 infection is a zoonosis (Sharp *et al.*, 1999).

By contrast, HIV-1 is most closely related to SIV cpz isolated from the chimpanzee subspecies *Pantroglodytes troglodytes* (*P.t.t.*) (Corbet *et al.*, 2000; Gao *et al.*, 1999; Hahn *et al.*, 2000; Peeters *et al.*, 1997). The most diverse forms of HIV-1 are found in the geographic region corresponding to the range of *P.t.t.* in West equatorial Africa (Charneau *et al.*, 1994; De Leys *et al.*, 1990; Gurtler, 1996; Simon *et al.*, 1998), and HIV-1 groups and

(Thailand variant of subtype B) and C, and CRF01_AE, respectively. Strains (a–f) are "outliers" that are not assigned to any known HIV-1 genotypes (subtypes/CRFs). They turned out to be novel HIV-1 intergenotype recombinants, that is, "unique recombinant forms" (URFs). The data outputs obtained from various recombination breakpoint analyses, including bootscanning plot analysis (B) and subregion tree analysis (C) for "outlier" strain (c) are schematically illustrated. The results indicate that the strain (c) is a novel HIV-1 URF composed of subtype B′ and CRF01_AE of Thailand origins. The strain (c) shows the structural similarity to CRF15_01B, but is not exactly identical (Takebe *et al.*, 2003). (See Color Plate Section.)

SIV cpz sequences are interspersed in phylogenetic trees, suggesting that there are shared viral lineages in human and chimpanzees (Corbet *et al.*, 2000; Gao *et al.*, 1999; Hahn *et al.*, 2000; Peeters *et al.*, 1997). Each group of HIV-1 and HIV-2 is believed to represent a distinct cross-species transmission of the viruses from its chimpanzee and sooty mangabey reservoirs, respectively (Hahn *et al.*, 2000). However, genetic survey of SIVs in African primate species in central Africa using fecal specimens identified HIV-1 group O-like viruses in wild gorilla (*Gorilla gorilla gorilla, G.g.g.*) (Van Heuverswyn *et al.*, 2006). Collectively, it could be speculated that SIV-cpz*Ptt* have crossed at least twice in humans, resulting in the AIDS pandemic by HIV-1 group M in one instance and infection of a few individuals in Cameroon by group N in another, and that the third HIV-1 lineage group O appears to be evolved from a virus from wild gorilla (*G.g.g*), while this virus (SIV gor) forms monophyletic lineages within SIV-cpz*Ptt* radiation (Sharp *et al.*, 2005). The plausible origins and routes of cross-species transmissions of HIVs are illustrated in the left side of Fig. 2.

B. Dating the Origin of Pandemic HIV-1 Strains

Korber *et al.* (2000) estimated the date of the most recent common ancestor (MRCA) of HIV-1 group M to be 1931 [95% confidence interval (CI): 1915–1941], suggesting that HIV-1 group M began its expansion in human population roughly 70 years ago. The phylogenetic analyses assuming molecular clock suggested that the founder of subtype B in the United States originated in 1967 (95% CI: 1960–1971). Similarly, the MRCA of CRF01_AE in Thailand was dated 1986 (95% CI: 1978–1989) (Korber *et al.*, 2000). By contrast, according to Lemey *et al.* (2003), the date of the MRCA of HIV-2 group A strains was estimated to be 1940 ± 16, and that of HIV-2 group B strains was estimated to be 1945 ± 14 in Guinea, Bissau. Taken together, zoonotic transfers of HIVs occurred in early or the first-half of the twentieth century and subsequently spread globally, generating the pandemic observed today. The origins and plausible route of dissemination of HIV-1 strains responsible for epidemic in Asia is schematically illustrated in Fig. 6.

VII. Biological Significance of HIV-1 Variability and Recombination

A. HIV-1 Subtypes and Disease Progression

It has been suggested that HIV-1 subtypes could influence viral transmissibility and pathogenecity. However, the existence of many other factors makes it difficult to establish the true effect of viral subtypes. A study in Thailand showed that the disease progression in the patients infected with

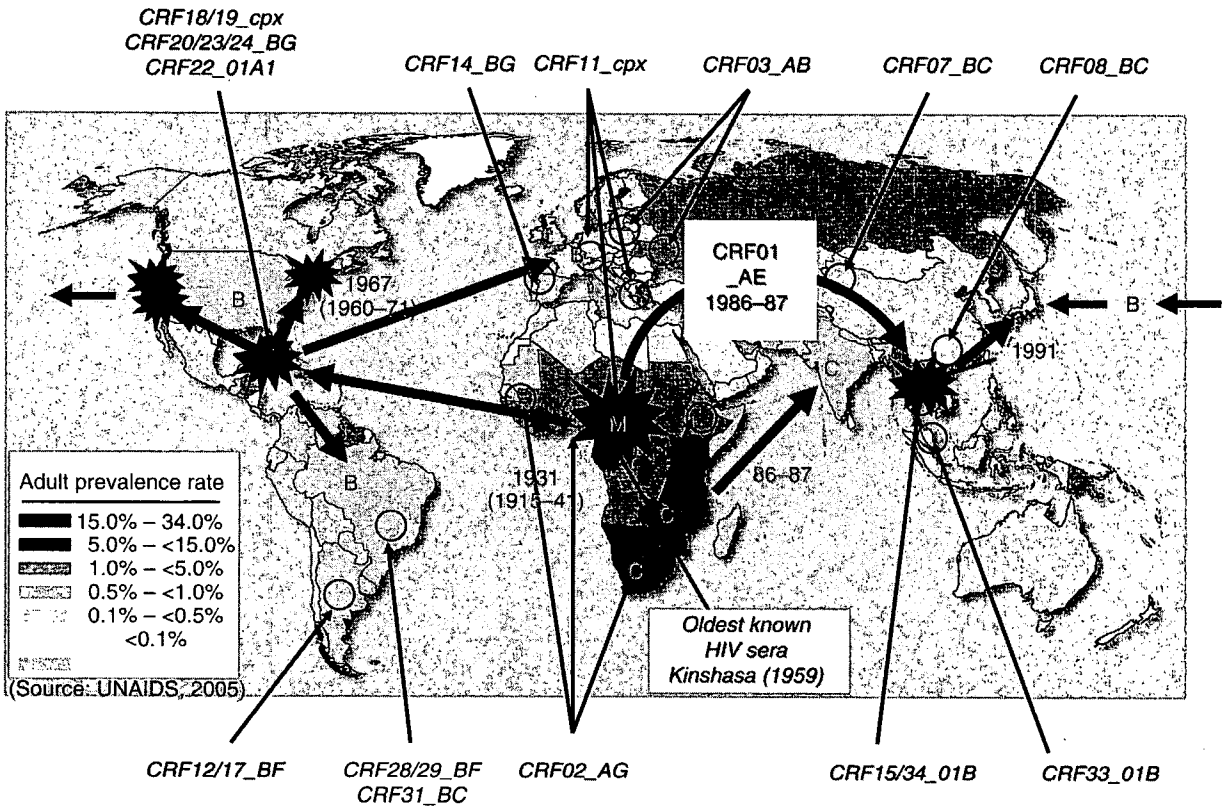

FIGURE 6 Origin of HIV-1 group M and plausible routes of the spread of the HIV-1 strains responsible for epidemic in Asia. The epidemic focuses of selected CRFs of geographical relevance are shown. The illustrations are superimposed on the world map with estimated adult HIV prevalence in different countries (UNAIDS/WHO, 2005). (See Color Plate Section.)

CRF01_AE is similar to those observed in subtype B-infected populations in the West (Amornkul *et al.*, 1999; Kilmarx *et al.*, 2000).

In contrast, several studies showed that HIV-1 subtypes differ in rates of progression to AIDS: (1) A prospective study of female prostitutes in Senegal showed that women infected with subtypes C, D, or G were eightfold more likely to develop AIDS than subtype A (Kanki *et al.*, 1999); (2) The cohort study in Kenya, where subtypes A, C, and D cocirculate, plasma RNA levels were found to be highest in subtype C (Neilson *et al.*, 1999); (3) A study in Tanzania suggests that subtypes A and C and recombinant viruses are more likely to be transmitted perinatally than subtype D (Renjifo *et al.*, 2001), suggesting that maternal subtype may play a role in vertical transmission; (4) The response to the proinflammatory cytokine tumor necrosis factor alpha (TNF-α) is increased in subtype C long terminal repeat (LTR) with nuclear factor κB (NFκB) configuration, suggesting that subtype C may have a replication advantage in individuals with chronic immune activation (Montano *et al.*, 2000); (5) A matched case-control study showed that viruses containing subtype C LTRs were six times more likely to be transmitted than those with subtype D (Blackard *et al.*, 2001). The study in Uganda showed subtype D was associated with faster progression to death and with a lower CD4 cell count than subtype A (Kaleebu *et al.*, 2002).

In contrast, a study from Sweden showed no differences in disease progression in subtypes A, B, C, or D (Alaeus *et al.*, 1999). However, it is not clear whether such differences are due to the environmental factors such as the prevalence of other infectious diseases that may induce the systemic "immune activation," including sexually transmitted diseases (STDs) and parasitic diseases. Indeed, several studies suggest faster disease progression in the persons infected in Africa, compared with those infected in the United States or Western Europe (Galai *et al.*, 1997; Kanki *et al.*, 1999). Long-term prospective studies in recent seroconverters will be needed to elucidate the relationship between HIV-1 genotypes and clinical disease progression.

B. HIV-1 Dual Infection, Superinfection, and Recombination

1. Dual Infection: Mechanism and Prevalence

Dual infections are the prerequisite for the generation of recombinants. When a single cell that is infected with genetically distinct viruses produces progeny virions with RNAs from each virus, recombination could occur between the two copackaged heterologous RNAs through strand switching during the next replication cycle (Malim and Emerman, 2001). Therefore, dual infection with more than one lineage of HIV-1 strains within an individual is a source of rapid viral evolution by recombination. Over the last decade, a number of cases of dual infections with the same or different HIV-1 subtypes through various transmission routes, including vertical

transmission (Janini *et al.*, 1998; Mellquist *et al.*, 1999), sexual transmission (Jost *et al.*, 2002; Zhu *et al.*, 1995), and blood transfusion or injection drug use (Diaz *et al.*, 1995; Ramos *et al.*, 2002; Sala *et al.*, 1994, 1995) have been documented.

2. Distinction Between Coinfection and Superinfection

By the temporal mode of the acquisitions of different HIV-1 strains, dual infections are divided into two categories: coinfection (simultaneous) and superinfection (sequential). Coinfection is defined as an infection with two heterologous strains ether simultaneously or within a brief period of time (arbitrarily within the first month of infection) before infection with the first strain has been established and an immune response has developed. In contrast, superinfection is defined as an infection with a second strain after the immune response to the first infection has been established (Smith *et al.*, 2005). As of August 2005, 16 published cases of superinfections have been reported worldwide (Smith *et al.*, 2005). In several reported cases, superinfection has resulted in recombination between the initial and the secondary strains (Fang *et al.*, 2004; McCutchan *et al.*, 2005; Yang *et al.*, 2005).

3. Superinfection: Implications for Vaccine Development

The majority of superinfection appears to has occurred in the early stage of infection. In contrast, several population-based studies reported the rarity of superinfection during chronic infections (Gonzales *et al.*, 2003; Tsui *et al.*, 2004). The reported rarity of superinfection during chronic HIV-1 infection may reflect the time required for the immune responses to mature and may suggest that immune responses in the infected host could protect against superinfection. This may offer hope for an effective vaccine against HIV-1. However, results from several published studies appear to indicate that even strong $CD8^+$ T-cell-mediated responses against the initial infection may not be sufficient for the protection against superinfection (Altfeld *et al.*, 2002; Jost *et al.*, 2002; Yang *et al.*, 2005). Moreover, in most reported cases of superinfection, patients have experienced a decrease in $CD4^+$ cell count and increase in HIV load, accelerating disease progression (Gottlieb *et al.*, 2004; Jost *et al.*, 2002; Smith *et al.*, 2005). The knowledge of superinfection is thus vital to understand the changes in viral pathogenesis and the host immunity and provides the important insights into future vaccine strategies.

C. Biological Implications of Recombination

Recombinant viruses may have certain advantages over the parental strain, including modifications in tropism and replication efficiency ("viral fitness"). Under selection pressure imposed by antiretroviral drugs, recombination between strains with different drug sensitivity resulted in new HIV-1 variants with dual or multiple drug resistance (Moutouh *et al.*, 1996). The

discovery of large numbers of recombinant strains clearly suggests that coinfection with different HIV-1 strains is not rare as once thought. The dual infections with different subtypes have been reported in the regions where multiple variants are cocirculating. Furthermore, as described in the previous section, a study showed that HIV-1 superinfection can occur in the setting of a strong and broadly directed virus-specific $CD8^+$ T-cell response (Altfeld *et al.*, 2002; Jost *et al.*, 2002), suggesting that the host immunological responses are not efficient against divergent strains. These findings would provide important implications for vaccine development as well as for the prevention efforts from public health viewpoints.

VIII. Conclusions

Global dissemination of HIVs represents a dramatic and deadly example of recent genome emergence and expansion. As reviewed in this chapter, recent studies revealed that a pandemic HIV strain, HIV-1 group M, began its expansion in human population roughly 70 years ago (early twentieth century), it has been diversifying rapidly, now comprising a number of different subtypes and CRFs, and that new recombinant strains are arising continually, becoming a powerful force in global HIV-1 spread. Studies also provide information to delineate the mechanism of viral evolution and for the studies on biological features of HIV strains related to pathogenecity and disease progression. However, the biological significance of the global diversity of HIV-1 strains remains to be defined. Although the immune correlates for protection are still incompletely understood, the extensive variation of HIVs could probably be important in the formulation of the vaccine immunogens. In conclusion, molecular epidemiological information on the HIV strains is critically important to elucidate the dynamics of HIV spread and to formulate future vaccine and other prevention strategies.

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Chronology of the HIV-1 CRF07_BC expansion in East Asia

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The HIV-1 epidemic among injecting drug users (IDU) in Taiwan is caused primarily by CRF07_BC infections. Evolutionary analyses, which utilize outgroup reference strains from northwestern China (Xinjiang), reveal that CRF07_BC was introduced into southern Taiwan in 1998–2001 and spread to central–northern Taiwan in 2001–2003, causing the largest HIV/AIDS epidemic in Taiwan. The separate introduction of CRF07_BC into Xinjiang occurred in 1992–1995. This study illustrates the temporal dynamics of CRF07_BC spread among IDU across east Asia.

The HIV-1 circulating recombinant form 07_BC (CRF07_BC) is a recombinant comprised of HIV-1 subtype B' (Thailand genotype of subtype B) and subtype C. This strain accounts for most infections among injecting drug users (IDU) in northwestern China (Xinjiang province), where an outbreak was detected in 1996 [1], although the most plausible origin of CRF07_BC as a whole is probably Yunnan province [2,3]. Outside mainland China, CRF07_BC is associated with a dramatic increase in HIV/AIDS cases in Taiwan; it was detected among IDU in prisons in southern Taiwan (Tainan) in 2002 and later detected in 2003–2004 in central (Nantou) and northern (Taipei) Taiwan [4]. By

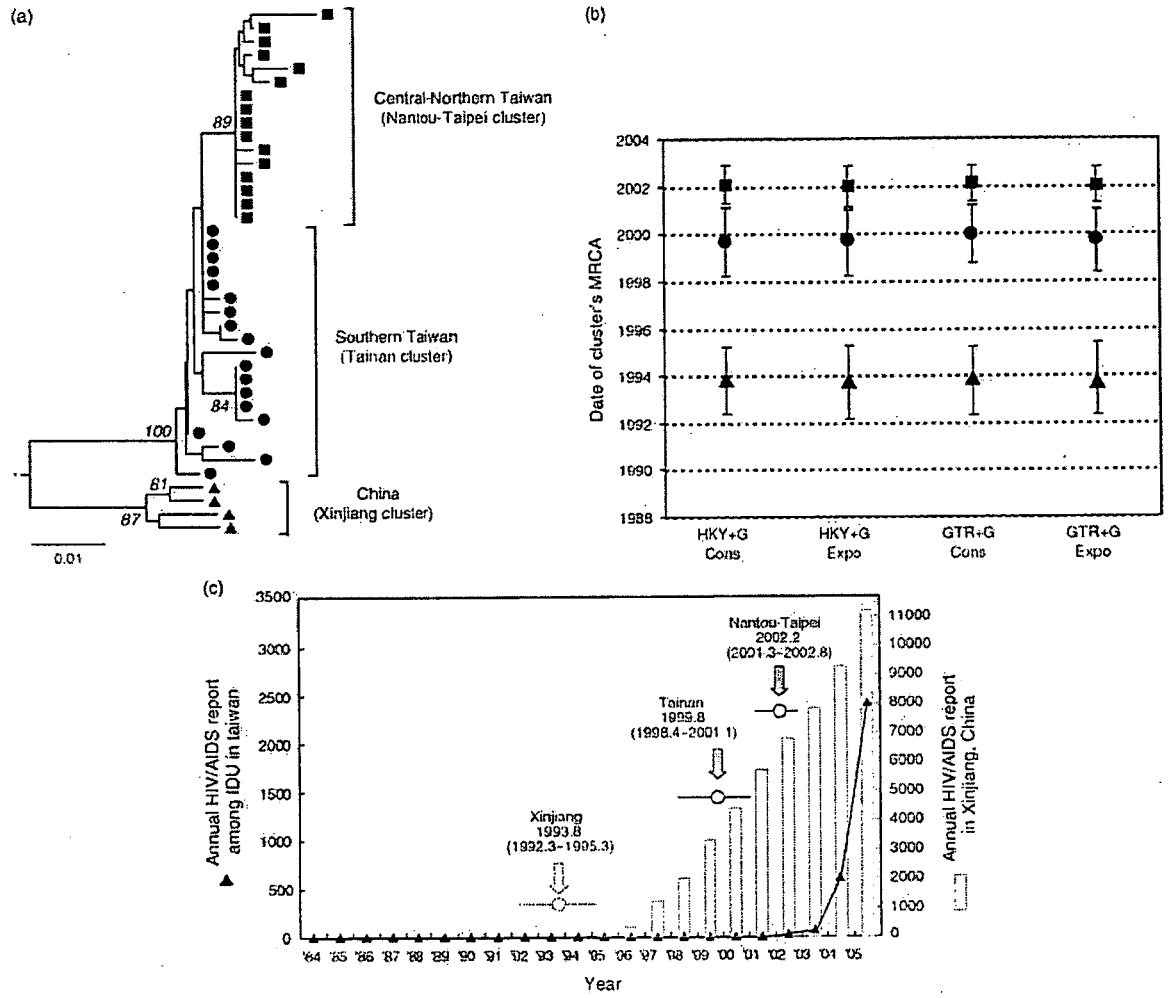

Fig. 1. Phylogenetic and evolutionary analysis of HIV-1 sequences from injection drug users in mainland China and Taiwan. (a) Estimated phylogeny for the *env* gene of HIV-1 CRF07_BC (HXB2 7077–7665 nt). For visual clarity, sequences are labeled with symbols according to location of isolation. (b) Estimated dates of the most recent common ancestors (MRCA) of CRF07_BC sequences from China (Xinjiang), southern Taiwan (Tainan), and central–northern Taiwan (Nantou–Taipei). Vertical lines denote the 95% highest posterior density credible intervals. Dates were estimated under various nucleotide substitution and evolutionary models: HKY, Hasegawa–Kishino–Yano model; GTR, general time reversible model; G, gamma distributed among-site rate heterogeneity; Cons, constant population size; Expo, exponential population growth. ■ Central–northern (Nantou–Taipei) cluster; ● southern Taiwan (Tainan) cluster; ▲ China (Xinjiang) cluster; (c) Change in annual HIV/AIDS cases in Xinjiang, China (□) and Taiwan (▲) between 1984 and 2005. The time to the MRCA of CRF07_BC clusters detected in the indicated locales are depicted with 95% highest posterior density credible intervals (in parentheses).

the end of September 2007, 15 183 HIV cases have been reported in Taiwan since 1984 and the majority of infections occurred relatively recently among IDU [5]. CRF07_BC accounts for 98% of the infections among IDU in Taiwan [4].

To estimate the timescale of CRF07_BC spread in mainland China (Xinjiang) and in Taiwan, we performed phylogenetic and Bayesian coalescent analyses on *env* sequences obtained from GenBank. The CRF07_BC *env* sequences used in this study are from Xinjiang province, China ($n=4$; sampled in 1997–1998), and from three cities in Taiwan; in particular, Tainan (southern; $n=19$), Nantou (central; $n=8$) and Taipei (northern; $n=8$), all sampled in 2004 (accession numbers EF078077–EF078079, EF078082–EF078105 and EF078107–EF078114). As shown in Fig. 1a, the CRF07_BC sequences group into three distinct phylogenetic clusters, denoted Xinjiang (China), Tainan (southern Taiwan) and Nantou-Taipei (central and northern Taiwan). Using the molecular clock approach implemented in BEAST v1.4 [6], we estimated the rate of evolution of the hypervariable region-stripped *env* gene (HXB2 7077–7665 nt) from an independent dataset of 41 HIV-1 subtype C strains with known sampling dates that ranged from 1989 to 2005. The

evolutionary rate estimate obtained ($4.7-5.0 \times 10^{-3}$ substitutions per site per year) was then incorporated as a prior probability distribution in the analysis of the CRF07_BC sequences [7]. A Bayesian Markov chain Monte Carlo method was used to estimate the dates of the most recent common ancestors (MRCA) of CRF07_BC in Xinjiang and Taiwan under various nucleotide substitution and evolutionary models. As illustrated in Fig. 1b, the likely year of origin of CRF07_BC from Xinjiang, China, was August 1993 [95% credible region (CR) March 1992–March 1995]. MRCA of CRF07_BC strains from southern and central-northern Taiwan were dated to August 1999 (95% CR April 1998–January 2001) and February 2002 (95% CR March 2001–August 2002), respectively. The evolutionary and statistical assumptions used have almost no effect on the estimated dates (Fig. 1b).

This study indicates that CRF07_BC was introduced into the IDU population in Xinjiang in the early to mid 1990s (1992–1995). The strain also spread into IDU in southern Taiwan in the late 1990s (1998–2001), and subsequently disseminated northward to IDU in central–northern Taiwan in the early 2000s (2001–2003), resulting in the largest ever HIV/AIDS epidemic in Taiwan. HIV/AIDS surveillance detected a dramatic upsurge of HIV cases in Xinjiang in the mid-1990s (1995–1996), and in 2003–2004 in Taiwan (Fig. 1c), suggesting that CRF07_BC may have been present among IDU for a year or two in each region before the epidemic spread and subsequent detection of the infection. It is most likely that both the Xinjiang and Taiwan outbreaks trace their origins back to Yunnan province, thought to be the geographical origin of CRF07_BC [2,3]. We note, however, that MRCA dates can also be more recent than the date of outbreak discovery, either because extant virus diversity has been incompletely sampled, or because the founding lineages of the outbreak have since gone extinct [8,9]. These results illustrate the history of the regional and international spread of HIV-1 CRF07_BC in east Asia.

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Potent Inhibition of HIV-1 Replication by Novel Non-peptidyl Small Molecule Inhibitors of Protease Dimerization*

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Dimerization of HIV-1 protease subunits is essential for its proteolytic activity, which plays a critical role in HIV-1 replication. Hence, the inhibition of protease dimerization represents a unique target for potential intervention of HIV-1. We developed an intermolecular fluorescence resonance energy transfer-based HIV-1-expression assay employing cyan and yellow fluorescent protein-tagged protease monomers. Using this assay, we identified non-peptidyl small molecule inhibitors of protease dimerization. These inhibitors, including darunavir and two experimental protease inhibitors, blocked protease dimerization at concentrations of as low as 0.01 μM and blocked HIV-1 replication with IC_{50} values of 0.0002–0.48 μM. These agents also inhibited the proteolytic activity of mature protease. Other approved anti-HIV-1 agents examined except tipranavir, a CCR5 inhibitor, and soluble CD4 failed to block the dimerization event. Once protease monomers dimerize to become mature protease, mature protease is not dissociated by this dimerization inhibition mechanism, suggesting that these agents block dimerization at the nascent stage of protease maturation. The proteolytic activity of mature protease that managed to undergo dimerization despite the presence of these agents is likely to be inhibited by the same agents acting as conventional protease inhibitors. Such a dual inhibition mechanism should lead to highly potent inhibition of HIV-1.

AQ: A

AQ: B

Highly active antiretroviral therapy has had a major impact on the AIDS epidemic in industrially advanced nations. However, eradication of human immunodeficiency virus, type 1 (HIV-1)[2] does not appear to be currently possible, in part due to the viral reservoirs remaining in blood and infected tissues. Moreover, a number of challenges have been encountered, which include various adverse effects, only partial and limited immunologic restorations achieved, and occurrence of various cancers as consequences of survival elongation with highly active antiretroviral therapy (1). Moreover, such limitations of highly active antiretroviral therapy are exacerbated by the development of drug-resistant HIV-1 variants (2). Thus, the identification of new classes of antiretroviral drugs that have one or more unique mechanisms of action and produce no or minimal adverse effects remains an important therapeutic objective.

Fn2

Dimerization of HIV-1 protease subunits is an essential process for the acquisition of proteolytic activity of HIV-1 protease, which plays a critical role in the maturation and replication of the virus (3, 4). Thus inhibition of protease dimerization by chemical reagents is likely to abolish proteolytic activity and inhibit HIV-1 replication. However, for possible development of HIV-1 protease dimerization inhibitors, better understanding of the nature and dynamics of protease dimerization is crucial. The monomer subunits are connected by polar and non-polar interactions to form the dimer. Hydrophobicity of Leu-89, Leu-90, and Ile-93 and several other residues have been considered important in the folding of a protease monomer as well as in dimer stabilization (5, 6). For a systematic analysis of the conserved network of hydrogen bonds, termed "fireman's grip," Strisovsky *et al.* (7) have mutated the active site Thr-26 to a Ser, Cys, or Ala and have shown that T26A substitution reduced protease dimer stability, thus virtually nullifying the proteolytic activity of protease. Indeed, in our present study, T26A substitution effectively disrupted protease dimerization (see below), corroborating the results by Strisovsky *et al.* The flexibility of monomeric and dimeric HIV-1 protease and the feasibility of a stable protease monomer have also been studied

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[2] The abbreviations used are: HIV-1, human immunodeficiency virus, type 1; FRET, fluorescence resonance energy transfer; CFP, cyan fluorescent protein; YFP, yellow fluorescent protein; BCV, brecanavir; DRV, darunavir; CHX, cycloheximide; PI, protease inhibitor; bis-THF, bistetrahydrofuranylurethane; TPV, tipranavir; Fluc, firefly luminescence; Rluc, *Renilla* luminescence.