Chapter 1

General introduction

The expression of genes encoded by human immunodeficiency virus type-1 (HIV-1) is regulated by the interaction of cellular factors and a viral *trans*-activation protein, Tat, with specific regulatory elements in the long terminal repeat (LTR) of HIV-1 (Gaynor, 1992). The HIV-1 regulatory protein Tat binds to one of the regulatory elements in the LTR region, which is called the *trans*-activating response region, TAR (Fig. 1A; Rosen et al., 1985; Dayton et al., 1986; Fisher et al., 1986). This region is located immediately downstream from the site of initiation of transcription at the 5'-end of all the viral transcripts (Berkhout et al., 1989). It is an RNA element consisting of 59 nucleotides (nts), which is the minimal motif that is sufficient for formation of a stable hairpin structure that allows binding of Tat *in vivo* (Rosen et al., 1985; Feng and Holland, 1988; Jakobovits et al., 1988). Tat effectively stimulates transcription after its binding to TAR RNA (Cullen, 1986; Peterline et al., 1986; Rice and Mathews, 1988). Deletion studies of TAR RNA revealed that so-called bulge residues are obligatory both for the specific binding to Tat and for *trans*-activation, whereas loop sequences are necessary for *trans*-activation but are not essential for the binding of Tat *in vitro* (Feng and Holland, 1988; Berkhout and Jeang, 1989; Dingwell et al., 1989; Cordingly et al., 1990; Roy et al., 1990; Weeks et al., 1990).

Tat of HIV-1 is a small cysteine-rich nuclear protein consisting of 86 amino acids (Fig. 1B). It has two major domains, a cysteine-rich region and basic region (Arya et al., 1985; Sodroski et al., 1985). The cysteine-rich region is essential for the function of this protein (Garcia et al., 1988; Kubota et al., 1988) and it has a metal-binding domain that probably mediates the metal-linked dimerization of Tat (Frankel et al., 1988). The basic region is responsible for the specific binding to TAR RNA (Weeks et al., 1990), as well as for nuclear localization (Dang and Lee, 1989; Endo et al., 1989). Tat belongs to a family of RNA-binding

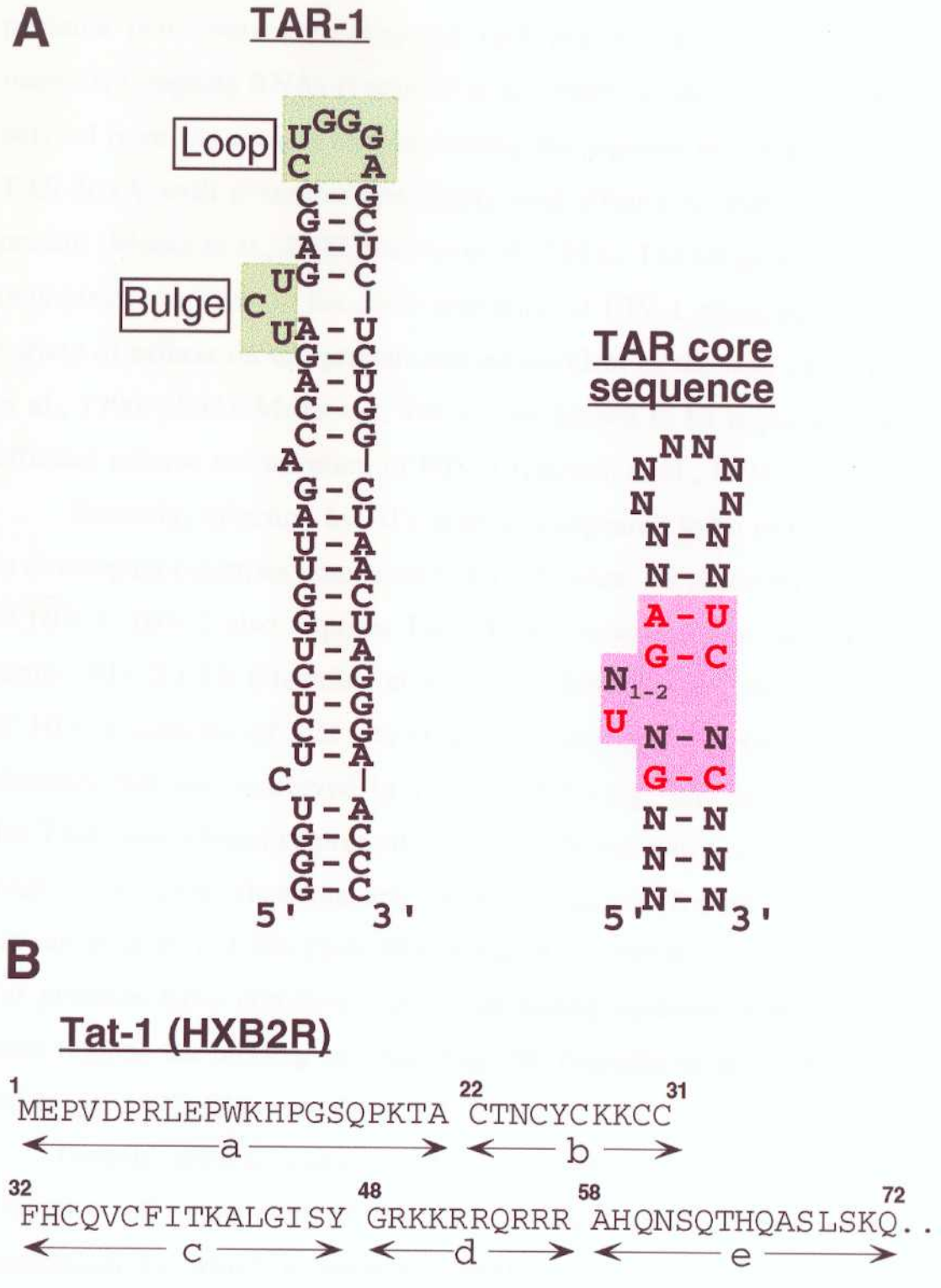

Figure 1. The Sequences of TAR RNA and Tat protein of HIV-1. A, TAR-1 RNA and the core element of TAR RNA. TAR motif is boxed and the core element that is required for binding to Tat protein is showed by red letters. B, The Sequence of Tat-1 protein. a. Amino Terminus, b. Cys-Rich Region, c. Core Region, d. Basic Region, e. Gln-Rich Region.

proteins that contain an arginine rich region for recognition of the respective cognate RNAs (Lazinski et al., 1989). A short peptide which is derived from Tat protein and containing the arginine rich region binds to TAR RNA with a similar specificity and affinity to that of the intact protein (Weeks et al., 1990; Calnan et al., 1991). The *tat* gene product not only plays a key role in the *trans*-activation of HIV-1 genes but also has a variety of effects on the growth and metabolism of the host cells (Ensoli et al., 1990, 1993). Moreover, Tat is now known to be important for the efficient reverse transcription of HIV-1 (Harrish et al., 1997).

Recently, infection by HIV-type 2 is appeared to be more prevalent in developing countries compared to HIV-1 cases. As observed in the case of HIV-1, HIV-2 also requires Tat-2/TAR-2 interaction at the activation center, HIV-2 LTR (Hannibal et al., 1993). Interestingly, the TAR region of HIV-2 consists of 123 nts (Fig. 2A) and contains two TAR core elements that are conserved in TAR-1 RNA (Fig. 1A, right). Although the TAR core elements present in two different hairpin location, both TAR-1 like core elements are, indeed, required for efficient *trans*-activation in HIV-2 life cycle (Rhim and Rice, 1993). HIV-1 and HIV-2 Tat proteins have common regions including cysteine-rich, core, and basic regions for binding to TAR (Fig. 2B; Guyader et al., 1987; Chang and Jeang, 1992; Rhim and Rice, 1993).

Despite several studies on the stimulation by Tat of the *trans*-activation of expression of the HIV genome, the precise molecular mechanism by which it operates remains obscure. The rates of viral mRNA and protein synthesis induced by Tat in mammalian cells were estimated to be 100-fold higher than control rates (Hauber and Cullen, 1988). It has been reported that Tat functions as an anti-terminator, an elongation factor in transcription and an enhancer of the initiation of transcription (for reviews, see Vaishav and Wong-Staal, 1991; Cullen,

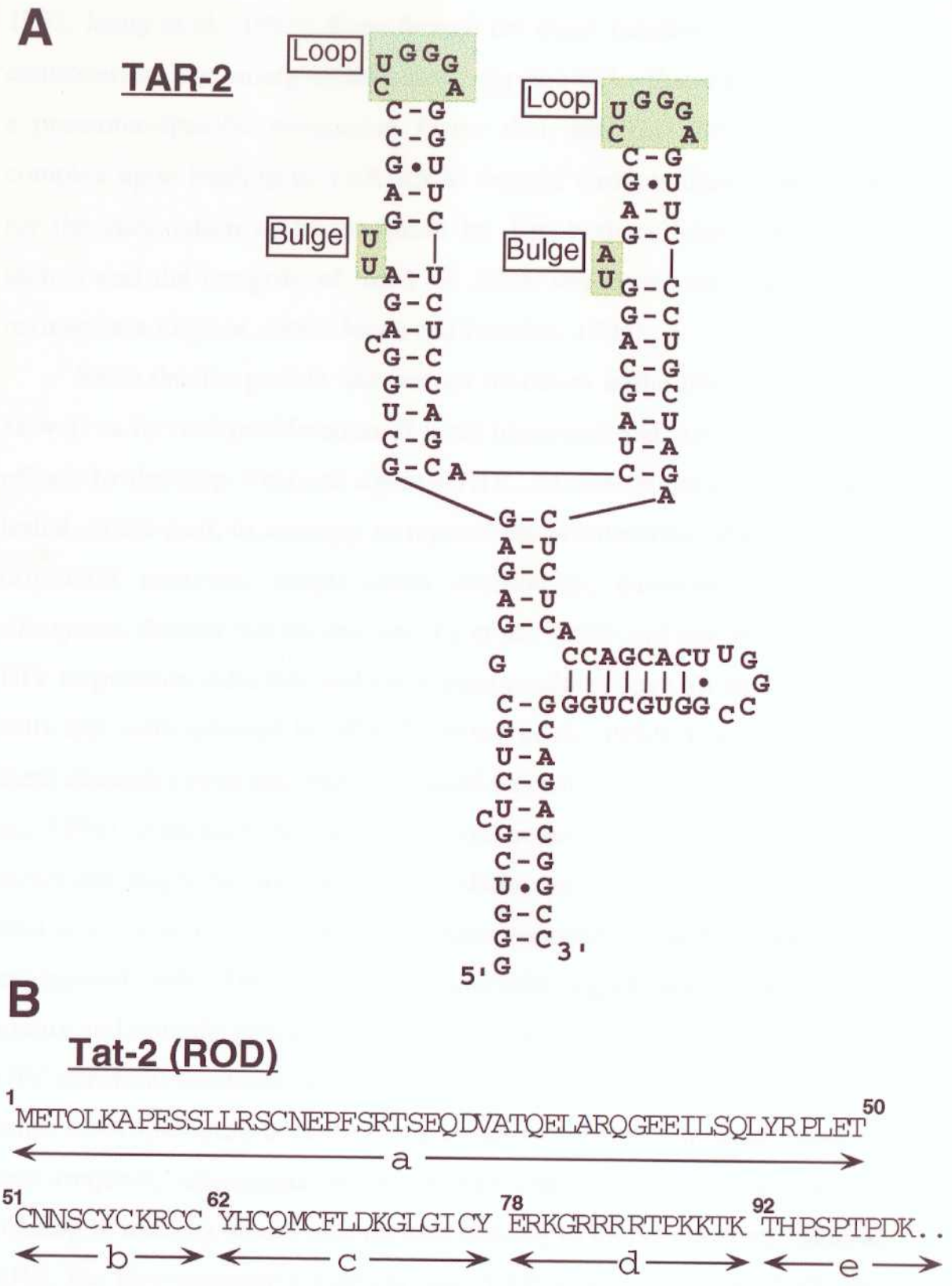

Figure 2. The Sequences of TAR RNA (A) and Tat protein (B) of HIV-2. a. Amino Terminus, b. Cys-Rich Region, c. Core Region, d. Basic Region, e. Gln-Rich Region.

1992; Jeang et al., 1993). Even though the exact function of Tat remains controversial, the emerging consensus appears to be that Tat functions as a promoter-specific elongation factor that modifies the transcription complex upon binding to TAR RNA. Several early studies showed that, for the stimulation of transcription by Tat, both cellular transcription factors and the integrity of the TAR RNA sequences are essential (for reviews, see Gaynor, 1992; Jones and Peterlin, 1994).

Since the Tat protein has various functions in the life cycle of HIV, as well as in viral proliferation, it is an important and attractive target in efforts to develop weapons against HIV. Several strategies have been tested, in the past, in attempts to repress the proliferation of HIV. *Trans*-dominant proteins, single-chain antibodies, antisense molecules, ribozymes, decoys (for review, see Yu et al., 1994) and use of the LTR of HIV to produce inducible and toxic gene products have all been tested in cells that were infected by HIV (Harrison et al., 1992). Combinations of these strategies have also been examined (Yuyama et al., 1994; Yamada et al., 1996). Although the expression and regulation of such therapeutic molecules might be possible *in vivo*, their constitutive expression could lead to cellular toxicity or to an immune response by the host against the engineered cells. This problem is especially significant in the case of toxins and suicide genes. Among various RNA-based strategies against HIV infection, the decoy strategy has a potential advantage over the use of other RNA inhibitors because the generation of escape mutants might be less frequent: alterations in Tat or Rev (HIV-1 protein) that prevent binding to a decoy would also prevent binding to native elements (such as RRE, the Rev-responsive element, and TAR sequences). Both RRE and TAR RNAs have been exploited as decoys and, in cell cultures, these decoys inhibited the replication of HIV (Graham and Maio, 1990; Sullenger et al., 1990; Lisziewicz et al., 1993).

Although decoys might act as much more efficient inhibitors than other molecules, decoys might potentially be toxic to cells if they were to sequester cellular factors, in particular when the decoy RNA happens to include regions that can interact with cellular proteins. Several previous studies showed that cellular factors bind efficiently to TAR RNA. Despite these studies, the effects of TAR RNA on the cellular machinery of the host cells have not been analyzed in detail either *in vitro* or *in vivo*.

Since authentic TAR RNA of HIV-1 interacts with several cellular factors within the cell, the TAR RNA might not be the most suitable antagonist and specific inhibitor of Tat. Alternatively, the binding sites of cellular factors that are identified may be substituted to other sequences in TAR RNA and such variant construct can be used directly to sequester the Tat protein specifically. However, such a variant TAR RNA construct might not be functional as efficient decoy as demonstrated by the cyclin T1 studies (Wei et al., 1998). Thus, the future application of Tat decoy mediated inhibition of HIV replication depends upon the novel source of nucleic acids.

The next challenge is not only to find a novel RNA molecule that binds efficiently with high specificity to the Tat protein of HIV in the absence of cooperative interaction with cellular factors, but also should have higher affinity over the combined affinity achieved by the natural TAR RNA with cellular factors (for example, cyclin T1) and Tat protein. In the past, *in vitro* genetic selection method has been used to isolate higher affinity nucleic acid aptamers to various proteins and thus, serve as a potential source for higher affinity RNA motifs (for reviews, see Gold et al., 1995; Osborne and Ellington, 1997). I used a similar method to find high affinity RNA aptamers to the Tat protein of HIV-1 and isolated high affinity aptamer that binds efficiently in the absence of any cellular factors.