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Structural approach of HIV-1 genome RNA Packaging

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ABSTRACT

The human immunodeficiency virus type 1 (HIV-1) selectively packages two copies of its unspliced genome RNA, both of which are utilized for strand-transfer-mediated recombination during reverse transcription a process that enables rapid evolution under environmental and chemotherapeutic pressures. The viral genome RNA appears to be selected for packaging as a dimerized form, and there is evidence that dimerization and packaging are mechanistically coupled. Both processes are mediated by interactions between the nucleocapsid domains of a small number of assembling viral gag precursor protein (called as p55) and full-length RNA elements within the 5′-untranslated region of the genome RNA. A number of secondary structures have been predicted for regions of the genome that are responsible for packaging, and high-resolution structures have been determined for a few small RNA fragments and RNA-p55 complexes. The secondary structure for the genome RNA packaging signal of HIV-I is investigated using RNA mapping techniques and extensive computer analyses. Recent reports suggest that the RNA sequence containing the packaging signal conforms to a stable stem-loop structure and that a portion of this structure is conserved among twenty independent HIV-I isolates. The heterogeneity of the RNA packaging sequence was not correlated with the amino acid sequence variability of the viral nucleocapsid protein. Here, we review efforts that have been made to identify the molecular determination and mechanism of HIV-1 genome RNA packaging. Research findings have critical implication in understanding the viral particle maturation process.

KEYWORDS : HIV-1, RNA packaging, Viral particle

Introduction

HIV-I shares many common features with other retroviruses.1,2.3 The assembly of HIV-I viral components into virions occurs on the surface of infected cells in parallel with the budding process during or after which the virions mature.4 When viral components of RNA virus assemble into virions, precursor p55 gag protein containing nucleic acid binding motifs (zinc finger motifs) incorporates the viral genome RNA by interacting with the specific sequence (called packaging signal).5,6,7 Since this protein can distinguish the unspliced viral genome RNA from other viral and cellular mRNAs, RNA-p55 interaction is considered to be sequence and structure specific.8,9 Efforts to elucidate the structural determinants and mechanisms that regulate these activities have been made using a variety of biochemical, *in vivo* genome RNA packaging, and computational approaches, and there is general consensus that some activities are controlled by well-defined hairpin-loop structures within the HIV-1 5′-UTR.9,10 A number of secondary structures have been predicted for regions of the genome that are responsible for packaging, and high-resolution structures have been determined for a few small RNA fragments and RNA-p55 complexes. However, there is less agreement regarding the structures that regulate HIV-1 genome RNA packaging. Here, we review efforts that have been made to understand the mechanism of HIV-1 genome RNA selection with emphasis on RNA-p55 complex that appear to play important roles.

Structural approach of viral HIV-1 genome RNA packaging signal

Retroviral Ψ element, which is called as genome RNA packaging signal is a cis-acting viral RNA element identified in the unspliced viral genome RNAs of the retrovirus HIV-1 and Simian immunodeficiency virus (SIV).11,12,13 It is involved in regulating the essential process of packaging the retroviral genome RNA into the viral capsid core during replication.14,15,16,17 The final virion contains a dimer of two identical unspliced copies of the viral genome RNA. In HIV-1, theΨ element is 75-160 nucleotides in length, and located at the 5′ end of the viral genome RNA just upstream of the *p55 gag* initiation codon.9,10,18 It has a known secondary structure composed of four hairpin structures called Stem-loop type 1 (*SL-1*) to *SL-4*, which are connected by relatively short linkers. The four stem-loop strutures are important for viral genome RNA packaging and each of the stem-loops *SL-1*, *SL-2*, *SL-3* and *SL-4* has been independently expressed and structurally characterised.18,19,20,21,22 Computational approaches demonstrated that *SL-1*, which is also referred to as HIV-1dimer initiation site (DIS) consists of a conserved hairpin structure with a palindromic loop sequence which was predicted and confirmed by genome mutagenesis.23,24 This palindromic loop is known as the primary DIS as it is believed to promote dimerization of the viral genome RNA through formation of a kissing dimer intermediate.23,25 It has been shown that *SL-1* may provide a secondary binding site for Regulator of expression of virion (Rev) protein.26 The Rev protein is an essential HIV-1 regulatory protein which increases the stability and transport of the unspliced viral genome RNA.27 *SL-2* (also referred to as HIV-1 *SD*) consists of a highly conserved 19 nucleotides stem-loop which has been shown by genome mutatagenis to modulate the splicing efficiency of HIV-1 mRNAs.28 *SL-3* consists of a highly conserved 14 nucleotides stem-loop which was predicted and confirmed by genome mutagenesis analysis and mass spectrometric detection (MSD). *SL-3* of HIV-1 RNA is sufficient by itself to induce heterologous RNA into virus-like particles but its deletion does not completely eliminate encapsidation.28 *SL-4* of HIV RNAs consists of a highly conserved 14 nucleotides stem-loop structure that is located just downstream of the *p55 gag* start codon. The structure of RNA stem-loop was confirmed by mutagenesis and has an NMR and MSD.28 It also may have coding and non-coding roles. A renewed and coordinated commitment to basic discovery research is required for the development of a safe and effective clinical therapeutic approach against AIDS.29.30.31

Conclusion

Conformational heterogeneity is likely to present problems for any RNA structure determination method that cannot distinguish between multiple equilibrium species. New methods that combine NMR-derived highresolution local structural information, which can distinguish between slowly interconverting RNA conformers, with low-resolution global structural information derived by single-molecule cryoelectron tomography should prove useful for future studies of larger and functionally relevant fragments of the HIV-1 genome RNA. The incorporation of viral genomic RNA into the viral particles is one of the crucial steps for production of infectious virions. Protein-RNA interaction between p55 gag precursor protein and the stable stem-loop structure within the viral RNA packaging signal could be a good target for therapy against AIDS. Moreover, the information obtained from this study should also be useful for the development of an HIV-1 vaccine using a novel retrovirus vector based on HIV-I.

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REFERENCES

1. Cullen BA. (1995) Regulation of HIV gene expression. AIDS 9 Suppl A: S19-32. | 2. Wong-Staal F, Haseltine WA. (1992) Regulatory genes of human immunodeficiency viruses. Mol Genet Med. 2, 189-219.. | 3. Kohl NE, Enimi EA, Schleif WA, Davis LF, Heimbach JC, Dixon RAF, Scolmck EM, Signal IS. (1988) Active human immunodeficiency virus protease is required for viral infectivity. Proc. Natl. Acad. Sci. USA 85, 4686-4690. | 4. Greene WC, Peterlin BM. (2002) Charting HIV's remarkable voyage through the cell: Basic science as a passport to future therapy. Nat Med. 8, 673-80. | 5. De Rocquigny H, Gabus C, Vincent A, Fournie-Zaluski MC, Roques B, Darlix JL. (1992) Viral RNA annealing activities of human immunodeficiency virus type 1 nucleocapsid protein require only peptide domains outside the zinc fingers. Proc. Natl. Acad. Sci. USA 89, 6472-7476. | 6. Dupraz P, Spahr PF. (1992) Specificity of Rous sarcoma virus nucleocapsid protein in genomic RNA packaging. J. Virol. 66, 4662-4670. | 7. Gorelick RJ, Nigida SM, Bess JW, Arthur LO, Henderson LE, Rein A. (1990) Noninfectious human immunodeficiency virus type 1 mutants deficient in genomic RNA. J. Virol. 64, 3207-3211. | 8. Harrison GP, Lever AM. (1992) The human immunodeficiency virus type 1 packaging signal and major splice donor region have a conserved stable secondary structure. J. Virol. 66, 4144-4153. | 9. Hayashi T, Shioda T, Iwakura Y, Shibuta H. (1992) RNA packaging signal of human immunodeficiency virus type 1. Vtrology 188, 590- 599. | 10. Aldovini A, Young. RA. (1990) Mutations of RNA and protein sequences involved in human immunodeficiency virus type 1 packaging result in production of noninfectious virus. J. Virol. 64, 1920-1926. | 11. Baba S, Takahashi K, Noguchi S, Takaku H, Koyanagi Y, Yamamoto N, Kawai G. (2005) Solution RNA structures of the HIV-1 dimerizatio n initiation site in the kissing-loop and extended-duplex dimers. J. Biochem. 138, 583-592. | 12. Lever A, Gottlinger H, Haseltine W, Sodroski J. (1989) Identification of a sequence required for efficient packaging of human immunodeficiency virus type 1 RNA into virions. J. Virol. 63, 4085-4087. | 13. Strappe PM, Greatorex J, Thomas J, Biswas P, McCann E, Lever AM. (2003) The packaging signal of simian immunodeficiency virus is upstream of the major splice donor at a distance from the RNA cap site similar to that of human immunodeficiency virus types 1 and 2. J. Gen. Virol. 84, 2423-2430. | 14. McBride MS, Panganiban AT. (1997) Position dependence of functional hairpins important for human immunodeficiency virus type 1 RNA encapsidation in vivo. J. Virol. 71, 2050-2058. | 15. McBride MS, Schwartz MD, Panganiban AT. (1997) Efficient encapsidation of human immunodeficiency virus type 1 vectors and further characterization of cis elements required for encapsidation. J. Virol. 71, 4544-4554. | 16. McBride MS, Panganiban AT. (1996) The human immunodeficiency virus type 1 encapsidation site is a multipartite RNA element composed of functional hairpin structures. J. Virol. 70, 2963-2973. | 17. Lever AM. (2007) HIV-1 RNA packaging. Adv. Pharmacol. 55, 1-32. | 18. Lawrence DC, Stover CC, Noznitsky J, Wu Z, Summers MF. (2003) Structure of the intact stem and bulge of HIV-1 Psi-RNA stem-loop SL1. J. Mol. Biol. 326, 529-542. | 19. Amarasinghe GK, De Guzman RN, Turner RB, Chancellor KJ, Wu ZR, Summers MF. (2000) NMR structure of the HIV-1 nucleocapsid protein bound to stem-loop SL2 of the psi-RNA packaging signal. Implications for genome recognition. J. Mol. Biol. 301, 491-511. | 20. Zeffman A, Hassard S, Varani G, Lever A. (2000) The major HIV-1 packaging signal is an extended bulged stem loop whose structure is altered on interaction with the Gag polyprotein. J. Mol. Biol. 297, 877-893. | 21. Pappalardo L, Kerwood DJ, Pelczer I, Borer PN. (1998) Three-dimensional folding of an RNA hairpin required for packaging HIV-1. J. Mol. Biol. 282, 801-818. | 22. Kerwood DJ, Cavaluzzi MJ, Borer PN. (2001) Structure of SL4 RNA from the HIV-1 packaging signal. Biochemistry 40, 14518-14529. | 23. Hayashi T, Ueno Y, Okamoto T. (1993) Elucidation of a conserved RNA stem-loop structure in the packaging signal of human immunodeficiency virus type 1. FEBS Letter 327, 213-218. | 24. Berkhout B; Van Wamel JL. (1996) Role of the DIS hairpin in replication of human immunodeficiency virus type 1. J. Virol. 70, 6723-6732. | 25. Skripkin E; Paillart JC; Marquet R; Ehresmann B; Ehresmann C. (1994) Identification of the primary site of the human immunodeficiency virus type 1 RNA dimerization in vitro. Proc. Natl. Acad. Sci. USA 91, 4945-4949. | 26. Gallego J, Greatorex J, Zhang H, Yang B, Arunachalam S, Fang J, Seamons J, Lea S, Pomerantz RJ, Lever AM. (2003) Rev binds specifically to a purine loop in the SL1 region of the HIV-1 leader RNA. J. Biol. Chem. 278, 40385-40391. | 27. Felber BK, Hadzopoulou-Cladaras M, Cladaras C, Copeland T, Pavlakis GN. (1989) Rev protein of human immunodeficiency virus type 1 affects the stability and transport of the viral mRNA. Proc. Natl. Acad. Sci. USA 86, 1495-1499. | 28. Abbink TEM, Berkhout B. (2007) RNA Structure Modulates Splicing Efficiency at the Human Immunodeficiency Virus Type 1 Major Splice Donor. J. Virol. 82, 3090-3098. | 29. Scanlan CN, Offer J, Zitzmann N, Dwek RA. (2007) Exploiting the defensive sugars of HIV-1 for drug and vaccine design. Nature 446, 1038-1045. | 30. Barouch DH. (2008) Challenges in the development of an HIV-1 vaccine. Nature 455, 613-619. | 31. Haase AT. (2010) Targeting early infection to prevent HIV-1 mucosal transmission. Nature 464, 217-223. |