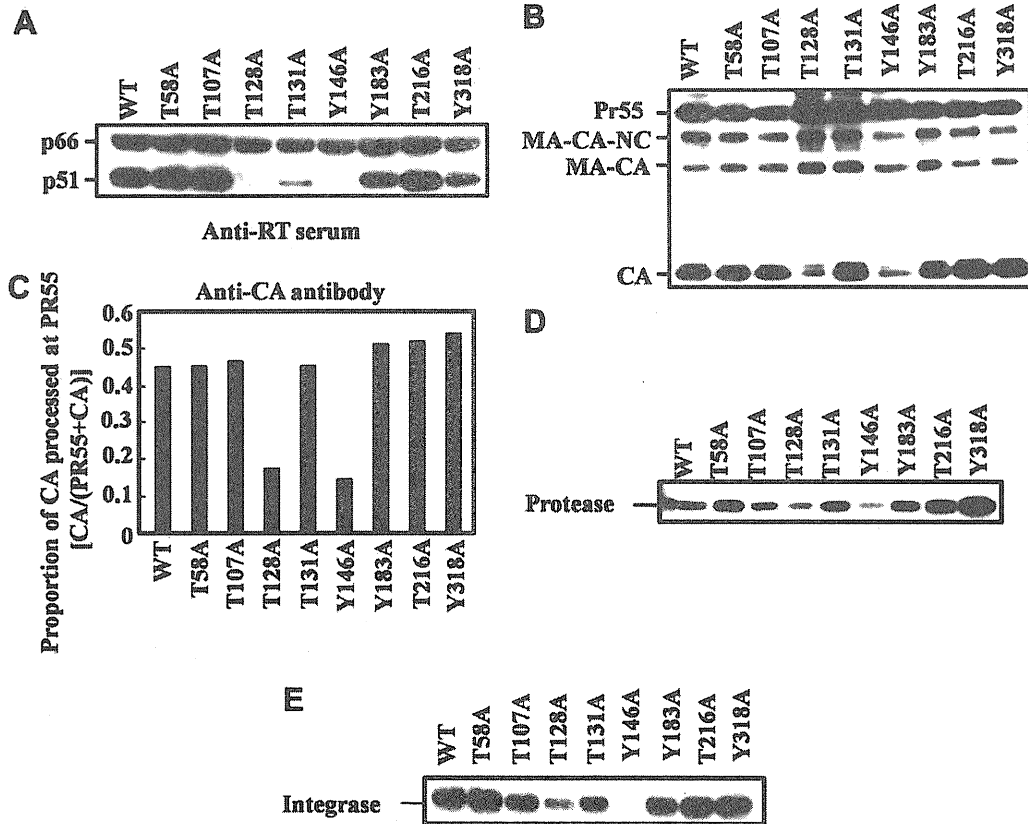

Fig. 2. The effect of RT mutations on the processing of HIV-1 Gag–Pol. 293T cells were transfected with 0.2 μg of each RT mutant pNL4-3. At 48 h post-transfection, the supernatants were harvested and subjected to Western-blot analysis. The Gag–Pol cleavage products were detected using anti-RT sera (A), an anti-CA antibody (B), an anti-protease antibody (D) and an anti-integrase antibody (E). (C) The proportion of CA cleaved at the Pr55-Gag cleavage site was calculated from the integrated band intensities.

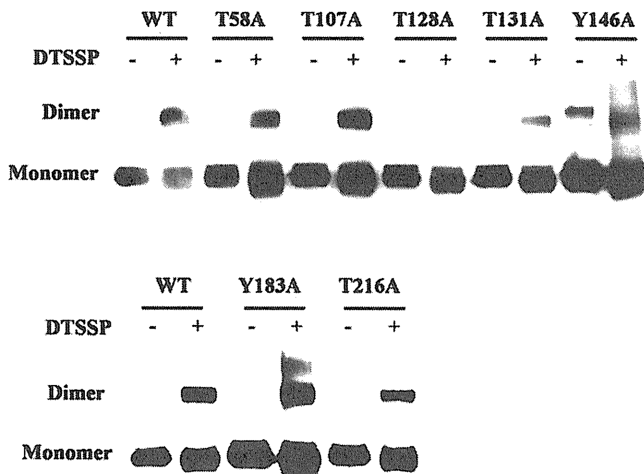

Fig. 3. Evaluation of RT dimerization. 293T cells were transfected with pLenti-p66-V5 or the RT mutants. At 48 h post-transfection, the cells were lysed with CSK buffer containing 0.5% NP-40. The cell lysates were incubated in either the absence or presence of 0.2 mM DTSSP at room temperature for 20 min and analyzed by Western blotting using an anti-V5 antibody.

the L264 and E302 residues are located in the two α-helixes of Thumb domain (Fig. S2A). Because helices are often critical in formation of a stable core structure of a protein, the L264S or E302Q mutations could critically influence proper folding and stability of the Thumb domain. Such effects could affect RT dimerization because the thumb of the p51 subunit extensively interacts with the RNase H domain. Simultaneously, the effects could increase susceptibility of mutants to PR in a virion via exposure of improperly folded domain. In contrast, the T128 and Y146 residues in p51 are positioned at the base of the β7–β8 loop, whose tip is embedded in a small cleft of p66 surface and constituted of a direct interaction surface between p51 and p66 (Fig. S2B). Therefore mutations at T128 and Y146 could influence orientation of the β7–β8 loop and alter stability of the RT dimer. Meanwhile, the mutations would not influence critically the stability of the RT, because the loop is positioned on protein surface. The failure of detection of RT p51 subunit in these mutants (Fig. 2A) may suggest that p66 dimerization is prerequisite for the processing. These structural insights are well consistent with the present experimental findings. However, further studies will be required to clarify the structure–function relationship with regard to the susceptibility of RT to PR degradation.

The mutation of T128 or Y146 in RT impaired the protease-mediated Gag–Pol processing. It is possible that substitutions in RT significantly change the conformation of the Gag–Pol precursor and decrease the accessibility of the targets for the protease. However, the three-dimensional (3-D) model suggests that the T128 and Y146 residues are not positioned near the inherent cleavage sites flanking RT, i.e., N- and C-terminal end of the RT (Fig. S2). In addition, reported electron microscopy studies suggest that Gag precursor has a rodlike structure [18,19], by which the T128 and Y146 residues are probably located far from the cleavage site and other proteins. Therefore, the mutations at the T128 and Y146 are less likely to influence critically the protease accessibility into the inherent cleavage sites of the Gag/Pol precursor protein, although the influences are formally not ruled out at present. An

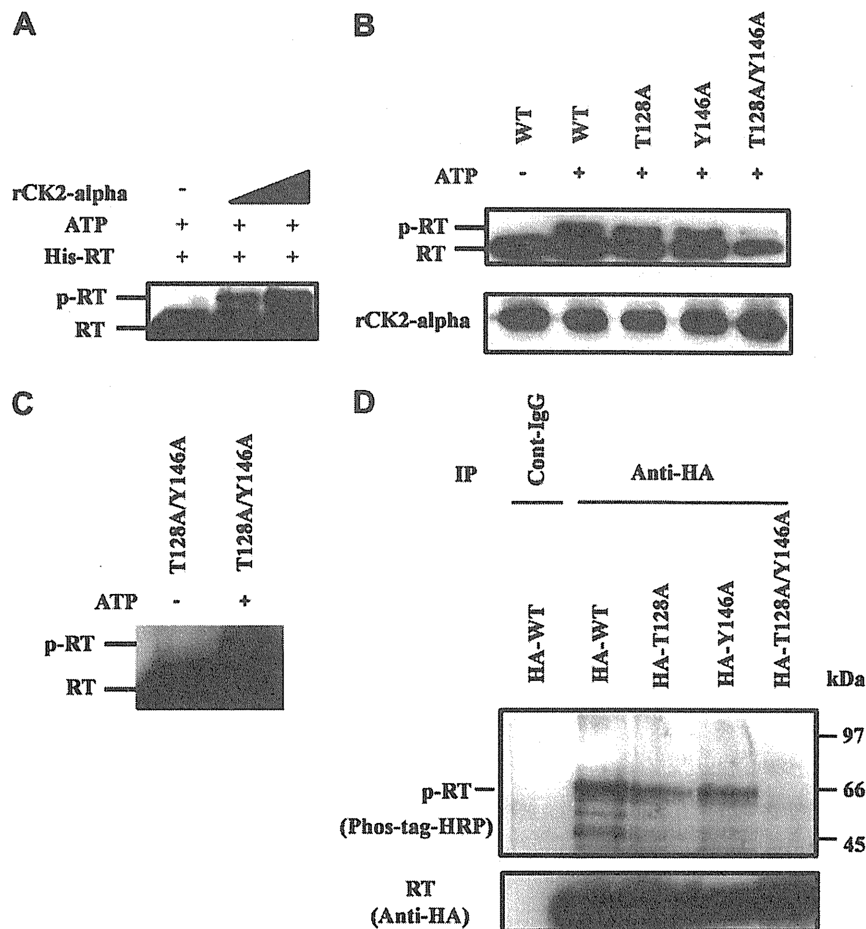

Fig. 4. The phosphorylation of recombinant RT in vitro. (A) Initially, 0.1 μg of recombinant His-RT-WT was incubated with 0.5 mM ATP in the absence or presence of 0.05 or 0.2 μg of recombinant His-CK2-alpha. The phosphorylation of recombinant His-RT was detected using Western immunoblot analysis after Phos-tag SDS–PAGE. The phosphorylation of the recombinant His-RT was indicated as a shifted band. (B, C) 0.1 μg (B) or 1.0 μg (C) of indicated recombinant His-RT was incubated with 0.2 μg of recombinant His-CK2-alpha in the absence or presence of 0.5 mM ATP. The phosphorylation of His-RT was detected as indicated in Fig. 4A. (D) 293T cells were transfected with pcDNA-HA-RT or its RT mutants. At 48 h after transfection, the cells lysates were immunoprecipitated with anti-HA antibody and phosphorylated proteins were detected using Phos-tag-Biotin and Streptavidin-conjugated HRP.

alternative possibility is that the mutations at the T128 and Y146 impaired the protease-mediated Gag–Pol processing via attenuation of Gag/Pol dimerization for PR activation. Further study is necessary to address each of these issues.

These observations are consistent with the hypothesis that antiviral drugs targeting RT might inhibit HIV-1 replication without necessarily inhibiting the catalytic function of the RNA-dependent DNA polymerase.

Acknowledgments

We thank Dr. I.S.Y. Chen for providing pNL4-3lucΔenv and Dr. H. Miyoshi for providing pMD.G-VSV-G. This work was supported by a Grant-in-Aid for AIDS research from the Ministry of Health, Labor, and Welfare, Japan, a Grant-in-Aid for High Technology Research (HTR) from the Ministry of Education, Science, Sports, and Culture, Japan, and a Grant from the Strategic Research Foundation Grant-aided Project for Private Universities from the Ministry of Education, Culture, Sport, Science, and Technology, Japan (MEXT).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2011.09.034.

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