Disturbance of genetic information by a ribonucleotide analogue

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ABSTRACT
The synthetic base analogue, 6H,8H-3,4-dihydropyrimido[4,5-c][1,2]oxazin-7-one (P), can efficiently base pair with A and G. We have previously demonstrated that the deoxyribonucleoside of P (dP) is highly mutagenic and that this is due to the ambiguous base pairing ability of P. In this work, we have shown that the ribonucleoside triphosphate of P (rPTP) induces C to U mutation on an in vitro model of retroviral genomic RNA replication pathway. This mutation induction by rPTP may be specific to retroviruses, since host genomic DNA should not be affected by such a ribonucleotide analogue, although temporary transcription-translation errors may occur.

INTRODUCTION
A number of base and deoxyribonucleoside analogues are able to base pair with more than two of the natural bases (1). When these analogues are metabolized to DNA precursors in cells, they can disturb the genetic information because of this ambiguous base pairing. Ribonucleoside analogues with such base pairing ability may be mutagenic since ribonucleosides can be metabolized to DNA precursors by the activity of ribonucleoside 5'-diphosphate reductase (2). Without doubt they are also converted to triphosphate RNA precursors. Their ambiguous nature should reduce the accuracy of transcription. However, there are only a small number of reports about the incorporation of such ambiguous RNA precursors during transcription (3).

The base analogue P forms stable base pairs with both A and G because its tautomeric constant is 10-100 (Figure 1) (4,5). Deoxyribonucleoside of P is one of the most potent nucleoside analogue mutagens known (6). We have also synthesized and studied the ribonucleoside triphosphate of P, rPTP, as an ambiguous RNA precursor. rPTP is efficiently incorporated into transcripts by various RNA polymerases (7,8).

The propagation of genetic information in retroviruses is based on transcription and reverse-transcription, whilst that of the host cell is by DNA replication. For this reason, the incorporation of an ambiguous RNA precursor like rPTP into transcripts may disturb the genetic information only of retrovirus, but not of host cell. In this work, we show that rPTP induces mutations in an in vitro model of retroviral transcription reverse-transcription machinery.
RESULTS AND DISCUSSION

One cycle of the propagation model, which consists of in vitro transcription and RT-PCR, is illustrated in Figure 2. Trans-activation responsive region (TAR) of HIV-1 was chosen for this model. This was carried out for five rounds in the absence or presence of rPTP, then the cDNA of fifth generation RNA was sequenced. The results are shown in Figure 3. When the cycles were run in the presence of rPTP, there were obvious T signals at every C peak on the cDNA sequencing pattern (Figure 3 (b)). Approximately 10% of every C was changed to U during the five cycles. rP incorporated opposite G would direct dATP incorporation during the next cDNA synthesis. As a consequence, C to U mutations was induced on RNA (C to T on cDNA). U to C mutation was not observed in this experiment, but in a different system this transition mutation by rPTP was observed at a low level (unpublished data).

In host cells, because transcripts are not used for the propagation of genetic information to the next generation, an ambiguous RNA precursor like rPTP will not induce the mutations in their genomic DNA although temporary transcription-translation errors may be induced. For this reason, ambiguous RNA precursors can be said to be specific mutagens to retroviruses.

As excess mutations are harmful to the species, these type of ribonucleotide analogues are possible candidates for new anti-retroviral drugs.

References