NOTES

Redesignation of the RNase D Activity Associated with Retroviral Reverse Transcriptase as RNase H*

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In the presence of Mn²⁺, reverse transcriptase of both human immunodeficiency virus and murine leukemia virus hydrolyzes duplex RNA. However, Designating this novel activity RNase D conflicts with Escherichia coli RNase D, which participates in tRNA processing. On the basis of its location in the RNase H domain, we propose that this novel retroviral activity be redesignated RNase H.

It has been recently reported that retroviral reverse transcriptas (RT) can cleave double-stranded RNA (1–3). Original studies of Ben-Artzi et al. (1), with substrate representing a tRNA₃U₃₃ : primer binding site complex, suggested that this activity, which the authors designated RNase D, might be required at certain stages during replication. However, the similarity in cleavage specificities between the RT-associated RNase D and Escherichia coli RNase III raised the possibility that RNase D activity might reflect low-level contamination with the bacterial enzyme. These contentions were strengthened by the findings of Hostomsky et al. (6) that preparations of p51 and several RNase H-deficient forms of human immunodeficiency type 1 (HIV-1) RT were also capable of cleaving double-stranded RNA, including a bacteriophage RNA substrate conventionally used for analysis of RNase III.

The RNases employed by Ben-Artzi et al. (1) and Hostomsky et al. (6) involved cleavage of a radiolabeled substrate and then fractionation of the products by high-resolution gel electrophoresis. Alternatively, DNA polymerase and RNase H activities of RT can be assessed by in situ methods with polycrylamide gels in which a radioactive substrate is embedded. While the latter approach is more qualitative, it has the advantage that activity is assigned by molecular mass, i.e., for HIV-1 and HIV-2 RT, the polymerase and RNase H activities were associated with the 66-kDa polypeptide (5, 8). Subsequent data from Ben-Artzi et al. (2) has indeed confirmed that their original HIV-1 RT preparation (1) contained contaminating E. coli RNase III. However, work by the same authors with HIV RT polypeptides purified by metal chelate affinity chromatography (p51, p66, and p66/p51) (7) revealed that an RNase activity capable of digesting double-stranded RNA was in fact associated with the p66 subunit of both homo- and heterodimer. Loss of this function with p66 HIV RT containing an inactivating mutation in the RNase H domain (Glu-478→Gln-478) verified RNase D as a bona fide activity and showed that it was tightly coupled with previously identified RNase H function. The ability of murine leukemia virus RT to hydrolyze duplex RNA supports observations with the HIV enzyme (2, 3). Blain and Goff present strong evidence that the RNase H domain is required for this activity, but there are distinct requirements for hydrolysis of RNA/DNA and RNA/RNA hybrids (3).

In light of these findings, assigning a new name (RNase D) to this RNase H-associated activity seems misleading. Such an assignment is more appropriately reserved for the E. coli enzyme involved in tRNA processing (4). By analogy with the convention for certain restriction enzymes, we propose RNase H* as a more suitable designation. Although RNase H* activity has been clearly demonstrated in RT, its relevance in vivo remains obscure. In this context, it is worth noting that the HIV enzyme requires Mn²⁺ to cleave double-stranded RNA (2). In contrast, both the DNA polymerase and RNase H activities of this enzyme show a strong preference for Mg²⁺.

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