

Unusual Reverse Transcriptases*

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Reverse transcriptase (RTase),¹ rather than being unique to retroviruses as once appeared likely, is encoded in many DNA genomes (1, 2). RTase genes occur within 1) the retrovirus-like class I retrotransposons that have long terminal repeats (e.g. Ty of yeast), 2) class II (or LINE-like) retrotransposons that lack terminal repeats of any kind and typically have a dA-rich stretch at the 3'-end of the sense DNA strand, and 3) unusual retroelements of chromosomal and organellar origin. Telomerase is also an RTase (3). Molecular phylogenetic analyses indicate that the RTases encoded by the class II retrotransposons and retroelements (called here type 2 RTases) are more like one another in predicted amino acid sequence than they are like the RTases (type 1) encoded by retroviruses and class I retrotransposons (4–7).

Soon after the discovery of the coding sequences predicting the type 2 RTases, many investigators recognized that these enzymes would be mechanistically distinctive from the already well known type 1 enzymes (8–12); the complex series of events that assure replication of long terminal repeats, including internal primer binding sites on the RNA template, priming by tRNAs, and template switching, is unnecessary. Another difference between type 1 and type 2 RTases is the apparent absence, in most type 2 coding sequences, of segments that predict an RNase H. As summarized in this review, a few type 2 enzymes and telomerase have now been studied and, remarkably, each of them has a distinctive priming mechanism. Moreover, none of them appears to utilize as primer a nucleotide covalently bound to the RTase protein as does the type 1 hepatitis B virus RTase (13).

The RTases of LINE-like Retrotransposons

The R2Bm RTase—Two types of LINE-like elements, R1 and R2, are found inserted at specific positions in some copies of the 28 S rRNA genes of many insect species (14, 15). An *Escherichia coli* expression plasmid containing the entire single open reading frame (ORF) (predicting a 123-kDa protein) of the *Bombyx mori* R2 element (R2Bm) produces a 120-kDa protein with integrase (endonuclease) and RTase activity. The integrase produces staggered, site-specific double strand breaks at the observed insertion site in 28 S rDNA yielding 5'-phosphate and 3'-hydroxyl termini and 2-base pair long 5'-overhangs (16, 17); the initial nick is between a C and an A residue (5'-(A/C)C-) on the 28 S rDNA coding strand. RNA is required for optimal activity. In the presence of RNA, the integrase rapidly catalyzes the initial nick to form relaxed plasmid circles and then

slowly converts these to full-length linear duplexes. However, in the absence of RNA only the nicked, relaxed circles are produced.

When dNTPs and *in vitro* synthesized R2Bm RNA are added to the integrase reaction mixtures, reverse transcription occurs and is dependent upon the presence of the rDNA target site. Analysis of the cDNA product demonstrated that the 3'-hydroxyl at the first nick in the DNA target is the primer for the RTase; the template is R2Bm RNA (17) (Fig. 1a). These findings indicated that target site cleavage and reverse transcription are coupled, as predicted by early models for LINE-like element insertion (9–12).

The relative rates at which the reaction products accumulate are as expected if second strand cleavage follows reverse transcription. Thus, the initial product is a branched chain in which the cDNA copy of the R2Bm RNA is covalently linked to the 28 S rDNA and hydrogen bonded to the RNA. Significantly, although an RNA chain lacking the R2Bm sequences can act as cofactor for second strand cleavage, only RNAs containing the 3'-end of R2Bm RNA can serve as the template for the RTase; it appears that sequences within the 250-nt long 3'-untranslated region of R2Bm RNA are recognized by the enzyme and, together with 5–10 residues in the primer end of the 28 S rDNA, permit chain synthesis (18).

All known genomic R2Bm elements have 4 As at the 3'-junction with 28 S rDNA. Experiments with *in vitro* synthesized R2Bm RNA containing varying numbers of terminal As indicate that the efficiency of cDNA synthesis decreases as the terminus changes from 4 to 1 to 8 As (17, 18). Substitution of the four As with other bases can yield efficient templates; many of the cDNA products initiate at the RNA terminal residue. When the (A)₄ terminus is reduced in length, occasional internal initiations and frequent additions of extra nontemplated residues occur. These observations may reflect constraints imposed by the necessity of the enzyme to position itself accurately on both the RNA template and the 28 S rDNA (18). Virtually nothing is known about second strand DNA synthesis or the fate of the RNA template except that the two overhanging bases left after cleavage of the second 28 S rDNA strand are removed.

RTases of Other Class II Retrotransposons—Several other LINE-like elements have been shown to encode active RTases, but the reaction mechanisms have not been studied. The coding region of the *Drosophila melanogaster jockey* element yields, in *E. coli*, active RTase associated with a 92-kDa polypeptide (19). RTase activity, accompanied by L1Hs RNA, has been reported to occur in the microsomal fraction of human NTera2D1 cells, but it remains to be proven that it is encoded by L1Hs (20). In more definitive experiments, L1Hs (21) or CRE1 (of *Crithidia fasciculata*) (22) RTase was detected in partly purified virus-like particles formed in yeast cells transformed with class I retrotransposon Ty1 in which the RTase gene was replaced by the L1Hs or CRE1 RTase coding region. The RTases encoded by L1Hs (23), CRE1,² and R2Bm³ have also been detected with an indirect genetic assay for the RTase-dependent formation, in yeast, of processed pseudogenes (24). In the case of L1Hs, the complex structures of the newly inserted sequences suggest

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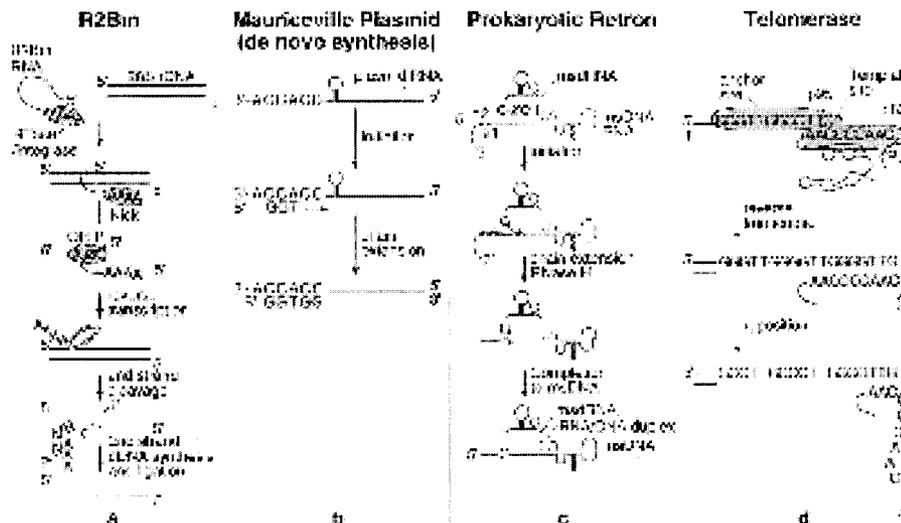
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¹ The abbreviations used are: RTase, reverse transcriptase; ORF, open reading frame; nt, nucleotide(s); RNP, ribonucleoprotein; msDNA, multicopy single-stranded DNA; msdRNA, RNA attached to msDNA.

² A. Gabriel, personal communication.

³ T. Eickbush, personal communication.

FIG. 1. Schematic representations of four RTase reactions described in the text: *a*, R2Bm; *b*, Mauriceville plasmid; *c*, bacterial retron; *d*, telomerase. Red is RNA, black is DNA, and green is newly synthesized DNA. Refer to the text for explanations and references.



that the RTase readily switches from one template to another (23).

The Prokaryotic Group of RTases

A subset of type 2 RTases can be defined based on the similarity of predicted amino acid sequences (6, 7). This subset includes the enzymes encoded by mitochondrial plasmids found in some strains of *Neurospora*, bacterial retrons, and group II introns and is called the prokaryotic group, reflecting the prokaryote origin of mitochondria and chloroplasts (7, 25).

The de Novo Synthesis of DNA by the RTases of a *Neurospora* Mitochondrial Plasmid—The Mauriceville plasmid (3.6 kilobase pairs) found in *Neurospora* mitochondria encodes a 710-amino acid long polypeptide in its single ORF (25). Ribonucleoprotein (RNP) particles isolated from the mitochondria contain full-length, plus strand transcripts of the plasmid and RTase activity associated with a homodimer of the 81-kDa protein that is encoded by the ORF (25, 26). The RTase within the RNPs copies the endogenous RNA to yield an RNA-cDNA duplex with a full-length (minus strand) cDNA (26, 27). After a lag, plus strand DNA is synthesized; an RNase H present in the RNP but not encoded in the plasmid can degrade the RNA (28).

The 3'-end of the plasmid transcript has striking similarities, in sequence and secondary structure, to the tRNA-like 3' termini of plant RNA virus genomes including the CCA 3' termini (Fig. 1*b*) (25, 29). And like the RNA-dependent RNA polymerases encoded by RNA viruses, many of the (minus strand) cDNAs synthesized in the RNPs begin (5'-end) with a G corresponding to the penultimate C in the RNA (27). Another large group of the cDNAs utilize DNA primers unrelated to the 3'-end of the RNA template and begin copying from the 3'-terminal A in the template. Experiments with purified RTase clarified these findings.

The RTase can be released from the RNPs with micrococcal nuclease and purified (30). When partially purified enzyme was incubated with an RNA template synthesized *in vitro* and containing 5'-truncated plasmid sequences, the cDNA products were about 20 nucleotides longer than the template RNA. The extra nucleotides were at the 5'-end of the cDNA and derived from priming oligodeoxynucleotides that were bound to the RTase. The primers were each different in sequence and length, and most appeared to be short cDNAs copied from plasmid or mitochondrial RNA. Moreover, these primers were all joined directly to the 5'-TGG sequence copied from the 3'-ACC end of the template RNA (as were some of the primed cDNAs synthesized within RNPs and described above) and

could be cleaved from the RNA-DNA duplex with S1 nuclease. Assuming that the plasmid RTase catalyzed synthesis of these primers, then it seems that the enzyme is capable of switching templates, in analogy with the type 1 RTases.

When the RTase is freed of the bound primers by treatment with polyethyleneimine (31), it can utilize either exogenously supplied oligodeoxynucleotides or the 3'-end of the RNA template itself as primer; that is, the primer can be either DNA or RNA. Most interestingly, however, the RTase is also efficient in *de novo* cDNA synthesis (Fig. 1*b*) and is the first DNA polymerase known to initiate DNA chains. A template containing only the 3'-terminal 26 residues of the plasmid RNA is sufficient to direct specific, *de novo* initiation, primarily opposite the penultimate C residue (as in the other major group of cDNAs synthesized in RNPs). The 5'-terminal G residue can be supplied by free deoxyguanosine, dGMP, or dGTP. If the 3'-terminal residues of the template are missing or if extra nucleotides are added to the 3'-end, copying occurs but not *de novo* synthesis; instead, the 3'-end of the RNA serves as a primer. The similarity between this RTase and the RNA-dependent RNA polymerases of Q β and bromo mosaic virus is underscored by their common ability to recognize the 3'-terminal tRNA-like structure of the template and initiate synthesis by copying the penultimate residue.

RTases of Bacterial Retrongs—Retrons, so-called by Temin (32), have been described in the chromosomes of myxobacteria, a few strains of *E. coli*, and several other bacteria (33–35). These DNA elements (from 1.3 to 3 kilobase pairs long) vary in detailed structure and sequence, but all include a single chromosomal transcription unit containing, from 5' to 3', sequences encoding msdRNA, msDNA, and RTase. Current understanding of the RTase is derived from structural analysis of the *in vivo* products and *in vitro* analysis of a retron RTase purified to homogeneity after expression from a recombinant plasmid in *E. coli* (36). It was the discovery and characterization of the product of the reverse transcription, msDNA (for multicopy single-stranded DNA), that led to the discovery of the RTase.

The msDNAs in various bacteria differ in length and sequence but share common features (Fig. 1*c*, bottom): 1) the 5'-end of the DNA (65–163 nt) is linked, through a 2',5'-phosphodiester, to a guanosine within a short RNA (fewer than 100 nt), the msdRNA; 2) from 6 to 8 nt at the 3'-end of both the msDNA and msdRNA are complementary and form a duplex; and 3) both msDNA and msdRNA assume stable secondary structures.

Although the retron RTases vary in size from about 300 to 590 amino acids (34, 35), the enzyme in cell extracts sediments in association with msDNA as a large complex and has an apparent molecular mass of 600–700 kDa on molecular sieves (33). The folded RNA of the retron transcription unit provides both the template and primer for the enzyme (Fig. 1c, top) and may also serve as mRNA for RTase translation although this has not been proved. Among the important features of the folded RNA is a duplex stem formed by the inverted repeats a2 and a1 that bracket the msdRNA and msDNA region of the retron transcript and several stem/loop structures. A G residue in the short single-stranded msdRNA segment just 3' of the a2-a1 stem provides the 2'-hydroxyl that primes reverse transcription. The residue in the msDNA region that marks the start of the template segment also occurs just after the end of the a2-a1 duplex. Thus, the a1-a2 stem brings the primer and the template in close proximity. With the purified enzyme and the folded RNA as template, msDNA is synthesized and its 5'-end is linked to the 2'-hydroxyl of the expected guanosine residue in the msdRNA region (36). Elongation of the DNA proceeds along the template, and the efficiency of chain extension depends on the absence of any stable secondary structures, but not on the particular sequence, within the msDNA template region (37). In most instances, copying stops and the chain terminates after addition of the 6–8 nucleotides that form the duplex between the 3'-ends of the msdRNA and msDNA segments; the mechanism of this specific chain termination has not been determined. In contrast to the lack of specificity for the RNA sequence in the msDNA template region, the msdRNA region must be from the same retron as the RTase; when the msdRNA regions of two different retrons are exchanged, msDNA synthesis does not occur (38).

With one possible exception, the RTase coding regions of retrons do not predict RNase H segments. Nevertheless, the formation of msDNA by the proposed scheme requires the removal of the RNA in the msDNA region of the template. Experiments with *E. coli* cells carrying mutations in chromosomal RNase H genes indicate that cellular RNase H is likely to play a role in msDNA synthesis (39).

The RTase of a Group II Intron—Group II introns were first found in the genomes of fungal and plant organelles (40) and more recently in certain cyanobacteria and proteobacteria (41). They have attracted attention because of 1) their ability to self-splice *in vitro* (though apparently not *in vivo*), 2) a splicing mechanism like that used by eukaryotic nuclear genes, 3) their mobility, 4) the presence, in some of them, of coding regions for genetically defined proteins, maturases, that are required for *in vivo* splicing, and 5) the presence, in some of them (group IIA), of a coding region that predicts a RTase. RNPs isolated from yeast mitochondria support the synthesis of cDNAs complementary to RNA transcribed from the *cox1* mitochondrial gene and containing sequences from the first and second (group IIA) introns in the gene, aI1 and aI2 (42). The aI2 enzyme can also utilize poly(rA)-oligo(dT). Analysis of mutant alleles of *cox1* indicated that RTase activity depends on the ORF of either aI1 or aI2, depending on the strain. The cDNAs initiated by aI2 RTase start at multiple sites near the 3'-end of aI2 or within exon 3, and their structures are such that the templates were either excised introns or unspliced or partially spliced mRNAs. Neither the priming mechanism nor the template requirements are understood, although the cDNA structures would be consistent with *de novo* chain initiation.

Telomeres and Telomerase

The formation of telomeric structures is associated with RTase in two known ways. Telomerase, which adds characteristic G-rich repeats to the ends of chromosomes in many eu-

karyotes, is an RTase (3), and *D. melanogaster's* distinctive telomeric structures are made up of multiple copies of two LINE-like elements, HeT-A (43, 44) and TART (45, 46), both of which can transpose onto *Drosophila* telomeres.

Telomerases are RNPs containing an uncapped RNA that acts as template (Fig. 1d). In the purified *Tetrahymena thermophila* enzyme, 80- and 95-kDa polypeptides bind the RNA and the telomeric primer DNA, respectively (47). There is limited homology between the amino acid sequences predicted from the cloned gene (and cDNA) for p95 and viral RNA-dependent RNA polymerases; otherwise, the telomerase proteins are different from other RTases and from each other (47). The telomerase RNAs vary in length (from 159 nt in *Tetrahymena* to 1300 in yeast (48)) and sequence, but each includes a sequence complementary to the species-specific, 3' single strand overhanging, G-rich telomeric repeat; a conserved secondary structure among the protozoan telomerase RNAs includes a generally single-stranded, although partly constrained, configuration for the template region (49–51). Active enzyme can be reconstituted after removal of the RNA by nuclease treatment (47, 52).

In the first step in telomere extension, the 3' G-rich overhang base pairs with a few nucleotides in the telomerase RNA (Fig. 1d). The RTase then copies the rest of the basic repeat unit (*e.g.* GGGTTG in *Tetrahymena*) and pauses and shifts relative to the telomere so that the new terminus can be repositioned for addition of the next repeat (3, 52). The mechanism whereby copying pauses and the primer shifts once the repeat segment is complete is unknown; as already mentioned, a similar question arises in the synthesis of retron msDNA. Other experiments indicate that the rate and processivity of polymerization *in vitro* are strongly dependent on the nucleotides just 5' of the terminal G-rich repeat on the primer (53, 54); it is likely that this represents a secondary binding site, the anchor site, for p95 and that such binding contributes to processivity.

Comments

Recent discussions about the origin of life on Earth postulate an early, "RNA" world and the conversion of that RNA world to one in which genetic information is stored in DNA through the mediation of a primitive RTase (55–57). Thus, special interest has focused on the widespread type 2 RTases. It has been suggested that they may 1) have evolved from a primitive RNA-dependent RNA polymerase such as those now associated with some RNA viruses, 2) represent the most primitive RTases yet observed and thus may be ancestral to type 1 RTases and DNA polymerases, and 3) be the closest relatives we know to the RTase required by the "RNA world" hypothesis (27, 31, 55–57).

Molecular phylogenies suggest that all the type 2 RTases may be more closely related than the organisms that harbor them and thus have, in part, an independent evolutionary history (4–7). This could be the consequence of conservation of the RTase coding sequence within the separate genomes over long periods of evolutionary history or could reflect horizontal transfer of elements across species and even phyla. There is evidence for horizontal transfer of *jockey* among distantly related members of the *Drosophila* genus (58). Apart from the similar RTase coding sequences, certain other features recur among the type 2 RTases and even telomerase. These include the importance of template secondary structure, the recognition by the enzymes of the 3'-terminal structure of the RNA template, the initiating role of guanosine nucleotides, and the association of the enzymes with RNPs. Thus, while each of the enzymes has evolved to develop distinctive properties, their similarities hint at a common ancestor.

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