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MINIREVIEW

Retrons and Multicopy Single-Stranded DNA

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BACTERIAL RETROELEMENTS

The central dogma of biology, which states that genetic information is transferred from DNA to RNA and then to protein, was challenged in 1970 by the finding that in certain viruses genetic information is stored in RNA which is then converted to DNA (1, 41). This reverse flow, or retroflow, of genetic information is carried out by an enzyme called reverse transcriptase (RT), and RNA viruses containing the RT gene are thus designated retroviruses.

The reverse flow of genetic information may not be, after all, just an exception to the central dogma, but may have played an essential role at the origin of life. It has been suggested that life started from an RNA world, which eventually evolved to the current DNA-based life (23, 44). During the 20 years since the discovery of RT, genetic elements encoding RT and other associated components, such as integrase and virion structural components, have been found in diverse eukaryotes, from unicellular organisms such as yeasts to insects, plants, and animals. It is probable that every eukaryotic cell carries such retroelements in one form or another. For example, they exist as proviruses or in the form of long-terminal repeat (LTR)-containing retrotransposons, non-LTR retroposons such as LINE (long interspersed repeated sequences)-like elements, or mitochondrial group II introns or mitochondrial plasmids (for reviews see references 5 and 45). In addition, pseudogenes are abundant in eukaryotic genomes and appear to have been generated as a consequence of RT function in the cell.

Thus, it is likely that RT not only played an essential role in the early evolution of life, by converting RNA to DNA, but is also playing a major evolutionary role in diversification of eukaryotic genomes. Given the importance of RT in the evolution of the eukaryotic genome, it had been speculated that the prokaryotes should contain RT or have had RT. However, except for the facts that *Escherichia coli* DNA polymerase I has RT activity (35) and that RT activity has been detected in some bacteria (4, 25, 43), there has been, until recently, no obvious clue to indicate that the prokaryotes have RT or retroelements evolutionarily related to eukaryotic retroelements.

The first clue to the existence of RT in the prokaryotes was suggested in 1987 from experiments on the biosynthesis of a peculiar RNA-DNA complex called multicopy single-stranded DNA (msDNA) (10). Two years later, it was demonstrated that indeed *Myxococcus xanthus*, a gram-negative soil bacterium (20, 26), and some natural isolates of *E. coli* (28, 33) contain RTs having sequence similarities to retroviral RTs and that these RTs are essential for msDNA biosynthesis.

This minireview will discuss major issues and unresolved questions relating to bacterial retroelements, in light of recent findings. The reader is referred to recent (18, 21) and earlier (17, 27, 32, 40, 42) reviews for more detailed and comprehensive discussions of msDNA and bacterial RT.

BIOSYNTHESIS OF msDNA

The DNA and RNA strands of msDNA are widely varied in length, depending upon the bacterial species and strain. Figure 1A shows the structure for msDNA-Ec107, which was recently discovered to exist in 8 of 72 wild strains of *E. coli* (ECOR collection) (14, 24). The features common to all msDNAs so far characterized are as follows. (i) A short, single-stranded DNA (65 to 163 bases; 107 bases for msDNA-Ec107) is linked to the 2′-OH group of an internal rG residue by a 2′,5′-phosphodiester linkage. (ii) Stable secondary structures are present in both DNA and RNA molecules. (iii) The complementary 3′-end sequences (5 to 8 bases; 6 bases for msDNA-Ec107) of the DNA and RNA molecules form a stable DNA-RNA hybrid. (iv) A few hundred copies of msDNA exist extrachromosomally in a cell.

To date, seven msDNAs have been identified: two from an *M. xanthus* strain, DZF1 (msDNA-Mx162 [10] and msDNA-Mx65 [9]), one from another myxobacterial species, *Stigmatella aurantiaca* (msDNA-Sa163 [13]), and four different msDNAs from four independent isolates of *E. coli* (msDNA-Ec67 [28], msDNA-Ec86 [33], msDNA-Ec73 [39], and msDNA-Ec107 [14]). Except for msDNA-Mx162 and msDNA-Sa163, the msDNAs are extensively diverse in terms of the lengths and primary nucleotide sequences of both DNA and RNA molecules.

The genetic locus required for msDNA synthesis is a component of the bacterial chromosome and consists of a single element called a retron. The retron is composed of three genetic components: *msr* for the RNA (msdRNA)-coding region, *msd* for the DNA-coding region, and the gene for RT (Fig. 1B). The *msr* and *msd* regions are in opposite orientations, overlapping by 5 to 8 bases at their 3′ ends. All three elements are under a single promoter for RNA polymerase, which is responsible for the production of a long RNA transcript containing the msDNA-coding sequence and the mRNA for RT. Notably, the mRNA contains two sets of inverted repeat sequences: b1 and b2, corresponding to the stem structure of msDNA, and a1 and a2 (Fig. 1B).

The a1 and a2 inverted repeat sequences exist in all retrons so far characterized and are situated as shown in Fig. 1B. Since a1 is immediately upstream of *msd* and a2 is immediately upstream of the branched G residue within *msr*, the RNA transcript is predicted to form a secondary struc-

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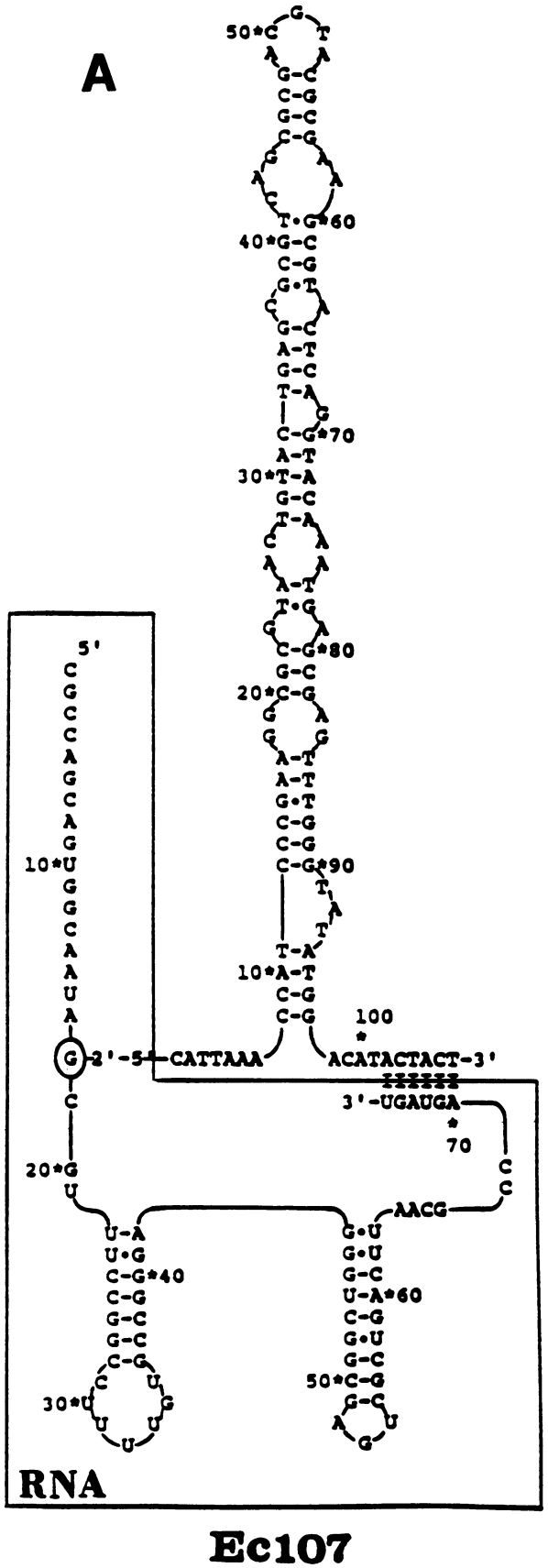
ture depicted in step 2 in Fig. 1B. As a result, the branched rG residue is placed at the end of the a1-a2 stem structure. If one postulates that the rG residue could serve as a primer for DNA synthesis, then elongation by RT would produce a DNA strand joined to the RNA transcript at this branched point (Fig. 1B; 10, 26). As DNA synthesis proceeds (step 3), the RNA template may be processed by RNase H, and DNA chain elongation then terminates at a specific site, leaving the RNA-DNA overlapping region at their 3′ ends (step 4). Thus, the branched RNA-linked msDNA is formed. It should be noted that the RNase H action is not necessarily coupled with the DNA chain elongation reaction. The msDNA synthesis may occur even without RNase H. Interestingly, all known bacterial RTs except for RT-Ec67 do not have the RNase H domain. Therefore, the RNase H activity used for msDNA synthesis (step 3) is considered to be provided from the chromosomal RNase H that is not associated with retrons.

The model explains well the absolute requirement for RT for msDNA synthesis. When the RT-coding sequence is removed from a retron, msDNA synthesis is completely blocked. However, if the RT gene is placed under a separate promoter and expressed in *trans* in the same cell, msDNA production is restored (26). In a cell-free system, elongation of msDNA synthesis has been demonstrated to proceed base by base, using an RNA template (26).

IS THE 2′-OH PRIMING REACTION UNIVERSAL?

The model proposed in Fig. 1B also accounts for the formation of the 2′,5′-phosphodiester linkage in msDNA. The priming reaction or the addition of the first deoxynucleoside base to the RNA template has been demonstrated in a cell-free system with total RNA isolated from cells containing only the *msr-msd* region of retron-Ec67 (no RT gene) and partially purified RT-Ec67 (unpublished results). In this cell-free system, only dTTP (but not dATP, dGTP, or dCTP) was incorporated into a 132-base precursor RNA, yielding a 133-base compound. This specific dT incorporation could be altered to dA or dC by simply substituting a dT or dG for the dA residue in the template RNA corresponding to the first base (base X in Fig. 1B), indicating that the priming reaction is a specific template-directed event. Once the priming reaction was completed, RT was able to elongate the DNA chain further, yielding the same sequence as msDNA-Ec67.

At present, the exact nature of the G residue in the precursor RNA molecule from which msDNA is primed is unknown. It is tempting to speculate that RT is capable of the 2′-OH priming reaction de novo. It should be noted that the structure around the branched G residue is highly conserved (Fig. 1B). The A · U base pair at the end of the a1-a2 stem, a G residue at the opposite side of the branched rG residue, and (except for msDNA-Ec67) a C residue at the 3′ end of the branched G residue are conserved among sequenced retrons. The branched G residue cannot be replaced with a C or A residue (16). When three base substitutions were introduced into the a1-a2 stem structure, a secondary structure immediately upstream of the branched G residue, the synthesis of msDNA-Mx162 was completely blocked. However, when three additional base substitutions were made on the other strand of the a1-a2 stem to restore the complementary base pairing, msDNA synthesis was restored (16). This supports the importance of the formation of the stem structure by the a1 and a2 inverted repeat sequences (Fig. 1B) and suggests that there may be no requirement for a specific primary sequence in the stem



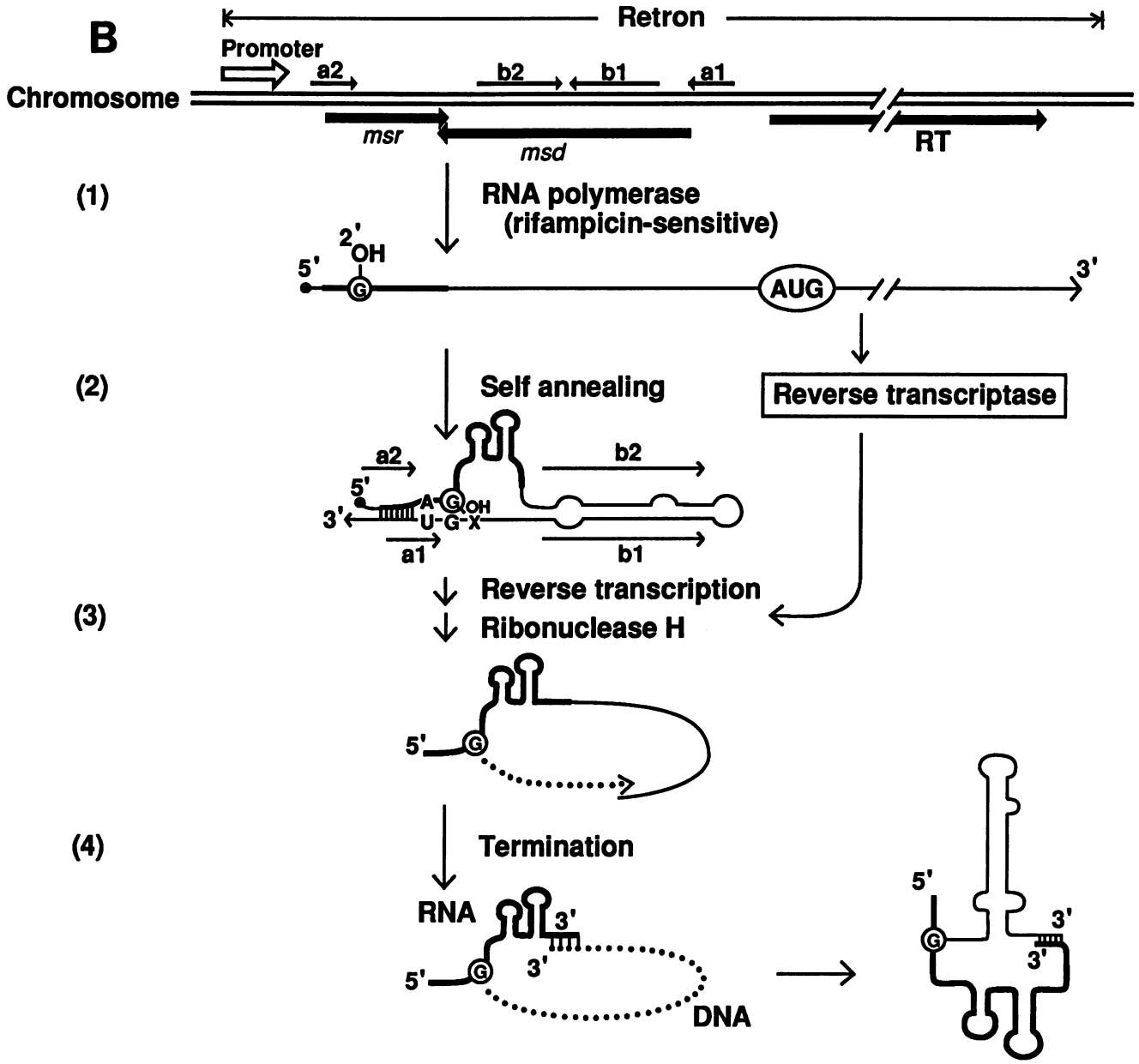


FIG. 1. Structure of msDNA and mechanism of msDNA biosynthesis. (A) Proposed secondary structure of msDNA-Ec107 (14). msdRNA is boxed, and the branched rG residue is circled. (B) Biosynthetic pathway of msDNA synthesis. See details in the text. Thick lines in the mRNA transcript represent the RNA which is left attached to the msDNA molecule.

structure. Similarly, there is no requirement for a specific deoxynucleoside triphosphate as the first base to be added to the branched rG residue. It is determined simply by complementarity to the X residue in Fig. 1B.

Interestingly, the 2′-OH priming reaction appears to be unique for each RT; msDNA-Ec67 can be synthesized by the RT from its own retron (retron-Ec67) but not by RT from retron-Ec73 or vice versa (unpublished results). This specificity may be dependent upon the secondary structure of the msdRNA molecule in the vicinity of the branched rG residue, which is unique for individual msdRNAs. This stringent requirement of a specific RT for the priming reaction for each msDNA is in sharp contrast to the fact that bacterial RT can use different templates (RNA or DNA) or primers for the chain elongation reaction (29). It is interesting that the priming reaction for retroviral minus strand DNA synthesis requires a tRNA specific for an individual retrovirus. For example, human immunodeficiency virus requires $tRNA^{Lys3}$, and its 3′-end 18-base sequence, including the CCA sequence at the 3′ terminus, is considered to form a duplex with the retrovirus RNA at the so-called primer-binding sequence (2, 3). It is generally believed that the 3′-OH group of the 3′-end rA residue is used for the priming reaction. However, surprisingly, the formation of a 3′,5′-phosphodiester linkage between the rA residue and the 5′ end of minus strand DNA has not been unambiguously demonstrated, since all the experiments have been carried out by using alkaline digestion to cleave the linkage between

the tRNA primer and minus strand DNA (for example, see reference 12).

Since retroviral RTs are evolutionarily related to bacterial RTs (20, 46), it is probably not farfetched to postulate that retroviral minus strand DNA synthesis may also be primed from the 2′-OH group of the 3′-end rA residue of a tRNA molecule. If the 2′-OH priming reaction is used for all RTs, the priming reaction would be a novel target for antiretroviral drugs. It therefore is urgent to reexamine the exact linkage between the tRNA primers and the 5′ ends of retroviral minus strand DNAs. It is very intriguing to note the recent finding that in a yeast mutant defective in the debranching enzyme required for intron turnover, Ty1 transposition frequency is significantly reduced (6), suggesting that a 2′,5′-phosphodiester linkage may be formed during DNA synthesis. In addition, retroviral RTs are able to bind to specific tRNAs used for cDNA priming (7, 8, 36), indicating that the minus strand DNA-priming reaction is highly specific, as in the case of the msDNA primer reaction. In addition, the 3′-terminal A residue of the primer tRNA has been shown to be released from the template-primer complex by RNase A (7), suggesting that the A residue may be placed in a nonconventional manner on the template RNA.

Recently, Lease and Yee (30) proposed an alternate model for msDNA synthesis in which a single-stranded DNA corresponding to the DNA portion of msDNA is first synthesized in a conventional manner (by a 3′-to-5′ priming reaction). This DNA strand is then ligated to the 2′-OH group of the branched rG residue of msdRNA at its 5′ end, forming a 2′,5′-phosphodiester linkage. This model, however, is highly unlikely on the basis of the facts described above. It is difficult to comprehend how several hundred copies of Okazaki-like DNA fragments are produced from a specific chromosomal region. Furthermore, the model fails to explain why RT is required for msDNA synthesis. When retron-Ec67 was placed under the *GAL10* promoter, msDNA-Ec67 was found to be synthesized in *Saccharomyces cerevisiae* (unpublished results). Thus, msDNA-Ec67 production was again exclusively dependent upon RT-Ec67.

ORIGIN OF RETRONS

Comparisons of amino acid sequences deduced from the DNA sequences of seven bacterial RTs reveal extensive differences in sizes (316 to 586 amino acid residues) and domain structures (18, 21). When the RT domains of approximately 230 residues of these polymerases are compared, one can again find extensive sequence diversity among them. Except for the high similarity (73%) between RT-Mx162 and RT-Sa163 (RTs required for the synthesis of msDNA-Mx162 of *M. xanthus* and msDNA-Sa163 of *S. aurantiaca*, respectively; unpublished results), identities between all other bacterial RTs are less than 50%. For example, the identity between RT-Mx162 and RT-Mx65 from the same bacterium is only 35%, and the identities of RT-Ec73 to all the other known bacterial RTs are 25 to 30%.

In considering the origin of retrons, it is important to point out that there are two distinctly different distributions of retrons in bacteria: in myxobacteria, retron-Mx162 of *M. xanthus* and retron-Sa163 of *S. aurantiaca* are very similar and ubiquitous, while in *E. coli*, four highly diverse retrons have been found in a minor population of wild *E. coli* strains (13% of natural isolates; 18, 21). Therefore, in contrast to *E. coli* retrons, which are considered to have been horizontally transmitted as discussed in the next section, retron-Mx162 and retron-Sa163 are likely to be derived from a common progenitor retron, which was most likely acquired before the two myxobacterial species diverged. From a phylogenetic analysis of myxobacteria with 16S rRNA, the maximum age of the myxobacteria is estimated to be approximately 10^9 years (see reference 38 for a review). Although the establishment of the subgroups for *Myxococcus* and *Stigmatella* spp. is thought to have occurred much later, these subgroups and another major subgroup of myxobacteria, *Nannocystis exedens*, are believed to have diverged approximately 9×10^8 years ago (34). Recently, it was found that *N. exedens* also contains msDNA (25a). If the *N. exedens* retron is related to retron-Mx162, these retrons are likely to be older than any known eukaryotic retroelements.

It is important to note that the determination of evolutionary distances between prokaryotic and eukaryotic RTs, and probably even between the eukaryotic enzymes themselves, is problematic. Evolutionary distances between two homologous proteins are estimated on the basis of amino acid substitution caused by spontaneous mutations during evolution. However, if mutation rates are different depending upon the sources of the proteins, it is not easy to make the estimation. Retron-Mx162 and retron-Sa163 are a part of the chromosome which is duplicated by DNA polymerase with high fidelity (10^{-10} to 10^{-11} mutation rate per base replication; 11). In contrast, retroviral RT genes are duplicated by highly error-prone RTs (10^{-4} per base; 37). Therefore, the error rates in retroviral retroelements are much higher (by 10^6 to 10^7 times) than those in elements which are duplicated by DNA polymerase. A phylogenetic tree which has been constructed for 82 different RTs from both prokaryotes and eukaryotes by Xiong and Eickbush (46) thus probably does not accurately reflect evolutionary relationships of these RTs. These authors propose that non-LTR retrotransposons are the oldest group of retroelements and are the ancestors of bacterial RTs. However, it is equally tempting to speculate that myxobacterial RTs and retrons are the oldest and that they acquired other retroelement structures, such as *gag* and *int*, during evolution, resulting in non-LTR retrotransposons.

WHY ARE *E. COLI* RETRONS SO DIVERSE?

In contrast to myxobacterial retrons, *E. coli* retrons are likely to have been acquired in the *E. coli* genome more recently, after the *E. coli* lineages were established. This notion is based on the following facts: (i) only a minor fraction of *E. coli* wild strains contain retrons; (ii) four highly diverged retrons have been found; (iii) these retrons are integrated into different sites on the *E. coli* chromosome; and (iv) codon usages in the RT genes are substantially different from the average codon usage in the *E. coli* genome. Before these retrons were integrated into the *E. coli* genome, they were probably replicated like retroviruses and retrotransposons in a highly error-prone system, using RTs for their reproduction. It is a very challenging task to identify this highly error-prone source(s) of *E. coli* retrons. Retrons appear to be widely distributed in prokaryotes; they are also found in *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Rhizobium trifolii* (25a). Characterization of these retrons may provide a clue to identify the lineages of retrons. It is also an interesting question whether retrons exist in any eukaryotic organisms.

IS THE RETRON A MOBILE ELEMENT?

Retron-Ec73 consists of a 2.7-kb element which is part of a prophage related to bacteriophage P4 (39). The genome of this prophage is 12.7 kb in size and is integrated into the *selC* gene (for selenocysteyl-tRNA), which maps at 82 min on the *E. coli* chromosome. Surprisingly, the prophage can be excised from the *E. coli* chromosome upon infection with helper phage P2 and can produce an infectious P4-like virion (22). This virion, called retronphage φR73, is able to integrate into the *selC* gene of a new host cell, thus enabling the newly formed lysogen to produce msDNA-Ec73. Retronphage φR73 is likely to be duplicated as a double-stranded DNA phage, like phage P4, under normal circumstances. However, an intriguing possibility is that the retronphage may also be able to duplicate through a retroviruslike cDNA cycle catalyzed by RT encoded by the phage genome. In this case, the 3′ end of selenocysteyl-tRNA or the 3′ end of msDNA could be used as a primer. Another similarity with retroviruses is that retronphage φR73 has its own integrase which recognizes a specific 29-bp sequence including the 3′-end CCA sequence of the selenocysteyl-tRNA gene.

Retron-Ec67 is approximately 2.5 kb in length. It is a part of a 34-kb foreign DNA fragment integrated at 19 min on the *E. coli* chromosome and flanked by 26-bp direct repeats (15). The partial sequence of the 34-kb fragment indicates that it is also a prophage closely related to phage 186. Retron-Ec86 also maps at 19 min, and it also appears to be associated with a similar prophage (31). Therefore, all three retrons described above somehow became integrated into the phage genomes and are probably able to be transferred from one cell to another by using bacteriophages as vectors.

A clue may be provided by another *E. coli* retron, retron-Ec107 (14). This retron is the shortest of the four *E. coli* retrons, consisting of a 1.3-kb fragment. The most interesting aspect of retron-Ec107 is that it is inserted into the *E. coli* genome by replacing a 34-bp intergenic sequence between the *pyrE* and *ttk* genes, located at 82 min on the *E. coli* chromosome. This retron was found in isolates representing three divergent branches in the well-established *E. coli* phylogenetic tree, suggesting that the retron was integrated into the *E. coli* genome probably as (part of) a mobile element. Retron-Ec107 is the retron most frequently found in wild strains of *E. coli*. But how could it exist in three independent branches in the phylogenetic tree and have integrated into the *E. coli* genome in the identical manner? The integrated foreign fragment contains only the retron containing the *msr-msd* region and the RT gene but does not contain a gene for integrase. It remains to be tested, therefore, whether retron-Ec107 is mobile.

POSSIBLE FUNCTIONS OF RETRONS

Another challenging question is the function of retrons. Bacterial RTs might have played an important role in diversification of bacterial genomes via cDNA production, as speculated for the eukaryotic genomes (45). However, what is the function of msDNA? *E. coli* cells are certainly able to live without msDNA. It has also been shown that both retron-Mx162 and retron-Mx65 can be deleted without any effects on vegetative growth and fruiting-body formation for *M. xanthus*, under the laboratory conditions tested (19). However, all 20 natural strains of *M. xanthus* independently isolated from various places in the world contain msDNA-Mx162 without exception (26a). These cells contain approximately 500 copies of msDNA per cell, which corresponds to approximately 1% of the total cellular DNA. Thus, it is tempting to speculate that msDNA may still play a role under certain environmental conditions. For example, it may serve as a primer for cDNA synthesis using the 3′ end of msDNA, or it may form a complex with proteins to exert a function required under a certain growth condition.

Because of its stability and high copy number in the cell, msDNA may provide a unique vector to produce artificial single-stranded RNA and DNA, which can be utilized for antisense RNA or DNA. When retron-Ec67 was placed under the yeast *GAL10* promoter, msDNA-Ec67 was indeed produced in yeast cells (unpublished results), suggesting that retrons could be used as vectors in the eukaryotic cell. At present msDNA is a mysterious DNA, but it will certainly be challenging and exciting to attempt to elucidate the functions of msDNA and retrons, the precise mechanism of msDNA biosynthesis, and the origin of RT.

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REFERENCES

1. **Baltimore, D.** 1970. Viral RNA-dependent DNA polymerase. Nature (London) **226:**1209–1211.
2. **Barat, C., S. F. J. LeGrice, and J.-L. Darlix.** 1991. Interaction of HIV-1 reverse transcriptase with a synthetic form of its replication primer, $tRNA^{Lys3}$. Nucleic Acids Res. **19:**751–757.
3. **Barat, C., V. Lullien, O. Schatz, G. Keith, M. T. Nugeyre, F. Gruninger-Leitch, F. Barre-Sinoussi, S. F. J. LeGrice, and J. L. Darlix.** 1989. HIV-1 reverse transcriptase specifically interacts with the anticodon domain of its cognate primer tRNA. EMBO J. **8:**3279–3285.
4. **Beljanski, M., and M. Beljanski.** 1974. RNA-bound reverse transcriptase in *Escherichia coli* and *in vitro* synthesis of a complementary DNA. Biochem. Genet. **12:**163–180.
5. **Boeke, J. D., and V. G. Cories.** 1989. Transcription and reverse transcription of retrotransposons. Annu. Rev. Microbiol. **43:** 403–434.
6. **Chapman, K. B., and J. D. Boeke.** 1991. Isolation and characterization of the gene encoding yeast debranching enzyme. Cell **65:**483–492.
7. **Colicelli, J., and S. P. Goff.** 1987. Isolation of a recombinant murine leukemia virus utilizing a new primer tRNA. J. Virol. **57:**37–45.
8. **Cordell, B., S. R. Weiss, H. E. Varmus, and J. M. Bishop.** 1978. At least 104 nucleotides are transposed from the 5′ terminus of the avian sarcoma virus genome to the 5′ termini of smaller viral mRNAs. Cell **15:**79–91.
9. **Dhundale, A., M. Inouye, and S. Inouye.** 1988. A new species of multicopy single-stranded DNA from *Myxococcus xanthus* with conserved structural features. J. Biol. Chem. **263:**9055–9058.
10. **Dhundale, A., B. Lampson, T. Furuichi, M. Inouye, and S. Inouye.** 1987. Structure of msDNA from *Myxococcus xanthus*: evidence for a long, self-annealing RNA precursor for the covalently linked, branched RNA. Cell **51:**1105–1112.
11. **Drake, J. W.** 1969. Comparative rates of spontaneous mutation. Nature (London) **221:**1132.
12. **Faras, A. J., J. M. Taylor, W. E. Levinson, H. M. Goodman, and J. M. Bishop.** 1973. RNA-directed DNA polymerase of Rous sarcoma virus: initiation of synthesis with 70S viral RNA as template. J. Mol. Biol. **79:**163–183.
13. **Furuichi, T., S. Inouye, and M. Inouye.** 1987. Biosynthesis and structure of stable branched RNA covalently linked to the 5′ end of multicopy single-stranded DNA of *Stigmatella aurantiaca*. Cell **48:**55–62.
14. **Herzer, P. J., S. Inouye, and M. Inouye.** Retron-Ec107 is inserted into the *Escherichia coli* genome by replacing a palin-

dromic 34-bp intergenic sequence. Mol. Microbiol., in press.
15. **Hsu, M.-Y., M. Inouye, and S. Inouye.** 1990. Retron for the 67-base branched RNA-linked multicopy DNA from *Escherichia coli*: a potential transposable element encoding both reverse transcriptase and Dam methylase functions. Proc. Natl. Acad. Sci. USA **87:**9454–9458.
16. **Hsu, M.-Y., S. Inouye, and M. Inouye.** 1989. Structural requirements of the RNA precursor for the biosynthesis of the branched RNA-linked msDNA of *Myxococcus xanthus*. J. Biol. Chem. **264:**6214–6219.
17. **Inouye, M., and S. Inouye.** 1991. Retroelements in bacteria. Trends Biochem. Sci. **16:**18–21.
18. **Inouye, M., and S. Inouye.** 1991. msDNA and bacterial reverse transcriptase. Annu. Rev. Microbiol. **45:**163–186.
19. **Inouye, S., P. J. Herzer, and M. Inouye.** 1990. Two independent retrons with highly diverse reverse transcriptase in *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA **87:**942–945.
20. **Inouye, S., M.-Y. Hsu, S. Eagle, and M. Inouye.** 1989. Reverse transcriptase associated with the biosynthesis of the branched RNA-linked msDNA in *Myxococcus xanthus*. Cell **56:**709–717.
21. **Inouye, S., and M. Inouye.** Bacterial reverse transcriptase. *In* S. Goff and A. Skalka (ed.), Reverse transcriptase, in press. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Inouye, S., M. G. Sunshine, E. W. Six, and M. Inouye.** 1991. Retronphage ϕR73: an *E. coli* phage that contains a retroelement and integrates into a tRNA gene. Science **252:**969–971.
23. **Joyce, G. F.** 1989. RNA evolution and the origins of life. Nature (London) **338:**217–224.
24. **Kawaguchi, T., P. J. Herzer, M. Inouye, and S. Inouye.** Sequence diversity of the 1.3 kb retron (retron-Ec107) among three distinct phylogenetic groups of *Escherichia coli*. Mol. Microbiol., in press.
25. **Kohiyama, M., M. Nakayama, and K. B. Mahrez.** 1986. DNA polymerase and primase-reverse transcriptase from holobacterium. Syst. Appl. Microbiol. **7:**79–82.
25a. **Lampson, B.** Personal communication.
26. **Lampson, B. C., M. Inouye, and S. Inouye.** 1989. Reverse transcriptase with concomitant ribonuclease H activity in a cell-free system of msDNA of *Myxococcus xanthus*. Cell **56:** 701–707.
26a. **Lampson, B. C., M. Inouye, and S. Inouye.** 1991. Survey of multicopy single-stranded DNAs and reverse transcriptase genes among natural isolates of *Myxococcus xanthus*. J. Bacteriol. **173:**5363–5370.
27. **Lampson, B. C., S. Inouye, and M. Inouye.** 1991. msDNA of bacteria. Prog. Nucleic Acid Res. Mol. Biol. **40:**1–24.
28. **Lampson, B. C., J. Sun, M.-Y. Hsu, J. Vallejo-Ramirez, S. Inouye, and M. Inouye.** 1989. Reverse transcriptase in a clinical strain of *E. coli*: its requirement for the production of branched RNA-linked msDNA. Science **243:**1033–1038.
29. **Lampson, B. C., M. Viswanathan, M. Inouye, and S. Inouye.** 1990. Reverse transcriptase from *Escherichia coli* exists as a complex with msDNA and is able to synthesize double-stranded DNA. J. Biol. Chem. **265:**8490–8496.
30. **Lease, R. A., and T. Yee.** 1991. Early events in the synthesis of the multicopy single-stranded DNA-RNA branched copolymer of *Myxococcus xanthus*. J. Biol. Chem. **266:**14497–14503.
31. **Lim, D.** 1991. Structure of two retrons of *Escherichia coli* and their common insertion site. Mol. Microbiol. **5:**1863–1872.
32. **Lim, D., and W. K. Maas.** 1989. Reverse transcriptase in bacteria. Mol. Microbiol. **3:**1141–1144.
33. **Lim, D., and W. K. Maas.** 1989. Reverse transcriptase-dependent synthesis of a covalently linked, branched DNA-RNA compound in *E. coli* B. Cell **56:**891–904.
34. **Ludwig, W., K. H. Schleifer, H. Reichenbach, and E. Stackebrandt.** 1983. A phylogenetic analysis of the myxobacteria *Myxococcus fulvus*, *Stigmatella aurantiaca*, *Cytobacter fuscus*, *Sorangium cellulosum* and *Nannocystis exedens*. Arch. Microbiol. **135:**58–62.
35. **Modak, M. J., S. L. Marcus, and L. F. Cavalieri.** 1973. DNA complementary to rabbit globin mRNA made by *E. coli* polymerase I. Biochem. Biophys. Res. Commun. **55:**1–7.
36. **Peters, G., and J. E. Dahlberg.** 1979. RNA-directed DNA synthesis in Moloney murine leukemia virus: interaction between the primer tRNA and the genome RNA. J. Virol. **31:**398–407.
37. **Preston, B. D., B. J. Poiesz, and L. A. Loeb.** 1988. Fidelity of HIV-1 reverse transcriptase. Science **242:**1168–1171.
38. **Shimkets, L. J.** 1990. Social and developmental biology of the myxobacteria. Microbiol. Rev. **54:**473–501.
39. **Sun, J., M. Inouye, and S. Inouye.** 1991. Association of a retroelement with a P4-like cryptic prophage (retronphage ϕR73) integrated into the selenocysteyl tRNA gene of *Escherichia coli*. J. Bacteriol. **173:**4171–4181.
40. **Temin, H. M.** 1989. Retrons in bacteria. Nature (London) **339:**254–255.
41. **Temin, H. M., and S. Mizutani.** 1970. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature (London) **226:**1121–1213.
42. **Varmus, H. E.** 1989. Reverse transcriptase in bacteria. Cell **56:**721–724.
43. **Vorob'eva, N. V., L. T. Nebrat, V. A. Potapov, A. G. Romashchenko, R. I. Salganik, and L. F. Yushkova.** 1983. Reverse transcription of heterologous RNA with the help of RNA-dependent DNA polymerase of *Escherichia coli*. Mol. Biol. (Moscow) **17:**770–775.
44. **Waldrop, M. M.** 1989. Did life really start out in an RNA world? Science **246:**1248–1249.
45. **Weiner, A. M., P. L. Deininger, and A. Efstratiadis.** 1986. Nonviral retroposons: genes, pseudogenes, and transposable elements generated by the reverse flow of genetic information. Annu. Rev. Biochem. **55:**631–661.
46. **Xiong, Y., and T. H. Eickbush.** 1990. Origin and evolution of retroelements based upon their reverse transcriptase sequences. EMBO J. **9:**3353–3362.