Reverse Transcriptase from Escherichia coli Exists as a Complex with msDNA and Is Able to Synthesize Double-stranded DNA*

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Reverse transcriptase required for the synthesis of msDNA Ec67 in an Escherichia coli strain was purified as a large molecular weight complex with msDNA. The complex sedimented in a glycerol gradient at an s value greater than 19. The predominant protein species co-purifying with reverse transcriptase activity in the complex had a molecular weight estimated at 65,000 which is close to the expected size of 67,227 for the Ec67 reverse transcriptase. In addition, the large complex also contained msDNA-Ec67. The purified complex was able to synthesize cDNA using 5 S rRNA as a template (annealed to a synthetic DNA primer), and a double-stranded DNA using a synthetic DNA template (annealed to a synthetic DNA primer). When msDNA-Ec67 was used as a natural template, the purified complex produced two major products: a 103-base single-stranded DNA by extending the 3' end of msDNA using msdRNA as a template, and a 60-base double-stranded DNA product resulting from the reverse reaction in which the 3' end of msdRNA is extended using msDNA as a template. The results suggest that bacterial reverse transcriptase is capable of producing single-stranded cDNA and possibly double-stranded DNA as well. Possible implications of these findings on the biology of the msDNA-retron system are discussed.

A reverse transcriptase (RT),1 resembling the polymerases found in retroviruses (Baltimore, 1970; Temin and Mizutani, 1970), was recently discovered in the soil bacterium Myxococcus xanthus (Inouye et al., 1989), in a clinical isolate of Escherichia coli, strain CI-1 (Lampson et al., 1989b), and in E. coli B (Lim and Maas, 1989). These RTs are responsible for the production of an unusual, extrachromosomal RNA-DNA molecule known as msDNA. The msDNA-synthesizing systems of bacteria appear to represent a primitive form of retroelement, and thus the term “retron” has been proposed for this system (see review by Temin, 1989).

msDNA was originally discovered in M. xanthus (Yee et al., 1984), and this molecule (msDNA-Mx162) consists of a 77-base, single-stranded RNA which is linked to the 5' end of a 162-base, single-stranded DNA. Branching out from the 2' position of an internal guanosine residue, a unique 2',5'-phosphodiester bond joins the RNA strand to the 5' end of the DNA strand (Furiuchi et al., 1987; Dhundale et al., 1987). Similar msDNA molecules have been found in other myxobacteria (Dhundale et al., 1985) as well as a number of E. coli strains (Sun et al., 1989). Although these various msDNAs share little, if any, primary nucleotide sequences, they all share conserved secondary structural features. These include (a) a branched, internal G residue which forms the 2', 5' linkage with the 5' end of the DNA strand; (b) secondary stem-loop structures in both the RNA and DNA chains; and (c) a short DNA-RNA hybrid structure at the 3' ends of the DNA and RNA strands.

The ability of the host cell to produce msDNA requires a single-copy chromosomal locus which contains a gene mrs which codes for the RNA strand (msdRNA) and a gene msd which encodes the DNA strand of the msDNA molecule. The mrs and msd genes are situated in opposite orientation on the chromosome such that their respective 3' ends overlap by 6 to 11 bases. Immediately upstream of msd (downstream of mrs) is an open reading frame (ORF) encoding the RT. All known loci which code for the production of msDNAs have a similar gene organization (Lim and Maas, 1989). The ORF encodes a 495-amino acid RT for msDNA-Mx162 from M. xanthus (Inouye et al., 1989), a 586-amino acid RT for msDNA-Ec67 (Lampson et al., 1989b), and a 320-amino acid RT for msDNA-Ec86 of E. coli B (Lim and Maas, 1989). Although differing in size and sequence, all ORFs show significant similarities between their amino acid sequences and sequences found in retroviral RTs including the highly conserved polymerase consensu sequence YXDD. Based on sequence comparisons, the RT from the clinical E. coli strain appears to have an RNase H domain at the C-terminal end of the ORF with the polymerase domain at the N-terminal region. This is similar to the arrangement of functional domains in retroviral RTs (Johnson et al., 1986). The RTs from E. coli B and M. xanthus do not appear to have an RNase H domain. The requirement of an ORF encoding a protein similar to retroviral RTs to produce msDNA and the unusual gene organization of the region encoding msDNA supports a model in which the msDNA molecule is synthesized from an RNA precursor by reverse transcription (Lampson et al., 1989a).

This paper describes the purification of the RT from the clinical E. coli strain CI-1, designated Ec67-RT, which co-purifies with its product, msDNA-Ec67, in a large molecular weight complex. The purified Ec67-RT is also shown to

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§ The abbreviations used are: RT, reverse transcriptase; ORF, open reading frame; AMV, avian myeloblastosis virus; PIPES, 1,4-piperazinediethanesulfonic acid.

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EXPERIMENTAL PROCEDURES

Bacterial Strains and Other Materials—E. coli strain C2110 (his rha polA1) was obtained from M. Roth and S. Goff (Columbia University). Restriction enzyme HindII was purchased from New England Biolabs. Nuclease S1 was from Bethesda Research Laboratories Life Technologies, Inc. Avian myeloblastosis virus (AMV) reverse transcriptase was from Boehringer Mannheim. [α-32P]dCTP (3000 Ci/mmol) was from Amersham. Protein molecular weight standards (myoglobin, adenosine deaminase, and thyroglobulin) were from Sigma.

Expression of Ec67. RT—From 10 liters of a stationary phase culture of strain C2110 harboring plasmid pCl-1EP5b, cells were harvested, washed in 50 mM Tris (pH 8.0), and resuspended in lysozyme buffer (50 mM Tris (pH 7.5), 10% sucrose, 0.3 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride). Fresh lysozyme was added to a final concentration of 2 mg/ml. The suspension was incubated on ice for 15 min followed by a quick freeze at -70 °C, then thaw on ice. Lysis was enhanced by the addition of 2 volumes of buffer M (50 mM Tris (pH 7.0), 1 mM EDTA, 1 mM dithiothreitol, 0.2% Nonidet P-40, 10% glycerol, and 25 mM NaCl) followed by incubation on ice, then a quick freeze-thaw. A cleared lysate was obtained by centrifugation at 38,000 × g (SW 50.1 rotor) for 30 min. The cleared lysate was fractionated by ammonium sulfate precipitation (0-60%, 60-70%, and 70-90%), followed by dialysis overnight (4 °C) for each fraction against buffer M. Ammonium sulfate fractions, 50-70% and 70-90%, showed RT activity and were pooled, then applied to a DEAE-column (2.5 × 50 cm, DE52 Whatman) equilibrated with buffer M. The DE52 column was washed, and RT activity eluted from the column at a range of 300 to 350 mM NaCl. The DE52 fractions showing RT activity were pooled, concentrated by membrane ultrafiltration (Amicon) and then loaded onto a Sephacryl S-300 column (Pharmacia LKB Biotechnology Inc., 1.5 × 75 cm) equilibrated with buffer M. The column was developed with the same buffer, and the elution profile is shown in Fig. 2. Agains, fractions from the S-300 column having RT activity were pooled and concentrated, and 0.7 ml was loaded onto a 16-30% glycerol density gradient. The glycerol gradient were set up and run as described previously (Viswanathan et al., 1989). The purified Ec67. RT (fractions 7, 8, and 9) was stored as separate glycerol fractions at -20 °C.

Template-Primer for the RT Assay—The 5.8 S rRNA template-primer was prepared by mixing 50 pmol of E. coli 5.8 S RNA (Boehringer Mannheim) with 120 pmol of a synthetic 15-base, oligo(DNA) (5′-ATGCTTGGCAGTTC-3′) complementary to the 3′ end of 5.8 S RNA. The mixture was dried, then resuspended in 30 μl of a formamide solution (80% formamide, 20 mM PIPES (pH 6.5), 0.4 mM NaCl). The mixture was then heated at 90 °C for 10 min, transferred to 37 °C for 2 to 5 h, followed by room temperature for 30 min. The annealed template-primer was then precipitated with ether and lyophilized. The synthetic 50-base oligo(DNA) template (5′-CGTAAAACCTCCTAACCTGGTGCACCTCCGTG- GGCAACACCCGTGAAAA-3′) was annealed to complementary, 20-base oligo(DNA) primer (5′-TTTCCCGCTGTGCTCCAA-3′) in a similar manner. A total RNA prepared from 1.2 ml of an overnight culture of E. coli C2110/pCl-1EP5b was used for a reaction in which msDNA served as a template-primer. RNA was prepared by the hot phenol method according to Maniatis et al. (1982).

Reverse Transcriptase Assay Reaction—50 pmol of template-primer (5′-CGTAAAACCTCCTAACCTGGTGCACCTCCGTG- GGCAACACCCGTGAAAA-3′) or oligo(DNA) was added to a 50-μl reaction mixture containing: 50 mM Tris-HCl (pH 7.8), 10 mM dithiothreitol, 60 mM NaCl, 0.05% Nonidet P-40, 10 mM MgCl2, 5.0 μM concentration each of dATP, dTTP, and dCTP, and 0.3 μM [α-32P]dGTP. For msDNA, E. coli, about 40 μg of total RNA from strain C2110/pCl-1EP5b was added to the reaction. The reaction is started by adding 15 to 20 μl (about 1/10 of the volume of the glycerol gradient fraction containing the purified Ec67. RT to the above mixture (or 10 units of AMV-RT). The reaction mixture was incubated at 37 °C for 45 min, followed by phenol/chloroform extraction and ethanol precipitation. The newly synthesized DNAs were analyzed by electrophoresis on 6% acrylamide, 8 M urea sequencing gels followed by autoradiography.

RESULTS

Expression of the Ec67. RT in E. coli K12—The Ec67. RT gene was cloned on a 5-kilobase pair, PstI (a)-EcoRI fragment from the chromosome of the clinical E. coli strain CI-1. This restriction fragment contains the genes mer, mod, and the closely linked Ec67. RT ORF and supplies all the information needed to produce msDNA when expressed in a pUC9 vector in an E. coli K12 strain (Lampson et al., 1989b). No msDNA molecules are produced in K12 with the vector plasmid alone, and there are no chromosomal sequences which cross-hybridize with either msDNA-Ec67 or the Ec67. RT gene (Sun et al., 1989). The Ec67. RT was purified by detecting RT activity using the msDNA molecule as a natural, intramolecular template-primer (Lampson et al., 1989b; Lim and Maas, 1989; see below). For this purpose, the PstI(a)-EcoRI fragment from the clinical E. coli Cl-1 chromosome was cloned and expressed in a polA-, K12 strain, C2110 (Tanese et al., 1986). Since DNA polymerase I can utilize RNA templates to synthesize DNA (Loeb et al., 1973; Modak et al., 1973), a polA strain was used to eliminate background activity in the RT assays. Because a polA strain is used, the PstI(a)-EcoRI fragment was cloned into the pSC101-derived vector, pGDB, to give the recombinant plasmid pCl-1EP5b, which does not require host cell DNA polymerase I for replication (Churchward et al., 1984; Linder et al., 1985). Fig. 1A presents a map of the pCl-1EP5b plasmid. Expression of the Ec67. RT ORF is predicted to occur from its native promoter located upstream from the msr gene (Lampson et al., 1989b) and is confirmed by the ability of strain C2110 harboring pCl-1EP5b (C2110/pCl-1EP5b) to produce msDNA (Fig. 1B, lane 1).

Purification of the Ec67. RT and Detection of Activity—Crude extracts were prepared from a 10-liter culture of C2110/pCl-1EP5b grown to stationary phase. Extracts were prepared by detergent lysis, after lysozyme treatment, with the nonionic detergent Nonidet P-40. Crude extracts were then assayed for RT activity by detecting the ability of RT to synthesize new DNA using the msDNA-Ec67 molecule as a natural template-primer (see Fig. 6). When crude extract containing the bacterial Ec67. RT is added to a reaction mixture containing the msDNA-Ec67 molecule as a substrate, a ribonuclease A (RNase A)-resistant product of about 103 bases in size is produced as a result of reverse transcription (Fig. 1C, lane 1, arrow). However, when extract crude from strain C2110 harboring only the vector plasmid pGB2 is added to a similar reaction mixture, no DNA products are detected, indicating an absence of RT activity (Fig. 1C, lane 2). The purification steps for the Ec67. RT are described in detail under “Experimental Procedures.”

Purified Ec67. RT Exists As a Large Complex with msDNA—Following elution from a DE 50 column, fractions displaying RT activity were pooled, concentrated, and then applied to a Sephacryl S-300 gel filtration column. Fig. 2 shows the elution profile of Ec67. RT activity and its relative molecular size. RT activity (inset, Fig. 2) appears in fraction numbers 18 to 21 with peak activity in fractions 19 and 20. These two fractions (19 and 20) elute just prior to elution of the largest protein molecular weight standard, apoferritin, at 443,000 (fractions 22 and 23). The Ec67. RT appears to elute from the column as a very large complex of roughly 600,000 to 700,000 molecular weight.

The Ec67. RT was further purified by sedimentation in a glycerol gradient. Pooled S-300 fractions having RT activity were concentrated by membrane ultrafiltration, then applied to a Sephacryl S-300 gel filtration column. Fig. 2 shows the elution profile of Ec67. RT activity and its relative molecular size. RT activity (inset, Fig. 2) appears in fraction numbers 18 to 21 with peak activity in fractions 19 and 20. These two fractions (19 and 20) elute just prior to elution of the largest protein molecular weight standard, apoferritin, at 443,000 (fractions 22 and 23). The Ec67. RT appears to elute from the column as a very large complex of roughly 600,000 to 700,000 molecular weight.
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activity in fractions 8 and 9. This band migrates at a relative molecular weight of about 65,000 (arrow, Fig. 3B) which is in good agreement with the predicted molecular weight of 67,227 for the Ec67-RT (Lampson et al., 1989b). In addition, when each glycerol fraction was examined for DNA content by electrophoresis of ethanol precipitates on a 5% acrylamide gel followed by staining with ethidium bromide, msDNA-Ec67 served as a primer for the polymerase. The annealed template-primer was added to a reaction mixture containing dNTPs and [α-32P]dCTP, and msDNA-Ec67 was added as template-primer (see Figs. 4 and 6); lane 2, crude extract from C2110 containing just the vector plasmid pGB2; lane S, pBR322 digested with MspI as molecular weight markers. Part C shows the detection of RT activity in crude cell extracts. Extracts were assayed for RT activity by adding a small aliquot of extract (equivalent to 50 μg of protein) to a reaction mixture containing dNTPs, [α-32P]dCTP, and msDNA-Ec67 as template-primer. After incubation at 37 °C, the reaction mixture was precipitated with ethanol and treated with RNase A, and the labeled DNA products formed were examined on a 4% polyacrylamide-8 urea gel followed by autoradiography (inset). Based on the elution profile of RT activity relative to the protein standards (arrow, plot), the molecular weight was roughly estimated at about 600,000 to 700,000.

Synthesis of cDNA from RNA or DNA Templates—The ability of purified Ec67-RT complex to synthesize DNA from various templates composed of random sequences was examined using three different template-primer systems. E. coli 5 S RNA was annealed to a synthetic 15-base oligo(DNA) (15-mer) complementary to the 3’ end of E. coli 5 S RNA which served as a primer for the polymerase. The annealed template-primer was added to a reaction buffer (pH 7.8) containing dNTPs and [α-32P]dCTP. An aliquot from the glycerol gradient fraction (fraction 8) containing the purified Ec67-RT was added to the reaction mixture and incubated at 37 °C for 45 min. The products were treated with RNase A before analysis by gel electrophoresis. Complete extension of DNA synthesis from the 3’ end of the primer, using 5 S RNA as a template, should give a DNA product of 120 nucleotides. A predominant band migrates at about 120 bases which is resistant to treatment with RNase A. A band of similar size is also produced when avian myeloblastosis virus reverse transcriptase (AMV-RT) is substituted for the bacterial enzyme in the reaction mixture (arrow, Fig. 4, lane 4). Although there are several intermediate size products formed, the bacterial enzyme, like the retroviral polymerase, can synthesize a full length cDNA of 120 nucleotides with the 5 S RNA as a template with a 15-mer DNA as a primer. The Ec67-RT can also polymerize DNA using DNA as a template. In this reaction, a 50-base, synthetic DNA was annealed to a synthetic 20-mer DNA primer complementary to its 3’ end. This oligo(DNA) template-primer was allowed to react with the Ec67-RT, and the resulting products formed are shown in Fig. 4, lane 2. A small band appears at the basis of optical density at 260 nm and the calculated protein content.

The Ec67-RT can also polymerize DNA using DNA as a template. In this reaction, a 50-base, synthetic DNA was annealed to a synthetic 20-mer DNA primer complementary to its 3’ end. This oligo(DNA) template-primer was allowed to react with the Ec67-RT, and the resulting products formed are shown in Fig. 4, lane 2. A small band appears at the
The ability of the Ec67 RT to synthesize a full length cDNA from the 3' end of the primer would be expected to incorporate the DNA strand and the 3' end of the RNA strand (see Fig. 4). This hybrid region serves as a natural, intramolecular primer which is efficiently used by AMV-RT with the RNA strand serving as a template (Lampson et al., 1989b; Lim and Maas, 1989). Like AMV-RT, when purified Ec67 RT is added to a reaction mixture containing msDNA, Ec67 molecule as a substrate, an RNase A-resistant product of 103 bases is produced (arrow, Fig. 4, lane 3). An additional RNase A-resistant band is also apparent at a size of about 60 bases (arrow, Fig. 4). This 60-base DNA product does not appear to be produced when AMV-RT is used in the reaction (Fig. 4, lane 6).

As presented in Fig. 6, the Ec67 RT is able to utilize the 3' end of the DNA strand of the msDNA molecule to initiate polymerization of DNA. A new strand of DNA is synthesized by extending the 3' end of msDNA using the RNA strand (msdRNA) as a template. The newly polymerized DNA continues to extend along the RNA template until it stops at the branched rG residue (Fig. 6, reaction A). This reaction produces a single-stranded DNA of 103 bases which is resistant to RNase A treatment. Indeed, the band that appears at 103 bases in size (Fig. 7, lane 1) is sensitive to digestion by S1 nuclease confirming that it is a single-stranded DNA (Fig. 7, lane 2) and is the product of reaction A (Fig. 6). Sensitivity to S1 nuclease without prior treatment with RNase A, also suggests that msdRNA, serving as the template, is removed from the 3' end of the primer.
FIG. 5. The nucleotide sequence and the location of HinfI restriction sites of msDNA-Ec67. The complete nucleotide sequence of the DNA strand and the RNA strand was determined previously (Lampson et al., 1989b). Two predicted HinfI restriction sites exist in the msDNA-Ec67 molecule and are shown in the boxed regions. Arrows denote the point of cleavage by the endonuclease. Cleavage occurs only if the 60-base double-stranded DNA product is formed by the purified Ec67-RT (see reaction B, Fig. 6).

msDNA-Ec67

as the 3' end of msDNA is extended by the Ec67-RT (Fig. 6, reaction A). Removal of the RNA template could be due to RNase H activity associated with the Ec67-RT as predicted from the amino acid sequence (Lampson et al., 1989b).

Interestingly, the 60-base DNA product is completely resistant to S1 nuclease treatment indicating that it is a double-stranded DNA (Fig. 7, lane 2). In contrast to the synthesis of the 103-base DNA which is the result of RNA-dependent polymerization, the formation of the 60-base product is a DNA-dependent polymerization as depicted in Fig. 6, reaction B. Here, the Ec67-RT initiates synthesis of DNA using the 3' end of the msdRNA chain as a primer and extending the new DNA (dashed line, Fig. 6) by using the msDNA strand as a template. This yields a double-stranded DNA which migrates under the denaturing conditions of a sequencing gel as a 32P-labeled, 60-base band (Fig. 7, lane 1). To confirm that the newly formed 60-base DNA product is a double-stranded DNA, the reaction products were treated with the restriction endonuclease HinfI. The restriction enzyme should cleave the double-stranded product at the two HinfI sites on msDNA (Fig. 5). As a result, three fragments of 36, 22, and 2 bases are expected to be produced. The 2-base fragment is predicted to retain a sequence of RNA at its 5' end. As shown in Fig. 6, lane 3, two labeled bands are clearly detected at 36 and 22 bases as expected. The 2-base band is not labeled with [α-32P]dCTP and is thus not detected. It should be noted that the restriction enzyme did not cleave the 103-base, single-stranded DNA product. The results demonstrate that the 60-base product is the result of DNA-dependent, DNA polymerization as shown in reaction B (Fig. 6).

Other msDNA Template:Primers—As demonstrated in the
...dNTPs, [32P]dCTP, and msDNA-Ec67 (total RNA preparation from E. coli C2110/pCl-EP5b) as the template:primer. The reaction mixture was incubated at 37 °C for 45 min, and the resulting DNA products were precipitated with ethanol. Aliquots from the reaction were treated as described for each lane followed by electrophoresis in a 6% acrylamide, 8 M urea sequencing gel. Lane s, pBR322 digested withMspI as molecular weight markers; lane 1, reaction products treated with RNase A followed by S1 nuclease; and lane 3, the reaction products digested with Hinfl followed by treatment with RNase A. The DNA product migrating at 103 bases (lane 1, arrow) represents single-stranded DNA and is produced by reaction A illustrated in Fig. 6. The DNA product migrating at 80 bases (lane 2) is an S1-resistant, double-stranded DNA which can be cleaved by Hinfl to give the fragments of 36 and 22 bases (lane 3, arrows). The double-stranded DNA product is produced by reaction B as shown in Fig. 6.

The experiments described above, the purified Ec67-RT is capable of using its own retron-encoded msDNA molecule as a template:primer substrate to synthesize new DNA in vitro. To examine if the Ec67-RT can utilize other, heterologous msDNAs as a template:primer, msDNA molecules encoded by other bacteria were isolated. RNA preparations containing either msDNA-Ec86 (from E. coli B) or msDNA-Mx162 (from M. xanthus) were added to the standard reaction mixture described containing the purified Ec67-RT. Ec67-RT produces a DNA extension product of 143 bases (Fig. 8, lane 1), identical in size with one produced with AMV-RT, when msDNA-Ec86 is used as a template:primer (lane 3). This is the size expected for a DNA product, synthesized by reaction A depicted in Fig. 8, using msDNA-Ec86 as a substrate. Similarly, an RNase A-resistant product of the expected size of 211 bases is produced by the Ec67-RT (lane 2) and AMV-RT (lane 4) when msDNA-Mx162 is used as a template:primer. It should be noted that an extra DNA band is observed at 103 bases in all the reactions containing the Ec67-RT (lanes 1 and 2). This band appears to be identical with the product observed when msDNA-Ec67 (the endogenously produced msDNA) is added as the template:primer as previously shown in Figs. 4 and 7. This product can be observed, even without the addition of an exogenous template:primer (see "Discussion").

**DISCUSSION**

The msDNA-RT Complex—The Ec67-RT is encoded by a retron element found on the chromosome of a clinical E. coli strain, Cl-1, and is required to synthesize the msDNA-Ec67 molecule (Lampson et al., 1989b). Purification of the bacterial RT revealed a predominant protein species of about 65,000 molecular weight which co-purifies with RT activity and is associated with msDNA-Ec67 molecules. It is noted that although RT activity clearly co-purifies with the 65,000 molecular weight protein and msDNA, the level of RT activity, as measured by the gel assay used, does not strictly correlate with the quantity of protein and msDNA observed. This may be explained by an observed decrease in RT activity when the RT (M, = 65,000 protein) is complexed with msDNA since preliminary experiments indicate that the Ec67-RT purified free of associated msDNA has higher specific RT activity. The results, however, strongly suggest that the retron-encoded Ec67-RT exists as a large molecular weight complex with its endogenous msDNA which sediments as a particle, greater than 19 S, in glycerol density gradients. The molar ratio of RT molecules to an msDNA molecule in the complex is estimated to be about 4 to 6. It is not clear at present how this complex, with an apparent molecular weight of 700,000, is formed. It is interesting to note that msDNA (msDNA-Mx162) from M. xanthus also appears to exist as a complex with proteins (Viswanathan et al., 1989). The fact that the Ec67-RT is isolated as a complex with msDNA also implies that synthesis of msDNA may involve a large complex composed of RT and the long precursor mRNA which serves as template and primer.

RNA-dependent and DNA-dependent DNA Polymerization—As demonstrated from experiments presented in this
paper, the Ec67-RT resembles retroviral RTs in its ability to utilize different templates and primers in vitro. A purified preparation of the Ec67-RT is able to synthesize a cDNA by extending the 3' end of a synthetic DNA primer annealed to the 3' end of 5 S RNA serving as a template. The Ec67-RT also synthesizes DNA using a DNA template annealed to a DNA primer. In addition to using a DNA primer, the Ec67-RT can also utilize RNA as a primer. The Ec67-RT can synthesize DNA by extending the 3' end of msdRNA using the msDNA strand as a template when the msDNA-Ec67 molecule is used as a natural template:primer (reaction B, Figs. 6 and 7). Conversely, when the Ec67-RT utilizes the msDNA molecule as an template:primer, it can also synthesize single-stranded DNA, by extending the 3' end of the msDNA strand using msdRNA as a template (reaction A, Figs. 6 and 7). In this case, the polymerization reaction may accompany RNase H activity similar to the RNase H activity associated with retroviral RT (Gerard, 1981; Moelling et al., 1971; Ratray and Champoux, 1989). When Ec67-RT extends the 3' end of the msDNA strand to produce a 103-base single-stranded DNA product (Fig. 7), it appears that the bacterial polymerase may remove the msdRNA template since the final 103-base DNA product is sensitive to S1 nuclease, a nuclease which exclusively digests single-stranded substrates (Vogt, 1973). An analysis of the amino acid sequence of the Ec67-RT revealed a region at the C-terminal end of the protein which shows a similarity to the RNase H domains of retroviral RTs (Lampson et al., 1989b). Further work is in progress to confirm this finding and establish if RNase H activity is associated with the Ec67-RT and is required to remove the msdRNA template during synthesis of DNA.

Possible Functions of msDNA—msDNA is proposed to be synthesized by reverse transcriptase from a long, primary mRNA transcript of the retron-encoded locus (Dhundale et al., 1987; Lampson et al., 1989a). The primary transcript initiates upstream of the ms gene encoding msdRNA and extends beyond the msd gene encoding the msDNA chain. Due to the location of two inverted repeat sequences, the primary transcript folds into a stable secondary structure which serves as both a primer and template for the synthesis of the msDNA chain. The Ec67-RT is believed to initiate synthesis of the DNA strand from the 2'-position of the rG residue of msdRNA forming the branch linkage, then extending the DNA chain along the folded mRNA serving as a template. Synthesis of the msDNA-Ec67 strand terminates at 67 bases, leaving a 58-base, unused template RNA strand remaining with the molecule. It is not clear how this precise termination of msDNA synthesis occurs in vivo. However, it is clear from experiments presented here and elsewhere (Lim and Maas, 1989) that when the msDNA-Ec67 molecule is isolated from the host cell (free of protein) and then used as a substrate, the Ec67-RT is able to further extend the DNA strand in vitro until it reaches the branched rG residue (reaction A, Fig. 6). These results clearly indicate that a factor(s) inhibit further extension of msDNA in vivo. msDNA-Ec67 appears to exist in a complex with its endogenous RT. Indeed, when the purified Ec67-RT is added to a standard reaction mixture to assay activity, a small amount of single-stranded DNA is synthesized even though no exogenous template has been added. These DNA products (Fig. 8) are similar in size to the 103-base single-stranded DNA product observed in Fig. 7 and is presumably due to the extension of the 3' end of msDNA still complexed with the purified Ec67-RT (reaction A, Fig. 6). Termination signals which normally stop synthesis of msDNA-Ec67 at the 67th base may be a combination of secondary structures in the template RNA and complexed protein(s) which are lost or altered during purification.

The ability of the Ec67-RT to synthesize cDNA from both RNA and DNA templates suggests other functions for the Ec67-RT in the cell. For example, msDNA could serve as a primer to produce cDNA from an mRNA if the mRNA contains a sequence complementary to the 3' end of msDNA. The retron-encoded RT could then convert the cDNA to double-stranded DNA. The ability of Ec67-RT to synthesize double-stranded DNA could promote the retron's ability to transfer and integrate its own element or other genes into a new location on the chromosome. The possibility that retrons were transmitted into the E. coli genome as movable elements or by some other means is supported by an analysis of the codon usage employed by the Ec67-RT gene which suggests that this gene is not native to E. coli and was probably acquired late in the evolution of this species (Inouye et al., 1989).

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REFERENCES

Baltimore, D. (1970) Nature 226, 1200-1211
Churchward, G., Belin, D., and Nagamine, Y. (1984) Gene (Amst.) 31, 165-171
Dhundale, A., Furuichi, T., Inouye, S., and Inouye, M. (1985) J. Bacteriol. 164, 914-917
Dhundale, A., Lampson, B., Furuichi, T., Inouye, M., and Inouye, S. (1986) Cell 41, 1105-1112
Furuichi, T., Dhundale, A., Inouye, M., and Inouye, S. (1987) Cell 48, 47-53
Gerard, G. F. (1981) Biochemistry 20, 256-265
Huber, P. W., and Wool, I. G. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 322-326
Inouye, S., Hau, M.-Y., Eagle, S., and Inouye, M. (1989) Cell 56, 709-717
Johnson, M. S., McClure, M. A., Feng, D.-F., Gray, J., and Doolittle, R. F. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 7648-7652
Lampson, B. C., Inouye, M., and Inouye, S. (1989a) Cell 56, 701-707
Lampson, B. C., Sun, J., Hsu, M.-Y., Vallejo-Ramirez, J., Inouye, S., and Inouye, M. (1989b) Science 243, 1033-1038
Lim, D., and Maas, W. K. (1989) Cell 56, 891-904
Linder, P., Churchward, G., Guixian, X., Yi Yi, Y., and Caro, L. (1985) J. Mol. Biol. 181, 383-393
Loeb, L. A., Tartof, K. D., and Travaglini, F. C. (1973) Nature New Biol. 242, 66-69
Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: a Laboratory Manual Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
Modak, M. J., Marcus, S. L., and Cavalleri, L. F. (1973) Biochem. Biophys. Res. Commun. 55, 1-7
Moelling, K., Bolognesi, D. P., Bauer, H., Busen, W., Plassmann, H. W., and Hansen, F. (1971) Nature 234, 240-244
Ratray, A. J., and Champoux, J. J. (1989) J. Mol. Biol. 208, 445-456
Sun, J., Herzer, P. J., Weinstein, M. P., Lampson, B. C., Inouye, M., and Inouye, S. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 7208-7212
Tanese, N., Sodroski, J., Haseltine, W. A., and Goff, S. P. (1986) J. Virol. 59, 743-745
Temin, H. M. (1989) Nature 339, 254-255
Temin, H. M., and Mizutani, S. (1970) Nature 226, 1211-1213
Viswanathan, M., Inouye, M., and Inouye, S. (1989) J. Biol. Chem. 264, 13663-13671
Vogt, V. M. (1973) Eur. J. Biochem. 33, 192-195
Yee, T., Furuichi, T., Inouye, S., and Inouye, M. (1984) Cell 38, 203-209
Reverse transcriptase from Escherichia coli exists as a complex with msDNA and is able to synthesize double-stranded DNA.

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J. Biol. Chem. 1990, 265:8490-8496.

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