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8. Six- to eight-week-old C57BL/6 mice (five per group) were injected subcutaneously and intraperitoneally with 0.5-ml doses containing 50 μg of the peptide-carrier conjugate emulsified (1:1) in complete Freund's adjuvant (CFA). Each group received booster injections 4 weeks later with antigen in incomplete Freund's adjuvant. Seven days later, blood was withdrawn, and the blood samples were pooled and clotted at 4°C overnight. The sera were stored at −80°C until tested. Rabbits were immunized subcutaneously and intramuscularly with 1.0-ml doses containing 2.5 mg of the region II-KLH conjugate emulsified 1:1 in CFA. Animals received booster injections of the homologous antigen in CFA 8 weeks later. For the final booster injection, unconjugated region II [2.5 mg in 0.5 ml of phosphate-buffered saline (PBS)] was incubated with 5 μl of 25 percent glutaraldehyde for 1 hour at room temperature. Rabbits received booster injections of this cross-linked region II in incomplete Freund's adjuvant at week 11. Blood was withdrawn 1 week later, and sera were stored at −80°C. Antibody to the peptides was detected by ELISA (enzyme-linked immunosorbent assay) (*6*, *22*). The screening antigen was homologous peptide conjugated to BSA. Rabbit antisera were also tested by ELISA as described (*6*, *22*) except that goat antibody to rabbit IgG conjugated to horseradish peroxidase (Bio-Rad) was substituted for the goat antiserum to mouse IgG.
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23. For IFA, salivary gland sporozoites were suspended in Medium 199 containing 2.5 percent BSA at a concentration of 2×10^4 to 5×10^4 per milliliter. Portions (10 μl) were spread onto each well of a multiwell printed IFA slide, air-dried at room temperature, and stored at −80°C. Portions (10 μl) of sera diluted 1:100 in PBS were spread on each spot of a thawed IFA slide and incubated for 20 minutes at room temperature. Sera were then aspirated and the slides washed with two drops of PBS. Portions (20 μl) of goat antiserum to mouse IgG conjugated to fluorescein isothiocyanate (Kirkegaard and Perry, Gaithersburg, Md.) diluted 1:40 in blocking buffer (1.0 percent BSA, 0.5 percent casein, 0.005 percent thimerosal, and 0.0005 percent phenol red in PBS) plus 0.4 percent ethidium bromide was then added to each spot. After a second 20-minute incubation the spots were washed with two drops of PBS, mounted in glycerol, and examined under ultraviolet light at 500× magnification for fluorescence.
24. Circumsporozoite protein from *P. falciparum* sporozoites isolated from the salivary glands of *Anopheles freeborni* mosquitoes was extracted as described (*6*). Protein from 10^5 sporozoites in sodium dodecyl sulfate (SDS) sample buffer containing 2 percent mercaptoethanol was separated by SDS–polyacrylamide gel electrophoresis according to the method of Laemmli [*Nature (London)* **227**, 680 (1970)] with an 8 to 12 percent gradient gel. Western blot analysis was performed according to a modification of the method of H. Towbin *et al.* [*Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979)]. The air-dried filter was cut into 4-mm strips and reacted with antisera to repeat peptides (8 and 16 mer), regions I, II, or a pool of five monoclonal antibodies (2E6.4, 2F1.1, 4D9.1, 4D11.6, and 5G5.3) diluted 1:1000 with PBS containing 0.05 percent Tween-20 (PBS-Tw 20). Filter-bound mouse antibody was incubated with ^{125}I-labeled sheep antiserum prepared against whole mouse antibody (2×10^5 cpm/ml in PBS-Tw 20). Rabbit antibody to region II was incubated with ^{125}I-labeled staphylococcal protein A (diluted 3×10^5 cpm/ml in PBS-Tw 20). Antibody was detected by autoradiography with a 48-hour exposure.

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A Catalytic RNA and Its Gene from *Salmonella typhimurium*

Abstract. *The gene for the RNA subunit (M1 RNA) of ribonuclease P from* Salmonella typhimurium *directs the synthesis of an RNA that can cleave transfer RNA precursor molecules. The mature M1 RNA coded for by* Salmonella typhimurium *is 375 nucleotides long and has six nucleotide changes in comparison to M1 RNA from* Escherichia coli. *The regions for promotion and termination of transcription are closely conserved, but adjacent regions of nucleotide sequences show considerable drift.*

RNA molecules that catalyze the cleavage or formation (or both) of covalent bonds have been identified in extracts of *Escherichia coli* (*1*, *2*), *Bacillus subtilis* (*1*), and *Tetrahymena thermophila* (*3*). Ribonuclease P, an enzyme essential for the processing of the 5′ termini of transfer RNA (tRNA) molecules (*4*), is composed of two subunits, an RNA and a protein; the RNA subunit (M1 RNA) is responsible for catalysis. The gene coding for M1 RNA in *E. coli* has been characterized (*5*). We isolated and characterized the corresponding gene and its transcript from *Salmonella typhimurium* to determine (i) whether the catalytic capabilities of the RNA subunit of ribonuclease P are conserved and reflected in extensive primary sequence homology or common higher order structure of the RNA and (ii) whether the regulatory regions adjacent to the gene in *E. coli* are utilized in a closely related organism.

Table 1. Comparison of some single-copy oligonucleotides in fingerprints of M1 RNA from *Salmonella typhimurium* and *Escherichia coli*. The oligonucleotide designations refer to the fingerprints shown in Fig. 1, C (*E. coli*, EC) and D (*S. typhimurium*, ST). Oligonucleotide EC 8 is grouped for comparative purposes with ST P and ST 8 because they are at the same location in their respective M1 RNA sequences. Similarly, EC 35 is grouped with ST 35X. EC 26 is grouped with ST Q because they are similar in composition and chromatographic mobility. Composition of the *E. coli* nucleotides was determined previously (*5*). Composition of the *S. typhimurium* oligonucleotides was determined as described in the text and was checked against the DNA sequence. U, uracil; other abbreviations for bases are as given in the text.

Oligonucleotide	Composition
EC 8	$UUUCACCU_{OH}$
ST P	$UUUCACUU_{OH}$
ST 8	$UUUCACU_{OH}$
EC 35	UCCUCUUCG
ST 35X	UCCUUUCG
EC 26	AACCCG + CAACAG
ST Q	CCCACG

The gene for M1 RNA was isolated by probing a phage library (*6*) containing a digest of the *S. typhimurium* LT2 genome with isotopically labeled M1 RNA from *E. coli* (*5*). We had previously determined that a single DNA fragment in an Eco RI digest of genomic *S. typhimurium* DNA hybridized with the probe even under high stringency conditions. This fragment, about 8.5 kilobases (kb) in length, was isolated from the phage library and cloned into the Eco RI site of pBR329 by standard techniques to make the plasmid pSa17.

We first determined whether pSa17 directed the synthesis of a gene transcript in *E. coli* similar in size to that of M1 RNA from *E. coli*. Cells harboring plasmids pSa17, pRR1 (carrying the gene for M1 RNA from *E. coli*), and pBR329 (carrying a wheat-storage protein gene 12-15) were treated with ^{32}P-labeled phosphate. The RNA was extracted with phenol and analyzed by polyacrylamide gel electrophoresis and autoradiography (Fig. 1A). A transcript with about the same mobility as M1 RNA from *E. coli* (lanes 1 and 2) was seen for pSa17. No

corresponding M1 RNA was seen for pBR329 (lane 3). In addition to the band corresponding to mature M1 RNA, another fainter band was visible in the original autoradiograph in lanes 1 and 2. We have previously shown for *E. coli* that this band represents the precursor to M1 RNA and is the primary gene transcript (*5*). We assume that the corresponding band in lane 1 represents the precursor to *S. typhimurium* M1 RNA, but we have not characterized this RNA any further. This assumption is strengthened, however, by the nucleotide sequence of the M1 RNA gene from *S. typhimurium*, which indicates that the signals for initiation and termination of transcription are similar to those in *E. coli*.

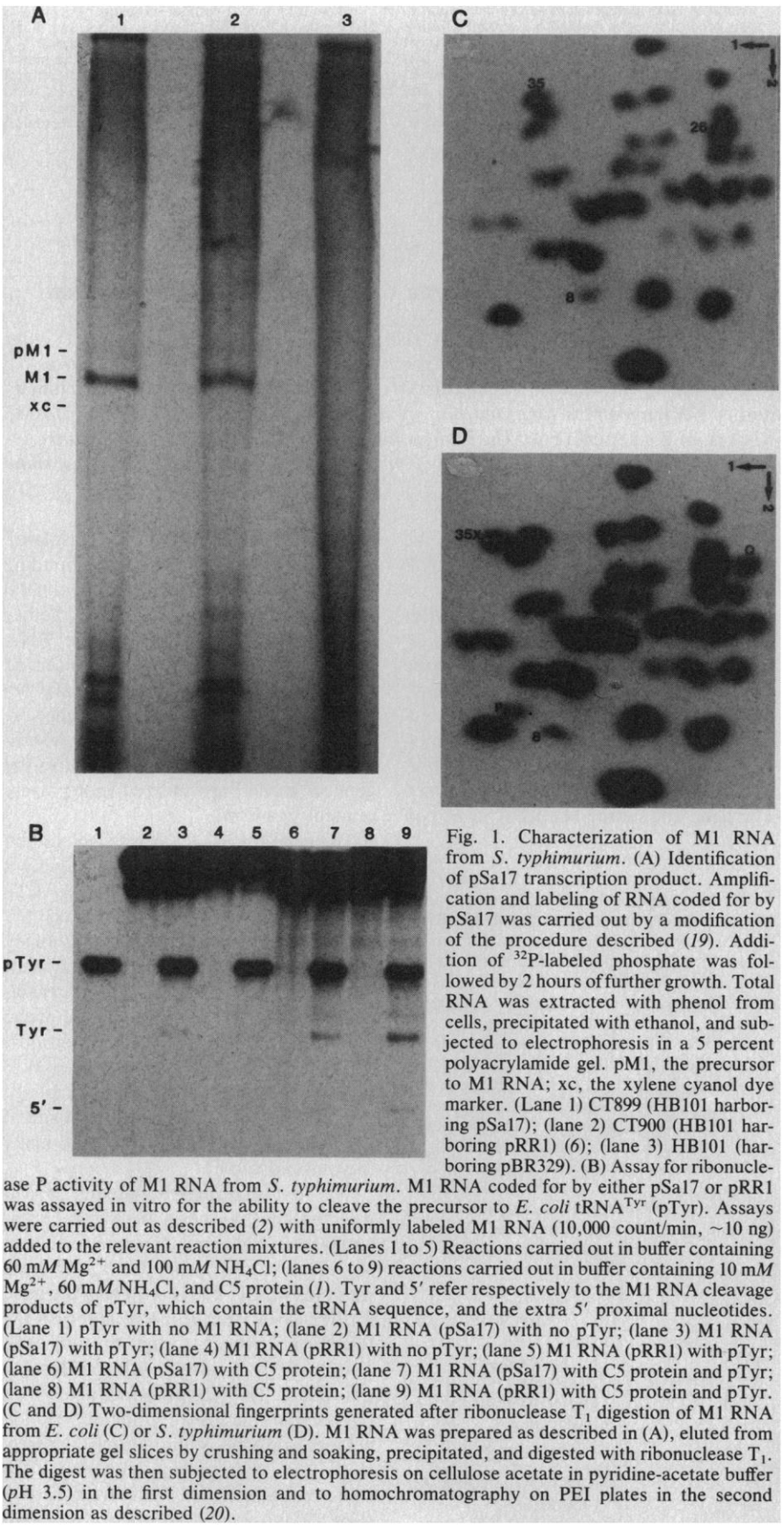

Fig. 1. Characterization of M1 RNA from *S. typhimurium*. (A) Identification of pSa17 transcription product. Amplification and labeling of RNA coded for by pSa17 was carried out by a modification of the procedure described (*19*). Addition of ^{32}P-labeled phosphate was followed by 2 hours of further growth. Total RNA was extracted with phenol from cells, precipitated with ethanol, and subjected to electrophoresis in a 5 percent polyacrylamide gel. pM1, the precursor to M1 RNA; xc, the xylene cyanol dye marker. (Lane 1) CT899 (HB101 harboring pSa17); (lane 2) CT900 (HB101 harboring pRR1) (*6*); (lane 3) HB101 (harboring pBR329). (B) Assay for ribonuclease P activity of M1 RNA from *S. typhimurium*. M1 RNA coded for by either pSa17 or pRR1 was assayed in vitro for the ability to cleave the precursor to *E. coli* $tRNA^{Tyr}$ (pTyr). Assays were carried out as described (*2*) with uniformly labeled M1 RNA (10,000 count/min, ~10 ng) added to the relevant reaction mixtures. (Lanes 1 to 5) Reactions carried out in buffer containing 60 m*M* Mg^{2+} and 100 m*M* NH_4Cl; (lanes 6 to 9) reactions carried out in buffer containing 10 m*M* Mg^{2+}, 60 m*M* NH_4Cl, and C5 protein (*1*). Tyr and 5′ refer respectively to the M1 RNA cleavage products of pTyr, which contain the tRNA sequence, and the extra 5′ proximal nucleotides. (Lane 1) pTyr with no M1 RNA; (lane 2) M1 RNA (pSa17) with no pTyr; (lane 3) M1 RNA (pSa17) with pTyr; (lane 4) M1 RNA (pRR1) with no pTyr; (lane 5) M1 RNA (pRR1) with pTyr; (lane 6) M1 RNA (pSa17) with C5 protein; (lane 7) M1 RNA (pSa17) with C5 protein and pTyr; (lane 8) M1 RNA (pRR1) with C5 protein; (lane 9) M1 RNA (pRR1) with C5 protein and pTyr. (C and D) Two-dimensional fingerprints generated after ribonuclease T_1 digestion of M1 RNA from *E. coli* (C) or *S. typhimurium* (D). M1 RNA was prepared as described in (A), eluted from appropriate gel slices by crushing and soaking, precipitated, and digested with ribonuclease T_1. The digest was then subjected to electrophoresis on cellulose acetate in pyridine-acetate buffer (*p*H 3.5) in the first dimension and to homochromatography on PEI plates in the second dimension as described (*20*).

To test for ribonuclease P function in vitro, we eluted both M1 RNA species identified in Fig. 1 from gel slices and assayed them with the precursor to *E. coli* $tRNA^{Tyr}$ as substrate. The assays were performed (i) in buffers containing 60 m*M* Mg^{2+} (ionic conditions that allow *E. coli* M1 RNA to carry out the cleavage reaction without added protein cofactor) and (ii) in buffers containing 10 m*M* Mg^{2+} and the *E. coli* protein cofactor (C5 protein, which is essential for M1 RNA function under these ionic conditions) (*1*). Under both sets of conditions, M1 RNA from *S. typhimurium* showed specific activity similar to that of the corresponding M1 RNA from *E. coli* (Fig. 1B). We conclude, therefore, that this RNA represents a ribonuclease P activity in *S. typhimurium* and that it can form a functional ribonuclease P nucleoprotein complex with the protein cofactor from *E. coli*.

In addition to the tests for function in vitro, pSa17 was used to transform A49 (*7*), a mutant of *E. coli* with a temperature-sensitive ribonuclease P phenotype that can be complemented by excess wild-type M1 RNA. We found that pSa17 complements A49 under restrictive conditions as well as does pRR1 (*8*).

For rigorous identification of the M1 RNA species eluted from the gel slices, both the isotopically labeled *S. typhimurium* and *E. coli* RNA's were digested with ribonuclease T_1, and the resulting oligonucleotides were analyzed by two-dimensional chromatography (fingerprinting) (Fig. 1, C and D). In the fingerprint of *S. typhimurium* M1 RNA (Fig. 1D), there is a change in the mobility of oligonucleotide 8, there are three new oligonucleotides (P, Q, and 35X), and oligonucleotide 35 is missing compared to the fingerprint of M1 RNA from *E. coli* (Fig. 1C) (*5*). In completing the identification of the M1 RNA from *S. typhimurium*, a DNA restriction fragment (a product of Sal I digestion) approximately 3 kb in length containing the gene for the *S. typhimurium* M1 RNA was subcloned from pSa17, and the nucleotide sequence of the gene and its surrounding region was determined as described (*9*). The sequence was compared to the data

from the ribonuclease T_1 fingerprint of the *S. typhimurium* M1 RNA and to the results of the secondary analysis of the oligonucleotides [(*10*) and Table 1]. The position and composition of the variant *S. typhimurium* oligonucleotides, as well as the loss in the *S. typhimurium* M1 RNA fingerprint of *E. coli* M1 RNA oligonucleotide 35, could be explained by an examination of the DNA sequence derived from *S. typhimurium*.

Transcription of the M1 RNA gene apparently initiates at the same position, seven nucleotides from the end of the promoter (*5*), in both organisms. This placement of transcription initiation is consistent with the observation of pppGp (G, guanine) in digests of M1 RNA (*11*) and with the molar yields for AAG and CCG (A, adenine; C, cytosine) in the mature *S. typhimurium* M1 RNA molecule. Analysis of the oligonucleotide containing the 3′ terminus of both the *E. coli* and *S. typhimurium* M1 RNA's placed the 3′ terminal nucleotide of the *S. typhimurium* M1 RNA 375 nucleotides from its 5′ end.

A total of two deletions and four transitions was found in the sequence of *S. typhimurium* M1 RNA compared with its counterpart from *E. coli* (Fig. 2). Thus the primary sequences of the two genes coding for the mature M1 RNA molecules are highly conserved. Also, the primary promoter sequence, the region between the primary promoter and the first nucleotide of the M1 gene transcript, the -35 sequence, and the transcription terminator stem and loop sequences are all conserved between the two organisms. The stem of the terminator is 3 base pairs (bp) shorter in *S. typhimurium* than in *E. coli*. In the small region between the end of the mature M1 RNA sequence and the beginning of the transcription terminator, there is a comparatively large number of differences between the *S. typhimurium* and the *E. coli* DNA sequences. Several base changes are also present in the region between the -35 sequence and the promoter and also in the space between the end of the terminator and an ATG (T, thymine), which marks the beginning of an open reading frame [nucleotide 424 (*12*)] similar to the one beyond the terminator of M1 RNA in *E. coli* (*13*) (Fig. 2). A possible Shine-Dalgarno sequence also appears in the *S. typhimurium* M1 DNA sequence beyond the terminator and before the beginning of the open reading frame, but unlike the sequence in *E. coli*, the *S. typhimurium* sequence contains only three contiguous purines, the minimum number required for ribosome binding (*14*). The sequence of the first 20 amino acids in the protein beyond the terminator has only one change compared to the protein in *E. coli*. Beyond

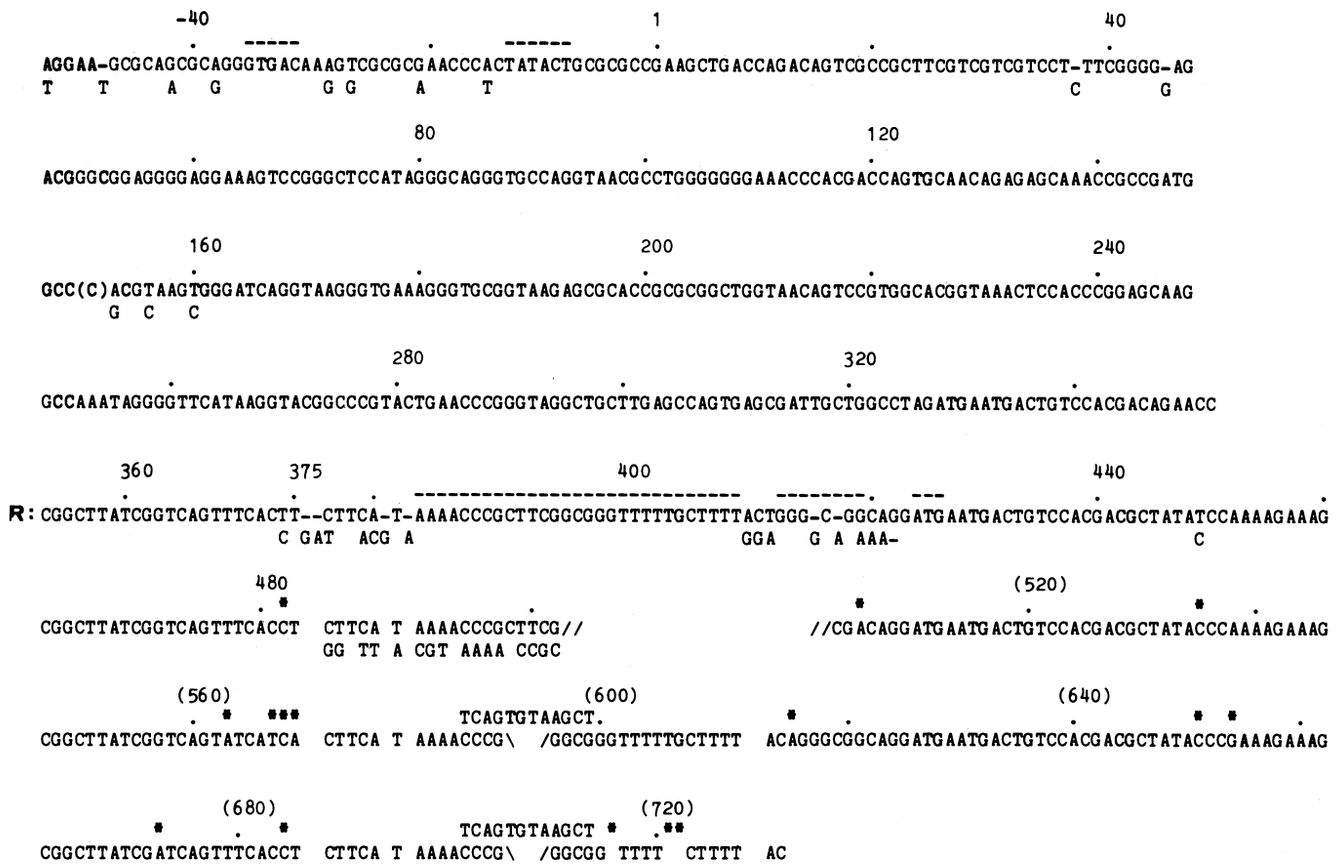

Fig. 2. Nucleotide sequence of the *S. typhimurium* gene for M1 RNA and its flanking regions. The sequence of the antisense strand is shown. The mature M1 RNA sequence starts at nucleotide 1 and ends at nucleotide 375. The -35 sequence, promoter, terminator of transcription (nucleotides 382 to 410), possible ribosome-binding site (nucleotides 414 to 419), and adjacent initiator codon of an open reading frame are all indicated by lines above the sequence. Differences in the sequence compared to the corresponding *E. coli* gene are shown below the line of the *S. typhimurium* sequence up to nucleotide 502. A dash indicates that no corresponding base is present in one or the other sequence. One ambiguity is indicated by parentheses at nucleotide 152. The bottom four lines of the sequence starting at the R next to nucleotide 353 are arranged to show the matching, repeated nature of the sequence downstream. Base changes in the repeats downstream compared to the first repeat are indicated by asterisks and by the insertions (nucleotides 584 to 595 and 700 to 711) above the sequence. A gap of about 130 to 150 nucleotides (as determined by restriction mapping) exists in the sequence analysis starting at nucleotide 502. Beyond this point, the sequence is numbered as if there were no interruption, but the numbers are in parentheses. Because the sequences can be matched on either side of the gap, the repeated sequence must occur at least 4.5 times. The drift in the repeats (number of nucleotides changed or deleted with respect to the first repeat) is of the same order as that found in *E. coli* except for the small insertions in repeats 4 and 5.

this point the sequences diverge. The high degree of conservation of the nucleotide sequence of mature M1 RNA is consistent with the observation that another set of stable RNA's, tRNA's, also has almost perfect sequence conservation between *S. typhimurium* and *E. coli* (*15*). However, there is considerable drift in the *trp* operon messenger RNA (although it is mostly in the third position of degenerate codons) and in nearby spacer sequences when this operon is compared in the two organisms (*16*).

The M1 RNA gene in *E. coli* contains 3.5 repeats of a 113-bp sequence (*13*). The repeated DNA begins with the last 23 bp of the mature M1 RNA coding region. The *S. typhimurium* DNA also contains repeated sequences. The first repeat begins at nucleotide 353, 23 bp from the 3′ end of the mature M1 coding sequence (Fig. 2), and ends at nucleotide 460 just before the beginning of the second repeat 108 bp downstream. Analysis of regions downstream from the *S. typhimurium* M1 gene revealed the presence of at least three more repeats of this sequence (Fig. 2).

Our results provide further evidence that the catalytic subunit of the enzyme ribonuclease P is an RNA molecule. The catalytic RNA subunit and the protein cofactor of ribonuclease P from *E. coli* and *S. typhimurium* are able to form a functional, heterologous complex, as are the subunits from *E. coli* and *B. subtilis* (*1*). In comparing the gene structures for M1 RNA from *E. coli* and *S. typhimurium*, we found conservation of the transcriptional start and stop signals but no common primary sequence adjacent to the 3′ terminal site, where processing is needed for the formation of mature M1 RNA from its precursor molecule. Nevertheless, M1 RNA synthesis directed by pSa17 in an *E. coli* host is efficient. Therefore, some aspect of M1 RNA secondary or tertiary structure (or both) must be important for processing of the M1 RNA precursor molecule, and this signal must be recognizable by the appropriate enzymes from both *E. coli* and *S. typhimurium*.

Of the six differences between the mature M1 RNA's from *E. coli* and *S. typhimurium*, two are clustered near position 40 and three near position 160. Recent data indicate that the structural features of M1 RNA (*17*) near the differences may be important for M1 RNA function, but further comparative sequence information is needed before more can be said about the relation between sequence and function. The conserved organization of the genome in the flanking regions of M1 RNA indicates that the repeated sequences play some role in expression of the genome in both *E. coli* and *S. typhimurium*.

Note added in proof: There may exist conformations of *E. coli* M1 RNA other than the one that is most consistent with the data from digestion with nucleases (*17*). In one such additional conformation, four of the six differences in the nucleotide sequence of M1 RNA from *S. typhimurium* can be explained as compensatory changes that preserve secondary structure. For example, deletions at positions 37 and 45 remove a GC base pair in a hairpin structure in which C-20 pairs with G-59 at the base of the stem. Furthermore, the changes A-153 and T(U)-160 preserve a base pair in a hairpin structure in which G-148 pairs with C-166 at the base of the stem. Cimino *et al.* (*18*) have recently found evidence in studies of the intramolecular cross-linking of *E. coli* M1 RNA that a region of the molecule from about nucleotides 140 to 170 can be in a structure different from that proposed (*17*).

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11. We observed the tetraphosphate, pppGp, in ribonuclease T_2 digests of both M1 RNA's after chromatography on PEI plates in 0.75*M* phosphate buffer (*p*H 3.5).
12. A complete open reading frame begins with ATG in the third repeat at nucleotide 510 and ends with TGA in the fourth repeat at nucleotide 627. This result, together with the data from *E. coli*, suggests that the first open reading frame must end in the gap in the nucleotide sequence and that the second one must end with the TGA at nucleotide 511.
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The Stability of DNA in Human Cerebellar Neurons

Abstract. *Human tissues have carbon-isotope ratios ($^{13}C/^{12}C$) that reflect dietary ratios. This observation has been used to determine the extent of metabolic turnover of DNA in cells of the adult human cerebellum (90 percent of which are neuronal). If adult human neuronal DNA were metabolically stable, its $^{13}C/^{12}C$ would reflect that in the maternal diet during fetal development as nearly all neurons are formed during maturation of the fetal brain and do not undergo cell division thereafter. The $^{13}C/^{12}C$ ratios in the food chains and body tissues of Europeans differ from corresponding American ratios by about 50 parts per million on the average. Therefore, turnover was studied by comparing $^{13}C/^{12}C$ ratios in cerebellar DNA of American-born Americans, European-born Americans, and European-born Europeans. The $^{13}C/^{12}C$ ratios in cerebellar DNA from European-born Americans were closer to $^{13}C/^{12}C$ ratios in cerebellar DNA from European-born Europeans than from American-born Americans, indicating that there was little or no turnover of neuronal DNA.*

Stable carbon-isotope ratios, $^{13}C/^{12}C$, are slightly but consistently greater in North Americans than in Europeans. The difference is attributed to the relative proportions of two photosynthetic pathways in the food chains of these populations (*1–4*). Human migration from Europe to North America provides a basis for a natural stable carbon-isotope tracer experiment that can detect turnover of long-lived body constituents such as neuronal DNA (*5*) without exposure to radioactive tracers.

Tritium-labeled mouse brain DNA has an average biological half-life of 1 to 4 years (*6*, *7*). A substantial proportion of mammalian whole brain DNA is in glial and endothelial cells which undergo mi-