RNase E is an important regulatory enzyme that governs the principal pathway for mRNA degradation in *Escherichia coli*. This endonuclease controls its own synthesis via a feedback mechanism in which the longevity of *rne* (RNase E) mRNA is modulated by a cis-acting sensory element that responds to changes in cellular RNase E activity. Previous research has shown that this element is an RNA stem-loop (hp2) within the 5′-untranslated region of the *rne* transcript. Here we report studies involving mutational analysis and phylogenetic comparison that have identified the features of *rne* hp2 important for its function. These comprise an internal loop flanked on one side by a 2-bp stem and a hairpin loop and on the other side by a longer stem whose sequence is inconsequential. A search of bacterial genome sequences suggests that regulation by an hp2-like element may be a unique evolutionary adaptation of the *rne* transcript that is not shared by other mRNAs.

Messenger RNA degradation is an important mechanism for controlling gene expression in all organisms. The rate of mRNA decay directly affects the steady-state concentration of mRNA, thereby influencing rates of protein synthesis. mRNA lifetimes can differ markedly within a single cell. In *Escherichia coli*, for example, mRNA half-lives can be as short as a fraction of a minute or as long as an hour, with a typical half-life being 2–4 min (1). The stability of a given message need not be fixed and may vary in response to growth conditions (2–5).

In *E. coli*, mRNA decay generally involves the sequential action of endonucleases and 3′-exonucleases (1). For most *E. coli* mRNAs, degradation begins with internal cleavage by RNase E (6–11). This endonuclease, 1061 amino acids in length, possesses broad cleavage-site specificity, cutting RNA in a variety of single-stranded regions that are AU-rich (12, 13). The rate at which RNase E cleaves mRNA is strongly influenced by RNA features that are distinct from its sites of cleavage. These features include structural elements within the 5′-untranslated region (UTR), 5′-terminal phosphorylation, and bound ribosomes (14–16).

In *E. coli*, RNase E is an essential protein whose underproduction or overproduction can impair cell growth (17, 18). Synthesis of this important endonuclease is tightly controlled by an autoregulatory mechanism that modulates the longevity of the transcript of the RNase E gene (*rne*) in response to changes in cellular RNase E activity (19). Feedback regulation of RNase E synthesis is mediated in cis by the 361-nucleotide (nt) *rne* 5′-UTR, which can confer a comparably high degree of sensitivity to cellular RNase E levels onto heterologous mRNAs to which it is fused (19, 20). Thus, expression of a reporter gene comprising the *rne* 5′-UTR and the initial portion of the *rne* coding region joined in-frame to *lacZ* can differ by more than 30-fold in cells containing low versus high RNase E activity, and this sensitivity to RNase E is virtually abolished when all of the 5′-UTR upstream of the ribosome binding site is deleted (19, 20). The *rne* 5′-UTR contains six structural domains (Fig. 1), two of which, the stem-loop structures hp2 and hp3, are important for feedback regulation (20). Of these two stem-loops, hp2 is the more potent sensor of cellular RNase E activity. When fused directly upstream of the *rne* ribosome binding site, hp2 alone is sufficient to direct efficient feedback regulation by RNase E (20).

To better understand the mechanism of RNase E autoregulation, we have used mutational analysis and phylogenetic comparison to examine the features of *rne* hp2 that are important for its ability to mediate feedback control. These studies indicate that an 8-nt internal loop near the top of hp2 plays a central role in this regulatory process.

**Experimental Procedures**

**Strains and Plasmids—**The isogenic *E. coli* strains CH1827 (MC1061, *rne*::zce-726::Tn10) and CH1828 (MC1061, *ams-1::zce-726::Tn10) are derivatives of MC1061 (*araD39 stra, leu7987, ΔlacX74 galU, galK* *hvr* *ham* stra) (22). CH1828 carries a chromosomal *rne* missense mutation (*ams-1*) that reduces cellular RNase E activity at 37 °C (19). Plasmid pRNE101 is a pACYC177 derivative bearing the wild-type *E. coli* *rne* gene; the presence of this multicopy plasmid in CH1748 causes RNase E to be overproduced at ~2.8 times its normal cellular concentration (19).

Plasmid pEZ3114–337 is a pSC101 derivative bearing an *rne-lacZ* fusion that comprises the *rne* promoter, all of the 361-nt *rne* 5′-UTR except nucleotides 114–337, and the first 181 *rne* codons fused in-frame to the tenth codon of *lacZ* (20). To construct plasmid pEZ8, a unique *Apal* restriction site was created in pEZΔ114–337 by inserting two guanidylate residues between nucleotides 53 and 54 and two cytidylate residues between nucleotides 106 and 107 of the *rne* 5′-UTR, resulting in two additional G-C base pairs at the base of hp2. Plasmid pEZ8hp2Hinf was constructed by inserting a DNA fragment encoding hp2 of *Haemophilus influenzae* rne mRNA (G GCCCTTGTTGTTGA- AACTCAATGCAAGTTGCAATACGTTACA) between the *Apal* and *BsrGI* restriction sites of pEZ8. In plasmid pEZ8hp2, the segment between the *Apal* and *BsrGI* sites of pEZ8, including hp2 and ss2, was replaced with a shorter linker (GGGCC-GGGCGCCGATTTAGGTACA), whereas in pEZ8SoI, the same pEZ8 segment was replaced with a different linker (G GCCCCGTCGCTA). Plasmids pEZ8hp2mut1T, pEZ8hp2mut2T, pEZ8 hp2mut3T, and pEZ8hp2mut2B are derivatives of pEZ8 that each encode mutations in the hairpin loop at the top of *rne* hp2 (AAUG →

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1 The abbreviations used are: UTR, untranslated region; nt, nucleotides; X-gal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside.
Key Features of rne hp2

RNAob (available at www.genetics.wustl.edu/eddy/software/#rnabob) was used to search for elements resembling rne hp2 in the genomes of *E. coli*, *Yersinia pestis*, and *Haemophilus influenzae* (genomic sequence files downloaded from ncbi.nlm.nih.gov/genomes/Bacteria). The search parameters specified an 8-nt internal loop consisting of the sequence CD—RUHAGA (where D = A, G, or U; R = A or G; H = A, C, or U), flanked below by a ≥3-bp stem and above by a 2-bp stem and a 4- to 6-nt hairpin loop whose sequence was not constrained. Strict search criteria allowed only canonical base pairs in the 3- and 2-bp stems, as observed in nature. Only elements present on the coding strand of each gene were considered.

RESULTS

Evolutionary Conservation of rne hp2 Function—Autoregulation of RNase E synthesis is mediated by rne hp2, an mRNA stem-loop that functions post-transcriptionally as a sensor of cellular RNase E activity (20). Elucidating the essential features of this 57-nt 5′-UTR element would be useful not only for understanding the mechanism of RNase E feedback regulation but also for efforts to identify other genes whose expression might be similarly regulated by this important ribonuclease.

Despite extensive sequence divergence, the 5′-UTR of the *Haemophilus influenzae* and *E. coli* rne transcripts share significant secondary-structure homology (20). We first wished to address whether hp2 of *Haemophilus influenzae* rne mRNA could substitute functionally for the corresponding *E. coli* stem-loop in mediating RNase E feedback regulation in *E. coli*. These studies were performed in the context of an rne-lacZ reporter (exΔ114–337, Fig. 2) in all of the *E. coli* rne 5′-UTR, except hp3, and the first 181 codons of the rne coding region were fused in-frame to lacZ. Expression of exΔ114–337 in *E. coli* is very sensitive to the cellular level of RNase E activity, which modulates the longevity of the reporter transcript (19, 20). Previous experiments have shown that, in the absence of hp3, feedback regulation of rne-lacZ expression is almost entirely dependent on the presence of hp2, thereby allowing the effects of hp2 mutations to be fully manifested (20).

To facilitate the construction of hp2 substitution mutants, we first introduced two additional G-C base pairs at the 5′ end of hp2 to create a unique *ApaI* restriction site (GGGCCG) in the reporter gene (Fig. 2). These additional base pairs did not significantly affect the sensitivity of the reporter mRNA to feedback regulation by RNase E, as determined by comparing β-galactosidase production from this new reporter (ex8) in isogenic lacZ* E. coli* host strains with low (CH1828) or high (CH1827 + pRNE101) levels of cellular RNase E activity. The observed repression ratio (*R*, the ratio of β-galactosidase activity in the CH1828 host versus the CH1827 + pRNE101 host), which is a direct measure of the degree to which expression of the rne-lacZ fusion can be inhibited by RNase E, was approximately the same for ex8 (R = 11.0 ± 2.0) and the original exΔ114–337 reporter (R = 11.1 ± 2.1). As expected, when hp2 was replaced instead with an unrelated stem-loop (UUGGCC-CGCCGCGCCAG), sensitivity to cellular RNase E activity was nearly abolished (R = 2.4 ± 0.2 for ex8hp2).

We then replaced hp2 of ex8 with the corresponding stem-loop of the *H. influenzae* rne transcript (ex8hp2Hinf, Fig. 2). Despite numerous differences in sequence and secondary structure between the *Haemophilus* and *E. coli* stem-loops, this hp2 replacement had almost no effect on feedback regulation (*R* = 9.8 ± 2.3). This finding suggests that the features of hp2 that are critical for feedback regulation in *E. coli* are conserved in the *Haemophilus* stem-loop.

To determine which of the conserved features of rne hp2 are most important for feedback regulation, this stem-loop was divided conceptually into three subdomains. One was an 8-nt internal loop near the top of hp2, a region of the stem-loop whose sequence is especially well conserved between *Hae-
mophilus and E. coli (Fig. 2). The other two subdomains were the hairpin loop and 2-bp stem above this internal loop and the long imperfect stem below it. Each of these subdomains was examined by mutational analysis.

**Sequence Requirements Within the Internal Loop of rne**

To identify which nucleotides in the 8-nt internal loop of E. coli rne hp2 (CA—GUAAGA) are most important for feedback regulation, we created two combinatorial libraries in which the sequence of each half of the internal loop was randomized in the context of the ez8 reporter (hp2IL-top: C N2—N3N4N5AGA and hp2IL-bot: N1A—GUAN6N7N8). We chose to divide the internal loop into halves, as the maximum complexity of each library would equal 256 (44), a relatively manageable number. In contrast, simultaneously randomizing all eight positions in a single library would greatly increase the potential complexity of the library to 65,536 (48), making it much less likely that all sequence combinations would be represented.

The libraries were each transformed into E. coli cells containing a high concentration of RNase E due to the presence of a multicopy rne plasmid (pRNE101). The high level of RNase E activity in these cells allowed reporter mRNAs susceptible to feedback repression to be identified on the basis of the pale color of bacterial colonies grown on LB-agar plates containing the chromogenic β-galactosidase substrate X-gal. The color of these transformants was compared with that of colonies with an rne-lacZ reporter either containing (ez8) or lacking (ez8Δhp2) a functional hp2 sequence. Reporter plasmids were purified from colonies that were as pale as those containing the parent plasmid (pEZ8) and retransformed into isogenic E. coli strains containing low or high RNase E activity. For each plasmid, a repression ratio was calculated by comparing levels of β-galactosidase activity in the two host strains. A total of 1202 colonies were screened from the hp2IL-bot library (CA—GUAAGA — N1A—GUAN6N7N8). Nearly all of these colonies were as blue as colonies expressing a reporter deficient for feedback regulation (ez8Δhp2). This finding seemed to imply that the sequence requirements for the bottom half of the internal loop were strict. Indeed, only two colonies in this combinatorial library contained reporters with repression ratios similar to that of a reporter (ez8) that has a wild-type internal loop sequence (R = 12.7 ± 1.9 for hp2IL-bot#7 and 15.2 ± 3.4 for hp2IL-bot#8 versus 10.4 ± 2.0 for ez8; Fig. 3).

DNA sequencing revealed that the internal loops of these two
FIG. 3. Analysis of a combinatorial library of rne-lacZ reporters bearing a randomized sequence in the lower half of the hp2 internal loop. Members of the hp2IL-bot library that appeared to be sensitive to feedback regulation on the basis of colony color were introduced into the isogenic E. coli strains CH1828 (low RNase E activity) and CH1827 + pRNE101 (high RNase E activity). Cellular β-galactosidase activity and repression ratios were measured as described in Fig. 2. Among the 1202 library members that were screened, only two (clone #7 and #8) were found to be sensitive to feedback regulation, and both of these had an internal loop that was identical in sequence to that of wild-type E. coli rne hp2. To the right is a diagram of the upper portion of hp2; the nucleotides in the internal loop that were randomized in the hp2IL-bot library (N1, N5, N7, and N8) are enclosed in boxes.

FIG. 4. Analysis of a combinatorial library of rne-lacZ reporters bearing a randomized sequence in the upper half of the hp2 internal loop. Members of the hp2IL-top library that appeared to be sensitive to feedback regulation on the basis of colony color were introduced into the isogenic E. coli strains CH1828 (low RNase E activity) and CH1827 + pRNE101 (high RNase E activity). Cellular β-galactosidase activity and repression ratios were measured as described in Fig. 2. Nucleotides that deviated from the sequence of wild-type E. coli rne hp2 are underlined. To the right is a diagram of the upper portion of hp2; the nucleotides in the internal loop that were randomized in the hp2IL-top library (N3, N5, N7, and N8) are enclosed in boxes. Four hp2 variants (IL-2G, IL-2U, IL-3A, and IL-5C) were isolated from the library on two independent occasions.

reporters were identical in sequence to that of wild-type E. coli hp2. These results indicate that only the wild-type sequence of nucleotides is tolerated at positions N1 (C), N6 (A), N7 (G), and N8 (A) of the internal loop.

In contrast to the hp2IL-bot library, the hp2IL-top library (CA—GUAAGA → CN2—N5N7N8AGA) gave rise to colonies with a wide range of color intensities on X-gal plates. Among a total of 3580 colonies, several contained reporter genes that were functional for feedback regulation (defined as having a repression ratio greater than 6.0), with repression ratios ranging from 7 to 15. This variation in the repression ratios suggested more relaxed sequence requirements in the upper half of the internal loop. Sequencing of eleven library members that were functional for feedback regulation confirmed this prediction (Fig. 4). Although the nucleotide at position N5 (U) was invariant, sequence variation was observed at positions N1 (A, G, or U), N3 (A or G), and N6 (A, C, or U). Although the wild-type E. coli sequence was not isolated in this screen, the repeated isolation of four variants (hp2IL-2G, hp2IL-2U, hp2IL-3A, and hp2IL-5C) suggested that we had identified most internal loop sequences in the library that were competent for feedback regulation.

Additional information about the key features of the internal loop was gleaned from close inspection of the repression ratios for each functional sequence variant within the hp2IL-top library and from the creation of additional site-directed mutants. For example, the potential for Watson-Crick base pairing between positions N2 and N3 appears to partially impair the ability of hp2 to mediate feedback regulation. In the double mutant hp2IL-2U,3A (CA—GUAAGA → CU—AUAAGA), where Watson-Crick base pairing is possible between uracil at N2 and adenine at N3, there is a 35% reduction in the repression ratio relative to the wild-type sequence (R = 6.9 ± 0.4 for hp2IL-2U,3A versus 10.4 ± 2.0 for ez8; Fig. 4). In contrast, the same substitutions made individually at N2 or N3 lack the potential for Watson-Crick base pairing and have no detrimental effect on feedback regulation (R = 10.6 ± 1.3 for hp2IL-2U and 12.6 ± 0.8 for hp2IL-3A; Fig. 4).

In none of the functional sequence variants was cytosine observed at N2. Although cytosine at this position would be capable of pairing with a wild-type guanine base at N3, it would not be able to form a Watson-Crick base pair with adenine at N3, a nucleotide substitution that is itself well-tolerated (hp2IL-3A in Fig. 4). To determine the effect of cytosine at N2 in either of these sequence contexts, we constructed two additional variants: hp2IL-2C (CA—GUAAGA → CC—GUAAGA) and hp2IL-2C,3A (CA—GUAAGA → CC—AUAAGA). In the context of guanine at N3, the presence of cytosine at N2 severely impaired feedback regulation, causing the reporter to be scarcely more sensitive to cellular RNase E activity than a reporter lacking hp2 altogether (R = 3.6 ± 0.2 for hp2IL-2C versus 2.4 ± 0.2 for ez8hp2; Fig. 5). Eliminating the potential for Watson-Crick base pairing between N2 and N3 by substituting both cytosine at N2 and adenine at N3 resulted in only a partial restoration of feedback regulation (R = 4.7 ± 0.4 for hp2IL-2C,3A versus 3.6 ± 0.2 for hp2IL-2C and 12.6 ± 0.8 for hp2IL-3A; Figs. 4 and 5). These findings indicate that cytosine is intrinsically not well tolerated at position N2, quite apart...
from its base pairing potential.

Position N₅ shows considerable sequence flexibility, with adenine (wild-type), cytosine, or uracil present at this position in functional transcripts. The reporter transcript with cytosine at position N₅ was fully sensitive to feedback repression by RNase E (R = 10.7 ± 0.6 for hp2IL-5C; Fig. 4). The presence of uracil at this position only partially impaired feedback regulation when this substitution was accompanied by guanine at position N₂, which by itself does not affect feedback regulation (R = 6.8 ± 0.7 for hp2IL-2G,5U versus 14.7 ± 1.2 for hp2IL-2G; Fig. 4). The same uracil substitution had a more deleterious effect in the context of the naturally occurring adenine at position N₁ (R = 4.1 ± 0.2 for hp2IL-5U; Fig. 5). Introducing guanine at position N₅ severely impaired feedback regulation (R = 1.9 ± 0.2 for hp2IL-5G; Fig. 5), as expected from the absence of this substitution among the functional reporters in the hp2IL-top library (Fig. 4). Other nucleotide substitutions that did not appear among the functional hp2 variants in the hp2IL-top and hp2IL-bot libraries (IL-4G, IL-6U, IL-7U, IL-7A) were likewise found to be deleterious for feedback regulation (Fig. 5).

The Hairpin Loop at the Top of rne hp2—Other features of rne hp2 that may contribute to feedback regulation include the hairpin loop (AAUG) and 2-bp stem at the top. The importance of the hairpin loop was evaluated by mutational analysis (Fig. 6). Mutating all four nucleotides of this loop (hp2mut4; AAUG → GGAA) significantly impaired but did not abolish feedback regulation (R = 4.7 ± 0.4 for hp2mut4 versus 2.4 ± 0.2 for ez8hp2), as did replacing only the top two nucleotides of the loop with two dissimilar nucleotides (hp2mut2T; AAUG → AGAG; R = 4.8 ± 0.5). Nonetheless, there is no sequence feature of the hairpin loop that is absolutely required. Thus, substituting three dissimilar nucleotides for the top two nucleotides of the loop (hp2mut3T; AAUG → AUUCG) did not impair feedback regulation (R = 9.9 ± 1.0), and mutating the bottom two nucleotides of the loop (hp2mut2B; AAUG → GAUA) caused only a mild reduction in the repression ratio (R = 8.4 ± 0.8). These findings indicate that the hairpin loop at the top of rne hp2 makes a significant contribution to feedback regulation by RNase E but in a manner that cannot readily be predicted by merely examining the loop sequence. A significant regulatory defect was also observed when the 2-bp stem that separates the hairpin loop from the 8-nt internal loop was doubled in length (R = 4.0 ± 0.5 for hp2+2bp), suggesting that the distance and orientation of the hairpin loop with respect to the internal loop are also important.

The Lower Stem of rne hp2—The internal loop and hairpin loop of E. coli rne hp2 sit atop a tall, imperfectly base-paired stem. To determine whether the sequence of this lower portion of hp2 contributes to feedback regulation, we replaced all of hp2 below the internal loop (a total of 41 nucleotides, including 14 Watson-Crick and wobble (G-U) base pairs and three small internal loops) with 13 base pairs differing in sequence from wild-type at all but the three bottom-most positions and the position immediately beneath the internal loop (hp2bot-syn; Fig. 7). The resulting reporter transcript remained fully sensitive to feedback regulation (R = 12.1 ± 0.3). The ez8 reporter also retained its sensitivity to feedback regulation when the G-C base pair directly below the internal loop was mutated to a C-G pair (hp2CG; R = 9.1 ± 1.0). These findings indicate that neither the sequence of the stem below the internal loop of rne hp2 nor the base-pairing imperfections within this lower stem are important for efficient feedback regulation in E. coli.

Phylogenetic Analysis of rne hp2—The mutational studies described above identify features of rne hp2 that are important for RNase E autoregulation in E. coli. Natural sequence variation among bacterial species provides an independent source of information as to the key features of this stem-loop. We
Crick base pairing between these two nucleotides). Except for the absence of phylogenetic variation at N5, this consensus sequence agrees perfectly with the results of mutational analysis. Also well conserved in length and sequence is the 2-bp stem above this internal loop, consistent with our finding that feedback regulation is impaired when this short stem is extended. The hairpin loop at the top of rne hp2 shows somewhat greater evolutionary variation, ranging in size from four to five nucleotides with the consensus sequence AN(N/NG) (where N is a nucleotide of unspecified identity). The lack of sequence variation at the first and last positions of the hairpin loop is unexpected in view of our finding that these two nucleotides can be mutated without adversely affecting feedback regulation in E. coli. Except for the highly conserved G-C base pair immediately beneath the internal loop, the lower portion of hp2 shows the greatest variability in sequence and secondary structure, ranging in size from 32 nt (including 12 base pairs) to 41 nt (including 17 base pairs) and bearing diverse internal loops at disparate locations. This high degree of variability is in agreement with our finding that the sequence and secondary structure of the lower stem of hp2 can be changed significantly without impairing feedback regulation.

**DISCUSSION**

An important step in elucidating post-transcriptional gene regulation is to identify the features of mRNAs that determine their lifetimes in vivo. Previous studies have shown that RNase E tightly autoregulates its synthesis in E. coli via a mechanism in which rne hp2, a sensory element within the 5'-UTR, modulates rne mRNA longevity in response to changes in cellular RNase E activity (19, 20). We have now identified evolutionarily conserved features in the upper portion of rne hp2 that are crucial for its ability to mediate efficient feedback regulation of RNase E synthesis. These include an 8-nt internal loop and the 2-bp stem and hairpin loop above it.

The core recognition element within rne hp2 is an internal loop comprising two nucleotides on one strand and six nucleotides on the other. Point mutations within this internal loop can abolish hp2 function in E. coli. At five of the eight positions (N1 = C; N2 = U; N6 = A; N7 = G; N8 = A), no sequence variation is tolerated, whereas at three other positions (N2 = A, G, or U; N3 = A or G; N6 = A, C, or U) some sequence variation is permitted (Fig. 9A). Consistent with these findings is the limited sequence variation that occurs naturally for this rne internal loop in several related bacterial species, where the sequence is strictly conserved at six of the eight positions and observed to vary only at positions N2 (A, G, or U) and N3 (A or G) (Fig. 9B).

Above the internal loop of rne hp2, between nucleotides N2 and N6, are a 2-bp stem and a 4–5 nt hairpin loop that also contribute to hp2 recognition. In the rne transcripts of related bacterial species, the sequence of this hairpin loop varies at all but the first and last positions (Fig. 9B). Nonetheless, nucleotide substitutions at these two conserved positions can have little effect on hp2 function in E. coli, whereas substitutions at the nonconserved positions can significantly reduce the efficiency of feedback regulation when they deviate from variations observed naturally in other bacterial species (Figs. 6 and 8; note that the hairpin loop of hp2mut3T is identical to that of rne hp2 in Actinobacillus actinomycetemcomitans, whereas the hairpin loop of hp2mut2T is unlike any known hp2 variant in nature). The length of the short stem between the hairpin loop and internal loop is critical, hp2 function being markedly impaired by increasing the size of this stem from 2 to 4 bp (Fig. 6). On the other hand, the sequence of this 2-bp stem is consequential (20) despite its evolutionary conservation. Together,
Besides hp2, the only other domain of the rne 5'-UTR that is known to contribute appreciably to feedback regulation is rne hp3 (Fig. 1); however, this branched stem-loop is not required, and its role is not known (20). Previous experiments have shown that a putative RNase E cleavage site in the single-stranded segment (ss1) that precedes hp2 can be removed without impairing regulation (19, 20). Likewise, the rne coding region appears not to contribute in cis to feedback regulation, because an rne-lacZ reporter in which the fifth rne codon has been fused to lacZ is tightly regulated by RNase E (23).

In comparison to a number of other E. coli genes, expression of the rne gene is unusually sensitive to the cellular level of RNase E activity (19). The identification of the key sequence features of rne hp2 provides a basis for searching the genomes of E. coli and related microorganisms for other genes encoding similar elements that may render those genes particularly sensitive to feedback regulation by RNase E. Using strict search criteria based on the essential features of hp2 to examine the sequences of the E. coli, Versinia pestis, and H. influenzae genomes (see “Experimental Procedures”), rne was the only gene found to encode a hp2-like stem-loop in all three species. Two other mRNAs in E. coli and one in Y. pestis have the potential to form a similar stem-loop, but in each case this potential is of questionable significance due to its lack of conservation in either of the other two bacterial species that were examined. Using more relaxed criteria, additional candidates for possible hp2 homologs were identified, but again none were conserved in all three species. We conclude that hp2 most likely is a unique evolutionary adaptation of the rne gene that enables its expression to be tightly regulated by cellular RNase E activity.

Examination of the RNase E genes of bacterial organisms that are only distantly related to E. coli suggests that their rne transcripts may lack a recognizable homolog of hp2 upstream of the coding region. Either RNase E synthesis is not autoregulated in those species, or its autoregulation is mediated in cis by an element distinct from hp2.

The precision with which E. coli cells regulate RNase E synthesis is crucial for properly controlling rates of mRNA decay and for sustaining cell growth (18). Knowing the critical features of rne hp2 should be of considerable value in elucidating the molecular mechanism by which this regulatory stem-loop senses the cellular concentration of RNase E and modulates rne gene expression through changes in rne mRNA longevity.

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