In Escherichia coli, programmed cell death is mediated through the system called “addiction module,” which consists of a pair of genes encoding a stable toxin and a labile antitoxin. The pemI-pemK system is an addiction module present on plasmid R100. It helps to maintain the plasmid by post-segregational killing in E. coli population. Here we demonstrate that purified PemK, the toxin encoded by the pemI-pemK addiction module, inhibits protein synthesis in an E. coli cell-free system, whereas the addition of PemI, the antitoxin against PemK, resums the protein synthesis. Further studies reveal that PemK is a sequence-specific endoribonuclease that cleaves mRNAs to inhibit protein synthesis, whereas PemI blocks the endoribonuclease activity of PemK. PemK cleaves only single-stranded RNA preferentially at the 5' or 3' side of the A residue in the “UAH” sequences (where H is C, A, or U). Upon induction, PemK cleaves cellular mRNAs to effectively block protein synthesis in E. coli. The pemK homologue genes have been identified on the genomes of a wide range of bacteria. We propose that PemK and its homologues form a novel endoribonuclease family that interferes with mRNA function by cleaving cellular mRNAs in a sequence-specific manner.

In Escherichia coli, programmed cell death is proposed to be mediated through the system called “addiction module,” which consists of a pair of genes encoding a toxin and an antitoxin. The addiction module has the following properties. (a) The toxic protein is stable, whereas the antitoxin is a labile protein. (b) The toxin and the antitoxin are coexpressed from an operon and interact with each other to form a stable complex. (c) Their expression is auto-regulated either by the toxin-antitoxin complex or by the antitoxin alone. When the co-expression is inhibited under stress conditions, the antitoxin is degraded by proteases, enabling the toxin to act on its target. In E. coli, some extrachromosomal elements are known to contain addiction modules causing the bacterial programmed cell death by the so-called post-segregational killing effect. The most studied extrachromosomal addiction modules are the phd-doc system on bacteriophage P1 (2–5), the ccdA-cddB system on factor F (6–9), the kis-kid system on plasmid R1 (10–13), and the pemI-pemK system on plasmid R100 (14–17). Interestingly, the E. coli chromosome also contains several addiction module systems, such as the relBE system (18–21), the mazEF system (22–25), and the chpB system (26–28).

The cellular effects of the toxins in the addiction modules have been studied quite extensively. CcdB, the toxin in the ccdA-cddB system, interacts with DNA gyrase to block DNA replication (7, 29), and RelE, the toxin in the relBE system, is not able to degrade free RNA but cleaves mRNA in the ribosome A site with high codon specificity (21). Recently, it was demonstrated that the A-site mRNA cleavage can occur in the absence of RelE (30). The mechanism of the A-site mRNA cleavage is still unknown. It has been proposed that MazF (ChpAK), the toxin encoded by the mazEF system, and ChpBK, the toxin encoded by chpB system, inhibit translation by a mechanism very similar to that of RelE in a ribosome-dependent and codon-specific manner (28). However, we have recently demonstrated that MazF is a sequence-specific endoribonuclease functional only for single-stranded RNA, which preferentially cleaves mRNAs at the ACA sequence in a manner independent of ribosomes and codon, and is therefore functionally distinct from RelE (31).

The pemI-pemK system and the kis-kid system are involved in the stable maintenance of two closely related inc/FII low copy plasmids, plasmid R100 (14, 15) and plasmid R1 (10, 32), respectively. These two systems turned out to be identical (1). It has been demonstrated that Kid (PemK) inhibits the ColE1 replication acting at the initiation of DNA synthesis but does not inhibit P4 DNA replication in vitro (12). It has not been shown whether Kid (PemK) inhibits the chromosome DNA replication. Toxin Kid (PemK) and antidote Kis (PemI) not only function in bacteria but also function efficiently in a wide range of eukaryotes. Kid (PemK) inhibits cell proliferation in yeast, Xenopus laevis, and human cells, and the inhibition was released by Kis (PemI) (33). It has also been demonstrated that Kid (PemK) triggers apoptosis in human cells (33). These results suggest that there is a common target for Kid (PemK) in both prokaryotes and eukaryotes. In this paper, we demonstrate that PemK is an endoribonuclease, which effectively blocks protein synthesis by cleaving cellular mRNAs in a sequence-specific manner. We propose that PemK and its homologues constitute a novel endoribonuclease family that interferes with mRNA function.

**Experimental Procedures**

Strains and Plasmids—E. coli BL21 (DE3) and BW25113 cells were used. The pemIK gene was amplified by PCR with plasmid R100 as template and cloned into the NdeI-Xhol sites of pET21cc (Novagen) to create an in-frame translation with a (His)6 tag at the PemK C terminus. The plasmid was designated as pET21cc-IKHis6. The pemI gene was cloned into the NdeI-BamHI sites of pET28a (Novagen), creating plasmid pET28a-IHis6. PemI was expressed as a fusion with an N-terminal (His)6 tag followed by a thrombin cleavage site named (His)6PemI. The pemK gene was cloned into pBAD (34), creating plasmid pBAD-K. E. coli mazG gene was cloned into NdeI-BamHI sites of pET11a (New England Biolabs), creating plasmid pET11a-MazG. The mazG gene was cloned into a pIIIIV vector (35), creating plasmid pIN-MazG. E. coli era gene was cloned into the Scal-Xhol sites of...
pET28a, creating plasmid pET28a-Era. The era gene was also cloned into pNIII vector to create plasmid pN-ExpEera.

Protein Purification—For purification of (His)_{6}PemI, pET28a-(His)_{6}I was introduced into E. coli BL21(DE3) strain and (His)_{6}PemI expression was induced with 1 mM IPTG for 4 h. (His)_{6}PemI protein was purified with use of nickel-nitrilotriacetic acid resin (Qiagen). pET21c-IR(His)_{6} was also introduced into E. coli BL21(DE3) strain. The co-expression of PemI and PemK(His)_{6} was induced in the presence of 1 mM IPTG for 4 h. The PemI-PemK(His)_{6} complex was purified with the use of nickel-nitrilotriacetic acid (Qiagen). To purify PemK(His)_{6} from the purified PemI-PemK(His)_{6} complex, PemI in the purified PemI-PemK(His)_{6} complex was dissociated from PemK(His)_{6} in 5 mM guanidine HCl. PemK(His)_{6} was retracted on nickel-nitrilotriacetic acid resin (Qiagen) and then eluted and refolded by stepwise dialysis.

Assays of Protein and DNA Syntheses in Vivo—E. coli BW25113 cells containing pBAD-K were grown in modified M9 medium with 0.5% glycerol (no glucose) and an amino acid mixture (1 mM each) without methionine. When the A_{600} of the culture reached 0.6, arabinose was added to a final concentration of 0.2% to induce PemK expression. Cell cultures (1 ml) were taken at the time points indicated and mixed with 5 μCi of [35S]methionine (protein synthesis) or 2 μCi of [methyl-^3H]thymidine (DNA synthesis). After 1-min incubation at 37 °C, the rates of DNA replication and protein synthesis were determined as described previously (36). To prepare the samples for SDS-PAGE analysis of the total cellular protein synthesis, [35S]methionine incorporation reaction mixture was prepared by partial alkaline hydrolysis of the 5'-32P]ATP using T4 polynucleotide kinase and used as the substrate to detect the PemK-mediated inhibition of protein synthesis and protein synthesis. Interestingly, the addition of the antitoxin PemI blocked the PemK-mediated inhibition of protein synthesis and resumed MazG and (His)_{6}Era synthesis in the presence of increasing amounts of PemK (Fig. 2A, lanes 2–5). Both MazG and (His)_{6}Era synthesis were blocked by PemK in a dose-dependent manner (Fig. 2A). These results demonstrate that PemK inhibits protein synthesis, consistent with the PemK-mediated inhibition of protein synthesis observed in vivo (Fig. 1, B and C). The delayed PemK-mediated inhibition of DNA replication observed in vivo (Fig. 1A) thus is speculated to be due to a secondary effect of the inhibition of cellular protein synthesis. Interestingly, the addition of the antitoxin PemI blocked the PemK-mediated inhibition of protein synthesis and resumed MazG and (His)_{6}Era synthesis in a PemI dose-dependent manner (Fig. 2B). It should be noted that preincubation of the E. coli cell-free system with PemK for 15 min at

1 The abbreviations used are: IPTG, isopropyl-β-D-thiogalactopyranoside; ORF, open reading frame; d, oligodeoxynucleotide.
37°C did not have a significant adverse effect on (His)_6Era synthesis if PemI was added together with the plasmid DNA after the 15-min preincubation (Fig. 2C, compare lanes 1 and 3). However, in the absence of PemI, no protein was produced (Fig. 2C, lane 2). (His)_6Era synthesis was resumed regardless of whether PemI was added after the 15-min preincubation with PemK (Fig. 2C, lane 3), or it was added together with PemK during the 15-min preincubation (Fig. 2C, lane 4). These results suggest that the primary target of PemK is mRNAs but not tRNA, ribosomes, or any other factors that are required for protein synthesis in the cell-free system.

Endoribonuclease Activity of PemK—The DNA fragment containing a T7 promoter and the mazG gene was obtained by PCR amplification using the plasmid pET11a-MazG as a template as described under “Experimental Procedures.” Similarly, another DNA fragment containing a T7 promoter and the era

**Fig. 1.** Effects of PemK on DNA and protein synthesis in vivo. A, effect of PemK on DNA synthesis. E. coli BW25113 cells containing pBAD-K were grown at 37°C in M9 medium with glycerol as carbon source. When the A_600 of the culture reached 0.6, arabinose was added to a final concentration of 0.2% to induce PemK expression. The rates of DNA replication were measured by detecting the [methyl-^3H]thymidine incorporation at various time points after the induction of PemK as described under “Experimental Procedures.” B, effect of PemK on protein synthesis. The rates of protein synthesis were measured by detecting the [^35S]methionine incorporation at various time points after the induction of PemK as described under “Experimental Procedures.” C, SDS-PAGE analysis of the total cellular protein synthesis after the induction of PemK. Cell culture (1 ml) was taken at the time point after the induction of PemK as indicated and mixed with 5 μCi of [^35S]methionine. After a 1-min incorporation at 37°C, the [^35S]methionine incorporation reaction mixture (500 μl) was taken into a chilled test tube containing 25 μl of 100% trichloroacetic acid solution and 100 μg/ml non-radioactive methionine. Cell pellets were collected by centrifugation and subjected to SDS-PAGE followed by autoradiography. The band indicated with an arrow is proposed to be PemK (~12 kDa). Pre-stained colored protein molecular weight standards (Invitrogen) were used in the SDS-PAGE.

37°C did not have a significant adverse effect on (His)_6Era synthesis if PemI was added together with the plasmid DNA after the 15-min preincubation (Fig. 2C, compare lanes 1 and 3). However, in the absence of PemI, no protein was produced (Fig. 2C, lane 2). (His)_6Era synthesis was resumed regardless of whether PemI was added after the 15-min preincubation with PemK (Fig. 2C, lane 3), or it was added together with PemK during the 15-min preincubation (Fig. 2C, lane 4). These results suggest that the primary target of PemK is mRNAs but not tRNA, ribosomes, or any other factors that are required for protein synthesis in the cell-free system.

**Fig. 2.** Effects of PemK and PemI on the cell-free protein synthesis. A, inhibition of cell-free protein synthesis by PemK. Protein synthesis was performed at 37°C for 1 h in the E. coli T7 S30 extract system (Promega). MazG was expressed from pET11a-MazG, and (His)_6Era was expressed from plasmid pET28a-Era. Lane 1, control without the addition of PemK(His)_6; lanes 2–5, 0.125, 0.25, 0.5, and 1 μg of PemK(His)_6 were added, respectively. B, release of PemK-mediated inhibition of protein synthesis in the cell-free system by PemI. Lane 1, control without the addition of PemK(His)_6; lane 2, with 1 μg of PemK(His)_6; lanes 3–5, 0.5, 1, and 2 μg of (His)_6PemI were added together with 1 μg of PemK(His)_6, respectively. C, effect of preincubation of the cell-free system with PemK on protein synthesis. The cell-free system was preincubated with or without PemK(His)_6 for 15 min at 37°C before the addition of pET28a-Era plasmid. The protein synthesis was then performed for another 1-h incubation at 37°C. Reaction products were analyzed by SDS-PAGE followed by autoradiography. Lane 1, control preincubated without PemK(His)_6; lane 2, preincubated with 1 μg of PemK(His)_6 followed by adding pET28a-Era plasmid; lane 3, preincubated with 1 μg of PemK(His)_6 followed by adding pET28a-Era plasmid and 1 μg of (His)_6PemI; lane 4, preincubated with 1 μg of PemK(His)_6 and 1 μg of (His)_6PemI together followed by the addition of pET28a-Era plasmid.
gene was obtained with the plasmid pET28a-Era as a template. The mazG mRNA and the era mRNA were then prepared from these two DNA fragments, respectively, using the T7 large-scale transcription kit (Promega). The mazG mRNA was digested into smaller fragments after incubation with PemK at 37 °C for 15 min (Fig. 3A, lane 2), whereas the addition of PemI inhibited the cleavage of mazG mRNA in a dose-dependent manner (Fig. 3A, lanes 3–6). PemI itself had no effect on the mazG mRNA (Fig. 3A, lane 7). A similar result was obtained with the era mRNA as substrate (data not shown). These results demonstrate that PemK is an endoribonuclease that cleaves mRNA to inhibit protein synthesis and that PemI functions as an antitoxin to block the endoribonuclease activity of PemK.

The fact that the digestion products of mazG mRNA by PemK form distinct bands on a 3.5% polyacrylamide gel (Fig. 3A) indicates that PemK cleaves RNA at specific sites. The mazG mRNA was partially digested by PemK and then subjected to primer extension using five different oligodeoxyribonucleotide primers, G1–G5, as described under “Experimental Procedures.” A number of specific cleavage sites along the mazG mRNA were detected on a 6% sequencing gel compared with the controls without PemK treatment (data not shown).

Partially digested era mRNA by PemK was also subjected to primer extension using four different primers, E1–E4, as described under “Experimental Procedures” to detect the PemK cleavage sites along the era mRNA. To determine the exact sequence around the PemK cleavage sites, each primer extension product was analyzed on a 6% sequencing gel with the DNA sequencing ladder prepared with the same primer (Fig. 3B). The RNA sequences around the major cleavage sites in the era mRNA detected with primers G1 (B) and G2 (C) and the PemK(His)6 cleavage sites in the era mRNA detected with primers E1 (D) and E4 (E).

The mRNA sequences around PemK cleavage sites (indicated by arrows) in the mazG mRNA (from pET11a-MazG), the era mRNA (from pET28a-Era), and the lpp mRNA (from E. coli chromosomal DNA, see Fig. 5D) are shown. The conserved UA dinucleotides are shown in boldface. The numbers show the positions of the nucleotides in mRNA taking the A residue in the initiation codon AUG as +1.

**TABLE I**

| Gene names | Primer | mRNA sequences around the cleavage sites |
|------------|--------|----------------------------------------|
| mazG       | G1     | CUGCGGAAGUAGACUCAAACUUUGGGAUAG               |
|            | G2     | CGGGCAAAUGUGUGUGUGUGUGUGUGU                |
|            | G3     | CUGGCGCAAAUUGUGUGUGUGUGUGU               |
|            | G4     | CUGGCGCAAAUUGUGUGUGUGUGUGU               |
|            | G5     | CUGGCGCAAAUUGUGUGUGUGUGUGU               |
| era        | E1     | AGUAGAAGAGUAGAUAGAUAGAUAGAUAGAUAGAUAGAUAG |
|            | E2     | AGUAGAAGAGUAGAUAGAUAGAUAGAUAGAUAGAUAGAUAG |
|            | E3     | AGUAGAAGAGUAGAUAGAUAGAUAGAUAGAUAGAUAGAUAG |
|            | E4     | AGUAGAAGAGUAGAUAGAUAGAUAGAUAGAUAGAUAGAUAG |
| lpp        | lppC   | CUGCGGAAGUAGACUCAAACUUUGGGAUAG            |

**FIG. 3**

**Endoribonuclease activity of PemK.** A, cleavage of the mazG mRNA by PemK and the inhibitory effect of PemI on the PemK-mediated RNA cleavage. Lane 1, control, the mazG mRNA alone; lane 2, the mazG mRNA (1.5 μg) incubated with 0.2 μg of PemK(His)6; lane 3–6, the mazG mRNA (1.5 μg) incubated with 0.05, 0.1, 0.2, and 0.4 μg of (His)6PemI, respectively; lane 7, the mazG mRNA (1.5 μg) incubated with 0.4 μg of (His)6PemI. The reactions were performed at 37 °C for 15 min, and the reaction products were analyzed by 3.5% native PAGE with TAE buffer (40 mM Tris acetate, 1 mM EDTA). B–E, primer extension analyses of PemK cleavage sites in the mazG mRNA and the era mRNA. Primer extension experiments were performed as described under “Experimental Procedures.” Each primer extension product was analyzed on a 6% sequencing gel running with the DNA sequencing ladder prepared with the same primer. The RNA sequences complementary to the DNA sequence ladders around the PemK(His)6 cleavage sites are shown at the right side, and the cleavage sites are shown by arrows. Shown in this figure are the PemK(His)6 cleavage sites in the mazG mRNA detected with primers G1 (B) and G2 (C) and the PemK(His)6 cleavage sites in the era mRNA detected with primers E1 (D) and E4 (E).
in a dose-dependent manner (Fig. 4B, lanes 3–7). A similar result was obtained when the 30-base RNA substrate formed a duplex with its complementary DNA (data not shown). These results indicate that the PemK cleavage sites in the 30-base RNA substrate became protected in the RNA-RNA and RNA-DNA duplexes. Therefore, we conclude that PemK is a sequence-specific endoribonuclease for single-stranded RNA.

**In Vivo mRNA Cleavage upon the Induction of PemK**—To examine the effect of PemK on mRNAs in vivo, Northern blot and primer extension analyses were performed with the total cellular RNA extracted at different time points after the induction of PemK as described under “Experimental Procedures.” The 16 and 23 S rRNAs were stable against PemK in vivo, because no significant changes were observed in their band intensities in 1% agarose gel electrophoresis in the total cellular RNA samples during the 60-min period after the induction of PemK (data not shown). This finding indicates that in vivo both 16 and 23 S rRNA are well protected from PemK cleavage. Fig. 5A shows the Northern blot analyses of the mazG, era, and lpp mRNAs at the various time points with or without the induction of PemK. The mazG mRNA and the era mRNA were produced, respectively, from pIN-MazG and pIN-Era in the presence of 1 mM IPTG for 30 min before the addition of arabinose (the final concentration of 0.2%) to induce PemK expression. The lpp mRNA was transcribed from the *E. coli* chromosome. All of the three mRNAs were degraded after a 10-min induction of PemK expression, whereas no changes were observed during the 60-min incubation without the induction of PemK (Fig. 5A). In comparison with the mazG and lpp mRNAs, the era mRNA was mostly converted to a smaller distinct band, which was comparatively stable during the 60-min induction of PemK. The nature of this stable mRNA cleavage product is unknown.

Primer extension experiments were also carried out to determine the PemK cleavage sites in mRNAs in vivo. One cleavage site for each mRNA is shown in Fig. 5, B–D, for mazG, era, and lpp, respectively. In all of the cases, a band appeared at 10 min after the induction of PemK (lane 2 in Fig. 5, B–D) whose intensity further increased during the 60-min induction of PemK (lanes 2–6). Importantly, the band was hardly detected at 0 min (lane 1), clearly demonstrating that the observed cleavages were caused by the induction of PemK. Both the mazG and lpp mRNAs were cleaved between the A and C residues in the UAC sequence, whereas the era mRNA was cleaved between the U and A residues. The mazG mRNA was cleaved at the identical site in vivo and in vitro (compare Fig. 3C and Fig. 5B). The in vivo cleavage of the era mRNA also occurred at the same site as detected in vitro with use of the same primer (compare Fig. 3D with Fig. 5C). The cleaved UAC sequences in the mazG and the era mRNAs are in the reading frame of the both ORFs, encoding Tyr-41 in MazG and Tyr-7 in Era, whereas the cleaved UAC sequence in the lpp mRNA is between two adjacent codons, GCU for Ala-73 and ACU for Thr-74. The in vivo mRNA cleavages by PemK were very specific because no other cleavages were detected in Fig. 5, B–D.

Therefore, unlike RelE that stimulates codon-specific mRNA cleavage at the A site on ribosomes (20, 21, 30), PemK is a sequence-specific endoribonuclease inhibiting protein synthesis by cleaving mRNAs in a manner independent of ribosomes and codon-reading frames.

**DISCUSSION**

In this paper, we demonstrate that PemK, the toxin encoded by the *pemI-pemK* addiction module, is a sequence-specific endoribonuclease. Both in vitro and in vivo studies support that PemK inhibits protein synthesis by cleaving mRNAs at specific sites. Purified PemK inhibits protein synthesis in an *E. coli* cell-free system, whereas the addition of PemI is able to block the inhibitory effect of PemK and resume protein synthesis. Further, we demonstrate that mRNAs are degraded by PemK and that the PemK-mediated mRNA cleavage is inhibited by PemI. Therefore, PemI functions as an antitoxin to block the
PemK, a Sequence-specific Endoribonuclease

endoribonuclease activity of PemK by the formation of a Pemi-PemK complex.

PemK was found to be highly specific to single-stranded RNA, because the PemK-mediated RNA cleavage was blocked when an RNA substrate was annealed with its antisense RNA or complementary DNA to form an RNA-RNA or RNA-DNA duplex. The present results demonstrate that PemK cleaves preferentially at the 5′ or 3′ side of the A residue in UAH sequences (where H is C, A, or U). It remains to be determined how the phosphodiester bond is cleaved either at the 3′ end of the bond or at the 5′ end of the bond. Our results also show that the RNA cleavage by PemK is independent of ribosomes, which is distinctly different from RelE, the toxin encoded by the relBE addiction module. RelE is not able to cleave free RNA but stimulates mRNA cleavage at the ribosome A site with a high codon specificity (20, 21, 30).

In the previous study on the kis-kid system, which is an addiction module identical to the pemI-pemK system, it has been reported that Kid (PemK) inhibits the in vitro ColE1 replication at the initiation stage but has no significant effect on the P4 DNA replication. DnaB has been proposed as the target for the inhibitory action of Kid (PemK) (12). However, so far there have been no data to support the interaction between Kid (PemK) and DnaB. It is interesting to note that the ColE1 replication is initiated by RNA II and inhibited by RNA I (39, 40), whereas the P4 DNA replication is mainly regulated by α protein (41). RNases involved in the metabolism of RNA I and RNA II are expected to play a key role in the control of the ColE1 plasmid replication (42). RNA II contains several UAC sequences, two of which exist in the loop regions of the first and second stem-loop structures (43, 44). Therefore, the inhibition of ColE1 DNA replication by Kid (PemK) is probably because of degradation of RNA II by its endoribonuclease activity. Furthermore, the fact that Kid (PemK), a toxin in bacteria, inhibits the growth of various eukaryotic cells (33) can be readily explained by its endoribonuclease activity against cellular mRNAs rather than by its interaction with DnaB.

PemK homologues are identified in a wide range of bacteria. MazF (ChpAK) and ChpBK are the two PemK-like proteins in E. coli (26–28). MazF (ChpAK), the toxin encoded by the mazEF addiction module, is 25% identical with PemK, ChpBK, the toxin encoded by the chpB addiction module, is 41% identical with PemK. It had been demonstrated that MazF (ChpAK) and ChpBK inhibit translation by cleaving mRNAs in a manner similar to RelE (28). On the other hand, we have recently demonstrated that MazF is an endoribonuclease that acts independently of ribosomes and inhibits protein synthesis by sequence specifically cleaving single-stranded mRNA (31). MazF preferentially cleaves mRNA between A and C residues at the ACA sequence (31). In this paper, we demonstrated that Kid (PemK), a toxin in bacteria, inhibits the growth of various eukaryotic cells (33) can be readily explained by its endoribonuclease activity against cellular mRNAs rather than by its interaction with DnaB.

The crystal structure of Kid (PemK) protein has been determined as a homodimer (11, 45). Although the structure of MazF has not been determined, Kamada et al. (24) have reported the crystal structure of the MazE-MazF complex (MazF2-MazE2-MazF2), which was formed by two MazF homodimers and one...
mRNAs. It is well known that the small RNAs, such as miRNA, act as inhibitors by interfering with the function of cellular proteins, and siRNA (48), interfere with the function of the specific target RNAs. The ribozyme also acts on the target RNA specifically and sequence-specifically.

Kid (PemK) triggers apoptosis in human cells, because PemK and the PemK homologues form a novel endoribonuclease family with a new mRNA-interfering mechanism by cleaving mRNAs at specific sequences, which could be termed as “mRNA interferase.” mRNA interferases may be useful to detect the second structures in RNA, because both PemK and MazF are able to cleave only single-stranded RNAs. As reported previously, Kid (PemK) triggers apoptosis in human cancer cells, whereas Kis (Peml) inhibits the toxic effect of Kid (PemK) (39). Therefore, this new regulatable mRNA-interfering system could be used for the gene therapy for human diseases.

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