upwelling period (17 July through 1 August) was 31.6% lower than during the other four periods (mean ± SEM = 2.48 ± 0.32 sea stars/m^2, n = 12 versus 3.63 ± 0.26 sea stars/m^2, n = 48) and was consistently higher at Pigeon Reef than at the other two sites (mean ± SEM = 4.36 ± 0.38 sea stars/m^2, n = 20 versus 2.92 ± 0.25 sea stars/m^2, n = 40).

16. I used multiple regressions to test whether variation in mean interaction strengths among time periods and sites was associated with (i) water temperature (the mean during 27 high tides per period), (ii) potential heat stress (the mean of maximum low tide air temperature on the five warmest days per period), or (iii) wave stress (the mean of maximum force per day on 5 to 7 days per period). Per capita interaction strength was associated with water temperature (P < 0.001) but was unrelated to potential heat stress (P = 0.18) or wave stress (P = 0.53). Similarly, population interaction strength was correlated with water temperature (P < 0.001) but not with potential heat stress (P = 0.13) or wave stress (P = 0.74). Site variables were significant in both models because both per capita and population interaction strength were consistently higher at Pigeon Reef, the site with higher sea star density (*15*). Together, water temperature and site explained 80.9% of the variation in mean per capita interaction strength and 82.4% of the variation in mean population interaction strength.

17. In early June 1996, 48 sea stars (wet weight, 118 to 138 g) were collected from Neptune State Park. Four individuals were randomly assigned to each of 12 closed 110-liter tanks held in a cold room, and heaters with controllers self-regulated treatments to ±0.1°C. Water was circulated by two pumps in each tank, and water quality was maintained by filters and weekly water changes. Salinity was maintained at 36 ± 1 parts per thousand, and the experiments were conducted under a schedule of 12 hours of light and 12 hours of darkness. All sea stars were initially acclimated without food at 11°C for 10 days, and then treatments were randomly assigned (n = 4 tanks per treatment).

18. *Mytilus trossulus* was used because this species is the most common prey item in *Pisaster's* diet at these sites. I quantified the diet of actively feeding sea stars (n = 1664) on 14 dates during the summer of 1995 and 1996. The percents of individuals feeding on a given prey species were as follows: mussels (*M. trossulus*, 56.0%; *M. californianus*, 5.0%), barnacles (*Pollicipes polymerus*, 41.8%; *Balanus glandula*, 6.0%; *Semibalanus cariousus*, 3.2%; *Chthamalus dalli*, 1.4%; *B. nubilus*, 0.7%), whelks (*Nucella* spp., 1.5%), and limpets (*Lottia* spp., 0.5%). The total exceeds 100% because *Pisaster* often feeds on several prey species at a time.

19. Results are presented for the first three periods of the experiment. Thereafter, sea stars became temporarily satiated on the ad libitum diet, and feeding rates declined sharply in all treatments. After 4.5 months, sea stars used in the experiment had energy stores (pyloric ceca) much larger than those of field animals. Thus, the initial phase of the experiment, with sea stars recently collected from the field, best reflects the effects of temperature on *Pisaster* with natural levels of energy reserves.

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21. *Pisaster ochraceus* ranges from at least Punta Baja in Baja California to Prince William Sound in Alaska, and populations near these geographic limits regularly experience water temperatures >20°C and <4°C, respectively.

22. I thank L. C. Ryan for field assistance and support; P. Sanford for engineering expertise; J. Lubchenco, B. Menge, G. Allison, E. Berlow, D. Bermudez, M. Bertness, J. Burnaford, B. Grantham, P. Halpin, M. Hixon, S. Navarrete, K. Nielsen, and G. Somero for helpful discussions and reviews; and L. Weber for laboratory space at Hatfield Marine Science Center. This research was supported by a NSF Predoctoral Fellowship, the Lerner-Gray Fund for Marine Research, and a National Wildlife Federation Climate Change Fellowship, as well as NSF grants to B. Menge and funds provided to J. Lubchenco and B. Menge by the Andrew W. Mellon and Wayne & Gladys Valley Foundations.

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A Cytotoxic Ribonuclease Targeting Specific Transfer RNA Anticodons

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The carboxyl-terminal domain of colicin E5 was shown to inhibit protein synthesis of *Escherichia coli*. Its target, as revealed through in vivo and in vitro experiments, was not ribosomes as in the case of E3, but the transfer RNAs (tRNAs) for Tyr, His, Asn, and Asp, which contain a modified base, queuine, at the wobble position of each anticodon. The E5 carboxyl-terminal domain hydrolyzed these tRNAs just on the 3′ side of this nucleotide. Tight correlation was observed between the toxicity of E5 and the cleavage of intracellular tRNAs of this group, implying that these tRNAs are the primary targets of colicin E5.

A variety of proteinaceous toxins inhibit protein synthesis; they have been used to elucidate complicated cell mechanisms. Ribosomes are one of the most sophisticated targets and are susceptible to many toxins, including plant-derived ricin, bacterial Shiga toxin and Shiga-like toxins, and a fungal α-sarcin (*1*). Colicin E3 and cloacin DF13 are special ribonucleases (RNases) that cleave 16*S* ribosomal RNA (rRNA) at the 49th phosphodiester bond from the 3′ end (*2*). Colicins comprise a treasury of cytotoxins with well-defined structures and modes of action.

Among the E-group colicins, which share receptor BtuB for the initial step of killing, E3 to E6 quickly stop amino acid incorporation into treated cells, suggesting inhibition of protein synthesis. On the basis of analogy with E3, E4 to E6 have been thought to be RNases (*3*). The nuclease type colicins exhibit high conservation in their NH_2-terminal large regions required for receptor binding and membrane transfer, and their nuclease activities are exclusively due to their small COOH-terminal domains, where sequence variations are concentrated (*4*, *5*). In this respect, E4 and E6 are in fact E3 homologs (*6*); however, the COOH-terminal region of E5 exhibits no sequence similarity to E3 (*7*). Moreover, these colicins are accompanied by specific inhibitors, Imm proteins, which account for the immunity of colicinogenic cells. Here again ImmE5 is excluded from the homology shared by ImmE3, ImmE4, and ImmE6. We thus suspected that E5 has a different target site on ribosomes, or even outside of ribosomes, for possible interference with protein synthesis.

To examine E5 activity in vitro, we focused on its COOH-terminal nonhomologous domain; a plasmid ColE5-099 DNA segment encoding both the COOH-terminal 115 amino acids of E5 (E5-CRD) and ImmE5 was cloned under the colicin E3 promoter (*8*). The NH_2-terminal sequencing of the purified ImmE5 revealed that the *imm* gene starts 78 base pairs upstream of the location previously speculated (*7*) and produces a 108–amino acid protein (*9*).

E5-CRD in fact caused a substantial decrease of the MS2 RNA-dependent amino acid incorporation in a cytoplasmic fraction separated at 30,000*g* (S-30) of *E. coli* (*10*) (Fig. 1B). This decrease was not due to any contamination by nucleases because the decrease was completely prevented by preincubation with ImmE5, as in the case of E3-CRD and ImmE3 (Fig. 1A). Curiously, however, the inhibitory effect of E5-CRD, unlike that of E3-CRD, was not observed when the incorporation of [^{14}C]Phe was measured with polyuridylate [poly(U)] as the template (Fig. 1, C and D), suggesting different modes of action of E3 and E5.

The action of E5-CRD on RNA was examined (Fig. 2A). E5-CRD degraded protein-free *E. coli* rRNAs, and this degradation was effectively inhibited by ImmE5, excluding the pos-

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sibility of contaminating nucleases. Under the same conditions, E3-CRD did not cause obvious RNA degradation in spite of good ribosome inactivation activity (*11*, *12*). To find possible alterations of ribosomes, we incubated an *E. coli* ribosome fraction (*10*) with E5-CRD or E3-CRD and extracted RNA fractions and analyzed them by a low-resolution gel after 5′ labeling (Fig. 2B). The RNA fraction from the E3-CRD treatment gave the so-called E3 fragment of 49 bases derived from the 3′ end of 16*S* rRNA. On the other hand, the E5-CRD treatment gave a shorter distinct RNA band, corresponding to about 42 nucleotides, as well as a fainter band similar to the E3 fragment. Both bands disappeared on the addition of ImmE5, indicating that the changes were due to the action of E5-CRD itself.

The 5′-labeled fragments were extracted from the gel and subjected to direct sequencing (*13*); the minor RNA observed for the E5-CRD treatment was in fact a 3′ fragment of the 16*S* rRNA (*14*), but the major band comprised a mixture of some RNA fragments, which could be separated into three distinct RNAs by careful electrophoresis on a native gel (*15*). These materials were not derived from ribosomes but were surprisingly coincident with the 3′ half fragments of the tRNAs for His, Asn, and Asp (*11*).

The tRNAs for His, Asn, and Asp, which may be sensitive to E5-CRD, decode NAY (where N is any nucleoside and Y is pyrimidine) in the third column of the codon table. But the most noticeable feature is a unique modified base, queuine or Q, as the first letter

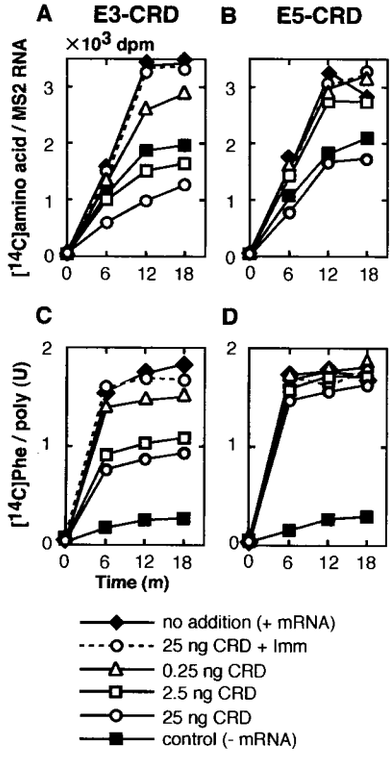

Fig. 1. MS2 RNA-dependent amino acid incorporation (**A** and **B**) and poly(U)-dependent Phe incorporation (**C** and **D**) of the S-30 fraction after incubation with E3-CRD (A and C) or E5-CRD (B and D). Two absorbance at 260 nm (A_{260}) units of S-30 fraction preincubated for 7 min was further incubated with E3-CRD or E5-CRD for 10 min in 20 μl of 5 mM tris-HCl (pH 7.8), 5 mM $MgAc_2$ (where Ac is acetyl), 25 mM KCl, and bovine serum albumin (0.1 mg/ml). In one portion, 25 ng of E3-CRD or E5-CRD was preincubated for another 10 min with 20 times the molar amount of ImmE3 or 15 times the molar amount of ImmE5, respectively, before mixing with the S-30 fraction. Either 30 μg of MS2 RNA and a [^{14}C]amino acid mixture or 60 μg of poly(U) and [^{14}C]Phe was then mixed with the S-30 fraction to give a final volume of 60 μl, from which 10 μl was withdrawn at intervals to follow the radioactivity incorporated into the acid-insoluble fraction. All reactions were carried out at 37°C.

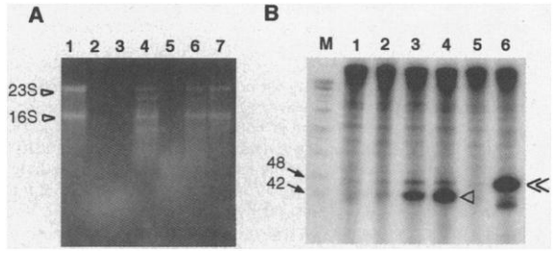

Fig. 2. RNA hydrolysis by E5-CRD (**A**) and small RNA fragments obtained on incubation of the ribosome fraction with E5-CRD (**B**). (A) Two micrograms of 23*S* and 16*S* RNA of *E. coli* MRE600 (Boehringer Mannheim) was incubated without (lane 1) or with 200 ng (lane 2), 20 ng (lane 3), or 2 ng (lane 4) of E5-CRD for 30 min at 37°C in 20 μl of buffer A [10 mM tris-HCl (pH 7.8), 10 mM $MgAc_2$, and 50 mM KCl]. Two hundred nanograms of E5-CRD was preincubated with 0.8, 1.5, or 3.8 times the molar amount of ImmE5 (lanes 5, 6, and 7, respectively) for 15 min before starting the reaction. The samples were electrophoresed on a 1% agarose gel and visualized with ethidium bromide. (B) 0.3 A_{260} units of ribosomes was incubated without (lane 1) or with 0.02 ng (lane 2), 0.2 ng (lane 3), or 2 ng (lane 4) of E5-CRD or with 2 ng of E3-CRD (lane 6) in 50 μl of buffer A for 30 min at 37°C. For lane 5, 2 ng of E5-CRD was preincubated with 15 times the molar amount of ImmE5 for 15 min at 37°C before mixing with the ribosomes (*29*). ImmE5 did not inhibit the E3-CRD activity at all (*11*). M, 5′ ^{32}P-labeled size markers of ϕX174 DNA/Hin fI, with 48 and 42 bases indicated. The triangle indicates the about 42-base RNA band specific to the E5-CRD treatment, which is distinct from the colicin E3 fragment of 49 bases (double arrowhead). The 5′-labeled RNA samples were electrophoresed on a 10% polyacrylamide gel containing 7 M urea.

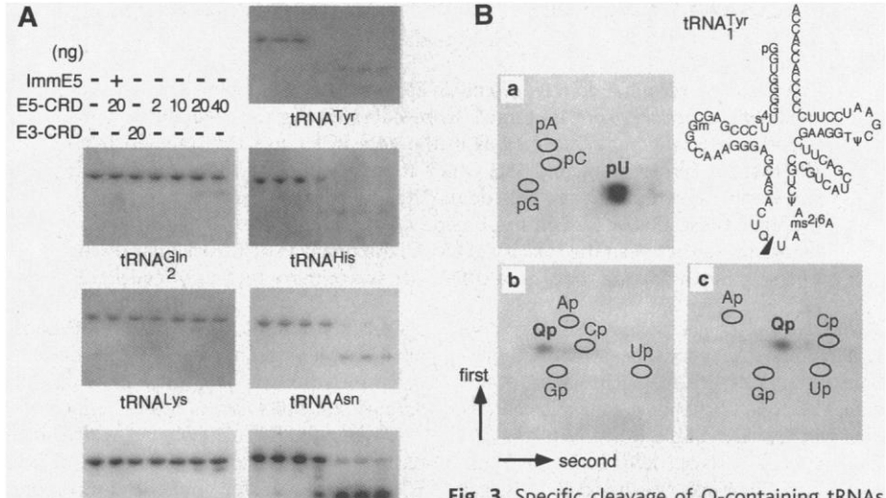

Fig. 3. Specific cleavage of Q-containing tRNAs by E5-CRD (**A**) and end analysis of the tRNA$_1^{Tyr}$ cleaved by E5-CRD (**B**). (A) Sixty-seven micrograms of the total tRNA (*E. coli* MRE600; Boehringer Mannheim) was incubated with the indicated amounts of E5-CRD or E3-CRD for 30 min at 37°C in 50 μl of buffer A. Fifteen times the molar amount of ImmE5 was preincubated with E5-CRD for 15 min at 37°C before the tRNAs were added. Four micrograms of RNAs was electrophoresed on a 10% polyacrylamide gel containing 7 M urea and transferred for Northern blot analysis. (B) 8.4 μg of *E. coli* tRNA$_1^{Tyr}$ (Sigma) was digested with 128 ng of E5-CRD in buffer A, and the products were gel purified. (**a**) The 3′ half fragment was 5′^{32}P-labeled and completely digested with nuclease P1, which gave labeled uridine 5′-phosphate (pU) on TLC. (b and c) The dephosphorylated 5′ half fragment was labeled with [^{32}P]pCp and T4 RNA ligase and completely digested with RNase T2, which gave labeled queuosine 3′-phosphate (Qp) by two different solvent systems (*30*). The clover leaf structure of tRNA$_1^{Tyr}$ with the cleavage site (arrowhead) is shown. pA, pC, pG, and pU, respective ribonucleoside 5′-phosphates; Ap, Qp, Cp, Up, and Gp, respective ribonucleoside 3′-phosphates.

of anticodons, corresponding to the wobble site pyrimidine (*16*). The remaining members based on the above criteria are the pair, $tRNA_1^{Tyr}$ and $tRNA_2^{Tyr}$, differing only in two adjacent nucleotides in their variable loops. Because of the greater lengths of these loops, the 3′ half fragments cleaved off by E5-CRD, if any, would have moved more slowly on electrophoresis.

Here we raise two questions. First, does the E5-CRD activity toward this group of tRNAs require ribosomes? Second, are the two $tRNA^{Tyr}$ molecules susceptible to E5-CRD, as the other three Q-containing tRNAs are? To address these questions, we incubated an authentic *E. coli* tRNA mixture with E5-CRD or E3-CRD in the absence of ribosomes and then analyzed it by Northern (RNA) blot hybridization (Fig. 3A). We prepared synthetic 30-mer DNA probes complementary to the 3′-terminal regions of $tRNA^{Tyr}$, $tRNA^{His}$, $tRNA^{Asn}$, and $tRNA^{Asp}$, as well as ones for $tRNA_2^{Gln}$, $tRNA^{Lys}$, and $tRNA^{Glu}$ as control tRNAs decoding NAR (where R is purine) in the third column of the codon table (*17*).

It is shown in Fig. 3A that the Q-containing tRNAs, including $tRNA^{Tyr}$ (the probe was designed for $tRNA_1^{Tyr}$ but does not distinguish between the two $tRNA^{Tyr}$ molecules under the conditions used), were cleaved by E5-CRD in the absence of ribosomes, whereas the control tRNAs were fully resistant. These activities were effectively inhibited by ImmE5, again excluding the possibility of contaminating RNases. Thus, E5-CRD cleaves Q-containing *E. coli* tRNAs irrespective of the presence or absence of ribosomes. The susceptible tRNAs in the ribosome fraction (Fig. 2B) may have been bound to ribosomes or merely contained in the fraction, which was prepared by simple centrifugation only (*10*).

To identify the product or products of $tRNA^{Tyr}$ on cleavage by E5-CRD, we 5′ labeled the total *E. coli* tRNAs incubated with E5-CRD and then resolved them by polyacrylamide gel electrophoresis. Besides the major material of about 42 bases corresponding to the 3′ half fragments of the above three E5-CRD–sensitive tRNAs, a distinct band of about 50 bases was also observed. This fragment was isolated and further separated by native PAGE into two close bands, which were confirmed to represent the 3′ halves of $tRNA_1^{Tyr}$ and $tRNA_2^{Tyr}$ by sequencing (*11*, *13*).

The cleavage site of $tRNA_1^{Tyr}$ with E5-CRD and the 5′ and 3′ end forms of the products were analyzed by two-dimensional thin-layer chromatography. Although the 5′ end of the 3′ half fragment of $tRNA_1^{Tyr}$ could be directly kinased, 3′ labeling of the 5′ half fragment with [5′-^{32}P]cytidine 3′,5′-bis(phosphate)([^{32}P]pCp) by T4 RNA ligase required preceding dephosphorylation with T4 polynucleotide kinase (*18*). This could not be attained with alkaline phosphatase, suggesting that the 3′ end of the cleavage site forms 2′,3′-cyclic phosphate (*19*). Thus, the 3′-labeled 5′ fragment gave 3′-labeled queuosine phosphate after RNase T2 digestion, and the 5′-labeled 3′ fragment gave 5′-labeled uridine phosphate after nuclease P1 digestion (Fig. 3B). This result shows that E5-CRD hydrolyzed the phosphodiester bond between Q34 and U35, that is, just on the 3′ side of the wobble position. We also confirmed that E5-CRD cleaves the corresponding site of $tRNA^{Asp}$ (*11*).

It is important to determine whether the in vitro activity of E5-CRD toward the tRNAs shown above reflects the physiological action of colicin E5 leading to cell death in a distinguishable way from that of colicin E3. *Escherichia coli* strains (*20*) grown to the logarithmic phase were challenged with colicin E5 or E3 (*21*), and RNA fractions were extracted from the cells for Northern blot analyses with synthetic DNA probes for tRNAs and for the 3′-terminal sequence of 16*S* rRNA. As can be seen by comparison of the second lanes in the panels of Fig. 4A, only the Q-containing tRNAs are cleaved by colicin E5 in vivo, which is consistent with the substrate specificity of E5-CRD, as an RNase (Fig. 3A). These changes depended on the presence of the functional colicin receptor (Fig. 4A, third lanes), suggesting that these are all physiological events caused by colicin E5. On the other hand, colicin E3 did not affect tRNAs in spite of cleaving 16*S* rRNA effectively. Under the same conditions, colicin E5 did not act on 16*S* rRNA at all, in interesting contrast to the results of in vitro incubation of ribosomes with E5-CRD. The minor action of E5-CRD toward the E3 target site of 16*S* rRNA observed in vitro (Fig. 2B) should not be essential, if at all, for the physiological action of colicin E5.

Toxicity of various amounts of colicin E5

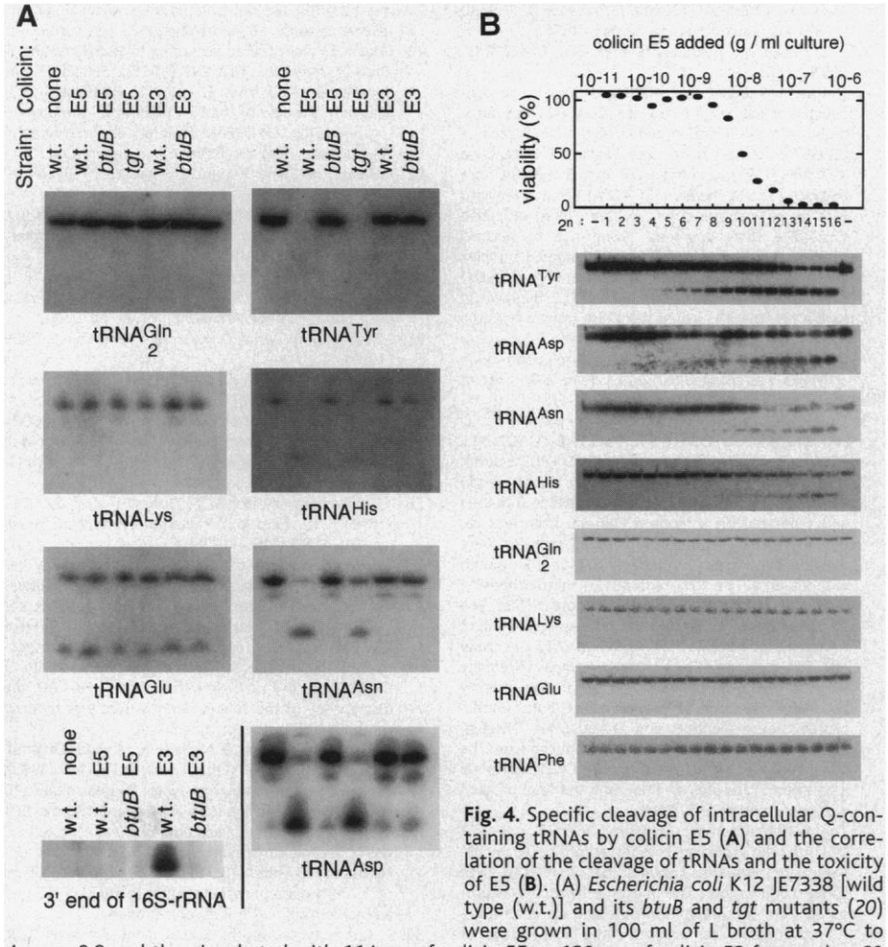

Fig. 4. Specific cleavage of intracellular Q-containing tRNAs by colicin E5 (**A**) and the correlation of the cleavage of tRNAs and the toxicity of E5 (**B**). (A) *Escherichia coli* K12 JE7338 [wild type (w.t.)] and its *btuB* and *tgt* mutants (*20*) were grown in 100 ml of L broth at 37°C to A_{660} = 0.8 and then incubated with 114 μg of colicin E5 or 120 μg of colicin E3 for another 60 min. RNAs were extracted from the harvested cells according to (*31*). Electrophoresis and Northern blot analysis were performed as in Fig. 3A. A DNA probe for the 3′ end sequence of 16*S* rRNA was also used (*17*), showing that the cleavage is specific to E3. (B) Logarithmic phase cultures of *E. coli* K12 W3110 in 20 ml of L broth were incubated for 30 min with colicin E5, which had been serially diluted by a factor of two as indicated. −, control samples without colicin. The cells were harvested to compare their colony-forming activities and the cleavage of intracellular tRNAs, which was determined by Northern blot hybridization.

to *E. coli* cells was precisely compared with the extents of cleavage of intracellular tRNAs (Fig. 4B). Substantial fractions of Q-containing tRNAs, in particular the $tRNA^{Tyr}$ molecules and $tRNA^{Asp}$, were cleaved even by the smallest amount of colicin E5 affecting cell viability, providing convincing evidence that these tRNAs are the primary targets of colicin E5. Figure 4B also shows the E5 resistance of $tRNA^{Phe}$, which explains the E5-CRD resistance of the S-30 fraction in the poly(U)-dependent Phe incorporation (Fig. 1D).

Queuine is a guanine analog and is incorporated into specific tRNAs as a precursor through a base change of the gene-coded G by tRNA-guanine transglycosylase (TGT), followed by further modification to Q (*16*). So in TGT-defective cells, the tRNAs concerned have an inherent G at the wobble position. Nonetheless, *tgt* mutant strains were as sensitive to colicin E5 as the wild-type strain (*11*). Consistently, the tRNAs concerned within the *tgt* mutant were cleaved by E5 (Fig. 4A), suggesting that Q itself is not absolutely required for cleavage by E5, although Q-containing tRNAs are selected as natural substrates. In this regard, E5-CRD and TGT could recognize tRNAs in a similar manner, but sequence homology was not found between them (*22*).

E5-CRD is unique not only as a cytotoxin but also as an RNase. The end forms of the RNAs cleaved by E5-CRD (Fig. 3B) might suggest an enzymic mechanism similar to those of well-known RNases A or T1 (*23*). But this is not the case because, besides the lack of sequence homology with them, E5-CRD has no histidine in its molecule, which is an indispensable general acid for the catalytic mechanisms of traditional RNases (*23*, *24*).

In a certain clinical *E. coli* strain, an anticodon nuclease, PrrC, is induced on phage T4 infection and cleaves its own $tRNA^{Lys}$ at the 5′ side of the anticodon, leading to suicide, which interferes with propagation of the phage (*25*). In spite of the apparent similarity in their actions toward tRNAs, no sequence homology was found between E5-CRD and PrrC, and E5-CRD does not cleave $tRNA^{Lys}$ either in vitro or in vivo (Figs. 3A and 4). Angiogenin belonging to the RNase A superfamily was claimed to digest tRNAs preferably when incubated in vitro or artificially microinjected into *Xenopus* oocytes (*26*). However, there is no evidence that it cleaves a specific site of specific tRNAs like E5-CRD.

The most conspicuous feature common to the E5-sensitive tRNAs, disregarding base modifications, is the UGU at positions 33 to 35 (*27*). E5-CRD should recognize this sequence, something like "an RNA restriction enzyme," possibly in some structural context. E5-CRD tightly binds to the cognate inhibitor protein, ImmE5. Also of interest is whether E5-CRD mimics specific codons and ImmE5 mimics a part of the corresponding anticodons, as in the intriguing case of translation release factors possibly recognizing termination codons by mimicking tRNA structures (*28*).

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8. The plasmid producing E5-CRD and ImmE5 was constructed as follows. A Nco I site (CCATGG) was introduced into the initiation codon of the *colE3* gene of pSH357 [H. Masaki, A. Akutsu, T. Uozumi, T. Ohta, *Gene* **107**, 133 (1991)], and the *colE3-immE3* segment from this Nco I site to the Bcl I (TGATCA) site was replaced with a polymerase chain reaction (PCR)–amplified ColE5-099 DNA fragment, giving rise to plasmid pTO501, which produces the E5-CRD/ImmE5 complex in response to SOS inducers. For this purpose, an Nco I site was created at the 5′ end of the PCR fragment in such a way that the Asp at the 116th residue from the COOH-terminus of E5 was changed to an fMet, where the ATG is within the Nco I site. The termination codon of *immE5* was changed to TGA to create a Bcl I site at the 3′ end of the PCR fragment.
9. The E5-CRD/ImmE5 complex was prepared from *E. coli* K12 RR1 [pTO501] with a DEAE-TOYOPEARL 650S column (Tosoh) and a Mono S FPLC column (Pharmacia). E5-CRD and ImmE5 were dissociated in 20 mM sodium acetate (pH 5.0) containing 6 M urea and separated on a Mono S column. Urea was removed by dialysis, and the refolded proteins were individually further purified on a Mono S column without urea. The NH_2-terminus of the purified E5-CRD was confirmed to be Ala, next to the fMet. The ImmE5 protein, as limited to this paper, proved to have a mutation of Ile^{62} (ATA) to Met (ATG), possibly introduced during PCR, but no functional difference was detected from the wild type, which was recently purified. NH_2-terminal sequences of both ImmE5 proteins were identical and lacked fMet. Through similar construction, E3-CRD was produced from the truncated *colE3* gene encoding the COOH-terminal 110 amino acids plus an fMet, and the final product was confirmed to lack fMet.
10. The S-30 fraction was prepared from *E. coli* K12 W3110 cells grown in L broth according to M. W. Nirenberg [*Methods Enzymol.* **6**, 17 (1963)] with some modifications. The ribosome fraction was prepared from the precipitate obtained on centrifugation at 100,000*g* for 4 hours of the S-30 fraction of *E. coli* K12 A19 cells and resuspended in 10 mM tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM KCl, and 1 mM dithiothreitol.
11. T. Ogawa *et al.*, data not shown.
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14. Figure 2B suggests almost the same size for the E3 fragment and the minor RNA band by E5-CRD, but in fact, just the 5′ end was not determined in the sequencing of the E5-CRD minor band (*11*). The new high-resolution gel suggests that the E5-CRD minor band is 1 base smaller (48 bases?) than the major E3 fragment (49 bases). The 3′ next sequence of the E3-sensitive site, A50-G49, is G49-U48 (numbers being from the 3′ end) in 16*S* rRNA. If E5-CRD preferentially cleaves this GU in 16*S* rRNA, this interestingly resembles the cleavage site, GU (QU), within the E5-sensitive tRNA anticodons (Fig. 3B).
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21. Colicin E3 was purified according to H. Masaki and T. Ohta [*J. Mol. Biol.* **182**, 217 (1985)]. Colicin E5 was partially purified from *E. coli* K12 RR1(ColE5-099) cells as in the case of the E5-CRD/ImmE5 complex up to the DEAE-TOYOPEARL column chromatography (*9*). Its purity was densitometrically estimated.
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