Quorum Sensing Extracellular Death Peptides Enhance the Endoribonucleolytic Activities of *Mycobacterium tuberculosis* MazF Toxins

Akanksha Nigam,a Sathish Kumar,a,* Hanna Engelberg-Kulka

aDepartment of Microbiology and Molecular Genetics, IMRIC, the Hebrew University-Hadassah Medical School, Jerusalem, Israel

**ABSTRACT**  
*mazEF* is a toxin-antitoxin module located on chromosomes of most bacteria. MazF toxins are endoribonucleases antagonized by MazE antitoxins. Previously, we characterized several quorum sensing peptides called "extracellular death factors" (EDFs). When secreted from bacterial cultures, EDFs induce interspecies cell death. EDFs also enhance the endoribonucleolytic activity of *Escherichia coli* MazF. *Mycobacterium tuberculosis* carries several *mazEF* modules. Among them, the endoribonucleolytic activities of MazF proteins mt-1, mt-3, and mt-6 were identified. MazF-mt6 and MazF-mt-3 cleave *M. tuberculosis* rRNAs. Here we report the *in vitro* effects of EDFs on the endoribonucleolytic activities of *M. tuberculosis* MazFs. *Escherichia coli* EDF (EcEDF) and the three *Pseudomonas aeruginosa* EDFs (PaEDFs) individually enhance the endoribonucleolytic activities of MazF-mt6 and MazF-mt3 and overcome the inhibitory effect of MazE-mt3 or MazE-mt6 on the endoribonucleolytic activities of the respective toxins. We propose that these EDFs can serve as a basis for a novel class of antibiotics against *M. tuberculosis*.

**IMPORTANCE**  
*Mycobacterium tuberculosis* is one of the leading causes of death from infectious disease. *M. tuberculosis* is highly drug resistant, and drug delivery to the infected site is very difficult. In previous studies, we showed that extracellular death factors (EDFs) can work as quorum sensing molecules which participate in interspecies bacterial cell death. In this study, we demonstrated the role of different EDFs in the endoribonucleolytic activities of *M. tuberculosis* MazFs. *Escherichia coli* EDF (EcEDF) and the three *Pseudomonas aeruginosa* EDFs (PaEDFs) individually enhance the endoribonucleolytic activities of MazF-mt6 and MazF-mt3. The current report provides a basis for the use of the EDF peptides EcEDF and PaEDF as novel antibiotics against *M. tuberculosis*.

**KEYWORDS** extracellular death peptides, *Mycobacterium tuberculosis*, quorum sensing

**T**oxin-antitoxin (TA) modules are abundant on the chromosomes of most bacteria (1–8), including pathogens such as *Staphylococcus aureus* (7) and *Mycobacterium tuberculosis*, which carries at least 88 putative TA systems (8). Each of these modules consists of a pair of genes, usually transcribed as operons, in which the downstream gene encodes a stable toxin and the upstream gene encodes an unstable antitoxin (1–8). Among them, the most extensively studied and the first to have been described is the *Escherichia coli mazEF* system (9). *E. coli* MazF functions as a stable toxin, and MazE functions as an antitoxin degraded by ClpPA protease (9). *E. coli* MazF toxin is a sequence-specific endoribonuclease cleaving at ACA site (10) that was initially described to preferentially cleave single-stranded mRNAs and therefore was designated an mRNA interferase (11). However, subsequently, *E. coli* MazF was also shown to target the 16S rRNA within the 30S ribosomal subunit at the decoding center, thereby

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Address correspondence to Hanna Engelberg-Kulka, hanita@cc.huji.ac.il.

* Present address: Sathish Kumar, Department of Microbiology and Immunology, Stanford University, Stanford, California, USA.

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significant increase in the fluorescence signal corresponding to FAM (6-carboxyfluorescein [FAM]) at its 5’ end and a quencher molecule (BHQ1) at its 3’ end. Cleavage of this constructed substrate by MazF-mt6 led to an increase in the distance between the fluorophore and the quencher, causing a significant increase in the fluorescence signal corresponding to FAM (Fig. 1a). Adding EcEDF to this reaction mixture led to an increase in MazF-mt6 activity. Adding 4 μM

**RESULTS**

**EcEDF enhances the in vitro endoribonucleolytic activity of the** *M. tuberculosis* **toxin** MazF-mt6. **We started by studying the effects of each of the several EDF peptides on the endoribonucleolytic activity of the** *M. tuberculosis* **MazF toxin MazF-mt6. Using affinity chromatography, as we have described previously (19), we prepared highly purified MazF-mt6. To measure MazF-mt6 activity, we used the continuous fluorometric assay (20) for the quantification and kinetic analysis of MazF endoribonucleolytic activity in real time. As mentioned above, the MazF-mt6 target site is UU ↓ CCU (13). Therefore, as a substrate for MazF-mt6, we used a chimeric oligonucleotide composed of RNA bases (rU), including UU ↓ CCU, flanked by DNA nucleotides, and labeled with a fluorophore molecule (6-carboxyfluorescein [FAM]) at its 5’ end and a quencher molecule (BHQ1) at its 3’ end. Cleavage of this constructed substrate by MazF-mt6 led to an increase in the distance between the fluorophore and the quencher, causing a significant increase in the fluorescence signal corresponding to FAM (Fig. 1a). Adding EcEDF to this reaction mixture led to an increase in MazF-mt6 activity. Adding 4 μM
EcEDF led to an increase in MazF-mt6 activity of 40%, and adding 8 μM EcEDF led to an increase of 55% (Fig. 1b).

The ability of EcEDF to enhance the in vitro activity of MazF-mt6 was dependent on the specific amino acid sequence of the EcEDF molecule. We wondered about the contribution to the enhancement of MazF-mt6 activity of each of the amino acids in the pentapeptide EcEDF. We constructed and tested seven derivatives of NNWNN, the wild-type EcEDF, in which we either replaced each of the original amino acids with a glycine residue (G) or changed the length of the peptide. Replacing each of the amino acids of the wild-type sequence significantly interfered with the ability of EcEDF to enhance the in vitro activity of MazF-mt6 (Fig. 2a to e). Replacing the central tryptophan (W) with glycine (G) (Fig. 2a) or removing the asparagine residues (N) from each end (NWN) (Fig. 2g) eliminated the peptide-induced stimulation of MazF-mt6 which had been observed with the native peptide. Adding N residues at each end to create the EcEDF septapeptide NNNWNNN did not significantly interfere with the ability of EcEDF to enhance MazF-mt6 activity (Fig. 2f).

EcEDF overcomes the inhibitory effect of MazE-mt6 on the endoribonucleolytic activity of MazF-mt6. We also asked whether EcEDF could overcome the inhibitory effect of the antitoxin MazE-mt6 on the endoribonucleolytic activity of MazF-mt6. To this end, we carried out an experiment under conditions in which MazE-mt6 almost completely inhibited MazF-mt6 activity. As shown, this inhibitory effect was almost completely reversed by adding EcEDF (Fig. 3a). We also tested the importance of each of the amino acids of EcEDF in overcoming the inhibitory effect of MazE-mt6. We found that only the central amino acid, tryptophan, played a critical role. When tryptophan (W) was replaced with glycine (G), the EcEDF derivative no longer overcame inhibition by MazE-mt6 (Fig. 3b). In contrast, replacing each of the other amino acids of EcEDF still permitted the derivatives to overcome the inhibitory effect of MazE-mt6 (Fig. 3c to 3e). In addition, lengthening of EcEDF to NNNWNNN partially overcame the inhibitory effect.
of MazE-mt6 (Fig. 3f), and shortening the length of EcEDF to NWN did not permit the herein described overcoming effect (Fig. 3g; for a summary, see Table 1).

As in the case of MazEF-mt6, EcEDF both enhanced the endoribonucleolytic activity of *M. tuberculosis* toxin MazF-mt3 and overcame the inhibitory effect of MazE-mt3 on MazF-mt3. Like MazF-mt6, MazF-mt3 is an *M. tuberculosis* MazF endori-
FIG 3  EcEDF overcomes the inhibitory effect of MazE-mt6 on MazF-mt6 endoribonucleolytic activity in vitro. (a) A mixture of (0.5 μM) MazE-mt6 and (4 μM or 8 μM) EcEDF (NNWNN) was added to preparations of (0.5 μM) MazF-mt6. (Left) The in vitro activity of MazF-mt6 was inhibited in the presence of MazE-mt6. (Right) Percent (%) increase in MazF-mt6 activity after the addition of EcEDF to wells containing both MazF-mt6 and MazE-mt6. (b) Data were determined as described for panel a, but instead of NNWNN, NNGNN was added. (c) Data were determined as described for panel a, but instead of NNWNN, GNWNN was added. (d) Data were determined as described for panel a, but instead of NNWNN, NGWNN was added. (e) Data were determined as described for panel a, but instead of NNWNN, NNWGN was added. (f) Data were determined as described for panel a, but instead of NNWNN, NNWNG was added. (g) Data were determined as described for panel a, but instead of NNWNN, a lengthened, modified EDF to which a new N residue was attached at each end (NNNWNNN) was added. (h) Data were determined as described for panel a, but instead of NNWNN, a shortened, modified EDF from which an N residue has been removed from each end (NWN) was added. The data represent means ± standard deviations of results from three experiments performed in triplicate. *, P < 0.01; **, P < 0.001; ***, P < 0.0001 (statistical significance compared to the control data).
While the target site for MazF-mt6 is UUCCU (13), the target site for MazF-mt3 is UUCUU (14). We asked whether EcEDF would affect MazF-mt3 activity as it affected MazF-mt6 activity. As we did for MazF-mt6, we analyzed the activity of a highly purified preparation of MazF-mt3 using the continuous fluorometric assay (20).

As in the case of MazF-mt6 (Fig. 1b), adding MazF-mt3 to the reaction mixture led to an increase in fluorescence (see Fig. S1b in the supplemental material). Adding 4 μM EcEDF to this reaction mixture led to an ~30% increase in MazF-mt3 activity, and adding 8 μM EcEDF led to an ~55% increase in MazF-mt3 activity; thus, as in the case of MazF-mt6, the increase in MazF-mt3 activity caused by the addition of EcEDF was concentration dependent (Fig. S1b). Moreover, EcEDF also overcome the inhibitory effect of MazE-mt3 on the endoribonucleolytic activity of MazF-mt3 (Fig. S3a). Thus, the addition of EcEDF affected MazEF-mt6 (Fig. 3a) and MazEF-mt3 (Fig. S3a) similarly.

Adding 8 μM EcEDF to the reaction mixtures led to nearly identical responses of MazE-mt6 and MazE-mt3: in each case, the antitoxin function of the MazE molecules was almost completely overcome. Note that the only residue of EcEDF that was certainly required for overcoming the inhibitory effects of both MazE-mt6 (Fig. 3b) and MazE-mt3 (Fig. S3b) was the central tryptophan residue (W). The activity of MazE-mt3 was not affected by altered EcEDF when any of the other amino acid residues was replaced (Table 1; see also Fig. S3c to f). However, no such complete similarity between the effects of the EDF peptides on the MazEF-mt6 and MazEF mt-3 systems occurred regarding their enhancement effect on the endoribonucleolytic activity of the two MazF toxins. With the exception of the first asparagine, the presence of each amino acid residue of EcEDF was required for enhancing the effects of both MazF-mt6 (Fig. 2b) and MazF-mt3 (Fig. S2b) was the central tryptophan residue (W). The activity of MazE-mt3 was not affected by altered EcEDF when any of the other amino acid residues was replaced (Table 1; see also Fig. S3c to f). However, no such complete similarity between the effects of the EDF peptides on the MazEF-mt6 and MazEF mt-3 systems occurred regarding their enhancement effect on the endoribonucleolytic activity of the two MazF toxins. With the exception of the first asparagine, the presence of each amino acid residue of EcEDF was required for enhancing the effects of both MazF-mt6 (Fig. 2b) and MazF-mt3 (Fig. S2b) was the central tryptophan residue (W). The activity of MazE-mt3 was not affected by altered EcEDF when any of the other amino acid residues was replaced (Table 1; see also Fig. S3c to f). However, no such complete similarity between the effects of the EDF peptides on the MazEF-mt6 and MazEF mt-3 systems occurred regarding their enhancement effect on the endoribonucleolytic activity of the two MazF toxins.

**The effects of EDFs from *P. aeruginosa* (PaEDFs) on the endoribonucleolytic activities of *M. tuberculosis* toxins MazF-mt6 and MazF-mt3.** We also studied the effects of each of the three *P. aeruginosa* EDFs, PaEDF-1, PaEDF-2, and PaEDF-3, on the *M. tuberculosis* MazF-mt6 and MazF-mt3 toxins. The addition of each of these PaEDFs at a concentration of 4 μM or 8 μM led to an increase in the endoribonucleolytic activities of both MazF-mt6 and MazF-mt3 (Fig. 4). The greatest increases in activity were caused by the addition of PaEDF-3 (Fig. 4c and f); however, for both MazF-mt6 and MazF-mt3, those levels were lower than those caused by the addition of EcEDF (Fig. 1; see also Fig. S1).

**PaEDF overcomes the inhibitory effect of MazE-mt6 on the endoribonucleolytic activity of MazF-mt6 and the inhibitory effect of MazE-mt3 on the endoribonucleolytic activity of MazF-mt3.** We also asked whether PaEDF could overcome the inhibitory effect of the antitoxin MazE-6 on MazF-6. To this end, we carried out an experiment under conditions in which MazE almost completely inhibited MazF activity.

### TABLE 1

| Sequence of EcEDF | Enhancement of effect of: | Overcoming of effect of: |
|-------------------|--------------------------|--------------------------|
|                   | MazF-mt3 | MazF-mt6 | MazE-mt3 | MazE-mt6 |
| GNWNN             | +        | −        | +        | +        |
| NgWNN             | −        | −        | +        | +        |
| NNGNN             | −        | −        | −        | −        |
| NNWGN             | −        | −        | +        | +        |
| NNWNG             | −        | −        | +        | +        |

*The data for MazF-mt6 were taken from Fig. 2 and 3. The data for MazF-mt3 were taken from Fig. S2 and S3. “−” indicates that the amino acid was required. “+” indicates that the amino acid was not required.*
As shown, this inhibitory effect was almost completely reversed by adding all three PaEDFs (PaEDF-1, PaEDF-2, or PaEDF-3) (Fig. 5a to c). Moreover, all three PaEDFs also overcome the inhibitory effect of MazE-mt3 on the endoribonucleolytic activity of MazF-mt6 (Fig. 5d to f). Thus, the addition of PaEDFs affected MazEF-mt6 (Fig. 5a to c) and MazEF-mt3 (Fig. 5d to f) similarly.

**DISCUSSION**

Previously, we discovered extracellular death factors (EDFs) in several unrelated bacteria: EcEDF in *E. coli* (17), BsEDF in *B. subtilis* (18), and three PaEDFs in *P. aeruginosa* (18). Of the many *mazEF* systems identified in *M. tuberculosis* so far, three, MazEF-mt6, MazEF-mt3, and MazEF-mt1, have been characterized and their cleaved sites identified (13, 14, 21). Here we studied how each of these various EDF peptides affected the *in vitro* activities of MazEF-mt6 (Fig. 1 to 5), MazEF-mt3 (see in the supplemental material), and MazEF-mt3 (Fig. 5d to f) similarly.
and MazEF-mt1 (Fig. S4). Our work (summarized in Table 2) revealed the following. (i) The EcEDF peptide similarly activated two systems, MazF-mt3 and MazF-mt6. It enhanced the endoribonucleolytic activities of MazF-mt3 (Fig. S1b) and of MazF-mt6 (Fig. 1b); moreover, EcEDF overcame the inhibitory effects of MazE-mt3 on the endoribonucleolytic activity of MazF-mt3 (Fig. S3a) and of MazE-mt6 on the endoribonucleolytic activity of MazF-mt6 (Fig. 3a). (ii) Unexpectedly, the exact sequence of wild-type EcEDF (NNWNN) was more significant for enhancing the activities of the MazF toxins than for overcoming the effects of the MazE antitoxins (Table 1). Each of the wild-type amino acid residues was required to increase MazF-mt6 activity (see the left sides of the columns in Table 1; see also Fig. S2b). In contrast, only the central tryptophan residue of the

FIG 5 PaEDFs partially overcomes the inhibitory effect of MazE-mt6 on MazF-mt6 and of MazE-mt3 on MazF-mt3 endoribonucleolytic activity in vitro. (a) A mixture of (0.5 μM) MazE-mt6 and (4 μM or 8 μM) PaEDF-1 was added to preparations of (0.5 μM) MazF-mt6. (Left) The in vitro activity of MazF-mt6 was inhibited in the presence of MazE-mt6, and PaEDF-1 overcomes this inhibitory effect. (Right) Percent (%) increase in MazF-mt6 activity after the addition of PaEDF-1 to wells containing both MazF-mt6 and MazE-mt6. (b) Data were determined as described for panel a, but instead of PaEDF-1, PaEDF-2 was added. (c) Data were determined as described for panel a, but instead of PaEDF-1, PaEDF-3 was added. (d) A mixture of (0.5 μM) MazE-mt3 and (4 μM or 8 μM) PaEDF-1 was added to preparations of (0.5 μM) MazF-mt3. (Left) The in vitro activity of MazF-mt3 was inhibited in the presence of MazE-mt3 and PaEDF-1 overcomes this inhibitory effect. (Right) Percent (%) increase in MazF-mt3 activity after the addition of PaEDF-1 to wells containing both MazF-mt3 and MazE-mt3. (e) Data were determined as described for panel d, but instead of PaEDF-1, PaEDF-2 was added. (f) Data were determined as described for panel d, but instead of PaEDF-1, PaEDF-3 was added. The data represent means ± standard deviations of results from three experiments performed in triplicate. *, P < 0.01; **, P < 0.001; ***, P < 0.0001 (statistical significance compared to the control data).
Peptides Enhance Activities of *M. tuberculosis* MazF Toxins

### MATERIALS AND METHODS

**Strains and plasmids.** We used *E. coli* strains BL21(DE3) (Invitrogen, Carlsbad, CA) and TG1 (our strain collection). We constructed plasmids pET28a-mazF-mt1(His)6, pET28a-mazF-mt3(His)6, and pET28a-mazF-mt6(His)6, from pET28a (Novagen, San Diego, CA) to express MazF(His)6 under the control of the T7 promoter. We also constructed plasmids pET28a-mazE-mt3(His)6, pET28a-mazF-mt3(His)6, and pET28a-mazE-mt6(His)6, from pET28a (Novagen, San Diego, CA) to express MazE(His)6.

**Synthetic oligonucleotides.** To study MazF-mt1 cleavage, we used an oligonucleotide with the sequence 5′-5′/FAM/AAGTCrU and ACTCAG/3′BHQ_1/-3; for MazF-mt3 cleavage, we used an oligonucleotide with the sequence 5′-5′/FAM/AAGTCrU and CCrURUCAG/3′BHQ_1/-3; and for MazF-mt6 cleavage, we used an oligonucleotide with the sequence 5′-5′/FAM/AAGTCrU and CACrUCAG/3′BHQ_1/-3. Here, “r” represents an RNA base. These oligonucleotides are labeled with 6-carboxyfluorescein (FAM) on the 5′ end and with black hole quencher-1 (BHQ_1) on the 3′ end (18), and the corresponding oligonucleotide cleavage fragments of MazF-mt1 (5′-5′/FAM/AAGTCrU and ACTCAG/3′BHQ_1/-3), MazF-mt3 (5′-5′/FAM/AAGTCrU and CACrURUCAG/3′BHQ_1/-3), and MazF-mt6 (5′-5′/FAM/AAGTCrU and CACrURUCAG/3′BHQ_1/-3) were also used. These oligonucleotides were purchased from IDT.

**Production of (His)6 MazF and (His)6 MazEs of *M. tuberculosis*.** To produce MazF(His)6, we transformed E. coli BL21(DE3) with pET28a-mazF-mt1(His)6, or pET28a-mazF-mt3(His)6, or pET28a-mazF-mt6(His)6. Transforms were grown overnight, and then the culture was diluted 1:50 in LB medium containing kanamycin (25 mg/ml) and 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside). These cultures were then grown at 30°C to an optical density at 600 nm (OD600) of 0.5, and 1.0 mM IPTG was added.

### TABLE 2 The effect of each of the EDF peptides studied on the endoribonucleolytic activities of the *M. tuberculosis* toxins MazF-mt1, MazF-mt3, and MazF-mt6.

| *M. tuberculosis* toxin | EcEDF (NNWNN) | BsEDF (RGQQNE) | PaEDF-1 (INEQTVVT) | PaEDF-2 (VEVSDDGSSGNTLSS) | PaEDF-3 (APKLSDGAAAGYVTKA) |
|------------------------|---------------|----------------|--------------------|---------------------------|-----------------------------|
| MazF-mt1               | –             | –              | –                  | –                         | –                           |
| MazF-mt3               | +             | –              | ±                  | ±                         | +                           |
| MazF-mt6               | –             | –              | ±                  | ±                         | +                           |

**Note:** “+” indicates a significant increase in the endoribonucleolytic activity of the EDF. “-” indicates a moderate increase in the endoribonucleolytic activity of the EDF. “-” indicates no effect on the endoribonucleolytic activity of the EDF.
after which the bacteria were allowed to grow for an additional 2 h. Bacteria were harvested by centrifugation at 4,000 rpm at 4°C for 15 min. The bacterial pellets were stored at −80°C for no more than 2 weeks. To produce MazE(\text{His})_6, we transformed E. coli BL21(DE3) with pET28a-mazE-mt1(\text{His})_6 or pET28a-mazE-mt3(\text{His})_6 or pET28a-mazE-mt6(\text{His})_6. Transformants were grown overnight in LB medium as described above for MazF purification, except that we added 0.5 mM IPTG at an OD$_{600}$ of 0.4, after which the bacteria were allowed to grow for an additional 2 h.

**Purification of MazF(\text{His})_6 and MazE(\text{His})_6 of M. tuberculosis.** We purified MazF(\text{His})_6 and MazE(\text{His})_6 at equal levels as follows. (i) Pellets of BL21(DE3) expressing either MazF(\text{His})_6 or MazE(\text{His})_6 were thawed at room temperature for 20 min and resuspended in 200 ml cold binding buffer (50 mM Tris-HCl, 300 mM NaCl, 10 mM imidazole, pH 8.0). Subsequent steps were performed at 4°C. (ii) Cells were incubated with lysozyme (0.25 mg/ml) for 30 min and then sonicated for 10 s three times at 30-s intervals. (iii) Lysates were centrifuged at 8,000 rpm for 30 min. (iv) To trap the proteins on resin, 4 ml nickel-nitrilotriacetic acid (Ni-NTA) resin (Adar Biotech, Rehovot, Israel) was mixed with the supernatant, and the mixture was then incubated with gentle shaking for 1 h. (v) Centrifugation was then performed at 8,000 rpm and 4°C for 10 min, and the resin was loaded with the protein on the column and left untreated for 10 min. (vi) The resin was washed with 20 ml binding buffer and then with 10 ml binding buffer containing 8 M urea, followed by seven additional washes using 10 ml binding buffer in which the concentration of urea was decreased by 1 M for each wash; finally, a wash was performed with 10 ml wash buffer (50 mM Tris-HCl, 300 mM NaCl, and 20 mM imidazole at pH 8.0). (vii) Elution of the proteins was performed using 5 ml elution buffer (50 mM Tris-HCl, 300 mM NaCl, and 250 mM imidazole at pH 8.0). (viii) The protein was collected in 10 aliquots, which were stored with 20% (final concentration) glycerol at −80°C. Protein concentrations were determined by using the Bradford assay (Bio-Rad, Germany).

**Determination of MazF activities by measuring the cleavage of fluorescent chimeric labeled substrate.** To determine MazF endoribonucleolytic activity quantitatively, we used a procedure previously described by Wang and Hergenrother (20). Wells of a black 96-well plate (Nunc, Thermo Fisher Scientific, Denmark) were filled with TE (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) and 0.5 \( \mu \)M labeled fluorescent oligonucleotide, and 0.5 \( \mu \)M MazF was added. Cleavage by MazF of the fluorescent oligonucleotide was measured by determination of an increase in FAM fluorescent emission (Fig. 1a). To study the effects on MazF activity of EDF (GenScript Corp., Piscataway, NJ) and its derivatives, we added to this reaction mixture 8 \( \mu \)M tested peptide or an equivalent volume of the peptide buffer as a control. In each well, we measured fluorescence using an excitation filter (485 ± 15 nm) and an emission filter (530 ± 15 nm) 30 times at intervals of 60 s for MazF-mt3 and MazF-mt6. On the other hand, we measured fluorescence for MazF-mt1 40 times at intervals of 60 s and considered the tenth cycle to represent the first cycle, because such reactions start at min 10 (Spark multiplate reader; Tecan). We assigned a value of 100% to the level of MazF activity seen in the absence of EDF. To determine the ratios of MazF activities in the presence or absence of EDF or mutated EDF, we calculated the slopes corresponding to the 15th and 25th readings of each reaction and compared those values to the 100% MazF value. We carried out experiments for each experimental peptide with various MazF productions at least three times; from the results of these experiments, we calculated the average activities and determined the standard deviations. The relative MazF activities are shown in the figures. A calibration plot in which fluorescence unit (FU) values were converted to product concentration values (expressed in picomoles) was constructed by the use of a 1:1 ratio of the synthetic oligonucleotide cleavage products.

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at https://doi.org/10.1128/mBio.00685-18.

**TEXT S1**, DOCX file, 0.1 MB.
**FIG S1**, TIF file, 19.9 MB.
**FIG S2**, TIF file, 19.9 MB.
**FIG S3**, TIF file, 19.9 MB.
**FIG S4**, TIF file, 19.9 MB.
**FIG S5**, TIF file, 19.9 MB.

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A.N. performed experiments and wrote the paper; S.K. performed the cloning; H.E.-K. led the project and wrote the paper.
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