Escherichia coli ZiPD is the best characterized protein encoded by the elac gene family and is a model for the 3′-pre-tRNA processing endoribonucleases (tRNase Z). A metal ligand-based sequence alignment of ZiPD with metallo-β-lactamase domain proteins of known crystallographic structure identifies a ZiPD-specific sequence insertion of ~50 residues, which we will refer to as the ZiPD exosite. Functionally characterized ZiPD homologs from Bacillus subtilis, Methanococcus janaschii, and human share the presence of the ZiPD exosite, which is also present in the amino-terminal, but not in the carboxyl-terminal, domain of ElaC2 proteins. Another class of functionally characterized tRNase Z enzymes from Thermotoga maritima and Arabidopsis thaliana lack characteristic motifs in the exosite but possess a sequence segment with clustered amino acid residues. As an experimental attempt to investigate the function of the exosite we constructed a ZiPD variant that lacks this module (ZiPDΔ). ZiPDΔ has almost wild-type-like catalytic properties for hydrolysis of the small, chromogenic substrate bis(p-nitrophenyl)phosphate. Removal of the ZiPD exosite only affects $k_{cat}$, which is reduced by less than 40%, whereas both $K$ and the Hill coefficient (measures of the substrate affinity and cooperativity, respectively) remain unchanged. Hence, the exosite is not required for the intrinsic phosphodiesterase activity of ZiPD. Removal of the exosite also does not affect the dimerization properties of ZiPD. In contrast to the wild-type enzyme, ZiPDΔ does not process pre-tRNA, and gel shift assays demonstrate that only the wild-type enzyme, but not ZiPDΔ, binds mature tRNA. These findings show that the exosite is essential for pre-tRNA recognition. In conclusion, we identify a ZiPD exosite that guides physiological substrate recognition in the ZiPD/ElaC protein family.

Precise trimming of tRNA precursors is a vitally important step in tRNA maturation that involves the removal of the 5′- and 3′-extensions by specific nucleases (1). The endonuclease that generates the mature tRNA 3′-end, the tRNase Z (E.C. number 3.1.26.11) was recently identified (2) and is encoded by the ubiquitous elac gene. The tRNase Z homolog from Escherichia coli has been shown to be a zinc-dependent phosphodiesterase (ZiPD) and has been characterized in detail (3–6). The phosphodiesterase activity of E. coli ZiPD was originally discovered for two chromogenic substrates, thymidine-5′-p-nitrophenyl phosphonate and bis(p-nitrophenyl) phosphate (bpNPP) (3). ZiPD processes pre-tRNA in vitro (Ref. 7 and this report); however, a physiological function as a tRNase Z has not yet been shown (see also below) (5). The availability of large amounts of pure recombinant protein together with detailed knowledge about its biochemical and spectroscopic features makes E. coli ZiPD an excellent model system for the tRNase Z enzymes and the whole ElaC protein family.

A number of eukaryotes possess two elac genes, elac1 and elac2, the latter being approximately twice the size of ZiPD (an elac1 homolog). The ElaC2 proteins are regarded as a fusion of two ElaC1 monomers (8). However, only the carboxyl-terminal domain retains its catalytic competence, whereas the amino-terminal domain lacks amino acid residues required for coordination of the catalytically essential metal ion (8, 9). Human elac2 is a putative prostate cancer susceptibility gene (8), and its Caenorhabditis elegans homolog is involved in germ line proliferation (10). tRNase Z activity was demonstrated for ElaC homologs from various species, including E. coli (7, 11), Arabidopsis thaliana and Methanococcus janaschii (2), human ElaC1 and ElaC2 (9), Saccharomyces cerevisiae ElaC2 (9), Drosophila melanogaster ElaC2 (12), Thermotoga maritima (7), Haloferax volcanii (13), and Bacillus subtilis (14).

Several studies suggest that bacterial tRNase Z enzymes process pre-tRNAs that lack the CCA triplet at the acceptor stem (7, 14–16). In bacteria, a number of tRNA genes code for the CCA triplet, which is otherwise added by the tRNA nucleotidyl transferase. The proportion of bacterial tRNA genes with encoded CCA is species-dependent: in E. coli this is the case for all tRNA genes, whereas in B. subtilis only two thirds of tRNA genes encode the CCA triplet (14). The tRNase Z activity of E. coli ZiPD has been shown for exogenous CCA-less pre-tRNAs. In B. subtilis, the gene coding for the ZiPD homolog protein is essential for viability (14), whereas a comprehensive characterization of an E. coli Δelac strain revealed wild-type-like viability and unaffected transcriptome and proteome. The CCA triplet appears to be an antideterminant for tRNase Z activity, with the exception of the ElaC protein from T. maritima, which processes pre-tRNAs not after the discriminator but 3′ to the genetically encoded CCA triplet (7).

The tRNase Z enzymes indifferently process various kinds of pre-tRNA even across species borders. Structural features of the pre-tRNA molecule have a vast influence on substrate
recognition and processing. Reduced and elongated acceptor stem lengths decrease cleavage efficiency (16) as well as long 5′-extensions (17). The tRNase Z from H. volcanii requires the T-arm and the D-arm for pre-tRNA processing, whereas deletion of the anticodon arm had only a small effect on cleavage efficiency (13).

ZiPD shares the metallo-β-lactamase fold (3) consisting of external α-helices that enclose two layers of β-sheets. The metallo-β-lactamase superfamily is continuously expanding and includes numerous hydrolytic enzymes as well as several redox enzymes (17). A common feature of the metallo-β-lactamaise family is the presence of a binuclear metal binding site that is essential for catalysis. The metal binding residues of E. coli ZiPD have only recently been identified (4). In the present study, we used this knowledge for metal ligand-based sequence alignment of E. coli ZiPD with metallo-β-lactamase domain proteins of known crystallographic structure and identified a specific ZiPD sequence insertion. The characterization of an E. coli ZiPD variant lacking the insertion (ZiPDΔ) shows that this sequence module is required for tRNA binding and pre-tRNA processing, but not for the phosphodiesterase activity with the small chromogenic substrate bis(5-nitrophenyl) phosphate. We conclude that ZiPD proteins possess a specific exosite module that directs physiological substrate recognition.

Comparison of this sequence region to functionally characterized tRNase Z proteins gives rise to three subgroups.

**EXPERIMENTAL PROCEDURES**

Materials—Except when stated otherwise, all fine chemicals were purchased from Sigma. Restriction enzymes were from New England Biolabs (Frankfurt, Germany). Oligonucleotides were synthesized by Genex (Paris, France) and MWG-Biotech (Ebersberg, Germany). DNA sequencing was performed by MWG.

Construction of ZiPDΔ—For construction of the ZiPD deletion mutant, the complete plasmid pETM-ZiPD (3) excluding the coding region for amino acids 153–203 was amplified by PCR using the primers ZiPD152 (5′-TACTAGTTTCTTCAATGATAGCATAC-3′) and ZiPD204 (5′-AAGTCTTGGAAACGCTGCTATTTTT-3′) introducing restriction sites for SpeI (underlined). PCR product was digested with KOD Hot Start polymerase (Novagen). The linear PCR product was digested with SpeI and subsequently circularized by ligation. Transformation of E. coli DH5α yielded vector pETM-ZiPDΔ. The coding sequence of ZiPDΔ was verified by DNA sequencing. The resulting plasmid encodes a ZiPD variant with residues 153–203 replaced by Thr-Ser.

Expression and Purification of ZiPDwt and ZiPDΔ—Expression and purification of the amino-terminal His-tagged proteins followed the published procedure (3, 4). The molecular mass of the purified protein was determined by mass spectrometry to 31026.4 Da (data not shown), which corresponds nicely to the calculated molecular mass of 31024.3 Da.

Cloning, Expression, and Purification of S. cerevisiae tRNase Z, Trz1—The gene encoding the tRNase Z from S. cerevisiae (YKR079c) was obtained by reverse transcription-PCR using primers Y1Eco (5′-TAATAGATGGCGGAGTGGATGCAGATAC-3′) and Y2Stop (5′-ATATTACCGGATATCTTCTTCTTCTTTTCTTATTCATCTCTACCTG-3′) introducing the restriction sites for EcoRI (underlined) and Y2Stop (5′-ATATAGATGGCGGAGTGGATGCAGATAC-3′) introducing the restriction site for XhoI (underlined) and total RNA isolated from S. cerevisiae. The PCR product was digested with EcoRI and XhoI and cloned into pET32a, yielding pET32a-Trz1. The clone was sequenced to confirm that no mutations were induced by the reverse transcription-PCR. The truncated insert was cloned into Rosetta(DE3)pLysS (Novagen) cells and purified using S-protein-

RESULTS

ZiPD Has a Specific Sequence Insertion Module—Because of a generally low overall sequence similarity, standard multiple sequence alignments of E. coli ZiPD with other members of the metallo-β-lactamase family are not conclusive and even fail to align the highly conserved metallo-β-lactamase sequence signature (21) with the small chromogenic substrate bis(5-nitrophenyl) phosphate. Hence, the structure-based sequence alignment identifies a ZiPD-specific sequence insertion module of ~50 amino acids, located between the zinc ligands His-141 and Asp-212 (numbering for E. coli ZiPD). Within the metallo-β-lactamase superfamily, this insertion module is an exclusive feature for ZiPD-like proteins. We will refer to it as the “ZiPD exosite” because this report shows that it functions as an exosite that guides physiological substrate selectivity.

To investigate whether a sequence module similar to the ZiPD exosite is a general feature of the ElaC protein family, we performed a multiple sequence alignment with functionally characterized tRNase Z proteins. We found three subgroups, depending on characteristic sequence signatures at the location of the ZiPD exosite. Very similar sequence segments are found in the ZiPD homologs from E. coli ZidA and M. janaschii as well as in the human ElaC1 protein. The characteristic feature of the ZiPD-type exosite is a glycine- and proline-rich segment (Fig. 1B, GP motif, yellow box). ElaC2 proteins share the ZiPD exosite only in the amino-terminal domain, and it is completely absent in the carboxy-terminal domain. The ElaC2-type exosite shares the GP motif and is considerably longer than the ZiPD- and ElaC2-type exosites. Interestingly, the third subgroup lacks the GP motif and contains a cluster of 4–5 basic amino acid residues (Fig. 1B, blue box). This Thermotoga maritima (TM)-type exosite is significantly shorter than the ZiPD- and ElaC2-type exosites.

ZiPD Exosite Is Not Required for Phosphodiesterase Activity and Cooperativity—The phosphodiesterase activity of E. coli ZiPD was first detected with the small, chromogenic substrate bpNPP (3). Later, E. coli ZiPD was also found to have pre-tRNA 3′-processing activity (7). The bpNPP kinetic parameters for ZiPDΔ (insertion sequence replaced by Thr-Ser) are highly
The alignment of the ZiPD exosite region with functionally characterized trRNase Z proteins is shown. The alignment comprises sequences from \( \text{ZiPD} \) \( \text{Bacillus subtilis} \), the metallo-
\( \text{ZiPD} \) \( \text{ElaC1} \) \( \text{At1ElaC1} \), \( \text{Caenorhabditis elegans} \) \( \text{ElaC2} \) \( \text{DmElaC2} \), \( \text{S. cerevisiae} \) \( \text{ElaC2} \) \( \text{Trz1} \), nuclear \( \text{A. thaliana} \) \( \text{ElaC1} \) \( \text{At1ElaC1} \), mitochondrial \( \text{A. thaliana} \) \( \text{ElaC1} \) \( \text{At2ElaC1} \), and \( \text{T. maritima} \) \( \text{ElaC1} \) \( \text{TmElaC1} \). The alignment is shown between the metal ligating residues His-141 and Asp-212 (numbering for \( \text{ElaC2} \)-type are colored yellow, the names of the \( \text{ElaC2} \)-type are colored orange). The TM-type sequence names are colored blue. The yellow box indicates the GP motif, where glycine and proline residues are colored yellow. The blue box indicates the basic cluster in the TM-type exosite, where basic residues are colored in blue.

### Kinetic parameters of ZiPDwt and ZiPDΔ towards the small, chromogenic substrate bpNPP

| Sample   | Relative \( k_{\text{cat}} \) | \( k_{\text{cat}}/K \) | \( N_H \) |
|----------|-------------------------------|------------------------|---------|
|          | \( \text{E. coli} \)          | \( \text{ZiPDwt} \)     | \( \text{ZiPDΔ} \) |
| Wild-type| 100                           | 60 ± 6                 | 9.9     |
| ZiPDΔ    | 62                            | 37 ± 9                 | 8.8     |

Substrate-dependent activity of purified enzymes was measured continuously with a UV/Vis spectrophotometer and analyzed according to the Hill equation. Values were determined for three independent enzyme preparations. \( n_H \), Hill coefficient.

ZiPD Exosite Is Essential for Pre-tRNA Processing—E. coli ZiPD has a specific sequence insertion. In the multiple sequence alignments conserved residues are shaded in grayscale. The position in the amino acid sequence is indicated after the name. A, manually refined metal ligand-based sequence alignment of metallo-\( \beta \)-lactamase domain proteins, comprising \( \text{E. coli} \) ZiPD (ZiPD), human glyoxalase II (h GlxII), rubredoxin:oxygen oxidoreductase from Desulfovibrio gigas (Dg ROO), and the metallo-\( \beta \)-lactamases from \( \text{Bacillus cereus} \) (Be \( \beta \)-lac) and \( \text{Stenotrophomonas maltophilia} \) (Sm \( \beta \)-lac) and the metal ligating residues are boxed in blue. The ZiPD exosite is marked in red. B, three types of exosites exist in the ElaC family. Multiple sequence alignment of the ZiPD exosite region with functionally characterized trRNase Z proteins is shown. The alignment comprises sequences from \( \text{E. coli} \) ZiPD (ZiPD), \( \text{Bacillus subtilis} \) ElaC1 (BsElaC1), \( \text{Methanococcus jannaschii} \) ElaC1 (MjElaC1), \( \text{human ElaC1} \) (hElaC1), \( \text{human ElaC2} \) (hElaC2), \( \text{Caenorhabditis elegans} \) ElaC2 (CeElaC2), \( \text{Drosophila melanogaster} \) ElaC2 (DmElaC2), \( \text{S. cerevisiae} \) ElaC2 (Trz1), nuclear \( \text{A. thaliana} \) ElaC1 (At1ElaC1), mitochondrial \( \text{A. thaliana} \) ElaC1 (At2ElaC1), and \( \text{T. maritima} \) ElaC1 (TmElaC1). The alignment is shown between the metal ligating residues His-141 and Asp-212 (numbering for \( \text{E. coli} \) ZiPD). The sequence names belonging to the ZiPD-type exosite are colored yellow, the names of the ElaC2-type are colored orange, and the TM-type sequence names are blue. The yellow box indicates the GP motif, where glycine and proline residues are colored yellow. The blue box indicates the basic cluster in the TM-type exosite, where basic residues are colored in blue.

ZiPD Exosite Is Not Required for Dimerization—E. coli ZiPD is a dimer in its native state (3). The influence of the ZiPD exosite on the dimerization properties of ZiPD was analyzed by gel filtration and glutaraldehyde cross-linking (Fig. 2, A and B). Recombinant ZiPDwt has a molecular mass of \( \sim 36 \text{ kDa} \), ZiPDΔ of \( \sim 31 \text{ kDa} \) per monomer. In analytical gel filtration, ZiPDwt elutes at a volume corresponding to \( \sim 70 \text{ kDa} \), whereas ZiPDΔ elutes at a volume corresponding to \( \sim 60 \text{ kDa} \). Peaks corresponding to the monomers were not detected. In reducing SDS-PAGE, ZiPDwt migrates to \( \sim 36 \text{ kDa} \) and ZiPDΔ to \( \sim 31 \text{ kDa} \). Glutaraldehyde cross-linking followed by reducing SDS-PAGE leads to a fraction of ZiPDwt migrating to \( \sim 70 \text{ kDa} \) and a fraction of ZiPDΔ migrating to \( \sim 60 \text{ kDa} \). Both analytical gel filtration and glutaraldehyde cross-linking therefore show that ZiPDwt and ZiPDΔ persist as homodimers in solution. In good accordance, both ZiPDwt and ZiPDΔ have a 2-fold cooperativity with the substrate bpNPP (Table I).

### Table I

| Sample   | Relative \( k_{\text{cat}} \) | \( k_{\text{cat}}/K \) | \( N_H \) |
|----------|-------------------------------|------------------------|---------|
| Wild-type| 100                           | 60 ± 6                 | 9.9     |
| ZiPDΔ    | 62                            | 37 ± 9                 | 8.8     |

Substrate-dependent activity of purified enzymes was measured continuously with a UV/Vis spectrophotometer and analyzed according to the Hill equation. Values were determined for three independent enzyme preparations. \( n_H \), Hill coefficient.

ZiPD Exosite Is Essential for Pre-tRNA Processing—E. coli ZiPD has 3'-pre-tRNA processing endoribonucleolytic activity (7). In this study, we probed 3'-pre-tRNA processing activity with pre-tRNA\(^{\text{TYr}}\) from \( \text{O. bacteria} \), which has been widely used to study tRNA maturation (e.g. Refs. 13, 16, 23). The ElaC2 protein from \( \text{S. cerevisiae} \) (Trz1) served as a positive control (9). ZiPDwt processes pre-tRNA\(^{\text{TYr}}\) in a similar manner to Trz1. Both the mature tRNA\(^{\text{TYr}}\) and the removed 3'-trailer are detected (Fig. 3). Electrophoretic analysis of the cleavage products reveals that \( \text{E. coli} \) ZiPDwt and ZiPDΔ cut pre-tRNA\(^{\text{TYr}}\) at similar positions. Detection of both the 3'-trailer and the mature tRNA prove the specific, endoribonucleolytic activity and rules out a further exoribonucleolytic action. ZiPDΔ does not process pre-tRNA\(^{\text{TYr}}\) (Fig. 3). Neither mature tRNA\(^{\text{TYr}}\) nor the removed 3'-trailer was detected under experimental conditions that allowed for the processing of a large pre-tRNA\(^{\text{TYr}}\) fraction by ZiPDwt and Trz1. In comparison to ZiPDwt, the \( k_{\text{cat}} \) value of ZiPDΔ with the substrate bpNPP is decreased by \( \sim 40\% \). However, even a similarly decreased activity versus pre-tRNA\(^{\text{TYr}}\) is expected to lead to a significant fraction of processed tRNA\(^{\text{TYr}}\) under these experimental conditions. In conclusion, ZiPDΔ lost the pre-tRNA processing properties of ZiPDwt, demonstrating that the ZiPD insertion is essential for pre-tRNA recognition.
ZiPD Exosite Is Essential for tRNA Binding—The cytoplasmic ElaC1 protein from Arabidopsis thaliana (called nuz) binds mature tRNA (24), and in the case of the native wheat tRNase Z, tRNA affinity chromatography was used for purification (2). In the present study, we used electrophoretic mobility shift assays to probe wheat tRNA binding to Trz1, ZiPDwt, and ZiPDΔ/H9004. (Fig. 4). Trz1 has a high affinity for mature tRNA. Upon incubation with Trz1, a large fraction of the wheat tRNA migrated to a higher molecular size than in the absence of Trz1. tRNA binding was also detected for ZiPDwt. However, a larger protein amount (750 ng of ZiPD in comparison to 200 ng of Trz1) was required to detect tRNA mobility shifts; despite this elevated protein level, only a small fraction of the total tRNA bound to ZiPD. This indicates that ZiPDwt has a significantly weaker affinity to this tRNA than Trz1. No tRNA mobility shifts were detected with ZiPDΔ/H9004 under identical experimental conditions as with ZiPDwt. Thus, deletion of the ZiPD insertion abolished tRNA binding to ZiPD. The complex of tRNA bound to Trz1 or ZiPDwt migrates to very similar positions despite different molecular sizes of the hook proteins. Trz1, an ElaC2 protein, has a molecular size of ~97 kDa, ZiPDwt of ~37 kDa, and mature tRNA of ~20 kDa. Highly similar migration of the protein-tRNA complex for Trz1 and ZiPDwt is best explained with Trz1 being a monomer in solution and binding one tRNA molecule, whereas in the ZiPD homodimer each monomer binds one tRNA molecule. This model is in agreement with our find-
The ZiPD exosite is located opposite to the metal site. Ribbon representation of human glyoxalase II (19), which shares the metal coordination with ZiPD (4). The 2 glycine residues of the glyoxalase structure that mark the putative position of the ZiPD exosite in a typical metallo-β-lactamase domain are indicated.

Besides their prominent role in 3′-pre-tRNA processing, substrate selectivity and recognition of the elaC-encoded tRNase Z proteins have only been marginally understood. Here we identified for ZiPD, the ElaC protein from E. coli, an exosite that is essential for pre-tRNA processing and tRNA binding but whose removal affects neither dimerization nor the phosphodiesterase activity toward the small substrate bpNPP.

Almost unaltered bpNPP hydrolytic activity and unaffected dimerization properties of the exosite deletion mutant ZiPDΔ underline that the overall fold remains intact. In good agreement, the structure-based sequence alignment suggests that the ZiPD exosite is inserted in the metallo-β-lactamase fold motif without disrupting this general structure, e.g. in the form of an extended loop. Based on the sequence alignment, we mapped the position of the ZiPD exosite exemplary on the structure of the enzyme glyoxalase II (Fig. 5). This model proposes that the exosite is inserted as an extended loop between β-sheets 9 and 10, opposite to the binuclear active site. The same picture arises with the other metallo-β-lactamase domain proteins of known structures. This picture agrees nicely with the biochemical data of the present report, in particular with the barely altered bpNPP hydrolytic activity of ZiPDΔ. Because both ZiPDwt and ZiPDΔ exist as homodimers in solution, it will be of interest to investigate whether the exosite of one subunit comes into proximity with the active site of the other subunit. The high number of conserved proline and glycine residues in the ZiPD exosite suggests low secondary structure content and a high degree of structural flexibility.

The term exosite is most commonly used for protease substrate determinants that lie outside the active site. Matrix metalloproteases (MMPs) are examples of proteases possessing exosites. Here, the non-catalytic hemopexin domains participate in substrate binding and guide substrate selectivity (25). The ZiPD exosite (−50 residues) is significantly smaller than the MMP hemopexin domains (−200 residues). In addition, the ZiPD exosite appears inside the ZiPD sequence, whereas the MMP exosites are located carboxy-terminal to the catalytic domain. This work is the first report about exosite features of any metallo-β-lactamase domain protein. This exceptional characteristic distinguishes ZiPD-like proteins from other metallo-β-lactamase domain proteins.

The presence of the ZiPD exosite divides the tRNase Z protein family into three different subgroups. The ZiPD-type exosite is present in the functionally characterized ElaC1 proteins from E. coli, B. subtilis, and human. ElaC2 proteins possess a ZiPD exosite-like feature only in the amino-terminal domain, which lacks the catalytic metal binding site. The carboxy-terminal domain, on the other hand, contains the metal binding site and completely lacks the exosite. There is one report showing that the carboxy-terminal domain of human ElaC2 is sufficient for 3′-pre-tRNA processing (9), which indicates a different mechanism of tRNA recognition. However, a second report by the same group points toward significantly decreased tRNA processing activity of the carboxy-terminal domain alone (11). Both reports agree that the amino-terminal domain alone does not contain the tRNase Z activity. The ElaC2-type exosite has previously been described as a putative nucleotide binding site due to weak sequence similarity to a P-loop sequence motif (8). Recently, the ElaC2 amino-terminal domain was found to be essential for the so-called RNase65 activity, a term that describes specific endoribonucleolytic cleavage of small target RNAs in the presence of 3′-truncated tRNA that lacks several residues at the acceptor stem (11). The target RNA is thought to hybridize with the truncated tRNA, finally forming a pre-tRNA-like complex with a 3′-extension. This observation suggests that the amino-terminal ElaC2 exosite is also involved in substrate recognition. A third group of comparably smaller ElaC1 proteins lacks the GP motif and possesses instead a shorter exosite with clustered basic residues. For one member of this TM-type exosite-containing group, the T. maritima tRNase Z, an extraordinary 3′-pre-tRNA processing feature was shown. This enzyme processes CCA-containing pre-tRNA and removes the 3′-trailer after the CCA triplet instead of cutting after the discriminator (7). The functionally characterized enzyme from A. thaliana, which also possesses the TM-type exosite, removes the 3′-trailer after the discriminator (2) but also cleaves off the CCA triplet from mature tRNA (16). This activity was also found for the ZiPD homolog from M. jannaschii, which possesses the characteristic ZiPD-type exosite. It appears that the ZiPD exosite is responsible for overall tRNA recognition, whereas the precise localization of the cleavage site and the antideterminant effect of the CCA motif are guided by other segments of the protein. The organization of the ElaC2 proteins with the exosite only in the amino-terminal domain and the catalytic metal binding site in the carboxy-terminal domain suggests a similar functional interaction in the ZiPD homodimer. According to this model, the substrate that is recognized by the exosite of one monomer is processed at the metal site of the other monomer.

ZiPD-like proteins act as highly specific endoribonuclease in pre-tRNA maturation. Numerous functional studies on ZiPD homologs from various sources revealed endoribonuclease removal of the 3′-trailer from the precursor tRNA while both the mature tRNA and the trailer were left intact. The remarkable substrate and cleavage specificity requires complex substrate recognition, which underlines the physiological significance of the ZiPD exosite in tRNA recognition and substrate selectivity.

Addendum—After submission of this manuscript a report was published showing the crystal structure of the tRNase Z from B. subtilis (de la Sierra-Gallay, I. L., Pellegrini, O., and Condor, C. (2005) Nature 433, 657–661). The authors describe a flexible arm in the structure that is identical to the exosite we describe here. From a model with bound tRNA the authors suggest a role of the exosite in substrate recognition, which perfectly complements our biochemical data.

REFERENCES
1. Morl, M., and Marchfelder, A. (2001) EMBO Rep. 2, 17–20
2. Schiffer, S., Rosch, S., and Marchfelder, A. (2002) EMBO J. 21, 2769–2777
3. Vogel, A., Schilling, O., Niecke, M., Bettmer, J., and Meyer-Klaucke, W. (2002) J. Biol. Chem. 277, 29078–29085
4. Vogel, A., Schilling, O., and Meyer-Klaucke, W. (2004) Biochemistry 43, 10379–10386
5. Schilling, O., Rugeberg, S., Vogel, A., Rittner, N., Weichert, S., Schmidt, S., Doig, S., Andrews, S. C., Benes, V., Franz, T., Baum, M., and Meyer-
Klaucke, W. (2004) Biochem. Biophys. Res. Commun. 320, 1365–1373

6. Schilling, O., Vogel, A., Kostelecky, B., Natal da Luz, H., Spemann, D., Späth, B., Marchfelder, A., Troger, W., and Meyer-Klaucke, W. (2005) Biochem. J. 385, 145–153

7. Minagawa, A., Takaku, H., Takagi, M., and Nashimoto, M. (2004) J. Biol. Chem. 279, 16688–16697

8. Tavitjyan, A., Simard, J., Teng, D. H., Abtin, V., Baumgard, M., Beck, A., Camp, N. J., Carillo, A. R., Chen, Y., Dayananth, P., Desrochers, M., Dumont, M., Farnham, J. M., Frank, D., Frye, C., Ghaffari, S., Gupte, J. S., Hu, R., Iliiev, D., Janecki, T., Kurt, E. N., Lacity, K. E., Leavitt, A., Leblanc, G., McArthur-Morrison, J., Pederson, A., Penn, B., Peterson, K. T., Reid, J. E., Richards, S., Schroeder, M., Smith, R., Snyder, S. B., Swedlund, B., Swensen, J., Thomas, A., Tranchant, M., Woodland, A. M., Labrie, F., Skolnick, M. H., Neuhausen, S., Rommens, J., and Cannon-Albright, L. A. (2001) Nat. Genet. 27, 172–180

9. Takaku, H., Minagawa, A., Takagi, M., and Nashimoto, M. (2003) Nucleic Acids Res. 31, 2272–2278

10. Smith, M. S., and Levitan, D. J. (2004) Dev. Biol. 266, 151–160

11. Takaku, H., Minagawa, A., Takagi, M., and Nashimoto, M. (2004) Nucleic Acids Res. 32, 4429–4438

12. Dubrovsky, E. B., Dubrovskaya, V. A., Levinger, L., Schiffer, S., and Marchfelder, A. (2004) Nucleic Acids Res. 32, 255–262

13. Schierling, K., Rösch, S., Rupprecht, R., Schiffer, S., and Marchfelder, A. (2002) J. Mol. Biol. 316, 895–902

14. Pellegrini, O., Nezzar, J., Marchfelder, A., Putzer, H., and Condon, C. (2003) EMBO J. 22, 4534–4543

15. Mohan, A., Whyte, S., Wang, X., Nashimoto, M., and Levinger, L. (1999) RNA 5, 245–256

16. Schiffer, S., Rösch, S., and Marchfelder, A. (2003) Biol. Chem. 384, 333–342

17. Aravind, L. (1999) In Silico Biol. 1, 69–91

18. Kunzman, A., Brennicke, A., and Marchfelder, A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 108–113

19. Cameron, A. D., Riddetstrom, M., Olin, B., and Mannervik, B. (1999) Structure Fold. Des. 7, 1067–1078

20. Frazao, C., Silva, G., Gomes, C. M., Matias, P., Coelho, R., Sicker, L., Macedo, S., Liu, M. Y., Oliveira, S., Teixeira, M., Xavier, A. V., Rodrigues-Pousada, C., Carreiro, M. A., and Le Gall, J. (2000) Nat. Struct. Biol. 7, 1041–1045

21. Carfi, A., Pares, S., Duse, E., Galleni, M., Duez, C., Frère, J. M., and Dideberg, O. (1995) EMBO J. 14, 4914–4921

22. Ullah, J. H., Walsh, T. R., Taylor, I. A., Emery, D. C., Verma, C. S., Gamblin, S. J., and Spencer, J. (1998) J. Mol. Biol. 284, 125–136

23. Mayer, M., Schiffer, S., and Marchfelder, A. (2000) Biochemistry 39, 2096–2105

24. Schiffer, S., Rösch, S., Späth, B., Englert, M., Beier, H., and Marchfelder, A. (2005) in Handbook of RNA Biochemistry (Westhof, E., Bindereif, A., Schön, A., and Hartmann, R. K., eds), pp. 667–675, Wiley VCH, Weinheim, Germany

25. Overall, C. M. (2001) Methods Mol. Biol. 151, 79–120
Exosite Modules Guide Substrate Recognition in the ZiPD/ElaC Protein Family
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