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Translation Initiation with Initiator tRNA Charged with Exotic Peptides

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The translation system evolved to polymerize 20 specific kinds of proteinogenic L-α-amino acids with extremely high accuracy according to the sequence information encoded in mRNA. Exclusion of nonproteinogenic amino acids from the polymerization is achieved by sophisticated mechanisms involving the multistep selection of correctly charged aminoacyl-tRNAs.[1] Despite the fact that techniques allowing researchers to manipulate the genetic code, so-called genetic code expansion[2] or genetic code reprogramming,[3] have been developed and many successes in incorporating nonproteinogenic L-α-amino acids into nascent peptide chain have been reported, nonproteinogenic amino acids containing more drastically altered structures (referred to as exotic amino acids), such as D- and β-amino acids, are notoriously difficult or often impossible to be elongated.[2a−c,3c,4] On the other hand, recent investigations have shown that the initiation apparatus is more tolerant to exotic amino acids than elongation event. In fact, it has been reported that "nonmethionine" amino acids, for example, various L-α-amino acids[5a] and some D-α-amino acids[5b] with or without *N*-acyl groups, were able to initiate translation, whereas formylmethionine (fMet) is a sole initiator in the ordinary prokaryotic translation system (Figure 1A).[6] The observed tolerance of initiation has prompted

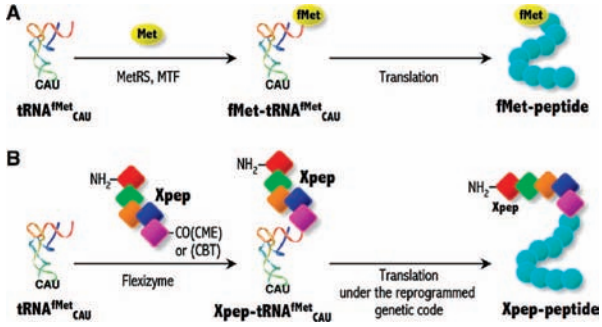

Figure 1. Reprogramming of the initiation event with exotic peptides. (A) Initiation in an ordinary prokaryotic translation system. The initiator tRNA (tRNA$^{fMet}_{CAU}$) is methionylated by methionyl-tRNA synthetase (MetRS) and its α-amino group is formylated by methionyl-tRNA formyltransferase (MTF) to afford fMet-tRNA$^{fMet}_{CAU}$. Initiation with fMet-tRNA$^{fMet}_{CAU}$ yields a peptide containing fMet at the N-terminus (fMet-peptide). (B) Outline of translation initiation with exotic peptides reported here. Flexizyme charges a short exotic peptide (Xpep) onto the tRNA$^{fMet}_{CAU}$. Reprogramming of the initiation event allows us to prime the translation reaction with Xpep-tRNA$^{fMet}_{CAU}$, yielding a peptide bearing several exotic amino acids at the N-terminus (Xpep-peptide).

us to further explore the repertoire expansion. Here we report the reprogramming of translation initiation with "exotic peptides" containing several exotic amino acids (Figure 1B).

To facilitate the reprogramming of initiation event, we utilized our original methodology in the combination of flexizyme and *w*PURE systems. The former system consists of a tRNA aminoacylation ribozyme, referred to as flexizyme, enabling us to charge a wide variety of nonproteinogenic amino acids activated with certain ester or thioester groups onto the 3′ hydroxyl group of any desired tRNAs, including the initiator tRNA$^{fMet}_{CAU}$.[3c] The latter system is based on a reconstituted *E. coli* cell-free translation system, so-called protein-translation using recombinant elements (PURE),[7] in which methionine (Met) is *withdrawn* to make the start codon vacant and thus it is called a *w*PURE system.[5,8] By using the integrated systems, that is, by adding an acylated tRNA$^{fMet}_{CAU}$ prepared by flexizyme to the *w*PURE system, the start codon AUG was reassigned from fMet to desirable non-fMet initiators.[5]

Although the flexizyme system has provided us a nearly unlimited opportunity for the synthesis of tRNA charged with nonproteinogenic amino acids (Xaa-tRNA),[3c,5,8] it is unknown if it can be applicable to tRNA peptidylation. We therefore first verified whether exotic peptides (Xpep) could be charged onto tRNA$^{fMet}_{CAU}$ by means of flexizyme. We designed eleven short peptides containing various combinations of proteinogenic amino acids and exotic amino acids including D-phenylalanine (^{D}Phe), D-glutamic acid (^{D}Glu), D-lysine (^{D}Lys), *N*-methyl-L-phenylalanine (^{Me}Phe), *N*-methyl-L-glutamic acid (^{Me}Glu), 4-aminobenzoic acid (^{4}Abz) and β-alanine (βAla) (the individual structures are shown in Supporting Information, Figure S1). To make the peptides to be accessible to the flexizyme system, those containing the C-terminal ^{L}Phe, ^{D}Phe, ^{Me}Phe, or ^{L}Met were derived to the cyanomethyl esters (CME) (Table 1, entries 1−8, 10, and 11), while one containing the C-terminal ^{L}Gln was derived to 4-cholorobenzyl thioester (CBT)

Table 1. The Sequences of Peptide Initiators Used in This Study and Their Acylation Yields and Translation Efficiencies

entry	initiator sequence[a]	activating group for flexizyme[b]	acylation yield (%)[c]	translation efficiency (%)[d]
1	^{D}Phe−^{L}Phe	CME	85	88
2	^{D}Phe−^{D}Phe−^{L}Phe	CME	44	68
3	^{D}Phe−^{4}Abz−^{L}Phe	CME	30	133
4	^{D}Phe−βAla−^{L}Phe	CME	69	115
5	^{D}Phe−^{Me}Glu−^{L}Phe	CME	47	39
6	^{D}Phe−βAla−^{D}Phe	CME	68	26
7	^{D}Phe−βAla−^{Me}Phe	CME	66	88
8	^{D}Phe−βAla−^{L}Met	CME	48	91
9	^{D}Phe−βAla−^{L}Gln	CBT	38	19
10	^{D}Glu−^{D}Lys−^{L}Phe	CME	55	25
11	^{D}Glu−^{D}Lys−^{D}Glu−^{D}Lys−^{L}Phe	CME	55	12

[a] ^{D}Xaa, ^{L}Xaa, ^{Me}Xaa, ^{4}Abz, and βAla denote D-amino acids, L-amino acids, *N*-methyl-*L*-amino acids, 4-aminobenzoic acid, and β-alanine, respectively. [b] CME and CBT denote cyanomethyl ester and 4-chlorobenzyl thioester activating groups, respectively. [c] Yields of the acylation were calculated based on the band intensity in acid PAGE (see Figure S2 for the detailed description). [d] Relative translation efficiencies of Xpep-peptides were determined by comparing with the band intensity of fMet-peptide in tricine-SDS PAGE (see Figure 2B). The expression quantity of the fMet-peptide was determined to be 7.5 pmol/μL based on the method reported elsewhere.[5a,8b]

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(Table 1, entry 9). For verification of the peptidylation ability of flexizyme, we used our conventional assay system using a tRNA analogue, microhelix RNA.[3c] The di-, tri-, and pentapeptide substrates were incubated with the microhelix RNA in the presence of the flexizyme, and the products were analyzed by denaturing acid polyacrylamide gel electrophoresis (PAGE). In all cases, a single mobility-shifted band corresponding to the individual Xpep-RNA was observed (Figure S2), indicating that the flexizyme system is compatible to the peptidyl substrates for tRNA peptidylation. Importantly, the observed yields were in the range of 30−69% (Table 1), which were sufficient to carry out the translation experiment based on our previous experimental knowledge.[3c] We thus next investigated whether the translation apparatus could accept these Xpep-tRNA$^{fMet}_{CAU}$ as an initiator.

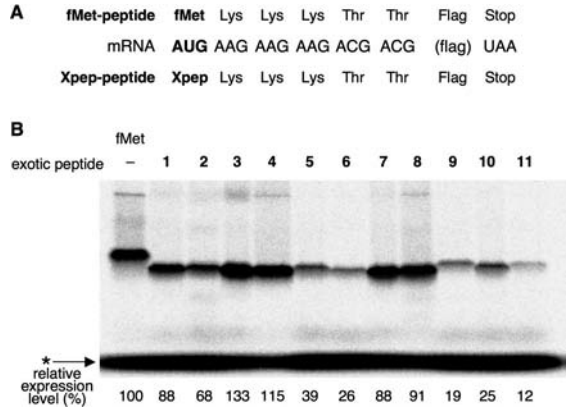

Figure 2. Translation initiation with exotic peptides. (A) Sequences of a mRNA template and expressed fMet/Xpep-peptides. Flag in the parentheses indicates the RNA sequence encoding a Flag peptide (DYKDDDDK). (B) Tricine-SDS PAGE of the translation products initiated with various exotic peptides. The product of the *w*PURE translation reaction containing each Xpep-tRNA$^{fMet}_{CAU}$ and [^{14}C]-Asp was analyzed by tricine-SDS PAGE (lanes 1−11 corresponding the exotic peptides shown in Figure S1). "fMet" indicates the wild-type expression in the ordinary PURE system in which fMet acted as the initiator. Each expression level relative to the fMet-peptide is determined by a mean score of triplicates or more. The band indicated by the asterisk corresponds to the remaining [^{14}C]-Asp that was not incorporated into the peptide.

We designed a mRNA template that encoded a 14-mer peptide, in which the C-terminus contained a Flag peptide sequence for the convenience of radiolabeling-detection using [^{14}C]-Asp as well as the peptide isolation (Figure 2A). Each peptide was expressed in the presence of the respective Xpep-tRNA$^{fMet}_{CAU}$ in the *w*PURE system, where the initiator was reassigned to the short exotic peptide. The translation product was analyzed by tricine-SDS PAGE (Figure 2B) and its relative translation efficiency was determined by the comparison with the wild-type expression where fMet acted as the initiator in the ordinary PURE system (Table 1). In addition, the expressed peptide was subjected to mass spectrometry for identification of the translation product (Figure S3). To our surprise, all Xpep-tRNAs could initiate the translation. The expression efficiencies were depending upon the peptides, ranging from 12% to 133% relative to the fMet-peptide expression. Although we could not define a general trend that correlated the sequence compositions to the observed expression levels, the longest peptide **11** was least efficient so that the long length of peptide might hamper the initiation event. Given the lower efficiency in relatively hydrophilic peptides (**5**, **9**, **10**, and **11**) and the previous observation that hydrophilic amino acids could initiate translation less efficiently than hydrophobic ones,[5] the hydrophilicity of initiator might also affect the initiation efficiency. Nonetheless, it is clear that exotic peptides containing consecutive D-amino acids or the combination of exotic amino acids charged onto tRNA$^{fMet}_{CAU}$ could initiate the translation to afford peptides containing a stretch of exotic amino acids at the N-terminus.

Here we have reported that ribosome is able to accept tRNA$^{fMet}_{CAU}$charged with nonstandard peptides as an initiator, and thus peptides containing a variety of exotic amino acids at the N-terminus could be expressed. To the best of our knowledge, this work represents the first demonstration of translation initiation with peptidyl molecules regardless of natural or non-natural building blocks. In contrast to the fact that elongation of exotic amino acids has often encountered difficulties, the initiation reprogramming approach enables us to synthesize peptides containing exotic amino acids at the N-terminus. Particularly, we envisage that the integration of this approach with appropriate peptide cyclization techniques[5a,8c] will open a new avenue for the ribosomal synthesis of unusual peptides. Moreover, the reported method facilitating the synthesis of desired peptidyl-tRNAs with proteinogenic or exotic amino acids by means of flexizyme system would provide a new tool for studying the mechanism of initiation and/or elongation in translation.

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Supporting Information Available: Detailed structure of short peptides used here, acid PAGE analysis, mass spectra, and experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Söll, D. *Experientia* **1990**, *46*, 1089–1096. (b) Sankaranarayanan, R.; Moras, D. *Acta Biochim. Pol.* **2001**, *48*, 323–335.
(2) (a) Roesser, J. R.; Xu, C.; Payne, R. C.; Surratt, C. K.; Hecht, S. M. *Biochemistry* **1989**, *28*, 5185–5195. (b) Bain, J. D.; Diala, E. S.; Glabe, C. G.; Wacker, D. A.; Lyttle, M. H.; Dix, T. A.; Chamberlin, A. R. *Biochemistry* **1991**, *30*, 5411–5421. (c) Ellman, J. A.; Mendel, D.; Schultz, P. G. *Science* **1992**, *255*, 197–200. (d) Link, A. J.; Mock, M. L.; Tirrell, D. A. *Curr. Opin. Biotechnol.* **2003**, *14*, 603–609. (e) Wang, L.; Xie, J.; Schultz, P. G. *Annu. Rev. Biophys. Biomol. Struct.* **2006**, *35*, 225–249.
(3) (a) Forster, A. C.; Tan, Z.; Nalam, M. N.; Lin, H.; Qu, H.; Cornish, V. W.; Blacklow, S. C. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6353–6357. (b) Josephson, K.; Hartman, M. C.; Szostak, J. W. *J. Am. Chem. Soc.* **2005**, *127*, 11727–11735. (c) Murakami, H.; Ohta, A.; Ashigai, H.; Suga, H. *Nat. Methods* **2006**, *3*, 357–359.
(4) (a) Starck, S. R.; Qi, X.; Olsen, B. N.; Roberts, R. W. *J. Am. Chem. Soc.* **2003**, *125*, 8090–8091. (b) Tan, Z.; Forster, A. C.; Blacklow, S. C.; Cornish, V. W. *J. Am. Chem. Soc.* **2004**, *126*, 12752–12753. (c) Dedkova, L. M.; Fahmi, N. E.; Golovine, S. Y.; Hecht, S. M. *Biochemistry* **2006**, *45*, 15541–15551.
(5) (a) Goto, Y.; Ohta, A.; Sako, Y.; Yamagishi, Y.; Murakami, H.; Suga, H. *ACS Chem. Biol.* **2008**, *3*, 120–129. (b) Goto, Y.; Murakami, H.; Suga, H. *RNA* **2008**, *14*, 1390–1398.
(6) (a) Kozak, M. *Microbiol. Rev.* **1983**, *47*, 1–45. (b) Gold, L. *Annu. Rev. Biochem.* **1988**, *57*, 199–233. (c) Gualerzi, C. O.; Pon, C. L. *Biochemistry* **1990**, *29*, 5881–5889.
(7) Shimizu, Y.; Inoue, A.; Tomari, Y.; Suzuki, T.; Yokogawa, T.; Nishikawa, K.; Ueda, T. *Nat. Biotechnol.* **2001**, *19*, 751–755.
(8) (a) Ohta, A.; Murakami, H.; Higashimura, E.; Suga, H. *Chem. Biol.* **2007**, *14*, 1315–1322. (b) Sako, Y.; Goto, Y.; Murakami, H.; Suga, H. *ACS Chem. Biol.* **2008**, *3*, 241–249. (c) Sako, Y.; Morimoto, J.; Murakami, H.; Suga, H. *J. Am. Chem. Soc.* **2008**, *130*, 7232–7234. (d) Kawakami, T.; Murakami, H.; Suga, H. *Chem. Biol.* **2008**, *15*, 32–42.

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