Timing and specificity of co-translational nascent protein modification in bacteria

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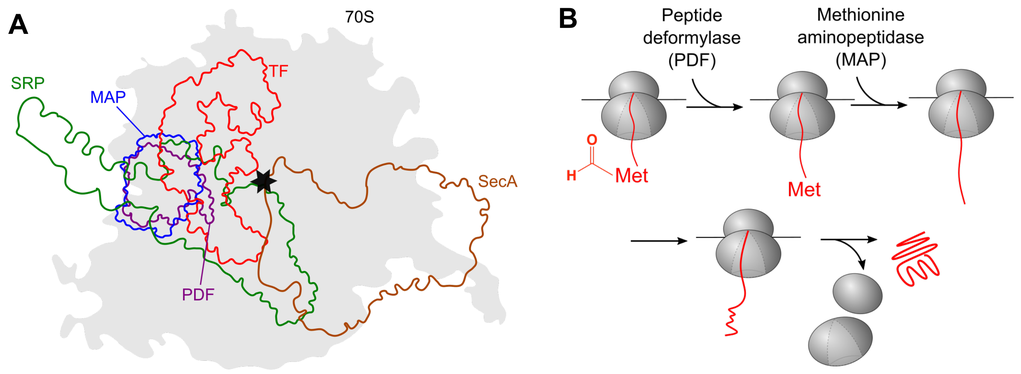
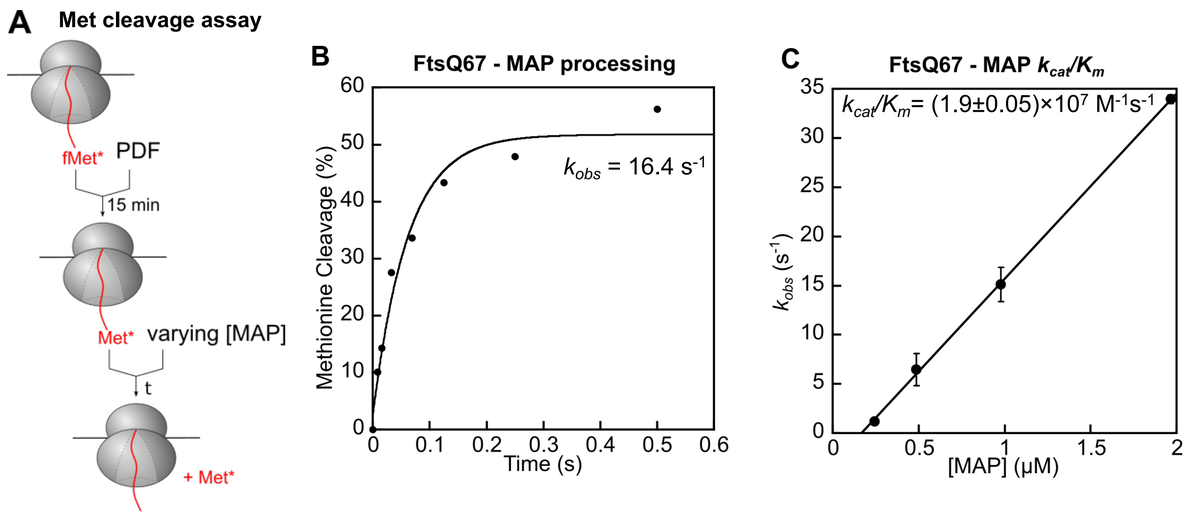
Introduction

The nascent polypeptide exit site of the ribosome is a crowded environment where multiple ribosome-associated protein biogenesis factors (RPBs) compete for the nascent polypeptide to influence their localization, folding, or quality control **(A)**. N-terminal methionine excision (NME), mediated by peptide deformylase (PDF) and methionine aminopeptidase (MAP), is a ubiquitous nascent protein modification that impacts >50% of the proteome **(B)**. How timely and selective NME occurs at the crowded ribosome exit is unclear.

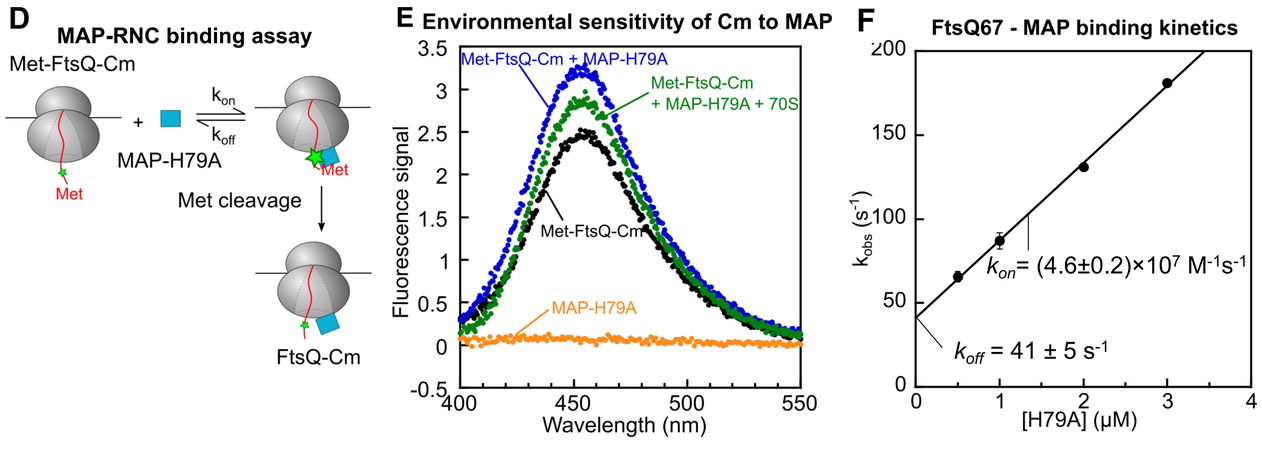


Results

MAP-mediated Met cleavage of nascent chains on the ribosome is diffusion-limited.

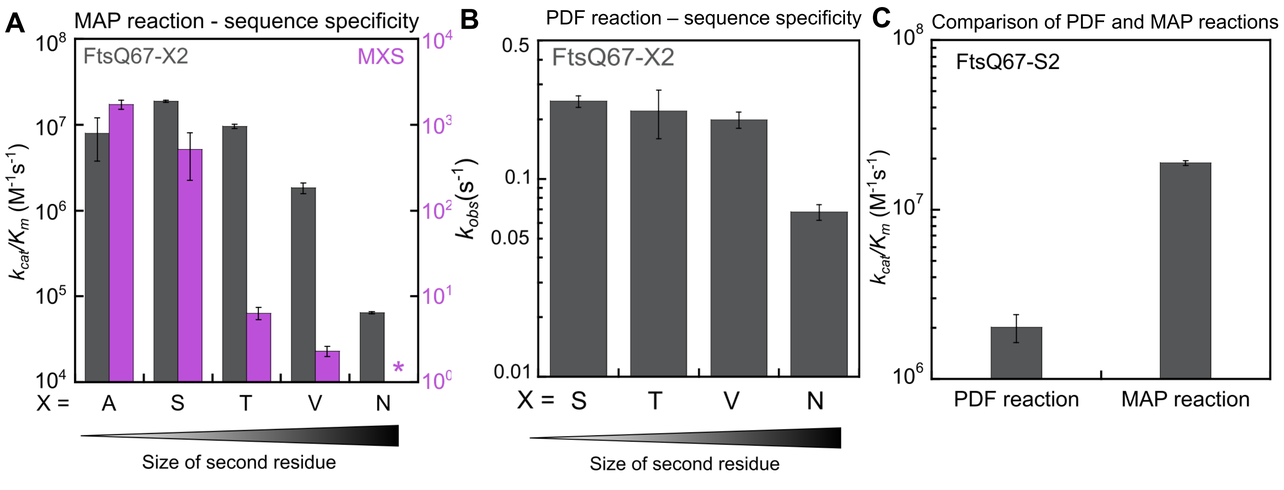


(A) Scheme of the methionine cleavage assay to measure the rate constant of the MAP reaction. **(B)** A representative time trace for cleavage of 10 nM $RNC_{Met\text{-}FtsQ67}$ by 1 μM MAP. **(C)** The observed rate constants for methionine cleavage of $RNC_{Met\text{-}FtsQ67}$ were plotted as a function of MAP concentration to give the k_{cat}/K_m value of the MAP reaction.



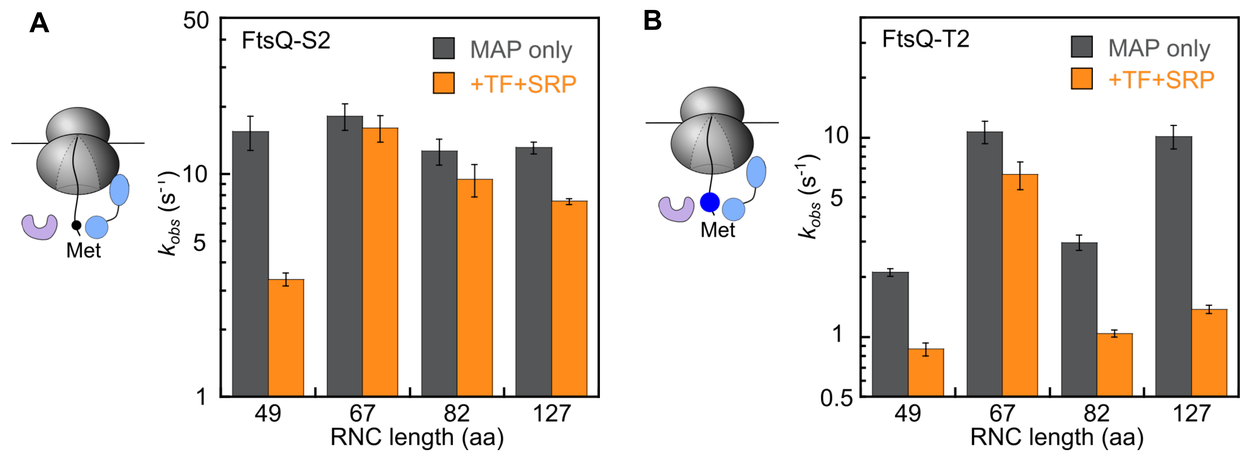
(D) Scheme of the fluorescence-based assay to measure the binding between $RNC_{Met\text{-}FtsQ67}^{Cm}$ and MAP. The FtsQ67 nascent chain was labeled with coumarin (Cm) at the fifth residue. **(E)** Fluorescence emission spectra to demonstrate the enhancement of Cm fluorescence upon binding of $RNC_{Met\text{-}FtsQ67}^{Cm}$ to MAP-H79A. **(F)** Observed rate constants (k_{obs}) of RNC-MAP association plotted against [MAP-H79A] to obtain k_{on} and k_{off} values.

Rate enhancement by ribosome interaction and promiscuous PDF reaction leads to compromised specificity.



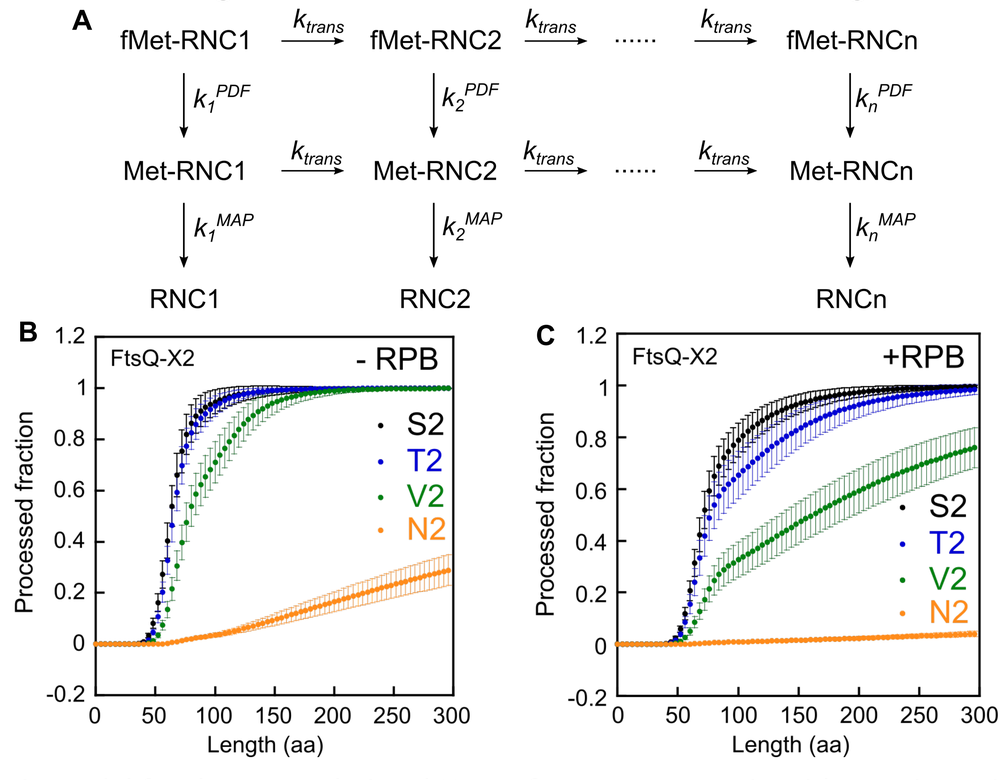
(A) Comparison of MAP specificity toward the second residue for reactions with $RNC_{FtsQ67\text{-}X2}$ versus tripeptide substrates (MXS; replotted from Frottin et al.) **(B)** Observed deformylation rate constants of $RNC_{fMet\text{-}FtsQ67}$ with indicated amino acids at the second residue. **(C)** Comparison of k_{cat}/K_m value for deformylation of $RNC_{fMet\text{-}FtsQ67}$ by PDF, with that for methionine excision of $RNC_{Met\text{-}FtsQ67}$ by MAP.

RPBs impose a limited time window for MAP reaction on suboptimal substrates



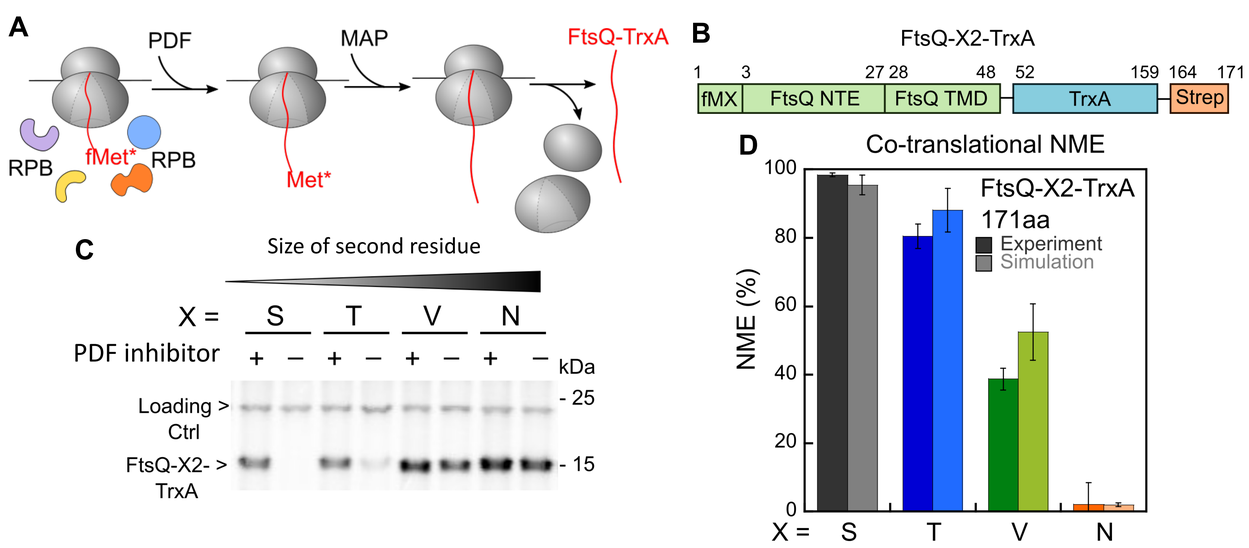
Effects of TF and SRP on the methionine cleavage of RNCs bearing FtsQ-S2 **(A)** and FtsQ-T2 **(B)** nascent chains of the indicated lengths. Observed MAP rate constants were measured with 10 nM RNCs and 1 μM MAP.

Kinetic simulation integrates NME reactions with translation elongation.



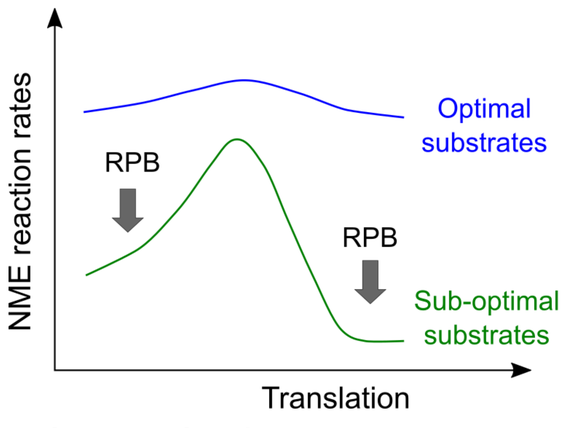
(A) A kinetic model for the cotranslational NME of a nascent protein with *n* amino acids. k_{trans} is the translation elongation rate. The PDF and MAP rate constants at nascent chain length i (k_i^{PDF} and k_i^{MAP}) are experimentally determined. **(B)** and **(C)** Simulated reaction profiles for cotranslational deformylation and methionine cleavage of FtsQ-X2 in the absence (B) and presence (C) of RPBs.

Kinetic simulation predicts co-translational NME in cell extracts.



(A) Scheme of the cotranslational NME assay. FtsQ-X2-TrxA was translated in the S30 extract and processed by endogenous PDF and MAP during translation. **(B)** Scheme of the model substrates. **(C)** and **(D)** Representative SDS/PAGE (C) and autoradiography analysis (D) of the cotranslational NME of FtsQ-X2-TrxA in the S30 extract.

Model



The reaction of MAP on ribosome-bound nascent chains approaches diffusion-limited rates, allowing timely NME for optimal substrates before translation termination. Specificity is achieved by kinetic competition of NME with translation elongation and by regulation from other RPBs, which selectively narrow the processing time window for suboptimal substrates.

Acknowledgement

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