Using Hydrogen-Deuterium Exchange Mass Spectrometry to Investigate Cotranslational Folding

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INTRODUCTION

Cotranslational folding and chaperone activity are critical for efficient protein biogenesis, but how they direct the maturation pathway of nascent proteins remains poorly understood. Here we have used hydrogen-deuterium exchange (HDX) mass spectrometry (MS) to investigate, at peptide resolution, the cotranslational chaperone-assisted folding pathway of E. coli dihydrofolate reductase. DHFR constructs studied

RESULTS AND DISCUSSION

Example UPLC separation Ribosome:Trigger factor:nascent chain complex

1984 peptides identified (1886 for dimeric protein)

DHDHFR peptides available for HDX MS analyses

This map was created based on all possible peptides seen in all RNC constructs and all possible peptides are seen in the HDX MS data for every construct.

Peptide 9-28 was common to all RNC constructs

Deuterium incorporation into peptide 9-28 of DHFR as a function of deuterium exposure time and NC length.

10 second HDX for DHFR RNC peptides followed

HDX RNC activity

Relative deuterium uptake of DHFR peptides after 10 s exposure to deuterium.

HDX MS differences due to nascent chain lengths mapped onto the cartoon structure of monomeric TF bound to the ribosome

HDX protection in WT TF RNC compared to WT TF at the indicated time points

1000 sec difference data plotted on the hypothetical structural model of trigger factor bound to the bacterial ribosome. Ribosomal proteins are shown in cyan. Ribosomal protein L23 is identified. The approximate nascent chain exit is indicated with a purple star.

Schematic biogenesis pathway of DHFR, based on HDX MS analysis of stalled RNCs

HDX MS differences in ribosomal protein L23

D$_{TF}$-D$_{PDB}$

Protected from HDX in complexes without TF

1000 sec difference data plotted on both TF and ribosomal protein L23.

Schematics showing the exit tunnel and ribosomes. The approximate nascent chain exit is indicated with a purple star.

CONCLUSIONS

On the ribosome, the nascent polypeptide folds along an unpaved pathway, via structured intermediates not populated during refolding from denaturant. Association with the ribosome allows these intermediates to form, as otherwise destabilizing C-terminal sequences remain confined in the ribosome exit tunnel, suggesting a general role for the tunnel in facilitating folding.

We find that partially-folded nascent chains recruit the endogenous chaperone Trigger factor, which uses a large composite hydrophobic-hydrophilic interface to engage folding intermediates without disrupting their structure. As a result, the nascent chain bound by Trigger factor is poised to complete folding immediately upon emergence of the C-terminus from the exit tunnel.

We further mined our HDX MS data to study ribosomal protein dynamics at the peptide level. We computationally map interactions between the nascent chain and several ribosomal proteins, tracing the path of the emerging polypeptide during synthesis. Our results provide a peptide-level description of de novo protein folding dynamics, thereby revealing new mechanisms by which cellular factors shape the conformational search for the native state.