Mapping the Co-translational Folding Energy Landscape of the ddFln5 Immunoglobulin Domain

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Efficient protein synthesis and folding, avoiding the population of misfolded or aggregation-prone states, is critical to healthy cell function. During protein synthesis, nascent polypeptides are gradually extruded through the ribosome exit tunnel, and folding may therefore be initiated at the N-terminus of the protein and proceed in a vectorial manner before the C-terminus has fully emerged from the ribosome. This process cannot be described by a single free energy landscape or folding funnel, for the configurational space accessible to the nascent chain increases with the length of the polypeptide chain, and intermediate states may become favored and disfavored as the chain emerges. Therefore, the co-translational 'landscape' must instead be conceptualized as a nested series of landscapes spanning increasingly large conformational spaces. Developing a quantitative understanding of these surfaces, and understanding the perturbations that arise from the presence of the ribosome and associated factors, is currently a major experimental challenge that is currently being addressed by a variety of methods. Within our own group, we have pioneered NMR spectroscopy as a tool to study the length-dependent folding of stalled ribosome-nascent chain complexes [1], focussing particularly on the ddFln5 immunoglobulin domain from the *Dictyostelium discoideum* tandem repeat protein ABP-120 [2].

A complementary experimental approach to the study of stalled ribosome-nascent chain complexes is the use of N-terminal protein fragments to create a ribosome-free model of co-translational folding [3]. This has the particular advantage that the polypeptide fragments may be studied at high concentrations using high-resolution spectroscopic and biophysical methods to accurately characterize the onset of folding in structural and thermodynamical detail. Here we present such a series of N-terminal fragments of ddFln5, revealing a series of folding intermediates that we have characterized extensively using a combination of CD spectroscopy, real-time NMR, EXSY, diffusion and ^{15}N relaxation and relaxation dispersion measurements, and RDC and chemical shift based structure determination. These intermediates are related to the formation of long-range contacts within the protein, and to the isomerisation of a highly conserved native state *cis*-proline residue. These results provide an essential description of the 'ground state' co-translational landscape, against which perturbations in stalled ribosome-nascent chain complexes may be discerned and more fully understood.

References

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