Viewing folding of nascent polypeptide chains from ribosomes

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"By harnessing the power of ribosome profiling technique, this so-called folding-associated cotranslational sequencing provides a unique view of the folding competency of the nascent chain attached to the ribosome."

Regulation of gene expression occurs at multiple stages and ultimately determines the protein levels in cells. The last decade has witnessed impressive progress in the development of approaches to measuring changes in cellular transcription and protein complement. However, techniques monitoring protein synthesis have lagged far behind. Current proteomic studies gauge the steady state levels of intracellular protein and rely on mass spectrometry, which has a low dynamic range and rarely samples more than 10% of the proteome [1]. RNA-seq is a sensitive approach for assessing transcriptional changes but the transcriptome correlates poorly with changes in protein levels [2,3]. Recent data suggest that the regulation of gene expression within cells occurs predominantly at the level of translation and that protein abundance correlates closely with translation rates [3]. Therefore, monitoring protein synthesis is crucial to understanding cellular homeostasis. Additionally, regulation of protein synthesis at the level of translation permits the cell to respond swiftly to adverse conditions [4]. Therefore, translational control is an integral part of cellular stress response.

An intricate process associated with protein synthesis is folding. Proteins must achieve proper tertiary structure to acquire their designated properties and functions. Our current understanding of protein folding is predominantly based on *in vitro* refolding of denatured full-length proteins [5]. However, under native intracellular conditions, protein folding could occur concurrently with the synthesis of primary polypeptide chains on the ribosomes [6,7]. Cotranslational folding of partially synthesized nascent chains differs from refolding of full length polypeptides owing to the vectorial nature and the relatively slow elongation speed of the translation process. However, it is challenging to capture cotranslational folding events of nascent chains attached to the translating ribosome. Several approaches have been developed to dissect this process but most of them rely on *in vitro* systems with limited resolution [7,8]. There is thus an urgent need to establish new approaches to monitor cotranslational folding processes *in vivo* with high resolution. One solution is to look at the folding status of nascent polypeptide chains from the perspective of translating ribosomes.

Ribosome: the birth place of nascent polypeptide chains

Ribosomes are conserved macromolecular enzyme complexes composed of two subunits. The small ribosome subunit decodes mRNA and the large subunit catalyzes polypeptide formation. In eukaryotes, the small (40S) and large (60S) subunits associate to form the 80S ribosome complex upon the initiation of translation [9]. The nascent polypeptide chain begins its journey at the peptidyltransferase center, travels through the peptide tunnel, emerges from the ribosome exit site, finds appropriate binding partners and releases into a

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new environment. Cotranslational folding may start within the ribosome tunnel because a diameter of 10–20 Å is wide enough to accommodate a secondary structure such as an α helix. The length of tunnel (100 Å) is expected to bury at least 30 amino acids when the elongating polypeptide is in a fully extended conformation [10]. Owing to the constraints imposed by the ribosome tunnel, the cotranslational formation of tertiary structures only occurs outside of the ribosome.

Upon extrusion out of the ribosome tunnel, nascent polypeptide chains face a formidable task in the crowded environment of the cell. Most newly synthesized polypeptides undergo substantial interaction with other proteins such as chaperones [11]. Not only do molecular chaperones prevent nascent chains from aggregation, they also assist cotranslational folding of polypeptides attached to the ribosome. The role of chaperones in cotranslational folding is best characterized in *Escherichia coli*. For instance, the trigger factor appears to be the first player in the folding of nascent chains [12]. In eukaryotes, much remains to be learned regarding ribosome-associated chaperones and their substrate specificity during cotranslational folding. In particular, it is unclear whether the chaperone assistance is continuously needed alongside the translation process.

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In addition to ribosome-associated chaperones, elongation speed also influences the cotranslational folding process [13]. Translation elongation is not in a uniform rate but punctuated with frequent ribosome pausing. There is substantial correlation between the kinetics of ribosome translation and the location of rare codon clusters along the mRNA [7]. It has been suggested that the local discontinuous translation temporally separates the synthesis of polypeptide segments and co-ordinates their cotranslational folding [14]. However, this view has been challenged recently [15]. Nevertheless, it remains possible that fine-tuning the translation speed might facilitate the folding process of newly synthesized polypeptides. A recent study reported that slowing the translation speed of *E. coli* ribosomes enhances the GFP folding efficiency [16]. Considering the fact that the folding speed (on the microsecond scale) is much faster than the elongation (~5 amino acids per second in mammalian cells), many details of the cotranslational folding pathway remains to be defined.

Viewing the behavior of nascent chains by ribosome profiling

Several key issues need to be addressed in understanding cotranslational folding. First, how early in translation are any tertiary structures formed? Second, does the folding process follow a sequential order? Third, is the folded conformation stable during elongation? A major hurdle to the capture of cotranslational folding events is the heterogeneous nature of newly synthesized polypeptides. One way to overcome this obstacle is to isolate ribosomes bearing recognizable structures and determine the ribosome positions on mRNAs. Because the length of newly synthesized polypeptides can be inferred precisely from the ribosome positions on mRNAs, it is possible to link cotranslational folding to stages of ribosome elongation. Taking into account the approximately 30 amino acid occlusion within the ribosome tunnel, the minimal length of nascent chains capable of folding can be estimated accordingly.

Ribosome profiling, based on deep sequencing of ribosome protected mRNA fragments, provides a wealth of information about ribosome positions and densities across the entire transcriptome [17]. After RNase I digestion of nonprotected mRNAs, ribosomes bearing specific nascent polypeptides can be enriched from the ribosome pool. Indeed, using an NH_2-terminal tag antibody, ribosomes translating an engineered multidomain fusion protein were captured successfully [18]. Importantly, the ribosome density pattern before and after pull-down was nearly identical except the first 50 codon region that has no ribosome density after affinity purification. This corresponds well with the length of growing polypeptide chain buried within the ribosome tunnel.

By capitalizing specific folding sensitive affinity reagents, one can acquire deep insight into cotranslational folding by capturing the folding status of ribosome-attached nascent chains. As a proof-of-principle, the folding status of FKBP12-rapamycin binding domain (FRB) was probed by its binding partner FKBP in the presence of rapamycin. The results revealed an efficient cotranslational folding immediately after the emergence of the full domain sequence of FRB [18]. By harnessing the power of ribosome profiling technique, this so-called folding-associated cotranslational sequencing (FactSeq) provides a unique view of the folding competency of the nascent chain attached to the ribosome.

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With the increasing number of available conformation-specific antibodies, FactSeq is readily applicable to cellular proteins including viral proteins. Viruses use the host cell translation machinery for their synthesis and the epitopes can be formed cotranslationally. Influenza represents an important target not only because of its public health relevance, but also owing to the fact that lots of neutralizing antibodies recognize conformational determinants of influenza proteins [19]. Using monoclonal antibodies against distinct epitopes of the receptor binding domain of hemagglutinin, FactSeq revealed several unexpected features of cotranslational folding [18]. First, different epitopes within the same domain are formed in a domain-wise manner rather than in a sequential order. Second, both epitopes show discontinuous antibody accessibility. Third, a mutation in one epitope

modulates the folding process of the other, further supporting the domain-wise global folding pathway during translation elongation.

Conclusion

A growing body of evidence suggests that cotranslational folding occurs from *E. coli* to mammalian cells, in particular for multidomain proteins [7]. Nevertheless, the nascent chain attached to the ribosome may adopt folding pathway dissimilar to the same chain refolded in solution due to the constraint imposed by ribosomes. FactSeq has the potential to fill in gaps in our understanding of cotranslational folding. Besides the tremendous sensitivity and resolution, FactSeq takes snapshots of nascent chains with various lengths at one time during the elongation, allowing direct comparison of their folding status quantitatively. As a sensitive tool, FactSeq can be applied to investigate cellular factors controlling cotranslational folding and to measure the folding efficiency under pathological conditions. In addition to cotranslational folding, FactSeq can be used to monitor other fates of nascent polypeptide chains, such as cotranslational degradation and chaperone interaction [20].

Current and emerging sequencing technology provides an unprecedented platform for life sciences. Unlike traditional methods directly detecting polypeptides, FactSeq monitors nascent polypeptide chains from ribosomes. It allows extensive investigation of individual polypeptides with high coverage but relatively low cost. Like many other novel approaches, the prototype of FactSeq still has limitations. One potential challenge is to monitor cotranslational folding inside cells rather than in cell lysates. Additionally, new approaches are required to illuminate real-time folding kinetics of nascent chains. A deeper understanding of cotranslational events will shed light on protein homeostasis that is essential to cellular functions and organismal growth.

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References

1 Bantscheff M, Schirle M, Sweetman G, Rick J, Kuster B. Quantitative mass spectrometry in proteomics: a critical review. *Anal. Bioanal. Chem.* 389(4), 1017–1031 (2007).

2 Rogers S, Girolami M, Kolch W *et al.* Investigating the correspondence between transcriptomic and proteomic expression profiles using coupled cluster models. *Bioinformatics* 24(24), 2894–2900 (2008).

3 Schwanhäusser B, Busse D, Li N *et al.* Global quantification of mammalian gene expression control. *Nature* 473(7347), 337–342 (2011).

4 Sonenberg N, Hinnebusch AG. Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* 136(4), 731–745 (2009).

5 Sosnick TR, Barrick D. The folding of single domain proteins – have we reached a consensus? *Curr. Opin. Struct. Biol.* 21(1), 12–24 (2011).

6 Kramer G, Boehringer D, Ban N, Bukau B. The ribosome as a platform for co-translational processing, folding and targeting of newly synthesized proteins. *Nat. Struct. Mol. Biol.* 16(6), 589–597 (2009).

7 Komar AA. A pause for thought along the co-translational folding pathway. *Trends Biochem Sci.* 34(1), 16–24 (2009).

8 Johnson AE. The co-translational folding and interactions of nascent protein chains: a new approach using fluorescence resonance energy transfer. *FEBS Lett.* 579(4), 916–920 (2005).

9 Jackson RJ, Hellen CU, Pestova TV. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat. Rev. Mol. Cell Biol.* 11(2), 113–127 (2010).

10 Fedyukina DV, Cavagnero S. Protein folding at the exit tunnel. *Annu. Rev. Biophys.* 40, 337–359 (2011).

11 Frydman J. Folding of newly translated proteins *in vivo*: the role of molecular chaperones. *Annu. Rev. Biochem.* 70, 603–647 (2001).

12 Bukau B, Weissman J, Horwich A. Molecular chaperones and protein quality control. *Cell* 125(3), 443–451 (2006).

13 O'Brien EP, Vendruscolo M, Dobson CM. Prediction of variable translation rate effects on cotranslational protein folding. *Nat. Commun.* 3, 868 (2012).

14 Zhang G, Hubalewska M, Ignatova Z. Transient ribosomal attenuation coordinates protein synthesis and co-translational folding. *Nat. Struct. Mol. Biol.* 16(3), 274–280 (2009).

15 Ingolia NT, Lareau LF, Weissman JS. Ribosome profiling of mouse embryonic stem cells reveals the complexity and dynamics of mammalian proteomes. *Cell* 147(4), 789–802 (2011).

16 Siller E, DeZwaan DC, Anderson JF, Freeman BC, Barral JM. Slowing bacterial translation speed enhances eukaryotic protein folding efficiency. *J. Mol. Biol.* 396(5), 1310–1318 (2010).

17 Ingolia NT, Ghaemmaghami S, Newman JR, Weissman JS. Genome-wide analysis *in vivo* of translation with nucleotide resolution using ribosome profiling. *Science* 324(5924), 218–223 (2009).

18 Han Y, David A, Liu B *et al.* Monitoring cotranslational protein folding in mammalian cells at codon resolution. *Proc. Natl Acad. Sci. USA* 109(31), 12467–12472 (2012).

19 Yewdell JW. Monoclonal antibodies specific for discontinuous epitopes direct refolding of influenza A virus hemagglutinin. *Mol. Immunol.* 47(5), 1132–1136 (2010).

20 Oh E, Becker AH, Sandikci A *et al.* Selective ribosome profiling reveals the cotranslational chaperone action of trigger factor *in vivo*. *Cell* 147(6), 1295–1308 (2011).