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Translational machinery and protein folding: Evidence of conformational variants of the estrogen receptor alpha

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Abstract

As an approach to understand how translation may affect protein folding, we analyzed structural and functional properties of the human estrogen receptor alpha synthesized by different eukaryotic translation systems. A minimum of three conformations of the receptor were detected using limited proteolysis and a sterol ligand-binding assay. The receptor *in vitro* translated in rabbit reticulocyte lysate was rapidly degraded by protease, produced major bands of about 34 kDa and showed a high affinity for estradiol. In a wheat germ translation system, the receptor was more slowly digested. Two soluble co-existing conformations were evident by different degradation patterns and estradiol binding. Our data show that differences in the translation machinery may result in alternative conformations of the receptor with distinct sterol binding properties. These studies suggest that components of the cellular translation machinery itself might influence the protein folding pathways and the relative abundance of different receptor conformers.

Keywords: *In vivo* protein folding; Protein biosynthesis; Limited proteolysis; Estrogen receptor; *In vitro* translation; Rabbit reticulocyte lysate; Wheat germ extract

Newly translated protein adopts characteristic folded structures within the complex and crowded cellular environment. Although protein secondary structure can be determined largely by its amino acid sequence, protein folding within the cell is affected by a range of factors beyond its composition of amino acids. Protein conformation can result from transient interactions with other proteins and ligands, such as chaperones, ancillary chaperonins, and folding catalysts. Also, the translation machinery can affect the folded state of its protein product by modulating the rate of translation elongation [1,2] (for a review [3]).

The folded states adopted by newly synthesized protein involve both co- and post-translational mechanisms, *i.e.*, those acting during or after protein biosynthesis. The integration of proposed mechanisms into a coherent view of the process of protein folding *in vivo* has been addressed experimentally mainly in a number of *in vitro* systems [4]. Co-translational folding has been proposed to play an essential role in the biosynthesis of many proteins, and especially for large multi-domain proteins [3]. The native conformation of the protein is adopted during its biosynthesis on the ribosome in the co-translational protein folding model. Evidence for this model was obtained in several systems involving the vectorial biosynthesis of polypeptides from their amino to their carboxyl terminus, and in showing that polypeptides still bound to the ribosome had functional properties of the mature protein, *e.g.*, enzymatic

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activity; binding of conformational antibodies, cofactors, or ligands (for a review [4]).

The chaperones are thought to prevent newly synthesized protein from misfolding and aggregating by impeding undesired hydrophobic interactions and allowing for alternative folding pathways [5]. The ribosome environment and the translation elongation rate could also affect the nascent peptide folding process and the ability to interact with chaperones [6,7]. Translation elongation proceeds in a step-by-step manner, and polypeptide emerges at the exit site of the ribosome into the cytoplasm across the ribosome channel. The rate of protein translation elongation is not uniform as the mRNA is read, and ribosomes are seen to pause as well as stack-up in specific regions or sites along the mRNA [8–14]. Two features of the mRNA molecule enable it to provide more information than the primary sequence of the peptide to be formed, both of which can affect the rate of translation elongation and thus the protein folding pathway. Polynucleotide secondary structure or its absence within the mRNA can alter the speed at which it can be translated by the ribosome [10]. The other feature is the occurrence of so-called slow codons or codons that direct the binding of anticodons on isoacceptor tRNAs present in low relative abundance in the cell [7,8,13]. It has been shown that codon usage is a major determinant for differences in translation rate, and correspondingly the relative composition of available isoacceptor tRNA pools is of primary importance [7,8]. Concerning the predicted secondary structure of a peptide, it was reported that segments with α-helical structures tend to be encoded by sequences of fast codons in the mRNA, whereas predicted β-strands and coil regions are often encoded by slow codons [14,15].

Our study addressed the possible effects of the translation machinery on protein folding and thus the conformation adopted by a protein that is known to exhibit conformational variation, the human estrogen receptor alpha (ERα)[1] [16,17]. The experimental strategy involves the use of two well established *in vitro* translation systems derived from evolutionarily distant eukaryotes, cell-free extracts of wheat germ and rabbit reticulocyte. ERα is a member of a large family of nuclear receptors that activate or repress the transcription of specific target genes. Regulation is achieved through recruitment of the receptor to DNA response elements within target gene promoters, either directly through interaction with DNA elements or through protein/protein interaction with other transcriptional factors [16,17]. Upon binding its natural ligand estradiol, the receptor undergoes an activating conformational change allowing it to interact with specific cofactors for binding to DNA. This protein–DNA complex acquires the ability to activate or repress different sets of genes depending on both the cell and the promoter context [18,19]. In particular, it has been shown that in addition to classical agonists and antagonists, the transcriptional activity can be regulated by selective estrogen receptor modulators, whose relative agonist/antagonist activity is determined by the cell [19,20]. The differential regulatory effect appears as tissue specific responses to a given ligand, but the mechanisms involved in the modulation of the regulatory activity are not well defined. Understanding these tissue-specific effects is of major importance for the study and design of selective estrogen receptor modulators for treatment of diseases such as breast cancer and osteoporosis.

Until recently it was generally believed that co-activators would be expressed in a cell specific manner and that recruitment of cofactors was determined by the concentrations of these proteins. However, it has been reported that with a few exceptions, the majority of cofactors are widely expressed in similar amounts in most cells [20]. It follows then that the binding affinity or the availability of specific binding sites for cofactors likely plays a role in regulating ERα activity. Recently, Mérot et al. investigated the influence of the differentiated state of the cell on ERα trans-activation and interestingly the authors showed that transcriptional activity varied with the cell developmental stage [18].

As an approach to understand whether differences in the translation machinery could lead to alternative conformations and contribute to cell-specific receptor activity, we analyzed the influence of two translation systems on nascent ERα structure. Our study suggests that the cellular context of biosynthesis may affect the folding pathway leading to alternative or co-existing ERα conformations with distinct functional activities.

Materials and methods

In vitro transcription of the ERα

The human ERα coding sequence (accession number NM_00125) was previously cloned in the expression vector pSG5 (Stratagene, CA, USA). The transcription reactions were done with transcription *in vitro* systems (Promega, CA, USA) following manufacturer's instructions manual with minor modifications: in 50 μl (total volume) of 80 mM Tris–HCl (pH 8.0), 20 mM $MgCl_2$, 2 mM spermidine, 5 mM dithiothreitol (DTT), 50 μg/ml BSA, 1.5 mM ATP, 1.5 mM GTP, 1.5 mM UTP, 1.5 mM CTP, 2.5 μl (100 U) of RNasin (Promega), 5 μg of SalI-linearized pSG5-ERα DNA template and 80 U of T7 RNA polymerase (Promega). Reactions were carried out at 37 °C for 2 h. The product was treated with RNAse-free DNase at 37 °C for 30 min for DNA template degradation. The transcripts were purified using the RNAeasy Mini Column Kit (QIAGEN). The purity and integrity of the RNA were checked by 1% agarose gel electrophoresis.

Cell-free protein synthesis

Cell-free translation of the ERα was done using either the rabbit reticulocyte lysate (RRL) or the wheat germ extract (WGE) *in vitro* translation systems (Promega) in the presence of [^{35}S]methionine (15 mCi/ml, Amersham). The final concentration of ERα mRNA in both systems was 30 μg/ml. *In vitro* translation was done at 30 °C, for 1.5 h in the RRL

[1] *Abbreviations used:* ERα, estrogen receptor alpha; E2, 17β-estradiol; DES, diethylstilbestrol; RRL, rabbit reticulocyte lysate; WGE, wheat germ extract.