Gene Regulation Lecture No 5: Protein folding and Degradation

1-Protein folding

For a given protein to be fully functional it has to be correctly folded. The correct folding enables proteins to have their three dimensional shape which help them performing their function. For example, if the protein has a catalytic function (i.e: an enzyme which mediates a specific reaction), its 3D structure has to contain specific grooves suit its substrates to fit in and the reaction to successfully complete.

Failure of the protein to correctly fold "protein mis-folding" was found to be associated with a number of pathological conditions. One of the well known protein mis-folding associated diseases is Parkinson Disease (PD). The molecular basis of PD appears to be tightly coupled to the aggregation of α-synuclein and the factors that affect its conformation. α-Synuclein attracted significant interest in 1997 after a mutation in its gene was found to be associated with the familial cases of early-onset PD, and its aggregates were found to be the major components of Lewy bodies, the hallmarks of PD (Breydo, *et al*., 2012).

Endoplasmic reticulum is the place where folding occurs for proteins destined for both intracellular organelles and the cell surface. Recent studies have identified specific signaling pathways that emanate from the ER to regulate mRNA translation. These pathways prevent the accumulation of unfolded protein in the ER by decreasing the load, increasing the ER folding capacity, and increasing the degradation of misfolded proteins. It is becoming apparent that these signaling pathways that determine the rate of polypeptide synthesis represent an essential component of cell differentiation as well as specific responses to viruses, hormones, growth factors, nutrients and other external stimuli. In this section of the lecture we will discuss scientific evidences support that proteins are being translocated to the ER in the unfolded state and that the folding occurs in the ER.

Proteins are trans located into the ER lumen in an unfolded state

The ER is the site of biosynthesis for sterols, lipids, membrane-bound and secreted proteins, and glycoproteins. Approximately one-third of all cellular protein synthesis occurs on the membrane of the rough ER. For specialized cells that function to secrete proteins, such as plasma cells, hepatocytes and pancreatic cells, 90% of the translated polypeptides are trans-located into the ER. Inhibition of translation initiation serves as an effective means to limit the flow of proteins into the ER. The targeting of proteins destined for the secretory pathway is regulated at the level of translational elongation. In higher eukaryotes, trans-location of nearly all proteins across the ER membrane occurs co-translational and is usually directed by an N-terminal signal peptide. Upon exposure of the signal peptide from the 60S ribosome, the signal recognition particle (SRP) binds and imposes a translational elongation arrest until the ribosome docks at the ER membrane. Docking of the SRP to the SRP receptor and transfer of the nascent polypeptide into the Sec61 channel in the ER membrane relieves the translation elongation block so that the polypeptide is simultaneously synthesized and trans-located across the lipid bilayer. This mechanism prevents the polypeptide from improper localization in the cytosol.

The minimal mammalian ER translocon is composed of an aqueous pore formed by the Sec61 complex. Immunoglobulin-binding protein/glucose-regulated protein of 78 kDa (BiP/GRP78) is a soluble, ER-resident, ER-stress-inducible member of the heat shock proteins (hsp)70 family that is also associated with the translocon. BiP has a peptide-dependent ATPase activity that is used to seal the luminal side of the aqueous pore to maintain the permeability barrier between the ER and the cytosol when the ribosome is not tightly attached. BiP also functions in post-translational translocation as a molecular ratchet to ensure unidirectional translocation of the elongating polypeptide across the ER membrane.

Given that BiP is the first member of a welcoming committee of molecular chaperones (a group of proteins playing important roles in protein folding and will be discussed later in this lecture) to interact with the nascent polypeptide translocating into the ER lumen.

Polypeptide folding, assembly and trafficking occurs in the ER

Because the protein concentration in the ER lumen is 100 mg/m, it is essential that protein chaperones facilitate protein folding by preventing aggregation of protein folding intermediates and by correcting misfolded proteins. These energy-consuming processes ensure high fidelity protein folding in the oxidizing environment of the ER lumen. For example, the most abundant ER chaperone – BiP/GRP78 – uses the energy from ATP hydrolysis to facilitate folding by preventing aggregation of proteins within the ER. Only those polypeptides that are properly folded and assembled in the ER can transit to the Golgi compartment, a process termed quality control. Proteins that are misfolded in the ER are retained and eventually translocated back through the Sec61 channel into the cytosol for degradation by the 26S proteasome. This ER-associated degradation (ERAD) requires retrograde transport of unfolded or misfolded proteins through the Sec61 complex out into the cytosol, where they are deglycosylated by an N-glycanase activity, ubiquitinated, and degraded by the 26S proteasome. By contrast, properly folded proteins are packaged into transport vesicles for transport to the Golgi.

Polypeptide folding status in the ER is communicated to the cytosol and nucleus

Because protein translation occurs in the cytosol, and folding occurs in the ER lumen, it is essential that the status of polypeptide folding in the lumen be communicated to the protein synthesis machinery in the cytosol. There are highly conserved specific signaling pathways that ensure the protein folding capacity in the ER is not overwhelmed. Upon accumulation of unfolded proteins in the ER lumen, these adaptive pathways are activated to: (i) reduce the amount of new protein translocation into the ER lumen, (ii) increase ERAD, and (iii) increase the protein folding capacity and secretion potential of the ER. These pathways are collectively termed the unfolded protein response (UPR). The UPR is orchestrated by a general attenuation of translation initiation, a selective translation of a small subset of mRNAs encoding adaptive functions, and transcriptional activation of a large set of genes. In multicellular organisms, if these translational and transcriptional adaptive responses are not sufficient to relieve the unfolded protein load (ER stress), the cell enters one of the cell-death pathways of apoptosis or necrosis.

The complex network of signaling responses to ER stress is regulated by only a few ER transmembrane proteins, inositol-requiring 1 (IRE1), dsRNA-activated protein kinase-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6) (Fig 1). Signaling through IRE1, PERK and ATF6 regulates the production and/or quality of basic leucine-zipper (bZIP)-containing transcription factors that can form homo- and heterodimers. Combinatorial interactions of these factors generate diversity for transcriptional induction of different subsets of UPR-responsive genes. The UPR sensors IRE1, PERK and ATF6 all have luminal domains that sense the presence of unfolded proteins within the ER.

A model was proposed where these UPR transducers are regulated by a common mechanism, the level of free BiP. BiP is a negative regulator of the UPR and interacts with IRE1, PERK and ATF6 under non-stressed conditions. When unfolded proteins accumulate in the ER, BiP is released from IRE1, PERK and ATF6. It is believed that the unfolded proteins bind and sequester BiP to prevent its interaction with IRE1, PERK and ATF6. Essentially, any protein that binds to BiP will activate the UPR if expressed at a sufficiently high level. Proteins that do not bind BiP, even though they are improperly folded, do not induce the UPR. Binding of IRE1 and PERK to BiP prevents their dimerization.

The release of BiP from IRE1 and PERK permits a spontaneous homo-dimerization mediated by their luminal domains, leading to autophosphorylation. Auto-phosphorylation of IRE1 activates its RNase activity. It cleaves the X-box-binding protein 1 (XBP1) mRNA to remove a small intron, resulting in a translational frameshift to yield a more potent transcriptional activator. Simultaneously, PERK dimerization leads to its activation to phosphorylate eukaryotic initiation factor 2 (eIF2) on the a-subunit at Ser51 to reduce the frequency of AUG codon recognition. As eIF2a phosphorylation reduces the functional level of eIF2, the general rate of translation initiation is reduced. However, selective mRNAs, such as ATF4 mRNA and cationic amino acid transporter 1 (CAT1) mRNA, are preferentially translated under these conditions.

By contrast, BiP interaction with ATF6 retains ATF6 in the ER. BiP release permits ATF6 transport to the Golgi compartment where it is cleaved by site-1 protease and site-2 protease to generate a cytosolic fragment that migrates to the nucleus to activate UPR transcription (Kaufman, 2004).

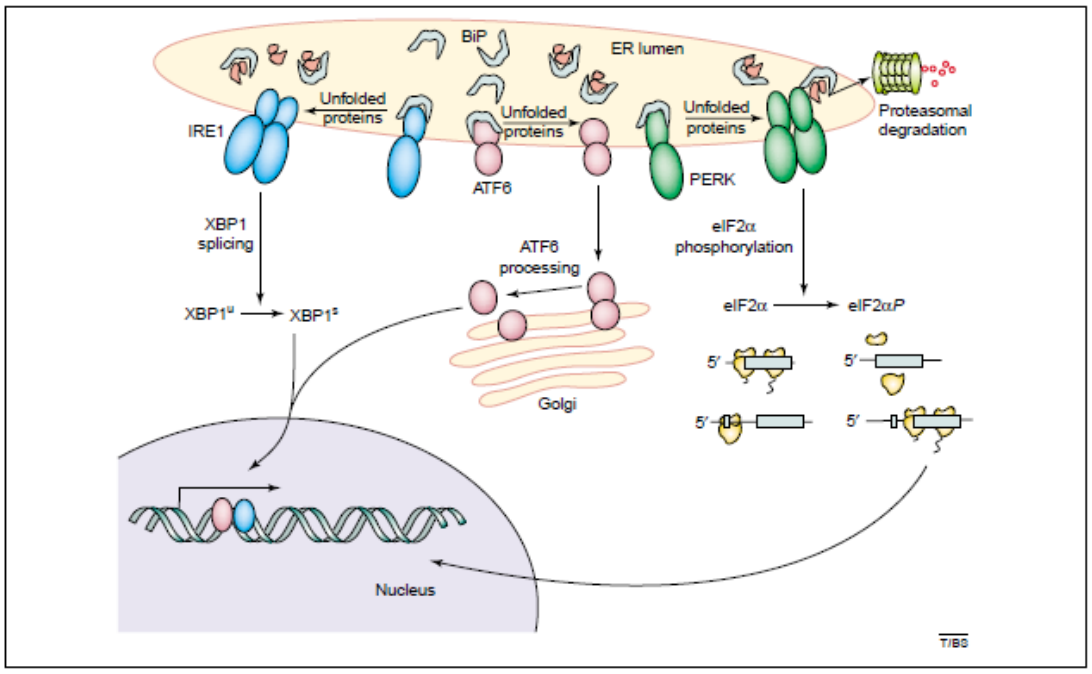

Fig 1. Signaling the unfolded protein response (UPR) in eukaryotes (Kaufman, 2004).

Mechanism of protein folding

As a newborn polypeptide emerges into the world, its first contacts with the cellular environment may be critical for determining its fate. Ribosome-bound nascent polypeptides are confronted by a unique set of dangers that must be avoided on the way to achieving a mature, native conformation. Fortunately, a remarkable mechanism involving molecular chaperones has evolved to safeguard the folding of nascent chains. Although chaperones are clearly important for protein folding and cellular viability, it has been argued that only a few essential proteins require chaperones to fold correctly, whereas the majority of proteins fold spontaneously. An alternative possibility stems from the broad specificity of chaperone binding *in vitro*: as nearly every unfolded polypeptide has the potential to bind chaperones, all newly translated polypeptides might transiently associate with chaperones. A number of new studies have now addressed this problem experimentally and have begun to define the role of chaperones in the folding of newly translated polypeptides (Frydman and Feldman, 2000).

The folding problems of newly translated polypeptides

It is known that the information which is essential to determine the native 3D structure of a protein is present in its complete amino acid sequence; however, efficient, reversible folding and unfolding do not occur in most proteins. This is because the hydrophobic nature of the newly synthesized polypeptide chains. Under the physiological conditions, the very high concentration of macromolecules creates conditions of crowding. This, together with the hydrophobic nature of the polypeptide chains, highly favors aggregation. The N-terminal portion of a nascent polypeptide could, in principle, fold spontaneously as it emerges from the ribosome, however, the cooperative nature of the interactions that stabilizes folded structures requires that a complete folding domain (50–200 amino acids) be available for productive folding. Furthermore, translation occurs on a timescale of seconds (in bacteria) to several minutes (in eukaryotes), much slower than the millisecond timescale of hydrophobic collapse. Thus the extended polypeptide chains have to be protected, somehow, from being collapsed by the action of their hydrophobic nature until a complete folding domain gets exposed from the ribosomal unit.

Mounting evidence now indicates that molecular chaperones interact with and stabilize nascent and translocating polypeptides *in vivo* and prevent nonproductive reactions, such as aggregation. Two major classes of ATP-dependent chaperones, the Hsp70s and the chaperonins, have been implicated in *de novo* protein folding in the cytosol of eukaryotic and prokaryotic cells, as well as in organelles such as ER, mitochondria and chloroplasts.

The Hsp70s, in conjunction with co-chaperones of the DnaJ/Hsp40 family, bind and release short linear peptide segments with a net hydrophobic character; such hydrophobic regions are probably present in all unfolded polypeptides. Association with an Hsp70 results in the stabilization of a polypeptide in an extended conformation, thereby preventing its aggregation. In many instances, the Hsp70-bound substrate must be transferred to a chaperonin complex for productive folding.

The chaperonins are large cylindrical protein complexes consisting of two stacked rings of seven to nine subunits each. The chaperonin of the eukaryotic cytosol, termed TRiC or CCT (for TCP-1 ring complex or chaperonin- containing TCP-1, respectively, where TCP-

1 is tailless complex polypeptide-1), forms a cage-like structure, but it is hetero-oligomeric, containing eight different subunits per ring. Unlike Hsp70s, chaperonins appear to interact with nonlinear hydrophobic determinants exposed in compact folding intermediates. Early studies of Hsp60 function in mitochondria and chloroplasts suggested that chaperonins play an important role in mediating protein folding and assembly. Several associated proteins continue to interact with chaperonins throughout the course of their lifetime, indicating that, in addition to folding, the chaperonin may also play an important role in the structural maintenance of mature cellular proteins.

The substrate spectrum of the eukaryotic cytosolic chaperonin TRiC/CCT has been a matter of controversy. It has been suggested that TRiC is a specialized chaperone that folds only a few cytoskeletal proteins. In contrast, direct examination of the substrate spectrum of TRiC/CCT using pulse-chase analysis in mammalian cells demonstrated that 9–15% of newly synthesized proteins transit through the chaperonin. The identity and structural features that characterize cellular TRiC substrates remain to be defined. On the basis of the structure of these known examples, TRiC substrates may have a complex domain organization that results in folding intermediates with a higher tendency to aggregate; alternatively, they may share a requirement for binding to either a cofactor or an oligomeric partner in order to complete folding. Given that most of the heterogeneity among TRiC subunits resides in the putative substrate-binding domain, it is possible that different subunits in the complex have evolved to recognize different motifs in substrate proteins. Figure 2 shows a simplified diagram for how chaperons and chaperonins regulate protein folding (Fig 2) (Frydman and Feldman, 2000).

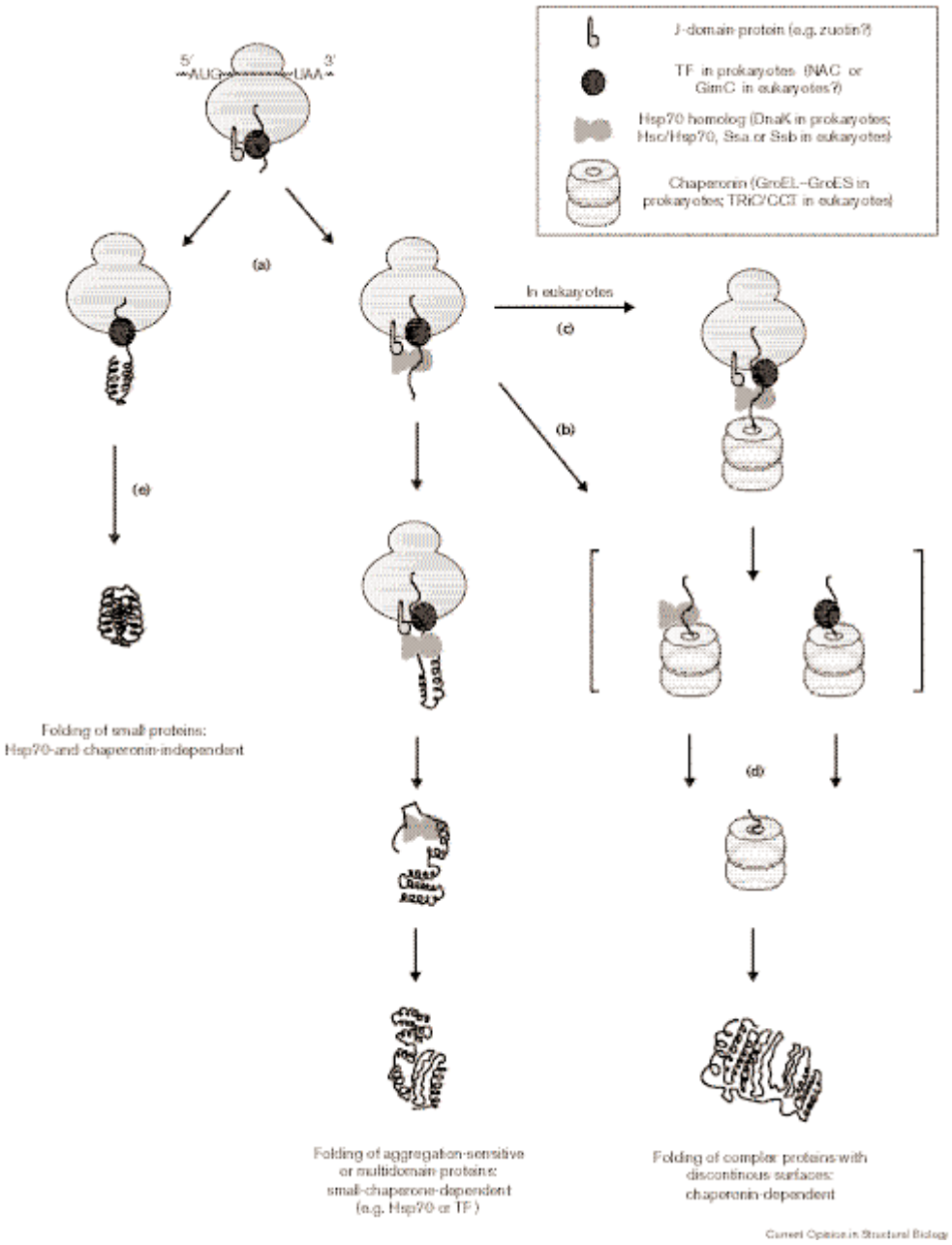

Fig 2: Schematic representation of de novo protein folding in the cytosol of prokaryotic and eukaryotic cells. The model emphasizes the evolutionarily conserved characteristics of the folding process; however, some aspects are specific to either prokaryotic or eukaryotic cells. For instance, co-translational domain folding, as well as association of the chaperonin complex with nascent chains, is favored in eukaryotes (Frydman and Feldman, 2000).

Protein Ubiquitination

The protein ubiquitination part of this lecture is attached as a pdf file. It is a very nice review. Thus all credits go to the authors of this review. The reason why I have not changed it is because this review contains all the necessary information about Ubiqutination which is needed in this stage. Also it is well written and easy to follow.

The review:

Review

The ubiquitin-mediated protein degradation pathway in

cancer: therapeutic implications

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Received 14 April 2004; received in revised form 16 June 2004; accepted 6 July 2004

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