The Lifecycle of an Integral Membrane Protein

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Integral membrane proteins are important for the transfer of information, nutrients, and energy between cells and organelles, as well as cellular binding. There are three types of integral membrane proteins: single pass, α-helical, and β-barrel. The major steps of the life cycle of a membrane protein can be organized into several major steps: transcription, RNA processing, translation, trafficking, membrane insertion, operation, and degradation. Various pathways have been identified within eukaryotic membrane protein in vivo production and between eukaryotes and prokaryotes. The different pathways will be described, with priority to eukaryotic membrane protein production.

In general, integral membrane proteins experience similar transcription and translation processes. Depending on the location of the signal sequence on the nascent protein, translation can either be halted or go to completion. In order to provide a comprehensive review of transcription and translation and to mitigate tangents due to steps where various pathways can be observed, both processes will be described going to completion. The different types of integral membrane proteins will be bundled with their corresponding trafficking and insertion pathways.

General Transcription – The first step in the life cycle of a membrane protein is transfer of genetic information from DNA to a complementary messenger RNA (mRNA) by transcription. There are three major steps in transcription: initiation, elongation, and termination. In general, the initiation step occurs when a pre-initiation complex, composed of RNA polymerase and transcription factors, binds to a specific promoter sequence of the DNA coding sequence (1). Elongation is the process of unzipping the DNA helix, copying the DNA strand to form a single strand of RNA (1). Transcription termination occurs when a termination sequence is reached or in the presence of a termination factor and the transcription complex is dissociated (1). The processes of eukaryotic and prokaryotic transcription are similar but the enzymes and proteins used are different. Eukaryotic transcription will be described in the following paragraph. Prokaryotic transcription will be described later as it occurs simultaneously with translation.

Eukaryotic Transcription – In eukaryotes, transcription occurs in the nucleus. Transcription of genes encoding mRNA is catalyzed by RNA polymerase II and several transcription factors (1). The RNA polymerase II and the transcription factors, TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH, form a pre-initiation complex that binds to a specific promoter sequence, the TATA box, of the DNA coding strand (1). The TATA box is a consensus sequence: T-A-T-A-A/T-A-A/T (1). The promoter sequence also contains an initiation site, where transcription is initiated (7 Life: The Science of Biology Sinauer Associates). Assembly of the pre-initiation complex occurs in several steps. The TATA box binding protein, TFIID, identifies and binds to the TATA box, creating a foundation to help form a stable protein-DNA complex (1). TFIID binding also partially unwinds the DNA helix by inserting phenylalanine between the two DNA strands (1). TFIIB coordinates with TFIID, creating a stable TFIIB-TFIID-DNA complex (1). TFIIA further stabilizes the complex by making contact with the DNA backbone, on the opposite surface of the double helix that TFIIB is interacting with, upstream of the TATA box (1). Non-phosphorylated RNA polymerase II, TFIIF, TFIIE, and TFIIH finish the pre-initiation complex

in that order (1). The transcription factor TFIIH performs two important steps necessary for transcription: unwinding the DNA helix around the initiator site and phosphorylation of RNA polymerase II (6). Phosphorylation of RNA polymerase II and activation by activator proteins that are bound to enhancer regions in the DNA, releases RNA polymerase II from the initiation complex and begins the elongation phase of transcription (1).

Elongation is the process of the RNA polymerase II reading the DNA coding strand and generating a complimentary mRNA strand. As RNA polymerase II travels along the DNA strand, it reads the DNA coding strand, and adds the complimentary RNA nucleotides to the growing mRNA strand are read, and a complimentary RNA nucleotide is added to the growing mRNA strand (7). The RNA continues to travel along the DNA strand, creating a single stranded RNA, until a transcription termination sequence is met on the DNA strand (7).

Eukaryotic RNA Processing – The newly formed RNA, considered pre-mRNA, needs to be converted to mature mRNA through RNA processing. In eukaryotes, RNA processing takes place in the nucleus and includes three parts: capping, polyadenylation, and splicing. The processing helps stabilize the mature mRNA strand, to initiation translation, and to facilitate nuclear export (1). A 5′-cap, modified GTP, is added to the 5′ end of the pre-mRNA to facilitate binding to the ribosome and to protect mRNA from ribonuclease digestion (7). Between 50 and 200 adenine nucleotides are added to the 3′ end of the pre-RNA to create a poly-A tail to facilitate nuclear export and stabilize the mature mRNA strand (1). Introns, or segments of the pre-RNA sequence that do not code for the protein of interest, are removed from the pre-mRNA through a splicing process performed by spliceosomes (7). Spliceosomes are complexes consisting of four small nuclear ribonucleoproteins (snRNPs) (1). The snRNPs identifies specific consensus sequences of the pre-mRNA: the exon-intron boundaries and a branch point in the intron sequence (1). The intron is excised through a process of boundary sequence recognition, rearrangement of the pre-mRNA, splicing at the 5′ splice site, lariat structure generation and removal (1). After RNA processing, the mRNA exits the nucleus through the nuclear pore and into the cytoplasm where translation takes place.

General Translation – The mRNA is converted to protein in a process called translation. Translation occurs in three major steps, initiation, elongation, and termination, and involves two important biological molecules: the ribosome and transfer RNAs (tRNA). Initiation factors assemble the initiation complex, which consists of the ribosome and charged tRNA with methionine, around the mRNA initiation site (1). The mRNA codons are converted to amino acids during elongation. The ribosome ratchets along the mRNA strand to expose one codon at a time, in which a charged tRNA, with the proper anticodon, will bind to the codon. A tRNA molecule is considered charged when it contains its specific amino acid. The ribosome ratchets to the next mRNA codon, allowing the amino acid attached to the tRNA to bind to the growing amino acid chain. Elongation continues until a stop codon is reached, in which termination factors will dissociate the translation complex and excise the protein. The details for the translation of membrane proteins are more complex and will be elucidated in the following discussion.

Eukaryotic Translation – In eukaryotes, translation occurs freely in the cytoplasm or across the endoplasmic reticulum membrane (1). Initiation factors, IF-1, IF-2, IF-3, assist in assembling the

initiation complex that includes the small and large ribosomal subunits, the methionine charged tRNA, and the mRNA initiation site (1). The small subunit of the eukaryotic ribosome, 40S, binds to the 5′ cap of the mRNA (1). The small ribosomal subunit moves down the mRNA strand until it identifies the start codon, AUG (7). A special formylmethionine charged tRNA containing the matched anticodon, UAG, binds to the mRNA start codon (7). The large ribosomal subunit, 60S, joins the initiation complex and aligns the charged tRNA in the P site (7). The large ribosomal subunit contains 3 tRNA binding sites, A, P, and E, which play important roles as the ribosome traverses the mRNA (7). The initiation complex is complete and elongation can be performed. The mRNA codon in the A site is exposed to be matched with the proper charged tRNA anticodon. Charging of tRNA molecules is a highly specific process: tRNA will bind to a specific amino acid through an ATP dependent reaction catalyzed by a specific synthetase (7). The roles of synthetases are two-fold, synthesis and editing, to ensure that the proper amino acid is bound to the tRNA and ultimately that the correct amino acid is added to the polypeptide chain (Reference class notes). An elongation factor, EF-Tu, shuttles charged tRNAs to the A site of the ribosome (1). If the anticodon of the charged tRNA compliments the RNA codon exposed at the A site, the anticodon binds to the RNA codon and EF-Tu is excised through GTP hydrolysis (1). A peptidyl transferase reaction occurs between the methionine attached to the tRNA in the P site and the amino acid attached to the tRNA in the A site, which forms a peptide bond between the two amino acids, leaving an uncharged tRNA in the P site and an extended tRNA in the A site (1). This reaction is catalyzed by highly conserved rRNA molecules in the large ribosomal subunit (1). Once the peptide bond is formed, the large ribosomal subunit ratchets in the 3′ direction of the mRNA, which moves the extended tRNA to the P site, the uncharged tRNA moves to the E site and dissociates from the complex, and the next codon is exposed in the A site (1).

Elongation continues and the growing nascent protein chain travels through the peptide exit tunnel of the large ribosomal subunit (8). The exit tunnel can accommodate a linear protein chain approximately 30 amino acids in length (8). Alternatively, interactions inside the exit tunnel can promote the formation of a α-helical protein structure consisting of up to 60 amino acids, which is common for membrane proteins (8). Once the nascent protein emerges from the exit tunnel, it interacts with several molecules that assist in co-translational processing events, such as modifications, folding, and targeting of the nascent protein chain (8). Co-translational processing is performed by but not limited to chaperones, signal recognition particles (SRP), peptide deformylase (PDF), and methionine aminopeptidases (MAPs) (8). Since nascent protein chains typically emerge from the exit tunnel in an unfolded state, they are susceptible to aggregation and degradation. Molecular chaperones assist in co-translational folding and protection of nascent protein chains (8). Eukaryotes contain multiple chaperone systems (Hsp70, J-protein-based systems, and heterodimeric nascent polypeptide-associated complexes) due to the increase complexity of large protein folding (8). Chaperones typically bind to the ribosome in a way that they are the first molecules that the emerging nascent protein interacts with, providing a protective cradle for the protein (8). Signal recognition particles (SRP) recognize specific signal peptide sequences on the nascent protein, associates with the nascent protein-ribosome complex, and shuttles it to the proper destination where the protein can be integrated into the membrane (1). Translation of integral membrane proteins is arrested when the signal peptide is recognized by the SRP, which initiates trafficking and concurrent translation-

membrane integration (8). SRP signal peptide recognition and trafficking will be described in following sections.

PDF and MAP perform N-terminal methionine excision when the nascent protein exits the ribosome (8). Several other enzymes interact with the exit site, in order to perform modifications on the growing nascent protein, such as glycosylation (8). The nascent protein continues to grow throughout elongation and interacts with the molecules surrounding the exit tunnel.

The elongation process continues until a stop codon, UAA, UAG, or UGA, is revealed in the A site, initiating the termination process (1). Protein release factors RF-1 and RF-2 recognize the stop codons and bind to the large ribosomal subunit, which is enhanced by RF-3 (1). Binding of the release factor complex to the large ribosomal subunit initiates a peptidyl transferase reaction that results in the release of the nascent protein chain (1). This completes eukaryotic translation.

Concurrent Prokaryotic Transcription/Translation – In prokaryotes, transcription and translation occur concurrently in the cytoplasm. Since prokaryotes do not contain a nucleus, concurrent transcription/translation is necessary to mitigate any deleterious interactions between the nascent mRNA and protein with the harsh conditions in the cytoplasm. In general, the processes of prokaryotic transcription and translation are very similar with most differences manifesting in the molecules that drive the transfer of information.

The process of transcription follows the same general process as outlined in the General Transcription section. The prokaryotic RNA polymerase used to catalyze mRNA production is more versatile than the eukaryotic RNA polymerase II, as it can also catalyze the production of tRNA and rRNA (1). The RNA polymerase binds to the Pribnow box promoter sequence, which consists of highly conserved nucleotide sequences at 10 and 35 base pairs upstream of the initiation site (1). No initiation factors are used in prokaryotic transcription; however specificity factors are used to assist the process (1). There are two modes of transcription termination: termination by a terminator sequence and a termination factor (7).

Prokaryotic RNA processing is rarely observed. Since the mRNA does not need to pass through the nuclear pore and contains a translation initiation sequence, capping and poly-adenylation does not need to be performed (7). Also, introns are generally not present in prokaryotes (7). The mRNA needs to be prepared for simultaneous translation and intron excision would not be efficient.

The process of translation follows the same general process as outline in the General Translation section. The two subunits of the prokaryotic ribosome used to catalyze polypeptide production are smaller than the eukaryotic ribosomal subunits but perform the same function similarly (1). Due to the lack of a 5′ cap on the mRNA, the prokaryotic mRNA contains an initiation sequence, the Shine-Dalgarno sequence, approximately 10 base pairs upstream of the start site where the ribosome binds (1). Similar initiation, elongation, and release factors are used in prokaryotic translation, as well as co-translational molecules. One important difference between prokaryotic and eukaryotic co-translational molecules is prokaryotes have one chaperone: Trigger factor. Trigger factor is a well understood molecular chaperone that preferentially associates to the exit

site of the prokaryotic large ribosomal subunit and provides a cradle for nascent protein folding and protease protection (Maier et al 2005).

Integral Membrane Protein Signals – The primary sequence of integral membrane proteins provides the cell with information on trafficking and membrane integration. The primary sequence contains multiple segments that assist in these processes: the signal peptide sequence (n-terminal or c-terminal), signal-anchor sequence, reverse signal-anchor sequence, and the start-transfer/stop-transfer sequences (1). This section and following sections will focus on eukaryotic endoplasmic reticulum and mitochondrial integral membrane protein trafficking and membrane integration.

The signal peptide sequence in an intrinsic signal that guides protein sorting and the composition is target dependent. Typically, the signal peptide sequence is close to the n-terminus of the growing nascent protein. This allows the signal to be recognized by recognition molecules prior to translation termination. When the nascent protein contains a signal peptide sequence close to the n-terminus, it is called a signal-anchored protein (10). For integral membrane proteins destined to eukaryotic endoplasmic reticulum, the signal peptide sequence is completely hydrophobic stretches of protein approximately 20-25 residues long (1). Conversely, eukaryotic mitochondrial membrane proteins are typically positively charged amphipathic α-helices approximately 15-50 residues long (12). The signal sequence is cleaved upon arriving to the destination by signal peptidase (1). However, if the signal sequence is sufficiently close to the c-terminus of the nascent protein, the signal peptide sequence will not be recognized until translation is complete (10). The c-tail-anchored proteins are trafficked to their destinations post-translation through the secretory pathway (9). In general, the intracellular membranes targeted in the secretory pathway are limited and the pathway is not well understood (11). Also, membrane insertions mechanisms for c-tail-anchored proteins have been identified (e.g. TRC40/Get3 mediation and unassisted insertion) but are not fully understood (11).

Trafficking and membrane integration pathways between the signal-anchored protein and the c-tail-anchored proteins differ. The topic of c-tail-anchored proteins trafficking and membrane integration is not well understood and was not discussed in the Protein Chemistry lectures. Description of the c-tail-anchored protein processes will not be discussed.

In general, the other sequences determine the transmembrane domains of the integral membrane protein. The start-transfer/stop-transfer sequences are hydrophobic sequences flanked by positively charged residues (1). The composition of the sequences allows the threading transmembrane domains through the membrane (1). These segments promote and halt travel through the translocon membrane complex (1). Depending on the number of start-transfer/stop-transfer sequences, a bitopic (single pass) or polytopic (α-helical and β-barrel) integral membrane protein can be generated.

Integral Membrane Protein Signal Recognition and Trafficking – As previously discussed, several co-translational molecules compete for interaction with the nascent protein chain as it emerges from the exit site of the ribosome (8). For integral membrane proteins destined to the endoplasmic reticulum, the interplay between SRP and molecular chaperones (e.g. Trigger factor) is crucial to the proper trafficking and translocation into the membrane (8). It has been

shown that SRP and Trigger factor compete for the same docking site on the ribosome (8). However, once the signal anchor sequence emerges from the exit tunnel, SRP association with the ribosome is greatly increased at the docking site as well as several other sites on the ribosome (8).

The signal recognition particle (SRP) associates with a signal peptide sequence of the nascent protein chain of endoplasmic reticulum membrane proteins (1). It is critical that the signal peptide sequence be entirely hydrophobic, as any charged residue will eliminate an association with the SRP (1). SRP is a RNA-protein complex that is found in every organism (1). There are major differences in the composition of eukaryotic and prokaryotic SRPs but they all contain a homologous domain that is critical to signal peptide recognition: the M site (1). The M site is a methionine rich, mainly α-helical domain that is critical to signal sequence and RNA binding (1). SRPs also contain GTPases that catalyze the binding of the nascent protein-ribosome complex to the signal receptor (SR) of the target membrane, where SRP is hydrolyzed (1). The SRP-mediated pathway shuttles integral membrane proteins to the endoplasmic reticulum of eukaryotes and the plasma membrane of prokaryotes (1).

For integral membrane proteins destined to the mitochondria, Hsp70 is the key chaperone in the molecular chaperone complex that binds to the nascent protein and assists in delivery it to the translocase (12). Hsp70 is a heat shock protein composed of three domains, an ATPase domain, a peptide binding domain, and a lid, that form a chamber for protein association (13). An ATP dependent mechanism of opening and closing of the lid allows for reversible tight and loose association with hydrophobic patches on the nascent protein (13). For mitochondrial protein transport, the chamber formed by Hsp70 protects the nascent protein and stabilizes the partially unfolded state (12). This allows the nascent protein to remain unfolded as the signal peptide sequence, or the presequence, identifies and interacts with the translocase receptors at the surface of the mitochondria (12).

Endoplasmic Reticulum Membrane Integration – The first step to membrane integration is the interaction of SRP and the signal receptor on the surface of the endoplasmic reticulum. The SRP is bound to the nascent protein-ribosome complex and interacts with a SRP receptor. Interactions are localized on homologous NG domains present on both the SRP and SRP receptor (1). GTP hydrolysis excises the SRP from complex and the nascent protein-ribosome complex is transferred to the receptor (1). The receptor then shuttles the complex to the translocon where translation resumes (1).

The translocon is responsible for translocation and integration of the integral membrane protein in the endoplasmic reticulum (9). The translocon is a dynamic protein complex, composed of three integral membranes, that creates a channel in the membrane that allows nascent proteins to enter the lumen (1). The dynamic nature of the translocon is important to proper membrane integration: it has the ability to expand into the membrane (9). Other proteins associate with the translocon that perform important roles in membrane integration. The TRAM, or translocating-chain-associated membrane protein translocates the protein across the membrane (9). Signal peptidases cleave the signal peptide sequence and oligosaccharyl transferase adds sugars to the nascent chain as it passes through the translocon (9).

Membrane integration is performed primarily using the start-transfer/stop-transfer sequences on the nascent protein as well as intraprotein and interprotein interactions (9). When hydrophobic patches are identified by the translocon, translocation is halted and the hydrophobic segment is inserted into the membrane (9). Intraprotein interactions can occur between the transmembrane sequences, which provide forces to get the transmembrane domain into the proper secondary and tertiary structures and orientation in the membrane (9). Other proteins have been showed to assist in transmembrane domain folding and integration: prior protein, ductin, etc. (9). Chaperones are used to protect the nascent chain from signal peptidases (9).

Mitochondrial Membrane Integration – In general, prior to integration into either the outer or inner membrane of the mitochondria, the nascent protein-Hsp70 must be imported through the translocase of the outer membrane: Tom (1). Tom (translocation outer membrane) is a translocase system composed of several integral membrane proteins (1). Tom contains a large oligomeric β-barrel integral protein, Tom40, which forms two to three pores that allow passage of unfolded nascent protein chains (12). Additionally, three integral Tom proteins extend into the cytosol and act as preprotein receptors: Tom20, Tom22, and Tom70 (12). Tom20 cooperates with Tom22 to recognize and bind signal peptide sequences, while Tom70 acts independently to preferentially bind to nascent proteins with internal targeting information, e.g. hydrophobic or membrane proteins (1). The other minor Tom proteins perform transport, assembly, and dissociation (1). Once the signal peptide sequence or other targeting sequences is recognized by the receptors, the protein is inserted through the Tom70 channel linearly, with the n-terminal going first, or in a loop formation (12). Tom40 forms a very stable structure within the membrane and is not as dynamic as the translocon described in the endoplasmic reticulum membrane integration section. Thus, additional systems are needed to integrate the integral membrane protein into the outer and inner membranes.

There are multiple pathways into the outer membrane of the mitochondria and the pathways are dependent on the integral membrane to be inserted: β-barrel and α-helical (12). For β-barrel integral membrane sorting, the initial step is targeting and passage through the Tom translocase system, which is described above (12). Once the nascent protein chain emerges into the intermembrane space they bind to chaperone complexes (Time9-Tim10 and Tim8-Tim13) to protect from aggregation/degradation and to stabilize the unfolded conformation (12). The chaperones shuttle the nascent protein chain to the SAM complex (12). The SAM complex is composed of several integral membrane proteins (12). Sam50 considered the core of the SAM complex and is a β-barrel integral membrane protein that forms a pore that is sensitive to the signal sequence of the nascent protein (12). Other Sam proteins that play major roles are: Sam35, which binds to the signal sequence, and Sam 37, which releases the nascent protein (12). The mechanism of lateral release of the nascent protein into the membrane is not well understood but several pathways have been proposed (12). The Sam complex may independently move the nascent protein into the membrane by using Sam50 as a scaffold or forming a channel into the membrane (12). Alternative pathways have included outer membrane proteins that can promote integral membrane protein assembly, such as Mdm10 or Mim1 (12). Even though the pathway for membrane assembly has not been elucidated, the initial steps are well understood for β-barrel integral membrane protein insertion into the outer membrane of the mitochondria.

There are several pathways that promote the integration of α-helical membrane proteins into the mitochondrial outer membrane (12). The pathway taken is dependent on the signal composition on the nascent protein (12). For signal-anchored bitopic integral membrane proteins, Tom interaction and passage is not required and Mim1 promotes the integration (12). Nascent proteins with an internal signal peptide sequence will interact with the Tom22 receptor, which will direct it to the Sam complex (12). The Sam complex also contains α-helical substrates to promote α-helical integral membrane assembly (12). The mechanisms for c-tail anchored and polytopic α-helical integral membrane proteins have not been identified (12).

The TIM22 complex is the key player in the pathway for the integration of membrane proteins into the inner mitochondrial membranes. After passage through the Tom translocase system, the nascent protein chain binds to the Tim9-Tim10 chaperone complex which directs it to the carrier translocase, TIM22 (12). The chaperone complex associates with Tim12 to form a larger complex on the surface of the TIM22 complex (12). The nascent protein chain is inserted into one of the TIM22 channels and laterally released into the membrane (12). The mechanism of lateral release has not been identified (12).

Roles of Integral Membrane Proteins – Integral membrane proteins can interact with a multitude of molecules throughout its life cycle. The interactions depend on the type of integral membrane protein but also the role it plays. There are three general types of integral membrane proteins: single pass, α-helical, and β-barrel. Since integral membrane proteins contain both hydrophobic and hydrophilic domains it can carry out several different roles, such as material transport, act as an anchor point, a receptor for signal transduction, and a catalysis site for reactions. As a material transporter, integral membrane proteins can facilitate diffusion of materials across a membrane, usually through binding sites at the surface of the membrane protein, where substances can bind and/or ATP can hydrolyze to impact conformation and allow material passage. The membrane protein can act as an anchor point for cellular adhesion. As a receptor at the membrane surface, the integral membrane can transduce intracellular or intercellular signals that are important to promote various cellular events. The hydrophilic domain(s) of the integral membrane proteins can act as enzymes to catalyze various reactions. Integral membrane proteins allow for transport of materials and signals while allowing the membrane to compartmentalize the cell for increased efficiency.

Endoplasmic Reticulum-Associated Degradation – Once the integral membrane protein has reached end-of-life, it must be tagged, removed from the membrane, and shuttled to the cytoplasm to undergo degradation (ubiquitylation and proteasome degradation). The pathway is complex and includes several steps just to remove the integral membrane protein.

The initial step in the degradation pathway is protein recognition as a degradation substrate. This step is largely facilitated by chaperones and occurs simultaneously with targeting to the retrotranslocon (15). Multiple chaperones have been identified for the recognition phase. In general, chaperones bind to lesions on the exposed hydrophilic domains of integral membrane proteins (15). Hsp70 is the main chaperone for degradation substrate recognition and recruitment of ubiquitin ligase (15). Multiple co-chaperones assist in the ATP dependent binding of Hsp70, such as Hsp40, Hsp 90, and various nucleotide exchange factors (13). Additional chaperones are used if the protein has been post-translationally modified. If the exposed

domains are glycosylated, calnexin and calreticulin act as chaperones (15). If the exposed domains contain disulfide bonds, due to the oxidizing environment in the endoplasmic reticulum lumen, protein disulfide isomerases are used (15).

In most cases protein recognition and targeting to the retrotranslocon occur concurrently, through three potential pathways, depending on the location of the domain: ERAD-C, ERAD-L, and ERAD-M (15). The ERAD-C and ERAD-L pathways are specific for cytoplasmic or luminal exposed domains, specifically, and use different ubiquitin ligase complexes (15). The ERAD-M pathway is specific for the transmembrane domain but has not been identified (15).

The retrotranslocon removes the transmembrane domain from the membrane and moves the luminal domain into the cytoplasm (15). The machinery is not fully understood but multiple candidates have been identified for retrotranslocation. Evidence has suggested that the Sec61 translocon, used for translocation and membrane integration of endoplasmic reticulum integral membrane proteins, can be a retrotranslocon channel (15). Integral membrane proteins from the derlin family can also be used (15). Ubiquitin ligase membrane proteins that are used to initiate ubiquitylation can form retrotranslocaiton channels (15). Vembar et al proposed that a retrotranslocation channel may not be needed and degradation may be mediated by an unknown protease pathway (15). The protein is now unfolded and in the cytoplasm, ready for ubiquitylation and proteasome degradation.

The degradation substrate is tagged for degradation via ubiquitylation (1). Ubiquitylation is an enzymatic process where ubiquitin is added to target proteins (1). The composition of ubiquitin is highly conserved and contains a c-terminus sequence, Arg-Gly-Gly, which is critical to function (1). The c-terminal glycine forms a peptide bond with the ε-amino group of lysines in the target protein (1). Three enzymes catalyze the peptide bond formation: E1, E2, and E3 (1). Through a two-step ATP dependent reaction, E1 enzymes catalyze the formation of active ubiquitin and then bind to the c-terminal glycine through a thiolester linkage (1). E2 enzymes transfer ubiquitin to the lysine of the target protein (1). E3 enzymes recruit E2 bound ubiquitin and catalyze the peptide bond formation between ubiquitin and lysine (1). Further ubiquitination forms a peptide bond between an ubiquitin bound to the target protein and a second ubiquitin and this continues as a ubiquitin chain grows (1). Poly-ubiquitination is necessary to enhance proteasome recognition (1).

The proteasome is a highly conserved multicatalytic protease complex that degrades ubiquitin tagged proteins. The proteasome is composed of a 20S core that consists of four stacked rings (1). The stacked rings are composed of 14 α subunits, which make up the top and bottom homoheptameric rings, and 14 β subunits, which make up the two middle homoheptameric rings (1). The active site is located on the β subunit, which is oriented towards the interior of the ring to perform peptide bond cleavage (1). The α subunits are important for proper translocation of the substrate through the catalytic center (1). A gating system is used to control entry into the 20S core, which is performed by two 19S regulatory complexes that are located at the top and bottom entry/exit sites (1). The 19S regulatory complexes are important in removing ubiquitin from the ubiquitin tagged protein (1).

The ubiquitin tagged protein binds to the cap assembly, ATP drives the opening of the cap and hydrolysis of ubiquitin, and the cap assembly unfolds the protein (1). The cap assembly assists in moving the protein into the 20S core, where protein is degraded (1). Various catalytic sites in the interior of the core perform peptide bond scission after hydrophobic, basic, and acidic residues (1). The degraded protein exits through either entry/exit site of the proteasome (1). This allows the protein to reuse the building blocks for production of new proteins (1).

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