Review
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# Tyrosinase maturation through the mammalian secretory pathway: bringing color to life

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## Summary

**Tyrosinase has been extensively utilized as a model substrate to study the maturation of glycoproteins in the mammalian secretory pathway. The visual nature of its enzymatic activity (melanin production) has facilitated the identification and characterization of the proteins that assist it becoming a functional enzyme, localized to its proper cellular location. Here, we review the steps involved in the maturation of tyrosinase from when it is first synthesized by cytosolic ribosomes until the mature protein reaches its post-Golgi residence in the melanosomes. These steps include protein processing, covalent modifications, chaperone binding, oligomerization, and trafficking. The disruption of any of these steps can lead to a wide range of pigmentation disorders.**

Key words: calnexin/calreticulin/lectin chaperones/BiP/endoplasmic reticulum

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## Introduction

Tyrosinase is a multifaceted protein that has garnered the attention of a wide range of disciplines. Bioinorganic chemists have used it as a model protein for the study of oxygen centers of metallo-oxygenases (Solomon et al., 1996). The major histocompatability complex (MHC) presentation of tyrosinase peptides has been extensively studied by immunologists to extend our understanding of antigen presentation (Engelhard et al., 2002). Cell biologists have utilized its colorimetric activity to identify cellular machinery responsible for the sorting and trafficking of proteins to post-Golgi organelles (Odorizzi et al., 1998). Understanding the maturation and activities of tyrosinase has also been of keen interest to medical scientists as abnormalities with the above processes and functions are associated with a variety of human disease states including albinism, Hermansky–Pudlak syndrome (HPS) and melanoma (Oetting and King, 1999). In this review, we focus on the current state of our understanding of tyrosinase maturation in the cell. We will highlight the steps the protein goes through from when it is first synthesized by cytosolic ribosomes until the mature and active protein reaches its ultimate home in melanosomes.

## Tyrosinase activity and function

To understand the maturation of tyrosinase, one must first have an appreciation for its activity and function within the cell. Tyrosinase (monophenol, L-dopa: oxygen oxidoreductase, EC 1.14.18.1) is a copper-containing enzyme that catalyzes several steps in the synthesis of melanin. It is found in epidermal melanocytes, as well as the pigment epithelia of the retina, iris, and ciliary body of the eye. Melanin is widely distributed in bacteria, fungi, plants, and animals. It is synthesized in two major forms known as eumelanin and pheomelanin, which are black-brownish and red-yellowish pigments, respectively. The different combination and intensity of these two melanins determine mammalian skin, eye, and hair coloration.

Melanin synthesis is a highly cooperative process carried out by tyrosinase family proteins, which include tyrosinase, tyrosinase-related protein 1 (Tyrp1 or TRP1) and tyrosinase-related protein 2 (Tyrp2, DCT or TRP2) (Figure 1). Tyrosinase is the best-studied multicopper oxygenase. It uses its binuclear copper center to catalyze the hydroxylation of tyrosine to dihydroxyphenylalanine (DOPA) (tyrosine hydroxylase activity) (Lerner et al., 1949). This step is considered to be the rate-limiting step in melanin production. Tyrosinase also catalyzes the subsequent oxidation of DOPA to DOPAquinone (DOPA oxidase activity) and further downstream products 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) into the eumelanin precursors indole-5,6-quinone and indole-5,6-quinone carboxylic acid, respectively (Körner and Pawelek, 1982; Lerner et al., 1949).

The process of melanin synthesis is auto-regulated. Tyrosinase is autocatalytically activated by its own substrate and co-factor tyrosine and DOPA. DOPA accelerates the oxidation of tyrosine to DOPAquinone by

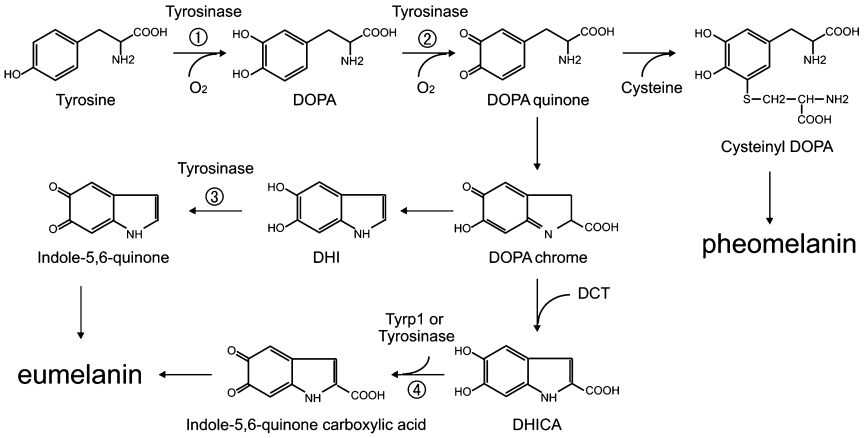


**Figure 1.** Melanin synthesis pathway regulated by tyrosinase, tyrosinase family proteins Tyrp1 and DCT/Tyrp2. Tyrosinase catalyzes the first two rate-limiting steps (1 and 2) in melanin synthesis of tyrosine hydroxylation to DOPA and DOPA oxidation to DOPAquinone, and also the oxidation of the downstream intermediate DHI to indole-5,6-quinone (3) and DHICA to indole-5,6-quninone carboxylic acid (4). Tyrp1 and DCT/Tyrp2 catalyze the oxidation of DHICA to indole-5,6-quinone carboxylic acid and tautomerization of DOPAchrome to DHICA. The above processes lead to the synthesis of eumelanin and the pathway for pheomelanin synthesis is still less understood. Tyrp1/2, tyrosinase related protein 1/2; DHI, 5,6-dihydroxyindole; DHICA, 5,6-dihydroxyindole-2-carboxylic acid.

removing an initial lag period observed for tyrosine hydroxylation (Riley, 2000). Furthermore, DHI can inhibit the oxidation of tyrosine to DOPA, and tyrosine can inhibit the conversion of DHI to indole-5,6-quinone (Körner and Pawelek, 1982). The activity of tyrosinase is also strongly affected by pH. Mammalian tyrosinase displays optimal enzymatic activity at neutral pH with little activity below pH 5 (Saeki and Oikawa, 1978, 1980, 1983, 1985). This pH requirement is surprising as in mammalian cells tyrosinase resides in a post-Golgi lysosomal organelle termed melanosomes, which possess an acidic lumen (Bhatnagar et al., 1993). However, melanosomes mature to contain a less acidic environment (Raposo et al., 2001). Overall, the catalytic activity of tyrosinase is tightly controlled by its substrates, co-factors, and cellular environment.

## Tyrosinase structure

Human tyrosinase is a type I membrane glycoprotein that contains 529 amino acid including an 18 amino acid N-terminal signal sequence (Figure 2). The human (NP_000363) and mouse (NP_035791) tyrosinase proteins are highly conserved, possessing 85% sequence identity (Kwon et al., 1988a,b). The mature protein can be divided into three domains: (i) the N-terminal 455 amino acid lumenal ectodomain; (ii) a single transmembrane domain; and (iii) a C-terminal cytoplasmic tail.

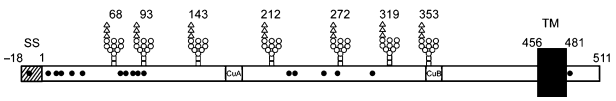


**Figure 2.** Structure of human tyrosinase. Tyrosinase is a type I membrane glycoprotein with an N-terminal signal sequence and a C-terminal transmembrane domain. The seven glycosylation sites (branched structure), three cysteine (filled circles) clusters, and two copper-binding sites are indicated.

The ectodomain of human tyrosinase possesses the enzymatic activity. It has seven consensus N-linked glycosylation sites and 17 Cys residues. One Cys residue is located in its cleaved signal sequence and another in the cytoplasmic tail, leaving 15 Cys available for disulfide bonding under the oxidizing conditions of the endoplasmic reticulum (ER) lumen. Six of the seven glycosylation sites are conserved in mouse tyrosinase while all the lumenal Cys are conserved. The N-linked glycosylation sites are spaced throughout the lumenal domain, while the Cys residues are concentrated in three Cys-rich clusters.

Both human and mouse tyrosinase have a single 26 amino acid hydrophobic transmembrane domain which is approximately 70% conserved. The transmembrane domain anchors tyrosinase in the melanosome membrane with the N-terminal ectodomain facing the lumen. Mouse tyrosinase is four amino acid longer than human tyrosinase, because of an extension of the C-terminal cytoplasmic tail. The C-terminal tails of both human and mouse tyrosinase possess two well-studied intracellular targeting signals. These signals include a di-leucine (LL) motif and a tyrosine-based motif (YXXB, where B is a hydrophobic residue) that will be discussed in more detail below (Blagoveshchenskaya et al., 1999; Calvo et al., 1999; Honing et al., 1998; Simmen et al., 1999).

The numbering of tyrosinase residues has previously been based on starting with the initiating Met residue as amino acid number 1. Therefore, the signal sequence comprised residues 1 to 18. However, traditionally proteins that contain cleavable N-terminal signal sequences designate the first residue in the processed

protein as the number 1 residue (for tyrosinase His-1) and the signal sequence residues are designated with negative numbers. Therefore, the initiating Met would be referred to as Met-18 and Gly at the site of cleavage as Gly-1. Accordingly, the traditional secretory protein designations will be employed in this review.

Deficiencies in tyrosinase activity can cause depigmentation and are associated with human Oculocutaneous Albinism type I (OCAI) (Oetting et al., 1996, 2003). Amelanotic phenotypes are associated with over 100 tyrosinase mutations (Albinism database: http://albinismdb.med.umn.edu/). These mutations include a large number of missense, nonsense frameshift and deletion mutations. Some of these mutations involve alteration in Cys (C71R) residues or deletions of glycosylation sites (T355K), which disrupt the proper maturation of tyrosinase (Halaban et al., 2000), while others involve active site residues directly (H349Y) (Spritz et al., 1997).

Although the primary structure of tyrosinase has been known for many years, the higher order structure as a whole and for the individual domains remain unresolved. Its transmembrane domain and glycans have hindered crystallographic studies. However, recent advances in overexpression and purification of tyrosinase should aid in the acquisition of future structural information (Hearing, 1987; Kohashi et al., 2004; Naraoka et al., 2003).

The known crystallographic structures of two related proteins hemocyanin and plant catecholoxidase permit predictions of tyrosinase secondary structure. Tyrosinase is a type 3 copper-binding protein that contains a binuclear copper active site, which brings together the molecular oxygen and the phenolic substrates tyrosine or DOPA. Each of the copper-binding sites in a type 3 copper protein is coordinated by three His residues. The CuA site of tyrosinase is comprised of His162, 184, and 193, while the CuB site involves His345, 349, and 371 (Huber and Lerch, 1988; Muller et al., 1988; Spritz et al., 1997). These six His residues are conserved in tyrosinase through a variety of species. The CuA site of bacterial and human tyrosinase display 42% and 53% sequence identity and similarity and the CuB site have 42% and 73% sequence identity and similarity, respectively (van Gelder et al., 1997). Chemical and spectroscopic studies on hemocyanin and tyrosinase have demonstrated that both proteins have similar active sites (Jolley et al., 1972, 1974). Secondary structure predictions and homology to copper proteins with known structures indicated that the CuA and CuB regions contain an $\alpha$-helical bundle creating a hydrophobic pocket near the protein surface (Garcia-Borron and Solano, 2002; van Gelder et al., 1997).

## ER maturation

For proteins that traverse the secretory pathway, the ER provides a protective folding environment by coupling a series of foldases, molecular chaperones, folding sensors, and covalent modifiers, which work in concert to assist with the proper maturation of nascent proteins. Due to the visual nature of its enzymatic activity and its connection to human disease states, tyrosinase has been extensively employed as a model substrate for studies of protein maturation (Francis et al., 2003; Petrescu et al., 1997, 2000; Újvári et al., 2001).

Protein maturation in cells commences during translation, and for proteins that pass through the secretory pathway, these events are also coupled to translocation into the ER. Beginning the maturation process co-translationally has been proposed to help optimize the fidelity of the overall process by temporally and spatially controlling the acquisition of higher order structure (Hardesty et al., 1999). The Sec61 translocon provides a conduit by which the protein is co-translationally inserted into the ER. The co-translational and co-translocational maturation events include signal sequence cleavage, glycosylation, disulfide bond formation, and chaperone binding (Chen et al., 1995; Daniels et al., 2003; Hebert et al., 1997; Kowarik et al., 2002; Molinari and Helenius, 2000; Schnell and Hebert, 2003). The maturation process continues post-translationally in the ER until a fully folded and assembled protein is packaged into vesicles that exit the ER for the Golgi.

### Signal sequence cleavage

The translocation of tyrosinase into the ER lumen is guided by its N-terminal cleavable signal sequence. The signal sequence binds to the signal recognition particle (SRP), which targets the ribosome nascent chain complex to the ER membrane by binding to its cognate SRP receptor. This binding positions the ribosomal protein exit site onto the Sec61 channel to provide a direct route from the biosynthetic machinery into the ER lumen. The hydrophobic nature of the signal sequence creates an N-terminal membrane tether or an additional transmembrane domain for tyrosinase prior to its cleavage by the signal sequence peptidase. Therefore, the timing of signal sequence cleavage can greatly affect the folding and maturation of a protein.

The early maturation events for tyrosinase were studied by simulating the co-translational processes using ribosomal-arrested chains of increasing lengths (Wang et al., 2005). The in vitro translation of abbreviated transcripts lacking a stop codon created ribosome-arrested nascent chains. Coupling the translation with a semi-permeabilized melanocyte system permitted the ribosome-nascent chain complex to be targeted to the ER membranes. This study found that the signal sequence of tyrosinase was first cleaved when the cleavage site was located 142 amino acid away from the ribosomal P-site (Wang et al., 2005). This demonstrated that signal sequence cleavage was one of the first events to take place in the ER during tyrosinase maturation.

## Glycosylation of tyrosinase

The majority of proteins synthesized in the ER are modified with multiple asparagine (Asn)-linked (N-linked) glycans ($Glc_3Man_9GlcNAc_2$) on the consensus sequence Asn-X-(Thr/Ser), where X is any amino acid except Pro. The consensus sequence must form a turn so that the Ser/Thr hydroxyl can increase the nucleophilic properties of the Asn side chain to aid in the transfer reaction (Bause, 1983; Imperiali and Shannon, 1991). A rigid Pro residue in the X positions inhibits this process. A preassembled glycan is transferred en bloc by the oligosaccharide transferase, generally co-translationally, once the Asn is located 12–14 amino acids into the ER lumen (Nilsson and von Heijne, 1993). The addition of multiple glycosylation sites greatly effects the maturation of tyrosinase by adding bulky 2.5 kDa hydrophilic modifications, as well as dictating interactions with a variety of carbohydrate-binding proteins that reside in the ER (Hebert et al., 2005; Helenius and Aebi, 2004).

Human tyrosinase is heterogenously glycosylated with either six or all seven of its glycosylation sites being occupied (Újvári et al., 2001). The hypoglycosylation at Asn-272 (Asn-Gly-Thr-Pro) is caused by the proximal Pro at position 275. Pro residues can also inhibit the transfer reaction when they immediately follow the consensus site. The glycosylation efficiency of Asn272 can be increased by slowing the translation rate both in live cells and by using a cell-free translation system. An accelerated translation rate observed in melanoma cells is associated with a higher level of hypoglycosylation and an amelanotic phenotype (Újvári et al., 2001).

Mouse tyrosinase lacks the hypoglycosylated site at Asn272. However, it has been reported that only four of the remaining six conserved glycosylation sites are occupied when tyrosinase is expressed in CHO cells (Branza-Nichita et al., 2000). This inefficient glycosylation was likely due to the heterologous expression in non-melanocytic cells, as mouse tyrosinase from melanocytes appears to possess glycans on all six remaining sites (Figure 3). Partial endoglycosidase digestion of $^{35}S$-labeled mouse tyrosinase from albino melan-c cells created seven evenly spaced tyrosinase bands. These bands corresponded to the completely deglycosylated protein, and glycosylated proteins containing from one to six glycans. This suggests that the efficiency of tyrosinase glycosylation is cell dependent.

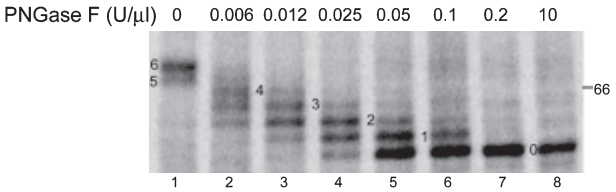


**Figure 3.** Glycosylation sites recognition in mouse melan-c cells. Mouse melanocytes harboring the albino C71S mutation of tyrosinase were pulse labeled with [$^{35}S$]-Met/Cys in the presence of *N*-butyldeoxynojirimycin and deoxymannojirimycin (DMJ) for 1 h. Tyrosinase was purified by immunoprecipitation with anti-tyrosinase antisera and partial digestion with varying concentrations of peptide: N-glycanase F (PNGase F). The number of glycosylations associated with each band is indicated.

Glycosylation is essential for tyrosinase activity, as inhibition of glycosylation with tunicamycin abolishes proper tyrosinase maturation and its subsequent enzymatic activity (Imokawa and Mishima, 1981; Mishima and Imokawa, 1983; Takahashi and Parsons, 1992b). To determine the requirement of the individual glycans for tyrosinase maturation, a systematic study of tyrosinase glycans was carried out by expressing 15 mouse tyrosinase glycan deletion mutants possessing one or more mutated glycosylation sites in CHO cells (Branza-Nichita et al., 2000). Glycans at Asn68 and Asn353 were found to be critical for proper folding, as only 30% of the total enzymatic activity was detected when these two glycosylation sites were removed. The requirement for Asn353 has also been found for human tyrosinase where the frequently and naturally occurring mutation Thr355Lys that abolishes the consensus site at Asn353 is associated with human albinism (Gershoni-Baruch et al., 1994). This protein is retained in the ER and subsequently degraded by the cytosolic proteasome (Halaban et al., 2000). Deletion of the glycans at Asn212 and Asn319 showed compatible enzyme activity and copper content to the wild-type enzyme (Branza-Nichita et al., 2000). It is evident that elective sites of glycosylation on tyrosinase are required for its proper maturation and stability, while others are dispensable.

## Chaperone binding

The ER contains two different classes of molecular chaperones that have been shown to interact with tyrosinase and assist its maturation (Figure 4). First, the ER Hsp70 family member BiP serves a variety of roles in protein maturation and quality control by recognizing unfolded regions containing hydrophobic residues (Fewell et al., 2001; Flynn et al., 1991). In sharp contrast to the binding activity of BiP are the carbohydrate binding or lectin chaperones of the ER, calnexin, and calreticulin. These chaperones bind directly to the hydrophilic N-linked modification. Together, these chaperones with their contrasting binding properties assist in the proper maturation of tyrosinase and also comprise part of a stringent quality control system that ensures correctly folded proteins are anterogradely exported through the secretory pathway.

BiP has been called the master regulator of ER function because of its many roles in the ER that include: (i) creating a seal on the protein conducting Sec61 channel to maintain the permeability barrier; (ii) helping to drive translocation using either a ratcheting or motoring mechanism; (iii) acting as a molecular chaperone protecting nascent chains from misfolding; (iv) an activator of the unfolded protein response (UPR); and (iv) targeting of aberrant proteins for degradation (Fewell et al.,

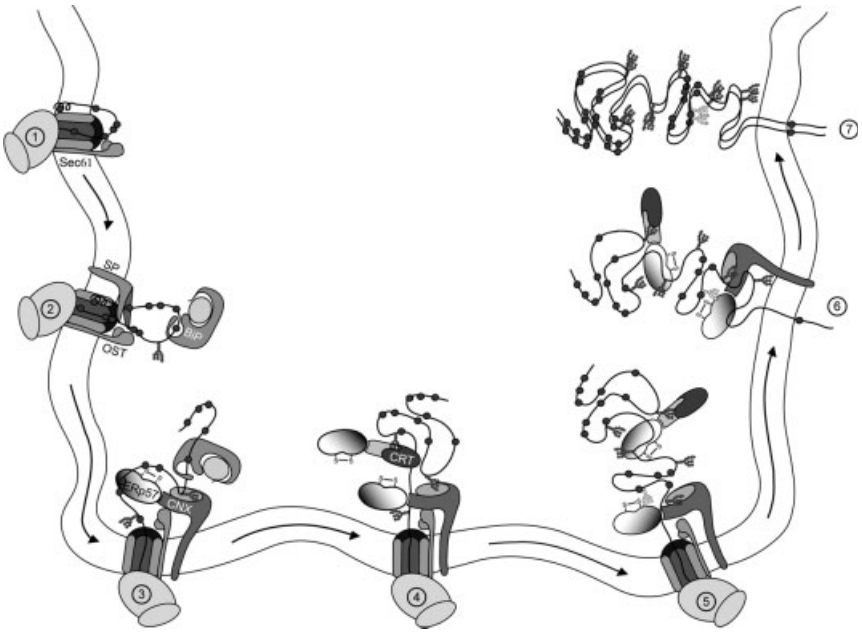


**Figure 4.** Co-translational tyrosinase maturation in the ER. (1) Tyrosinase is co-translationally translocated into the ER lumen through the Sec61 translocon. (2) The nascent protein is glycosylated by the oligosaccharide transferase (OST) and the signal sequence is cleaved by the signal sequence peptidase (SP). BiP initially associates with the non-glycosylated protein. (3,4) Calnexin (CNX), calreticulin (CRT) and its associated oxidoreductase ERp57 commence their interactions with tyrosinase when there are two or three glycans added. (5, 6) Calnexin, calreticulin and ERp57 associations continue with longer nascent chains until it is properly folded. (7) Full length tyrosinase forms homodimers.

2001; Hamman et al., 1998; Hendershot, 2004; Matlack et al., 1999; Schroder and Kaufman, 2005). BiP binds to hydrophobic amino acids, which are found in extended conformations (Blond-Elguindi et al., 1993). Exposed hydrophobic amino acids are the hallmark of a non-native protein. These regions make a protein highly vulnerable to the formation of non-productive aggregates. BiP binds to these vulnerable structures in an adenine nucleotide-dependent manner, thereby protecting the protein from aggregation (Bukau and Horwich, 1998). Prolonged BiP binding can lead to the activation of the UPR, ER retention and subsequent targeting of the protein for degradation.

BiP is the first chaperone that associates with tyrosinase during its maturation. It binds tyrosinase co-translationally at an early stage of translocation, when the N-terminus of tyrosinase is only 142–170 residues away from the P-site of the ribosome (Wang et al., 2005). The mature N-terminus of tyrosinase possesses several hydrophobic regions that would be expected to support binding in an unfolded conformation. BiP is partially localized to the Sec61 translocon by a membrane associated J-domain containing protein (Alder et al., 2005). This location allows it to maintain the ER permeability barrier, as well as positions it to bind to hydrophobic regions shortly after they emerge into the ER lumen, as observed with tyrosinase. BiP also interacts post-translationally with the albino melan-c mutant and soluble tyrosinase, which lacks its C-terminus, to support ER retention of these mutant proteins for their eventual degradation (Popescu et al., 2005; Toyofuku et al., 2001b).

The lectin chaperone system is comprised of the integral membrane chaperone calnexin, its soluble paralogue calreticulin and a series of glycosidases and transferases that control the chaperone-binding cycle (for reviews see Hebert et al., 2005; Helenius, 1994). Calnexin and calreticulin bind to proteins that possess monoglucosylated N-linked carbohydrates ($Glc_1Man_xGlcNAc_2$) (Hammond et al., 1994; Hebert et al., 1995; Ou et al., 1993). This composition is rapidly generated co-translationally through the sequential trimming of the transferred triglucosylated species ($Glc_3Man_9GlcNAc_2$) by the ER glucosidases I and II, respectively. Lectin chaperone binding is persistent until the final glucose is trimmed by glucosidase II. The unbound substrate is then free to fold and assemble to its native state (Hebert et al., 1997). If non-native or immature conformations exist, the protein will be reglucosylated by the UDP-glucose: glycoprotein glucosyltransferase (GT) to regenerate the monoglucosylated species (Sousa and Parodi, 1995; Sousa et al., 1992). Therefore, reglucosylation can support the ER retention of aberrant proteins by persistent binding to calnexin and/or calreticulin (Helenius, 1994). In contrast to BiP, the modifications that support lectin chaperone binding are generally solvent exposed in the native state of the protein. Regardless of these varying binding specificities, the

carbohydrate-binding chaperones serve overlapping functions with BiP by helping the folding process, protecting vulnerable nascent proteins from aggregation, and retaining non-native structures in the ER.

Calnexin and calreticulin binding is required for tyrosinase maturation to its native structure. Ablation of the calnexin-binding cycle in human MM96E or mouse B16 melanoma cells by treatment with the glucosidase inhibitors castanospermine, deoxynojirimycin or *N*-butyldeoxynojirimycin (DNJ) resulted in the generation of an inactive or less active tyrosinase (Petrescu et al., 1997; Takahashi and Parsons, 1992a). For B16 melanoma cells treated with DNJ, the activity of tyrosinase for tyrosine hydroxylase and DOPA oxidase were 15% and 5%, respectively, and the total melanin content represented only 2% of the total melanin of untreated cells. Inhibition of calnexin/calreticulin binding disrupted the proper folding and copper loading of tyrosinase but did not affect its transport to the melanosomes (Branza-Nichita et al., 1999; Petrescu et al., 1997). Evidently, lectin chaperone binding was also responsible for the retention of the inactive protein and disruption of the binding allowed the inactive protein to leak out of the ER. In addition, tyrosinase folded with an increased rate in B16 cells in the absence of calnexin/calreticulin binding; however, the accelerated folding reaction was less efficient (Branza-Nichita et al., 1999). Bypassing the calnexin cycle has also been shown to support tyrosinase aggregation and degradation in mouse melanocytes (Svedine et al., 2004). A critical role for calnexin in tyrosinase folding was further supported by following the maturation of tyrosinase in COS7 cells. Co-expressing human tyrosinase with calnexin doubled the activity of tyrosinase and the level of melanin produced when compared with expression of tyrosinase alone (Toyofuku et al., 1999). Together, these results clearly demonstrated that the lectin chaperones are required for efficient tyrosinase maturation.

Calnexin first interacted with nascent tyrosinase co-translationally when there were two glycans added and calreticulin associated after the addition of a third glycan (Wang et al., 2005). Binding to the chaperone then continued post-translationally until the protein was properly folded. A similar scenario has also been observed for influenza hemagglutinin, suggestive of a conserved binding order of calnexin and calreticulin (Daniels et al., 2003). The necessity for two glycans for lectin chaperone binding was previously thought to be a requirement of the co-immunoprecipitation procedure employed. However, recent results suggested that glucosidase II needs more than one glycan to recognize nascent glycoproteins to support its entry into the calnexin cycle (Deprez et al., 2005). Glucosidase II was believed to be recruited by the first glycan to the nascent chain through its mannose receptor homology domain, after which the enzyme trims the second glycan. This was also supported by the study using mouse tyrosinase glycan deletion mutants, which showed that two glycans were sufficient for calnexin association (Branza-Nichita et al., 2000).

Glycans appear to be the dominant factor in directing co- and post-translational chaperone binding to tyrosinase. As tyrosinase does not possess its first glycan until Asn68, the hydrophobic nature of the N-terminus of tyrosinase initially supported co-translational BiP binding. As the hydrophilicity of the nascent chains increases with the addition of the glycans, and these glycans are subsequently trimmed to their monoglucosylated state, the Hsp70 chaperone system hands tyrosinase off to the lectin chaperone system to assist with the later stages of tyrosinase folding until it reaches its native conformation. Crosslinking studies have found BiP to be present in a complex with PDI, GRP94, CaBP1, ERdj3, cyclophilin B, ERp72, GRP170, SDF2-L1, GT, but not calnexin, calreticulin or ERp57 (Meunier et al., 2002). The results with tyrosinase suggest that while calnexin/calreticulin are not found in the BiP complex, the two general chaperone systems can exchange substrates to optimize the folding reaction for tyrosinase.

**Disulfide bond formation**

Tyrosinase possesses 15 lumenal Cys residues, largely arranged in three Cys-rich clusters containing five Cys each. The role of these Cys is unknown. However, they are important for proper tyrosinase maturation as several mutations associated with OCA1 involve critical Cys residues such as C18Y, C37Y, C71R, and C271G/R/Y (King et al., 1991, 2003; Oetting et al., 1998; Passmore et al., 1999; Spritz et al., 1991; Takizawa et al., 2000). Although the disulfide bonding pattern for tyrosinase remains unsolved, it is clear that tyrosinase possesses multiple intramolecular disulfides that stabilize its final structure. Comparison of the mobility of oxidized and reduced tyrosinase by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) indicated that tyrosinase contains intramolecular disulfide bonds; however, the small difference in mobility suggested that these linkages are proximal and do not involve large covalent loops (Wang et al., 2005). Alkylation studies have shown that the majority of the Cys are unavailable for modification and few free thiols are found on the mature protein (Aroca et al., 1990; Wang et al., 2005).

Tyrosinase disulfide bonding begins co-translationally after the signal sequence was cleaved and when the mature N-terminus was 142 amino acids away from the ribosomal P-site (Wang et al., 2005). Disulfide bond formation is an oxidation reaction, which is catalyzed by the protein disulfide isomerase (PDI) protein family members of the ER. ERp57 is an ER oxidoreductase from the PDI family that is associated with calnexin and calreticulin by binding to their P-domain even in the

absence of substrates (Frickel et al., 2004; Oliver et al., 1997). Therefore, the binding of calnexin/calreticulin also recruits the oxidation catalyst ERp57 to the nascent chain.

The oxidation catalyst ERp57 was identified to interact with tyrosinase by chemical crosslinking during its co-translational maturation (Wang et al., 2005). This association was lectin chaperone dependent as the interaction was inhibited by DNJ. The initiation of the ERp57 interaction coincided with tyrosinase disulfide bond formation. ERp57 binding persisted with the full-length protein until it was fully oxidized. Therefore, ERp57 appears to serve as the catalyst for co- and post-translational oxidation of tyrosinase.

## Oligomerization

The quaternary structure of tyrosinase appears to involve both homo- and hetero-oligomeric interactions. Tyrosinase formed homodimers after properly folding in the ER, prior to being transported to the Golgi (Francis et al., 2003) (Figures 4 and 5). These homodimers were observed using an in vitro translation and semi-permeabilized melanocytes maturation system, which permitted proper maturation in the ER but not transport to the Golgi. Crosslinked tyrosinase dimers migrated at approximately 150 kDa by non-reducing SDS-PAGE and as a 7.5S complex by hydrodynamic studies using sucrose gradient density ultracentrifugation. These dimers were not naturally covalently linked. However, covalent linkages could be initiated through artificial oxidation of the cytosolic tail Cys482 of tyrosinase to form intermolecular disulfides or through crosslinking with the thiol reactive crosslinker bismaleimidohexane.

Tyrosinase dimerization required proper folding, as the tyrosinase misfolding mutant TYR(C71R) did not oligomerize (Francis et al., 2003). Coexpression of wild-type tyrosinase enhanced the maturation of temperature-sensitive tyrosinase mutants likely through the formation of mixed oligomers (Halaban et al., 2002a). Dimerization also appeared to require Tyrp1 as homodimerization did not occur in melan-b cells, which expressed a defective Tyrp1 protein (C86Y) (Francis et al., 2003). Furthermore, oligomers did not form in heterologous CHO cells, supporting the role of a melanocyte-specific factor in the proper maturation of tyrosinase. The conclusion that tyrosinase forms homodimers was further verified by the fact that purified tyrosinase behaved as homodimers by gel filtration chromatography and electrophoresis (Jimenez-Cervantes et al., 1998).

The melanocyte-specific proteins Tyrp1 and dopachrome tautomerase (DCT or Tyrp2) stabilize tyrosinase in the early secretory pathway (Kobayashi et al., 1998; Manga et al., 2000). These proteins appear to constitute a melanogenic complex that helps to increase the activity of tyrosinase (Manga et al., 2000). Large complexes containing tyrosinase, Tyrp1, and DCT multimers from approximately 200 to >700 kDa were isolated from murine melanocyte lysates by sucrose gradient density ultracentrifugation (Orlow et al., 1994). While tyrosinase forms higher order oligomers, the site of complex formation in the cell and the role of these complexes in tyrosinase maturation and its activity remains to be determined.

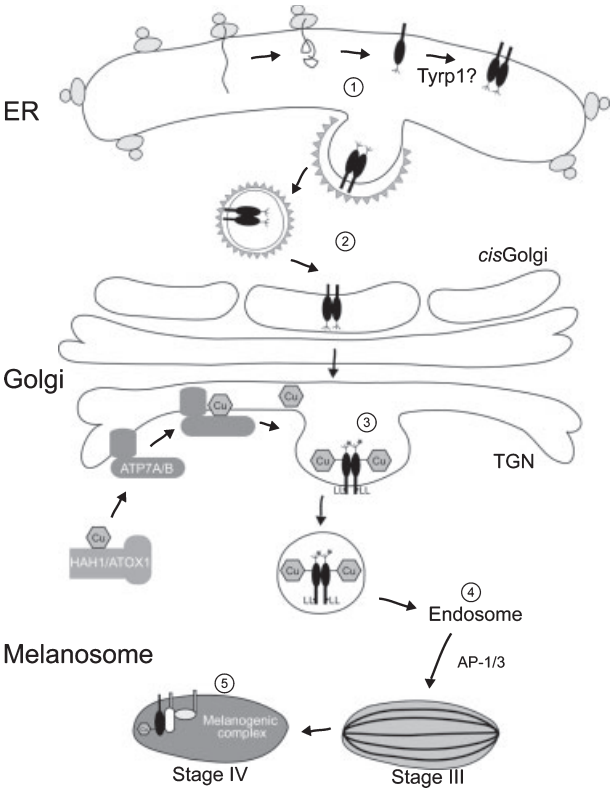


**Figure 5.** Tyrosinase maturation and trafficking through the secretory pathway. (1) Tyrosinase folds in the ER co-translationally and dimerizes. The quality control system in the ER ensures the folding and assembly are correct. (2) The export-competent tyrosinase is transported to the *cis*-Golgi network in COPII vesicles. (3) In the *trans*-Golgi network (TGN), the N-linked glycans are modified further to complex sugars and copper is loaded. (4) Tyrosinase is transported out of the TGN to the melanosomes. (5) The melanogenic complex is formed in melanosomes, which mature through the various stages.

## Transport

Once tyrosinase has properly matured in the ER, it is packaged into COPII-coated vesicles that bud from the smooth ER membrane. Tyrosinase then travels by way of the traditional anterograde pathway to the ER-Golgi intermediate compartment where it is subsequently sorted to the *cis*-Golgi. In the Golgi, additional mannose residues are removed by the Golgi mannosidases I and II (Helenius and Aebi, 2001). The newly mannose trimmed glycans then become substrates for the glycosyltransferases that transfer complex sugars. Tyrosinase enters the *cis*-Golgi as an Endo H-sensitive approximately

70 kDa protein and exits the *trans*-Golgi network (TGN) as an Endo H-resistant approximately 80 kDa protein due to the addition of these complex sugars (Halaban et al., 1997).

Copper appears to be first introduced into the secretory pathway at the TGN (Figure 5). It is an essential cofactor for tyrosinase required for its oxidative activity. Proper folding of tyrosinase is a prerequisite for copper binding (Spritz et al., 1997). The level of free copper in the cell is extremely low (Rae et al., 1999). Copper is almost completely chelated by copper binding proteins within the cell. Therefore, metallochaperones are required for trafficking and distributing copper to various intracellular sites (for review, see Huffman and O'Halloran, 2001).

The metallochaperone HAH1 or ATOX1 shuttles copper-to-copper transporters located in the TGN (Hung et al., 1998; Klomp et al., 1997). These transporters catalyze the movement of copper ions across Golgi membranes. The TGN possesses two P-type ATPase copper transporters termed ATP7A and ATP7B (Vulpe and Packman, 1995). Defects in ATP7A are associated with disorders of copper starvation called Menkes disease, which is lethal during early childhood (Dierick et al., 1997). ATP7A is often referred to as the Menkes protein or MNKp. It is required for the activation of tyrosinase (Petris et al., 2000). The expression of tyrosinase in immortalized Menkes fibroblasts produced an inactive enzyme. All together, these results suggest that tyrosinase receives its copper prior to its exit from the TGN. Therefore, tyrosinase likely becomes enzymatically active in the TGN unless additional downstream factors are required for its activation.

The sorting of membrane proteins from the TGN to lysosomal-related organelles such as melanosomes are generally mediated by signals localized to the C-terminal cytosolic tail of the cargo protein. Tyrosinase, as well as the other melanosomal proteins including Tyrp1, p-protein and Pmel17 (also called silver or gp100) possess di-leucine motifs in their cytoplasmic tails (Calvo et al., 1999; Vijayasaradhi et al., 1995). The deletion of the cytoplasmic tail of tyrosinase resulted in its mislocalization to the cell surface, producing an OCAI phenotype (Beermann et al., 1995). In addition, the expression of tyrosinase in non-melanocytic cells, such as HeLa cells, caused its trafficking to be directed to late-endosomes and lysosomes (Calvo et al., 1999). The removal of the di-leucine motif supported the cell surface localization of tyrosinase, underscoring the importance of the di-leucine motif in targeting tyrosinase to melanosomes.

Vesicle budding requires the formation of membrane coats to create the membrane curvature. Cargo selection to the membrane bud is determined by adaptor proteins that bind to both the membrane coat and the cytoplasmic tail of the cargo protein. The visual nature of the tyrosinase reaction product has been used effectively in mice and drosophila as a screen to identify proteins that are required for the proper localization of tyrosinase such as the adaptor protein-3 (AP-3). AP-3 binds to the di-leucine motif of tyrosinase in vitro and mediates its trafficking in melanocytes (Honing et al., 1998; Huizing et al., 2001). AP-3 is comprised of four subunits $\beta$3-, $\delta$3-, $\mu$3-, and $\gamma$3-adaptin. The garnet fly ($\delta$3-), and the mocha ($\delta$3-) and pearl ($\beta$3-) mice have defects in various subunits of AP-3 (Odorizzi et al., 1998). Defects in AP-3 are also associated in humans with HPS, a disease that displays pigmentation and blood clotting abnormalities, as well as immunodeficiencies (Dell'Angelica et al., 1999, 2000). These varying phenotypes demonstrate the importance of AP-3 in trafficking of proteins to melanosomes, platelet dense granules, and in antigen presentation. Recently, it was found that AP-1 also plays an AP-3 independent role in tyrosinase trafficking to premelanosomes (Theos et al., 2005). Additional genes including *HPS1*, *HPS4*, *beige* have also been identified that are associated with pigmentation defects; however, their precise role in pigmentation remains to be determined.

Glycosphingolipids are also required for the proper sorting of tyrosinase in the TGN. Melanocytes deficient in glycosphingolipids synthesis accumulated tyrosinase in the TGN (Sprong et al., 2001). This indicated that the composition of lipid bilayer is also important in the trafficking of tyrosinase from the TGN to endosomal or premelanosomal compartment.

The quality control for tyrosinase appears to extend beyond the ER to the Golgi where a variety of proteins and lipids are involved in its trafficking to the melanosomes. Once tyrosinase reaches the premelanosome compartment, these premelanosomes eventually mature into the pigment producing melanosomes through a series of morphologically defined stages (reviewed in Marks and Seabra, 2001).

## Melanocyte-specific factors

Several melanocyte-specific proteins have also been found to assist the maturation and trafficking of tyrosinase in the cell. These proteins include the tyrosinase-related proteins 1 (Tyrp1, gp75 or brown locus protein), and two multipass membrane proteins termed the p-protein and membrane-associated transporter protein (MATP).

Mutations in Tyrp1 are associated with OCAIII while there is no associated albinism caused by DCT/Tyrp2 defects. Tyrosinase and its related proteins possess conserved copper-binding domains, glycosylation sites, and Cys residues that are essential for the proper maturation of the individual proteins. However, the maturation of tyrosinase in the Tyrp1 mutant mouse melan-b cell was also greatly diminished demonstrating that tyrosinase was stabilized by Tyrp1 (Kobayashi et al., 1998). Tyrosinase could not oligomerize properly when translocated into the ER from melan-b cells (Francis

et al., 2003). Furthermore, isolated Tyrp1 can stabilize tyrosinase during heat denaturation further verifying its role in assisting tyrosinase (Hearing et al., 1992).

The p-protein is encoded by the mouse pink-eyed dilution gene (*p*) (Gardner et al., 1992; Rinchik et al., 1993). It is predicted to have 12 transmembrane domains. Mutations in the p-protein cause the most common form of tyrosinase-positive OCAII (Lee et al., 1994). The p-protein controls tyrosinase processing and intracellular transport. In melan-p1 cells that contain mutant p-protein, tyrosinase was partially retained in the ER and Golgi (Chen et al., 2002; Toyofuku et al., 2002). Tyrosinase was also found in vesicles secreted into the cell culture medium as a soluble form created by the cleavage. As p-protein possesses structural homology with ion transporters and treatment of melan-p1 cells with vacuolar $H^+$-ATPase inhibitor bafilomycin A1 or ammonium chloride rescues the retained tyrosinase, a role of p-protein in pH regulation has been proposed (Puri et al., 2000). However, further experimentation will be needed to understand how a putative melanosomal proton channel could affect the premelanosomal routing of tyrosinase.

The mouse underwhite gene (*uw*) encodes for another melanocyte protein with 12 predicted transmembrane regions (Newton et al., 2001). Mutations with *uw* are associated with human OCAIV, which affects approximately 1/20 000 people worldwide. The human homologues of the *uw* gene encodes for the MATP. Similar to the p-protein, mutations with the *uw* gene cause the secretion of tyrosinase-containing vesicles into the tissue culture medium because of an apparent defect in post-Golgi targeting (Costin et al., 2003). Therefore, the putative melanocyte membrane transporters p-protein and MATP appear to be critical for the proper maturation, processing and trafficking of tyrosinase to post-Golgi melanosomes.

## Tyrosinase quality control and degradation

Proteins that traverse the secretory pathway are closely monitored by a stringent quality control process, which begins in the ER. If a protein improperly matures, it is sorted for degradation by the cytosolic proteasome through the ER-associated protein degradation (ERAD) process (Ahner and Brodsky, 2004). Tyrosinase is also subjected to this evaluation process (Halaban et al., 1997; Svedine et al., 2004). The maturation of wild-type tyrosinase is inefficient with only approximately 50% of the protein passing the quality control test and reaching its mature form under optimal conditions (Halaban et al., 1997). Mutant tyrosinase from albino melanocytes can be completely retained in the ER and subsequently turned over through the ERAD pathway (Halaban et al., 2000; Svedine et al., 2004).

The N-linked glycans act as quality control tags that signal the status of the maturing glycoprotein (Hebert et al., 2005; Helenius, 1994). The composition of the glycan is controlled by the glycosidases and glycosyltransferases that line the secretory pathway. The glycan composition encodes a message that is deciphered by a variety of carbohydrate binding proteins that aid in the maturation and quality control processes.

As previously discussed, the ER resident chaperones calnexin and calreticulin bind to glycoproteins that possess monoglucosylated glycans. Calnexin and calreticulin binding supports the ER retention of glycoproteins with immature or non-native structures. Therefore, monoglucosylated glycans signal the retention of aberrant structures. Mutant tyrosinase from albino melanocytes is completely retained in the ER as the immature monoglucosylated 70 kDa glycoform bound to both calnexin and calreticulin (Berson et al., 2000; Halaban et al., 2000; Toyofuku et al., 2001a). The GT plays a pivotal role in the retention process, by supporting the reglucosylation of unglucosylated glycans that are linked to proteins that contain non-native structures (Sousa and Parodi, 1995; Trombetta and Parodi, 2003). GT is the only enzyme currently known that modifies the glycan state after analyzing the integrity of a protein. Persistent reglucosylation by the GT causes ER retention, which can eventually lead to degradation.

Soluble tyrosinase is also retained to a large extent in the ER in both melanogenic and non-melanogenic cells (Berson et al., 2000; Popescu et al., 2005). Frameshift mutations that introduce premature termination codons that delete or abbreviate the hydrophobic transmembrane domain are associated with albinism (Chintamaneni et al., 1991; Oetting et al., 2003). Soluble tyrosinase is retained through its interaction with the soluble ER chaperones, calreticulin and BiP (Popescu et al., 2005). Interactions were not observed between soluble tyrosinase and the integral membrane protein calnexin. In addition, soluble tyrosinase was secreted from cells lacking calreticulin or treated with DNJ, supporting a role for calreticulin in the ER retention of the soluble protein.

The retention of proteins in the ER has been hypothesized to be caused by interactions with an immobile ER matrix of chaperones or the formation of protein aggregates that are too large to be anterogradely transported to the Golgi (Ellgaard et al., 1999; Tatu and Helenius, 1997). Fluorescence correlation spectroscopy was used to measure the ER mobility of tyrosinase tagged with yellow fluorescent protein (YFP) (Kamada et al., 2004). Thermally misfolded tyrosinase-YFP was found to be highly mobile, while aberrant protein synthesized in the presence of glucosidase inhibitors displayed extensive immobilization. The appearance of tyrosinase aggregates was also observed for mutant mouse tyrosinase (C71S) in the absence of calnexin and calreticulin binding using sucrose gradient ultracentrifugation (Svedine et al., 2004). These aggregates colocalize with the ER markers PDI and BiP but not calnexin by immunofluorescence microscopy. Together, these

results verify the importance of calnexin and calreticulin in protecting tyrosinase from non-productive interactions that lead to its inactivation and aggregation.

Carbohydrates are also trimmed of mannose residues in the ER. Removal of $\alpha$-1,2-linked mannoses from the B chain by ER mannosidase I appears to generate a degradation tag that helps sort aberrant glycoproteins to the ERAD pathway in yeast and mammalian cells (Cabral et al., 2001; Jakob et al., 2001; Su et al., 1993). Pharmacological inhibition of mannose trimming delayed the ER-associated degradation of tyrosinase in both melanogenic and non-melanogenic cells demonstrating that mannose trimming also plays a role in tyrosinase degradation (Svedine et al., 2004; Wang and Androlewicz, 2000). The identity of a carbohydrate-binding quality control receptor that can recognize mannose-trimmed glycans on tyrosinase is currently unknown. ER degradation-enhancing alpha-mannosidase-like protein (EDEM) is a candidate to serve this function as it has been shown to extract misfolded proteins from the calnexin-binding cycle (Molinari et al., 2003; Oda et al., 2003; Wang and Hebert, 2003). However, further studies will be required to clarify this issue.

Proteins evaluated as defective are delivered to a translocon in the ER membrane in a translocation-competent state. Candidates for the dislocation channel include the Sec61p translocon and Derlin1/2 (Lilley and Ploegh, 2004; Pilon et al., 1997; Plemper et al., 1998; Wiertz et al., 1996; Ye et al., 2004; Zhou and Schekman, 1999). The dislocation of ERAD substrates from the ER lumen is driven from the cytosolic side by the AAA-ATPase P97 (Tsai et al., 2002; Ye et al., 2001). Once in the cytosol, proteins are ubiquitinated by E3 ligases and deglycosylated by the endoglycosidase pngase prior to degradation by the proteasome.

Mutant mouse tyrosinase is rapidly degraded by the proteasome with a half-life of 3 h, compared with 6 h for the wild-type protein (Svedine et al., 2004). Proteasomal inhibitors stabilize the expression of both wild type and mutant tyrosinase (Halaban et al., 1997). These inhibitors support the accumulation of tyrosinase as ubiquitinated and deglycosylated soluble proteins (Halaban et al., 1997; Svedine et al., 2004). Together these results support the usage of the established ERAD pathway for the turnover of tyrosinase.

Tyrosinase levels can be manipulated by regulating its stability and degradation through the ERAD pathway. Fatty acids modulate tyrosinase levels and control melanogenesis. The unsaturated fatty acids oleic acid (C18:1) and linoleic acid (C18:2) decrease tyrosinase activity by increasing its ubiquitination and proteasomal degradation (Ando et al., 1999, 2004). In contrast, the saturated fatty acids palmitic acid (C16:0) and stearic acid (C18:0) stabilize tyrosinase expression and increase pigmentation.

Phospholipase D2 (PLD2) activation also appears to lead to a decrease in pigmentation because of rapid tyrosinase ER-associated degradation (Kageyama et al., 2004). The exposure of mouse melanoma cells to 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) activates PLD and causes the accelerated degradation of tyrosinase. In addition, transforming growth factor-$\beta$1 (TGF-$\beta$1) also increases the level of tyrosinase degradation (Martinez-Esparza et al., 1997). These results indicate that PLD2 and TGF-$\beta$1 can regulate pigmentation by controlling the level of tyrosinase.

Wild-type tyrosinase is retained in the ER and rapidly turned over in human amelanotic melanoma cells causing the non-pigmented phenotype (Halaban et al., 1997). Although the tyrosinase gene is wild type in these cells, a hostile ER folding environment appears to contribute to the misfolding of tyrosinase, resulting in it being treated similarly to a mutant defective protein. Amelanotic melanoma cells display elevated proton export properties when compared with pigmented melanoma cells (Halaban et al., 2002b). This caused the acidification of the extracellular media and the inability to maintain a neutral intracellular pH. Tyrosinase levels and activity could be rescued by maintaining the cells in alkaline media. In addition, treatment of the melanoma cells with the proton ionophore monesin or the V-ATPase inhibitors concanamycin A or bafilomycin A1 also supported proper maturation of tyrosinase and pigmentation (Halaban et al., 2002b; Watabe et al., 2004). A pH imbalance may also explain why many melanomas are resistant to weak base chemotherapies, which would accumulate in the acidic organelles rather than reach the nucleus, their site of action (Simon, 1999).

Tyrosinase can also be stabilized in amelanotic melanomas by treatment with its own substrates, DOPA and tyrosine (Halaban et al., 2001). These substrates allow tyrosinase to fold properly in the ER and be transported to the Golgi thereby escaping the ERAD pathway. The use of small molecules as therapies to restore proper protein maturation has seen growing interest in recent years (Perlmutter, 2002). Small molecules can stabilize immature and flexible structures through their binding, allowing the bound protein to pass the quality control test in the ER. DOPA and tyrosine binding to tyrosinase appears to serve this role in amelanotic melanoma cells where their treatment allows the protein to reach a protease resistant state, exit the ER, have its glycans processed in the Golgi and become active in the production of melanin (Halaban et al., 2001; Ostankovitch et al., 2005).

In addition to the degradation of short-lived or defective proteins, the proteasome also creates antigenic peptides for MHC class I presentation at the cell surface. The proteasome-generated antigenic peptides are translocated into the ER by the transporter for antigen presentation (TAP) where they bind MHC class I complex liberating it for transport through the secretory pathway to the cell surface. Tyrosinase is an excellent source of class I MHC-restricted peptides (Slingluff,

1996). It has been used extensively as a model protein to help understand the processing and presentation of antigenic peptides from membrane proteins (Engelhard et al., 2002).

The Engelhard group elegantly demonstrated that tyrosinase antigenic peptides were created from glycosylated protein, which had previously resided in the ER as one of the presented peptides contained a deamidated Asp residue that had been changed from an original Asn residue (Mosse et al., 1998). This alteration occurred after the dislocation of the full-length glycoprotein to the cytosol, as a result of the cleavage of the Asn-linked glycan by pngase (Hirsch et al., 2003). The amount of MHC class I-restricted tyrosinase peptide presented at the cell surface was directly dependent upon the improper maturation of tyrosinase in the ER (Ostankovitch et al., 2005). Peptide presentation was increased for mutant protein, and decreased after DOPA and tyrosine treatment, which increased the efficiency of tyrosinase folding. Altogether, these results demonstrated that the level of tyrosinase misfolding is a critical determinant of the amount of MHC class I peptide created through the ERAD pathway.

Recently, the Ng laboratory has found that two tests of quality control surveillance are present in the yeast ER that result in the targeting of proteins to the ERAD pathway (Vashist and Ng, 2004; Vashist et al., 2002). The initial check point involves monitoring cytoplasmic domains of membrane proteins for defects. If a protein is determined to possess a cytosolic defect it is targeted directly to the ERAD pathway. This process has been termed the ERAD-cytosolic or ERAD-C pathway. If a protein passes this first test, it is then subjected to an analysis of it lumenal domain by the ERAD-lumenal (ERAD-L) machinery. Surprisingly, degradation of ERAD-L substrates requires ER-to-Golgi transport, and retrograde transport back to the ER prior to dislocation to the cytosol for proteasomal degradation. As the majority of the mutations in tyrosinase associated with albinism are due to defects in the lumenal ectodomain of tyrosinase (Oetting, 2000), tyrosinase mutants would be expected to be treated as ERAD-L substrates. However, currently these distinct checkpoints have only been observed in yeast. Additional studies using mammalian system will be required to establish the role of the ERAD-L pathway in higher eukaryotic systems.

The quality control of proteins that traverse the secretory pathway also extends beyond the ER and into the Golgi where the fidelity of the maturation process continues to be monitored (Arvan et al., 2002). Proteins determined to be non-native within the Golgi can either be sorted back to the ER for ER-associated degradation or sent to the lysosome for degradation. Tyrosinase also appears to be scrutinized in the Golgi as several factors have recently been shown to inhibit melanin production by derailing post-ER tyrosinase maturation.

Phenylthiourea (1-phenyl-2-thiourea; PTU) is an effective inhibitor of tyrosinase activity (Dieke, 1947). It has recently been shown to cause the lysosomal degradation of tyrosinase following its maturation in the Golgi (Hall and Orlow, 2005). PTU likely disrupts copper binding to tyrosinase in a manner similar to that, which has been found for catechol oxidase (Klabunde et al., 1998). 25-hydroxylcholesterol (25HC) also acts in the Golgi to inhibit melanogenesis by inducing tyrosinase degradation, however the protease source is currently unknown (Hall et al., 2004). Both PTU and 25HC appear to act in the Golgi to support the accelerated degradation of tyrosinase through diverse degradative pathways.

## Concluding remarks

Tyrosinase has served the role as the miner's canary for studies of the mammalian secretory pathway. Its proper maturation is highly sensitive to defects in the ER, Golgi, and post-Golgi, as well as the machinery that transports material between these locations. Tyrosinase is subjected to the surveillance by both ER and Golgi quality control systems. Defects in tyrosinase maturation are easily visualized by the absence of cellular pigmentation. The visual nature of the activity of tyrosinase has facilitated the discovery, identification, and characterization of cellular machinery involved in its maturation. These studies have illuminated the wide spectrum of proteins required for a protein to become functional and properly localized within the cell. These factors include general machinery that are likely involved in maturation of most of the proteins that traverse the secretory pathway, as well as melanocyte-specific proteins used specifically by tyrosinase. Although tyrosinase is arguably one of the most thoroughly studied proteins in regards to its cellular maturation, a more complete understanding of its maturation will be aided by solving its 3-D structure. All together, tyrosinase maturation studies have taught us that protein maturation is a delicate process that relies on the concerted action of a large number of cellular factors, and the disruption of any of these processes can lead to disease states.

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