Glycoprotein folding and quality-control mechanisms in protein-folding diseases

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ABSTRACT

Biosynthesis of proteins – from translation to folding to export – encompasses a complex set of events that are exquisitely regulated and scrutinized to ensure the functional quality of the end products. Cells have evolved to capitalize on multiple post-translational modifications in addition to primary structure to indicate the folding status of nascent polypeptides to the chaperones and other proteins that assist in their folding and export. These modifications can also, in the case of irreversibly misfolded candidates, signal the need for dislocation and degradation. The current Review focuses on the glycoprotein quality-control (GQC) system that utilizes protein N-glycosylation and N-glycan trimming to direct nascent glycopolypeptides through the folding, export and dislocation pathways in the endoplasmic reticulum (ER). A diverse set of pathological conditions rooted in defective as well as over-vigilant ER quality-control systems have been identified, underlining its importance in human health and disease. We describe the GQC pathways and highlight disease and animal models that have been instrumental in clarifying our current understanding of these processes.

KEY WORDS: N-glycosylation, Glycoprotein folding, ER quality control, ER-associated degradation, ER export

Introduction

Over the past 30 years, the pathogenesis of multiple human genetic disorders has been directly linked to the retention of misfolded proteins in the endoplasmic reticulum (ER), sometimes driven by mutations as modest as a single amino acid substitution (Welch, 2004). These diseases – α1-antitrypsin (α1AT) deficiency, cystic fibrosis, combined factor V and VIII deficiency (F5F8D), to name a few – have been termed ER storage diseases (Kim and Arvan, 1998; Rutishauser and Spiess, 2002; Schröder and Kaufman, 2005). Interestingly, some of these mutant proteins still possess the biological activity of their wild-type counterparts (Drumm et al., 1991; Bhargava et al., 2012), suggesting that improving the exit of the mutant protein from the ER might reduce disease pathology. The mechanism(s) by which proteins with a native conformation selectively transit the ER to the Golgi apparatus, whereas misfolded proteins are retained in the ER and/or are degraded, is termed ER quality control (ERQC) (de Silva et al., 1990; Araki and Nagata, 2011). Such a failsafe mechanism ensures that the assembly line of nascent proteins in the ER does not export defective material. Quality control occurs at every stage of protein biosynthesis in the ER – during co-translational translocation, post-translational modification, chaperone-assisted folding, assembly of multi-subunit complexes, trafficking and export – with mechanisms in place for immediate recognition of mutant and/or misfolded proteins for degradation. This Review focuses on one aspect of ERQC that utilizes N-glycosylation to ascertain the ‘foldedness’ of the ER itinerant, which we refer to as glycoprotein quality control (GQC).

In the first part, we describe the process of co-translational glycosylation of nascent polypeptides and the mechanism of GQC, including the role of N-glycan modification in assisting the folding, export and degradation of nascent glycopolypeptides. We then highlight how studies into the molecular mechanisms underlying GQC have provided insights into human diseases caused by defects in this pathway. In some cases, these insights could pave the way for therapeutic interventions that could alleviate disease.

Stages in protein N-glycosylation

Glycosylation has been described as the most common post-translational modification of proteins (Apweiler et al., 1999), with the majority of proteins produced in the ER bearing covalent attachment of an oligosaccharide to asparagine (Asn) side-chains (N-linked glycosylation). These oligosaccharides, termed N-linked glycans or simply N-glycans, are added to proteins in the ER, where they serve as an entry pass to an intricate glycoprotein-specific pathway – GQC – that ultimately enhances the export efficiency of high-quality glycoprotein products (Roitsch and Lehle, 1989).

Protein N-glycosylation occurs in two stages: (1) assembly of a glycan molecule and (2) transfer of the glycan onto a nascent protein. In stage 1, a tetradecaoligosaccharide consisting of three glucose, nine mannose and two N-acetylglucosamine residues (Glc3Man9GlcNAc2) is assembled on an ER-membrane-anchored protein-folding diseases
transferred onto the glycan using Dol-P-Man and Dol-P-Glc as four mannose residues and three glucose residues are sequentially luminal surface (Sanyal and Menon, 2009). Inside the ER lumen, ‘flipped’ across the ER membrane from the cytosolic side to the ER luminal side to allow assembly of the remainder of the glycan on the ‘flipped’ across the ER membrane from the cytosolic side to the ER luminal side to allow assembly of the remainder of the glycan on the luminal surface (Sanyal and Menon, 2009). Inside the ER lumen, four mannose residues and three glucose residues are sequentially transferred onto the glycan using Dol-P-Man and Dol-P-Glc as donors, respectively, generating a GlcManGlcNAcP-Dol tetradecaoligosaccharide.

In the second stage of N-glycosylation, the membrane-bound multi-subunit complex oligosaccharyltransferase (OST) associates with the translocon pore on the ER membrane and catalyzes the covalent linkage of the 14-sugar N-glycan to the Asn amide group of N-glycosylation sequons (Asn-X-Ser/Thr, where X can be any amino acid except proline) as they emerge into the lumen (Mohorko et al., 2011). This reaction generates Dol-P as a by-product, which is recycled for use in glycan assembly (stage 1).

**Glycoprotein folding**

A recurring theme in GQC (and protein quality control in general) is the close association of the nascent glycoprotein not only with chaperones that assist in its proper folding, but also with those that target it for degradation. This arrangement, despite being overzealous in certain instances, ensures that misfolded, aggregation-prone glycoproteins are readily degraded. For GQC, sequential cleavage of sugar residues from the N-glycans is a major factor in determining the fate of ER glycoproteins. One major question in cell biology for the last 25 years is why the cell expends energy to assemble the complex N-glycan core, only to then catalyze its sequential trimming. Studies over recent years suggest that the addition and modification of the N-glycan core is intimately linked to the folding, disulphide-bond formation and complex assembly of glycoproteins.

The α1,2 glycosidic linkage between the outermost glucose residues (‘M’–‘N’, Fig. 1) from the N-glycan of the nascent glycoproteins is cleaved almost immediately by the membrane-bound enzyme α-glucosidase I (GS-I), forming Glc2ManGlcNAc2 (Hubbard and Robbins, 1979). This step prevents re-binding of the processed N-glycan by OST (Helenius and Aebi, 2004) and promotes binding to a recently discovered membrane-anchored ER protein, called malectin (Schallus et al., 2008). Based on studies using the model substrates αLAT and hemagglutinin, malectin was found to preferentially bind misfolded proteins, preventing further progress along the folding pathway and directing them to the ER-associated degradation (ERAD) pathway (Chen et al., 2011; Galli et al., 2011).

Once the glycoprotein passes the malectin GQC checkpoint, a multi-subunit α-glucosidase – GS-II – removes the second glucose residue (‘M’, Fig. 1), generating GlcManGlcNAc2 glycan that have affinities in the submicromolar range for ER lectin-like chaperones calnexin (CNX) and calreticulin (CRT) (Michalak et al., 2002; Rutkevich and Williams, 2011; Wijeyesakare et al., 2013). Glycoprotein association with CNX or CRT marks the beginning of the ‘calnexin cycle’ (Hammond et al., 1994) (Fig. 2). CNX and CRT are thought to associate in similar ways with GlcManGlcNAc2-glycoproteins, but CNX is a membrane-anchored protein and CRT is a luminal homolog. Both proteins consist of: (1) a globular domain that harbors both the oligosaccharide- and calcium-binding sites; and (2) an elongated arm, also known as the P domain because of its proline-rich sequence motifs (Ellgaard et al., 2001; Schrag et al., 2001; Michalak et al., 2009; Kozlov et al., 2010a; Wang et al., 2012). Although the oligosaccharide-binding site in the globular domain of CRT has been well established (Kozlov et al., 2010a), how polypeptides might bind CNX and CRT is less clear. Recent structural studies have identified possible peptide-binding sites in both the lectin and arm domains of CRT (Chouquet et al., 2011; Pocanschi et al., 2011). The P domain binds to a luminal thiol-disulfide oxidoreductase, ERP57 (Frickel et al., 2002; Kozlov et al., 2006), and a peptidy l prolyl isomerase, cyclophilin B (CypB) (Kozlov et al., 2010b). Together, CNX or CRT (CNX/CRT), ERP57 and CypB assist the nascent glycoprotein in achieving a native conformation and correct disulfide pairings.

A second deglucosylation (removing residue ‘L’, Fig. 1) by GS-II prevents rebinding of the glycoprotein to the CNX/CRT-ERP57 complex (D’Alessio et al., 2010). At this stage, another key component of the CNX cycle springs into action: UDP-Glc:glycoprotein glucosyltransferase 1 (UGGT1) binds to fully deglucosylated N-glycans and ascertains whether the substrate has achieved the native conformation. Completely folded glycoproteins are promptly released by UGGT1 and are allowed to proceed along the ER-to-Golgi anterograde trafficking pathway. In contrast, substrate glycoproteins with non-native folds selectively bind to UGGT1 via solvent-exposed hydrophobic patches in conjunction with the deglucosylated N-glycan, and undergo reglucosylation using UDP-Glc as a Glc donor. This results in glycoproteins with monoglucosylated N-glycans that can once again enter the CNX cycle for folding assistance.

The ability of UGGT1 to discriminate folded from misfolded substrates is crucial for GQC. Studies using purified UGGT1 suggest the molecular mechanism by which UGGT1 recognizes its glycoprotein substrates is bipartite (Trombetta and Parodi, 1992; Sousa and Parodi, 1995). For glucosylation, the substrates must have at a minimum the innermost GlcNAc residue of the N-glycan, and...
Sousa and Parodi, 1995). Using artificially constructed heterodimers of folded and misfolded ribonuclease monomers, UGGT1 was found to reglucosylate N-glycans only on the misfolded half (Ritter and Helenius, 2000), suggesting that, for UGGT1-mediated reglucosylation, the N-glycan must be near the misfolded region of the substrate. Interestingly, for a different glycoprotein substrate, UGGT1 was reported to reglucosylate N-glycans that were more distant (40 Å) from local hydrophobic regions (Taylor et al., 2004). Despite these substrate-specific differences, it seems that UGGT1 can survey glycoprotein substrates for misfolded regions and reglucosylate attached N-glycans when appropriate (Fig. 2). Reglucosylation mediated by UGGT1 can promote association with CNX/CRT-ERp57 and the thiol-disulfide isomerase activity of ERp57 can promote proper disulfide bond formation (Zapun et al., 1998).

In addition to the CNX cycle, glycoprotein itinerants are serviced by the ER-resident molecular chaperone BiP. A member of the Hsp70 family of proteins, BiP is the most abundant chaperone in the ER, and consists of an N-terminal nucleotide-binding domain (NBD) and a C-terminal substrate-binding domain (SBD). BiP is a peptide-dependent ATPase that can either increase or decrease the folding rate of protein ligands (Bukau et al., 2006). ATP-bound BiP binds to hydrophobic patches in nascent polypeptides that get ‘locked’ into the SBD upon hydrolysis of ATP to ADP, a process that is accelerated by an ER co-chaperone of the DnaJ family (a so-called ERdj protein). Consequently, BiP ‘holds’ the substrate, allowing it to attain its native conformation, and then permits the substrate to assemble with other subunits, as well as promotes accessibility to other chaperones such as the protein disulfide isomerase (PDI) family members that generate and rearrange disulfide bonds that are properly paired. Seven ERdj proteins (ERdj1-ERdj7) have been characterized so far, with functions ranging from ensuring nascent polypeptides are serviced by BiP immediately after entering the ER, to targeting terminally misfolded proteins for degradation (Otero et al., 2010). The substrate protein is released from BiP upon exchange of ADP with ATP, triggered by the guanine nucleotide exchange factor BAP (BiP associated protein) (Chung et al., 2002; Bukau et al., 2006).

Glycoprotein export

How does the substrate protein escape the CNX cycle and the BiP-assisted cycles of protein folding? Once again, N-glycan trimming plays a crucial role in this process (Caramelo and Parodi, 2008). If the substrate protein does not attain its native conformation after repeated interactions with CNX/CRT, BiP or other chaperones of the ER, it is targeted for degradation by the ERAD pathway, described below. By contrast, if the substrate protein assumes a functionally competent three-dimensional structure, it traffics to the Golgi apparatus en route to its final destination. For forward transport of secretory proteins from the ER to the Golgi, two mutually non-exclusive mechanisms have been proposed: (1) bulk-flow and (2) receptor-mediated transport (Warren and Mellman, 1999).

Glycoproteins entering the Golgi through bulk flow are immediately demannosylated by one or more of the resident mannosidases: Golgi Man I (A, B, C) and Golgi Man II (Moremen, 2002). These Golgi mannosidases can remove mannoses from Glc3,Man8,9GlcNAc2 N-glycans, allowing processing of all glycoproteins arriving at the Golgi, including the ones that enter owing to abnormal or failed ER quality control (Gabel and Bergmann, 1985; Moremen, 2002). Natively folded, demannosylated substrates are glycosylated and trafficked to their final destination, whereas misfolded glycoproteins are recognized by quality-control systems that operate in the Golgi complex, and are delivered to lysosomes for degradation (Arvan et al., 2002).

Receptor-mediated trafficking in the ER utilizes N-linked glycans. For example, ERGIC-53 (along with VIPL and VIP36) is thought to act as a cargo receptor, transferring correctly folded substrates to COP-II vesicles for transport out of the ER to the Golgi (Barlowe, 2003; Kamiya et al., 2008; Kamiya et al., 2012). Similarly to
UGGT1, ERGIC-53 might have a bipartite recognition signal and the ability to sense folding status of its substrates (Appenzeller-Herzog et al., 2005). As an example, ERGIC-53 recognizes both the high-mannose type N-glycan and a β-hairpin loop structure present only in correctly folded cathepsin Z, an ERGIC-53 binding substrate (Appenzeller-Herzog et al., 2005). Significantly, the autosomal recessive bleeding disorder F5F8D is caused in two-thirds of patients by complete loss-of-function mutations in LMAN1, the gene encoding ERGIC-53 (Nichols et al., 1998). The other third of patients have mutations in the multiple clotting factor deficiency 2 (MCFD2) gene, which encodes a calcium-binding protein that interacts with ERGIC-53 to form a heterodimer required for trafficking of factors V and VIII from the ER to the Golgi (Zhang et al., 2003). F5F8D (described in more detail below) was the first human genetic disease identified that results from defective trafficking of proteins out of the ER.

Degradation of misfolded glycoproteins

ER-associated degradation (ERAD)

Trimming of mannose residues from N-linked glycans predicates productive transport of one subset of glycoproteins through the Golgi complex and another subset – typically irreversibly misfolded glycoproteins – to sequestration inside the ER and degradation by ERAD (Aebi et al., 2010; Hebert and Molinari, 2012; Olzmann et al., 2013) (Fig. 3). ERAD is a process in which misfolded proteins are retrotranslocated from the ER to the cytosol and subsequently degraded by the ubiquitin-proteasome system.

The archetypal enzyme that catalyzes the removal of the terminal mannose from the B chain of the N-glycan (residue ‘I’, Fig. 1) and initiates the events of export and/or ERAD is ER mannosidase I (ERMan I) (Gonzalez et al., 1999; Tremblay and Herscovics, 1999). Glycoproteins that are subject to degradation are further demannosylated by one or more of the following: (1) the mannosidase activity of the ER-degradation-enhancing mannosidase-like proteins (EDEM1, 2 or 3) (Kanehara et al., 2007; Olivari and Molinari, 2007); (2) multiple rounds of demannosylation by ERMan I (Hosokawa et al., 2003; Wu et al., 2003); or (3) the action of Golgi mannosidases during ER-to-Golgi cycling (Hosokawa et al., 2007; Kukushkin et al., 2011). Crucially, removal of the mannose residue ‘G’ (Fig. 1) precludes the substrate from UGGT1-mediated re-glucosylation and re-entry into the ER, ending any further attempts at protein folding. Removal of the terminal mannose residues from the B and C chains (residues ‘I’ and ‘K’, Fig. 1; Fig. 3), by contrast, allows binding of the N-glycan by the ERAD lectins OS-9 and XTP3-B, promoting degradation. OS-9 and XTP3-B are luminal proteins containing mannose-6-phosphate receptor homology (MRH) domains that specifically bind to terminal α-1,6-mannose residues to facilitate transfer of terminally misfolded glycoproteins to the membrane-associated ERAD complex for retrotranslocation and degradation (Kanehara et al., 2007; Christianson et al., 2008; Hosokawa et al., 2010).

Multiple protein complexes that are resident on the ER membrane have been proposed to be the sites of retrotranslocation of ERAD substrates and eventual entry into the ubiquitin proteasome pathway (Hoseki et al., 2010; Hampton and Sommer, 2012). One such complex is the HRD1-SEL1L ERAD complex (Fig. 3). HRD1 is an ER-membrane-localized E3 ubiquitin ligase that polyubiquitylates ERAD substrates on the cytoplasmic face of the ER membrane. SEL1L is a membrane-associated glycoprotein that interacts with an assortment of ERAD regulators, including OS-9, XTP3-B, EDEM1 and EDEM3, which facilitate substrate transfer from the protein-folding components to the ERAD complex (Mueller et al., 2008). An active area of investigation is the molecular definition of the adaptor proteins, their interactions and the mechanism for delivery to the ERAD complex (Araki and Nagata, 2011). Given that OS-9 and XTP3-B can bind to misfolded polypeptides devoid of any N-glycans, the MRH domains of OS-9 and XTP3-B might act to facilitate association with SEL1L through interaction with its N-glycans.

Degradation of ER protein complexes and aggregates

If ER glycoproteins are not delivered to the Golgi compartment or degraded through ERAD, they become susceptible to aggregation. These aggregates are often insoluble in non-ionic detergent lysis buffers, such as 1% Triton X-100 (Kaganovich et al., 2008; Buchberger et al., 2010). Multiple mutant glycoproteins are detected...
Fig. 4. Degradation of insoluble and soluble ER glycoproteins. Some misfolded glycoproteins form insoluble aggregates or ordered polymers in the ER, and UGGT1-mediated modification of the glucosylation status (monoglucosylated or unglucosylated) might play a role in limiting insolubility. The soluble-to-insoluble transition might occur via compartmentalization in the ERAC, and insoluble substrates might also be resolubilized. Soluble forms of both luminal and transmembrane glycoproteins tend to be degraded through ERAD, whereas insoluble forms tend to be degraded via autophagy, although the mechanism by which insoluble ER proteins get to the lysosome is not entirely clear. Luminal insoluble glycoproteins might be packaged into EDEM1-containing vesicles (EDEMasomes) and transported to the lysosome. Transmembrane insoluble glycoproteins could accumulate in the aggresome and then be degraded by the proteasome, or be targeted by an unknown mechanism to the lysosome for degradation.
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Diseases of GQC

Either insufficient or overvigilant GQC can cause a wide range of pathological conditions. Below, we describe a select few conditions that have formulated our current view of GQC because they are used as models to decipher the GQC pathways. Congenital disorders of glycosylation (CDGs) illustrate the importance of glycosylation in the normal health and functioning of various human organs and tissues. α1AT deficiency demonstrates how mutations in a cellular protein overburden the GQC machinery, resulting in the accumulation of protein aggregates in the ER. Furthermore, experiments using wild-type and mutant alleles of α1AT have been instrumental in uncovering the surveillance and scrutiny that substrates undergo during every step of GQC. Much of our current understanding of the ERQC pathways is thus based on such studies. Finally, we describe the consequences of the loss of glycan-mediated trafficking in F5F8D. This discovery demonstrates how genetic studies of a disease can uncover physiological functions of components of ERQC pathways that have evolved to service an exclusive clientele.

CDGs

CDGs are a set of rare human diseases that have very severe consequences and can always be traced back to either complete or partial loss of at least one of the components of the glycosylation pathway. Over 70 such diseases are now known, with abnormalities spanning almost every organ system (Freeze et al., 2012; Hennet, 2012; Freeze, 2013). The majority of these diseases are associated with mutations in the proteins of the N-glycosylation pathway. In particular, mutations in enzymes that catalyze the biosynthesis of the nucleotide sugars that form the substrates of N-glycan assembly as well as mutations affecting the enzymes that are responsible for the assembly of N-glycans are frequently observed. The convenience of using a simple diagnostic test that determines the N-glycosylation status of serum transferrin by isoelectric focusing is perhaps the reason for this over-representation of N-glycosylation pathway components in CDGs (Jaeken et al., 1984; Stibler and Jaeken, 1990; Hennet, 2012). With the advent of more sophisticated analytical methods, including next generation sequencing, consequences of the loss of more GQC components will very likely be discovered (Freeze, 2013). Recent reviews provide comprehensive analyses of various CDGs and their clinical manifestations (Freeze et al., 2012; Hennet, 2012; Freeze, 2013). Below, CDGs that are immediately relevant to GQC are highlighted.

TUSC3-CDG, MAGT1-CDG and DDOST-CDG result upon the loss of subunits in the OST complex, which catalyzes the en bloc transfer of the N-glycan core oligosaccharide onto the nascent polypeptide chain. Loss of TUSC3 or MAGT1 is known to cause intellectual disabilities (Garshasbi et al., 2008; Molinari et al., 2008; Garshasbi et al., 2011). Delayed psychomotor development (ability to walk) and failure to develop speech capacity were recently reported in a child lacking functional DDOST (Jones et al., 2012). GC1S-CDG (also known as CDG-IIb) arises when the affected individual lacks GS-I, the enzyme that cleaves the terminal glucose (Fig. 1; glucose ‘N’) from the N-glycan (De Praeter et al., 2000; Völker et al., 2002). The index case of GC1S-CDG was a compound heterozygote for two different GS-I missense mutations. Enzymatic analysis (of liver tissue and skin fibroblasts) demonstrated an almost complete lack of GS-I activity. The affected individual survived embryonic development but presented at about 2.5 months of age with multiple organ system failure, severe neurological defects (hypotonia, hypoventilation and seizures), dysmorphic features and progressive hepatomegaly (De Praeter et al., 2000). Although not yet categorized as a CDG, mutations in the β-subunit of GS-II had been previously linked with polycystic liver disease in humans (Drenth et al., 2003; Li et al., 2003; Janssen et al., 2010). Finally, MAN1B1-CDG is an autosomal recessive disease caused by the loss of ERMan I enzyme, and these patients also suffer from intellectual disability (Rafiq et al., 2011). Tissue-specific analyses have revealed that brain contains the largest N-glycoproteome (Zielinska et al., 2010), suggesting a potential connection to the frequent observation of intellectual disability as a consequence of the loss of N-glycosylation.

α1AT deficiency

α1AT is a serine protease inhibitor synthesized primarily in hepatocytes, and is secreted into the bloodstream where it acts as the primary inhibitor of elastase in the lungs. α1AT deficiency is an autosomal codominant genetic condition resulting from any of the ~120 variant alleles described to date (Stoller and Aboussouan, 2012). The severity of the disease varies based on the allele, but the most common allele (found in nearly 95% of patients) is the Z variant (ATZ), characterized by a Glu342Lys missense mutation in the wild-type protein of 394 amino acids. This mutation renders the ATZ molecule inherently unstable with a propensity to self-associate, resulting in the accumulation of protein aggregates in the ER. Thus, the effect of the ATZ allele is twofold: (1) loss of functional α1AT in the bloodstream resulting in compromised connective tissue of the lung causing chronic emphysema; and (2) the accumulation of toxic aggregates in the hepatocyte ER, causing hepatitis, cirrhosis and an increased risk for hepatocellular carcinoma (HCC) (Valastyan and Lindquist, 2014). The extensive biochemical analysis of α1AT variants has proven indispensable in deciphering GQC and ERAD pathways. In addition to the common disease allele ATZ, many of these studies have employed the null Hong Kong (NHK) allele that results in a truncated form of α1AT (Sifers et al., 1988). In turn, this clarified picture of α1AT folding in the ER promises to introduce more effective therapeutic approaches for patients (Bouchecareilh et al., 2010; Marciniak and Lomas, 2010).

Like all glycoproteins, the folding status of α1AT is appraised at every stage of its biosynthesis by a host of proteins inside the ER, and is guided through the folding, export and ERAD pathways, as appropriate. α1AT undergoes N-glycosylation at three distinct sites during its biosynthesis. Immediately after the terminal glucose residue is removed by GS-I, α1AT associates with maelectin, which specifically recognizes the Glc2Man9GlcNAc2 form of the N-glycan. The function of maelectin binding to the nascent glycopeptides was studied using wild-type and NHK alleles of α1AT (Chen et al., 2011; Galli et al., 2011). Malectin was found to stably associate with NHK, but not wild-type or unglycosylated NHK, and limit NHK degradation through the classical ERAD pathway (Johnston et al., 1998; Morito et al., 2008). It is very likely that a glycoprotein substrate with a propensity to misfold could be targeted to degradation by a combination of both the ERAD and autophagy pathways.

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interacts with ribophorin I, a subunit of the OST complex that has chaperone-like activity (Qin et al., 2012). It is likely that malectin binds to Glc2Man9GlcNAc2 glycans of all glycoproteins but retains only the misfolded molecules in a stable ternary complex with ribophorin I. Whether malectin itself has peptide-binding activity remains to be investigated.

Misfolded α1AT that escapes the malectin GQC checkpoint enters the CNX cycle after glucose trimming by GS-II. Indeed, Galli et al. demonstrated that malectin does not compete with CNX for substrate binding (Galli et al., 2011). Similarly to malectin and demonstrated that malectin does not compete with CNX for the CNX cycle after glucose trimming by GS-II. Indeed, Galli et al. however, indicates that XTP3-B can bind Man9GlcNAc2-NHK and 2008; Hosokawa et al., 2009; Mikami et al., 2010). A recent study, (Bernasconi et al., 2008; Christianson et al., 2008; Hosokawa et al., 2007). Finally, direct interaction of resident Golgi Man IA, IB or IC enzymes and returned to the ER for degradation (Hosokawa et al., 2007). Importantly, deglucosylation of NHK and ATZ molecules by UGGT1 improved secretion of the non-native form of NHK, it increased solubility differs depending on the mutant; whereas UGGT1 improved secretion of the non-native form of NHK, it reduced aggregation of ATZ and retained it in the ER in a soluble, misfolded form (Ferris et al., 2013). These findings indicate that even modulation of a single component of GQC can have different outcomes based on the particular mutation in the substrate.

Studies using α1AT alleles have helped formulate our current understanding of glycoprotein export and ERAD pathways. As substrate proteins exit the CNX cycle, misfolded candidates are demannosylated and targeted for degradation. The transmembrane region of CNX associates with EDEM1 and targets misfolded NHK molecules to the ERAD pathway (Oda et al., 2003). ERMan I, the principal candidate that primes substrate proteins for degradation, was characterized using NHK as a model (Cabral et al., 2000; Cabral et al., 2002; Wu et al., 2003; Karaveg and Moremen, 2005; Karaveg et al., 2005; Hosokawa et al., 2007; Avezov et al., 2008). For instance, degradation of luminal misfolded NHK was significantly hindered upon abrogation of ERMan I activity either by treating with the inhibitor kifunensine or by siRNA-mediated knockdown. The fraction of NHK that escaped scrutiny in the ER or entered the Golgi via bulk-flow was demannosylated by the resident Golgi Man IA, IB or IC enzymes and returned to the ER for degradation (Hosokawa et al., 2007). Finally, direct interaction of NHK with the ERAD lectins OS-9 and XTP3-B mediates delivery of misfolded NHK to the HRD1-SEL1L complex for degradation (Bernasconi et al., 2008; Christianson et al., 2008; Hosokawa et al., 2008; Hosokawa et al., 2009; Mikami et al., 2010). A recent study, however, indicates that XTP3-B can bind Man9GlcNAc2-NHK and inhibit its degradation (Fujimori et al., 2013). Nevertheless, these studies on α1AT have helped define the mechanisms of multiple back-up systems to ensure tight surveillance in GQC.

**Combined deficiency of F5 and F8 (F5F8D)**

Coagulation factors V and VIII (F5, F8) are homologous glycoproteins with a conserved domain organization. They require complex folding and post-translational processing to attain their final functional structures and be secreted. Both proteins are extensively glycosylated in the ER and handled by the GQC machinery before being exported to the Golgi. F5F8D is an autosomal recessive disorder distinct from hemophilia A (F8 deficiency) and parahemophilia (F5 deficiency) in that the genetic loci of F5 and F8 remain unperturbed. The molecular basis of F5F8D remained a mystery until forward genetic analyses uncovered mutations in the LMAN1 and MCFD2 genes (Nichols et al., 1998; Zhang et al., 2003). Lectin mannose-binding protein 1 (LMAN1) is a type I transmembrane protein originally identified as ERGIC-53, a 53 kDa protein of unclear function that localizes to the ER-Golgi intermediate compartment (ERGIC). MCFD2 is a soluble 16 kDa EF-hand protein that associates in a Ca^{2+}-dependent manner with LMAN1 to form the cargo receptor that cycles between the ER and ERGIC. Both LMAN1 and MCFD2 directly bind to both F5 and F8, LMAN1 through its lectin-like carbohydrate recognition domain and MCFD2 through its EF-hand domains, and deliver F5 and F8 to the ERGIC (Zhang et al., 2005). The normal levels of other plasma proteins in individuals with F5F8D suggests that LMAN1 and MCFD2 might have evolved to selectively assist trafficking of only F5 and F8. Thus, genetic analyses of F5F8D patients clarified two important aspects of protein folding and trafficking: (1) the role of LMAN1 as well as its partner, MCFD2, in the ER-to-Golgi trafficking pathway; and (2) that the efficient export of F5 and F8 requires specialized machinery for selective cargo, revising the presumed bulk-flow model.

Intriguingly, LMAN1 and MCFD2 are expressed in lower organisms that do not express either F5 or F8, leaving the possibility that they are involved in servicing additional proteins. Indeed, in vitro experiments using cell-culture systems have demonstrated that cathepsin C (Vollenweider et al., 1998), cathepsin Z (Appenzeller et al., 1999; Appenzeller-Herzog et al., 2004; Appenzeller-Herzog et al., 2005) and α1AT (Nyfeler et al., 2008; Zhang et al., 2011) are cargos for the LMAN1-MCFD2 complex. In contrast, mice deficient in LMAN1 do not display any discernible defect in the hepatic intracellular levels of cathepsins Z and C, or plasma levels of α1AT, although they do accumulate α1AT in the liver (Zhang et al., 2011). It is possible that, unlike other substrates studied so far, F5 and F8 proteins need to be exclusively serviced by the LMAN1-MCFD2 complex.

**Model systems to study GQC**

Studies on pathological conditions resulting from abnormal folding and/or quality control of cellular proteins facilitated the discovery of multiple ERQC pathways and their significance in cellular proteostasis and organismal health. The mechanistic intricacies of these pathways, however, have required cellular and animal models that could be manipulated in more defined scenarios. Thus, almost every component of the GQC has been systematically knocked down or ectopically expressed, followed by interrogation using model glycoproteins to study their specific functions in a cellular context. Additionally, animal models in which genes of the ERQC pathway are disrupted have proven invaluable in understanding the effects of such mutations at the physiological level. Indeed, several of these proteins are essential for embryonic development and survival, making studies on such gene mutations in humans impossible. Here, we briefly discuss a number of knockout mouse models related to GQC and important findings produced from their study.

Crt deletion in mice results in embryonic lethality due to irreversible disruption of Ca^{2+} homeostasis during cardiac development (Mesaedi et al., 1999; Guo et al., 2002). The mechanism for Crt^{−/−} embryonic lethality likely involves calcineurin activation and nuclear translocation of MEF2c (Lynch et al., 2005). Cnx deletion, by contrast, results in postnatal lethality, with half of the Cnx^{−/−} animals dying 48 hours after birth. Surviving Cnx^{−/−} mice have motor problems with decreased myelination of nerve fibers (Denzel et al., 2002). The dramatic phenotypes observed for...
Cnx and Crt deletions demonstrate the absolute requirement of these chaperones for mammalian survival, and suggest that, although homologous in structure and function in the GQC system, these proteins play distinctly vital roles that cannot be compensated for by the other. At the cellular level, deletion of Cnx and/or Crt was demonstrated to affect multiple aspects of protein folding, including the folding rate, efficiency and fidelity of model substrates (Molinari et al., 2004). Specifically, studies utilizing Crt−/− cells demonstrated a role for CRT in ensuring optimal assembly and antigen presentation by the MHC class I molecules (Gao et al., 2002; Howarth et al., 2004; Howe et al., 2009).

Embryonic lethality was also observed upon deletion of three other components of GQC: Grp78 (Hspa5; which encodes BiP)-deleted embryos die as early as E3.5 (Luo et al., 2006), Uggt1-null embryos die at E10.5 (Molinari et al., 2005) and ERP57 (Pdia3)-null embryos die at E13.5 (Coe et al., 2010). However, ablation of ERP57 specifically in B cells demonstrated its role in the process of antigen presentation; ERP57 is dispensable for the oxidative folding of substrates but is required for recruitment of MHC class I molecules to the antigenic peptide loading complex (Garbi et al., 2002; Garbi et al., 2004).

Upon discovering that mutations in the LMAN1 and MCFD2 genes lead to F5F8D, these genes were deleted in mice in order to further study the mechanism of F5F8D using a reverse genetics approach. As expected, plasma levels of F5 and F8 in the Lman1−/− animals decreased, to half of the normal levels (Zhang et al., 2011). Despite the absence of any apparent defects in the production of other plasma proteins or COPII-coated-vesicle formation, Lman1−/− hepatocytes displayed slightly distended ER with significant accumulation of α1AT and BiP, suggesting a disruption in proteostasis (Zhang et al., 2011).

Finally, transgenic models expressing mutant glycoproteins have also proved very useful in deciphering the GQC pathways and their role in the pathophysiology of human disorders. Thus, mice expressing human ATZ helped demonstrate the role of ER-localized accumulation of misfolded ATZ in causing hepatic fibrosis. Pharmacological activation of autophagy with carbamazepine partially ameliorated the fibrosis, and this might represent a potential therapeutic avenue to treat liver pathologies of α1AT deficiency (Hidvegi et al., 2010).

Conclusion and outlook

Protein folding is the most error-prone step in gene expression. As a consequence, cells have evolved sophisticated surveillance systems to limit protein misfolding and to eliminate irreversibly misfolded proteins. N-linked glycosylation of proteins in the ER initiates entry into a complex glycoprotein-specific quality-control system (GQC) that maintains the fidelity of the secretory and transmembrane proteome. GQC is quite sensitive, recognizing glycoproteins with even slight amino acid substitutions, and targeting them for ERAD (despite their potential to contribute a measure of bioactivity). The CNX cycle functions prominently in GQC, with the reglucosylating enzyme UG GTI serving as a primary folding sensor that dictates downstream decisions regarding folding, trafficking and degradation. How the GQC system functions to degrade terminally misfolded glycoproteins, and what happens when these degradation pathways are overwhelmed, are key questions for future exploration. Although many of the components of GQC have been identified, recent discoveries related to the targeting of misfolded proteins to the ERAD by malectin (Galli et al., 2011) and O-mannosylation of unfolded proteins (Xu et al., 2013) makes it likely that additional GQC components remain to be identified. The discovery of LMAN1 and MCFD2 illustrates how specialized systems have evolved to address folding and export of a limited set of related proteins. It also serves as a reminder of the complexity of ER-folding, quality-control and export pathways, for which, despite the strides made by the scientific community of late, many exciting discoveries lie ahead. Greater understanding of the mechanistic details of GQC will help identify potential therapeutic targets and inform the pathogenesis of the ever-increasing list of ER storage diseases.

This article is part of a review series on protein-folding diseases. See related articles at http://dmm.biologists.org/site/protein-folding-disease.xhtml.

Competing interests

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References

Aebi, M., Bernasconi, R., Clerc, S. and Molinari, M. (2010). N-glycan structures: recognition and processing in the ER. Trends Biochem. Sci. 35, 74-82.

Aebi, M., Bernasconi, R., Kappeler, F. and Hauri, H. P. (1999). The lectin ERGIC-53 is a cargo transport receptor for glycoproteins. Nat. Cell Biol. 1, 330-333.

Aebi, M., Bernasconi, R., Nyfeler, B., Burkhard, P., Santamaria, I., Lopez-Otin, C. and Hauri, H. P. (2004). pH-induced conversion of the transport lectin ERGIC-53 triggers glycoprotein release. J. Biol. Chem. 279, 12943-12950.

Aebi, M., Bernasconi, R., Nyfeler, B., Burkhard, P., Santamaria, I., Lopez-Otin, C. and Hauri, H. P. (2005). Carbohydrate- and conformation-dependent cargo capture for ER-exit. Mol. Biol. Cell 16, 1259-1267.

Apweiler, R., Hermjakob, H. and Sharon, N. (1999). On the frequency of protein glycosylation, as deduced from analysis of the SWISS-PROT database. Biochem. Biophys. Acts 1473, 4-8.

Arai, K. and Nagata, K. (2011). Protein folding and quality control in the ER. Cold Spring Harb. Perspect. Biol. 3, a007526.

Arvan, P., Zhao, X., Ramos-Castaneda, J. and Chang, A. (2002). Secretory pathway quality control operating in Golgi, plasmalemmal and endosomal systems. Traffic 3, 771-780.

Avezov, E., Frenkel, Z., Ehrlich, M., Herscovics, A. and Lederkremer, G. Z. (2008). Endoplasmic reticulum (ER) mannosidase I is compartmentalized and required for N-glycan trimming to Man5(6)GlcNAc2 in glycoprotein ER-associated degradation. Mol. Biol. Cell 19, 216-225.

Barlowe, C. (2003). Signals for COPII-dependent export from the ER: what’s the ticket out? Trends Cell Biol. 13, 295-300.

Benyair, R., Ron, E. and Lederkremer, G. Z. (2011). Protein quality control, retention, and cargo transport at the endoplasmic reticulum. Int. Rev. Cell Mol. Biol. 292, 197-280.

Bernasconi, R. and Molinari, M. (2011). ERAD and ERAD tuning: disposal of cargo and of ERAD regulators from the mammalian ER. Curr. Opin. Cell Biol. 23, 176-183.

Bernasconi, R., Pertel, T., Luban, J. and Molinari, M. (2008). A dual task for the Xbp1-responsive OS-9 variants in the mammalian endoplasmic reticulum: inhibiting secretion of misfolded protein conformers and enhancing their disposal. J. Biol. Chem. 283, 16446-16454.

Bhargava, A., Voronov, I., Wang, Y., Glogauer, M., Kartner, N. and Manolison, M. F. (2012). Osteopetrosis mutation R444L causes endoplasmic reticulum retention and misprocessing of vacuolar H+-ATPase a subunit. J. Biol. Chem. 287, 26829-26839.

Bouchecareilh, M., Conkright, J. J. and Balch, W. E. (2010). Proteostasis strategies for restoring alpha1-antitrypsin deficiency. Proc. Am. Thorac. Soc. 7, 415-422.

Buchberger, A., Bukau, B. and Sommer, T. (2010). Protein quality control in the cytosol and the endoplasmic reticulum: brothers in arms. Mol. Cell 40, 238-252.

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

Cabral, C. M., Choudhury, P., Liu, Y. and Sifers, R. N. (2000). Processing by endoplasmic reticulum mannosidases partitions a secretion-impaired glycoprotein into distinct disposal pathways. J. Biol. Chem. 275, 25015-25022.

Cabral, C. M., Liu, Y., Moremen, K. W. and Sifers, R. N. (2002). Organizational diversity among distinct glycoprotein endoplasmic reticulum-associated degradation programs. Mol. Biol. Cell 13, 2639-2650.

Caramelo, J. J. and Parodi, A. J. (2008). Getting in and out from calnexin/calreticulin cycles. J. Biol. Chem. 283, 10221-10225.

Chen, H., Hu, D., Yabe, R., Tateno, H., Qin, S. Y., Matsumoto, N., Hirabayashi, J. and Yamamoto, K. (2011). Role of malectin in Glc(2)Man(9)GlcNAc(2)-dependent protein folding. FEBS Lett. 584, 4195-4199.

Choudhury, P., Liu, Y., Bick, R. C. and Sifers, R. N. (1997). Intracellular association between UDP-glucose:glycoprotein glucosyltransferase and an incompletely folded variant of alpha1-antitrypsin. J. Biol. Chem. 272, 13446-13451.

Chouquet, A., Paidais, H., Ling, W. L., Frachet, P., Houen, G., Arlau, G. J. and Gaboriaud, C. (2011). X-ray structure of the human calreticulin globular domain reveals a peptide-binding area and suggests a multi-molecular mechanism. PLoS ONE 6, e17886.
Gabel, C. A. and Bergmann, J. E. (2006). Impaired glycoprotein glucosyltransferase-glucosidase II, the ying-yang of the ER quality control. Semin. Cell Dev. Biol. 21, 491-499.

de Silva, A. M., Balch, W. E. and Helenius, A. (1990). Quality control in the endoplasmic reticulum: folding and misfolding of vesicular stomatitis virus G protein in cells and in vitro. J. Cell Biol. 111, 857-866.

Denzel, A., Molinari, M., Trigueros, C., Martin, J. E., Velumagan, S., Brown, S., Stahl, D. J. (2002). Early postnatal death and motor disorders in mice congenitally deficient in calnexin expression. Mol. Cell. Biol. 22, 7398-7404.

De Praeter, C. M., Gerwig, G. J., Bause, E., Nyutnick, L. K., Vliegenthart, J. F., Breuer, W., Kamerling, J. P., Espeel, M. F., Martin, J. J. and Chan, N. (2000). A novel disorder caused by defective biosynthesis of N-linked oligosaccharides due to glucosidase I deficiency. Am. J. Hum. Genet. 66, 1744-1756.

Drenth, J. P., de Morsche, R. H., Smink, R., Bonifacino, J. S. and Jansen, J. B. (2010). UDP-Glc:glycoprotein glucosyltransferase-glucosidase II, the ying-yang of the ER quality control. Biochim. Biophys. Acta 1802, 1306-1317.

Hosokawa, N., Kamiya, Y., Kamiya, D., Kato, K. and Nagata, K. (2011). Mannose 4-epimerase required for glucose trimming of proteins. Glycobiology 21, 563-573.

Kim, P. S. and Arvan, P. (2008). Cardiac-specific expression of calcineurin reverses embryonic lethality in a newly recognized genetic syndrome. J. Clin. Invest. 111, 1327-1337.

Kamen, R., Aebi, M. (2004). Roles of N-linked glycan in the endoplasmic reticulum. Annu. Rev. Biochem. 73, 1019-1049.

Hosokawa, N., Kamiya, Y., Kamiya, D., Yamamoto, K., Nyfeler, B., Hauri, H. P. and Kato, K. (2011). The role of MRH domain-containing lectin-related N-glycan-dependent determination of glycoprotein fates in cells. J. Biochem. 150, 460-469.

Gallo, C., Bernascioni, R., Soldà, T., Calanca, V. and Molinari, M. (2011). Malectin participates in a backup glycoprotein quality control pathway in the mammalian ER. PLoS ONE 6, e16304.

Guo, L., Nakamura, K., Lynch, J., Opas, M., Olson, E. N., Agellon, L. B. and Michalak, M. (2002). Cardiac-specific expression of calcineurin reverses embryonic lethality in calreticulin-deficient mouse. J. Biol. Chem. 277, 50778-50779.

Hammond, C., Braakman, I. and Helenius, A. (1994). Role of N-linked oligosaccharide trimming in the budding and release of measles virus from infected cells. J. Biol. Chem. 269, 6725-6738.

D’Alessio, C., Caramelo, J. J. and Parodi, A. J. (2010). UDP-Glc-glycoprotein glucosyltransferase-glucosidase II, the ying-yang of the ER quality control. Semin. Cell Dev. Biol. 21, 491-499.
Morito, D., Hiroa, K., Oda, Y., Hosokawa, N., Tokunaga, F., Ciry, D. M., Tanaka, K., Iwai, K. and Nagata K. (2008). Gp78 cooperates with RMA1 in endoplasmic reticulum-associated degradation of CFTREdeltAf508. Mol. Biol. Cell 19,1328-1336.

Miller, B., Klemm, E. J., Spooner, E., Claessen, J. H. and Ploegh, H. L. (2008). SEL1L nucleates a protein complex required for dislocation of misfolded glycoproteins. Proc. Natl. Acad. Sci. USA 105, 12325-12330.

Nichols, W. C., Seligsohn, U., Zivelin, A., Terry, V. H., Hertel, C. E., Wheatley, M. A., Moussali, M. J., Hauri, H. P., Cavarella, N., Kaufman, R. J. et al. (1996). Mutations in the ER-Codi fibrinogen receptor alpha chain are responsible for a combined deficiency of coagulation factors V and VIII. J. Cell Biol. 135,61-70.

Nyfeler, B., Reiterer, V., Wendeler, M. W., Stefan, E., Zhang, B., Michnick, S. W. and Hauri, H. P. (2008). Identification of ERGIC-53 as an intracellular transport receptor for alpha-1-antitrypsin. Mol. Biol. Cell 19, 705-712.

Oda, Y., Hosokawa, N., Wada, I. and Nagata, K. (2003). EDEM as an acceptor of terminally misfolded glycoproteins released from calnexin. Science 299,1394-1397.

Olvari, S. and Molinari, M. (2007). Glycoprotein folding and the role of EDEM1, and EDEMs in degradation of folding-defective glycoproteins. FEBS Lett. 581,3658-3664.

Oltmann, J. A., Kopito, R. R. and Christianson, J. C. (2013). The mammalian endoplasmic reticulum-associated degradation system. Cold Spring Harb. Perspect. Biol. 5.a014839.
Valastyan, J. S. and Lindquist, S. (2014). Mechanisms of protein-folding diseases at a glance. Dis. Model. Mech. 7, 9-14.

Völkner, C., De Praeter, C. M., Hardt, B., Breuer, W., Kalz-Füller, B., Van Coster, R. N. and Bause, E. (2002). Processing of N-linked carbohydrate chains in a patient with glucosidase I deficiency (CDG type Iib). Glycobiology 12, 473-483.

Vollenweider, F., Kappeler, F., Iltin, C. and Hausr, H. P. (1998). Mistargeting of the lectin ERGIC-53 to the endoplasmic reticulum of HeLa cells impairs the secretion of a lysosomal enzyme. J. Cell Biol. 142, 377-389.

Wang, W. A., Groenendyk, J. and Michalak, M. (2012). Calreticulin signaling in health and disease. Int. J. Biochem. Cell Biol. 44, 842-846.

Warren, G. and Milliman, I. (1993). Bulk flow redux? Cell 96, 125-127.

Welch, W. J. (2004). Role of quality control pathways in human diseases involving protein misfolding. Semin. Cell Dev. Biol. 15, 31-38.

Wijeyesakere, S. J., Rizvi, S. M. and Raghavan, M. (2013). Glycan-dependent and -independent interactions contribute to cellular substrate recruitment by calreticulin. J. Biol. Chem. 288, 35104-35116.

Wu, Y., Whitman, I., Molmenti, E., Moore, K., Hippenmeyer, P. and Perlmutter, D. H. (1994). A lag in intracellular degradation of mutant alpha 1-antitrypsin correlates with the liver disease phenotype in homozygous PiZZ alpha 1-antitrypsin deficiency. Proc. Natl. Acad. Sci. USA 91, 9014-9018.

Wu, Y., Swulius, M. T., Moremen, K. W. and Sifers, R. N. (2003). Elucidation of the molecular logic by which misfolded alpha 1-antitrypsin is preferentially selected for degradation. Proc. Natl. Acad. Sci. USA 100, 8229-8234.

Xu, C., Wang, S., Thibault, G. and Ng, D. T. (2013). Futile protein folding cycles in the ER are terminated by the unfolded protein O-mannosylation pathway. Science 340, 978-981.

Zapun, A., Darby, N. J., Tessier, D. C., Michalak, M., Bergeron, J. J. and Thomas, D. Y. (1998). Enhanced catalysis of ribonuclease B folding by the interaction of calnexin or calreticulin with ERP57. J. Biol. Chem. 273, 6009-6012.

Zhang, B., Cunningham, M. A., Nichols, W. C., Bernat, J. A., Seligsohn, U., Pipe, S. W., McVey, J. H., Schulte-Overberg, U., de Bosch, N. B., Ruiz-Saez, A. et al. (2003). Bleeding due to disruption of a cargo-specific ER-to-Golgi transport complex. Nat. Genet. 34, 220-225.

Zhang, B., Kaufman, R. J. and Ginsburg, D. (2005). LMAN1 and MCFD2 form a cargo receptor complex and interact with coagulation factor VIII in the early secretory pathway. J. Biol. Chem. 280, 25881-25886.

Zielinska, D. F., Gnadt, F., Wiosniewski, J. R. and Mann, M. (2010). Precision mapping of an in vivo N-glycoproteome reveals rigid topological and sequence constraints. Cell 141, 897-907.

Zuber, C., Cormier, J. H., Guhl, B., Santimaria, R., Hebert, D. N. and Roth, J. (2007). EDEM1 reveals a quality control vesicular transport pathway out of the endoplasmic reticulum not involving the COPII exit sites. Proc. Natl. Acad. Sci. USA 104, 4407-4412.