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Double the Fun, Double the Trouble: Paralogs and Homologs Functioning in the Endoplasmic Reticulum

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Abstract

The evolution of eukaryotic genomes has been propelled by a series of gene duplication events, leading to an expansion in new functions and pathways. While duplicate genes may retain some functional redundancy, it is clear that to survive selection they cannot simply serve as a backup but rather must acquire distinct functions required for cellular processes to work accurately and efficiently. Understanding these differences and characterizing gene-specific functions is complex. Here we explore different gene pairs and families within the context of the endoplasmic reticulum (ER), the main cellular hub of lipid biosynthesis and the entry site for the secretory pathway. Focusing on each of the ER functions, we highlight specificities of related proteins and the capabilities conferred to cells through their conservation. More generally, these examples suggest why related genes have been maintained by evolutionary forces and provide a conceptual framework to experimentally determine why they have survived selection.

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INTRODUCTION

The evolution of complex organisms has been coupled to the expansion of genome size and gene number. Such an increase in complexity has often been achieved by whole-genome duplication (WGD) or small-scale duplications of genes that have expanded the potential for diversification of gene function and regulation. When looking at present-day genomes of even a simple eukaryote, it is clear that many of the genes are very similar. Evolutionarily, such homologous proteins could not have been selected for their backup function but rather for their capacity to lend plasticity to existing capabilities (1). While it is clear that the presence of such closely related pairs of genes has been beneficial at the organismal level (given their survival through natural selection), it is not always trivial at the experimental level to understand what the benefits are of maintaining such closely related proteins. Moreover, the presence of pairs or groups of homologous genes that regulate and execute each function confounds the capacity to study such processes. This is because, while the presence of such homologs can offer functional diversity, it often also provides backup in extreme conditions where one of the proteins is lost, which dramatically complicates the genetic study of protein functions using loss-of-function approaches. This may also be the reason why relatively few genes appear to be essential—a phenomenon observed in yeast, mice, and humans. It is therefore of interest to try and explore the unique as well as overlapping functions of gene pairs in cells.

Homolog: gene related by descent from a common ancestral DNA sequence; a homolog can be either an ortholog or a paralog

Half of all eukaryotic proteins reside in organelles. Part of the challenge of understanding organelle functions arises from the presence of duplicate proteins and protein families. In this review, we use one such organelle, the endoplasmic reticulum (ER), as an example of the diversity and regulatory capacity conferred to cells by maintaining duplicates, alongside the experimental challenges that duplicate genes pose to understanding cell biology.

The ER is the gateway of the secretory pathway, with about a third of the eukaryotic proteome targeted to this organelle (2, 3). For many of these proteins, the ER is not their final destination, and they will be trafficked along the secretory pathway, first to the Golgi apparatus and some further still to the endolysosomal system, the plasma membrane (PM), and beyond. Before being trafficked to the Golgi, each secretory cargo is subjected to a series of quality control processes that ensure its structural and functional integrity while maintaining cellular homeostasis. These processes are carried out by proteins that remain in the ER after being targeted there. ER-resident proteins perform several other functions, including lipid biosynthesis, ion transfer, and contact site formation.

Supplemental Material >

This review explores homologous pairs or groups of proteins within the functional context of the budding yeast (*Saccharomyces cerevisiae*, referred to here as yeast unless otherwise stated) and mammalian ER. The ER and its various functions provide a wonderful example of the diversity, plasticity, and robustness imparted to eukaryotic cells by gene duplicates and families. Even though experimental data directly comparing homologs is often lacking, the evidence collected here strongly supports the above-mentioned notion that, while homologs can provide backup, they also have each evolved distinct functions, likely to be essential in particular conditions, suggesting how selective pressure has maintained them in the genome. Since covering all protein pairs and families in the secretory pathway is impossible in the context of this review, we provide at least one example of a protein pair/group for each ER function (**Figure 1**). For simplicity, a list of protein abbreviations, full names, and identifiers is provided in **Supplemental Table 1**.

Gene family: a collection of multiple analogous genes, formed by duplication of a single original gene, that generally have comparable functions

The fact that there is so much diversity in ER-resident proteins has likely contributed to generating capabilities that are both robust and dynamic. While this complicates the study of protein functions, it is a fascinating endeavor to investigate how gene families have evolved to support optimal activity in a wide range of conditions and cellular requirements.

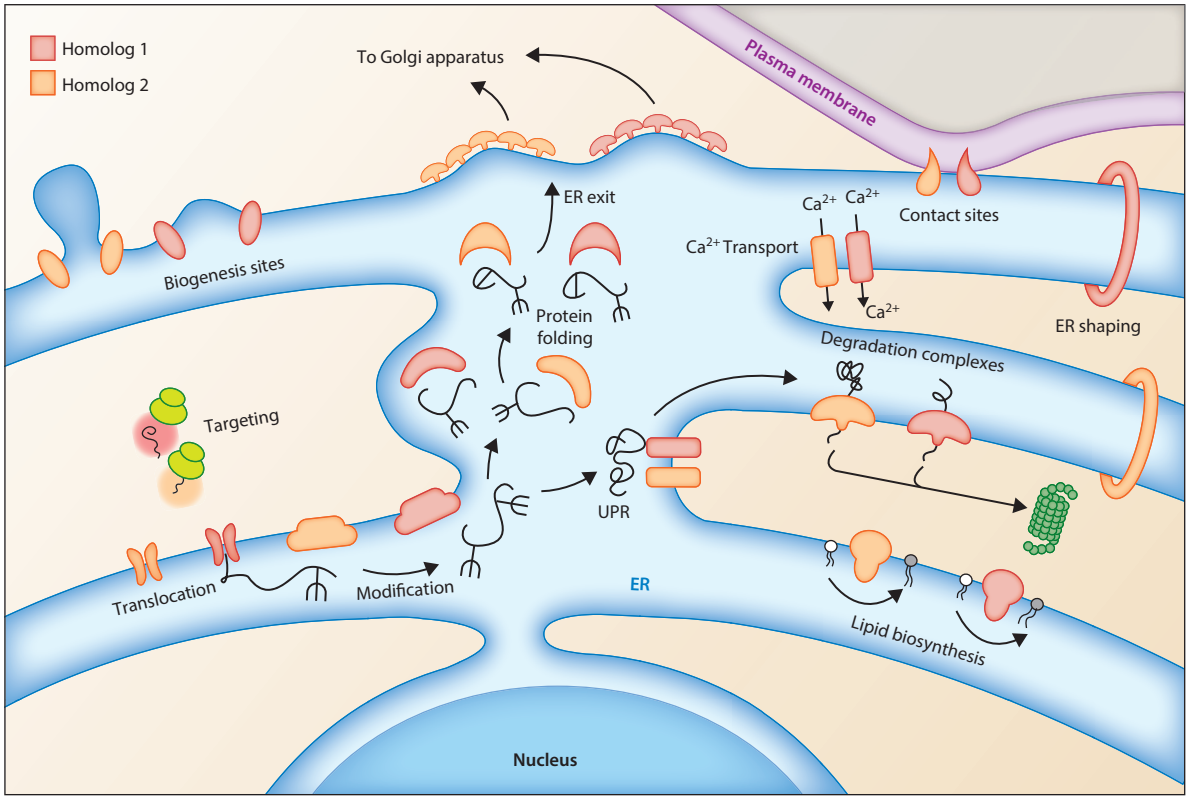

Figure 1

Homologs participating in each endoplasmic reticulum (ER) function. Within each function, homologs are depicted in red and orange. The functions include targeting and translocation, modification by glycosylation machinery, folding by chaperones and protein disulfide isomerases, export from the ER, activation of the unfolded protein response (UPR) upon sensing misfolded proteins, protein degradation, lipid biosynthesis, peroxisome/lipid droplet biogenesis, Ca^{2+} transport, contact site formation, and ER shaping.

TARGETING AND TRANSLOCATION

Hundreds of proteins reside in the ER membrane and lumen, and many more (thousands in human cells) traffic through the ER to organelles further downstream the secretory pathway and endolysosomal system. How all these different proteins are targeted to the ER and then translocated efficiently into its membranes or lumen has been the focus of intense research over the last four decades.

Targeting Pathways in Yeast

Three conserved targeting pathways (SRP, SND, GET) have been identified in yeast and humans (4). Interestingly, in most organisms studied, the pathways all consist of single-copy nonparalogous proteins. In plants, however, multiple GET3 homologs exist (5), and this suggests that other pathways may exist in more complex forms in other organisms.

Another way of reaching organelle membranes is through the binding of cytosolic Hsp70 chaperones that keep targeted cargo in a translocation-competent state. In yeast, the SSA and SSB Hsp70 subfamilies have both been proposed to play a role in ER targeting, and together they

comprise six cytosolic homologs: the paralogs Ssa1 and Ssa2, which share 98% sequence identity[1] (6); Ssa3 and Ssa4; and Ssb1 and Ssb2. The last two pairs are both ohnologs (**Supplemental Table 2**).

Paralog: homologous gene related by either whole-genome duplication or small-scale duplication

Ohnolog: paralogous gene formed specifically by a whole-genome duplication event

The first evidence that the Hsp70 proteins could influence targeting came from in vitro translocation experiments, where the addition of Ssa1 and Ssa2 increased the rate of protein translocation into ER microsomes (7). These data were later supported by in vivo work showing that, in *ssa*-deleted cells, there was an accumulation of the pretranslocated form of specific secretory proteins (8). Since the deletion of the whole SSA subfamily is lethal, this study used a *Δssa2/ssa3/ssa4* yeast strain, which contained a temperature-sensitive mutant of *ssa1*. Therefore, shifting the cells from the permissive to the restrictive temperature allowed the authors to analyze changes in secretory protein maturation in the absence of four out of the six homologs (8).

Recently, the crystal structure of a C-terminal peptide of Ssa1 together with a specific component of the auxiliary translocon [a complex that enables the Sec61 channel to import low-hydrophobicity signal peptide (SP)-containing proteins (9)] was solved, providing insight into how SSA proteins target nascent polypeptides to ER translocation sites (10). Interestingly, this study also demonstrated that Ssb1 is able to bind the same domain of the auxiliary translocon as Ssa1. SSB proteins are able to bind ribosomes and nascent chains to maintain newly synthesized proteins in a folding-competent state (11). Only in the double knockout (*Δssb1/2*) was there an increase in aggregation, which was used to highlight redundancy in this gene family (11). However, under native conditions, it is possible that all six Hsp70 homologs have their own specific subset of proteins to target. More sophisticated methods will be required to uncover their substrate specificity. Additional complexity arises from the fact that targeting to other organelles such as mitochondria (12) and peroxisomes (13) also seems to depend on these same chaperones, and how specificity and directionality are conferred is not clear. Furthermore, the mechanism of how these cytosolic chaperones engage the translocon, release their substrate, and get recycled back to the cytosol for another round of targeting remains unknown. Future work should therefore focus on elucidating the steps of this pathway and understanding if putative human homologs, such as HSPA1A, HSPA2, and HSPA8 (**Supplemental Table 2**), have conserved functionality.

Supplemental Material >

Translocation Channels in Yeast and Mammalian Systems

Once targeted to the ER membrane, client proteins must embed into or traverse the phospholipid barrier to enter the secretory pathway. In yeast, there are two paralogous pore-forming channels that enable this process: the canonical translocon, Sec61, and the alternative translocon, Ssh1. To function optimally, both proteins must be in a trimeric complex with a β and γ subunit. The β subunit is encoded by the ohnologs Sbh1 and Sbh2, which form unique interactions with Sec61 and Ssh1, respectively. The transmembrane protein Sss1 is present in both complexes as a shared γ subunit. The heterotrimeric Sec61 complex is conserved to humans, with SEC61α1, SECβ, and SECγ representing human homologs of the yeast Sec61, Sbh1, and Sss1, respectively (reviewed in 14).

The unique roles of each translocation channel are unclear. *SSH1*, unlike *SEC61*, is nonessential (15); however, both can support co- and post-translational translocation processes (16, 17). While Sec61 can compensate for the loss of Ssh1 (17, 18), it is clear that the Ssh1 complex has different roles in the cell. First, Ssh1 does not coprecipitate the same cofactors as Sec61 [for example, the auxiliary translocon components (15)], and it displays distinct interaction preferences

[1]Percentage identity or similarity between two amino acid sequences was calculated using EMBOSS Needle and is cited as Reference 6.

with different SP sequences (19). Furthermore, loss of Ssh1 gives rise to a decrease in respiration capacity not observed in *sec61* mutants (20), and finally, Sec71 was shown to be a specific substrate of Ssh1 (18).

Given the differences in phenotype, stable interactions with adaptor proteins, and transient interactions with substrates, it is clear that the Sec61 and Ssh1 translocons have unique functions. Future efforts should therefore focus on uncovering the native substrate ranges for both the canonical and alternative protein channel complexes. Similarly, humans express two *SEC61* genes: *SEC61α1* and *SEC61α2*. Although the former is well-characterized as a translocation channel, almost nothing is known about the latter, except for the fact that its expression level in most cell types is very low but is higher in brain tissues (21, 22; **https://www.proteinatlas.org/**). Hence it would be very interesting to establish if SEC61α2 can function as a translocase specifically in the brain and whether it plays a role in ER import.

Beyond the Conventional Translocon

Other proteins involved in ER protein translocation include the recently discovered human Oxa1 homologs: GET1, EMC3, and TMCO1 (23). These proteins are related to the Oxa1-like bacterial insertase, and all share similar architecture.

PROTEIN MODIFICATION

Once in the ER lumen, polypeptide chains are subject to a series of modifications to accelerate their folding and enable their targeting and quality control.

Yeast and Mammalian Paralogs in the Oligosaccharyltransferase Complex

One protein modification that occurs in the ER lumen is the addition of glycan decorations. The covalent linkage of oligosaccharides to a nucleophilic asparagine (N) residue within the N-X-serine/threonine motif is carried out by the oligosaccharyltransferase (OST) complex (reviewed in 24). This membrane-embedded, hetero-oligomeric structure is conserved from yeast to humans. In yeast, there are two exclusive complexes, each containing the catalytic subunit Stt3, an additional six core proteins (Ost1, Ost2, Ost4, Ost5, Swp1, and Wbp1), and one of either Ost3 or Ost6 (25, 26)—nonessential paralogous genes required to maximize the efficiency of glycosylation (25). Interestingly, the distinct complexes seem to be associated with the two different translocons, since Ost3 was found to interact with Sbh1 (adjacent to the Sec61 channel) and Ost6 with Sbh2 (a unique interactor of Ssh1) (27). Furthermore, mass spectrometry (MS) analysis of the glycopeptides from *Δost3/ost6* strains reconstituted with either Ost3 or Ost6 revealed different efficiencies in site-specific glycosylation (28).

The respective mammalian homologs of yeast Ost3 and Ost6 are MAGT1 and TUSC3, which share 72% sequence identity with each other (29). All four proteins share the same topology of four transmembrane domains (TMDs) with an ER-luminal N terminus, which harbors oxidoreductase activity. However, the role of these homologs in mammals is not yet clear. In contrast, substrate specificity in the mammalian OST complex is at least partially provided by the two homologs of the yeast catalytic subunit Stt3: STT3A and STT3B, which were found to coordinate co- and post-translational *N*-linked glycosylation, respectively (30). These homologs have unique interaction partners that specify their functions. For example, DC2 acts as an adaptor for STT3A, recruiting it to the SEC61 channel and poising it for cotranslational glycosylation (31). On the other hand, MAGT1 and TUSC3 are unique components of the STT3B complex (29, 32), and knocking out both of these oxidoreductases renders STT3B virtually inactive (33). Hence it should be determined whether MAGT1 and TUSC3 confer client specificity to STT3B.

Beyond Glycosylation

N-linked glycosylation is not the only modification of secretory proteins. One additional example is *O*-linked mannosylation carried out by ER-resident protein *O*-mannosyltransferases (PMTs). There are several yeast and human PMT family members with distinct functions and substrates (reviewed in 34).

PROTEIN FOLDING

The ER lumen is a specialized environment abundant in chaperones and foldases, which assist newly synthesized polypeptides in achieving their functional conformation.

The HSP40 Cochaperone Family in Humans

BIP (homolog of Kar2 in yeast) is the principal ER Hsp70 chaperone, which recognizes extended, hydrophobic stretches in its substrates (35). Cochaperones (also known as Hsp40s or J-domain proteins) stimulate the ATPase activity of BIP, which in turn induces conformational changes in its substrate binding domain. There are three BIP-interacting J-domain proteins facing the lumen of the yeast ER (Sec63, Scj1, and Jem1) and at least seven in the human ER (ERDJ1, 2, 4, and 7, which are all predicted to have 1 TMD, and ERDJ3, 5, and 6, which are soluble proteins). All ERDJ proteins, except for ERDJ1 and 2, are upregulated by ER stress, with ERDJ4 being the most strongly induced (36).

ERDJ1 and ERDJ2 share 30% amino acid sequence similarity (6) and appear homologous to yeast Sec63 (37). ERDJ1 simultaneously binds BIP and the ribosome exit tunnel via its luminal and cytosolic termini, respectively (38, 39). ERDJ2 (more commonly referred to as SEC63) is a component of the auxiliary translocon and enables conformational changes in the SEC61 channel that facilitate the translocation of weakly hydrophobic SP-containing proteins (40, 41). ERDJ3, on the other hand, seems to cater preferentially for unassembled substrates, including immunoglobulin heavy chain and unfolded light chains (37), whereas ERDJ4, 5, and 6 all bind misfolded proteins, with ERDJ4 and 5 playing a role in endoplasmic reticulum–associated degradation (ERAD) (42, 43). These functions of ERDJ3–6 are consistent with their upregulation by ER stress. The function of ERDJ7 is currently still unknown. Given the fact that hundreds of proteins are correctly folded in the ER, and that J-domain proteins generally provide substrate specificity to their cognate Hsp70s, future endeavors should focus on understanding the client range of each of these cochaperones, since very little is known about the unique function of these proteins. Understanding their dynamic regulation and whether any of these family members can compensate for one another during perturbations in ER homeostasis will also be of great interest.

Beyond Hsp40s

Two additional protein families that assist in folding are the protein disulfide isomerases (PPIs), which promote disulfide bond formation in client proteins, and prolyl peptidyl *cis-trans* isomerases (PPIases) which promote isomerization of peptide bonds preceding proline residues (reviewed in 44). Like the Hsp40s, there are several PDI and PPIase family members, and generally very little is known about their redundancies or specificities.

VESICULAR TRAFFICKING

Following targeting, translocation, glycosylation, disulfide bonding, and protein folding, secretory proteins will exit the ER and be trafficked to the Golgi apparatus, where further modifications can

take place and sorting to their final destination occurs. Transport from the ER to the Golgi begins at an ER-exit site, where COPII machinery ensnares the cargo and its surrounding membrane, inducing vesicle formation. Vesicles subsequently bud off the ER and fuse to Golgi acceptor membrane sites where the cargo is delivered. The COPII coat comprises five cytosolic subunits: Sar1, the Sec23/Sec24 dimer, and the Sec13/Sec31 tetramer (45).

Yeast and Mammalian COPII Cargo Recognition

Sec24 proteins function as the cargo adaptors of the COPII coat (46) by recognizing sorting sequences within cytosolic domains of secretory proteins. The ability of the COPII coat to transport a highly diverse repertoire of cargo is achieved not only through the different substrate binding sites of Sec24 (47) but also through its homologs, which have specific cargo preferences (48).

In yeast, Sfb2 (also called Iss1) and Sfb3 (also called Lst1) are the respective ohnolog and paralog of Sec24. Sfb3 was found in a screen designed to find synthetic lethal interactors of COPII mutants (49). The observation that the *Δsfb3* strain shared the same growth phenotype on a low-pH source as cells knocked out for the PM ATPase, Pma1, led to the discovery that Pma1 trafficking was dependent on Sfb3 (49). However, optimal packaging of Pma1 into COPII vesicles requires both Sfb3 and Sec24 to be present, and vesicles containing both paralogs are slightly larger than those formed by Sec24 alone (46, 50). Vesicles generated with only Sfb3 are devoid of SNARE proteins (which mediate fusion of vesicles to membranes) and consequently are unable to fuse with the Golgi membrane (46). The third homolog, Sfb2, can recruit SNAREs just like Sec24 (51); however, there is currently no known cargo that is strictly dependent on it.

In mammals there are four members of the SEC24 family, which can be divided into the SEC24a/b and SEC24c/d subfamilies. Both pairs share high sequence similarity (52, 53). To gain insight into whether each human SEC24 homolog has distinct cargo specificities, a set of glycosylatable ERGIC-53 reporters was used to assess ER to Golgi transport upon single or combined depletion of the different SEC24 proteins (54). The transport of ERGIC-53 with a cytosolic C-terminal di-leucine signal was only significantly blocked by the knockdown of SEC24a. Interestingly, however, the combined reduction of SEC24b and SEC24c levels also inhibited ERGIC-53 di-leucine transport, suggesting that these homologs can compensate for one another, at least to some extent (54). The different SEC24 subfamilies were also shown to have distinct preferences in sorting SNAREs, with SEC24a/b responsible for the recognition of an unassembled arginine (R)-SNARE protein, SEC22, and SEC24c/d for the glutamine (Q)-SNAREs syntaxin-5 and membrin/GS27 (55, 56). These results were recapitulated in a later study that additionally demonstrated that the differential recruitment of R- and Q-SNAREs is required to maintain them in a fusion-competent state (57). Interestingly, SEC24c has recently been shown to play a role in ER-phagy (58), an autophagy-mediated process that selectively degrades the ER. This function is conserved from the SEC24c yeast homolog, Sfb3, and highlights the possibility that each of the SEC24 proteins may also contribute to alternative processes independent of vesicular trafficking. In addition, mutations in individual *SEC24* homologs cause distinct disorders: Cranio-lenticulo-sutural dysplasia is caused by a *SEC24a* mutation (59), whereas Cole-Carpenter syndrome is mapped to two mutations in *SEC24d* (60).

Beyond SEC24s

Many more homologous protein pairs exist in trafficking, such as the Sec23 homologs (61) and other cargo receptors that provide specificity to recognition of cargo. One well-studied example is the family of p24 proteins. One role of p24s is to coordinate the COPII transport of

GPI-anchored proteins (62), which due to their topology lack the cytosolic motifs normally recognized by SEC24s. However, for this single role it is not clear why so many family members are required—there are 11 mammalian family members, 8 of which have yeast homologs (63). In yeast, deleting all 8 homologs (64) creates a viable strain that can serve as a tool to study specificity of a single, reintroduced p24 protein. Precisely how these proteins recognize their cargo is unknown, and like for the SEC24 proteins, future research will undoubtedly uncover differential cargo preferences for the different homologs.

STRESS RESPONSE AND HOMEOSTATIC REGULATION PATHWAYS

In some cases, proteins cannot be correctly folded and are hence not trafficked to the Golgi. At other times, such as during secretory cell development, the ER has to increase its folding capacity in response to secretory requirements. In both circumstances, the accumulation of un/misfolded proteins in the ER lumen activates a program termed the unfolded protein response (UPR). The UPR targets both transcriptional and post-transcriptional pathways aimed at restoring ER homeostasis, increasing ER folding capacity, or, in metazoan cells in the event of chronic stress, initiating apoptosis. The conserved and ancient UPR pathway is built around IRE1. However, the metazoan UPR signals through two additional ER membrane–embedded transducers: PERK and ATF6 (reviewed in 65). While these three pathways are not evolutionarily related, it is remarkable that they converge on highly similar mechanisms, which have unique but overlapping transcriptional targets.

Metazoan IRE1α and IRE1β

IRE1 is a single-pass ER membrane protein conserved from yeast to humans. It comprises an N-terminal luminal sensing domain, a single TMD, and a cytosolic domain, which harbors both RNase and kinase activities (66). ER stress triggers IRE1 dimerization (and later oligomerization), autophosphorylation, and activation of the RNase domain (67), which performs alternative splicing of the *XBP1* transcript generating spliced *XBP1* (*XBP1s*) (68). XBP1s is a potent transcription factor that upregulates a subset of prosurvival genes, including chaperones and components of the ERAD pathway.

In mammals, there are two paralogs, α and β (also termed ERN1 and ERN2). IRE1α is ubiquitously expressed across different tissue types and is essential for embryonic viability (69, 70). On the other hand, IRE1β expression is restricted to epithelial cells of respiratory tissues (71) and the gastrointestinal tract, and its knockout in mice is not lethal (72). The kinase and RNase domains of the two IRE1 homologs share 80% and 61% identity, respectively (73). While both paralogs are able to splice *XBP1* messenger RNA (mRNA), in vitro cleavage studies were used to demonstrate that IRE1α had a much higher activity toward the *XBP1* transcript relative to IRE1β (74). This is consistent with the fact that the majority of the research carried out on the IRE1-XBP1 axis has been done on the α form.

IRE1 has also been demonstrated to cleave other mRNAs such as transcripts of secretory proteins that are found proximal to the ER membrane and bear a consensus sequence similar to that found in *XBP1*. This process, termed regulated IRE1-dependent decay of mRNA (RIDD), was first described in the fruit fly (75) and later shown to be conserved in mammals (76, 77) and fission yeast (78). While RIDD was initially proposed to help restore ER homeostasis by reducing the load of incoming proteins into the ER (75, 77), it seems that this pathway is also required to orchestrate cell death following irremediable ER stress (76). RIDD activity is present in both IRE1α and IRE1β, and it is clear that at least five substrates are processed by both paralogs (79).

However, since they show distinct tissue expression patterns, to date no direct comparisons between IRE1α and IRE1β in appropriate physiological settings have been made to assess their substrate specificities.

Metazoan ATF6α and ATF6β

The UPR also signals through ATF6, a multipass membrane protein in the metazoan ER. Upon induction of ER stress, ATF6 is trafficked to the Golgi by a yet undefined mechanism and undergoes intramembrane proteolysis by site 1 and site 2 proteases (SP1 and SP2) (80). This results in the liberation of its N-terminal fragment, ATF6(N), which encodes a transcription factor that binds the ER stress response element motifs in the genome, causing the upregulation of a similar but distinct subset of prosurvival genes compared to IRE1 (81).

Two paralogs, ATF6α and β, are expressed in vertebrates, and both undergo the same processing event during ER stress (82). Despite initial reports showing the capacity of ATF6β(N) to induce expression of UPR target genes (82), it was noted that the transcriptional activation domain (TAD) of ATF6β differed significantly from that of ATF6α. Exogenously expressed ATF6α(N) can upregulate gene expression in a manner inversely proportional to the levels of ATF6β(N). Consistently, higher gene expression was reported in cells that were ATF6β-depleted relative to control cells. These observations suggested that the β variant with its divergent TAD sequence may actually have an inhibitory and not a stimulatory function on gene expression (83).

From these results, however, one could not exclude the possibility that the increased response in ATF6β-depleted cells could be the result of overcompensation by ATF6α, consistent with the original data arguing that this paralog pair has overlapping functions. Indeed, a later study confirmed that ATF6α and β must have at least partially redundant functions, since the double knockout was embryonic lethal in mice (84) and Japanese rice fish (85), whereas both single knockouts were viable. Yet despite this striking evidence, it is clear that both ATF6 homologs do not function identically. UPR-mediated gene expression was almost completely lost in *ATF6α*$^{-/-}$ mouse embryonic fibroblasts (MEFs) but essentially unperturbed in the *ATF6β*$^{-/-}$ cells. Additionally, the latter MEFs showed a very modest loss in viability upon induction of ER stress compared to the former ones, which were dramatically sensitive to the same conditions (84). Therefore, although ATF6β might be able to act as a limited backup for ATF6α, other approaches are required to understand the endogenous function of this protein.

UBIQUITINATION AT THE ENDOPLASMIC RETICULUM MEMBRANE

ERAD constitutes the quality control machinery that recognizes and degrades misfolded proteins and unassembled complex components, as well as regulating levels of metabolic proteins to meet cellular demands. The process of ERAD can be divided into four steps: (*a*) recognition of the substrate, (*b*) retrotranslocation into the cytosol, (*c*) substrate ubiquitination by E3 ubiquitin ligases (E3s), and (*d*) destruction by cytosolic 26S proteasomes (86). Although ERAD has been the main context within which ubiquitination at the ER membrane has been studied, the focus on nondegradative ubiquitination has been growing, and several examples are highlighted below.

Protein Families Acting in Ubiquitination and Degradation in Yeast and Humans

All E3s embedded in the ER membrane are members of the RING-type family. Yeast encodes only two ER-resident E3s: Hrd1, which ubiquitinates substrates containing misfolded luminal or intramembrane domains, and Doa10, which caters for substrates with misfolded cytosolic domains

(87) or preinserted proteins (88). Doa10 uses two E2 ubiquitin-conjugating enzymes (89): the membrane-spanning Ubc6, required for initializing ubiquitination, and Ubc7, a soluble enzyme that extends the ubiquitin chain (90). Hrd1, on the other hand, preferentially uses Ubc7 (91).

Ortholog: homologous gene related by speciation rather than duplication

The human genome encodes two yeast Ubc6 homologs, UBE2J1 and UBE2J2, which share high sequence similarity (92). Both enzymes are upregulated by ER stress-inducing agents (93, 94) consistent with their role in ERAD of misfolded proteins. However, only UBE2J1 is phosphorylated upon UPR activation (93), and it has recently been suggested that this modification contributes to limiting ER stress-induced cell death (94).

UBE2J1 and UBE2J2 also differ in their interactions with specific E3 ligases. For example, UBE2J1 interacts with RNF5 and HRD1 (a homolog of yeast Hrd1), promoting ubiquitination and degradation of mutant CFTR and misfolded MHC class I heavy chains, respectively (95, 96). UBE2J2 also plays a role in MHC class I molecule degradation; however, this is achieved through its interaction with another E3 ligase, TMEM129, and under the specific conditions of viral-mediated suppression of antigen presentation (97). Loss of UBE2J2 has also been shown to affect degradation of squalene epoxidase, a substrate of the MARCH6 E3 (a homolog of yeast Doa10), and an interaction between this E2–E3 pair was confirmed (98).

From these observations it is clear that the human Ubc6 family members contribute to the modification of different substrates by interacting with different E3s. In addition to the four E3s mentioned above, there are at least another 21 in humans (99, 100). The reason behind the expansion of RING-type ER E3s in humans is unknown; however, evidence suggests that while closely related members are likely to have individual functions, they may also be able to compensate for one another in specific conditions, thus lending robustness to ER quality control systems.

For example, human HRD1 and AMFR share 30% amino acid sequence similarity (6) and are commonly referred to as orthologs of yeast Hrd1 (101). While they may be able to compensate for one another in the degradation of certain model ERAD substrates (102), each E3 also ubiquitinates distinct client proteins. HRD1-specific substrates include the UPR sensor IRE1α (103) and the unassembled glycoprotein CD147 (104). Interestingly, all substrates of HRD1 or AMFR that were tested for ubiquitin chain type were shown to be modified with lysine (K)48-linked polyubiquitin, with just one exception. STING, an ER-localized immune signaling transducer, is K27 ubiquitinated by AMFR, and this modification is required to facilitate downstream signaling and elicit an appropriate antiviral response (105). HRD1 and AMFR are also controlled by different transcriptional programs, and during ER stress HRD1 expression strongly increases, whereas AMFR levels remain unchanged (106).

Similar lessons can be learned from other homologous ER E3s such as RNF5 and RNF185, which are 60% identical on the amino acid level (6). Like HRD1 and AMFR, they seem to have some degree of overlapping function, as evidenced by their shared contribution to the degradation of mutant CFTR (107, 108). Both are also reported to play a role in regulating STING signaling, although through opposing mechanisms, which involve the ubiquitination of two distinct substrates with different chain linkages (109, 110).

With the exception of the examples discussed above, most of the ER-resident E3 family remains poorly characterized in terms of both cofactors and substrates. Cofactors are not limited to E2-conjugating enzymes but also include a range of luminal, membrane-spanning and cytosolic binding proteins that mediate regulation, substrate recognition, retrotranslocation, and transfer to cytosolic proteasomes. Intriguingly, many of these cofactors are related family members and/or homologs such as the DERLIN, ERLIN, and EDEM proteins (reviewed in 111), which impart distinct functionalities on the different E3s they interact with. Future research should therefore focus on performing comparative studies within this E3 family to discover new cofactors and substrates for uncharacterized E3s and to understand which interactions are unique or shared between

several family members. This in turn will help elucidate unique and shared functions of this class of enzymes.

Isoenzyme (or isozyme): enzyme that differs in amino acid sequence from a similar enzyme but catalyzes the same or a similar chemical reaction

Beyond Endoplasmic Reticulum–Associated Degradation: Degradation in an Endoplasmic Reticulum Subcompartment

The ER is continuous with the outer nuclear membrane (ONM) and therefore also with the inner nuclear membrane (INM). In yeast, degradation of misfolded INM proteins was shown to depend on the ASI ubiquitin ligase complex, which is composed of the pair of ohnologs Asi1 and Asi3 as well as Asi2 (112). The human homologs responsible for this quality control mechanism remain unknown.

LIPID BIOSYNTHESIS

The majority of cellular lipid biosynthetic enzymes are embedded in the membrane of the ER—the central hub for generating phospholipids, sphingolipids, and sterols. The simplest phospholipid, phosphatidic acid, is de novo synthesized in the ER and is used to generate phosphatidylserine (PS), phosphatidylcholine (PC), and phosphatidylethanolamine (PE), the primary constituents of eukaryotic lipid bilayers. The specific composition of different phospholipids, sphingolipids, and sterols is critical in defining and regulating the function of each organelle's membrane (reviewed in 113).

Yeast Phosphatidylserine Decarboxylases

PS is usually present at relatively low concentrations in eukaryotic membranes, as it is efficiently converted to PE via the phosphatidylserine decarboxylase (PSD) pathway. The first enzyme to be discovered was named Psd1, and early fractionation studies were used to show that Psd1 localized to the inner mitochondrial membrane (114). The second PSD discovered was called Psd2 (115), and it was later found to be resident in endosomes (116). Although Psd1 and Psd2 are isoenzymes, they are not paralogs, as evidenced by their low sequence similarity (6). Furthermore, knockout of each gene yielded unique phenotypes, and the pools of PE generated by Psd1 and Psd2 are required to support the function of the mitochondria and vacuole, respectively (116, 117). It has also recently been shown that Psd1 is actually dually targeted to the ER and mitochondrial membranes and that $Psd1_{ER}$ is required for maintaining ER homeostasis (118). Therefore, de novo synthesis of PE by this isoenzyme pair distributed across three organelles ensures the maintenance of local membranes and functions. In humans, only one PSD is encoded (*PISD*), and it is localized to mitochondria. Although it undergoes alternative splicing, there is currently no evidence to suggest that this gives rise to differentially localized proteins.

Yeast and Mammalian Ceramide Synthases

Lag1 and Lac1 are the paralogous ceramide synthases of budding yeast, which were both thought to catalyze the conversion of dihydrosphingosine (DHS) into dihydroceramide (DHCer) and of phytosphingosine (PHS) into phytoceramide (PHCer) during sphingolipid biosynthesis. Curiously, however, the deletion of Lag1 caused a dramatic increase in replicative life span (119), a phenomenon not observed in Lac1-deleted cells (120).

To uncover any differences in substrate specificity, their activity was assayed on the background of a deletion in Sur2, which converts DHS to PHS and DHCer to PHCer and would hence mask

any effect of single Lag1 or Lac1 knockout strains. Under these conditions it was demonstrated that Lac1 is more effective in converting DHS to DHCer, while Lag1 is more effective in converting PHS to PHCer (the K_m of Lag1 for PHS was about four times lower than that of Lac1). Indeed, loss of Lag1 was demonstrated to correlate with the formation of a less effective diffusion barrier between mother and daughter cells known to depend on PHCer (121). This suggests that the life span phenotype in *Δlag1* is due to the overall reduction of PHCer synthesis, which negatively impacts barrier formation, causing mother cells to remain younger.

Isoform: a member of a set of highly similar proteins that originate from a single gene

Mammals have six ceramide synthases (CERS1–6), all of which are multipass membrane proteins. Each enzyme displays specificity toward particular acyl-CoA chain lengths, thereby determining the fatty acid structure of different ceramide species. CERS1 and CERS5 are only active toward C18 and C16 acyl-CoA chains, respectively, whereas the remaining CERS enzymes have broader substrate ranges and overlap in function (reviewed in 122). Recently, a stretch of 11 amino acids between the last two TMDs was found to specify the preference of these enzymes. The insertion of the 11-residue region of CERS2 into CERS5 caused the preference of CERS5 to change from long-chain acyl-CoA to very-long-chain acyl-CoA, the preferred target of CERS2 (123). Hence, in the case of these isoenzymes it is clear that while much overlap exists between their functions and while cellular pathways (such as Sur2) have evolved to help buffer any small changes in pathway flux, each enzyme still has a very unique substrate range that defines its physiological role.

Yeast and Human HMG-CoA Reductases

In humans, the enzyme HMGCR catalyzes a rate-limiting step at the beginning of the sterol biosynthesis pathway, converting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate. Yeast contain two homologous HMGCR genes, *HMG1* and *HMG2*, which are the result of a WGD event. This isoenzyme pair shares very high sequence similarity in their catalytic domains, the same topological organization, and a conserved motif called a sterol-sensing domain (reviewed in 124). Yet despite their similarities, early experimental evidence comparing mutant alleles of either isoenzyme concluded that Hmg1 provides over 80% of the reductase activity to this reaction (125). Despite being the less competent enzyme, it is Hmg2 that undergoes Hrd1-dependent ubiquitination and degradation upon accumulation of sterol-biosynthetic pathway intermediates (126) as part of a negative feedback cycle to avoid excessive sterol generation. This fate is not shared by Hmg1, a protein that is stable thanks to protective sequences at its extreme N terminus that are sufficient, if transplanted, to also render Hmg2 resistant to degradation (127). More work is required to clarify the disparity between the original observation placing Hmg1 as the major reductase and later data showing that the flux through the sterol biosynthetic pathway is controlled by the metabolic induction of Hmg2 degradation.

Although there is only one gene encoding human HMGCR activity, there exist at least two splice variants. The first, missing exon 13 (128), annotated as isoform 2, corresponds to the 90-kD form of HMGCR that was earlier found to localize to the peroxisomal compartment (129). Isoform 1, which is the full-length transcript, gives rise to the 97-kD protein found in the ER. Like yeast Hmg2, this isoform undergoes degradation to limit de novo cholesterol production in a process that depends on three ER-resident E3s: HRD1, AMFR, and RNF145 (130). Unlike isoform 1, isoform 2 turnover is not increased by the addition of sterol pathway intermediates (131). Furthermore, statins, which are a class of drugs that lower cholesterol levels by targeting HMGCR, have been shown to be less effective at inhibiting isoform 2 (132). Therefore, our understanding of the specific contribution of each of these isoforms in different cell/tissue types is crucial to developing more effective pharmaceutical agents to combat the global threat of cardiovascular disease.

And Beyond . . . More Lipid Biosynthesis Homologs

Other homologs in lipid biosynthetic enzymes include the yeast paralogs Pem1 and Pem2, which generate PC by methylating PE, and the human isoenzymes PSS1 and PSS2, which synthesize PS. Both pairs display distinct substrate preferences (133, 134). Additionally, yeast contain a paralogous pair of glycerol-3-phosphate acyltransferases (Sct1 and Gpt2) (135), of which there are four in mammals (GPAT1–4), each displaying a variety of unique features (136).

CALCIUM TRANSPORT

Another major function of the ER is to coordinate the transport and storage of ions. A plethora of different transporters are resident in the ER membrane, and in higher eukaryotes, the ER represents the largest cellular reserve of calcium (Ca^{2+}).

Ca^{2+} is an important second messenger, regulating several processes, including gene transcription, secretion, cell death, and muscle contraction. The ER membrane houses four separate transport systems, which collectively maintain Ca^{2+} homeostasis: the SERCA pumps; IP_3 receptors (IP_3Rs); ryanodine receptors (RYRs); and the STIM proteins, which can engage the ORAI Ca^{2+} channels in the PM (see below). None of these machineries have conserved homologs in yeast, potentially since the ER plays a less critical role in regulating Ca^{2+} homeostasis and instead the vacuole is the principal Ca^{2+} storage organelle.

SERCA pumps drive ATP-mediated Ca^{2+} import across an electrochemical gradient, thereby maintaining a much higher Ca^{2+} concentration in the ER relative to the surrounding cytosol (137). In vertebrates, three SERCA paralogs are encoded, *SERCA1–3* (or *ATP2A1–3*), all of which undergo alternative splicing. Collectively, this produces ten different isoforms, which have distinct developmental and tissue-specific expression profiles (reviewed in 138). Studies comparing the SERCA paralogs and isoforms have also revealed functional differences. For example, SERCA1 and SERCA2a, both of which are present in muscle cells, transport Ca^{2+} at a higher rate than the nonmuscle variant SERCA2b, and SERCA3 was shown to have a lower affinity for Ca^{2+} relative to the other tested variants (139). The different paralogs are also differentially regulated: SERCA2 activity is inhibited by the addition of unphosphorylated phospholamban (a small integral membrane protein), whereas the activity of SERCA3 is unchanged (140). Furthermore, the lactone thapsigargin inhibits the different SERCA pumps with varying effectiveness (141).

In mammals, there are three RYRs (RYR1–3), and there are three IP_3Rs in vertebrates (IP_3R1–3). Both sets are often referred to as isoforms; however, this terminology is incorrect since they are not the product of alternative splicing but are rather encoded by three separate homologous genes (see the sidebar titled Describing Related Genes and Proteins). As with the different SERCA

DESCRIBING RELATED GENES AND PROTEINS

The terminology used to describe homologous genes is not always correct and frequently goes unchallenged. Contrary to common misconceptions, while homologs in the same genome will always be paralogs, homologs in different organisms can have a paralogous or orthologous relationship. Since paralogs are the result of gene duplication, and orthologs arise due to speciation, it would initially seem that the term paralog would only be fit to describe homologs in the same genome; however, this is not the case (see 193). In cases where paralogy/orthology is not clear, the blanket term homolog should be used. Another common misconception is that the term isoform is interchangeable with homolog or paralog. Isoform should strictly be reserved for describing proteins that come from a single gene that is subject to variable promoter usage and/or post-transcriptional modification such as alternative splicing.

pumps, the three variants of RYRs and IP_3Rs have highly conserved sequences but show distinct tissue expression patterns and respond uniquely to specific regulators (reviewed in 142, 143). Conversely, the vertebrate STIM paralogs, STIM1 and STIM2, show comparable expression levels in a range of different tissues and cell lines (144). However, since STIM2 has a lower affinity toward Ca^{2+} than STIM1, it can promote Ca^{2+} import upon relatively minor decreases in ER Ca^{2+} levels, whereas STIM1 is required for responding to more dramatic decreases (145). The variety in expression, regulation, and function of the many forms of Ca^{2+} transporters in the ER membrane highlights the need for the diversification of genes that share common ancestors to create an adaptable system able to maintain organellar and cellular homeostasis.

CONTACT SITES

A membrane contact site is an area of close proximity between two organelle membranes that are tethered together by either protein–protein or protein–lipid interactions. One important role of contact sites is as hubs for nonvesicular transport of lipids, amino acids, and small molecules (146). The ER was the first organelle described to make contact sites, and it is now known to form contact sites with all organelles and the PM (147, 148). Considering that the ER is the primary site for synthesizing and modifying lipids, several different contact sites are required to support lipid exchange and thus the maintenance of specific biochemical and biophysical properties of other organellar membranes. These properties in turn are crucial for coordinating protein trafficking and signaling processes. The proteins that maintain contact sites have been dubbed tethers, and many tethers have already been described, most of which are part of large protein families.

Yeast and Human LAM Proteins

Lipid exchange at contact sites is carried out by lipid-transfer proteins (LTPs). The StARkin LTP superfamily has a subfamily of proteins called LAM (LTP anchored at membrane contacts). Humans have three LAM homologs (GRAMD1a, GRAMD1b, GRAMD1c), whereas yeast have six, which can be subdivided into two pairs of ohnologs (Lam1/Lam3 and Lam2/Lam4) and one pair of paralogs (Lam5/Lam6) (149). The LAM proteins all possess a structure consisting of an N-terminal pleckstrin homology (PH)-like GRAM domain, followed by one or two START domains and, lastly, a C-terminal TMD, which serves as a membrane anchor (149). All the LAM proteins are embedded in the ER membrane, with Lam1–4 residing at contact sites between the ER and PM (149), Lam5 at the ER–Golgi interface (150), and Lam6 at ER–mitochondria and ER–vacuole contacts (149, 151, 152). Lam6 is recruited to these two distinct contacts via interactions with either Tom70/71 or the ER–vacuolar tether, Vac8 (151, 152).

The ER–PM LAM proteins, Lam1–4, have been suggested to function as retrograde sterol transporters (149), trafficking sterols obtained from extracellular sources back to the ER, where they can be modified depending on the specific metabolic demand. On the other hand, Lam6 (which is also called Ltc1) is able to transport sterols away from the ER. This was demonstrated for the ER–vacuolar subpopulation of Lam6, which is required for sterol-enriched domain formation in the vacuolar membrane under stress conditions (152). It has recently been established that Lam6 has a specific PH domain fold, which is necessary for its localization to contacts between the ER and mitochondria (153). The function of the Lam6 paralog, Lam5, remains to be determined, although they appear nonredundant, since stress-induced vacuolar membrane domain formation was reduced upon the loss of Lam6 even though Lam5 was present (152).

The different human LAM proteins also have unique properties. GRAMD1a–c, homologs of yeast Lam4–6, localize to ER–PM contacts upon sterol treatment (154). Although all three

proteins have been suggested to transfer cholesterol from the PM (154), GRAMD1b is also suggested to transfer PI(4,5)P_2 and, to a lesser extent, PI(4)P (155). Furthermore, GRAMD1b is expressed in specific tissues (154) and localizes to ER–PM contacts distinct from those of GRAMD1a (156).

Beyond LAMs

In addition to the LAMs, many other tethers are part of larger protein families such as human VAPs, E-SYTs, VPS13 proteins, and ORPs (all of which have related homologs in yeast) (157). These contribute to the diversity of functions at contact sites. Future questions should therefore address how the different homologs and family members are recruited to specific contact sites and what their specific substrates for transfer are.

ENDOPLASMIC RETICULUM STRUCTURE

The ER is a highly dynamic and interconnected network that can occupy the majority of the cytosol in some cell types. Incredibly, the ER lumen is enclosed by a single, continuous lipid bilayer, which extends from the ONM. ER morphology can be classified into either tubular or cisternal structures. In higher eukaryotes, the sheet-like cisternae tend to be closer to the nucleus, whereas the tubules spread throughout the cytosol toward the periphery of the cell. In yeast, the peripheral ER resides beneath the PM and forms extensive contacts with it, and this is connected to the nuclear envelope by a few tubules (158). ER shape is maintained by ER shaping proteins, several of which are members of large families.

The Reticulon Family of Proteins

Within the last 15 years, several different protein families have been found to play a role in tubular network formation and maintenance. The first of these families to be identified were the reticulons (RTNs), which were discovered as targets of ER network formation inhibitors (159). This study focused in particular on vertebrate RTN4A and the two yeast ohnologs, Rtn1 and Rtn2. All of these homologs are localized specifically to tubular ER and excluded from the perinuclear area. Furthermore, they have a hairpin-like TMD arrangement, which stabilizes the high-curvature structure of ER tubules (159). ER sheet edges also represent domains of high curvature, and indeed human RTN4A and yeast Rtn1 were additionally shown to localize there (160). Although there are four human RTN proteins, all of which undergo alternative splicing, ER shaping has only been extensively explored in the context of RTN4 isoforms. The other RTN family members, with the exception of the uncharacterized RTN2, all have distinct functions independent of ER shaping, such as RTN3, the long isoform of which functions as a receptor for ER-phagy of ER tubules (161). One can therefore speculate that each RTN might play a unique role in controlling ER morphology, and this should be addressed in future research.

The REEP and Atlastin Protein Families

RTN4A was also shown to interact with DP1, the ortholog of the yeast protein Yop1 (159). DP1 is a member of the REEP family and was shown to share characteristics with the RTNs such as possessing a hairpin TMD structure and localizing specifically to tubular ER (159). There are six REEP family members in humans and metazoans, and they can be categorized into two subfamilies containing either REEP1–4 or REEP5–6 (162). Although DP1 (which is REEP5) had previously

been established to play a role in shaping the tubular ER network (159), antibodies raised against common domains in REEP1–4 inhibited ER network formation (162), thus at least some of the REEP1–4 proteins are also effectors of ER morphology.

Furthermore, REEP1 was shown to interact with the three human atlastin proteins (ATLs) (162), which comprise a family of dynamin-like GTPases that likely also adopt a hairpin-like wedge structure (163). ATL1–3 also interact with specific RTN isoforms, and their function is to interconnect ER tubules (163). The fact that only the combined knockdown of ATL2 and ATL3 generated a disconnected ER network (163) suggests that there might be some functional overlap within the ATL family. Further still, a synthetic sick phenotype was observed for yeast deleted for both *yop1* and *sey1* (the yeast ATL ortholog) (163), hinting that atlastins, with their hairpin structure, may also have a role in curvature stabilization and not only in tubule fusion.

In general, the studies on ER morphogenic proteins have not focused on comparing families or family members, and as a consequence the extent of redundancy and how each of these hairpin-domain proteins specifically affect the structure of the ER network remain to be determined. Understanding how these proteins function, interact, and cooperate with one another is an important step in gaining insight into neurological disorders such as hereditary spastic paraplegia, which is caused by mutations in different ATL and REEP family members (164).

Beyond Shaping Tubules

The ER membranes must be shaped not only at tubular invaginations. The biogenesis of lipid droplets (LDs) and peroxisomes from the ER also requires specific structural changes, and different paralogs are involved in these processes. For example, the ohnolog pair Pex30 and Pex31 is known to play a role in preperoxisomal vesicle formation, and the FIT homologs in yeast and mammals are critical for LD biogenesis (reviewed in 165).

FUNCTIONAL DETERMINATION OF PROTEIN HOMOLOG FUNCTION

Every functional aspect of the ER has homologs of proteins or entire families maintaining it. While this adds richness to regulatory capacity and dynamic distribution, it creates dramatic complexity for the study of each process. Below are some suggestions on how to elucidate homolog functions in such cases.

The examples highlighted above focus on pairs or families of proteins functioning inside the ER. However, they demonstrate a more general trend in the area of protein homologs, which is that studies tend to focus on either a single protein from a pair or family of homologs or on the function of one homolog in the absence of its relative(s) (such as for the yeast Hsp70s and Ost paralogs). Naturally, the latter approach is used to remove the potential masking effect of homologs, which might provide backup; however, this artificial situation is unlikely to fully uncover endogenous protein function. While it is not always easy to compare and contrast expression, regulation, substrate specificity, interactors, or post-translational modifications (PTMs), this lack of information on most protein homologs in eukaryotic genomes keeps us in the dark when it comes to understanding why a pair of proteins or an entire family has evolved.

Since in every cellular compartment, not just in the ER, homologs carry out diverse functions, it is important for the future of cell biology research to get better information on such protein pairs and families. Extrapolating from the above examples, we suggest that in most cases the evolutionary reason to evolve protein pairs or families in one organism falls into one (or more) of the following six categories (**Figure 2**): (*a*) targeting to different organelles to perform specific

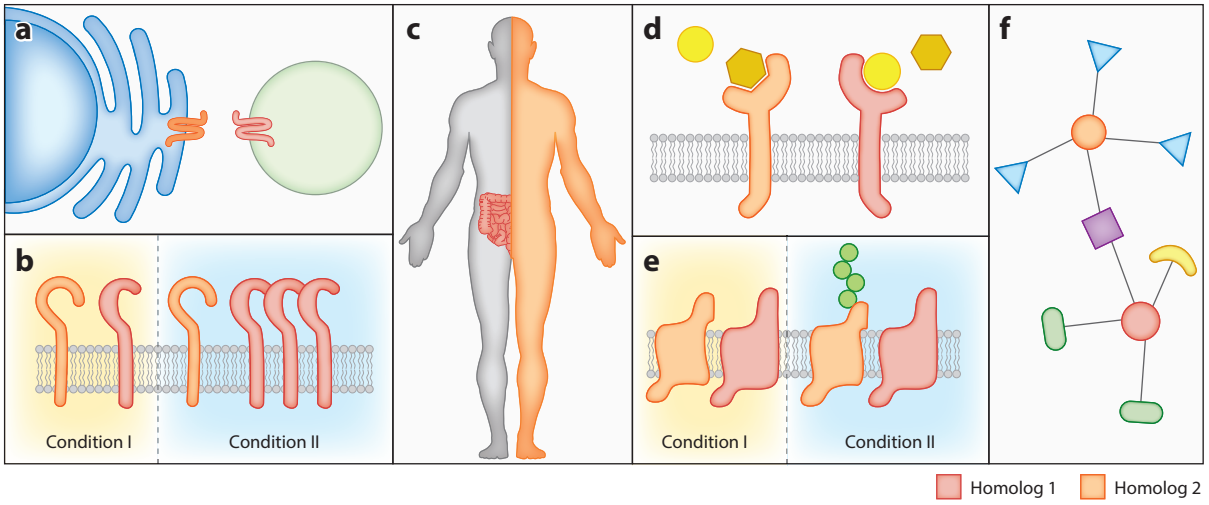

Figure 2

Ways in which homologs can differ from one another. In each of the six panels, homologs are depicted in red and orange. In panels *b* and *e*, the vertical, dotted line demarcates a change in conditions. The different homologs can (*a*) be targeted to different organelles; (*b*) have altered abundance as part of a response; (*c*) display cell/tissue-specific expression patterns or different developmental patterns [in this example, one of the homologs (*red*) is expressed only in the digestive tract, and the other (*orange*) is ubiquitously expressed throughout all tissues]; (*d*) have differential substrate preference; (*e*) undergo unique post-translational modifications; and (*f*) have distinct protein–protein interactions.

functions or be active in specialized biochemical environments, (*b*) differential expression in the same cell type by distinct transcriptional or post-translational mechanisms to enable response to different environmental/stress conditions or as part of a developmental program, (*c*) expression in different cell types or tissues important for maintaining functions in distinct environments, (*d*) preference toward distinct substrates, (*e*) unique PTMs that enable specific modulation of function under different conditions, and (*f*) distinct protein–protein interactions (PPIs) that impart different functionality.

We suggest here that our capacity to truly understand cell biology, development, and disease would be enhanced if papers addressing the function of a protein that is part of a pair or family of homologs would acknowledge the other proteins in the family and, where possible, address how they behave in the same system of study. Listed below are methodologies and resources that would enable comparison of the pairs and/or families in question.

Determining Subcellular Localization

Since targeting to different organelles or to different tissues is an important aspect of function diversification, finding out where proteins from a family localize is a crucial step in understanding each individual function and can help distinguish between protein paralogs. In yeast, the creation of whole-genome libraries in which green fluorescent protein (GFP) is appended to either the C terminus (166, 167) or the N terminus (3, 168) of each gene allows the comparison of the residence of each paralog pair. Data from these libraries, and therefore the localization of nearly the entire yeast proteome, are freely available for the community [**http://www.weizmann.ac.il/molgen/loqate** (169); **http://www.yeastrgb.org** (170)]. In other model organisms and cell lines, immunofluorescence (IF) can be used to visualize endogenous or tagged proteins. The Human

Protein Atlas (**https://www.proteinatlas.org/**) (21) has collected endogenous IF data for thousands of proteins so far.

Tracking Changes and Differences in Expression

Another important reason for preserving homologs or isoforms is so that they can be differentially expressed either in specialized cell types at specific developmental stages or in response to changes in intra/extracellular conditions. To compare transcript levels of a pair of homologs or members of a gene family, qRT-PCR (quantitative reverse transcription polymerase chain reaction) offers a sensitive and affordable method. For high-throughput experiments, microarrays were historically utilized, and large databases contain information on expression changes in multiple conditions such as for yeast [**https://spell.yeastgenome.org/** (171)]. RNA-sequencing is currently the preferred high-throughput technique, with data collected in databases (e.g., **https://www.ebi.ac.uk/gxa/home**) that serve as an open resource to extract expression data for almost any gene of interest. Additionally, FISH (fluorescence in situ hybridization) can be used as a method to uncover RNA localization in a tissue.

For tracking expression on the protein level in a high-throughput manner, MS is used. Although SILAC (stable isotope labeling with amino acids in cell culture) used to be the gold standard in quantitative proteomics, modern MS instruments reliably quantify peptide and protein levels from unlabeled samples. As with transcriptomics, proteomic data are stored in an online repository, PRIDE (172), through which protein abundance from all recent studies can be freely downloaded. Particularly useful studies include those that compare protein expression levels in a wide range of cells or tissues (173, 174). Additionally, some MS studies have attempted to map the half-life of the proteome (175), providing a useful resource for gaining insight into the stability of individual proteins. The LoQAtE website (**http://www.weizmann.ac.il/molgen/loqate**) also provides information on how localization and abundance of each protein in yeast changes under different external perturbations (169). Of course, protein expression and stability can be explored on a smaller scale using conventional Western blotting and translational shut-off/pulse-chase experiments.

Determining Metabolic Substrate Specificity

A vast repertoire of biochemical tools exist to elucidate substrate preference or the generation of specific products that may uncover differential functions of each homolog. It is now possible to use metabolomics to do this more globally or without a predetermined hypothesis. Metabolomics, which encompasses the subfield of lipidomics, is based on the detection, identification, and quantification of intermediate and end-product metabolites and the full complement of different metabolites in cells, tissues, and fluids. While MS is the principal analytical platform used in this field, nuclear magnetic resonance (NMR) is also used. NMR allows sample recovery after analysis, but it is less sensitive and requires larger amounts of starting material relative to MS techniques (176). Different methods for extraction and sample preparation as well as the different types of MS that can be used and their advantages have been reviewed elsewhere (177). MS methods ideally require the spike-in of isotope-labeled standards so that their conversion can be easily traced, as was done for monitoring sphingolipid and ceramide biogenesis in the Lag1/Lac1 study (see above and 121).

Uncovering Protein Modifications

Protein modification is a spatially and temporally controlled process, which is frequently used to activate, alter, or attenuate specific pathways as part of a response to a stimulus. Modification

can alter protein function, interaction profile, substrate preference, stability, localization, or any combination of these properties. Importantly, different paralogs can have unique PTM signatures, which functionally distinguish them from one another, as discussed above for the E2-conjugating enzymes UBE2J1 and UBE2J2 and the HMGCoA reductase variants.

Hundreds of PTMs exist, with some of the most pursued classes being acetylation, methylation, phosphorylation, and ubiquitination, all of which are reversible. Fortunately, recent developments in tandem MS (MS/MS) protocols and instrumentation mean that modification of amino acid residues within peptides produces a detectable mass shift sufficient to distinguish it from the unmodified form. For example, whole-proteome studies identified ubiquitination sites in ~5,000 different proteins (178). These data can be accessed using an online resource (**https://ggbase.hms.harvard.edu/**). Since this study, many other methods for studying the different types of ubiquitination have been developed (179). Similarly, phosphoproteomics has identified thousands of modifications and has been instrumental in discovering differential regulation of homologs. Progress has been made on developing high-sensitivity and multiplex workflows, which will enable hundreds of phosphorylation events to be monitored in different conditions (180, 181).

Discovering Protein–Protein Interactions

Paralogous protein pairs or members of the same protein family often form unique interactions that define their specific functions. Hence, when studying protein pairs or families, it would be wise to assess potential differences in their interaction partners. The recent expansion in large-scale interaction studies has produced an extensive network of PPI data, which in turn has significantly accelerated our discovery of novel protein regulators. There are two main methods for discovering PPIs: binary and cocomplex. The yeast two-hybrid (Y2H) approach is the classical binary technique, whereas affinity purification (AP) followed by MS represents the most frequently used cocomplex method (182). Two large-scale AP-MS projects found hundreds of yeast protein complexes (183, 184), and two years later, a genome-wide binary PPI screen using a protein-fragment complementation assay rather than Y2H identified nearly 3,000 interactions (185). In human cells, the largest-scale interaction study was performed using a pipeline based on a lentiviral system to express thousands of C-terminally FLAG-HA-tagged proteins, which were then subjected to AP-MS and comparative proteomic profiling analysis (186). All the data from these studies and others (both large- and small-scale) have been collected and are available on BioGRID (**https://thebiogrid.org/**).

Of course, putative interactors of bait proteins found by these high-throughput methods need to be validated and can represent either cofactors or substrates—both providing insight into specificity of a homolog. In general, substrates are more challenging to detect due to the transient nature of enzyme–substrate interactions. However, different tags that modify proteins proximal to them with a molecule that serves as a ligand for AP have been developed and will facilitate finding new substrates for different enzymes. These tags include BioID2 (187), APEX2 (188), and TurboID (189). Progress has also been made on generating trapping mutants, which lock onto their substrate upon interaction, such as the ubiquitin-activated interaction traps (UBAITs), modified E3s designed to irreversibly capture their substrates (190). Collectively, these methods can be used to discriminate the stable and transient interactomes of different protein homologs.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Throughout evolution, gene duplication has dramatically expanded the genome. While gene duplicates originally would have been identical, the combination of random changes and natural

selection has created a situation whereby, over time, they evolved unique features and characteristics, which in turn lend plasticity and robustness to a living system. This intrinsic feature often makes it very hard to understand the biological roles of individual proteins and their impact on cells and organisms. However, it is clear that they have a fundamental role in allowing complex biological traits to arise. While for many years there has been much focus on uncovering the role of single members of protein homologs or families, our next big frontier and what will now need to be explored is the exact role of each member of such families and their dynamic interplay. This review uses the ER as a platform to showcase the differences between genes that share a common ancestor. The specificities of each protein contribute to the capacity of the ER to handle such a broad range of functions and to its tunability in changing conditions.

In yeast, WGD has been successfully mapped, and a list of ohnologs is freely available [**http://ygob.ucd.ie/ygob/** (191)]. To provide a strong starting ground for those wishing to explore the various homologs of the ER and other organelles, we have created a resource in which each ohnolog pair is annotated with localization data (3) and corresponding human homologs (**Supplemental Table 2**). Protein localization and human gene cluster references were also added to a list of all yeast paralogs (192) (**Supplemental Table 2**).

Supplemental Material >

More globally, we suggest that future studies aimed at finding functions for homologous proteins should be careful when exploring what the function of one protein is in the absence of its homolog(s), as although this may help reveal potential redundancy, it is unlikely to uncover endogenous functional variation. Therefore, comparative approaches must be employed to truly understand the intricate roles of each homolog, how they enhance organelle dynamics, and therefore why genes and proteins with similar sequences have been preserved by evolution.

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LITERATURE CITED

1. Ihmels J, Collins SR, Schuldiner M, Krogan NJ, Weissman JS. 2007. Backup without redundancy: Genetic interactions reveal the cost of duplicate gene loss. *Mol. Syst. Biol.* 3(1):86
2. Ghaemmaghami S, Huh W-K, Bower K, Howson RW, Belle A, et al. 2003. Global analysis of protein expression in yeast. *Nature* 425(6959):737–41
3. Weill U, Yofe I, Sass E, Stynen B, Davidi D, et al. 2018. Genome-wide SWAp-Tag yeast libraries for proteome exploration. *Nat. Methods* 15:617–22
4. Aviram N, Schuldiner M. 2017. Targeting and translocation of proteins to the endoplasmic reticulum at a glance. *J. Cell Sci.* 130(24):4079–85

5. Xing S, Mehlhorn DG, Wallmeroth N, Asseck LY, Kar R, et al. 2017. Loss of GET pathway orthologs in *Arabidopsis thaliana* causes root hair growth defects and affects SNARE abundance. *PNAS* 114(8):E1544–53
6. Madeira F, Park YM, Lee J, Buso N, Gur T, et al. 2019. The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* 47(W1):W636–41
7. Chirico WJ, Waters MG, Blobel G. 1988. 70K heat shock related proteins stimulate protein translocation into microsomes. *Nature* 332(6167):805–10
8. Becker J, Walter W, Yan W, Craig EA. 1996. Functional interaction of cytosolic Hsp70 and a DnaJ-related protein, Ydj1p, in protein translocation in vivo. *Mol. Cell. Biol.* 16(8):4378–86
9. Ng DT. 1996. Signal sequences specify the targeting route to the endoplasmic reticulum membrane. *J. Cell Biol.* 134(2):269–78
10. Tripathi A, Mandon EC, Gilmore R, Rapoport TA. 2017. Two alternative binding mechanisms connect the protein translocation Sec71-Sec72 complex with heat shock proteins. *J. Biol. Chem.* 292(19):8007–18
11. Willmund F, del Alamo M, Pechmann S, Chen T, Albanèse V, et al. 2013. The cotranslational function of ribosome-associated Hsp70 in eukaryotic protein homeostasis. *Cell* 152(1–2):196–209
12. Craig EA. 2018. Hsp70 at the membrane: driving protein translocation. *BMC Biol.* 16(1):11
13. Cichocki BA, Krumpe K, Vitali DG, Rapaport D. 2018. Pex19 is involved in importing dually targeted tail-anchored proteins to both mitochondria and peroxisomes. *Traffic* 19(10):770–85
14. Zimmermann R, Eyrisch S, Ahmad M, Helms V. 2011. Protein translocation across the ER membrane. *Biochim. Biophys. Acta* 1808(3):912–24
15. Finke K, Plath K, Panzner S, Prehn S, Rapoport TA, et al. 1996. A second trimeric complex containing homologs of the Sec61p complex functions in protein transport across the ER membrane of *S. cerevisiae*. *EMBO J.* 15(7):1482–94
16. Wilkinson BM, Tyson JR, Stirling CJ. 2001. Ssh1p determines the translocation and dislocation capacities of the yeast endoplasmic reticulum. *Dev. Cell* 1(3):401–9
17. Jiang Y, Cheng Z, Mandon EC, Gilmore R. 2008. An interaction between the SRP receptor and the translocon is critical during cotranslational protein translocation. *J. Cell Biol.* 180(6):1149–61
18. Spiller MP, Stirling CJ. 2011. Preferential targeting of a signal recognition particle-dependent precursor to the Ssh1p translocon in yeast. *J. Biol. Chem.* 286(25):21953–60
19. Wittke S, Dünnwald M, Albertsen M, Johnsson N. 2002. Recognition of a subset of signal sequences by Ssh1p, a Sec61p-related protein in the membrane of endoplasmic reticulum of yeast *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 13(7):2223–32
20. Laborenz J, Hansen K, Prescianotto-Baschong C, Spang A, Herrmann JM. 2019. In vitro import experiments with semi-intact cells suggest a role of the Sec61 paralog Ssh1 in mitochondrial biogenesis. *Biol. Chem.* 400(9):1229–40
21. Thul PJ, Åkesson L, Wiking M, Mahdessian D, Geladaki A, et al. 2017. A subcellular map of the human proteome. *Science* 356(6340):eaal3321
22. Lang S, Benedix J, Fedeles SV, Schorr S, Schirra C, et al. 2012. Different effects of Sec61α, Sec62 and Sec63 depletion on transport of polypeptides into the endoplasmic reticulum of mammalian cells. *J. Cell Sci.* 125(Pt. 8):1958–69
23. Anghel SA, McGilvray PT, Hegde RS, Keenan RJ. 2017. Identification of Oxa1 homologs operating in the eukaryotic endoplasmic reticulum. *Cell Rep.* 21(13):3708–16
24. Breitling J, Aebi M. 2013. *N*-linked protein glycosylation in the endoplasmic reticulum. *Cold Spring Harb. Perspect. Biol.* 5(8):a013359
25. Spirig U, Bodmer D, Wacker M, Burda P, Aebi M. 2005. The 3.4-kDa Ost4 protein is required for the assembly of two distinct oligosaccharyltransferase complexes in yeast. *Glycobiology* 15(12):1396–406
26. Schwarz M, Knauer R, Lehle L. 2005. Yeast oligosaccharyltransferase consists of two functionally distinct sub-complexes, specified by either the Ost3p or Ost6p subunit. *FEBS Lett.* 579(29):6564–68
27. Yan A, Lennarz WJ. 2005. Two oligosaccharyl transferase complexes exist in yeast and associate with two different translocons. *Glycobiology* 15(12):1407–15
28. Schulz BL, Aebi M. 2009. Analysis of glycosylation site occupancy reveals a role for Ost3p and Ost6p in site-specific *N*-glycosylation efficiency. *Mol. Cell. Proteom.* 8(2):357–64

29. Kelleher DJ, Karaoglu D, Mandon EC, Gilmore R. 2003. Oligosaccharyltransferase isoforms that contain different catalytic STT3 subunits have distinct enzymatic properties. *Mol. Cell* 12(1):101–11
30. Ruiz-Canada C, Kelleher DJ, Gilmore R. 2009. Cotranslational and posttranslational *N*-glycosylation of polypeptides by distinct mammalian OST isoforms. *Cell* 136(2):272–83
31. Braunger K, Pfeffer S, Shrimal S, Gilmore R, Berninghausen O, et al. 2018. Structural basis for coupling protein transport and N-glycosylation at the mammalian endoplasmic reticulum. *Science* 360(6385):215–19
32. Cherepanova NA, Shrimal S, Gilmore R. 2014. Oxidoreductase activity is necessary for N-glycosylation of cysteine-proximal acceptor sites in glycoproteins. *J. Cell Biol.* 206(4):525–39
33. Cherepanova NA, Gilmore R. 2016. Mammalian cells lacking either the cotranslational or posttranslocational oligosaccharyltransferase complex display substrate-dependent defects in asparagine linked glycosylation. *Sci. Rep.* 6:20946
34. Loibl M, Strahl S. 2013. Protein *O*-mannosylation: what we have learned from baker's yeast. *Biochim. Biophys. Acta* 1833(11):2438–46
35. Blond-Elguindi S. 1993. Affinity panning of a library of peptides displayed on bacteriophages reveals the binding specificity of BiP. *Cell* 75(4):717–28
36. Melnyk A, Rieger H, Zimmermann R. 2015. Co-chaperones of the mammalian endoplasmic reticulum. In *The Networking of Chaperones by Co-chaperones: Control of Cellular Protein Homeostasis*, ed. GL Blatch, AL Edkins, pp. 179–200. Cham, Switz.: Springer
37. Shen Y, Hendershot LM. 2005. ERdj3, a stress-inducible endoplasmic reticulum DnaJ homologue, serves as a cofactor for BiP's interactions with unfolded substrates. *Mol. Biol. Cell* 16(1):40–50
38. Dudek J. 2002. A novel type of co-chaperone mediates transmembrane recruitment of DnaK-like chaperones to ribosomes. *EMBO J.* 21(12):2958–67
39. Dudek J, Greiner M, Müller A, Hendershot LM, Kopsch K, et al. 2005. ERj1p has a basic role in protein biogenesis at the endoplasmic reticulum. *Nat. Struct. Mol. Biol.* 12(11):1008–14
40. Haßdenteufel S, Johnson N, Paton AW, Paton JC, High S, Zimmermann R. 2018. Chaperone-mediated Sec61 channel gating during ER import of small precursor proteins overcomes Sec61 inhibitor-reinforced energy barrier. *Cell Rep.* 23(5):1373–86
41. Wu X, Cabanos C, Rapoport TA. 2019. Structure of the post-translational protein translocation machinery of the ER membrane. *Nature* 566:136–39
42. Dong M, Bridges JP, Apsley K, Xu Y, Weaver TE. 2008. ERdj4 and ERdj5 are required for endoplasmic reticulum-associated protein degradation of misfolded surfactant protein C. *Mol. Biol. Cell* 19(6):2620–30
43. Petrova K, Oyadomari S, Hendershot LM, Ron D. 2008. Regulated association of misfolded endoplasmic reticulum lumenal proteins with P58/DNAJc3. *EMBO J.* 27(21):2862–72
44. Ellgaard L, McCaul N, Chatsisvili A, Braakman I. 2016. Co- and post-translational protein folding in the ER. *Traffic* 17(6):615–38
45. Barlowe C. 1994. COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. *Cell* 77(6):895–907
46. Miller E, Antonny B, Hamamoto S, Schekman R. 2002. Cargo selection into COPII vesicles is driven by the Sec24p subunit. *EMBO J.* 21(22):6105–13
47. Miller EA, Beilharz TH, Malkus PN, Lee MCS, Hamamoto S, et al. 2003. Multiple cargo binding sites on the COPII subunit Sec24p ensure capture of diverse membrane proteins into transport vesicles. *Cell* 114(4):497–509
48. Geva Y, Schuldiner M. 2014. The back and forth of cargo exit from the endoplasmic reticulum. *Curr. Biol.* 24(3):R130–36
49. Roberg KJ, Crotwell M, Espenshade P, Gimeno R, Kaiser CA. 1999. LST1 is a SEC24 homologue used for selective export of the plasma membrane ATPase from the endoplasmic reticulum. *J. Cell Biol.* 145(4):659–72
50. Shimoni Y, Kurihara T, Ravazzola M, Amherdt M, Orci L, Schekman R. 2000. Lst1p and Sec24p cooperate in sorting of the plasma membrane ATPase into COPII vesicles in *Saccharomyces cerevisiae*. *J. Cell Biol.* 151(5):973–84

51. Kurihara T, Hamamoto S, Gimeno RE, Kaiser CA, Schekman R, Yoshihisa T. 2000. Sec24p and Iss1p function interchangeably in transport vesicle formation from the endoplasmic reticulum in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 11(3):983–98
52. Pagano A, Letourneur F, Garcia-Estefania D, Carpentier J-L, Orci L, Paccaud J-P. 1999. Sec24 proteins and sorting at the endoplasmic reticulum. *J. Biol. Chem.* 274(12):7833–40
53. Tang BL, Kausalya J, Low DYH, Lock ML, Hong W. 1999. A family of mammalian proteins homologous to yeast Sec24p. *Biochem. Biophys. Res. Commun.* 258(3):679–84
54. Wendeler MW, Paccaud J-P, Hauri H-P. 2007. Role of Sec24 isoforms in selective export of membrane proteins from the endoplasmic reticulum. *EMBO Rep.* 8(3):258–64
55. Mancias JD, Goldberg J. 2007. The transport signal on Sec22 for packaging into COPII-coated vesicles is a conformational epitope. *Mol. Cell* 26(3):403–14
56. Mancias JD, Goldberg J. 2008. Structural basis of cargo membrane protein discrimination by the human COPII coat machinery. *EMBO J.* 27(21):2918–28
57. Adolf F, Rhiel M, Reckmann I, Wieland FT. 2016. Sec24C/D-isoform-specific sorting of the preassembled ER-Golgi Q-SNARE complex. *Mol. Biol. Cell* 27(17):2697–707
58. Cui Y, Parashar S, Zahoor M, Needham PG, Mari M, et al. 2019. A COPII subunit acts with an autophagy receptor to target endoplasmic reticulum for degradation. *Science* 365(6448):53–60
59. Boyadjiev SA, Fromme JC, Ben J, Chong SS, Nauta C, et al. 2006. Cranio-lenticulo-sutural dysplasia is caused by a *SEC23A* mutation leading to abnormal endoplasmic-reticulum-to-Golgi trafficking. *Nat. Genet.* 38(10):1192–97
60. Garbes L, Kim K, Rieß A, Hoyer-Kuhn H, Beleggia F, et al. 2015. Mutations in *SEC24D*, encoding a component of the COPII machinery, cause a syndromic form of osteogenesis imperfecta. *Am. J. Hum. Genet.* 96(3):432–39
61. Paccaud JP, Reith W, Carpentier JL, Ravazzola M, Amherdt M, et al. 1996. Cloning and functional characterization of mammalian homologues of the COPII component Sec23. *Mol. Biol. Cell* 7(10):1535–46
62. Takida S, Maeda Y, Kinoshita T. 2008. Mammalian GPI-anchored proteins require p24 proteins for their efficient transport from the ER to the plasma membrane. *Biochem. J.* 409(2):555–62
63. Strating JRPM, Martens GJM. 2012. The p24 family and selective transport processes at the ER-Golgi interface. *Biol. Cell* 101(9):495–509
64. Springer S, Chen E, Duden R, Marzioch M, Rowley A, et al. 2000. The p24 proteins are not essential for vesicular transport in *Saccharomyces cerevisiae*. *PNAS* 97(8):4034–39
65. Karagöz GE, Acosta-Alvear D, Walter P. 2019. The unfolded protein response: detecting and responding to fluctuations in the protein-folding capacity of the endoplasmic reticulum. *Cold Spring Harb. Perspect. Biol.* 11(9):a033886
66. Walter P, Ron D. 2011. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 334(6059):1081–86
67. Korennykh AV, Egea PF, Korostelev AA, Finer-Moore J, Zhang C, et al. 2008. The unfolded protein response signals through high-order assembly of Ire1. *Nature* 457(7230):687–93
68. Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K. 2001. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 107(7):881–91
69. Iwawaki T, Akai R, Yamanaka S, Kohno K. 2009. Function of IRE1 alpha in the placenta is essential for placental development and embryonic viability. *PNAS* 106(39):16657–62
70. Ghosh R, Lipson KL, Sargent KE, Mercurio AM, Hunt JS, et al. 2010. Transcriptional regulation of VEGF-A by the unfolded protein response pathway. *PLOS ONE* 5(3):e9575
71. Martino MB, Jones L, Brighton B, Ehre C, Abdulah L, et al. 2013. The ER stress transducer IRE1β is required for airway epithelial mucin production. *Mucosal Immunol.* 6(3):639–54
72. Bertolotti A, Wang X, Novoa I, Jungreis R, Schlessinger K, et al. 2001. Increased sensitivity to dextran sodium sulfate colitis in IRE1β-deficient mice. *J. Clin. Investig.* 107(5):585–93
73. Iwawaki T, Hosoda A, Okuda T, Kamigori Y, Nomura-Furuwatari C, et al. 2001. Translational control by the ER transmembrane kinase/ribonuclease IRE1 under ER stress. *Nat. Cell Biol.* 3(2):158–64

74. Imagawa Y, Hosoda A, Sasaka S-I, Tsuru A, Kohno K. 2008. RNase domains determine the functional difference between IRE1α and IRE1β. *FEBS Lett.* 582(5):656–60
75. Hollien J, Weissman JS. 2006. Decay of endoplasmic reticulum-localized mRNAs during the unfolded protein response. *Science* 313(5783):104–7
76. Han D, Lerner AG, Vande Walle L, Upton J-P, Xu W, et al. 2009. IRE1α kinase activation modes control alternate endoribonuclease outputs to determine divergent cell fates. *Cell* 138(3):562–75
77. Hollien J, Lin JH, Li H, Stevens N, Walter P, Weissman JS. 2009. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *J. Cell Biol.* 186(3):323–31
78. Kimmig P, Diaz M, Zheng J, Williams CC, Lang A, et al. 2012. The unfolded protein response in fission yeast modulates stability of select mRNAs to maintain protein homeostasis. *eLife* 1:e00048
79. Maurel M, Chevet E, Tavernier J, Gerlo S. 2014. Getting RIDD of RNA: IRE1 in cell fate regulation. *Trends Biochem. Sci.* 39(5):245–54
80. Haze K, Yoshida H, Yanagi H, Yura T, Mori K. 1999. Mammalian transcription factor ATF6 is synthesized as a transmembrane protein and activated by proteolysis in response to endoplasmic reticulum stress. *Mol. Biol. Cell* 10(11):3787–99
81. Shoulders MD, Ryno LM, Genereux JC, Moresco JJ, Tu PG, et al. 2013. Stress-independent activation of XBP1s and/or ATF6 reveals three functionally diverse ER proteostasis environments. *Cell Rep.* 3(4):1279–92
82. Haze K, Okada T, Yoshida H, Yanagi H, Yura T, et al. 2001. Identification of the G13 (cAMP-response-element-binding protein-related protein) gene product related to activating transcription factor 6 as a transcriptional activator of the mammalian unfolded protein response. *Biochem. J.* 355(1):19–28
83. Thuerauf DJ, Morrison L, Glembotski CC. 2004. Opposing roles for ATF6α and ATF6β in endoplasmic reticulum stress response gene induction. *J. Biol. Chem.* 279(20):21078–84
84. Yamamoto K, Sato T, Matsui T, Sato M, Okada T, et al. 2007. Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6α and XBP1. *Dev. Cell* 13(3):365–76
85. Ishikawa T, Okada T, Ishikawa-Fujiwara T, Todo T, Kamei Y, et al. 2013. ATF6α/β-mediated adjustment of ER chaperone levels is essential for development of the notochord in medaka fish. *Mol. Biol. Cell* 24(9):1387–95
86. Olzmann JA, Kopito RR, Christianson JC. 2013. The mammalian endoplasmic reticulum-associated degradation system. *Cold Spring Harb. Perspect. Biol.* 5(9):a013185
87. Carvalho P, Goder V, Rapoport TA. 2006. Distinct ubiquitin-ligase complexes define convergent pathways for the degradation of ER proteins. *Cell* 126(2):361–73
88. Ast T, Aviram N, Chuartzman SG, Schuldiner M. 2014. A cytosolic degradation pathway, prERAD, monitors pre-inserted secretory pathway proteins. *J. Cell Sci.* 127(14):3017–23
89. Swanson R, Locher M, Hochstrasser M. 2001. A conserved ubiquitin ligase of the nuclear envelope/endoplasmic reticulum that functions in both ER-associated and Matα2 repressor degradation. *Genes Dev.* 15(20):2660–74
90. Weber A, Cohen I, Popp O, Dittmar G, Reiss Y, et al. 2016. Sequential poly-ubiquitylation by specialized conjugating enzymes expands the versatility of a quality control ubiquitin ligase. *Mol. Cell* 63(5):827–39
91. Bays NW, Gardner RG, Seelig LP, Joazeiro CA, Hampton RY. 2001. Hrd1p/Der3p is a membrane-anchored ubiquitin ligase required for ER-associated degradation. *Nat. Cell Biol.* 3(1):24–29
92. Lester D, Farquharson C, Russell G, Houston B. 2000. Identification of a family of noncanonical ubiquitin-conjugating enzymes structurally related to yeast UBC6. *Biochem. Biophys. Res. Commun.* 269(2):474–80
93. Oh RS, Bai X, Rommens JM. 2006. Human homologs of Ubc6p ubiquitin-conjugating enzyme and phosphorylation of HsUbc6e in response to endoplasmic reticulum stress. *J. Biol. Chem.* 281(30):21480–90
94. Elangovan M, Chong HK, Park JH, Yeo EJ, Yoo YJ. 2017. The role of ubiquitin-conjugating enzyme Ube2j1 phosphorylation and its degradation by proteasome during endoplasmic stress recovery. *J. Cell Commun. Signal.* 11(3):265–73

95. Younger JM, Chen L, Ren H-Y, Rosser MFN, Turnbull EL, et al. 2006. Sequential quality-control checkpoints triage misfolded cystic fibrosis transmembrane conductance regulator. *Cell* 126(3):571–82
96. Burr ML, Cano F, Svobodova S, Boyle LH, Boname JM, Lehner PJ. 2011. HRD1 and UBE2J1 target misfolded MHC class I heavy chains for endoplasmic reticulum-associated degradation. *PNAS* 108(5):2034–39
97. van de Weijer ML, Bassik MC, Luteijn RD, Voorburg CM, Lohuis MAM, et al. 2014. A high-coverage shRNA screen identifies TMEM129 as an E3 ligase involved in ER-associated protein degradation. *Nat. Commun.* 5:3823
98. Stefanovic-Barrett S, Dickson AS, Burr SP, Williamson JC, Lobb IT, et al. 2018. MARCH6 and TRC8 facilitate the quality control of cytosolic and tail-anchored proteins. *EMBO Rep.* 19(5):e45603
99. Neutzner A, Neutzner M, Benischke A-S, Ryu S-W, Frank S, et al. 2011. A systematic search for ER membrane-associated RING finger proteins identifies Nixin/ZNRF4 as a regulator of calnexin stability and ER homeostasis. *J. Biol. Chem.* 286(10):8633–43
100. Maruyama Y, Yamada M, Takahashi K, Yamada M. 2008. Ubiquitin ligase Kf-1 is involved in the endoplasmic reticulum-associated degradation pathway. *Biochem. Biophys. Res. Commun.* 374(4):737–41
101. Shmueli A, Tsai YC, Yang M, Braun MA, Weissman AM. 2009. Targeting of gp78 for ubiquitin-mediated proteasomal degradation by Hrd1: cross-talk between E3s in the endoplasmic reticulum. *Biochem. Biophys. Res. Commun.* 390(3):758–62
102. Bernasconi R, Galli C, Calanca V, Nakajima T, Molinari M. 2010. Stringent requirement for HRD1, SEL1L, and OS-9/XTP3-B for disposal of ERAD-L$_S$ substrates. *J. Cell Biol.* 188(2):223–35
103. Sun S, Shi G, Sha H, Ji Y, Han X, et al. 2015. IRE1α is an endogenous substrate of endoplasmic-reticulum-associated degradation. *Nat. Cell Biol.* 17(12):1546–55
104. Tyler RE, Pearce MMP, Shaler TA, Olzmann JA, Greenblatt EJ, Kopito RR. 2012. Unassembled CD147 is an endogenous endoplasmic reticulum-associated degradation substrate. *Mol. Biol. Cell* 23(24):4668–78
105. Wang Q, Liu X, Cui Y, Tang Y, Chen W, et al. 2014. The E3 ubiquitin ligase AMFR and INSIG1 bridge the activation of TBK1 kinase by modifying the adaptor STING. *Immunity* 41(6):919–33
106. Christianson JC, Olzmann JA, Shaler TA, Sowa ME, Bennett EJ, et al. 2012. Defining human ERAD networks through an integrative mapping strategy. *Nat. Cell Biol.* 14(1):93–105
107. Tcherpakov M, Delaunay A, Toth J, Kadoya T, Petroski MD, Ronai ZA. 2009. Regulation of endoplasmic reticulum-associated degradation by RNF5-dependent ubiquitination of JNK-associated membrane protein (JAMP). *J. Biol. Chem.* 284(18):12099–109
108. Khouri El E, Le Pavec G, Toledano MB, Delaunay-Moisan A. 2013. RNF185 is a novel E3 ligase of endoplasmic reticulum-associated degradation (ERAD) that targets cystic fibrosis transmembrane conductance regulator (CFTR). *J. Biol. Chem.* 288(43):31177–91
109. Zhong B, Zhang L, Lei C, Li Y, Mao A-P, et al. 2009. The ubiquitin ligase RNF5 regulates antiviral responses by mediating degradation of the adaptor protein MITA. *Immunity* 30(3):397–407
110. Wang Q, Huang L, Hong Z, Lv Z, Mao Z, et al. 2017. The E3 ubiquitin ligase RNF185 facilitates the cGAS-mediated innate immune response. *PLOS Pathog.* 13(3):e1006264
111. Christianson JC, Ye Y. 2014. Cleaning up in the endoplasmic reticulum: ubiquitin in charge. *Nat. Struct. Mol. Biol.* 21(4):325–35
112. Foresti O, Rodriguez-Vaello V, Funaya C, Carvalho P. 2014. Quality control of inner nuclear membrane proteins by the Asi complex. *Science* 346(6210):751–55
113. van Meer G, Voelker DR, Feigenson GW. 2008. Membrane lipids: where they are and how they behave. *Nat. Rev. Mol. Cell Biol.* 9(2):112–24
114. Kuchler K, Daum G, Paltauf F. 1986. Subcellular and submitochondrial localization of phospholipid-synthesizing enzymes in *Saccharomyces cerevisiae*. *J. Bacteriol.* 165(3):901–10
115. Trotter PJ, Voelker DR. 1995. Identification of a non-mitochondrial phosphatidylserine decarboxylase activity (PSD2) in the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* 270(11):6062–70
116. Gulshan K, Shahi P, Moye-Rowley WS. 2010. Compartment-specific synthesis of phosphatidylethanolamine is required for normal heavy metal resistance. *Mol. Biol. Cell* 21(3):443–55

117. Chan EYL, McQuibban GA. 2012. Phosphatidylserine decarboxylase 1 (Psd1) promotes mitochondrial fusion by regulating the biophysical properties of the mitochondrial membrane and alternative topogenesis of mitochondrial genome maintenance protein 1 (Mgm1). *J. Biol. Chem.* 287(48):40131–39
118. Friedman JR, Kannan M, Toulmay A, Jan CH, Weissman JS, et al. 2018. Lipid homeostasis is maintained by dual targeting of the mitochondrial PE biosynthesis enzyme to the ER. *Dev. Cell* 44(2):261–66
119. D'mello NP, Childress AM, Franklin DS, Kale SP, Pinswasdi C, Jazwinski SM. 1994. Cloning and characterization of LAG1, a longevity-assurance gene in yeast. *J. Biol. Chem.* 269(22):15451–59
120. Jiang JC, Kirchman PA, Allen M, Jazwinski SM. 2004. Suppressor analysis points to the subtle role of the LAG1 ceramide synthase gene in determining yeast longevity. *Exp. Gerontol.* 39(7):999–1009
121. Megyeri M, Prasad R, Volpert G, Sliwa-Gonzalez A, Haribowo AG, et al. 2019. Yeast ceramide synthases, Lag1 and Lac1, have distinct substrate specificity. *J. Cell Sci.* 132(12):jcs228411
122. Levy M, Futerman AH. 2010. Mammalian ceramide synthases. *IUBMB Life* 62(5):347–56
123. Tidhar R, Zelnik ID, Volpert G, Ben-Dor S, Kelly S, et al. 2018. Eleven residues determine the acyl chain specificity of ceramide synthases. *J. Biol. Chem.* 293(25):9912–21
124. Hampton RY, Garza RM. 2009. Protein quality control as a strategy for cellular regulation: lessons from ubiquitin-mediated regulation of the sterol pathway. *Chem. Rev.* 109(4):1561–74
125. Basson ME, Thorsness M, Rine J. 1986. *Saccharomyces cerevisiae* contains two functional genes encoding 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *PNAS* 83(15):5563–67
126. Gardner RG, Hampton RY. 1999. A highly conserved signal controls degradation of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase in eukaryotes. *J. Biol. Chem.* 274(44):31671–78
127. Gardner R, Cronin S, Leader B, Rine J, Hampton R. 1998. Sequence determinants for regulated degradation of yeast 3-hydroxy-3-methylglutaryl-CoA reductase, an integral endoplasmic reticulum membrane protein. *Mol. Biol. Cell* 9(9):2611–26
128. Johnson JM, Castle J, Garrett-Engele P, Kan Z, Loerch PM, et al. 2003. Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays. *Science* 302(5653):2141–44
129. Engfelt WH, Shackelford JE, Aboushadi N, Jessani N, Masuda K, et al. 1997. Characterization of UT2 cells: the induction of peroxisomal 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *J. Biol. Chem.* 272(39):24579–87
130. Menzies SA, Volkmar N, van den Boomen DJ, Timms RT, Dickson AS, et al. 2018. The sterol-responsive RNF145 E3 ubiquitin ligase mediates the degradation of HMG-CoA reductase together with gp78 and Hrd1. *eLife* 7:e40009
131. Aboushadi N, Shackelford JE, Jessani N, Gentile A, Krisans SK. 2000. Characterization of peroxisomal 3-hydroxy-3-methylglutaryl coenzyme A reductase in UT2* cells: sterol biosynthesis, phosphorylation, degradation, and statin inhibition. *Biochemistry* 39(1):237–47
132. Medina MW, Gao F, Ruan W, Rotter JI, Krauss RM. 2008. Alternative splicing of 3-hydroxy-3-methylglutaryl coenzyme A reductase is associated with plasma low-density lipoprotein cholesterol response to simvastatin. *Circulation* 118(4):355–62
133. Kodaki T, Yamashita S. 1989. Characterization of the methyltransferases in the yeast phosphatidylethanolamine methylation pathway by selective gene disruption. *Eur. J. Biochem.* 185(2):243–51
134. Tomohiro S, Kawaguti A, Kawabe Y, Kitada S, Kuge O. 2009. Purification and characterization of human phosphatidylserine synthases 1 and 2. *Biochem. J.* 418(2):421–29
135. Zaremberg V, McMaster CR. 2002. Differential partitioning of lipids metabolized by separate yeast glycerol-3-phosphate acyltransferases reveals that phospholipase D generation of phosphatidic acid mediates sensitivity to choline-containing lysolipids and drugs. *J. Biol. Chem.* 277(41):39035–44
136. Yu J, Loh K, Song Z-Y, Yang H-Q, Zhang Y, Lin S. 2018. Update on glycerol-3-phosphate acyltransferases: the roles in the development of insulin resistance. *Nutr. Diabetes* 8(1):34
137. Samtleben S, Jaepel J, Fecher C, Andreska T, Rehberg M, Blum R. 2013. Direct imaging of ER calcium with targeted-esterase induced dye loading (TED). *J. Vis. Exp.* 7(75):e50317
138. Altshuler I, Vaillant JJ, Xu S, Cristescu ME. 2012. The evolutionary history of sarco(endo)plasmic calcium ATPase (SERCA). *PLOS ONE* 7(12):e52617

139. Lytton J, Westlin M, Burk SE, Shull GE, MacLennan DH. 1992. Functional comparisons between isoforms of the sarcoplasmic or endoplasmic reticulum family of calcium pumps. *J. Biol. Chem.* 267(20):14483–89
140. Toyofuku T, Kurzydlowski K, Tada M, MacLennan DH. 1993. Identification of regions in the Ca^{2+}-ATPase of sarcoplasmic reticulum that affect functional association with phospholamban. *J. Biol. Chem.* 268(4):2809–15
141. Wootton LL, Michelangeli F. 2006. The effects of the phenylalanine 256 to valine mutation on the sensitivity of sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) Ca^{2+} pump isoforms 1, 2, and 3 to thapsigargin and other inhibitors. *J. Biol. Chem.* 281(11):6970–76
142. Lanner JT, Georgiou DK, Joshi AD, Hamilton SL. 2010. Ryanodine receptors: structure, expression, molecular details, and function in calcium release. *Cold Spring Harb. Perspect. Biol.* 2(11):a003996
143. Ivanova H, Vervliet T, Missiaen L, Parys JB, De Smedt H, Bultynck G. 2014. Inositol 1,4,5-trisphosphate receptor-isoform diversity in cell death and survival. *Biochim. Biophys. Acta* 1843(10):2164–83
144. Williams RT, Manji SSM, Parker NJ, Hancock MS, van Stekelenburg L, et al. 2001. Identification and characterization of the STIM (stromal interaction molecule) gene family: coding for a novel class of transmembrane proteins. *Biochem. J.* 357(3):673–85
145. Brandman O, Liou J, Park WS, Meyer T. 2007. STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca^{2+} levels. *Cell* 131(7):1327–39
146. Scorrano L, De Matteis MA, Emr S, Giordano F, Hajnóczky G, et al. 2019. Coming together to define membrane contact sites. *Nat. Commun.* 10(1):1287
147. Valm AM, Cohen S, Legant WR, Melunis J, Hershberg U, et al. 2017. Applying systems-level spectral imaging and analysis to reveal the organelle interactome. *Nature* 546(7656):162–67
148. Shai N, Yifrach E, van Roermund CWT, Cohen N, Bibi C, et al. 2018. Systematic mapping of contact sites reveals tethers and a function for the peroxisome-mitochondria contact. *Nat. Commun.* 9(1):1761
149. Gatta AT, Wong LH, Sere YY, Calderón-Noreña DM, Cockcroft S, et al. 2015. A new family of StART domain proteins at membrane contact sites has a role in ER-PM sterol transport. *eLife* 4:e07253
150. Weill U, Arakel EC, Goldmann O, Golan M, Chuartzman S, et al. 2018. Toolbox: Creating a systematic database of secretory pathway proteins uncovers new cargo for COPI. *Traffic* 19(5):370–79
151. Elbaz-Alon Y, Eisenberg-Bord M, Shinder V, Stiller SB, Shimoni E, et al. 2015. Lam6 regulates the extent of contacts between organelles. *Cell Rep.* 12(1):7–14
152. Murley A, Sarsam RD, Toulmay A, Yamada J, Prinz WA, Nunnari J. 2015. Ltc1 is an ER-localized sterol transporter and a component of ER-mitochondria and ER-vacuole contacts. *J. Cell Biol.* 209(4):539–48
153. Tong J, Manik MK, Im YJ. 2018. Structural basis of sterol recognition and nonvesicular transport by lipid transfer proteins anchored at membrane contact sites. *PNAS* 115(5):E856–65
154. Sandhu J, Li S, Fairall L, Pfisterer SG, Gurnett JE, et al. 2018. Aster proteins facilitate nonvesicular plasma membrane to ER cholesterol transport in mammalian cells. *Cell* 175(2):514–20
155. Horenkamp FA, Valverde DP, Nunnari J, Reinisch KM. 2018. Molecular basis for sterol transport by StART-like lipid transfer domains. *EMBO J.* 37(6):e98002
156. Besprozvannaya M, Dickson E, Li H, Ginburg KS, Bers DM, et al. 2018. GRAM domain proteins specialize functionally distinct ER-PM contact sites in human cells. *eLife* 7:e31019
157. Hariri H, Ugrankar R, Liu Y, Henne WM. 2016. Inter-organelle ER-endolysosomal contact sites in metabolism and disease across evolution. *Commun. Integr. Biol.* 9(3):e1156278
158. Shibata Y, Voeltz GK, Rapoport TA. 2006. Rough sheets and smooth tubules. *Cell* 126(3):435–39
159. Voeltz GK, Prinz WA, Shibata Y, Rist JM, Rapoport TA. 2006. A class of membrane proteins shaping the tubular endoplasmic reticulum. *Cell* 124(3):573–86
160. Shibata Y, Shemesh T, Prinz WA, Palazzo AF, Kozlov MM, Rapoport TA. 2010. Mechanisms determining the morphology of the peripheral ER. *Cell* 143(5):774–88
161. Grumati P, Morozzi G, Hölper S, Mari M, Harwardt M-LIE, et al. 2017. Full length RTN3 regulates turnover of tubular endoplasmic reticulum via selective autophagy. *eLife* 6:e25555
162. Park SH, Zhu P-P, Parker RL, Blackstone C. 2010. Hereditary spastic paraplegia proteins REEP1, spastin, and atlastin-1 coordinate microtubule interactions with the tubular ER network. *J. Clin. Investig.* 120(4):1097–110

163. Hu J, Shibata Y, Zhu P-P, Voss C, Rismanchi N, et al. 2009. A class of dynamin-like GTPases involved in the generation of the tubular ER network. *Cell* 138(3):549–61
164. Westrate LM, Lee JE, Prinz WA, Voeltz GK. 2015. Form follows function: the importance of endoplasmic reticulum shape. *Annu. Rev. Biochem.* 84:791–811
165. Joshi AS, Zhang H, Prinz WA. 2017. Organelle biogenesis in the endoplasmic reticulum. *Nat. Cell Biol.* 19(8):876–82
166. Huh W-K, Falvo JV, Gerke LC, Carroll AS, Howson RW, et al. 2003. Global analysis of protein localization in budding yeast. *Nature* 425(6959):686–91
167. Meurer M, Duan Y, Sass E, Kats I, Herbst K, et al. 2018. Genome-wide C-SWAT library for high-throughput yeast genome tagging. *Nat. Methods* 15(8):598–600
168. Yofe I, Weill U, Meurer M, Chuartzman S, Zalckvar E, et al. 2016. One library to make them all: streamlining the creation of yeast libraries via a SWAp-Tag strategy. *Nat. Methods* 13(4):371–78
169. Breker M, Gymrek M, Moldavski O, Schuldiner M. 2013. LoQAtE—Localization and Quantitation ATlas of the yeast proteomE. A new tool for multiparametric dissection of single-protein behavior in response to biological perturbations in yeast. *Nucleic Acids Res.* 42(D1):D726–30
170. Dubreuil B, Sass E, Nadav Y, Heidenreich M, Georgeson JM, et al. 2018. YeastRGB: comparing the abundance and localization of yeast proteins across cells and libraries. *Nucleic Acids Res.* 47(D1):D1245–49
171. Hibbs MA, Hess DC, Myers CL, Huttenhower C, Li K, Troyanskaya OG. 2007. Exploring the functional landscape of gene expression: directed search of large microarray compendia. *Bioinformatics* 23(20):2692–99
172. Vizcaíno JA, Csordas A, del-Toro N, Dianes JA, Griss J, et al. 2016. 2016 update of the PRIDE database and its related tools. *Nucleic Acids Res.* 44(D1):D447–56
173. Geiger T, Wehner A, Schaab C, Cox J, Mann M. 2012. Comparative proteomic analysis of eleven common cell lines reveals ubiquitous but varying expression of most proteins. *Mol. Cell Proteom.* 11(3):M111.014050
174. Geiger T, Velic A, Macek B, Lundberg E, Kampf C, et al. 2013. Initial quantitative proteomic map of 28 mouse tissues using the SILAC mouse. *Mol. Cell Proteom.* 12(6):1709–22
175. Larance M, Ahmad Y, Kirkwood KJ, Ly T, Lamond AI. 2013. Global subcellular characterization of protein degradation using quantitative proteomics. *Mol. Cell Proteom.* 12(3):638–50
176. Nagana Gowda GA, Djukovic D, Bettcher LF, Gu H, Raftery D. 2018. NMR-guided mass spectrometry for absolute quantitation of human blood metabolites. *Anal. Chem.* 90(3):2001–9
177. Cajka T, Fiehn O. 2015. Toward merging untargeted and targeted methods in mass spectrometry-based metabolomics and lipidomics. *Anal. Chem.* 88(1):524–45
178. Kim W, Bennett EJ, Huttlin EL, Guo A, Li J, et al. 2011. Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol. Cell* 44(2):325–40
179. Ordureau A, Münch C, Harper JW. 2015. Quantifying ubiquitin signaling. *Mol. Cell* 58(4):660–76
180. Lebesgue N, Megyeri M, Cristobal A, Scholten A, Chuartzman SG, et al. 2017. Combining deep sequencing, proteomics, phosphoproteomics, and functional screens to discover novel regulators of sphingolipid homeostasis. *J. Proteome Res.* 16(2):571–82
181. Humphrey SJ, Karayel O, James DE, Mann M. 2018. High-throughput and high-sensitivity phosphoproteomics with the EasyPhos platform. *Nat. Protoc.* 13(9):1897–916
182. De Las Rivas J, Fontanillo C. 2010. Protein–protein interactions essentials: key concepts to building and analyzing interactome networks. *PLOS Comput. Biol.* 6(6):e1000807
183. Krogan NJ, Cagney G, Yu H, Zhong G, Guo X, et al. 2006. Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. *Nature* 440(7084):637–43
184. Gavin A-C, Aloy P, Grandi P, Krause R, Boesche M, et al. 2006. Proteome survey reveals modularity of the yeast cell machinery. *Nature* 440(7084):631–36
185. Tarassov K, Messier V, Landry CR, Radinovic S, Molina MMS, et al. 2008. An in vivo map of the yeast protein interactome. *Science* 320(5882):1465–70
186. Huttlin EL, Bruckner RJ, Paulo JA, Cannon JR, Ting L, et al. 2017. Architecture of the human interactome defines protein communities and disease networks. *Nature* 545(7655):505–9

187. Kim DI, Jensen SC, Noble KA, Birendra KC, Roux KH, et al. 2016. An improved smaller biotin ligase for BioID proximity labeling. *Mol. Biol. Cell* 27(8):1188–96
188. Lam SS, Martell JD, Kamer KJ, Deerinck TJ, Ellisman MH, et al. 2014. Directed evolution of APEX2 for electron microscopy and proximity labeling. *Nat. Methods* 12(1):51–54
189. Branon TC, Bosch JA, Sanchez AD, Udeshi ND, Svinkina T, et al. 2018. Efficient proximity labeling in living cells and organisms with TurboID. *Nat. Biotechnol.* 36(9):880–87
190. O'Connor HF, Lyon N, Leung JW, Agarwal P, Swaim CD, et al. 2015. Ubiquitin-activated interaction traps (UBAITs) identify E3 ligase binding partners. *EMBO Rep*. 16(12):1699–712
191. Byrne KP, Wolfe KH. 2005. The Yeast Gene Order Browser: Combining curated homology and syntenic context reveals gene fate in polyploid species. *Genome Res*. 15(10):1456–61
192. Guan Y, Dunham MJ, Troyanskaya OG. 2007. Functional analysis of gene duplications in *Saccharomyces cerevisiae*. *Genetics* 175(2):933–43
193. Jensen RA. 2001. Orthologs and paralogs—we need to get it right. *Genome Biol*. 2(8):interactions1002.1

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Errata

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