Unraveling what makes a monoclonal antibody difficult-to-express: From intracellular accumulation to incomplete folding and degradation via ERAD

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Abstract
Although most therapeutic monoclonal antibodies (mAbs) can routinely be produced in the multigram per litre range, some mAb candidates turn out to be difficult-to-express (DTE). In addition, the class of more complex biological formats is permanently increasing and mammalian expression systems like Chinese hamster ovary (CHO) cell lines can show low performance. Hence, there is an urgent need to identify any rate limiting processing step during cellular synthesis. Therefore, we assessed the intracellular location of the DTE antibody mAb2 by fluorescence and electron microscopy (EM) and revealed an accumulation of the antibody, which led to an aberrant morphology of the endoplasmic reticulum (ER). Analysis of underlying cellular mechanisms revealed that neither aggregation nor antibody assembly, but folding represented the reason for hampered secretion. We identified that the disulfide bridge formation within the antibody light chain (LC) was impaired due to less recognition by protein disulfide isomerase (PDI). As a consequence, the DTE molecule was degraded intracellularly by the ubiquitin proteasome system via ER-associated degradation (ERAD). This study revealed that with the continuous emergence of DTE therapeutic protein candidates, special attention needs to be drawn to optimization processes to ensure manufacturability.

KEYWORDS
CHO cell line engineering, difficult-to-express mAbs, ER-associated degradation, incomplete domain folding, production bottleneck, protein disulfide isomerase

1 | INTRODUCTION

Monoclonal antibodies (mAbs) represent a class of therapeutic proteins, which are highly specific in activating, inhibiting, or blocking molecular targets to modulate diseases and can routinely be produced in the multigram per litre range using modern fed-batch cultivation processes (Kunert & Reinhart, 2016). However, the number of innovative and nonnaturally existing more complex biologicals such as multispecific formats has been increasing dramatically during the past years (Jost & Plückthun, 2014; Spiess, Zhai, & Carter, 2015). Since these artificially generated molecules have never undergone an evolutionary optimization, mammalian cellular expression systems often show low performance with cell lines displaying inferior product titer, low specific productivity, or insufficient product quality (Johari, Estes, Alves, Sinacore, & James, 2015; Lee et al., 2007). In addition, even some classical mAb candidates can be difficult-to-express (DTE), which might result in the termination of the
product development (Pybus et al., 2014). To provide access to sufficient amounts of innovative drugs, there is an urgent need to identify any rate limiting processing step during cellular synthesis and transport, which might represent a secretion bottleneck.

The biosynthesis, processing, and subsequent secretion of extracellular proteins is a complex and multistep procedure, which requires several different compartments within the respective cell to function highly efficiently. Each organelle thereby represents a tightly regulated and balanced network to provide the basis for performing specific tasks (Alberts et al., 2002; Lodish et al., 2000). Any disturbances in this equilibrium will impair the functionality as well as morphological appearance of the respective compartment. Therapeutic proteins which are assigned for secretion are ribosomally synthesized and cotranslationally translocated into the lumen of the endoplasmic reticulum (ER). Here, proper folding is facilitated by various resident chaperones such as members of the 70-kDa heat shock protein family like binding immunoglobulin (IgG) protein (BIP), lectins including calnexin and calreticulin as well as peptidyl—prolyl isomerases (Braakman & Hebert, 2013; Ellgaard & Helenius, 2003). After folding, the native protein structure is stabilized by disulfide bridges, which are simultaneously established via protein disulfide isomerases (PDIs) oxidizing free cysteine residues (Appenzeller-Braun, 2014; Wu & Rapoport, 2018). However, an accumulation of unfolded or misfolded proteins in the lumen of the ER (due to an overloading of the processing capacity or the ERAD machinery) can trigger the unfolded protein response (UPR; Hetz, 2012; Xu, Bailly-Maitre, & Reed, 2005). Since DTE proteins will gain in importance during the next years they might represent a secretion bottleneck.

2 | MATERIALS AND METHODS

2.1 | Fed-batch cultivation

Cells were seeded with a viable cell density (VCD) of 7 · 10^5 cells/ml in an initial volume of 60 ml Boehringer Ingelheim (BI, Biberach, Germany) proprietary production medium in a 250-ml shake flask (Corning, Oneonta, NY) and incubated at 34.5 °C in an orbital shaker incubator (Infors, Bottmingen, Switzerland) with 120 rpm (50 mm) and a CO₂ concentration of 10 % (v/v). The cultures were fed daily with 30 ml/L of culture start volume starting from Day 1 using BI proprietary feed medium. Since cultures were not fed on weekends, they received 60 ml/L of start volume feed on Fridays. Glucose levels were adjusted to 5 g/L via glucose bolus addition, if determined < 3 g/L. Relevant cellular and metabolic output parameters were determined daily: Cell concentration as well as viability was determined using the Cedex device (Roche Diagnostics, Mannheim, Germany) by means of trypan blue exclusion. A Biosen S-line analyser (EKF Diagnostics, Barleben, Germany) was used to determine glucose and lactate concentrations, pH and pO₂ were analyzed using a RAPiDlab®248 system (Siemens Healthcare, Erlangen, Germany). mAb concentration was determined by biolayer interferometry on an Octet®8 HTX system (Pall Life Science, Dreieich, Germany).

2.2 | Gene expression analysis

Total RNA was extracted from investigated CHO cells in triplicates using the RNA isolation Kit on the automated QIAsymphony® platform (Qiagen, Hilden, Germany). For library generation, 250 ng total RNA was processed with the TrueSeq Stranded mRNA Library Prep Kit (Illumina, San Diego, CA) according to the manufacturer’s instructions while using a total of 12 different unique indexes for multiplexing during analyses. Quality of isolated RNA and generated libraries was assessed using the High Sensitivity RNA analysis Kit (DNF-472, 15 nt) and Standard Sensitivity NGS Fragment Analysis Kit (DNF-473) on an Advanced Analytical Fragment Analyzer™ (Advanced Analytical Technologies, Heidelberg, Germany), respectively. The quantification of generated sequencing libraries was performed with the Quant-it™ ProGreen® dsDNA Kit (Invitrogen) analyzed with a Tecan Infinite® 200 Pro Reader (Tecan Group, Männedorf, Switzerland). By using the unique multiplexing indexes, each of the 12 libraries was pooled for sequencing on a HiSeq4000 instrument (Illumina) in single-end mode and 85 cycles. After demultiplexing, acquired data were quality trimmed using trimomatic (Bolger, Lohse, & Usadel, 2014) and mapped to the BI proprietary CHO K1 genome using STAR (Dobin et al., 2013). For quantification of mAb heavy chain (HC) and antibody light chain (LC) transcripts, data were mapped to the respective plasmids. Gene expression was quantified based on algorithms of the Cufflinks Suite (Trapnell et al., 2010) and standardized on fragments per kilobase million (FPKM) values. For statistical analysis of differentially expressed genes, the contrast model within the Profiler Genome software (Genedata, Basel, Switzerland) was used. Genes were considered to be significantly different when the Benjamini–Hochberg false discovery rate BHQ < 0.05 and a fold change (FC) > 1.2 and FC < 1/1.2, respectively. Functional annotation of significantly regulated genes was performed using David Bioinformatics Resources (Huang, Sherman, & Lempicki, 2009a; 2009b).

2.3 | Western blot (WB) analysis

For routine protein analyses, cells were lysed for 30 min on ice using Western blot lysis buffer (1 % Triton X-100 [v/v], 50 mM Tris (hydroxymethyl)-aminomethan (TRIS), 150 mM NaCl, pH = 7.5) and
vortexed approximately every 10 min. After centrifugation, lysates were separated within NuPAGE® 4–12 % or 10 % Bis-Tris Gels (Invitrogen, Carlsbad, CA) and transferred to a nitrocellulose membrane using the iBlot® 2 dry blotting system (Invitrogen). The antibodies used for HC (#Fab98616; Abcam, Cambridge, UK) as well as LC (#K3502, Sigma Aldrich, St. Louis, MO) detection were diluted 1:20,000 and 1:5,000, respectively. Secondary IRDye® 800CW-labeled donkey anti-mouse (#925–32212), IRDye® 680RD-labeled donkey anti-goat (#925–68074) and IRDye® 800CW-labeled donkey anti-rabbit (#926–32213) antibodies were diluted 1:5,000 (LI-COR, Lincoln, NE). An antibody against β-actin (#4970; Cell Signaling Technologies, Cambridge, UK) was used as loading control in a Odyssey Infrared Scanning System (LI-COR).

2.4 | Immunocytochemistry for confocal microscopic analysis

The procedure for immunocytochemistry was described elsewhere (Mathias et al., 2018).

2.5 | Transmission electron microscopy

CHO cells were fixed using prewarmed fixing solution (4 % paraformaldehyde [v/v], 2 % Glutaraldehyde [v/v, Sigma Aldrich], phosphate buffered saline [PBS]) for 30 min at 37 °C. Depleted fixing solution was replaced by ice-cold fixing solution and incubation continued for 1.5 hr on ice. The samples were washed three times with ice-cold PBS for 10 min each and stained with 1 % osmium tetroxide (v/v) for 1 hr on ice. Subsequently, specimens were dehydrated through a graded ethanol series (30 % [v/v], 50 % [v/v], 70 % [v/v], 90 % [v/v], 100 % [v/v]) and embedded into Glycid ether/Methylnadic/Dodecylsuccinic acid/Tris(dimethyl-aminoethyl)phenol epoxy resin (Serva, Heidelberg, Germany) overnight at 70 °C in an oven (Binder, Tuttlingen, Germany). On the next day, ultrathin sections were prepared with the ultramicrotome Ultracut UCT (Leica Microsystems, Wetzlar, Germany) and investigated on the transmission electron microscope LEO 912 AB (Zeiss, Oberkochen, Germany).

2.6 | Cell lysate separation using ultracentrifugation

Investigated CHO cells were lysed with UW lysis buffer (1 % Triton X-100 [v/v], 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES], 150 mM KCl, pH = 7.8) for 30 min on ice and subsequently separated into soluble and insoluble fractions by centrifugation at 100,000 g and 4 °C for 1 hr using the ultracentrifuge Optima XE-90 (Beckman Coulter, Bera, CA). The insoluble components were resuspended in the original sample volume with UW lysis buffer and solubilized using sonication (Bandelin, Berlin, Germany). Equal volumes of insoluble and soluble fraction were used for WB analyses.

2.7 | Gel mobility shift assay

CHO cells were lysed with WB lysis buffer supplemented with either 20 mM PEG5000-maleimide (Sigma Aldrich) or N-ethylmaleimide (Alfa Aesar) for 30 min on ice to modify free thiols. After centrifugation, an immunoprecipitation of the free mAb LC was performed using a mouse anti-human κ free LC antibody (MAB8461; Abnova, Taiwan), which was desthiobiotinylated with the DSB-X Biotin Protein Labeling Kit (D20655; Thermo Fisher Scientific) according to the manufacturer’s instructions. In brief, after addition of the desthiobiotinylated mouse anti-human κ free LC antibody to the centrifuged cell lysates, the samples were incubated overnight at 4 °C on a rotary shaker. Afterward, equilibrated Pierce™ Streptavidin Plus UltraLink™ Resin (Thermo Fisher Scientific) was added and the samples were further incubated for 1 hr at 4 °C. The beads were washed several times with ice-cold WB lysis buffer and subsequently subjected to WB analyses.

2.8 | Coimmunoprecipitation experiments

For coimmunoprecipitation experiments, CHO cells were harvested and lysed with UW lysis buffer. After centrifugation, a biotinylated anti-human IgG (HC+LC) antibody fragment (#709-066-149; Jackson ImmunoResearch) was added and samples subsequently incubated overnight at 4 °C on a rotary shaker. The immunocomplexes were purified as described earlier and washed several times with 50 mM ammonium bicarbonate pH = 8.5. Isolated proteins were directly digested on the beads using Trypsin (Roche, Penzberg, Germany). The resulting peptides were desalted using C18 stage tips (Supelco; Sigma Aldrich), separated using a 2 hr high performance liquid chromatography (HPLC) gradient and identified by a Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific). Obtained data were evaluated using Scaffold software version 4.8.6. (Proteome Software, Portland, OR).

2.9 | Glycan profile analysis

Purification of secreted mAbs was performed using a XK 16/20 column (GE Healthcare, Munich, Germany) run with MabSelect™ SuRe™ resin (GE Healthcare) on an ÄKTApurifier. Elution was performed with sodium citrate buffer pH = 2.8 and adjusted to pH = 6.0.

Intracellular antibody was purified using an anti-human IgG fragment crystallisable (Fc) antibody fragment, which was desthiobiotinylated with the DSB-X Biotin Protein Labeling Kit (D20655; Thermo Fisher Scientific) according to the manufacturer’s instructions. Purification of the immunocomplexes was performed as described earlier. The beads were washed several times with ice-cold sodium citrate pH = 6.0 and purified antibody eluted with 50 mM biotin, 25 mM TRIS, pH = 10. The buffer was afterwards exchanged to sodium citrate pH = 6.0 using 10 kDa MWCO PES Vivaspin 500 filter units (GE Healthcare).
For N-linked glycosylation analysis, samples were diluted in 0.1 % formic acid, reduced with 5 mM Tris(2-carboxyethyl) phosphin (TCEP), and separated on a LaChrom Ultra HPLC system (VWR, Radnor, PA) using a reverse-Phase MAbPac RP column (Thermo Fisher Scientific). Proteins were eluted with 0.1 % formic acid and 0.1 % formic acid in acetonitrile. Mass spectrometric analysis was performed with a micrOTOF-Q II (Bruker Daltonik, Billerica, MA). Spectra were summed, deconvoluted, and smoothed using DataAnalysis (Bruker Daltonik).

3 | RESULTS

3.1 Identification of an intracellular processing bottleneck in an immunoglobulin producing cell line during fed-batch cultivation

Although mAbs belong to a class of recombinant therapeutic proteins that can routinely be produced in the multigram per litre range, some molecules turn out to be DTE. A low productivity of stable cell lines represents a severe disadvantage of a biological drug candidate and can lead to termination of product development. To explore the intracellular fate of a DTE mAb, we assessed its location in a stable CHO production cell line.

The production capacity of a highly productive reference CHO cell line (mAb1) was compared with a DTE mAb2 producing CHO cell line. Both recombinant proteins were monoclonal IgG1 antibodies with x LCs (Figures S1 and S2). However, in a 12-day fed-batch cultivation process, both investigated cell lines showed comparable growth behavior with a peak VCD of approximately $1.0 \times 10^7$ cells/ml and $1.4 \times 10^7$ cells/ml, respectively, as well as high viability (Figure 1a). The VCD and viability of both cell lines started to decline from Day 11 onward. Final mAb concentrations in the supernatant of 4.75 g/L ± 0.29 g/L and 0.86 g/L ± 0.06 g/L were determined for the mAb1 and mAb2 producing cell line on Day 12, respectively (Figure 1b). Consequently, the calculated cell-specific productivity (qP) of the mAb2 producing cell line was > 80 % lower compared to the mAb1 producing cell line. To exclude that inefficient transcription and translation processes were the reason for inferior protein secretion in case of mAb2, classical biochemical analyses were performed. Even though the mAb2 producing cell line showed significantly reduced mRNA levels of both LC and HC in comparison to the mAb1 producing cell line (Figure 1c), WB analyses confirmed that the respective messenger RNAs (mRNAs) were sufficiently translated in both cell lines (Figure 1d). Interestingly, intracellular HC and LC protein concentrations were determined to be higher in the mAb2 producing CHO cell line. In addition, the N-glycosylation pattern of the secreted and subsequently Protein A purified mAbs revealed that mAb2 antibodies exhibited much less maturated glycans as indicated by a lower degree of galactosylation and increased amounts of high-mannose type glycans, which pointed toward an intracellular processing issue of mAb2 (Figure 1e,f). These data suggested that neither transcription nor translation processes sufficiently explained the particular low secretion of the DTE protein. However, increased intracellular levels of mAb2 protein and an impaired glycosylation pointed towards an intracellular processing bottleneck.

3.2 Immunofluorescence based cellular analyses revealed impaired endoplasmic reticulum structures in the mAb2 producing CHO cell line

After translocation into the ER, secreted proteins are processed in different organelle structures, therefore we decided to analyze the distribution of the investigated mAbs within these distinct compartments to identify unproductive or saturated organelles in respect to recombinant protein processing. Fluorescence microscopy of the LC (Figure 2) and HC (Figure S3) of the recombinant proteins and concurrent visualization of the distinct secretory organelles, ER and Golgi apparatus was performed. Interestingly, the ER of the highly productive mAb1 producing CHO cell line was composed of very thin tubules, which were distributed throughout the entire cytoplasm (Figure 2). In contrast, the mAb2 producing CHO cell line displayed a heavily altered ER morphology showing very large and possibly inflated but separated spherical structures, which colocalized with the staining pattern of the mAb2 protein and thus putatively included antibody deposits. This aberrant morphology was found in five individually analyzed clonal cell lines stably producing mAb2 (Figure S4).

After folding and assembly in the lumen of the ER, secreted proteins are transported to the Golgi apparatus consisting of stacks of membrane-enclosed cisternae. Here, proteins are further modified and packed into secretory vesicles for final secretion. Notably, the mAb2 producing cell line showed no or only weak colocalization of the Golgi marker and mAb fluorescence staining, whereas a very strong signal for the produced recombinant mAb was observed in the Golgi apparatus of mAb1 producing cells (Figure 2 and Figure S5). In conclusion, immunofluorescence based microscopic analyses revealed putative antibody deposits in the lumen of the ER possibly pointing toward intracellular aggregation of mAb2.

3.3 Intracellular aggregation of mAb2 and oxidative stress is not the causative for inefficient mAb2 secretion

Since immunofluorescence based microscopic analyses revealed putative antibody deposits and thus an aberrant ER morphology, we investigated the degree of intracellular protein aggregation of mAb2. In contrast to the control cell line, EM pictures confirmed a distinct ER morphology within the mAb2 producing cell line showing aberrant and possibly inflated or overloaded larger round-shaped structures (Figure 3a,b). However, no electron dense and thus aggregation indicative structures were identified inside the vesicular ER structures (Figure 3c). Furthermore, separation of cell lysates into soluble as well as insoluble
fractions using ultracentrifugation and WB analyses (Figure 3d) confirmed for both mAb producing cell lines that only marginal amounts of the HC could be found in the insoluble fraction of the cell lysate. In addition, it was shown that purified inflated vesicles can be dissolved completely using Triton X-100 (Figure S6) indicating that mAb2 did not form intracellular detergent resistant protein aggregates. Subsequent investigation of oxidative stress levels revealed no differences between both cell lines demonstrating that vesicularization of the ER is not a consequence of oxidative stress (Figure S7).

These data demonstrated that oxidative stress or intracellular protein aggregation of mAb2 within the lumen of the ER is not the reason for the reduced protein secretion.

3.4 Incomplete folding of the LC hampers mAb2 from efficient intracellular transport and secretion

In the ER, proteins assigned for final secretion are cotranslationally folded and assembled into multifunctional complexes. A fully assembled antibody of subtype γ is composed of two identical LCs and two identical
HCs, which are interconnected via disulfide bridges. To access antibody assembly, production cell samples were subjected to nonreducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS‐PAGE) and subsequent WB analysis. LC and HC displayed all possible antibody assembling entities, however, the total intracellular amount was increased for all entities in the mAb2 producing CHO cell line (Figure 4a), indicating that the DTE antibody mAb2 was as efficiently assembled as the highly secreted mAb1. However, in contrast to mAb1, free LC of the mAb2 antibody displayed two distinct bands with different intensities (Figure 4a). These two bands putatively indicated different folding states of the LC where individual intradomain disulfide bridges have not been formed due to incomplete or slow folding. As a consequence, the amount of completely folded and thus entirely oxidized cysteines was assessed using a gel mobility shift assay (Figure 4b). Free LC molecules were isolated from cell lysates, coupled to PEG5000‐maleimide (reacts specifically with cysteine residues) and subsequently subjected to SDS‐PAGE and WB

**FIGURE 2** Immunofluorescence based cellular distribution analyses of the produced monoclonal antibodies showed impaired endoplasmic reticulum (ER) structures in the mAb2 producing cell line. Confocal images of the investigated cell line visualizing the distribution of the produced recombinant protein (LC) within the secretory organelles ER and Golgi apparatus. For superior visualization of the distinct organelles, cells have been cultured adherently for this analysis only. Investigated cell lines were fixed and the produced recombinant proteins, the ER as well as the Golgi apparatus were visualized with immunofluorescent antibodies and are shown in red, green, and magenta, respectively. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; blue). Pictures were acquired at ×63 primary magnification. The scale bar represents 10 µm [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 3** Examination of the intracellular aggregation of the investigated monoclonal antibodies within the respective production cell lines indicated no antibody deposits. (a) Transmission electron microscopic image of the mAb1 producing cell line using a magnification of × 4,000. (b) Transmission electron microscopic image of the mAb2 producing cell line using a magnification of × 3,150. The dotted square is enlarged in (c) using a magnification of × 10,000. (d) Recombinant protein heavy chain (HC) and light chain (LC) detection using Western blot (WB) analyses of cell lysate samples which were separated in soluble and insoluble fraction by ultracentrifugation. M, marker [Color figure can be viewed at wileyonlinelibrary.com]
The detection of the LC. The three different potential folding intermediates are completely unfolded LC, partially folded, and entirely folded free LC molecules displaying five, three, and one free cysteine for efficient coupling, respectively (Figure 4c). Confirmatively, the highly secreted antibody mAb1 only showed marginal amounts of incompletely or partially folded free LC molecules, whereas the free LC of the DTE mAb2 was mainly partially folded or misfolded indicated by one disulfide bridge which has not been formed.

3.5 The LC of the DTE antibody mAb2 is less recognized by cellular disulfide bridge forming chaperones of the protein disulfide isomerase family

In eukaryotes, protein resident cysteines are oxidized by chaperones of the PDI family in the lumen of the ER. Therefore, coimmunoprecipitation experiments of both antibodies with subsequent mass spectrometry (MS) analyses were used to identify differentially bound proteins and chaperones (Figure 5a).

![Figure 4](wileyonlinelibrary.com) 

**FIGURE 4** Intracellular antibody assembly and light chain (LC) folding indicated that incomplete folding of the LC hampered mAb2 from efficient secretion. (a) Western blot (WB) analyses of antibody assembling entities by detection of heavy chain (HC) and LC under nonreducing but denaturing conditions, respectively. β-actin was used as loading control. (b) Gel mobility shift assay detecting not formed intradomain disulfide bridged in the antibody LC. In brief, available cysteines of the free LC were coupled to PEG₅₀₀₀⁻maleimide and the respective molecule subsequently purified and subjected to WB analyses. Representative result of three independent WBs. (c) Intermediate folding entities during intracellular LC folding. M, Marker; PEG, polyethylene glycol [Color figure can be viewed at wileyonlinelibrary.com]
Interestingly, PDIs (PDIA4, PDIA1, PDIA6) were found to bind more heavily or exclusively to mAb1 molecules indicating a possible inaccessibility of mAb2 for oxidation of its cysteines to form intradomain disulfide bridges. To validate these results, WB analyses of coimmunoprecipitation experiments were carried out and showed comparable binding of the molecular chaperone BIP (Figure S8) but less binding of PDI to the mAb2 LC indicating that it is not recognized correctly by PDIs putatively due to improper folding (Figure 5b). In addition, other protein classes were found to bind differentially to mAb1 and mAb2 molecules including members of the heat shock protein family (e.g., HSP90β1, HSPA8) binding more intensively to mAb1 but prolyl-peptidyl isomerases (PPIA, PPIB) binding exclusively to mAb2. In summary, incomplete formation of disulfide bridges may lead to incomplete folding hampering mAb2 from efficient secretion.

3.6 | mAb2 is partially degraded via the ERAD pathway

Since the DTE antibody mAb2 accumulated in the lumen of the ER probably due to incomplete folding of the LC, it was of interest to examine the adaptation process of the cell line. Using transcriptome analysis of both mAb2 and mAb1 cell lines and subsequent functional clustering of differentially transcribed genes, protein processing mechanisms in the ER were shown to be significantly affected by mAb2 production (Figure 6a). While regular protein processing pathways were downregulated in the mAb2 producing CHO cell line, proteins belonging to the ERAD pathway were predominantly enriched indicating enhanced protein degradation (Figure 6b). Especially chaperones recruiting misfolded proteins for degradation via ERAD and effector proteins of the respective ubiquitin ligase complex (Figure 6c) were transcriptionally upregulated. Finally, subunits of the proteasome for final protein degradation were also upregulated indicating that the incomplete folding and accumulation of mAb2 in the lumen of the ER possibly led to an enhanced clearance of the secretory organelle from adverse protein chains. Interestingly, the UPR was not explicitly triggered (Figure S9). To further investigate the intracellular fate of the antibody and access possible degradation of mAb2 via ERAD, the intracellular glycosylation of purified mAb2 was investigated (Figure 7a). Glycosylation was found to be mainly of the premature high-mannose type (Figure 7b, Man9-Man6) usually found in the early secretory pathway (Aebi, Bernasconi, Clerc, & Molinari, 2010; Hebert, Garman, & Molinari, 2005; Helenius & Aebi, 2004). In addition, intracellular trimmed glycan branches like Man7, Man6, and Man5 indicated degradation of the respective chains via ERAD (Aebi et al., 2010; Ferris, Kodali, & Kaufman, 2014; Hebert et al., 2005). Grouping of the individual glycan forms clearly highlighted that nearly half of the molecules of mAb2 are marked for proteosomal degradation via ERAD (Figure 7c).

![FIGURE 6](image-url) Gene expression analyses putatively indicated degradation of mAb2 via ER-associated degradation (ERAD). (a) Canonical pathways which are affected by the production of the difficult-to-express antibody mAb2 in comparison to mAb1. (b) Transcriptionally upregulated and downregulated proteins in respect to mAb2 production belonging to major biochemical pathways within the ER: unfolded protein response (UPR), general protein processing, and ERAD. (c) Detailed pathway map of the ERAD process and components of the respective ubiquitin ligase complex and proteasome where upregulated and downregulated genes are indicated by green and red arrows, respectively. The indication of an upregulation and downregulation of a respective protein means that the function is conducted by different proteins of which some were upregulated and downregulated during the analysis, respectively. A gene was considered to be regulated with a Benjamini–Hochberg false discovery rate BHQ < 0.05 and a fold change (FC) > 1.2 and FC < 1/1.2, respectively. The figure was modified from Kyoto Encyclopedia of Genes and Genomes (KEGG) Database with permission (Kanehisa & Goto, 2000; Kanehisa, Sato, Furumichi, Morishima, & Tanabe, 2017; 2019). A full list of proteins and FC is available in Table S2. Same analyses were performed for four additional mAb2 producing cell lines (Figures S10–S13). ER, endoplasmic reticulum; mAb, monoclonal antibody.
FIGURE 7 Glycan profile analysis of the intracellular accumulated antibody mAb2 validated massive degradation via ER-associated degradation (ERAD). (a) Abundance of different glycan forms were analyzed using mass spectrometry (MS). The antibody was purified as described and subsequently subjected to MS under reducing conditions. (b) Overview of different glycan forms and physiological meaning. (c) Summary of identified glycan forms and their physiological significance [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

Since mammalian expression systems can show low performance in the production of the sustainably emerging complex protein formats and DTE antibodies, there is an urgent need to identify any rate limiting processing step. Even though the DTE protein mAb2 producing cell line showed significantly reduced mRNA levels in comparison to the mAb1 producing cell line, it is highly likely that the detected amount of transcripts enables high protein production. While there is a significant correlation between the protein abundance and mRNA levels, it was also shown that only part of the regulation of a protein’s abundance can be explained by varying transcript levels, which is further complemented by mechanisms as degradation and translation rates (de Sousa Abreu, Penalva, Marcotte, & Vogel, 2009; Vogel & Marcotte, 2012). Therefore the reduced mRNA levels did not sufficiently explain the particular low production of mAb2 in CHO cells. However, immunofluorescence based microscopic analyses of the production cell lines revealed an aberrant ER morphology, in which the normally observed interconnected network of sacs and tubules collapsed to become large and distinct round-shaped structures (Schwarz & Blower, 2016). In other reports such an aberrant ER morphology has also been referred to as Russell Bodies (RBs), where IgGs aggregate in the lumen of the ER or derived vesicles thereof and can neither be degraded nor secreted (Kopito & Sitia, 2000). Similar structures can be found in plasma cells undergoing excessive synthesis of IgGs, especially as a manifestation of physiological abnormalities like multiple myeloma, inflammatory diseases, and autoimmune disorders (Decourt, Galea, Sirac, & Cogné, 2004; Jiang et al., 1997). Nevertheless, RBs have also been identified in recombinant IgG producing cell lines such as CHO and HEK293 (Hasegawa et al., 2017; Hasegawa, Woods, Kinderman, He, & Lim, 2014; Stoops, Byrd, & Hasegawa, 2012) as well as during over-expression of bspecific antibody formats in CHO cells (Mathias et al., 2018). Interestingly, this vesicularized phenotypic appearance of the ER has also been described independently in the context of several different and very diverse conditions including oxidative stress (Hendershot et al., 1995; Wei et al., 2012), downregulation of chaperones (Lee, Chu, Iwakoshi, & Glimcher, 2005; Li et al., 2008), and parts of the ERAD machinery (Sun et al., 2014). In contrast to these reports, we observed no evidence for oxidative stress (Figure S7) or downregulation of chaperones while analyzing CHO cells expressing the DTE antibody mAb2. In addition, it was clarified that
mAb2 did not form intracellular aggregates. The described aberrant ER morphology was detected in all investigated mAb2 producing cell lines (total number of five) even under different culture conditions as well as time points in cultivation and therefore did neither represent a clone-specific phenomenon nor was it exclusively induced by exogenous stress conditions (Figure S4).

However, when analyzing folding of the antibody, it became apparent that the disulfide bridge within the free mAb2 LC was not formed. As both antibodies share the same LC constant sequence (Figure S1), we speculated that it might be the variable domain of the mAb2 LC that hampers mAb2 from efficient transport and secretion. It may rapidly adopt an atypical folding state during translation and therefore is not readily recognized by ER resident chaperones catalyzing the formation of the respective disulfide bridge. Indeed, we showed that the incompletely folded mAb2 LC was less bound by the chaperone PDI, which may lead to a deficient catalysis of disulfide bridges. By expressing several noncognate antibody HC/LC pairs, Stoops et al. (2012) showed that a LC’s manufacturability was sufficient to promote RB formation, which finally resulted in low secretion of the respective antibody. But contrary to this, an LC’s manufacturability alone was not sufficient to ensure high secretion levels. The generation of noncognate mAb1 LC/mAb2 HC pairs and vice versa might further elucidate the detrimental properties of mAb2 LC.

Despite the heavily disrupted ER morphology, which was identified by microscopy, mAb2 production cells grew well and stayed viable under fed-batch cultivation pointing toward an at least partially functional ER. In addition, mAb2 producing CHO cells were still able to secrete approximately 10 pg/(cell·day) representing appreciable amounts of recombinant protein. Therefore, secretion might either be facilitated by not detectable but remaining amounts of functional ER or by secretion of residual mAb2 from affected RB-shaped ER structures. However, within these structures, we were able to show that also fully assembled entities of mAb2 were retained (Figure 4), which might be caused by the accidental assembly of some HCs with only partially folded LCs. It remains unknown whether those antibodies with partially folded LC and free LC can also be secreted into the production medium since proteins assigned for secretion displaying free cysteines are actively retained (Alberini, Bet, Milstein, & Sitia, 1990). However, it was shown that antibodies can escape cellular protein quality control mechanisms as some HCs possess constant HC domain 1 (CH1), which are inherently folded and thus secretion might occur without LC assembly (Stoyle et al., 2017).

As overproduction of mAb2 caused a massive aberrant ER phenotype, we analyzed effects on cellular pathways by transcriptome analysis to elucidate possible escape routes or survival mechanisms. The observed cellular reaction included a general downregulation of genes involved in processes such as protein synthesis and translocation as well as an upregulation of genes involved in protein degradation processes as ERAD (Figure 6). Again, it did not represent a clonal phenomenon as the ERAD pathway was upregulated in four additional and independently analyzed mAb2 producing cell lines (Figure S10–S13). Since misfolded proteins are tagged for ERAD by clipping of mannose residues (Ferris et al., 2014; Slominska-Wojewodzka & Sandvig, 2015), we subsequently determined the glycan profile of purified intracellular mAb2 and found increased number of glycan species which tag the protein for proteasomal degradation (Figure 7). Since intracellular mAb2 was purified using an Fc directed antibody, only fully assembled mAb2 and free HC were analyzed suggesting that also fully assembled antibody might be designated for degradation, possibly due to assembly with only partially folded LC. These amounts of proteins assigned for degradation via ERAD possibly overloaded the respective machinery and the resulting massive accumulation of proteins may in consequence represent the reason for the observed aberrant ER morphology in mAb2 producing CHO cells.

The UPR is a cellular stress response mechanism to an accumulation of unfolded proteins within the lumen of the ER (Hetz, 2012). It is enabled by the stress sensors activating transcription factor 6 (ATF6), protein kinase RNA-like ER kinase (PERK), and inositol-requiring protein 1α (IRE1α) by releasing BIP (Hetz, 2012; Hussain, Maldonado-Agurto, & Dickson, 2014; Ma & Hendershot, 2002). BIP is one of the central chaperones recognizing and interacting with exposed hydrophobic sequences of newly synthesized proteins actively maintaining its clients in a folding competent state (Behnke, Mann, Scruggs, Feige, & Hendershot, 2016; Pobre, Poet, & Hendershot, 2019). Interestingly, the UPR was not explicitly triggered in mAb2 producing CHO cells since ATF6, PERK, IRE1α downstream target genes were not upregulated (Figure S9), even though we clearly showed that mAb2 accumulated within the lumen of the ER and ER morphology was heavily disrupted. In addition, even if BIP was not upregulated in its expression, still no expected stronger interaction between BIP and the incompletely folded mAb2 LC in comparison to mAb1 was detected (Figure S8) and thus UPR not triggered. However, Hellman, Vanhove, Lejeune, Stevens, and Hendershot (1999) showed that the association with the chaperone BIP is not simply determined by the presence of sequences but also the folding state and protein stability. This again indicated that the mAb2 LC may adopt some kind of folded or stable state, which might impede terminal folding through PDIs. Subsequent investigations will show whether overexpression of PDI can rescue the respective phenotype.

It was demonstrated that mAb2 may be hampered from efficient secretion due to an only partially folded LC, which did not form all necessary intradomain disulfide bridges. Thus, this study revealed that with the continuous emergence of artificially designed and DTE therapeutic protein candidates more attention needs to be drawn to molecule design as well as detailed sequence investigations. Especially, LC sequences are of importance as the LC significantly contributes to the folding rate of the respective HC and thus the overall assembly and secretion of mAbs (Feige et al., 2009; Lee, Brewer, Hellman, & Hendershot, 1999).

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests. S. Mathias, A. Wippermann, I. Gorr, P. Schulz, S. Fischer, and M. Gamer are employees of Boehringer Ingelheim, which sells pharmaceuticals.

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REFERENCES

Aebi, M., Bernasconi, R., Clerc, S., & Molinari, M. (2010). N-glycan structures: Recognition and processing in the ER. Trends in Biochemical Sciences, 35(2), 74–82. https://doi.org/10.1016/j.tibs.2009.10.001
Alberini, C. M., Bet, P., Milstein, C., & Sita, R. (1990). Secretion of immunoglobulin M assembly intermediates in the presence of reducing agents. Nature, 347(18), 485–487. https://doi.org/10.1038/347485a0
Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). The Molecular Biology of the Cell. NY: Garland Pub.
Appenzeller-Herzog, C. (2011). Glutathione- and non-glutathione-based oxidant control in the endoplasmic reticulum. Journal of Cell Science, 124(6), 847–855. https://doi.org/10.1242/jcs.080895
Behnke, J., Mann, M. J., Scruiggs, F., Feige, M. J., & Hendershot, M. (2016). Members of the Hsp70 family recognize distinct types of sequences to execute ER quality control. Molecular Cell, 63(5), 739–752. https://doi.org/10.1016/j.molcel.2016.07.012

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics, 30(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Braakman, I., & Hebert, D. (2013). Protein folding in the endoplasmic reticulum. Cold Spring Harbor Perspectives in Biology, 5(5), a013201. https://doi.org/10.1098/R978-0-08-088504-9.00029-5

Decourt, C., Galea, H. R., Sirac, C., & Cogné, M. (2004). Immunologic basis for the rare occurrence of true nonsecretory plasma cell dyscrasias. Journal of Leukocyte Biology, 76(3), 528–536. https://doi.org/10.1189/jlb.0803382

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., ..., Gingeras, T. R. (2013). STAR: Ultrafast universal RNA-seq aligner. Bioinformatics, 29(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635

Ellgaard, L., & Helenius, A. (2003). Quality control in the endoplasmic reticulum. Nature Reviews Molecular Cell Biology, 4(3), 181–191. https://doi.org/10.1038/nrm1052

Feige, M. J., Grosscurth, S., Marcinowski, M., Shimizu, Y., Kessler, H., Hendershot, L. M., & Buchner, J. (2009). An unfolded CH1 domain controls the assembly and secretion of IgG antibodies. Molecular Cell, 34(5), 569–579. https://doi.org/10.1016/j.freeradbiomed.2008.10.025

Ferris, S. P., Kodali, V. K., & Kaufman, R. J. (2014). Glycoprotein folding and quality-control mechanisms in protein-folding diseases. Disease Models & Mechanisms, 7(3), 331–341. https://doi.org/10.1242/dmm.014589

Hasegawa, H., Hsu, A., Tinberg, C. E., Siegler, K. E., Aaron, A., & Tsai, M. (2017). Single amino acid substitution in LC-CDR1 induces Russell body phenotype that attenuates cellular protein synthesis through eIF2 α phosphorylation and thereby downregulates IgG secretion despite operational secretary pathway traffic. mAbs, 9(5), 854–873. https://doi.org/10.1080/19420862.2017.1314875

Hasegawa, H., Woods, C. E., Kinderman, F., He, F., & Lim, A. C. (2014). Russell body phenotype is preferentially induced by IgG mAb clones with high intrinsic condensation propensity: Relations between the biosynthetic events in the ER and solution behaviors in vitro. mAbs, 6(6), 1518–1532. https://doi.org/10.4161/mabs.36242

Hebert, D. N., Garman, S. C., & Molinari, M. (2005). The glycan code of the endoplasmic reticulum: Asparagine-linked carbohydrates as protein maturation and quality-control tags. Trends in Cell Biology, 15(7), 364–370. https://doi.org/10.1016/j.tcb.2005.05.007

Helenius, A., & Aebi, M. (2004). Roles of N-Linked glycans in the endoplasmic reticulum. Annual Review of Biochemistry, 73(1), 1019–1049. https://doi.org/10.1146/annurev.biochem.73.110303.073752

Hellman, R., Vanhove, M., Lejeune, A., Stevens, F. J., & Hendershot, L. M. (1999). The in vivo association of BiP with newly synthesized proteins is dependent on the rate and stability of folding and not simply on the presence of sequences that can bind to BiP. The Journal of Cell Biology, 144(1), 21–30. http://jcb.rupress.org/content/144/1/21.full.pdf+html

Hendershot, L. M., Wei, J. Y., Gaut, J. R., Lawson, B., Freiden, P. J., & Murti, K. G. (1995). In vivo expression of mammalian BIP ATPasE mutants causes disruption of the endoplasmic reticulum. Molecular Biology of the Cell, 6(3), 283–296. https://doi.org/10.1091/mbc.6.3.283

Hetz, C. (2012). The unfolded protein response: Controlling cell fate decisions under ER stress and beyond. Nature Reviews Molecular Cell Biology, 13(2), 89–102. https://doi.org/10.1038/nrm3270

Huang, D. W., Sherman, B. T., & Lemppicki, R. A. (2009a). Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Research, 37(1), 1–13. https://doi.org/10.1093/nar/gkn923

Huang, D. W., Sherman, B. T., & Lemppicki, R. A. (2009b). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature Protocols, 4(1), 44–57. https://doi.org/10.1038/nprot.2008.211

Hussain, H., Maldonado-Agurto, R., & Dickson, A. J. (2014). The endoplasmic reticulum and unfolded protein response in the control of mammalian recombinant protein production. Biotechnology Letters, 36(8), 1581–1593. https://doi.org/10.1007/s10529-014-1537-y

Jiang, Y., Hirose, S., Hamano, Y., Kodera, S., Tsurui, H., Abe, M., ..., Shirai, T. (1997). Mapping of a gene for the increased susceptibility of B1 cells to Mott cell formation in murine autoimmune disease. Journal of Immunology, 158(2), 992–997. http://www.ncbi.nlm.nih.gov/pubmed/8993021

Johari, Y. B., Estes, S. D., Alves, C. S., Sinacore, M. S., & James, D. C. (2015). Integrated cell and process engineering for improved transient production of a “difficult-to-express” fusion protein by CHO cells. Biotechnology and Bioengineering, 112(12), 2527–2542. https://doi.org/10.1002/bit.25687

Jost, C., & Plückthun, A. (2014). Engineered proteins with desired specificity: DARPinS, other alternative scaffolds and bispecific IgGs. Current Opinion in Structural Biology, 27(1), 102–112. https://doi.org/10.1016/j.sbi.2014.05.011

Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y., & Morishima, K. (2017). KEGG: New perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Research, 45(D1), D353–D361. https://doi.org/10.1093/nar/gkw1092
Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acid Research, 28(1), 27–30.

Kanehisa, M., Sato, Y., Furumichi, M., Morishima, K., & Tanabe, M. (2019). New approach for understanding genome variations in KEGG. Nucleic Acids Research, 47(D1), D590–D595. https://doi.org/10.1093/nar/gky962

Kopito, R. R., & Sitia, R. (2000). Aggresomes and Russell bodies. EMBO Reports, 1(3), 225–231. https://doi.org/10.1093/embo-reports/kvd052

Kunert, R., & Reinhart, D. (2016). Advances in recombinant antibody manufacturing. Applied Microbiology and Biotechnology, 100(8), 3451–3461. https://doi.org/10.1007/s00253-016-7388-9

Lee, A. H., Chu, G. C., Iwakoshi, N. N., & Glomcher, L. H. (2005). XBP-1 is required for biogenesis of cellular secretory machinery of exocrine glands. EMBO Journal, 24(24), 4368–4380. https://doi.org/10.1038/sj.emboj.7600903

Lee, G. W., Fecko, J. K., Yen, A., Donaldson, D., Wood, C., Tobler, S., … Leonard, M. (2007). Improving the expression of a soluble receptor:Fc fusion protein in CHO cells by coexpression with the receptor ligand. Cell Technology for Cell Products, 29–39. https://doi.org/10.1007/978-1-4020-5476-1_4

Lee, Y. K., Brewer, J. W., Hellman, R., & Hendershot, L. M. (1999). BiP and immunoglobulin light chain cooperateto control the folding of heavy chain and ensure the fidelity of immunoglobulin assembly. Molecular Biology of the Cell, 10(7), 2209–2219. https://doi.org/10.1091/mbc.10.7.2209

Li, J., Ni, M., Lee, B., Barron, E., Hinto, D. R., & Lee, A. S. (2008). The unfolded protein response regulator GRP78/BiP is required for endoplasmic reticulum integrity and stress-induced autophagy in mammalian cells. Cell Death & Differentiation, 15(9), 1460–1471. https://doi.org/10.1038/sj.cdd.4402576

Lodish, H., Berk, A., Zipursky, S. L., Matsudaira, P., Baltimore, D., & Darnell, J. (2000). Molecular Cell Biology (4th). New York: W.H. Freeman. https://doi.org/10.1002/bmb.8

Ma, Y., & Hendershot, L. M. (2002). The mammalian endoplasmic reticulum as a sensor for cellular stress. Cell Stress & Chaperones, 7(2), 222–229.

Mathias, S., Fischer, S., Hendrick, R., Fieder, J., Schulz, P., Bradl, H., … Otte, K. (2018). Visualisation of intracellular production bottlenecks in suspension-adapted CHO cells producing complex biopharmaceuticals using fluorescence microscopy. Journal of Biotechnology, 271(Feb-February), 47–55. https://doi.org/10.1016/j.jbiotec.2018.02.009

Olzmann, J. A., Kopito, R. R., & Christianson, J. C. (2013). The mammalian endoplasmic reticulum-associated degradation system. Cold Spring Harbor Perspectives in Biology, 5(9), 1–16. https://doi.org/10.1101/cshperspect.a013185

Pobrec, K. R. F., Poet, G. J., & Hendershot, L. M. (2019). The endoplasmic reticulum (ER) chaperone BiP is a master regulator of ER functions: Getting by with a little help from ERd friends. Journal of Biological Chemistry, 294(6), 2098–2108. https://doi.org/10.1074/jbc.REV118.002804

Pybus, L. P., James, D. C., Dean, G., Slidel, T., Hardman, C., Smith, A., … Field, R. (2014). Predicting the expression of recombinant monoclonal antibodies in Chinese hamster ovary cells based on sequence features of the CDR3 domain. Biotechnology Progress, 30(1), 188–197. https://doi.org/10.1002/btp.1839

Ruggiano, A., Foresti, O., & Carvalho, P. (2014). ER-associated degradation: Protein quality control and beyond. Journal of Cell Biology, 204(6), 869–879. https://doi.org/10.1083/jcb.201312042

Schwarz, D. S., & Blower, M. D. (2016). The endoplasmic reticulum: Structure, function and response to cellular signaling. Cellular and Molecular Life Sciences, 73(1), 79–94. https://doi.org/10.1007/s00018-015-2052-6

Slominska-Wojewodzka, M., & Sandvig, K. (2015). The role of lectin-carbohydrate interactions in the regulation of ER-associated protein degradation. Molecules, 20(6), 9816–9846. https://doi.org/10.3390/molecules20069816

de Sousa Abreu, R., Penalvaa, L. O., Marcotte, E. M., & Vogel, C. (2009). Global signatures of protein and mRNA expression levels. Molecular Systems Biology, 5(12), 1512–1526. https://doi.org/10.1039/b908315d

Spiess, C., Zhai, Q., & Carter, P. J. (2015). Alternative molecular formats and therapeutic applications for bispecific antibodies. Molecular Immunology, 67(2), 95–106. https://doi.org/10.1016/j.molimm.2015.01.003

Stoops, J., Byrd, S., & Hasegawa, H. (2012). Russell body inducing threshold depends on the variable domain sequences of individual human IgG clones and the cellular protein homeostasis. Biochimica et Biophysica Acta, 1823(10), 1643–1657. https://doi.org/10.1016/j.bbamcr.2012.06.015

Stoyle, C. L., Stephens, P. E., Humphreys, D. P., Heywood, S., Cain, K., & Bulleid, N. J. (2017). IgG light chain-independent secretion of heavy chain dimers: Consequence for therapeutic antibody production and design. Biochemical Journal, 474(18), 3179–3188. https://doi.org/10.1042/BCJ20170342

Sun, S., Shi, G., Han, X., Francisco, A. B., Ji, Y., Mendonca, N., … Qi, L. (2014). Sel1L is indispensable for mammalian endoplasmic reticulum-associated degradation, endoplasmic reticulum homeostasis, and survival. Proceedings of the National Academy of Sciences, 111(5), E582–E591. https://doi.org/10.1073/pnas.1318114111

Trapnell, C., Williams, B. a, Pertea, G., Mortazavi, A., Kwan, G., van Baren, M. J., … Pachter, L. (2010). Transcript assembly and abundance estimation from RNA-Seq reveals thousands of new transcripts and switching among isoforms. Nature Biotechnology, 28(5), 511–515. https://doi.org/10.1038/nbt.1621

Vogel, C., & Marcotte, E. M. (2012). Insights into the regulation of protein abundance from proteomics and transcriptomics analyses. Nature Reviews Genetics, 13(4), 227–232. https://doi.org/10.1038/nrg3185

Insights

Walsh, G. (2018). Biopharmaceutical benchmarks 18. Nature Biotechnology, 36(12), 1136–1145. https://doi.org/10.1038/nbt0706-769

Wei, P. C., Hsieh, Y. H., Su, M. I., Jiang, X. J., Hsu, P. H., Lo, W. T., … Lee, W. H. (2012). Loss of the oxidative stress sensor NPGPx compromises GRP78 chaperone activity and induces systemic disease. Molecular Cell, 48(5), 747–759.

Wu, X., & Rapoport, T. A. (2018). Mechanistic insights into ER-associated protein degradation. Current Opinion in Cell Biology, 53, 22–28. https://doi.org/10.1016/j.jceb.2018.04.004

Xu, C., Bailly-Maitre, B., & Reed, J. C. (2005). Endoplasmic reticulum stress: Cell life and death decisions. Journal of Clinical Investigation, 115(10), 2656–2664. https://doi.org/10.1172/JCI26373.2656

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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