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VIEWPOINT

Molecular chaperones: from proteostasis to pathogenesis

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Maintaining protein homeostasis (proteostasis) is essential for a functional proteome. A wide range of extrinsic and intrinsic factors perturb proteostasis, causing protein misfolding, misassembly, and aggregation. This compromises cellular integrity and leads to aging and disease, including neurodegeneration and cancer. At the cellular level, protein aggregation is counteracted by powerful mechanisms comprising of a cascade of enzymes and chaperones that operate in a coordinated multistep manner to sense, prevent, and/or dispose of aberrant proteins. Although these processes are well understood for soluble proteins, there is a major gap in our understanding of how cells handle misfolded or aggregated membrane proteins. This article provides an overview of cellular proteostasis with emphasis on membrane protein substrates and suggests host–virus interaction as a tool to clarify outstanding questions in proteostasis.

Introduction

Protein biogenesis is a highly complex and error-prone process. Cells maintain protein homeostasis via evolutionarily conserved protective mechanism called protein quality control (PQC), involving extensive chaperones and degradative pathways. When PQC encounters misfolded protein it is either repaired or disposed via the ubiquitin proteasomal system [1]. When this quality control fails, proteins can clump to form aggregates, which then undergo autophagic degradation [2]. Although vast amount of information regarding the cellular mechanism of soluble protein quality control is available, membrane proteins PQC process is poorly understood, especially in the context of how quality control factors coordinate to rectify the misfolded or aggregated membrane protein problem.

Viruses are outstanding tools to break new grounds in cell biology and disease mechanisms. In order to replicate and propagate, viruses are highly dependent on their host and they achieve this by hijacking host factors called 'cues'. Cues are receptors, enzymes, or chemicals, which directly or indirectly promote different stages of virus infection. The viruses, on the other hand trick these cues by either tuning or reprogramming their cellular role [3]. Detailed understanding of these cues have paved way for the development of crucial antiviral targets and also helped us understand the basic cellular processes [4]. Below, I will discuss our

Abbreviations
BiP, binding immunoglobulin protein; CFTR, cystic fibrosis transmembrane conductance regulator; DENV, Dengue virus; ERAD, ER-associated degradation; ER, endoplasmic reticulum; HCV, Hepatitis C virus; HPV, human papillomavirus; HSV-1, Herpes Simplex virus; IAV, Influenza A virus; NEF, nucleotide exchange factors; PQC, protein quality control; VACV, Vaccinia virus; ZIKV, Zika virus.

current knowledge and outstanding issues on proteostasis by comparing aberrant soluble versus membrane protein substrates, and also provide examples of host-virus interaction as a new strategy to tackle these issues.

Proteostasis

Aberrant soluble proteins: recognition, correction, and/or degradation

Nascent proteins are highly unstable and tend to misfold and/or entangle due to their chemical and physical properties [5]. PQC pathway deploys powerful molecular chaperones that recognize and triage misfolded clients (Fig. 1, Step 1). Different chaperones possess distinct modes of substrate recognition that determine their substrate range and specificity [6]. Among them the ubiquitous 70-kDa heat shock protein (Hsp70) family of chaperones is shown to be associated with plethora of misfolded and aggregated substrates, possibly selecting their targets for proteasomal or autophagy degradation. The Hsp70's activity, in turn, is regulated by a number of cofactors and cochaperones, together functioning as a 'machine' [7]. For instance, J-proteins prime the Hsp70's folding property by selecting and supplying the substrate to Hsp70 and also stimulate the Hsp70's ATPase activity, whereas nucleotide exchange factors (NEF) promote the exchange of ADP with ATP, to accelerate the cyclic reaction [8]. However, the identity of these machineries and its components can vary for different clients.

Besides recognizing and selecting the misfolded proteins, chaperones and associated factors also promote refolding, prevent aggregation, or triage these targets for degradation (Fig. 1, Step 2). For instance, the ATP-dependent refolding by chaperone binding and release involving Hsp70, a J-protein, and a NEF is well defined for several soluble proteins [7]. Among them the mostly widely understood are the model substrates processed in the endoplasmic reticulum (ER) lumen. The cellular organelle ER is the most crowed environment in the cell performing diverse cellular roles. Any dysfunction in the ER activity leads to accumulation of misfolded and/or unfolded proteins. Cells maintain ER proteostasis by deploying diverse array of ER-resident chaperones and enzymes, which process their client by correcting or priming them to degradative pathways. For example, in the case of misfolded secretory protein carboxypeptidase mutant CPY* and nonglycosylated pro-α-factor, the ER lumen Hsp70 called binding immunoglobulin protein (BiP) and its associated cochaperones efficiently process the misfolded proteins for ER-associated degradation (ERAD) (Fig. 1, Step 3 & 4) [9]. Similarly, ERAD of terminally misfolded α1-antitrypsin variant null Hong Kong and transthyretin mutant D18G are handled by BiP and a NEF, 170-kDa glucose-regulated protein (Grp170) [10] and processed by degradation pathways.

In case of aging diseases, when the above PQC system fails to repair or destroy severely damaged proteins, they tend to aggregate (Fig. 1, Step 5) causing diseases, such as Amyotrophic lateral sclerosis, Parkinson's disease, Huntington's disease, Alzheimer's

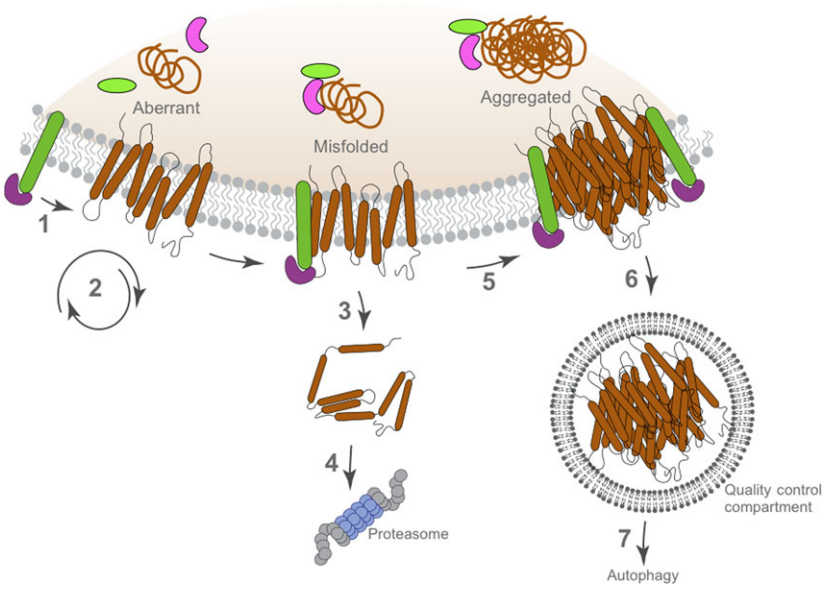

Fig. 1. The fate of aberrant proteins. Aberrant soluble or membrane proteins (brown) are recognized (1) by chaperones (green and magenta) and promote its refolding (2). When proteins misfold they are then extracted (3) into the cytosol and degraded (4) by proteasomal machinery. When proteins aggregate (5), it is then sequestered (6) into quality control compartments, and degraded (7) by autolysosomal pathway.

disease, type II Diabetes, etc. In most instances, the aggregate also recruits bystanders such as intermediately folded, and correctly folded species causing cytotoxicity and cell death [11]. Cells counteract these aggregates by sequestering them in special cytoplasmic quality control compartment (Fig. 1, Step 6) for refolding or autophagic degradation (Fig. 1, Step 7) [12]. Partitioning of misfolded proteins into compartments is an organized process that appears to be conserved from yeast to mammalian cells. Distinct compartments with specific characteristics have been observed, including 'aggresome' colocalizing with microtubule organizing center, 'perinuclear inclusion' that costain with ER markers, and 'insoluble inclusion' colocalizing with autophagic markers [13]. These structures serve several purposes, such as in concentrating toxic species, thereby reducing substrate burden on quality control systems, and orchestrating efficient repair.

For instance, in yeast, specific quality control compartments are reported to possess Hsp104 disaggregase activity. Although metazoans lack Hsp104 homolog, several recent reports have demonstrated the existence of a mammalian Hsp110-dependent disaggregase activity [14]. For example, Hsp110 is shown to stabilize Apolipoprotein from undergoing ERAD [15]; Hsp70 has been demonstrated to be transiently associated with polyQ protein aggregates, raising the possibility that it may be involved in disaggregating polyQ aggregates [16]. Similarly, overexpression of several Hsp40 family proteins along with Hsp70 has been shown to prevent accumulation of polyQ ataxia-1/3 in inclusions [17]. In the ER lumen, BiP prevents aggregation of a misfolded client by binding to its exposed hydrophobic patches until the client is delivered to the ERAD machinery [18]. Despite these findings, the normal cellular function of this machinery is poorly characterized, especially in the context of protein quality control.

Aberrant membrane proteins: recognition, correction, and/or degradation

All membrane proteins are synthesized in the ER and they comprise one-third of the human proteome. Synthesis of the membrane proteins is a highly complex and error-prone process, which includes insertion of membrane domain into the bilayer and organizing domains on either side of the membrane. Unsurprisingly, due to its complex organization, error in membrane protein synthesis, assembly, and delivery is associated with several diseases such as cystic fibrosis, retinitis pigmentosa, nephrogenic diabetes insipidus, hypogonadotropic hypogonadism, hypocalciuric hypercalcemia, etc. Cells counteract this problem by deploying powerful PQC machineries analogous to soluble proteins with overlapping components and mechanisms. For instance, the ER-resident ATP-dependent quality control involving Hsp70/BiP, a J-protein, and a NEF is defined for several membrane protein clients, such as rhodopsin [19], surfactant C [20], cystic fibrosis transmembrane conductance regulator (CFTR), etc. In the case of ΔF508 mutant of CFTR and gonadotropin-releasing hormone receptor, the ER membrane chaperone BAP31 [21] and DnaJ B12 [22] associate with their respective clients and promote its retrotranslocation and clearance from the ER. Similarly, in the cytosol, several proteostasis factors, such as Hsp70, Hsc70, Hsp90, and CHIP E3 ligase, are shown to promote PQC of Niemann–Pick disease type C-2 [23]. These aforementioned examples demonstrate the interplay of PQC components for their specific clients.

Another key question is how the protein quality control deals with the aggregated membrane proteins. Similar to soluble PQC compartments, increasing evidence indicates the existence of quality control structures for membrane proteins [24], but the formation and composition of these structures are poorly characterized. Recent studies have implicated requirement of certain PQC factors for the formation of these structures, including chaperones (Hsp70, DnaJB, Bag3), molecular motors, microtubules, and microtubule-associated factors (histone deacetylase; HDAC6) [25,26]. However, the basic formation mechanism of these structures is vague. Moreover, the manners in which substrates are recognized and targeted to aggresomes leading to autophagy are not known. Recent studies have supported the notion of ER membrane chaperones playing pivotal role in recognition and fate of aberrant clients. For instance, membrane-localized J-protein B12 along with cytosolic Hsp70 is reported as a potential factor for membrane client recognition [27,28]. Another Hsp70 cochaperone, Bag3, was also reported to be involved in targeting misfolded client to the quality control sites for further processing [26]. Also, an unbiased RNAi screening analysis toward aggresome substrate (synphilin-1) has identified RuvbL proteins as aggresome-forming proteins with disaggregase activity [29]. In addition, little is understood about the underlying mechanism of retrotranslocation of membrane clients during ERAD, with several groups suggesting direct interplay of membrane channels Hrd1 and Derlin-1 in client selection and retrotranslocation [30,31].

Table 1. List of viruses exploiting proteostasis pathways.

Family	Classification	Strain	Mechanisms of PQC factor exploitation
Entry and disassembly			
Polyomaviridae	Nonenveloped DNA	Simian vacuolating virus 40	PDI family members isomerizes VP1 disulfide bonds [34]; Cytosolic disaggregase machinery disassemble the virus [37]
Polyomaviridae	Nonenveloped DNA	Murine polyomavirus	PDI family members isomerizes VP1 disulfide bonds [32,33,38,49]
Polyomaviridae	Nonenveloped DNA	BK virus	PDI family members isomerizes VP1 disulphide bonds [50,51]
Papillomaviridae	Nonenveloped DNA	Human papillomavirus 16	Cytosolic and ER chaperones promote capsid disassembly [39,52]
Poxviridae	Enveloped DNA	Vaccinia virus	Host proteasome promotes mechanical core uncoating [41,53]
Orthomyxoviridae	Enveloped DNA	Influenza virus	Hijacks host aggresome and disassembly machinery [40]
Parvoviridae	Nonenveloped DNA	Adeno-associated virus 2/8	Ubiquitin-proteasome pathways is involved in uncoating [54,55]
Flaviviridae	Enveloped RNA	Dengue virus	Hsp70 chaperone and cochaperone promote entry [43]
Replication, assembly and morphogenesis			
Flaviviridae	Enveloped RNA	Hepatitis C virus	Replication site is enriched in chaperones of unknown function [42]
Flaviviridae	Enveloped RNA	Dengue virus	Chaperone form replication site and promote virion biogenesis [43]
Flaviviridae	Enveloped RNA	Zika virus	ER and cytosolic chaperones build virus replication compartment [44]
Herpesviridae	Enveloped DNA	Herpes simplex virus 1	Virus-induced chaperone enriched domain promotes infection [45]
Herpesviridae	Enveloped DNA	Varicella-zoster virus	Hsc70, Hsp90, and BAG3 facilitates virus replication [56]
Herpesviridae	Enveloped DNA	Hepatitis E virus	ERAD pathway to retrotranslocate ORF2 to the cytosol [57]
Reoviridae	Enveloped RNA	Rotavirus	ER-resident chaperones promote viral morphogenesis [47]
Coronaviridae	Enveloped RNA	SARS coronavirus	ERAD tuning vesicle-like structures serves as replication site [58,59]
Coronaviridae	Enveloped RNA	Mouse hepatitis virus	ERAD tuning vesicle-like structures serves as replication site [58,59]
Retroviridae	Enveloped DNA	Mouse mammary tumor virus	Viral protein Rem is processed in the ER and retrotranslocated [60]
Parvoviridae	Nonenveloped DNA	Minute virus of Mice	Ubiquitin-proteasome pathway and motor proteins are important
Parvoviridae	Nonenveloped DNA	Canine parvovirus	Ubiquitin-proteasome pathway and motor proteins are important

Proteostasis and viruses

Viruses hijack host factors called 'cues' by either exploiting their cellular role or modify to facilitate specific function [3]. Several viruses trick host PQC factors into performing novel functions to support infection, which in turn has helped us to learn about the function and molecular mechanism of these host factors. It is well established that viruses exploit host PQC factors for many aspects of their life cycle [4], including entry, replication, and assembly (Table 1). A detailed overview of viruses, which use different steps of proteostasis during infection (as shown in Fig. 2), is discussed below.

Proteostasis cues in virus entry

In the case of nonenveloped viruses host entry and genome delivery is poorly characterized. Polyomavirus family is the most studied nonenveloped virus whose host entry is well understood. During entry, the virus reaches the ER from the cell surface and co-opts ERAD factors to reach cytosol. Specifically, the PDI family of enzymes reduces and isomerizes the viral disulfide bonds that often expose hydrophobic epitopes [32,33]. These changes partially disassemble the virus and the particle now mimics a giant misfolded protein aggregate, which now recruits Hsp70 homolog BiP and its luminal cochaperones [34,35]. The restructured, hydrophobic virus is primed for membrane penetration, by exploiting molecular motor kinesin-1 to drive the reorganization of ER membrane chaperone B14 to form the virus membrane penetration site, called 'focus' [36]. The focus-localized virus is then extracted from the membrane by a B14-tethered cytosolic disaggregation machinery (B14, Hsc70, and Hsp110),

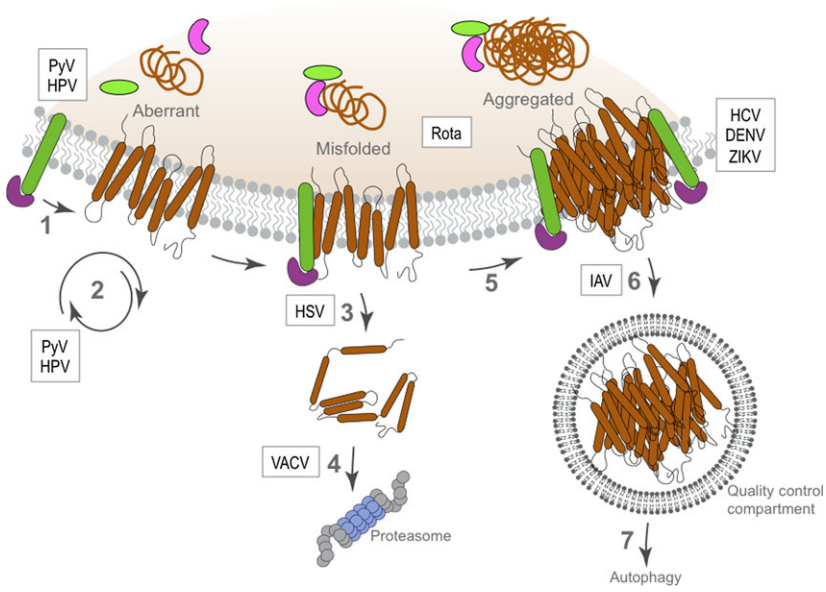

Fig. 2. Viruses hijacking proteostasis components. List of viruses using different steps of proteostasis during infection.

consequently reaching the cytosol [37]. In summary, studies on polyomavirus have unraveled the interplay of host PQC components in the ER lumen, ER membrane, and the cytosol.

A similar mechanism has been proposed for the entry and disassembly of human papillomavirus (HPV). For instance, several studies have proposed HPV reaching ER during host entry and utilizing ER-resident PDI family proteins [38]. In addition, Hsp70 chaperone system has been demonstrated to disassemble HPV *in vitro*, a mechanism similar to disassembly of polyomavirus [39]. But a detailed mechanistic understanding of the host membrane penetration and virus disassembly in HPV infection is poorly understood.

Another well-characterized example of host quality control machinery being utilized by a virus to promote its disassembly is Influenza A virus (IAV), an enveloped DNA virus. During host entry, the IAV capsid released from late endosome mimics as misfolded protein aggregate by carrying unanchored ubiquitin chains that activates histone deacetylase 6 (HDAC6) to recruit cytoskeleton motors that generate opposing physical forces to break apart the capsid and disassemble the virus [40]. Another example of an enveloped virus taking advantage of host PQC factors is in the case of Vaccinia virus (VACV), the prototypic poxvirus. VACV has evolved a complex multistep core disassembly and genome release process due to its shape and structure. After 'core activation', host proteasome activity is required for core degradation and genome release [41]. Overall, the examples illustrated above demonstrate how viruses hijack host protein quality control machinery and tweak them to promote virus entry and disassembly. Nonetheless, studies on these viruses have demonstrated the key components of the aggresome formation and disassembly machinery and also provided a broad understanding of host components and cellular processes.

Proteostasis cues in virus replication, assembly, and egress

Postentry into the host cell, viral genome is transcribed and translated to promote virus replication and assembly and for all viruses this step depends entirely on the host proteostasis machinery. Numerous viruses exploit host PQC factors to build site of replication and promote assembly. Several members of *Flaviviridae* family are reported to indirectly utilize ER membrane chaperones to build and sustain their replication site. For instance, during Hepatitis C virus (HCV) replication, virus induces ER membrane rearrangement to form a viral replication factory. Although, several chaperones (Hsp70, Hsp90, and calnexin) are implicated to play a role in virus replication, the exact composition and mechanism of replication factory formation is poorly defined and proposed to be closely related to PQC [42]. Similarly, recent study on Dengue virus (DENV) has illuminated the requirement of Hsp70 chaperone network that are required at distinct steps of the viral cycle, including entry, RNA replication, and virion biogenesis. More importantly, the role of Hsp70 at each step is specified by nine distinct DNAJ cofactors [43]. Of these, DnaJB11 relocalizes to virus-induced replication complex, while DnaJB6 facilitates virion biogenesis. Studies on recently emerged Zika virus

(ZIKV) has demonstrated widespread remodeling of intracellular membrane and formation of cytoplasmic vacuoles. Several ER and cytosolic chaperones are implicated in formation of these vacuoles, but a thorough understanding is needed to reveal the importance and formation of these vacuoles [44]. Globally, studies on flaviviruses have provided vital information on the membrane remodeling and role of chaperones during ER membrane-derived compartment formations.

Another instance of PQC factors that are subverted to promote virus infection is for Herpes Simplex virus (HSV)-1, an enveloped DNA virus. It has been proposed that the virus-induced replication compartment is enriched in chaperones such as Hsc70, Hsp90, Bag3, and proteosomes, which perhaps remodel viral replication and regulatory proteins to promote HSV-1 replication [45]. Although the virus-induced replication compartments have traces of PQC compartments, they vary in their protein composition and especially how they are built. Nevertheless, studies on HSV-1, similar to flaviviruses, have provided key information on formation, maintenance, and functioning of the PQC compartments.

In the case of enveloped RNA rotavirus, the final assembly of the viral particle takes place in the ER [46], where ER-resident chaperones Grp78, PDI, calnexin, and calreticulin are reported to promote morphogenesis of the viral particle. Specifically, these chaperones promote accurate trimming of the glycan chains on VP7 and NSP4, the correct formation of VP7 disulfide bonds, and the incorporation of properly folded VP7 into assembled rotavirus [47]. Overall, studies on rotavirus assembly and morphogenesis have provided vital information on the interplay of chaperones and protein homeostasis in the ER. In conclusion, the aforementioned example of viruses utilizing PQC factors as cues during infection has provided a broad understanding of host proteostasis mechanism.

Future perspective

Long-term research should focus on studying the quality control compartment for membrane protein aggregates. The key outstanding question is to understand the mechanism of membrane substrate recognition by the chaperone system. Specifically, pinpointing the identity of ER luminal, membrane, and cytosolic factors for a specific misfolded membrane client is vital. It is also important to clarify how misfolded/aggregated membrane substrates are refolded and sequestered and if not, how they are disaggregated and targeted toward degradation pathways. Studies on virus should guide our understanding of how aggregated membrane proteins are processed from the cell in order to maintain cellular proteostasis and understanding how proteostasis pathways are affected in the cells infected with viruses. Some of the experimental approaches should focus on unbiased proteomic analysis for specific membrane protein substrates to identify target PQC components. These targets should be further validated with gain and/or loss of function studies. From the virus perspective, the identities of the host quality control factors that influence the formation of virus-induced structures and also understanding how host proteostasis is impacted by formation of these structures are vital.

Currently several therapeutic options are explored for protein misfolding-related diseases, specifically targeting prevention, refolding, and degradation pathways [48]. Future research should be directed toward unlocking further secrets of cellular protein homeostasis in conjunction with virus infection and provide therapeutic targets to combat diseases caused by these toxic agents, and to illuminate novel cellular mechanisms. For instance, these insights should help us develop molecular and pharmacological chaperones to prevent formation of protein aggregates thereby delaying the onset of misfolded protein-associated diseases or even develop antiviral agents. An allosteric Hsp70 inhibitor, JG40, has been shown to potently block infection of different Flaviviruses (Dengue, yellow fever, West Nile and Japanese encephalitis viruses) without exerting toxicity to the host cells [43]. Thus, targeting host chaperone networks should provide a path for broad-spectrum antivirals.

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Conflict of interests

The author declares no conflict of interests.

References

1 Olzmann JA, Kopito RR & Christianson JC (2013) The mammalian endoplasmic reticulum-associated degradation system. *Cold Spring Harb Perspect Biol* **5**, a013185.

2 Bernales S, Schuck S & Walter P (2007) ER-phagy: selective autophagy of the endoplasmic reticulum. *Autophagy* **3**, 285–287.

3 Ravindran MS & Tsai B (2016) Viruses utilize cellular cues in distinct combination to undergo systematic priming and uncoating. *PLoS Pathog* **12**, e1005467.
4 Ravindran MS, Bagchi P, Cunningham CN & Tsai B (2016) Opportunistic intruders: how viruses orchestrate ER functions to infect cells. *Nat Rev Microbiol* **14**, 407–420.
5 Hartl FU & Hayer-Hartl M (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* **295**, 1852–1858.
6 Bukau B, Weissman J & Horwich A (2006) Molecular chaperones and protein quality control. *Cell* **125**, 443–451.
7 Kampinga HH & Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol* **11**, 579–592.
8 Craig EA & Marszalek J (2017) How do J-proteins get Hsp70 to do so many different things? *Trends Biochem Sci* **42**, 355–368.
9 Nishikawa S, Brodsky JL & Nakatsukasa K (2005) Roles of molecular chaperones in endoplasmic reticulum (ER) quality control and ER-associated degradation (ERAD). *J Biochem* **137**, 551–555.
10 Inoue T & Tsai B (2016) The Grp170 nucleotide exchange factor executes a key role during ERAD of cellular misfolded clients. *Mol Biol Cell* **27**, 1650–1662.
11 Yang H & Hu HY (2016) Sequestration of cellular interacting partners by protein aggregates: implication in a loss-of-function pathology. *FEBS J* **283**, 3705–3717.
12 Sontag EM, Vonk WI & Frydman J (2014) Sorting out the trash: the spatial nature of eukaryotic protein quality control. *Curr Opin Cell Biol* **26**, 139–146.
13 Kaganovich D, Kopito R & Frydman J (2008) Misfolded proteins partition between two distinct quality control compartments. *Nature* **454**, 1088–1095.
14 Nillegoda NB, Kirstein J, Szlachcic A, Berynskyy M, Stank A, Stengel F, Arnsburg K, Gao X, Scior A, Aebersold R *et al.* (2015) Crucial HSP70 co-chaperone complex unlocks metazoan protein disaggregation. *Nature* **524**, 247–251.
15 Hrizo SL, Gusarova V, Habiel DM, Goeckeler JL, Fisher EA & Brodsky JL (2007) The Hsp110 molecular chaperone stabilizes apolipoprotein B from endoplasmic reticulum-associated degradation (ERAD). *J Biol Chem* **282**, 32665–32675.
16 Kim S, Nollen EA, Kitagawa K, Bindokas VP & Morimoto RI (2002) Polyglutamine protein aggregates are dynamic. *Nat Cell Biol* **4**, 826–831.
17 Muchowski PJ, Schaffar G, Sittler A, Wanker EE, Hayer-Hartl MK & Hartl FU (2000) Hsp70 and hsp40 chaperones can inhibit self-assembly of polyglutamine proteins into amyloid-like fibrils. *Proc Natl Acad Sci USA* **97**, 7841–7846.
18 Kabani M, Kelley SS, Morrow MW, Montgomery DL, Sivendran R, Rose MD, Gierasch LM & Brodsky JL (2003) Dependence of endoplasmic reticulum-associated degradation on the peptide binding domain and concentration of BiP. *Mol Biol Cell* **14**, 3437–3448.
19 Athanasiou D, Bevilacqua D, Aguila M, McCulley C, Kanuga N, Iwawaki T, Chapple JP & Cheetham ME (2014) The co-chaperone and reductase ERdj5 facilitates rod opsin biogenesis and quality control. *Hum Mol Genet* **23**, 6594–6606.
20 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**, 2620–2630.
21 Wang B, Heath-Engel H, Zhang D, Nguyen N, Thomas DY, Hanrahan JW & Shore GC (2008) BAP31 interacts with Sec61 translocons and promotes retrotranslocation of CFTRDeltaF508 via the derlin-1 complex. *Cell* **133**, 1080–1092.
22 Houck SA, Ren HY, Madden VJ, Bonner JN, Conlin MP, Janovick JA, Conn PM & Cyr DM (2014) Quality control autophagy degrades soluble ERAD-resistant conformers of the misfolded membrane protein GnRHR. *Mol Cell* **54**, 166–179.
23 Nakasone N, Nakamura YS, Higaki K, Oumi N, Ohno K & Ninomiya H (2014) Endoplasmic reticulum-associated degradation of Niemann-Pick C1: evidence for the role of heat shock proteins and identification of lysine residues that accept ubiquitin. *J Biol Chem* **289**, 19714–19725.
24 Miller SB, Mogk A & Bukau B (2015) Spatially organized aggregation of misfolded proteins as cellular stress defense strategy. *J Mol Biol* **427**, 1564–1574.
25 Hageman J, Rujano MA, van Waarde MA, Kakkar V, Dirks RP, Govorukhina N, Oosterveld-Hut HM, Lubsen NH & Kampinga HH (2010) A DNAJB chaperone subfamily with HDAC-dependent activities suppresses toxic protein aggregation. *Mol Cell* **37**, 355–369.
26 Gamerdinger M, Kaya AM, Wolfrum U, Clement AM & Behl C (2011) BAG3 mediates chaperone-based aggresome-targeting and selective autophagy of misfolded proteins. *EMBO Rep* **12**, 149–156.
27 Grove DE, Fan CY, Ren HY & Cyr DM (2011) The endoplasmic reticulum-associated Hsp40 DNAJB12 and Hsc70 cooperate to facilitate RMA1 E3-dependent degradation of nascent CFTRDeltaF508. *Mol Biol Cell* **22**, 301–314.
28 Yamamoto YH, Kimura T, Momohara S, Takeuchi M, Tani T, Kimata Y, Kadokura H & Kohno K (2010) A novel ER J-protein DNAJB12 accelerates ER-associated degradation of membrane proteins including CFTR. *Cell Struct Funct* **35**, 107–116.
29 Zaarur N, Xu X, Lestienne P, Meriin AB, McComb M, Costello CE, Newnam GP, Ganti R, Romanova NV, Shanmugasundaram M *et al.* (2015) RuvbL1 and RuvbL2 enhance aggresome formation and disaggregate amyloid fibrils. *EMBO J* **34**, 2363–2382.
30 Sato BK, Schulz D, Do PH & Hampton RY (2009) Misfolded membrane proteins are specifically

recognized by the transmembrane domain of the Hrd1p ubiquitin ligase. *Mol Cell* **34**, 212–222.
31 Mehnert M, Sommer T & Jarosch E (2014) Der1 promotes movement of misfolded proteins through the endoplasmic reticulum membrane. *Nat Cell Biol* **16**, 77–86.
32 Magnuson B, Rainey EK, Benjamin T, Baryshev M, Mkrtchian S & Tsai B (2005) ERp29 triggers a conformational change in polyomavirus to stimulate membrane binding. *Mol Cell* **20**, 289–300.
33 Schelhaas M, Malmstrom J, Pelkmans L, Haugstetter J, Ellgaard L, Grunewald K & Helenius A (2007) Simian Virus 40 depends on ER protein folding and quality control factors for entry into host cells. *Cell* **131**, 516–529.
34 Inoue T, Dosey A, Herbstman JF, Ravindran MS, Skiniotis G & Tsai B (2015) ERdj5 reductase cooperates with protein disulfide isomerase to promote simian virus 40 endoplasmic reticulum membrane translocation. *J Virol* **89**, 8897–8908.
35 Geiger R, Andritschke D, Friebe S, Herzog F, Luisoni S, Heger T & Helenius A (2011) BAP31 and BiP are essential for dislocation of SV40 from the endoplasmic reticulum to the cytosol. *Nat Cell Biol* **13**, 1305–1314.
36 Ravindran MS, Engelke MF, Verhey KJ & Tsai B (2017) Exploiting the kinesin-1 molecular motor to generate a virus membrane penetration site. *Nat Commun* **8**, 15496.
37 Ravindran MS, Bagchi P, Inoue T & Tsai B (2015) A non-enveloped virus hijacks host disaggregation machinery to translocate across the endoplasmic reticulum membrane. *PLoS Pathog* **11**, e1005086.
38 Gilbert J, Ou W, Silver J & Benjamin T (2006) Downregulation of protein disulfide isomerase inhibits infection by the mouse polyomavirus. *J Virol* **80**, 10868–10870.
39 Chromy LR, Oltman A, Estes PA & Garcea RL (2006) Chaperone-mediated *in vitro* disassembly of polyoma- and papillomaviruses. *J Virol* **80**, 5086–5091.
40 Banerjee I, Miyake Y, Nobs SP, Schneider C, Horvath P, Kopf M, Matthias P, Helenius A & Yamauchi Y (2014) Influenza A virus uses the aggresome processing machinery for host cell entry. *Science* **346**, 473–477.
41 Mercer J, Snijder B, Sacher R, Burkard C, Bleck CK, Stahlberg H, Pelkmans L & Helenius A (2012) RNAi screening reveals proteasome- and Cullin3-dependent stages in vaccinia virus infection. *Cell Rep* **2**, 1036–1047.
42 Paul D, Madan V & Bartenschlager R (2014) Hepatitis C virus RNA replication and assembly: living on the fat of the land. *Cell Host Microbe* **16**, 569–579.
43 Taguwa S, Maringer K, Li X, Bernal-Rubio D, Rauch JN, Gestwicki JE, Andino R, Fernandez-Sesma A & Frydman J (2015) Defining Hsp70 subnetworks in dengue virus replication reveals key vulnerability in flavivirus infection. *Cell* **163**, 1108–1123.
44 Monel B, Compton AA, Bruel T, Amraoui S, Burlaud-Gaillard J, Roy N, Guivel-Benhassine F, Porrot F, Genin P, Meertens L *et al*. (2017) Zika virus induces massive cytoplasmic vacuolization and paraptosis-like death in infected cells. *EMBO J* **36**, 1653–1668.
45 Livingston CM, Ifrim MF, Cowan AE & Weller SK (2009) Virus-Induced Chaperone-Enriched (VICE) domains function as nuclear protein quality control centers during HSV-1 infection. *PLoS Pathog* **5**, e1000619.
46 Inoue T & Tsai B (2013) How viruses use the endoplasmic reticulum for entry, replication, and assembly. *Cold Spring Harb Perspect Biol* **5**, a013250.
47 Maruri-Avidal L, Lopez S & Arias CF (2008) Endoplasmic reticulum chaperones are involved in the morphogenesis of rotavirus infectious particles. *J Virol* **82**, 5368–5380.
48 Sweeney P, Park H, Baumann M, Dunlop J, Frydman J, Kopito R, McCampbell A, Leblanc G, Venkateswaran A, Nurmi A *et al*. (2017) Protein misfolding in neurodegenerative diseases: implications and strategies. *Transl Neurodegener* **6**, 6.
49 Walczak CP & Tsai B (2011) A PDI family network acts distinctly and coordinately with ERp29 to facilitate polyomavirus infection. *J Virol* **85**, 2386–2396.
50 Bennett SM, Jiang M & Imperiale MJ (2013) Role of cell-type-specific endoplasmic reticulum-associated degradation in polyomavirus trafficking. *J Virol* **87**, 8843–8852.
51 Jiang M, Abend JR, Tsai B & Imperiale MJ (2009) Early events during BK virus entry and disassembly. *J Virol* **83**, 1350–1358.
52 Campos SK, Chapman JA, Deymier MJ, Bronnimann MP & Ozbun MA (2012) Opposing effects of bacitracin on human papillomavirus type 16 infection: enhancement of binding and entry and inhibition of endosomal penetration. *J Virol* **86**, 4169–4181.
53 Kilcher S, Schmidt FI, Schneider C, Kopf M, Helenius A & Mercer J (2014) siRNA screen of early poxvirus genes identifies the AAA+ ATPase D5 as the virus genome-uncoating factor. *Cell Host Microbe* **15**, 103–112.
54 Yan Z, Zak R, Luxton GW, Ritchie TC, Bantel-Schaal U & Engelhardt JF (2002) Ubiquitination of both adeno-associated virus type 2 and 5 capsid proteins affects the transduction efficiency of recombinant vectors. *J Virol* **76**, 2043–2053.
55 Yan Z, Zak R, Zhang Y, Ding W, Godwin S, Munson K, Peluso R & Engelhardt JF (2004) Distinct classes of proteasome-modulating agents cooperatively augment recombinant adeno-associated virus type 2 and type 5-mediated transduction from the apical surfaces of human airway epithelia. *J Virol* **78**, 2863–2874.
56 Kyratsous CA & Silverstein SJ (2007) BAG3, a host cochaperone, facilitates varicella-zoster virus replication. *J Virol* **81**, 7491–7503.

57 Surjit M, Jameel S & Lal SK (2004) The ORF2 protein of hepatitis E virus binds the 5' region of viral RNA. *J Virol* **78**, 320–328.

58 Reggiori F, Monastyrska I, Verheije MH, Cali T, Ulasli M, Bianchi S, Bernasconi R, de Haan CA & Molinari M (2010) Coronaviruses Hijack the LC3-I-positive EDEMosomes, ER-derived vesicles exporting short-lived ERAD regulators, for replication. *Cell Host Microbe* **7**, 500–508.

59 Bernasconi R, Noack J & Molinari M (2012) Unconventional roles of nonlipidated LC3 in ERAD tuning and coronavirus infection. *Autophagy* **8**, 1534–1536.

60 Byun H, Das P, Yu H, Aleman A, Lozano MM, Matouschek A & Dudley JP (2017) Mouse mammary tumor virus signal peptide uses a novel p97-dependent and derlin-independent retrotranslocation mechanism to escape proteasomal degradation. *MBio* **8**, e00328–17.