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REVIEW

Quality Control: Proteins and Organelles

V. N. Luzikov

Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, 119899 Russia; fax: (095) 939-3181; E-mail: luzikov@genebee.msu.su

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Abstract—This review summarizes materials on the mechanisms of intracellular degradation of proteins whose topogenesis is disturbed at one stage or another. Chaperone and proteolytic systems involved in this process in the endoplasmic reticulum, mitochondria, and chloroplasts of eucaryotic cells as well as those in distinct subcellular compartments of procaryotic cells are considered. The available data suggest that living cells contain numerous systems keeping under control both folding of newly synthesized and newly imported polypeptide chains and their incorporation into heterooligomeric complexes. The point of view is elaborated that organelle formation is controlled not only at the level of individual protein molecules but also at the supermolecular level when whole organelles incapable of carrying out their integral key functions become targets for partial or total elimination. This type of control is realized through an autophagic mechanism involving lysosomes/vacuoles.

Key words: protein, folding, oligomerization, glycosylation, chaperones, proteases, quality control, ERAD, bacterial cells, mitochondria, chloroplasts, peroxisomes, autophagy

As early as some decades ago this topic might seem too revolutionary. At that time an *a priori* point of view was in general use that the cell would be over-wasteful if it eliminates newly synthesized proteins and newly formed organelles. However, experimental data favoring this idea has accumulated with time.

As applied to formation of protein molecules capable of full functioning, the term "quality control" appeared about ten years ago when it became obvious that it is by no means always that folding of nascent polypeptide chains (NPC) results in native protein molecules and all NPCs find their counterparts in heterooligomeric complexes. Cases are known when only 15-20% of NPCs take mature conformation [1] and only 5-10% of subunits of heterooligomeric complexes bind their counterparts, thus arranging themselves in a stable unit [2]. To estimate general scale of the deviations from regular folding, attendant covalent modifications, and oligomerization of the NPCs it will suffice to address a recent work [3] where it has been shown that about 30% of NPCs suffer the action of proteasomes, the targets of which are aberrant and unnecessary molecules. In fact, such molecules accumulated in the cell in ubiquitinated forms in the presence of proteasome inhibitors.

We used a similar approach about 20 years ago when studying the formation of the respiratory apparatus in yeast mitochondria [4]. It turned out that administration of inhibitors of vacuolar proteases into the yeast growth medium leads to about 25% increase in the contents of native components of the mitochondrial respiratory chain that is not followed by the proportional increase in the rate of cell respiration. This observation suggested that upon formation of the respiratory apparatus some amount of "mitochondrial material" (some regions of mitochondria or whole organelles) accumulates which are then degraded with the involvement of vacuoles.

The above examples should be considered just as a "priming" to the following text. By now a number of reviews on protein quality control have been published (see, e.g., references [5-7]). Unfortunately, not much is known about the control over formation and renewal of cell organelles [8-11]. This paper summarizes novel data in this field of endeavor and sets off aspects that have still not been considered with attention adequate to their importance.

1. PROTEOLYTIC CONTROL OVER FOLDING AND OLIGOMERIZATION OF NPCs IN THE ENDOPLASMIC RETICULUM

The ER is an intracellular compartment where individual proteins and protein complexes are formed and then directed to the plasma membrane via the exocytic pathway; some of them are sorted from the total protein flow into lysosomes/vacuoles or return to the ER. There

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is a point of view that ER is a major contributor of the targets for proteasomes [12] because it is there where NPC folding is complicated by covalent modifications, the main of which are glycosylation and formation of intra- and intermolecular disulfide bridges. The most complicated is the process of formation of the ER polytopic membrane proteins when modification and folding of NPCs occurs in parallel with the arrangement of their transmembrane segments in the lipid bilayer. Targets for degradation are mutant NPCs [13-17], NPCs lacking their counterparts in oligomeric complexes [18, 19], as well as native proteins the content of which no longer fits metabolic needs [20-22].

Since degradation of the targets is suppressed by specific inhibitors of proteasomes, one may assume that the latter are responsible for this process. Besides, degradation of improperly folded and unassembled NPCs is hindered in cells with mutant forms of proteasomes [23, 24]. One should emphasize that the degradation of NPCs proceeds after (or in the course of) their insertion into the lumen or the membrane of ER. This is particularly evidenced by the fact that the NPCs to be degraded are glycosylated, which might occur only in the ER interior [24-27]. Overall, this event is known in the literature as ERAD, which means Endoplasmic Reticulum Associated Degradation.

Analysis of yeast mutants defective with respect to ERAD revealed a number of proteins responsible for this process. In such mutants aberrant and unbound NPCs are not degraded and, as a rule, accumulated in the ER. By now the following proteins involved in the ERAD have been identified: Sec61p [25, 28, 29], Sec63p [29], Hrd1p(Der3p) [21, 30], Hrd3p [21, 31], Der1p [32], Ubc6p [33], Ubc7p [24, 34] Cue1p [34], and Kar1p [24].

Sec61p (Sec61α for mammals) is a key component of the channel for both cotranslational and posttranslocational translocation of NPCs into the ER (see reviews [35, 36]). Since ERAD is a Sec61p-dependent process, there are grounds to believe that aberrant NPCs are exported from the ER into the cytoplasm, where proteasomes are situated, through the Sec61-channel. Some components of the ERAD system are involved in one way or another in ubiquitination of the NPCs ranking among potential proteasome targets. It has been found that a cytoplasmic domain of Hrd1p, an integral membrane protein of the ER, bears a RING-H2 motif characteristic for the E3 ubiquitin ligases. The peculiarity of Hrd1p is that in the presence of its substrates the enzyme undergoes self-ubiquitination and subsequent degradation. These processes are prevented through stabilization of Hrd1p in a complex with Hrd3p [31, 37]. Ubc6p and Ubc7p are E2 ubiquitin conjugating enzymes. The former is an ER integral membrane protein, whereas the latter takes part in NPS ubiquitination through binding with Cue1p, a membrane protein [34]. Mutations in Ubc6p and Ubc7p lead to accumulation of the aberrant NCPs in the ER [24, 33, 34].

With these data in mind, one may assume the following basic ERAD stages: 1) aberrant or unbound NPCs are somehow captured by the Sec61-channel and exposed at the ER surface; 2) the NPCs are ubiquitinated with the involvement of Hrd1p, Ubc6p, and Ubc7p, thus being prevented from reverse translocation into the ER interior; 3) ubiquitinated NPCs lose their oligosaccharide chains under the action of cytoplasmic glycosidases and suffer digestion by proteasomes.

Selection of NPCs for ERAD is accomplished by specific ER chaperones which, on one hand, maintain the NPCs in a translocation-competent state and, on the other, assist in their delivery into the Sec61-channel. There are data confirming the involvement of the two ER chaperones, namely Kar2p(BiP) and calnexin, in ERAD. Preferential interaction with BiP is characteristic for protein molecules with vast regions enriched in hydrophobic amino acids (e.g., immunoglobulins). In addition to its part in the NPC folding, BiP interacts in an ATP-bound form with the Sec61-channel [38]. Thus, as long as an NPC·BiP complex exists, its binding with the translocation channel and following delivery of the NPCs to the ER cytoplasmic surface remain possible. Active involvement of BiP in the translocation of NPCs across the ER membrane can be confirmed by the observation that the ERAD depends on Sec63p [24], which, as one of the protein translocon constituents, controls the interaction of NPCs with BiP [39-41]. In fact, both release of mutant soluble proteins from the ER lumen and their degradation are disturbed in *kar2* mutants [42].

Calnexin is a single chaperone belonging among integral proteins of the ER membrane. Like other lectins, it is capable of reversible binding with unfolded NPCs bearing $GlcNAc_2Man_9Glc_1$ oligosaccharide chains. Quite comprehensive data on the role of oligosaccharide chains in selection of properly and improperly folded NPCs have recently become available. According to current views (see, e.g., [43, 44]), interaction of the NPCs complexed with calnexin is accompanied by splitting out of a Glc residue by glucosidase II, which leads to detachment of the NPC from the chaperone (Fig. 1). If the NPC assumes therewith a native form, its subsequent interaction with the chaperone does not occur anymore. Otherwise, a new Glc residue binds to the Man group in still unfolded NPC under the action of UDP-glucose glycoprotein transferase. As a result, the NPC becomes capable of binding with calnexin again, which gives it a chance for a new round of folding, probably with the involvement of other ER chaperones. The probability of repeated binding of the Glc residue to the Man_9 chain decreases in the case when the NPC becomes a target for the ER resident mannosidases I and II (ERMI and ERMII), which convert Man_9 into Man_8 and Man_7. As a consequence, the NPCs containing reduced Man-chains

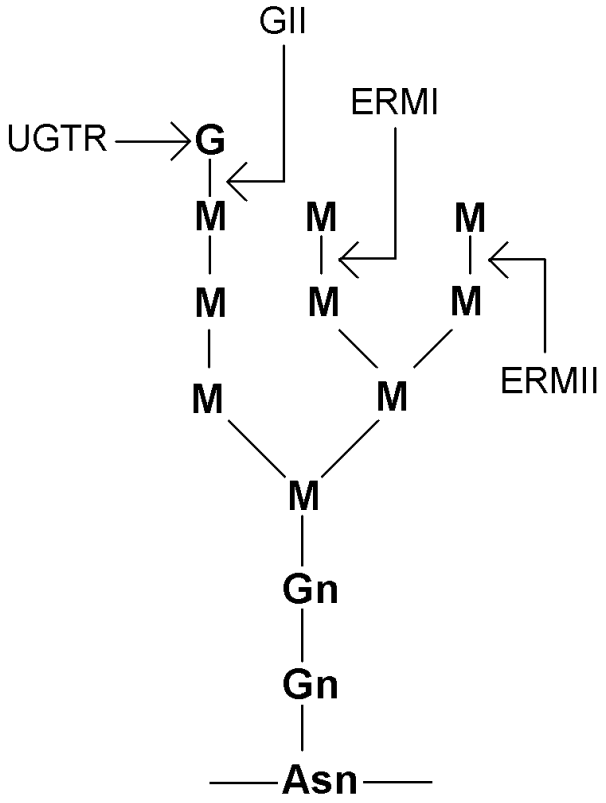

Fig. 1. Modification of hydrocarbon chains bound to Asn residues in nascent polypeptide chains in the endoplasmic reticulum. The points of action of glucosidase II (GII), UDP-glucose: glycoprotein transferase (UGTR), mannosidase I (ERMI), and mannosidase II (ERMII) are indicated. M, G, and Gn are mannose, glucose, and N-acetylglucosamine, respectively.

lose the ability to interact with calnexin and render the targets for the ERAD system [42, 43] (Fig. 2).

This particularly concerns the major complex histocompatibility class I heavy chain, a type I transmembrane protein [44] which, as well as influenza virus hemagglutinin [45], is stabilized by calnexin. The NPCs inserted into the ER membrane seem to find the way to the Sec61-channel for reverse translocation having no need of support on the part of calnexin.

On the other hand, calnexin stimulates degradation of a mutant form of α1-antitrypsin (α1-PIZ), a secreted protein, by means of binding this protein and exporting it for proteasomes at the ER cytoplasmic surface [46]. Upon α1-PIZ degradation calnexin operates jointly with Kar2p(BiP) [42]. Perhaps, Kar2p transports α1-PIZ to the Sec61-channel whereas calnexin participates in its insertion into the channel, suffering ubiquitination itself in so doing [46]. If transformation of Man_9 into Man_8 or Man_7 occurs the degradation of NPCs renders insensitive to proteasome inhibitors, which testifies to the existence of an alternative mechanism different from the protea-

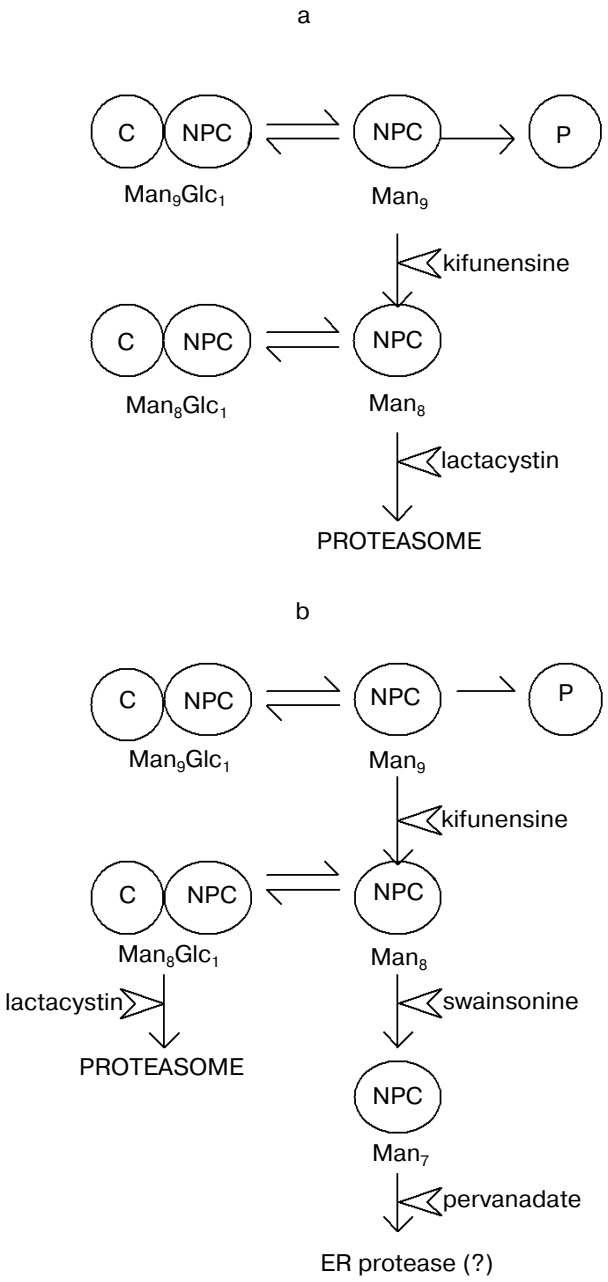

Fig. 2. Role of hydrocarbon chains in the ERAD of NPCs (adapted from [43, 44]). a) Degradation of NPCs stimulated by ERMI and inhibited by calnexin: in this case deficit of calnexin stimulates degradation and, consequently, calnexin is not involved in dislocation of NPCs to proteasomes. P, folded polypeptide chain; C, calnexin; kifunensine is specific inhibitor of ERMI; lactacystin is a proteasomal inhibitor. b) Degradation of NPCs stimulated by ERMI, ERMII, and calnexin. In this case, deficit of calnexin hinders degradation of NPCs in the presence of ERMII inhibitor (swainsonine) and, consequently, calnexin is involved in dislocation of NPCs to protease; kifunensine and swainsonine completely prevent degradation, lactacystin inhibits degradation partially.

some action [43]. Such a mechanism cannot be realized in case of low activities of ERMI and EMRII.

As a whole, analysis of the data on the influence of various ER chaperones on ERAD of aberrant NPCs shows that there are no universal regularities about it as well as there are no such about the interaction of the NPCs with chaperones in the ER. For instance, some secreted soluble proteins are dislocated from the ER into the cytoplasm and degraded further by proteasomes via a calnexin-independent pathway, which, however, requires preliminary transformation of Man_9 into Man_8 under the action of ERMI [47, 48]. In this case, it is unclear what driving force is responsible for dislocation of the proteasomal targets across the Sec-61-channel, why it is Man_8 protein forms that are degraded by proteasomes, etc.

The stage of NPC insertion into the channel is followed by the stage of its extraction to the cytoplasmic surface of the ER where the RING-2H domain of Hrd1p exhibiting ubiquitin ligase activity, as well as Ubc6p and Ubc7p with ubiquitin conjugating activity, are arranged. Retranslocation of the NPCs into the cytoplasm is stimulated by their ubitiquination after releasing from the Sec61-channel [49], which however takes place not in all cases [50]. Data are available showing that translocation of some NPCs into the cytoplasm is realized owing to their interaction with cytoplasmic chaperones (Hsp70 [51, 52] and Hsp90 [53]), which but again is not necessary [42]. An active part in the NPC reverse translocation can be played by proteasomes capable of extracting integral membrane proteins into the cytoplasm. Yeast mutants are known which degrade only cytoplasmic, not membranous segments of such proteins [23]. Perhaps in these mutants proteasomes cannot hydrolyze ATP, which attenuates their binding to NPCs. In other words, the energy of ATP hydrolysis is somehow utilized for pulling the NPCs through the Sec61-channel.

A special case is polytopic membrane proteins having several (up to 12) transmembrane segments. Obviously, their extraction from the ER membrane cannot be ordinary. Translocation of some ER polytopic membrane proteins into the cytoplasm was shown to be preceded by digestion of their segments facing the ER lumen under the action of an endogenous protease [18, 54-56]. It is anticipated that the polytopic membrane proteins are degraded through joint action of the ubiquitin–proteasome system in the cytoplasm and endogenous protease(s) in the ER interior.

Thus far experimental data have accumulated suggesting that in the ER membrane a special complex, including at least the Sec61-channel, Hrd1p(Der3p), Hrd3p, Cue1p, Ubc6p, and Ubc7p forms. Although such complex (the retranslocon) has not yet been isolated, its components are obviously associated with each other either directly or indirectly through common substrates, i.e., NPCs destined for degradation [37]. Hrd3p, an ER transmembrane protein, the lumenal domain of which controls interaction of Hrd1p with a substrate, is likely to play a regulatory part in the translocon formation. In the absence of Hrd3p its counterpart loses the ability to dissociate from the substrate at a proper moment and suffers auto-ubiquitination with subsequent degradation, which requires the involvement of the Sec61-channel [31]. The necessity of such regulation is evident since otherwise the bulk of channels could be involved in the retranslocation of aberrant NPCs, which would reduce the level of protein exocytosis in the living cell.

There are some exceptions in this reasonably fair picture of the ERAD. For example, degradation of NPCs can proceed along the path insensitive to the proteasomal inhibitors. For the α1-PIZ mutant form of α_1-antitrypsin this is caused by the action of ERMI and ERMII, which cleave Man groups of oligosaccharide chains of NPCs, thus preventing them from calnexin binding [43]. Degradation of Vph1p, a subunit of vacuolar ATPase, requires preliminary ubiquitination of the protein and involves proteasomes and cytosolic chaperones. However, Ubc6p and Ubc7p ubiquitin conjugating enzymes as well as the Hrd1p/Hrd3p complex do not take part in this process, which suggests that in this particular case the ERAD is realized through an alternative pathway [57].

Selection of mutant forms of some proteins for degradation is realized in the Golgi apparatus. This particularly concerns some proteins of the plasma membrane, namely ATPase (Pma1p) [58], yeast α-factor receptor (Ste2-3p), and arginine permease (Can1p) [59]. Their improperly folded polypeptide chains bypass the ERAD system but are intercepted in the Golgi and then directed to vacuoles through the endosomal compartment. There are grounds to believe that the proteins destined for degradation retrace the path of vacuolar carboxypeptidase Y (CPY), the receptor of which is known to be the Vsp10p protein. As the *vsp10* mutation suppresses degradation of the Ste2-3p mutant form, one may suggest that the latter also binds with Vsp10p before forwarding to the vacuole. Unfortunately, the details of this process (in particular, possible involvement of other proteins) are still unknown.

Notably, in nature a version of the ERAD mechanism is used by cytomegalovirus. US2 and US11 proteins of this virus bind with the native MHC class I heavy chains in the ER interior and direct them to the Sec61-channel for subsequent ubiquitination and degradation by proteasomes [25].

It has recently been found [60] that for some proteins the ERAD is controlled by a mechanism ensuring their retrieval from the pre-Golgi compartment, into which they are transported in a complex with BiP bearing the KDEL sequence. If retrograde transport of the complex is disturbed the protein destined for degradation accumulates in the pre-Golgi, thus escaping the contact with proteasomes.

2. ERAD AND UPR

From the preceding text, it is seen that ERAD is a constitutive system ensuring permanent control over folding and oligomerization of NPCs. However, when the functional capacity of the ERAD system proves to be too low (i.e., under heat shock conditions or upon addition of thiol compounds disturbing formation of disulfide bridges), aberrant NPCs accumulate in vast amounts. In such cases a special mechanism comes into action which makes the cell free of such NPCs. This mechanism was designated UPR (Unfolded Protein Response). The essence of this event is that the deficiency of Kar2p(BiP), caused by extremely high content of aberrant and unbound NPCs, results in at least two basic process: 1) activation of synthesis of a group of proteins responsible for folding of NPCs and providing their release from the cell, and 2) lowering the total level of protein synthesis [12, 61, 62].

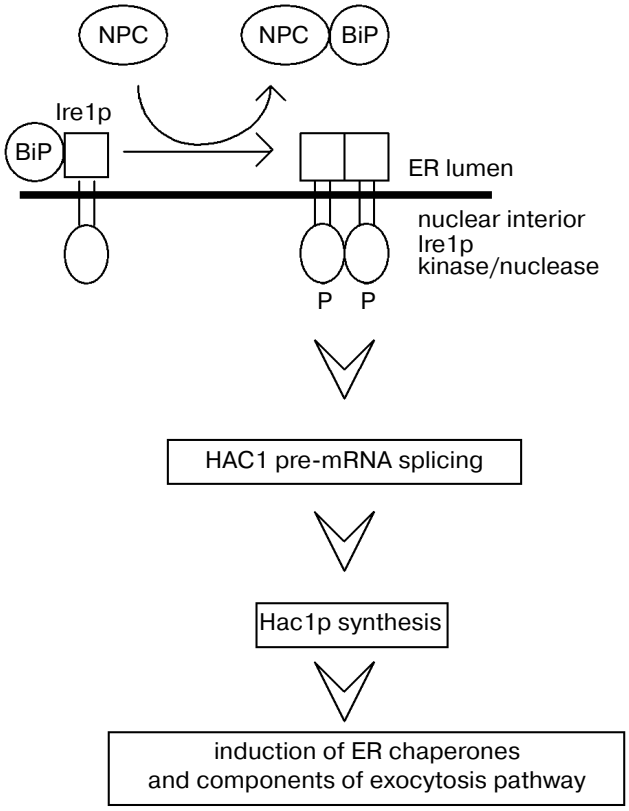

Fig. 3. Mechanism of UPR for yeast (adapted from [61]). Initially BiP becomes in deficit because of its binding with unfolded and uncomplexed NPCs. This results in oligomerization of the nuclear membrane Ire1p molecules through their N-terminal domains facing the ER lumen, which is followed by autophosphorylation of C-terminal domains exposed to the nuclear interior and stimulation of their nuclease activity.

For yeast, the former mechanism is well understood (Fig. 3). It has been shown that in the inner nuclear membrane there is Ire1p, a type I membrane protein facing the ER lumen, which under stress-induced Kar2p deficiency oligomerizes through its N-terminal lumenal domain. This induces autophosphorylation of the C-terminal domain exposed to the nuclear interior, which results in a rise of nuclease activity in this domain. In such a form Ire1p carries out splicing of HAC1 pre-mRNA, the mature form of which encodes transcription factor Hac1p. The latter induces expression of about 200 genes of the UPR system including components of the ERAD apparatus.

In mammalian cells there are several pathways of UPR realization (see review [61]). However, their mechanisms have not received much study. It is particularly unclear how the accumulation of aberrant NPCs is concerned with the induction of synthesis of components of the UPR system. On the other hand, for mammalian cells the mechanisms of lowering the total translation level under stress conditions have been revealed. One of them proceeds with the involvement of PERK, a type I ER membrane protein the cytoplasmic domain of which is about 20% homologous to the corresponding domains of the yeast Ire1p kinase/nuclease and its mammalian analogs IRE1α and IRE1β. Under shortage of BiP in the ER, PERK oligomerizes, its cytoplasmic domain being autophosphorylated therewith. In such a form it gains the capacity to phosphorylate the α subunit of eucaryotic translation factor 2 (eIF2-α), which disturbs interaction of mRNA with the ribosomal subunits. Another mechanism of lowering the level of translation in mammalian cells under stress conditions consists in the cleavage of 28S ribosomal RNA by the IRE1β kinase/nuclease [62].

3. CONTROL OF PROTEIN QUALITY IN BACTERIAL CELLS

Unlike the eucaryotic cell, bacterial cells have no need of special systems ensuring delivery of aberrant proteins to the place of their degradation. For this purpose various subcellular compartments contain their own proteolytic systems: OmpT (outer membrane), HrtA or DegP (periplasm), FtsH (cytoplasmic membrane), Lon or La, ClpAP or Ti, ClpXP, and HslUV (cytoplasm). Soluble proteases act jointly with chaperone systems of the cytoplasm (DnaK/DnaJ/GrpE and GroEL/GroES) and periplasm (i.e., DsbA). The properties and the mechanism of action of bacterial proteases are described in many reviews [63-69].

The main function of the majority of bacterial proteases is degradation of proteins suffering denaturation under stress conditions. As denaturation is accompanied by aggregation of polypeptide chains, their removal from the cell requires the involvement of chaperones and chap-

eronins. A similar situation takes place upon accumulation of aberrant proteins capable of forming inclusion bodies [70]. The fact of appearance of the latter, which occurs especially often upon hyperexpression of recombinant proteins, testifies to the limited functional capacity of the bacterial chaperone and proteolytic systems.

As pointed out above, when protein quality control is realized in the ER of eucaryotic cells, chaperones ensuring proper NPC folding and proteasomes cleaving aberrant and unassembled NPCs are spatially separated. Bacterial cells have complexly built proteases combining chaperone and proteolytic functions. ClpAP(ClpXP) and HslUV are such proteases where the ClpA(X) and HslU components prevent degradation of NPCs, thus giving them chance for proper folding [64]. The targets for degradation are those NPCs which prove to be associated with ClpA(X) or HslU for time long enough to provide binding of protease counterparts of the latter, i.e., ClpP and HslV, respectively [71].

One of the aspects of the ERAD which remained unclear until recently is how proteasomes degrade integral membrane proteins. It has been earlier mentioned that proteasomes are likely to extract NPCs from the lipid bilayer in the course of ERAD. A similar process has been demonstrated for bacterial FtsH, an AAA-protease situated in the cytoplasmic membrane [72]. Both ATP-binding and protease centers of FtsH are known to be exposed to the cytoplasm. However, FtsH is capable of effectively cleaving some polytopic membrane proteins, e.g., SecY (a basic component of the protein translocation channel of the cytoplasmic membrane) which undergoes degradation with half-life of about 2 min if other components of the translocon are absent. Remarkably, SecY-PhoA, a fusion protein with bulky PhoA segment facing the periplasm, is degraded under the action of FtsH if the cells are devoid of DsbA, a factor ensuring NPC folding in the periplasm. In the presence of this factor, degradation of the fusion protein proceeded only partially, with the periplasmic PhoA-segment remaining intact. Hence, it is only unfolded polypeptide chains that can be pulled by FtsH to its cytoplasmic active center through the lipid bilayer of the membrane.

4. PROTEIN QUALITY CONTROL IN MITOCHONDRIA

Mitochondria have their own systems controlling folding and assembly of NPCs both imported from the cytoplasm and synthesized in the mitochondrial matrix. This is evidenced by the fact that these organelles contain chaperones (mtHsp70) with associated co-chaperones (Mdj1p and Mge1p) as well as chaperonins (mtHsp78 and mtHsp60/mtHsp10) [73] similar to those in bacterial cells (DnaK/DnaJ/GrpE, ClpB, and GroEL/GroES). The chaperones operate in conjunction with the three so far known mitochondrial proteases, i.e., Pim1p, Yme1p, and Yta10p-12p (see reviews [67, 74, 75]). The Pim1p protease, similar to bacterial Lon protease, resides in the matrix while the two others are integral proteins of the mitochondrial inner membrane. The structure of Yme1p and Yta10p-12p resembles that of bacterial FstH protease [75]. They all are homo- or heterooligomers of about 1000 kD composed of subunits with one (Yme1p) or two (Yta10p and Yta12p) N-terminal transmembrane segments and bulky C-terminal segments facing the intermembrane space and the matrix, respectively (Fig. 4). These domains include ATP-binding and proteolytic segments as well as a coiled-coil segment for oligomerization of subunits of the protease. The proteolytic segment has the HEXGH sequence specific for metalloproteases.

Mitochondrial proteases carry out proteolysis of uncompleted mitochondrial translation products [76], improperly folded polypeptide chains [77, 78] as well as polypeptide chains lacking their counterparts in heterooligomeric complexes [78-83]. Perhaps selection of the targets for Pim1p, on one hand, and for membrane proteases, on the other, is determined not by the availability of specific recognition sites in the targets but rather by specific intramitochondrial location of the targets and their conformational state. In particular, a protein addressed in an unfolded form into the matrix or the inner membrane is degraded by Pim1p or Yta10p-12p, respectively. In other words, substrate specificities of Pim1p and Yta10p-12p exert overlapping [78].

The fact of existence of the ATP-binding sites in all mitochondrial proteases suggests their ability to carry out chaperone functions. It has been shown that Δ*yta10* and Δ*yta12* mutations result in the interruption of subunit incorporation into the ATP synthase and cytochrome *c* oxidase complexes [84]. On the other hand, such mutations can be suppressed by hyperexpression of a Pim1p protease mutant form with destroyed proteolytic and intact ATP-binding centers [85]. Direct evidences of the interaction of unfolded polypeptide chains with the ATP-binding site of the Yme1p protease are available [86]. Thus there are grounds to assume that mitochondrial proteases are capable of keeping the polypeptide chains in a state competent for their insertion into oligomeric complexes, i.e., carrying out the functions of integrating chaperones.

For a long time it remained unclear how Yme1p and Yta10p-12p proteases, the proteolytic and ATP-binding sites of which are in the intermembrane space and in the matrix, respectively, digest membranous proteins. Now we know [87] that they, like bacterial FtsH, can pull the substrates across the inner membrane. This requires that the segment of potential substrate situated on the membrane side opposite to that of location of the protease active center has to be in an unfolded form or could be unfolded easily. The target polypeptide chain travels through the lipid bilayer by using either a channel formed

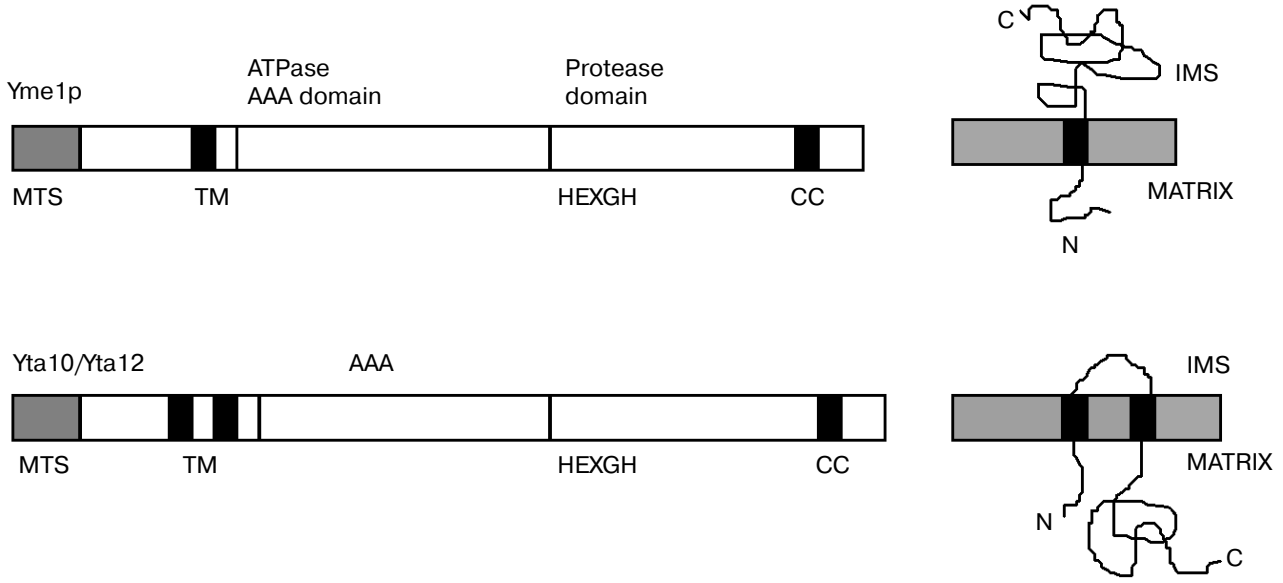

Fig. 4. Arrangement of functionally important segments in mitochondrial proteases Yme1p and Yta10p-12p as well as the ways of their insertion into the inner mitochondrial membrane (adapted from [75]). MTS, mitochondrial topogenic signal; TM, transmembrane domain; HEXGH, metal-binding (proteolytic) center; CC, fragment characterized by coiled-coil structure; IMS, intermembrane space.

by the protease itself or the protein translocon of the inner membrane. In nature specialization of Yme1p and Yta10p-12p proteases is such that the former degrades proteins exposed mainly in the intermembrane space (e.g., Cox II) while the targets of the latter face the matrix (e.g., F_1-ATPase).

As mentioned above, the substrates for both proteases include polypeptide chains lacking their counterparts in the respiratory chain complexes and F_0F_1-ATPase. Evidently, this is a way to control the stoichiometry of the complexes. On the other hand, by using their ATP-binding domains the mitochondrial proteases can confer the ability of selective oligomerization on polypeptide chains.

5. PROTEIN QUALITY CONTROL IN CHLOROPLASTS

Folding and oligomerization of proteins both imported into chloroplasts from the cytoplasm and synthesized in the organelles themselves are controlled by their chaperones and proteases. The stroma contains ch-hsc70 and ch-cpn60/ch-cpn10 [88-91] chaperones which interact with the precursor and mature polypeptide chains, thus ensuring their folding under various conditions. In the same compartment there are also the nClpC(Erd1p)/pClpP/nClpP proteolytic system much like the ClpA/ClpP system in the cytoplasm of bacterial cells [92-97], Erd1p being able to function both as a chaperone and a constituent part of the proteolytic system [96]. The latter includes the constituents encoded both in the nucleus (n) and in the chloroplasts/plastids (p), which suggests that multiple regulatory mechanisms can be involved in the control over proteolytic activity in the stroma.

Thylakoids have specific isoforms of hsp70 and cpn60/cpn10 [98]. DegP1 [99] and DegP2 [100], GTP-dependent proteases similar to bacterial HtrA, are associated with the thylakoid membrane. Active center of the former faces the thylakoid lumen while that of the letter is exposed to the stroma. The thylakoid membrane contains also an FtsH-like ATP-dependent protease [101-104] with the active center in the stroma.

The chloroplast proteases are responsible for degradation of aberrant polypeptide chains [93] as well as those lacking corresponding prosthetic groups, e.g., chlorophyll *a/b* [105]. One more putative function of the chloroplast proteases is regulation of assembly of the oligomeric enzyme complexes by elimination of their unassembled subunits. It has been revealed, that in case of disturbed synthesis of some individual components of photosystem II [106], the ATP-synthase [107], and the cytochrome b_6/f complex [108], other subunits of these complexes are also undetectable. This pleiotropic effect was considered to be the result of degradation of unassembled subunits. However, direct evidence of such degradation has been obtained in some cases only [108, 109].

Although there are data on the involvement of particular proteases in the degradation of particular chloroplast proteins under extreme conditions (e.g., degradation of the D1 protein of photosystem II by DegP2 and

FtsH proteases upon intense irradiation [100] or degradation of proteins of the cytochrome b_6/f complex by ClpP upon nitrogen starvation [95]), a real part of proteases in the biogenesis of chloroplasts still remains obscure. One of the possible approaches to the problem under consideration lies in studying the features of biogenesis in cases of deficiency of individual proteases. In particular, it has recently been shown that mutation of the gene encoding an FtsH-like protease results in the formation of pigment-free plastids with reduced lamellar structures [103].

6. CONTROL OVER FORMATION OF ORGANELLES

Thus, upon folding of NPCs in the cell their noticeable portion assumes aberrant forms, or does not find corresponding counterparts, or proves to be unnecessary in one or another metabolic process. Special mechanisms of degradation of such NPCs are designated. Obviously, mistakes are also inevitable upon formation of cell organelles when many proteins unite into segregated and functionally interconnected systems. In the course of this process definite stoichiometric relationships are established among individual enzymes and oligoenzyme complexes when superensembles of such complexes arranged in a strictly definite way with respect to each other are formed, etc. The question arises of how such processes are controlled.

A possible approach to this problems stems from our works of more than 20-years ago [4] where it has been shown that upon formation of the respiratory machinery in yeast cells some excessive respiratory complexes are formed which, although potentially active in the partial redox reactions, do not nevertheless contribute to cell respiration (*in vivo*) or to the respiratory activity of total mitochondrial fraction (*in vitro*). Such excess complexes can be revealed by addition of the inhibitors of vacuolar proteases in the yeast growth medium.

Another way to observe degradation of the respiratory complexes and the ATP-synthase calls for registration of their intracellular contents and activities in synchronously growing yeast [110, 111]. It turns out that during the cell cycle periodic accumulation of the enzyme complexes following by their degradation up to particular level occurs.

The question arises as to which complexes are targets for degradation. As it follows from the above-said, in case of assembly of the complexes themselves a principle of higher stability of the counterparts upon their binding with each other is held. In other words, a stable oligomeric product results from superposition of the two processes, namely assembly and degradation. What does happen to the complexes afterwards? It is known that effective interaction between the respiratory complexes requires their close enough mutual disposition in the membrane (see, e.g., [112]) and definite stoichiometric relationships determined by the turnover numbers. Data are available that the complexes are able to bind selectively with each other, thus forming superensembles [113-116]. The mystery is how the optimal stoichiometry of complexes and their spatial organization in the respiratory chain are achieved. If the assembly of individual complexes has to be controlled (perhaps with the involvement of proteases and chaperones), then how can formation of the functional superensembles be controlled?

According to an alternative model, the respiratory complexes are distributed in the membrane in a random way and interact with each other through diffusion of coenzyme Q and cytochrome *c* [117]. This model suggests that only those complexes can essentially contribute to the mitochondrial respiration that accumulate in the membrane in optimal combination and in close enough vicinity to each other. What is then the fate of the individual complexes that do not meet these conditions?

Obviously, upon respiratory chain formation, in some regions of the membrane a situation is possible when stoichiometry and arrangement of the complexes do not provide highly efficient electron transfer. In principle, such regions of the membrane and corresponding parts of the organelle might have both low transmembrane potential and low local ATP content. It is known that such conditions stimulate accumulation of free fatty acids, activation of mitochondrial phospholipase A_2, changes in the membrane permeability, etc. (see details in [118]). These processes inevitably lead to the local swelling of mitochondria, rupture of the membranes and extrusion of membrane fragments and soluble mitochondrial contents into the medium where they may be rendered targets for lysosomes/vacuoles. In fact, morphological observations show that lysosomes capture either whole mitochondria (see references in [8] and also in [119, 120]) or their limited parts [121], this process being somehow related to de-energization of the organelles.

In the context of the above data for mitochondria, one may speculate that the role of lysosomal/vacuolar apparatus in the biogenesis of cell organelles is universal. There are numerous examples of autophagy of the ER induced by changing the functional state of its resident enzyme systems. It is known that upon removal of the inducers/substrates of the monooxygenase system cytochrome P450s as well as functionally associated NADPH: cytochrome P450 reductases and cytochrome b_5 undergo degradation. Therewith the "excessive" smooth reticulum is selectively eliminated and its fragments can be detected in autophagosomes [122]. In the presence of leupeptin, an inhibitor of lysosomal cathepsins, cytochrome P450(2B-1) and NADPH: cytochrome P450 reductase are accumulated in autophagosomal vacuoles [123-125].

Little is known about autophagy of chloroplasts, especially about the relationship of this event to their bio-

genesis. Chloroplasts are degraded in vacuoles under extreme conditions, e.g., upon cold adaptation [126] and vitrification of the plants [127] as well as upon their adaptation to the higher illumination [128]. In the latter case a decrease in the number of chloroplasts per cell was noted. The take-up of chloroplasts by vacuoles through microautophagy, when the chloroplast envelope penetrated into the vacuole, was described [127].

Relatively well studied is autophagy of peroxisomes (pexophagy). This process is induced under conditions when these organelles become unnecessary in the context of changes in the cell metabolism (see reviews [9, 10, 129]). Such a situation is possible when yeast grown in medium with methanol or fatty acids (i.e., basic substrates for peroxisomes) are transferred into medium with ethanol or glucose. Depending on the kind of yeast and carbon source in the growth medium, degradation of peroxisomes proceeds in the form of either macroautophagy or microautophagy. Upon macroautophagy, peroxisomes are initially sequestered by a bilayer membrane capable of fusing with the vacuolar membrane. Owing to this the whole organelle becomes incorporated into the vacuole where it undergoes digestion.

Two *Hansenulla polymorpha* mutants, namely *pdd1* and *pdd2* (peroxisome degradation-deficient), characterized by definite disturbed stages of pexophagy have been obtained. In the former mutant peroxisomes are not coated with the electron dense membrane while in the latter the sequestrated peroxisomes are not captured by vacuoles. The molecular mechanisms of both stages are not yet established. It is only known (see review [10]) that the *PDD1* gene encodes a protein homologous to the Vps34 protein (*Saccharomyces cerevisiae*) that shows phosphatidylinositol-3-kinase activity and takes some part in sorting the vacuolar proteins. As the targets for degradation are whole organelles, not their individual proteins accumulating in the cytoplasm of the yeast mutants abnormal with respect to formation of these organelles, one may speculate that the peroxisomal membranes contain some "sensor" capable of inducing pexophagy under definite conditions.

Upon microautophagy, peroxisomes are captured by the vacuole without preliminary covering the organelle with the sequestering membrane. In the *Pichia pastoris* yeast this process can follow two different routes depending on the carbon source in the medium. Individual stages of microautophagy have been characterized morphologically and the mutants (*gsa* is glucose selective autophagy and *pag* is peroxisome autophagy) with disturbed stages of this process have been prepared. Selective pexophagy is controlled by the Gsa9 protein at the stage immediately preceding organelle drawing into the vacuole [130]. This peripheral membrane protein susceptible to oligomerization concentrates at the vacuole site contacting the peroxisome.

Although there is some progress in understanding morphological and genetic aspects of pexophagy, the molecular mechanisms of this event remain obscure. More impressible results were achieved upon elucidating those for starvation-induced cell autophagy (see reviews [10, 129, 131]). This process is mediated by a group of unique proteins (APG- and AUT-proteins) ensuring controlled formation of autophagosomes and their fusion with the lysosome/vacuole. Among 16 members of the APG/AUT group, eight are functional analogs of components of the ubiquitin system. Apg8 can covalently bind with phosphatidylethanolamine, while Apg12 conjugates with Apg5. Such primary conjugates associates then with Apg16 capable of oligomerizing, which overall results in multicomponent protein complexes associated with lipids. Thus, there are grounds to suggest that the autophagosomal membrane is formed *de novo* from unique components. Formation of autophagosomes is somehow controlled through phosphorylation of Apg13 and its interaction with Apg1 (Ser/Thr kinase), which in turn depends on the activity of Tor, a phosphatidylinositol kinase homolog.

It is still questionable whether the usual apparatus of cell autophagy is involved in pexophagy, although there is evidence that the *apg/cvt* mutants are also imperfect with respect to pexophagy [129]. An alternative mechanism of degradation of both peroxisomes and mitochondria is that at the initial stage of autophagy the target organelles are coated with a bilayer membrane originating from the ER. In such structures (autophagosomes) higher acidity is already maintained but hydrolytic enzymes are still absent. In due course, the latter accumulate in autophagosomes, which then convert into autolysosomes (see review [8]). The molecular mechanisms of these transformations are still unknown.

Pexophagy is a selective process. It proceeds under conditions when other organelles, e.g., mitochondria [132], survive. There are reasons to suppose a definite relationship between the functional state of peroxisomes and their stability (see review [9]). It has particularly been shown that degradation of peroxisomes is triggered by antilipolytic agents disturbing provision of peroxisomes with fatty acids [133]. On the other hand, clofibrate-induced degradation of peroxisomes in hepatocytes is suppressed by the addition of fatty acids [134]. Moreover, in yeast the degradation of peroxisomes is stimulated by cyanide, which inactivates alcohol oxidase, a key enzyme of peroxisomes [135]. *In vitro* experiments [136] have shown that, if the medium contains a substrate for oxidase reaction but does not contain a substrate for coupled transamination, the fine structural organization of peroxisomes suffers some disturbance manifesting in "loosened" matrix. Finally, one should mention that the degradation of peroxisomes is usually preceded by the loss of activity of its key enzymes. Although the relationship between stability of peroxisomes and their functional state

is evident, the nature of this event is obscure. How the cell gets to know that peroxisomes cease to function for one or another reason and have to be eliminated as a ballast material is also something of mystery. Perhaps in this case a disturbance in the asymmetry of the limiting membrane occurs which leads to exposing a sensor recognizable by the components of the autophagosomal system or by some special ER region. Something like that probably occurs upon capturing apoptose cells by macrophages when the target cells expose phosphatidylserine playing the part of a ligand for corresponding macrophage receptors (see, e.g., [137]).

In any event, it is obvious that formation of peroxisomes can be controlled according to the "principle of selection by a performance criterion" which has been dealt with in detail as applied to mitochondria [118].

Amongst the processes hindering the rise of perfect organelles, one should point out misaddressing of foreign proteins into them. Such an event is well known for mitochondria. It has particularly been shown that a mutated form of alanine: gluoxylate aminotransferase, a peroxisomal enzyme, is partially imported into mitochondria in the patients suffering primary hyperoxaluria type I [138, 139]. It is also known that some peroxisomal proteins are imported into mitochondria in case of their hypersynthesis [140] or in case of disturbed protein import into peroxisomes [141]. Moreover, model experiments with the use of many recombinant proteins indicate that about 10% of random N-terminal amino acid sequences can play the part of the mitochondrial transport signals [142], i.e., that the fidelity of selection of proteins for mitochondria from the cytoplasm does not exceed 90%. The same is true for the translocation of foreign proteins into the ER [142]. Unfortunately, we still have not much data on both the fate of the imported foreign proteins in the cell and their possible effects on the biogenesis of the corresponding recipient organelles. However, it is evident from general considerations that, in principle, the foreign proteins can compete with the indigenous ones of an organelle for chaperones and proteases [143], thus disturbing normal organelle formation. When folding of the foreign proteins is retarded, they undergo selective degradation [77, 78]. It is tempting to speculate that in case of normal folding and maturation the foreign proteins will accumulate in the organelle until they somehow influence its key function, thus causing elimination of the organelle.

Notice that the afore-said is unrelated to the case of natural double addressing of proteins into organelles. For instance, it has been shown that some cytochrome P450s have complex addressing signals and under definite conditions are imported not into the ER but into mitochondria, where they carry out definite metabolic functions [144].

7. CONCLUSIONS

The question will not fail to emerge of whether the mechanisms of selective elimination of proteins and organelles discussed in the preceding sections are of vital necessity. This question might be elucidated by switching off the lysosomal/vacuolar apparatus and by following some changes in the cell. Unfortunately, the potentialities of such approach are limited as, besides the quality control, proteasomes and vacuoles carry out many vital functions determining the ability of the cell to survive (see, e.g., reviews [8, 145]). As a result, such an approach allows observation of only short-term effects related to the disturbance in the quality control systems of the cell.

Another approach is violation of the natural balance between the rates of protein synthesis and degradation by means of significant stimulation of the former. In this context one may refer to work [146] where it has been shown that hyperexpression of the translation initiation factor 4E (eIF-4E) in HeLa cells, ensuring 3-9-fold increase in its intracellular content, leads to dramatic changes finally manifested in the formation of multinuclear cells. Unfortunately, this work was not subsequently developed, although the suggested model could provide important information about the role of quality control in cell life.

One more example of interrupted balance between the rates of protein synthesis and degradation is given in work [147] where the consequences of hyperexpression of Lon protease in the *E. coli* cells were in focus. It turned out that just 2-3-fold increase in the activity of this protease alone proved to be enough to markedly increase the rate of degradation of the total cell protein and to decrease the cell growth.

When considering the controlling part of proteases in protein folding/oligomerization and organelle formation, two aspects of this problem have intentionally been left uncovered. First, one could go into details of NPCs folding and oligomerization in the cytosol. Such control is known to take place and is realized with the involvement of proteasomes. However, this is the matter of detailed analysis of interaction of the NPCs with proteasomes, which is far from the author's interests. Second, during recent years it has become obvious that proteasomes are transported into the nucleus [148]. However the data on the their specific functions in this compartment are still too scanty for discussion.

Be it as it may, the available data undoubtedly show that mistakes occur at all the levels and stages of formation of the cell architectonics and that the mechanisms for their elimination are at work.

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REFERENCES

1. Fayadat, L., Siffroi-Fernandez, S., Lanet, J., and Franc, J.-L. (2000) *J. Biol. Chem.*, **275**, 15948-15954.
2. Minami, Y., Weissman, A. M., Samelson, L. E., and Klausner, R. D. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 2688-2692.
3. Schubert, U., Anton, L. C., Gibbs, J., Norbury, C. C., Yewdell, J. W., and Bennink, J. R. (2000) *Nature*, **404**, 770-778.
4. Galkin, A. S., Tsoi, T. V., and Luzikov, V. N. (1980) *Biochem. J.*, **190**, 145-156.
5. Kopito, R. R. (1997) *Cell*, **88**, 427-430.
6. Brodsky, J. L., and McCracken, A. A. (1997) *Trends Cell Biol.*, **7**, 151-156.
7. Wickner, S., Maurizi, M. R., and Gottesman, S. (2000) *Science*, **286**, 888-893.
8. Dunn, W. A. (1994) *Trends Cell Biol.*, **4**, 139-143.
9. Subramani, S. (1998) *Physiol. Rev.*, **78**, 171-188.
10. Scott, S. V., and Klyonsky, D. J. (1998) *Curr. Opin. Cell Biol.*, **10**, 523-529.
11. Luzikov, V. N. (1999) *FEBS Lett.*, **448**, 201-205.
12. Travers, K. J., Patil, C. K., Wodicka, L., Lockhart, D. J., Weissman, J. S., and Walter, P. (2000) *Cell*, **101**, 249-258.
13. Teckman, J. H., and Perlmutter, D. H. (1996) *J. Biol. Chem.*, **271**, 13215-13220.
14. Werner, E. D., Brodsky, J. L., and McCracken, A. A. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 13797-13801.
15. Ward, C. L., Omura, S., and Kopito, R. R. (1995) *Cell*, **83**, 121-127.
16. Jensen, T. J., Loo, M. A., Pind, S., Williams, D. B., Goldberg, A. L., and Riordan, J. R. (1995) *Cell*, **83**, 129-135.
17. Halaban, R., Svedine, S., Cheng, E., Smicun, J., Aron, R., and Hebert, D. N. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 5889-5894.
18. Beguin, P., Hasler, U., Straub, O., and Geering, K. (2000) *Mol. Biol. Cell*, **11**, 1657-1672.
19. Yu, H., and Kopito, R. R. (1999) *J. Biol. Chem.*, **274**, 36852-36858.
20. Ravid., T., Doolman, R., Avner, R., Harats, D., and Roitelman, J. (2000) *J. Biol. Chem.*, **275**, 35840-35847.
21. Hampton, R. Y., Gardner, R. G., and Rine, J. (1996) *Mol. Biol. Cell*, **7**, 2029-2044.
22. McGee, T. P., Cheng, H. H., Kumagai, H., Omura, T., and Simoni, R. D. (1996) *J. Biol. Chem.*, **271**, 25630-25638.
23. Mayer, T. U., Brown, T., and Jentsch, S. (1998) *EMBO J.*, **17**, 3251-3257.
24. Hiller, M. M., Finger, A., Schweiger, M., and Wolf, D. H. (1996) *Science*, **273**, 1725-1728.
25. Wiertz, E. J. H. J., Tortorella, D., Bogyo, M., Yu, J., Mothes, W., Jones, T. R., Rapoport, T. A., and Ploegh, H. L. (1996) *Nature*, **384**, 432-438.
26. Petaja-Repo, U. E., Hogue, M., Laperriere, A., Bhalla, S., Walker, P., and Bouvier, M. (2001) *J. Biol. Chem.*, **276**, 4416-4423.
27. Liao, W., Yeung, S.-C., and Chan, L. (1998) *J. Biol. Chem.*, **273**, 27225-27230.
28. Pilon, M., Schekman, R., and Römisch, K. (1997) *EMBO J.*, **16**, 4540-4548.
29. Plemper, R. K., Böhmler, S., Bordallo, J., Sommer, T., and Wolf, D. H. (1997) *Nature*, **388**, 891-895.
30. Bordallo, J., Plemper, R. K., Finger, A., and Wolf, D. H. (1998) *Mol. Biol. Cell*, **9**, 209-222.
31. Gardner, R. G., Swarbrick, G. M., Bays, N. B., Cronin, S. R., Wilhovsky, S., Seeling, L., Kim, C., and Hampton, R. Y. (2000) *J. Cell Biol.*, **151**, 69-82.
32. Knop, M., Finger, A., Braun, T., Hellmuth, K., and Wolf, D. H. (1996) *EMBO J.*, **15**, 753-763.
33. Sommer, T., and Jentsch, S. (1993) *Nature*, **365**, 176-179.
34. Biederer, T., Volkwein, C., and Sommer, T. (1997) *Science*, **278**, 1806-1809.
35. Rapoport, T., Jungnickel, B., and Kutay, U. (1996) *Ann. Rev. Biochem.*, **65**, 271-303.
36. Corsi, A. K., and Schekman, R. (1996) *J. Biol. Chem.*, **271**, 30299-30302.
37. Plemper, R. K., Bordallo, J., Deak, P. M., Taxis, C., Hitt, R., and Wolf, D. (1999) *J. Cell Sci.*, **112**, 4123-4134.
38. Hamman, B. D., Hendershot, L. M., and Johnson, A. E. (1998) *Cell*, **92**, 747-758.
39. Meyer, H.-A., Grau, H., Kraft, R., Kostka, S., Prehn, S., Kalies, K.-U., and Hartmann, E. (2000) *J. Biol. Chem.*, **275**, 14550-14557.
40. Tyedmers, J., Lerner, M., Bies, C., Dudek, J., Skowronek, M., Haas, I. G., Heim, N., Nastainczyk, W., Volkmer, J., and Zimmermann, R. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 7214-7219.
41. Misselwitz, B., Staeck, O., Matlack, K. E. S., and Rapoport, T. (1999) *J. Biol. Chem.*, **274**, 20110-20115.
42. Brodsky, J. L., Werner, E. D., Dubas, M. E., Goeckeler, J. L., Kruse, K. B., and McCracken, A. A. (1999) *J. Biol. Chem.*, **274**, 3453-3460.
43. Cabral, C. M., Choudhury, P., Liu, Y., and Sifers, R. N. (2000) *J. Biol. Chem.*, **275**, 25015-25022.
44. Wilson, C. M., Farmery, M. R., and Bulleid, N. J. (2000) *J. Biol. Chem.*, **275**, 21224-21232.
45. Hebert, D. N., Foellmer, B., and Helenius, A. (1996) *EMBO J.*, **15**, 2961-2968.
46. Qu, D., Teckman, J. H., Omura, S., and Perlmutter, D. H. (1996) *J. Biol. Chem.*, **271**, 22791-22795.
47. Tokunaga, F., Brostrom, C., Koide, T., and Arvan, P. (2000) *J. Biol. Chem.*, **275**, 40757-40764.
48. Fagioli, C., and Sitia, R. (2001) *J. Biol. Chem.*, **276**, 12885-12892.
49. De Virgilio, M., Weninger, H. H., and Ivessa, N. E. (1998) *J. Biol. Chem.*, **273**, 9734-9743.
50. Yu, H., and Kopito, R. R. (1999) *J. Biol. Chem.*, **274**, 36852-36858.
51. Fisher, E. A., Zhou, M., Mitchell, D. M., Omura, S., Wang, H., Goldberg, A. L., and Ginsberg, H. N. (1997) *J. Biol. Chem.*, **272**, 20427-20434.
52. Lüders, J., Demand, J., and Höhfeld, J. (2000) *J. Biol. Chem.*, **275**, 4613-4617.
53. Imamura, T., Haruta, T., Takata, Y., Usui, I., Iwata, M., Ishichara, H., Ishiki, M., Ishibashi, O., Ueno, E., Sasaoka, T., and Kobayashi, M. (1998) *J. Biol. Chem.*, **273**, 11183-11188.
54. Moriyama, T., Sather, S. K., McGee, T. P., and Simoni, R. D. (1998) *J. Biol. Chem.*, **273**, 22037-22043.
55. Plemper, R. K., Egner, R., Kuchler, K., and Wolf, D. (1998) *J. Biol. Chem.*, **273**, 32848-32856.
56. Loo, T. W., and Clarke, D. M. (1998) *J. Biol. Chem.*, **273**, 32373-32376.
57. Hill, K., and Cooper, A. A. (2000) *EMBO J.*, **19**, 550-561.

58. Chang, A., and Fink, G. R. (1995) *J. Cell Biol.*, **128**, 39-49.
59. Li, Y., Kane, T., Tipper, C., Spatrick, P., and Jenness, D. D. (1999) *Mol. Cell. Biol.*, **19**, 3588-3599.
60. Yamamoto, K., Fujii, R., Toyofuku, Y., Saito, T., Koseki, H., Hsu, V. W., and Aoe, T. (2001) *EMBO J.*, **20**, 3082-3091.
61. Mori, K. (2000) *Cell*, **11**, 451-454.
62. Iwawaki, T., Hosoda, A., Okuda, T., Kamigori, Y., Nomura-Furuwatari, C., Kimata, Y., Tsuru, A., and Kohno, K. (2001) *Nature Cell Biol.*, **3**, 158-164.
63. Goldberg, A. (1992) *Eur. J. Biochem.*, **203**, 9-23.
64. Gottesman, S., Maurizi, M. R., and Wickner, S. (1997) *Cell*, **91**, 435-438.
65. Larsen, C. N., and Finley (1997) *Cell*, **91**, 431-434.
66. Lupas, A., Flanagan, J. M., Tamura, T., and Baumaister, W. (1997) *Trends Biochem. Sci.*, **22**, 399-404.
67. Suzuki, C. K., Rep, M., van Dijl, J. M., Suda, K., Grivell, L. A., and Schatz, G. (1997) *Trends Biochem. Sci.*, **22**, 118-123.
68. Gottesman, S. (1996) *Annu. Rev. Genet.*, **30**, 465-506.
69. Herman, C., and D'Ari, R. (1998) *Curr. Opin. Microbiol.*, **1**, 204-209.
70. Betton, J.-M., Sassoon, N., Hofnung, M., and Lauren, M. (1998) *J. Biol. Chem.*, **273**, 8897-8902.
71. Hoskins, J. R., Pak, M., Maurizi, M. R., and Wickner, S. (1998) *Proc. Natl. Acad. Sci. USA*, **95**, 12135-12140.
72. Kihara, A., Akiyama, Y., and Ito, K. (1999) *EMBO J.*, **18**, 2970-2981.
73. Stuart, R. A., Cyr, D. M., Craig, E. A., and Neupert, W. (1994) *Trends Biochem. Sci.*, **19**, 87-92.
74. Langer, T., and Neupert, W. (1996) *Experientia*, **52**, 1069-1076.
75. Langer, T. (2000) *Trends Biochem. Sci.*, **25**, 247-251.
76. Pajic, A., Tauer, R., Feldmann, H., Neupert, W., and Langer, T. (1994) *FEBS Lett.*, **353**, 201-206.
77. Wagner, I., Arlt, H., van Dyck, L., Langer, T., and Neupert, W. (1994) *EMBO J.*, **13**, 5135-5145.
78. Savel'ev, A. S., Novikova, L. A., Kovaleva, I. E., Luzikov, V. N., Neupert, W., and Langer, T. (1998) *J. Biol. Chem.*, **273**, 20596-20602.
79. Wheeldon, L. W., Dianoux, A. C., and Vignais, P. V. (1974) *Eur. J. Biochem.*, **46**, 189-199.
80. Kalnov, S. L., Novikova, L. A., Zubatov, A. S., and Luzikov, V. N. (1979) *Biochem. J.*, **183**, 195-202.
81. Pearce, D. A., and Sherman, F. (1995) *J. Biol. Chem.*, **270**, 20879-20882.
82. Guzelin, E., Rep, M., and Grivel, L. A. (1996) *FEBS Lett.*, **381**, 42-46.
83. Glerum, D. M., and Tzagoloff, A. (1997) *FEBS Lett.*, **412**, 410-414.
84. Arlt, H., Tauer, R., Feldmann, H., Neupert, W., and Langer, T. (1996) *Cell*, **85**, 875-885.
85. Rep, M., van Dijl, J. M., Suda, K., Schatz, G., Grivell, L. A., and Suzuki, C. K. (1996) *Science*, **274**, 103-106.
86. Leonhard, K., Stiegler, A., Neupert, W., and Langer, T. (1999) *Nature*, **398**, 348-351.
87. Leonhard, K., Guiard, B., Pellecchia, G., Tzagoloff, A., Neupert, W., and Langer, T. (2000) *Mol. Cell*, **5**, 629-638.
88. Landry, S. J., and Bartlett, S. G. (1989) *J. Biol. Chem.*, **264**, 9090-9093.
89. Lubben, T. H., Donaldson, G. K., Viitanen, P. V., and Gatenby, A. A. (1989) *Plant Cell*, **1**, 1223-1230.
90. Tsugeki, R., and Nishimura, M. (1993) *FEBS Lett.*, **320**, 198-202.
91. Viitanen, P. V., Schmidt, M., Buchner, J., Suzuki, T., Vierling, E., Dickson, R., Lorimer, G. H., Gatenby, A., and Soll, J. (1995) *J. Biol. Chem.*, **270**, 18158-18164.
92. Maurizi, M. R., Clark, W. P., Kim, S. H., and Gottesman, S. (1990) *J. Biol. Chem.*, **265**, 12546-12552.
93. Shanklin, J., DeWitt, N. D., and Flanagan, J. M. (1995) *Plant Cell*, **7**, 1713-1722.
94. Sokolenko, A., Lerbs-Mache, S., Altschmied, L., and Herrmann, R. G. (1998) *Planta*, **207**, 286-295.
95. Majeran, W., Wollman, F.-A., and Vallon, O. (2000) *Plant Cell*, **12**, 137-149.
96. Weaver, L. M., Froehlich, J. E., and Amasino, R. M. (1999) *Plant Physiol.*, **119**, 1209-1216.
97. Nakabayashi, K., Ito, M., Kiyosue, T., Shinozaki, K., and Watanabe, A. (1999) *Plant Cell Physiol.*, **40**, 504-515.
98. Schlicher, T., and Soll, J. (1996) *FEBS Lett.*, **379**, 302-304.
99. Itzhaki, H., Naveh, L., Lindhal, M., Cook, M., and Adam, Z. (1998) *J. Biol. Chem.*, **273**, 7094-7098.
100. Haußühl, K., Andersson, B., and Adamska, I. (2001) *EMBO J.*, **20**, 713-722.
101. Lindahl, M., Tabak, S., Cseke, L., Pichersky, E., Andersson, B., and Adam, Z. (1996) *J. Biol. Chem.*, **271**, 29329-29334.
102. Ostersetzer, O., and Adam, Z. (1997) *Plant Cell*, **9**, 957-965.
103. Chen, M., Choi, Y., Voytas, D. F., and Rodermel, S. (2000) *Plant J.*, **20**, 303-313.
104. Seo, S., Okamoto, M., Iwai, T., Iwano, M., Fukui, K., Isogai, A., Nakajima, N., and Ohashi, Y. (2000) *Plant Cell*, **12**, 917-932.
105. Cuming, A. C., and Bennett, J. (1981) *Eur. J. Biochem.*, **118**, 71-80.
106. DeVitry, C., Olive, J., Drapier, D., Recouvreur, M., and Wollman, F.-A. (1989) *J. Cell Biol.*, **109**, 991-1006.
107. Lemaire, C., and Wollman, F.-A. (1989) *J. Biol. Chem.*, **264**, 10235-10242.
108. Kuras, R., and Wollman, F.-A. (1994) *EMBO J.*, **13**, 1019-1027.
109. Biekmann, S., and Freierabend, J. (1985) *Eur. J. Biochem.*, **152**, 529-535.
110. Edwards, S. W., Evans, J. B., Williams, J. L., and Lloyd, D. (1982) *Biochem. J.*, **202**, 453-458.
111. Lloyd, D., Edwards, S. W., Williams, J. L., and Evans, J. B. (1983) *FEMS Microbiol. Lett.*, **16**, 307-312.
112. Schwerzmann, K., Cruz-Orive, L. M., Eggman, R., Sänger, A., and Weibel, E. R. (1986) *J. Cell Biol.*, **102**, 97-103.
113. Luzikov, V. N., Rakhimov, M. M., and Berezin, I. V. (1968) *Doklady Akad. Nauk SSSR*, **178**, 471-474.
114. Boumans, H., Grivell, L. A., and Berden, J. A. (1998) *J. Biol. Chem.*, **273**, 4872-4877.
115. Schägger, H., and Pfeiffer, K. (2000) *EMBO J.*, **19**, 1777-1783.
116. Cruciat, C.-M., Brunner, S., Baumann, F., Neupert, W., and Stuart, R. A. (2000) *J. Biol. Chem.*, **275**, 18093-18098.
117. Höchli, M., Höchli, L., and Hackenbrock, C. R. (1985) *Eur. J. Cell Biol.*, **38**, 1-5.
118. Luzikov, V. N. (1985) *Mitochondrial Biogenesis and Breakdown*, Plenum Publisher Corporation, New York-London.
119. Yakota, S., Himeno, M., Roth, J., Brada, D., and Kato, K. (1993) *Eur. J. Cell. Biol.*, **62**, 372-383.

120. Stromhaug, P. E., Berg, T. O., Fengsrud, M., and Seglen, P. O. (1998) *Biochem. J.*, **335**, 217-224.
121. Mancini, M., Anderson, B. O., Caldwell, E., Sedghinasab, M., Paty, P. B., and Hockenbery, D. M. (1997) *J. Cell. Biol.*, **138**, 449-469.
122. Feldman, D., Swarm, R. L., and Becker, J. (1980) *J. Histochem. Cytol.*, **28**, 997-1006.
123. Masaki, R., Yamamoto, A., and Tashiro, Y. (1987) *J. Cell Biol.*, **104**, 1207-1215.
124. Ueno, T., Muno, D., and Kominami, E. (1991) *J. Biol. Chem.*, **266**, 18995-18999.
125. Ronis, M. J. J., Johansson, I., Hultenby, K., Lagercrantz, J., Glaumann, H., and Ingelman-Sundberg, M. (1991) *Eur. J. Biochem.*, **198**, 383-389.
126. Wu, J., Lightner, J., Warwick, N., and Browse, J. (1997) *Plant Physiol.*, **113**, 347-356.
127. Franck, T., Crevecocur, M., Wuest, J., Greppin, H., and Caspar, T. (1998) *Biothech. Histochem.*, **73**, 32-43.
128. Maxwell, K., Marrison, J. L., Leech, R. M., Griffiths, H., and Horton, P. (1999) *Plant Physiol.*, **121**, 89-95.
129. Klionsky, D. J., and Ohsumi, Y. (1999) *Ann. Rev. Cell Dev. Biol.*, **15**, 1-32.
130. Kim, J., Kamada, Y., Stromhaug, P. E., Guam, J., Hefner-Gravink, A., Baba, M., Scott, S. V., Ohsumi, Y., Dunn, W. A., and Klionsky, D. J. (2001) *J. Cell Biol.*, **153**, 381-396.
131. Ohsumi, Y. (2000) *Nature Mol. Cell Biol.*, **2**, 211-216.
132. Gunkel, K., van der Klei, I. J., Barth, G., and Veenhuis, M. (1999) *FEBS Lett.*, **451**, 1-4.
133. Locci, C. T., Masiello, P., Pollera, M., and Bergamini, E. (1985) *Biochim. Biophys. Acta*, **839**, 96-104.
134. Luiken, J. J. F. P., van den Berg, M., Heikoop, J. C., and Meijer, A. J. (1992) *FEBS Lett.*, **304**, 93-97.
135. Van der Klei, I. J., Veenhuis, M., Nicolay, K., and Harder, W. (1989) *Arch. Microbiol.*, **151**, 26-33.
136. Heupel, R., and Heldt, H. W. (1994) *Eur. J. Biochem.*, **220**, 165-172.
137. Fadok, V. A., de Cathelineau, A., Daleke, D. L., Henson, P. M., and Bratton, D. L. (2001) *J. Biol Chem.*, **276**, 1071-1077.
138. Purdue, P. E., Takada, Y., and Danpure, C. J. (1990) *J. Cell Biol.*, **111**, 2341-2351.
139. Leiper, J. M., Oatery, P. B., and Danpure, C. J. (1996) *J. Cell Biol.*, **135**, 939-957.
140. Sulter, G. J., Waterham, H. R., Vrieling, E. G., Goodman, J. M., Harder, W., and Veenhuis, M. (1993) *FEBS Lett.*, **315**, 211-216.
141. Sacksteder, K. A., Jones, J. M., South, S. T., Li, X., Liu, Y., and Gould, S. J. (2000) *J. Cell Biol.*, **148**, 931-944.
142. Raiser, C. A., and Botstein, D. (1990) *Mol. Cell. Biol.*, **10**, 3163-3173.
143. Saveliev, A. S., Kovaleva, I. E., Novikova, L. A., Isaeva, L. V., and Luzikov, V. N. (1999) *Arch. Biochem. Biophys.*, **363**, 373-376.
144. Boopathi, E., Anandatheerthavarada, K., Bhagwat, S. V., Biswas, G., Fang, J.-K., and Avadhani, N. G. (2000) *J. Biol. Chem.*, **275**, 34415-34423.
145. Hilt, W., and Wolf, D. H. (1996) *Trends Biochem. Sci.*, **21**, 96-102.
146. De Benedetti, A., and Rhoads, R. E. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 8212-8216.
147. Goff, S. A., and Goldberg, A. L. (1987) *J. Biol. Chem.*, **262**, 4508-4515.
148. Reits, E. A., Benham, A. M., Plougastel, B., Neefjes, J., and Trowsdale, J. (1997) *EMBO J.*, **15**, 6987-6094.