Aggresomes and Russell bodies

Symptoms of cellular indigestion?

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All cells are equipped with a proteolytic apparatus that eliminates damaged, misfolded and incorrectly assembled proteins. The principal engine of cytoplasmic proteolysis, the 26S proteasome, requires that substrates be unfolded to gain access to the active site; consequently, it is relatively ineffective at degrading aggregated proteins. Cellular indigestion occurs when the production of aggregation-prone proteins exceeds the cell's (or organelle's) capacity to eliminate them. Cellular pathways that resolve this indigestion exist, but appear to have limited capacities. Russell bodies and aggresomes are manifestations of cellular indigestion in the endoplasmic reticulum and cytoplasmic compartments, respectively, and are often associated with disease.

Introduction

In living cells, proteins are constantly degraded. Proteolysis is essential to maintain metabolic homeostasis; it regulates central biological phenomena including cell proliferation, signaling and antigen presentation (see Kirschner, 1999; Wickner *et al.*, 1999) and serves to eliminate potentially toxic, misfolded and damaged proteins. Proteasomes, barrel-shaped structures in which the active sites of various proteases face the interior cavity, are the major engine of cytoplasmic proteolysis. A cost of this arrangement is the requirement that substrates must be unfolded to be 'fed' into the narrow entrance to the interior cavity. Eukaryotic cells face an additional topological problem: since proteasomes are excluded from membrane-bound organelles (Rivett, 1998), these must be equipped with either a distinct proteolytic system within these organelles, or with machinery to export short-lived proteins into the cytosol for degradation.

Quality control in the ER: the cytoplasmic connection

Proteins destined for the extracellular space and the organelles of the central vacuolar system fold in the endoplasmic reticulum (ER). A sophisticated quality control system (Ellgaard *et al.*, 1999) ensures that folding or assembly intermediates are prevented from progressing to the Golgi apparatus, and that misfolded proteins are eventually retro-translocated, or 'dislocated', across the ER membrane to the cytosol for proteasomal degradation. The Sec61 complex, which forms a channel called a 'translocon' through which nascent proteins are translocated into the ER, also seems to be responsible for dislocation of misfolded proteins (Wiertz *et al.*, 1996; Pilon *et al.*, 1997). This unexpected 'cytoplasmic connection' between quality control in the ER and cytosolic proteasomes (Kopito, 1997) has provided an answer to the long debated problem of how myriads of nascent proteins might coexist in the ER lumen with an aggressive proteolytic system (Klausner and Sitia, 1990). At the same time, it raised a new wave of exciting questions related to: (i) the mechanisms that determine substrate selection and delivery to the dislocation apparatus; (ii) the vectorial nature of retrograde transport; (iii) the role of chaperones and enzymes in substrate unfolding and presentation to the proteasomes, and (iv) the nature of the timer that allows the dislocation of misfolded molecules while sparing nascent chains or folding and assembly intermediates (Brodsky and McCracken, 1999; Plemper and Wolf, 1999).

The main features of the process of ER-associated degradation (ERAD) are outlined in Figure 1. For transmembrane proteins such as the cystic fibrosis transmembrane conductance regulator (CFTR) variant $\Delta F508$, which is the principal cause of cystic fibrosis (Jensen *et al.*, 1995; Ward *et al.*, 1995), ubiquitylation of the cytosolic domain may be required for dislocation and degradation (Ward *et al.*, 1995). Targeting of membrane-spanning

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Fig. 1. Quality control and ER-associated degradation. Proteins that fail to successfully fold or assemble are retained in the ER and, after a given time period, dislocated through a Sec61-based channel to be degraded by cytosolic proteasomes. Substrates may be multiubiquitylated by ubiquitin-conjugating enzymes associated with the cytosolic face of the ER membranes. Oligosaccharides are removed from glycoproteins by cytosolic enzymes. Unfolding and reduction of inter- and intra-chain disulfide bonds are likely to precede dislocation. Mechanisms for the concentration of substrates near dislocon complexes and for the gating of these complexes are likely to exist.

substrates to proteasomes therefore seems to follow a similar mechanism to that described for cytosolic proteins. However, questions regarding how the luminal and transmembrane domains are extracted from the ER, and what initiates the degradation of membrane proteins that, like the α -subunit of the T-cell receptor, lack ubiquitylatable cytoplasmic domains (Yu *et al.*, 1997; Yu and Kopito, 1999), remain unanswered. Perhaps even more complex is the situation of soluble ER proteins, which are fully protected from the cytoplasmic ubiquitin/proteasome apparatus by the ER membrane. As these proteins are thought to have left the translocon (Plemper *et al.*, 1999), mechanisms for their recruitment into functional dislocons must exist.

Because integral membrane ERAD substrates are usually hydrophobic and therefore prone to aggregate upon exposure to the aqueous environment of the cytoplasm, their dislocation must be tightly coupled to proteolysis. This coupling could, in principle, be achieved by a 'ratchet' mechanism in which substrates are ubiquitylated or engaged by cytoplasmic factors co-dislocationally. Dislocation could also be achieved by active 'pulling' by proteasomes or other ATPases, which could engage their substrates while still associated with the dislocon (de

Virgilio *et al.*, 1998; Mayer *et al.*, 1998; Chillaron and Haas, 2000; Mancini *et al.*, 2000). The demonstration that substrates completely lacking lysine residues and, hence, internal ubiquitylation sites, are efficiently dislocated and degraded, argues against a requirement for co-dislocational ubiquitylation (Yu *et al.*, 1997). On the other hand, the fact that some deglycosylated ERAD substrates accumulate in the cytosol (often as high molecular weight aggregates) in the absence of functional proteasomes, suggests that coupling of dislocation to proteolysis is not obligatory. It is therefore likely that sophisticated tug-of-war games are played around the Sec61 complex when polypeptides are engaged in the exit from the ER lumen. The result of these games is likely to dictate vectorial nature, efficiency of transport, and the functional coupling of dislocation and degradation.

When things go wrong

If the rate of synthesis of a given protein exceeds the combined rates of folding and degradation, some of the protein will be unable to proceed along the secretory pathway and, inevitably,

Fig. 2. Cellular indigestion. Dislocation and degradation are critical steps for the disposal of misfolded proteins in the ER. Failure of the former may perturb homeostasis, leading to the accumulation and aggregation of proteins in the ER lumen. Aggregates, which may be ordered or not, are often sorted into Russell bodies—subregions of the rough ER that tend to exclude soluble chaperones and other normal proteins present in the ER lumen. Failure of the proteasome to degrade dislocated proteins leads to the accumulation of polyubiquitylated, deglycosylated proteins in the cytosol. Aggregates are sequestered in aggresomes by retrograde transport on microtubules (gray track), facilitated by cytoplasmic dynein (red)—dynactin (green) complexes.

will cause cellular indigestion in one or more compartment as it accumulates. This indigestion may be exacerbated by mutations that interfere with protein folding and by environmental stress. Two steps are critical in the disposal of ER-synthesized proteins: extraction from the lumen and degradation by the proteasome. While inefficiency in the former will result in accumulation in the ER, reduced degradation with normal dislocation will cause substrate deposition in the cytosol (Figure 2). A number of diseases with apparently unrelated causes are accompanied by the presence of dilated ER cisternae containing mutated proteins. This feature suggests a common pathogenesis for these ER storage diseases (ERSD, see Kim and Arvan, 1998 for a comprehensive review). On the other hand, the growing number of pathological conditions characterized by the presence of cytosolic protein depositions and aggregates manifest themselves in a variety of pathogenic outcomes.

Russell bodies

More than 100 years ago, Russell (1890) described peculiar intracellular structures that he thought were fungi representing the etiological agent of cancer. It is now generally accepted that Russell bodies (RB) are dilated ER cisternae containing condensed immunoglobulins (Ig). As to their biogenesis, it was shown that the synthesis of a mutated Ig, which is neither secreted nor degraded, is sufficient to induce RB formation in cells of different species and histotype (Valetti *et al.*, 1991). Structurally, RB are generally separated from the normal ER network (Figure 3A). While calnexin and ribosomes are associated with RB membranes, soluble ER resident proteins such as BiP, PDI and ERp72 tend to be excluded from the lumen (Valetti *et al.*, 1991; C. Valetti, C. Fagioli, L. Mattioli and R. Sitia, unpublished data), suggesting the existence of sorting mechanisms

Fig. 3. Russell bodies and aggresomes. (A) Electron micrographs of myeloma cells expressing mutated IgM in which the μ chain lacks the first constant domain (courtesy of C. Grossi, L. Mattioli, C. Tacchetti and C. Valetti, University of Genova, Italy). Ribosomes can be seen on the RB membrane (see also Valetti *et al.*, 1991). At higher magnification (see inset), two RB of different sizes (dark arrows) can be seen next to a normal ER cisterna (white arrow). (B) Electron micrograph of an aggresome in an HEK293 cell expressing $\Delta F508$ CFTR (Nu, nucleus; m, mitochondria). At higher magnification (see inset), the centrioles (white arrows) appear localized within the aggresomes. Photomicrograph by J.A. Johnston, Stanford University.

facilitating the segregation of insoluble aggregates that might otherwise disrupt the secretory pathway.

RB were originally described in plasma cells (Maldonado *et al.*, 1966), and their frequency in this cell type probably correlates with the fact that Ig genes undergo somatic hypermutation. However, dilated ER cisternae containing condensed aberrant proteins are found in secretory cells of different origin, suggesting condensation of transport-incompetent proteins in the ER as the common cause for this morphological feature. Many disease-linked cases of intraluminal protein accumulation have been described (Kim and Arvan, 1998) including thyrocytes of congenital goiter patients and hepatocytes of individuals carrying mutated $\alpha 1$ anti-trypsin alleles (PiZ).

The correlation between liver disease and PiZ luminal depositions (Carlson *et al.*, 1989) indicates that the latter can be harmful for cells, but the exact mechanisms that lead to toxicity are not known. Protein deposits in the ER may disturb membrane traffic, generate deleterious signals, or induce an unfolded protein response (UPR; see Travers *et al.*, 2000, and references therein). Not only the abundance, but also the nature of the substrate seems to be important. For example, transfectants that accumulate Factor VIII in the ER lumen induce a robust UPR (Dorner *et al.*, 1989). In contrast, chimeric proteins engineered to form conditional aggregates in the ER (by pharmacological treatment) do not show signs of ER stress (Rivera *et al.*, 2000), perhaps because they do not sequester chaperone molecules. Indeed, ER stress sensors, like PERK and IRE1, are apparently able to sense the levels of free BiP (Bertolotti *et al.*, 2000). In some cases, the anatomy of the ER is restored by relief of the restrictive condition, clearly indicating that cells can recover (Pacifci and Iozzo, 1988). Moreover, in plants, the formation of aggregates in the ER seems to be part of the normal developmental program for the storage of specific proteins (Galili *et al.*, 1998). RB are not the sole form of protein accumulation in specialized ER subregions (Sitia and Meldolesi, 1992). For instance, BiP relocates into a punctate pattern (BiP bodies) when ER–Golgi transport is perturbed in yeast (Nishikawa *et al.*, 1994). Recent evidence suggests that transport-competent cargo proteins may be concentrated by interaction with appropriate receptors prior to exiting the ER (see Hendershot, 2000, and references therein).

How are RBs and related structures disposed of? In pancreatic cells, which do not divide rapidly, intracisternal granules containing aggregated zymogens are eventually fused with lysosomes (Tooze *et al.*, 1990). This may also be the case for mutant PiZ retained in the endoplasmic reticulum (J.H. Teckman and D.H. Perlmutter, personal communication). Autophagic vacuoles may be formed when cell division is not sufficient to avoid levels of accumulation incompatible with the maintenance of the proper cellular architecture.

One could speculate that in the tug-of-war model described above, certain proteins, e.g. unassembled Ig heavy chains (Mancini *et al.*, 2000), might be intrinsically difficult to dislocate to the cytosol either because they establish interactions with ER chaperones, or because they form aggregates. As discussed above, mechanisms are likely to exist that concentrate substrates in the vicinity of the dislocon and favor their partial unfolding to facilitate passage through the ER membrane. When the dislocation machinery is insufficient or when mutations tip the balance away from the folding pathway, these mechanisms may generate conditions favoring substrate aggregation within the lumen, leading to cisternal dilation.

Aggresomes

If the tug-of-war across the ER membrane is won by the machinery on the cytoplasmic side of the membrane, 'dislocated' proteins are generally rapidly degraded by proteasomes, in a tightly coupled process that presumably exploits the physical association of proteasomes and the ubiquitylation machinery (Sommer and Jentsch, 1993; Biederer *et al.*, 1997) with the ER membrane. Dislocation of integral membrane and secretory proteins that is not coupled to rapid proteolysis poses

a serious risk of cytoplasmic aggregation when these often extremely hydrophobic proteins are transiently exposed to the aqueous, reducing conditions in the cytosol. For example, dislocation of highly expressed integral membrane proteins like the $\Delta F508$ mutant form of CFTR leads to the formation of indigestible cytoplasmic aggregates, which are sequestered within inclusion bodies called aggresomes (Johnston *et al.*, 1998). While RB reflect accumulation within the ER lumen, aggresomes are a symptom of cytoplasmic indigestion.

Aggresomes are formed around the microtubule organizing center (MTOC) by active minus end-directed transport of misfolded protein on microtubules (Figure 2). Their formation is prevented by disruption of the dynein–dynactin complex by overexpression of p50/dynactin, implicating cytoplasmic dynein as the motor in this transport process (Garcia-Mata *et al.*, 1999). Aggresomes are also distinguished by the presence of a cage of intermediate filament (IF) proteins, like vimentin, which are displaced from their normal cellular distribution upon loss of proteasome activity (Johnston *et al.*, 1998). Aggresomes are also variably enriched for proteasome subunits, ubiquitin and molecular chaperones (Johnston *et al.*, 1998; Garcia-Mata *et al.*, 1999; Wigley *et al.*, 1999), consistent with their formation in response to cellular stress.

Johnston *et al.* (1998) first proposed that aggresome formation is part of a general cytoplasmic response to aggregated protein. Such aggregates could form when the synthesis of aggregation-prone proteins exceeds the capacity of cytoplasmic chaperone and proteolytic systems, either as a result of overexpression, or by the acquisition of mutations that alter the balance between folding and misfolding. Thus, cytoplasmic indigestion can result from acquired or inherited deficiencies in the digestive machinery itself, from toxic food, or from simple gluttony.

Consistent with the role described above, aggresomes have been shown to form in response to the presence of aggregated forms of misfolded cytoplasmic proteins as well as to dislocated variants of transmembrane proteins such as CFTR and presenilin (Garcia-Mata *et al.*, 1999). Indeed, the formation of aggresome-like structures was previously described by Wojcik and co-workers as a response to proteasome inhibition (Wojcik *et al.*, 1996; Wojcik, 1997). These structures were characterized by deposits of proteinaceous material, ubiquitin and proteasome subunits around the microtubule organizing center of HeLa cells. Spontaneous aggresome-like inclusions containing reorganized vimentin IFs and Hsp70 surround the MTOC of Epstein–Barr virus (EBV)-transformed lymphoblastoid cells in the absence of any chemical proteasome inhibitors (Laszlo *et al.*, 1991). Intriguingly, the EBV-encoded nuclear antigen (EBNA1) has been shown to inhibit proteolysis by the ubiquitin/proteasome pathway (as part of the virus' strategy to evade presentation by class I MHC; Levitskaya *et al.*, 1997; Ploegh, 1998), suggesting that viral infection can promote cellular indigestion.

What is the difference between 'aggresomes' and 'inclusion bodies', which form in microorganisms upon overexpression of exogenous protein? Both types of structures reflect strategies to sequester misfolded proteins within the cytoplasm. However, the relatively large size of animal cells, together with the presence of a cytomatrix that restricts the diffusional mobility of large particles (Gershon *et al.*, 1985), suggests that non-diffusional mechanisms are required to segregate or sequester protein

aggregates within the cytoplasm. Formation of aggresomes, unlike generic inclusion bodies, exploits transport on microtubules; disruption of either the microtubule cytoskeleton or the retrograde motor leads to the deposition of dispersed inclusions throughout the cytoplasm (Garcia-Mata *et al.*, 1999). A second distinguishing feature of aggresomes is the presence of displaced type III IF like vimentin, which relocates from its normal extended cytoplasmic distribution, 'collapsing' to form a dense cage-like structure around the pericentriolar mass of aggregated protein. The normal function of vimentin is obscure; cells and even knock-out mice seem to survive well without it (Colucci-Guyon *et al.*, 1994). Although it is not clear whether the vimentin collapse contributes to the formation or the stabilization of aggresomes, it is a consistent feature that distinguishes aggresomes from other types of inclusion bodies.

Cytoplasmic inclusion bodies resembling aggresomes are common cytopathological features of neurons and glia in many human neurodegenerative diseases (Alves-Rodrigues *et al.*, 1998; Tran and Miller, 1999). These inclusions, like aggresomes, are enriched in proteasome subunits, molecular chaperones, ubiquitin conjugates and displaced intermediate filaments. While the vast majority of common neurodegenerative disorders are of idiopathic origin, some have been characterized and are caused by toxic 'gain of function' mutations in known genes that encode abundant cytoplasmic proteins such as α -synuclein, in the case of Parkinson's disease (Polymeropoulos *et al.*, 1997), and superoxide dismutase (SOD1) in the case of familial amyotrophic lateral sclerosis (Rosen *et al.*, 1993). The correlation between missense mutations in these genes and the formation of cytoplasmic inclusion bodies containing aggregated forms of their protein products establishes a tight linkage between cellular indigestion and neurodegeneration.

Perspectives

It is becoming evident that protein folding in cells is not highly efficient (Schubert *et al.*, 2000), and that full-time cooperation between proteolytic and chaperone systems in different cellular compartments provides a measure of immunity against toxic protein aggregates (Casagrande *et al.*, 2000; Gething, 2000; Travers *et al.*, 2000). Still, the cellular digestive tract can become overwhelmed or dysfunctional, resulting in manifestations of cellular indigestion including the formation of aggresomes and Russell bodies. Whether these structures are part of the pathology, or reflect protective 'containment' strategies, is food for thought and for future research.

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