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Department of Biology, New York University, New York, NY 10003, USA.
E-mail: kk52@nyu.edu

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Protein Quality Control: On IPODs and Other JUNQ

The accumulation of misfolded cytosolic or aggregation-prone proteins leads to cellular stress. To protect the cell, damaged or aggregated proteins are actively sequestered in two newly discovered quality control compartments, JUNQ and IPOD, which are highly conserved in evolution.

Katrin Bagola and Thomas Sommer

Quality control systems monitor the correct folding, assembly and functionality of cellular proteins. Proteins that are singled out by these systems generally exhibit altered functions and tend to form aggregates. Cells have developed different strategies to cope with defective proteins: if possible, chaperones refold aberrant proteins to restore their native conformation [1], but, if these unwanted proteins cannot be repaired, they are rapidly destroyed by the ubiquitin–proteasome pathway [2,3]. Defects in the breakdown of aberrant polypeptides may result in their aggregation. Formation of aggregates is tightly linked to several neurodegenerative disorders collectively termed 'protein folding diseases'. Current studies, however, suggest that the cellular toxicity is associated with non-native soluble protein oligomers and that the formation of large aggregates is instead cytoprotective [4].

In a recent study aimed at gaining a better understanding of the mechanisms that lead to the formation of protein aggregates, Kaganovich *et al.* [5] found that an increased incidence of misfolded proteins led to their accumulation in two distinct subcellular compartments in both yeast and mammalian cells. Kaganovich *et al.* expressed aggregation-prone proteins as well as substrates that were unable to mature properly due to different defects: in the case of Ubc9^{ts} misfolding is triggered by a thermal shift, whereas the actin E364K mutant fails to fold as a result of the point mutation. Another substrate tested was the von Hippel-Lindau protein VHL, which is expressed in the absence of partner proteins. Under stress conditions, immunofluorescence analysis revealed the formation of two distinct inclusions. Firstly, a juxtanuclear inclusion was formed as a consequence of protein overexpression or proteasome inhibition. Additional stress, like elevated temperature, then led to the development of a second, large perivacuolar inclusion at the cell periphery. Most interestingly, all disease-related amyloidogenic proteins Kaganovich *et al.* [5] tested (Rnq1, Ure2, and the disease-related Huntingtin mutant HttQ103, which contains an extended polyglutamine stretch) form an aggregate that exclusively colocalized with a perivacuolar peripheral compartment, without showing any colocalization with the juxtanuclear inclusion. In contrast to misfolded cytosolic proteins, aggregation of amyloidogenic proteins in the perivacuolar compartment occurred even in unstressed cells. These findings indicate that different classes of defective proteins are sequestered in distinct inclusions. Given that both newly discovered compartments can be found in yeast and mammalian cells, these inclusions thus seem to be evolutionarily conserved.

The ensuing analysis of the protein diffusion kinetics between the two quality control compartments and the cytoplasm revealed that the juxtanuclear inclusion largely harbors misfolded but soluble proteins that can exchange with the cytoplasmic pool. Therefore, this compartment is called 'juxtanuclear quality control' or JUNQ. In contrast, results for the perivacuolar peripheral compartment led to the suggestion that this inclusion contains mostly non-diffusing and probably aggregated substrates, which leads to its designation as 'insoluble protein deposit' or IPOD.

Interestingly, the two compartments have similarities regarding their development and function. The formation of both JUNQ and IPOD was found to depend on the formation of microtubules. Benomyl, a drug that depolymerizes microtubules, led to substrate accumulation in small puncta throughout the cytosol. This implies that both JUNQ and IPOD are formed by an active mechanism that requires the cellular transport machinery. The proposed function of both compartments as quality control compartments implies that molecular chaperones contribute to the formation of JUNQ and IPOD and in the partitioning of substrate proteins to these compartments. Indeed, the cytosolic chaperone Hsp104, which interacts with misfolded or aggregated proteins, colocalizes with both compartments where it may assist in solubilizing aggregated proteins to allow either their degradation or refolding [6]. It should be noted, however, that several *in vitro* studies demonstrated that Hsp104 can fulfill its function only in cooperation with Hsp70 [7–9]. Thus, it remains to be shown whether

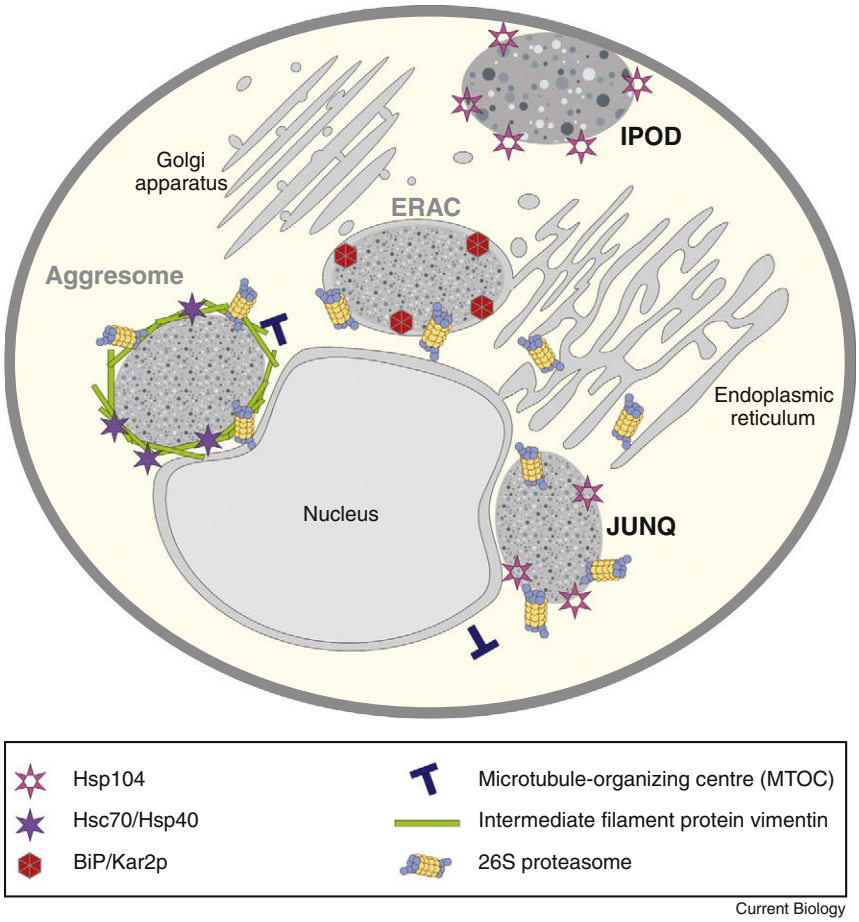

Figure 1. Quality control compartments in yeast or mammalian cells.

The IPOD compartment harbors aggregated, non-ubiquitylated proteins and is localized at the cell periphery. JUNQ, which contains ubiquitylated polypeptides, is found in an indentation of the nucleus where it is associated with proteasomes. The previously described aggresome and ERAC exhibit JUNQ-related functions but differ in compartment architecture.

Hsp70 also co-localizes with JUNQ and IPOD.

Besides these common attributes, the two inclusions also differ in certain respects: terminally misfolded proteins are degraded by the 26S proteasome. Indeed, Kaganovich *et al.* [5] observed the redistribution of proteasomes to the JUNQ compartment for all substrate proteins they examined. Conversely, proteasomes were not found in IPOD, indicating that ubiquitin-dependent protein degradation is associated with JUNQ. This is in line with the observation that, in yeast cells either lacking two stress-inducible ubiquitin-conjugating enzymes (Δ*ubc4/5*) or overexpressing the deubiquitylating enzyme Ubp4, substrate proteins exclusively accumulated in IPOD. Vice versa, it was possible to re-direct an IPOD substrate to the JUNQ compartment by artificially inducing its ubiquitylation.

In conclusion, JUNQ appears to serve as the temporary storage site for misfolded ubiquitylated proteins that cannot be folded or degraded because of the limited capacity of the ubiquitin–proteasome system under certain stress conditions. Contrary to this, IPOD is also found in non-stressed cells and results from the accumulation of aggregation-prone, mostly non-ubiquitylated substrates that are finally sequestered from the cytoplasm to protect the cell from the consequences of their potential toxicity.

It is tempting to speculate that IPOD is localized next to vacuoles and autophagic vesicles to facilitate the degradation of insoluble protein aggregates by the autophagosome. In line with this hypothesis, multilamellar structures that resemble autophagosomes are prevalent, for example, in degenerating neurons [10,11] or in cells exposed to proteasome inhibitors [12]. Previously published data indicate that certain aggregates utilize the autophagic pathway to eliminate aggregation-prone proteins that are not degraded by the ubiquitin–proteasome system [13]. But so far there is no direct proof for a functional link between the autophagosome and the IPOD described by Kaganovich *et al.* [5].

Over the past years, a number of cellular inclusions have been identified in different prokaryotic and eukaryotic organisms. Several intracellular compartments explored in yeast or mammalian cells seem to be linked to endoplasmic reticulum (ER) associated degradation of cytosolic or membrane proteins. Interestingly, previously described inclusions, for example the aggresome [12,14] and the ER-associated compartment (ERAC) [15], have features in common with the newly discovered JUNQ compartment (Figure 1). These inclusions are typically localized in a juxtanuclear region in close proximity to the ER. Moreover, they contain ubiquitylated misfolded or aggregated proteins, comparable to those found in JUNQ. In some cases, the formation of these inclusions also depends on the polymerization of microtubules [12]. Additionally, large amounts of chaperones (Hsc70/Hsp40 or BiP/Kar2p) and 26S proteasomes can be detected in these aggregates [12,15].

Future studies should address whether all of these inclusions represent related compartments or whether they have specialized functions. It remains to be analyzed how the diverse substrate proteins are recognized and destined for their different compartments. Are these proteins bound by molecular motor proteins and transported along microtubules? How do the microtubules know the destination of their transport? Furthermore, it would be interesting to clarify whether and how JUNQ and IPOD are separated from the cellular environment. The aggresome is ensheathed by the intermediate filament protein vimentin [12] whereas the ERAC is enclosed by a membrane [15]. At least in the case of JUNQ, such a boundary should be permeable for soluble misfolded

proteins and components of the quality control system to allow either refolding or proteolytic degradation. Since JUNQ and IPOD are at least partially dynamic it would be worth knowing if aggregate formation is a reversible process. Finally, the important observation that cytosolic and amyloidogenic proteins are differentially transported to distinct quality control compartments allows for a better understanding of the basic mechanisms and development of protein conformation diseases.

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Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Str.10, 13125 Berlin, Germany.
E-mail: tsommer@mdc-berlin.de

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Social Evolution: This Microbe Will Self-Destruct

Phenotypic noise and social evolution in microbes have recently attracted huge interdisciplinary interest. A new study highlights the interaction between these phenomena and its implications for self-destructive cooperation.

Andy Gardner and Rolf Kümmerli

The traditional view of the microbe is of a simple automaton, blindly following its genetic programming. This appears to leave little room for personal idiosyncrasies, let alone a social life. However, this view of the microbe as a generic loner has been increasingly challenged in recent years, which have seen an explosion of research on the topics of bacterial individuality and social behaviour.

Phenotypic noise is an inescapable fact of life. Although biologists commonly speak of a gene 'for' a particular trait, this is only ever intended in a statistical sense. Phenotypes vary even in the absence of genetic differences between individuals, and the phenomenon of bi-stability, whereby genetically-identical cells exhibit dramatically different phenotypes, has been of particular recent interest [1–3]. An example is bacterial persistence [4], whereby a fraction of cells enter a non-growing phase during which they are refractory to antibiotics. The quiescent cells that survive antibiotic treatment give rise to growing populations that exhibit no reduction in their antibiotic susceptibility, supporting the idea that they are genetically indistinguishable from cells that are killed. In many cases, bi-stability appears to emerge spontaneously, and a plausible mechanism for this differentiation is the coupling of stochastic gene expression with positive-feedback mechanisms, so that random noise at the molecular level is converted into distinct cell types at the phenotypic level [1].

In parallel to this work on bi-stability, there has been much recent interest in the social lives of microbes [5,6]. Here, the problems faced by human society are recast in miniature. Of particular interest is the question of why individuals should contribute effort for the greater public good, when this leaves them at a disadvantage relative to 'free-riders' who exploit the cooperation of others while giving nothing in return. For example, bacterial growth is often limited by access to iron, and cells that produce iron-scavenging molecules called siderophores help to increase the availability of iron for themselves and others; however, siderophores are costly to produce, and cooperative cells are in danger of being outcompeted by cheaters who invest nothing into this public good [7]. A major explanation for the survival of selflessness is kin selection [8]. Although cooperation can incur a personal cost, the gene for cooperation might enjoy an overall benefit if the public-good contributions accrue preferentially to relatives of the cooperator, who tend to carry copies of the same gene.

In a recent study, Ackermann *et al.* [9] combined the concepts of phenotypic noise and public-goods cooperation to study drastic cooperative behaviours that lead to a total loss of reproductive success for the cooperator. From a genetic-determinism point of view, such self-destructive cooperation is very puzzling: a gene that leads all its carriers to make the ultimate sacrifice should become extinct in a single