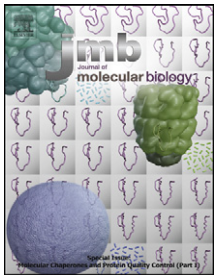
Review



Spatially Organized Aggregation of Misfolded Proteins as Cellular Stress Defense Strategy

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Abstract

An evolutionary conserved response of cells to proteotoxic stress is the organized sequestration of misfolded proteins into subcellular deposition sites. In *Saccharomyces cerevisiae*, three major sequestration sites for misfolded proteins exist, IPOD (*i*nsoluble *pro*tein *d*eposit), INQ (*i*ntra*n*uclear *q*uality control compartment) [former JUNQ (*ju*xta*n*uclear *q*uality control compartment)] and CytoQ. IPOD is perivacuolar and predominantly sequesters amyloidogenic proteins. INQ and CytoQs are stress-induced deposits for misfolded proteins residing in the nucleus and the cytosol, respectively, and requiring cell-compartment-specific aggregases, nuclear Btn2 and cytosolic Hsp42 for formation. The organized aggregation of misfolded proteins is proposed to serve several purposes collectively increasing cellular fitness and survival under proteotoxic stress. These include (i) shielding of cellular processes from interference by toxic protein conformers, (ii) reducing the substrate burden for protein quality control systems upon immediate stress, (iii) orchestrating chaperone and protease functions for efficient repair or degradation of damaged proteins [this involves initial extraction of aggregated molecules via the Hsp70/Hsp104 bi-chaperone system followed by either refolding or proteasomal degradation or removal of entire aggregates by selective autophagy (aggrephagy) involving the adaptor protein Cue5] and (iv) enabling asymmetric retention of protein aggregates during cell division, thereby allowing for damage clearance in daughter cells. Regulated protein aggregation thus serves cytoprotective functions vital for the maintenance of cell integrity and survival even under adverse stress conditions and during aging.

Introduction

The maintenance of protein homeostasis (proteostasis) even under adverse stress conditions, such as exposure to heat or intrinsic perturbation of the proteome, is a central achievement of cells critical to physiology and lifespan of an organism [1]. In a first line of defense, proteostasis is achieved by the activation of efficient protein quality control systems that detect non-functional and potentially harmful misfolded proteins, which are prone to aggregation, and that promote their refolding by chaperones or proteolytic degradation by the ubiquitin–proteasome system (UPS) and autophagy [2–4]. It was thought for a long time that stress-induced collapse of these protein quality control systems under severe or persistent stress leads to uncontrolled protein aggregation and stochastic formation of insoluble deposits at random sites inside cells. However, a number of recent studies provide compelling evidence that protein aggregation is an active, organized process in various model organisms, promoting the deposition of aggregates at specific cellular sites. The controlled sequestration of aggregated proteins represents a second line of defense against proteotoxic stress, which is suggested to have protective functions [5].

Protein aggregation of amyloidogenic proteins, however, has been linked to cellular toxicity caused by, for example, coaggregation and sequestration of essential components including molecular chaperones [6–8]. On the other hand, formation of amyloid aggregates can also protect cells from soluble oligomeric species, which are highly toxic [9–11]. Protein

aggregation is therefore a double-edged sword and its protective effects will depend on the inducing stress conditions, the particular sequestered substrate and the protein aggregates ultimately formed.

While the spatial sequestration of misfolded proteins is a strategy shared by all cells from bacteria to human, the subcellular localization of deposition sites differs between organisms. Furthermore, the pattern of aggregate formation shows stress-specific differences and depends on the particular aggregation-prone protein studied, which is typically fused to a fluorescent reporter. In this review, we will focus on stress-induced protein aggregation in *Saccharomyces cerevisiae*, a model organism for which extensive genetic and cell biological analyses exist. We will describe the different protein aggregate deposits and the sorting factors involved, and we will discuss the cytoprotective functions of controlled protein aggregation.

Three major classes of protein aggregates in yeast cells: INQ, IPOD and CytoQ

The first identification of distinct aggregate deposition sites in *S. cerevisiae* by Kaganovich *et al*. [12] was achieved through the analysis of cells subjected to heat shock combined with proteasome inhibition. With the use of constitutively (VHL) and conditionally (Ubc9ts) misfolding protein reporters tagged with fluorescent reporters, two separate deposition sites were described, the JUNQ (*ju*xta*n*uclear *q*uality control compartment) and the IPOD (insoluble protein deposit) (Fig. 1). Both compartments were proposed to be cytosolic, with JUNQ residing at an indentation of the nucleus while IPOD localizes next to the vacuole, typically adjacent to the preautophagosomal structure labeled with Atg8 [12,13]. IPOD also included overproduced prion proteins Rnq1 and Ure2, suggesting a common site for the deposition of misfolded and amyloidogenic proteins [12]. A recent study, however, revisited the subcellular localization of JUNQ. Employing electron microscopy of yeast thin sections and nuclear envelope markers, we demonstrated JUNQ to have an intranuclear localization adjacent to the nucleolus [14] and redefined it as INQ (*i*ntra*n*uclear *q*uality control compartment). INQ serves as deposit for both misfolded nuclear and cytosolic proteins (Fig. 1). For deposition at the INQ, misfolded cytosolic proteins are transported into the nucleus through the nuclear pore, involving the Hsp70 (Ssa) co-chaperone of the J-protein family, Sis1, and other, so far unknown import factors [6,14].

Further work indicated that the pattern of protein aggregation in yeast cells is more complex than initially suggested. Apart from the IPOD, an ensemble of multiple heat-stress-induced aggregates was described to exist in the cytosol, distant to the nucleus and hence referred to as peripheral aggregates [15]. Similar deposits were later described either as stress foci [13] or quality control bodies, termed Q-bodies [16]. To simplify the currently non-uniform nomenclature, we will refer to such cytosolic stress-induced aggregates as CytoQ (Fig. 1). The number of CytoQ deposits initially formed upon sudden

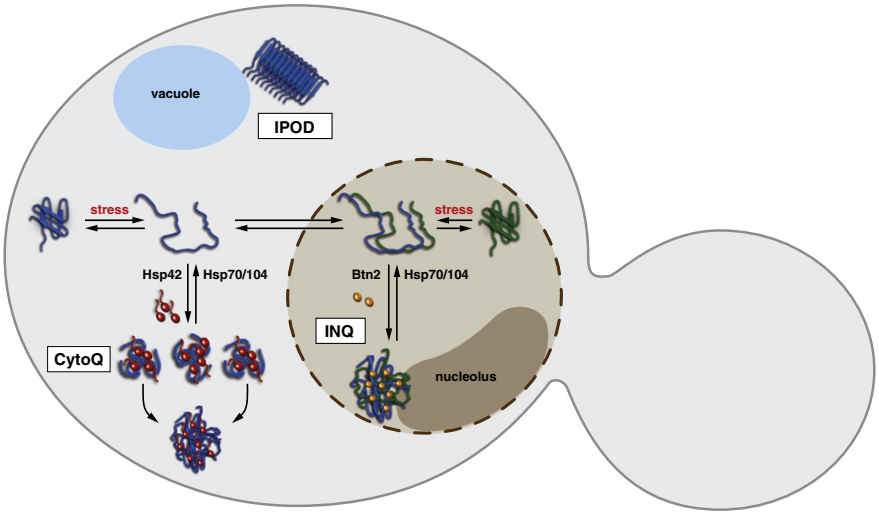

Fig. 1. IPOD, INQ and CytoQ represent specific deposits of misfolded proteins in yeast. Proteotoxic stress (e.g., heat shock) causes accumulation of misfolded proteins, which are sequestered at distinct cellular sites, CytoQ and INQ (former JUNQ). Sequestration at CytoQs relies on the sHsp Hsp42. The number of CytoQs is eventually reduced by fusion events. IPOD locates to the vacuole, can form constitutively and predominantly includes amyloidogenic proteins. INQ resides in the nucleus next to the nucleolus and harbors cytosolic and nuclear misfolded proteins. Protein aggregation inside the nucleus is triggered by Btn2. Misfolded proteins can be extracted from INQ and CytoQ by combined action of Hsp70 and Hsp104 chaperones, allowing for subsequent substrate refolding.