PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B

Phil. Trans. R. Soc. B (2011) **366**, 71–75
doi:10.1098/rstb.2010.0282

Review

Spatial protein quality control and the evolution of lineage-specific ageing

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Propagation of a species requires periodic cell renewal to avoid clonal extinction. Sexual reproduction and the separation of germ cells from the soma provide a mechanism for such renewal, but are accompanied by an apparently mandatory ageing of the soma. Data obtained during the last decade suggest that a division of labour exists also between cells of vegetatively reproducing unicellular organisms, leading to the establishment of a soma-like and germ-like lineage with distinct fitness and longevity characteristics. This division of labour in both bacteria and yeast entails segregation of damaged and aggregated proteins such that the germ-like lineage is kept free of damage to the detriment of the soma-like lineage. In yeast, this spatial protein quality control (SQC) encompasses a CCT-chaperonin-dependent translocation and merging of cytotoxic protein aggregates. This process is regulated by Sir2, a protein deacetylase that modulates the rate of ageing in organisms ranging from yeast to worms and flies. Recent data also demonstrate that SQC is intimately integrated with the machinery establishing proper cell polarity and that this machinery is required for generating a soma-like and germ-like lineage in yeast. Deciphering the details of the SQC network may increase our understanding of the development of age-related protein folding disorders and shed light on the selective forces that paved the way for polarity and lineage-specific ageing to evolve.

Keywords: replicative ageing; rejuvenation; spatial protein quality control; Sir2; polarity

1. INTRODUCTION

The proteome of an organism is defined as the entire pool of proteins residing inside cells and their membranes, and in extracellular fluids. Protein quality control (PQC) ensures that the individual proteins of the proteome are accurately produced, folded and compartmentalized (e.g. [1,2]). The PQC system consists of molecular chaperones and various proteases that recognize and repair damaged proteins or, alternatively, remove the aberrant proteins. Failure of the PQC to work properly severely affects cellular performance and has been shown to underlie various diseases, commonly referred to as protein conformational disorders [1]. Such disorders may be owing to the reduced activity of a specific aberrant protein or to misfolded proteins becoming cytotoxic. The latter phenomenon is referred to as proteotoxicity [1,3]. Proteotoxicity is often linked to damaged and misfolded proteins forming oligomers, aggregates, inclusion bodies, and, perhaps most treacherously, amyloids. The toxicity of such protein species may be due to, for example, proteasome clogging, chaperone sequestration, fibril pore formation, calcium overload, oxidative stress and aggregation-induced production of reactive oxygen species (e.g. [1–5]).

Apart from the fact that specific conformational disorders can be caused by defects in quality control, the efficiency of the PQC declines during 'normal' ageing, and accumulation of aberrant protein species in the form of carbonylated proteins, aggregates, amyloids and inclusion bodies arises in many tissues of aged organisms [6–8]. Most proteins have evolved to fold such that their amyloid-prone segments are effectively concealed but an increase in 'damaging' agents with age (e.g. reactive oxygen species) may affect such folding and cause sticky amyloid elements within proteins to emerge and seed the growth of potentially deadly fibrils [9–11]. Recent years have seen an immensely growing interest in the cellular mechanisms contributing to PQC, because genetic alterations in such systems have been shown to postpone the development of protein conformational disorders (e.g. [1,3,10,11]) and even extend the lifespan of some model organisms (e.g. [12]). Such an approach, using target-specific therapeutic drugs instead of genetic alterations, holds the potential to mitigate or postpone a large variety of age-related proteotoxic disorders, including neurodegeneration [1,10,11].

2. SPATIAL QUALITY CONTROL AND ITS LINKS TO AGEING

While the chaperones and proteolytic systems of the PQC provide the cell with a capacity for *temporal* quality control, i.e. a time-dependent repair or removal of damaged proteins, it has been discovered that cells possess the means of *spatially* controlling protein damage. In both yeast and mammalian cells, small aggregates can be transported on microtubules into a

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One contribution of 15 to a Discussion Meeting Issue 'The new science of ageing'.

juxtanuclear structure called the aggresome [13]. This spatial protein quality control (SQC) has been observed for aggregation-prone, aberrant protein species such as the cystic fibrosis transmembrane conducting regulator [13,14] and the huntingtin exon 1 with an expanded polyglutamine domain [15]. Aggresomes are formed via the microtubule network and dynein motors and are associated with the centrosome (spindle pole body in yeast [14,15]). The formation of aggresomes has been suggested to be a cytoprotective response that sequesters toxic misfolded proteins and facilitates their removal by autophagy, which concludes with lysosomal degradation. The formation and processing of aggresomes involve a variety of regulators, including E3 ubiquitin-protein ligase parkin, deubiquitinating enzymes such as ataxin-3 and ubiquilin-1, and histone deacetylase 6 [16–18].

Additional evidence for SQC comes from experiments demonstrating that oxidatively damaged (carbonylated) proteins do not diffuse freely but are controlled in space such that one cell is kept essentially free of damaged proteins during the process of cytokinesis [19]. This asymmetrical inheritance of damage was revealed using budding yeast as a model system for replicative ageing and follow-up experiments demonstrated that (i) oxidatively damaged proteins coalesce into aggregates recognized by the protein remodelling factor Hsp104p [20], (ii) Hsp104p-containing aggregates become tethered to the actin cables rather than the microtubule [21], and (iii) aggregates tethered to the cables do not enter the progeny because the flow of the actin cables is away from the daughter cell bud tip [21].

A genetic screen, using *Saccharomyces cerevisiae*, identified the protein Sir2p as a required component in retaining damage in the ageing mother cell [19,20,22,23]. Sir2p, the founding father of the family of sirtuins, is a conserved nicotinamide adenine dinucleotide-dependent protein (histone) deacetylase, shown to act as a modulator of ageing in a variety of organisms, including yeast, worms, flies, fish and mammals [24–27]. In yeast, Sir2p modulates replicative lifespan, in part, by removing histone H4 lysine 16 acetylation, leading to increased silencing [28]. By establishing the global genetic interaction network of *SIR2*, the polarisome at the daughter bud tip was identified as the machinery required for segregating protein aggregates recognized by Hsp104 [21]. Apart from being essential in inhibiting the transmission of damage into daughter cells, the Hsp104p/polarisome-dependent SQC machinery was demonstrated to allow the emerging daughter to move aggregates back into its progenitor. Thus, if damaged proteins find their way into the daughter compartment or if aggregates build up by transient external stresses, the daughter can clear itself of such damage by using actin cables as 'conveyor belts'. The retrograde flow of aggregates appears to be due to formin(Bni1p)-dependent actin nucleation at the daughter cell bud tip [21]. Sir2p affects this process by regulating the function and acetylation of the chaperonin CCT [21], which is required for folding actin and thus feeding the polarisome with properly folded substrates.

These results establish a new polarity mechanism that goes opposite to the flow associated with actin-based polarized secretion, which is directed by motor movement *along* actin cables. In addition, if protein aggregates are *bona fide* ageing factors, then this mechanism not only prevents the progeny from inheriting the ageing characteristics of its progenitor but also allows the daughter to actually reset the clock.

Like aggresome formation, actin cable-dependent SQC also encompasses the fusion of smaller aggregates into large inclusion bodies—a process that has been suggested to reduce the toxicity of such aggregates. This 'reversed' polarity-dependent fusion of aggregates requires actin nucleation at the septin ring by the Bnr1p formin rather than Bni1p-dependent actin nucleation at the bud tip [21]. Thus, the two formins of yeast have distinct roles in the polarity-dependent spatial management of protein aggregates; one, Bni1p, provides the organism with the means to retain (in mother cells) and translocate (into mother cells) aggregates, while the other allows aggregates to merge into a single large inclusion body.

It is not clear if the aggregates tethered on actin cables are in any way connected to the two intracellular compartments discovered for the sequestration of misfolded disease proteins [29]. Analysis of huntingtin and prion model proteins has revealed that aggregates can become partitioned into discrete compartments, which depends on their ubiquitination status and aggregation state. Soluble ubiquitinated misfolded proteins appear to accumulate in a juxtanuclear compartment (termed JUNQ) where proteasomes are concentrated, whereas terminally aggregated proteins are sequestered in a perivacuolar inclusion (called IPOD [29]). Whether JUNQ and/or IPOD aggregates are subjected to actin cable- and polarity-dependent SQC remains to be elucidated.

Intriguingly, SQC is not unique for cells dividing by budding, but also operates in the fission yeast, *Schizosaccharomyces pombe*, and is cytoskeleton- (both F-actin and microtubules), polarisome- (Tea1) and sirtuin-dependent [20,23]. This gives rise to a damage-enriched and a damage-free cell lineage despite the fact that cytokinesis is accomplished by binary fission [23]. Consistently, *S. pombe* has also been demonstrated to show signs of ageing in a lineage-specific fashion [30] and the ageing lineage was found to be the one accumulating protein damage [23].

3. SPATIAL QUALITY CONTROL AND AGEING IN BACTERIA

Binary fission of bacteria has been assumed to proceed with a non-conservative dispersion of both undamaged and damaged constituents. Thus, it has been understood that age-structured phenomena are absent in a bacterial population. However, Ackermann and co-workers [31] demonstrated that *Caulobacter crescentus* exhibits replicative ageing in a lineage-specific manner. This bacterium, however, displays an intrinsically asymmetrical cytokinesis in which a stalked cell gives rise to a motile swarmer cell. With each division, the stalked cell requires progressively longer times to produce swarmer cells, a phenomenon akin to

mother-cell ageing in budding yeast. Intriguingly, the second bacterium reported to show signs of replicative ageing was *Escherichia coli*, an organism that, in contrast to *C. crescentus*, divides by binary fission and, as far as one can tell, consists of a single lineage. However, by tracking the poles of *E. coli* cells during division and recording their growth, Stewart and co-workers calculated the generation time of individual cells and found that the growth rate decreased in cells inheriting old poles [32]. This suggests that *E. coli* cells, like those of *C. crescentus*, *S. cerevisae* and *S. pombe* are subjected to lineage-specific replicative ageing and that sibling-specific reduction in fitness may be more common in nature than previously anticipated. In addition, the data imply that cytokinesis during binary fission is also inherently asymmetrical. Indeed, previous to these studies it has been shown that cells of an *E. coli* population consist of two discrete populations with respect to damage: a damage-enriched and a damage-free population [33]. The damage-free cells remain reproductively competent in stationary phase, whereas the damage-enriched cells eventually become genetically dead (non-culturable).

Lindner *et al*. [34] took the damage analysis further and continuously followed the appearance and inheritance of spontaneously generated protein aggregates within lineages of *E. coli* cells grown under non-stressed conditions. The authors found that aggregates accumulated in cells with older poles; the cells previously shown to display signs of replicative ageing [32]. Apparently, bacteria, like yeast, use cues acting as indicators of polarity to faithfully segregate protein aggregates to an ageing cell lineages while leaving the other, rejuvenated, lineage free of such damage. How this polarity is established in bacteria and connected to the inheritance of the old cell poles remains to be determined.

4. ULTIMATE CAUSATION FOR DAMAGE ASYMMETRY IN UNICELLULAR ORGANISMS

The question that arises from data demonstrating damage segregation is whether there is a selective advantage of producing daughter cells of unequal reproductive potential in the population of a unicellular organism. In an attempt to elucidate the pros and cons of symmetrical and asymmetrical bacterial division, Watve *et al*. [35] modelled growth and the propagation of growth-limiting components of a unicellular system using a modified Leslie matrix framework. The model developed points to asymmetrical division favouring rapid growth, whereas symmetry results in slow growth but higher efficiency, that is a higher growth yield [35]. Similarly, using an individual-based simulation approach, Ackermann *et al*. [36] found that a differentiation between an ageing parental cell and a rejuvenated progeny readily evolves to cope with self-inflicted damage. Johnson & Mangel [37] using different modelling equations, came to a similar conclusion. Also, it is possible that upon elevated external stresses reaching lethal levels, an asymmetrical segregation of irreparable damage may permit survival of the clone at the expense of the 'mother-type' cells. Indeed, a mathematical model created to simulate the robustness and fitness of dividing systems displaying different degrees of damage segregation and size asymmetries indicated that systems dividing asymmetrically (size-wise) or displaying damage segregation withstand higher degrees of damage [23]. However, the model of Erjavec *et al*. [23] also suggests that damage retention may be a mixed blessing; it raises the upper limits for how much damage the system can endure before entering clonal senescence but becomes a selective disadvantage at low damage production rates. Thus, mutants with a crippled SQC are not, in this model, predicted to enter clonal senescence as long as the production of damage is relatively low. Indeed, wild-type and *sir2* mutant populations of *S. pombe* display the same fitness (growth rate) when cultured without stressors but hydrogen peroxide causes a much more pronounced fitness reduction in *sir2* mutants (lacking damage segregation) than wild-type cells [23].

Thus, very different types of models and simulations suggest that sibling-specific asymmetry may provide the system with a fitness advantage and that replicative ageing evolved early in the history of life. However, at present, the models and simulations are hampered by the fact that we know very little about the exact nature of the critical components (ageing factors) reducing cellular fitness and the mechanisms establishing their asymmetrical distribution. Elucidating these features will be critical in estimating the energetic costs for damage segregation versus damage removal (assuming that damage is, at least partially, responsible for unicellular ageing) and why segregation might, in some cases, be selected over damage repair/removal.

5. FUTURE PERSPECTIVES

The fact that Sir2p is a key member controlling the efficiency of SQC in unicellular yeasts is interesting in view of the fact that sirtuins, such as the mammalian Sir2 orthologue Sirt1, have been proposed to retard or postpone age-related neuronal degeneration. For example, attenuation of amyloid neuropathy through caloric restriction in animal models of Alzheimer's disease requires activation of Sirt1 [38] and increased sir-2.1 gene dosage (or activation of Sir2.1) alleviates neuronal dysfunction induced by the mutant huntingtin in *Caenorhabditis elegans* [39]. This link between sirtuins and proteotoxic disorders becomes even more intriguing in light of the data showing that the activity of the chaperonin CCT is controlled by Sir2p [21]. CCT is a member of the chaperonin subfamily of chaperones, which is an essential oligomer required for folding actin and tubulin *in vivo*. Apart from actin and tubulin, CCT substrates include polypeptides with β-strands prone to form fibrillar aggregates, such as amyloidogenic proteins. Consistently, reducing the levels of CCT subunits increase polyQ aggregation in *C. elegans* [40] and huntingtin (Htt)–polyQ aggregate formation and cellular toxicity in mammalian models [4,41]. Conversely, overexpression of CCT subunits has been demonstrated to mitigate Htt-polyQ aggregation [4,41]. The demonstration that CCT-dependent folding can be

modulated by *SIR2*-dependent deacetylation draws attention to the possibility that sirtuins may mitigate neurodegenerative, protein misfolding, pathologies by altering the efficiency with which CCT acts on misfolded substrates.

Another intriguing and important fact of the SQC discovered is that its efficiency can be boosted by external stimuli. For example, yeast mother cells subjected to a transient oxidative stress increase their ability to retain damage during subsequent cytokinesis—in other words, the youthfulness of the daughter cell is not affected by prior damaging stress to its progenitor [19]. In addition, resveratrol elevates (by 15%) the ability of mother cells to retain damage ([21]; although it is not yet clear if this effect of resveratrol acts through Sir2p). Finally, deleting UBP10, encoding a ubiquitin-specific protease, results in a more pronounced asymmetry in protein damage during cytokinesis [42], indicating that the SQC network is subjected to control also by ubiquitinylation. Moreover, the efficiency of SQC declines with the age of the mother cell [19]. Taken together, the data suggest that some members of the SQC machinery, or its regulators, are limiting—more so in aged cells—and may therefore be potential targets for novel drug treatments aimed at boosting SQC. One such limiting component may be Sir2p itself, as the levels of this protein have been shown to decline with the replicative age of yeast mother cells [28].

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