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SYMPOSIUM REVIEW

Mitochondrial proteostasis as a shared characteristic of slowed aging: the importance of considering cell proliferation

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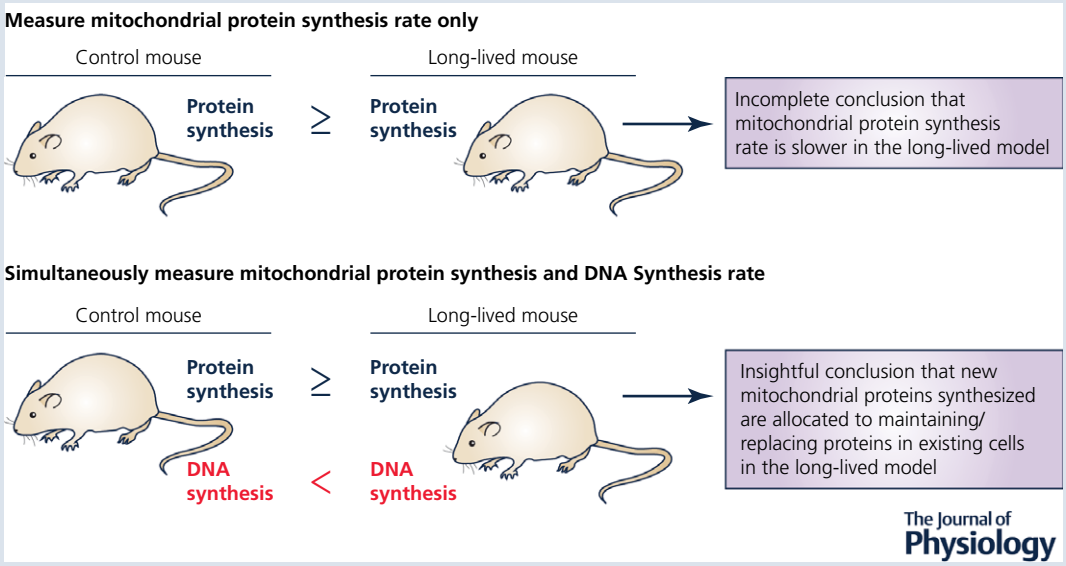

Abstract Proteostasis is one of the seven "pillars of aging research" identified by the Trans-NIH Geroscience Initiative and loss of proteostasis is associated with aging and age-related chronic disease. Accumulated protein damage and resultant cellular dysfunction are consequences of limited protein repair systems and slowed protein turnover. When relatively high rates of protein turnover are maintained despite advancing age, damaged proteins are more quickly degraded and replaced, maintaining proteome fidelity. Therefore, maintenance of protein turnover represents an important proteostatic mechanism. However, measurement of protein

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synthesis without consideration for cell proliferation can result in an incomplete picture, devoid of information about how new proteins are being allocated. Simultaneous measurement of protein and DNA synthesis provides necessary mechanistic insight about proteins apportioned for newly proliferating cells versus for somatic maintenance. Using this approach with a number of murine models of slowed aging shows that, compared to controls, energetic resources are directed more toward somatic maintenance and proteostasis, and away from cell growth and proliferation. In particular, slowed aging models are associated with heightened mechanisms of mitochondrial proteostatic maintenance. Taking cell proliferation into account may explain the paradoxical findings that aging itself and slowed aging interventions can both be characterized by slower rates of protein synthesis.

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Abstract figure legend Proteostasis is one of the seven 'pillars of ageing research' identified by the Trans-NIH Geroscience Initiative, and loss of proteostasis is associated with ageing and age-related chronic disease. Maintenance of protein turnover represents an important mechanism for preserving proteome fidelity. However, measurement of protein synthesis without consideration for cell proliferation can result in an incomplete picture, devoid of information about how new proteins are being allocated. Simultaneous measurement of protein and DNA synthesis provides critical insight about proteins apportioned for newly proliferating cells *versus* for somatic maintenance. Using this approach with murine models of slowed ageing shows that mitochondrial proteostatic maintenance is a characteristic shared among these slowed ageing models.

Introduction

People aged 65 years and older represented about 14.5% of the United States population in 2014 and this number is expected to grow to 21.7% of the population by 2040 (US Census Bureau), whereas in the United Kingdom the population aged 65 and over had grown by 47% since mid-1974 to make up nearly 18% of the total population in 2014 (Office for National Statistics). Despite the well-recognized fact that the world population is aging, defining aging is not entirely straightforward. Medawar, for example, defined aging as the collection of changes that render human beings progressively more likely to die (Medawar, 1952). The definition of aging from Rose's book on the evolution of aging (Rose, 1991) is 'a persistent decline in the age-specific fitness components of an organism due to internal physiological degeneration', which seems well suited to the discussion in this Symposium Review from Experimental Biology 2017.

Healthspan is a term used to describe the 'health life expectancy' or period of life spent free of chronic disease (Kennedy *et al.* 2014). A trans-National Institutes of Health (NIH) initiative called the Geroscience Initiative has focused on strategies to increase healthspan. The premise of the initiative is that since aging is a major risk factor and driver of chronic diseases, understanding the molecular and cellular mechanisms responsible for aging holds promise for simultaneously decreasing all age-related chronic diseases. Members of the Geroscience Initiative identified seven highly interrelated 'pillars of aging' that are critical for understanding and treating the aging process (Kennedy *et al.* 2014). Protein homeostasis (proteostasis) was one of the seven pillars of aging identified because protein dyshomeostasis has emerged is a common feature of aging and chronic disease as summarized in a number of excellent reviews (Balch *et al.* 2008; Labbadia & Morimoto, 2014; Kaushik & Cuervo, 2015; Labbadia & Morimoto, 2015). In this Symposium Review, we briefly summarize evidence that improved mechanisms of proteostatic maintenance – in particular mechanisms promoting mitochondrial proteostasis – are shared among experimental models of extended healthspan. Additionally, we will address what we believe are important considerations for designing studies to interrogate the potential for targeting mitochondrial proteostatic maintenance for interventions to increase healthspan. Specifically, we discuss the importance of assessing protein turnover in the context of cell proliferation, the impact of growth compared to somatic maintenance on proteostatic assessment, and how model systems can influence the interpretation of proteostasis.

Aging, proteostasis, and protein turnover: the influence of cell proliferation

Proteostasis is a term used to refer to a network of dynamic processes contributing to maintenance of proteome fidelity (Balch *et al.* 2008; Labbadia & Morimoto, 2014; Kaushik & Cuervo, 2015; Labbadia & Morimoto, 2015). Regulation of protein biogenesis, folding, targeting, quality control and degradation must be orchestrated in response to rapidly changing intrinsic

and extrinsic signals. In light of the dynamic network of processes required to maintain a functional proteome, it seems more appropriate to refer to the process of maintaining proteome fidelity as proteodynamics (Basaiawmoit & Rattan, 2010). A challenge to studying protein maintenance with aging and aging interventions is the complex and dynamic nature of the proteostatic network since it is difficult to simultaneously assay all components of the network. Here we provide evidence that protein turnover is a critical proteostatic mechanism and that cell proliferation is a key consideration when assessing protein turnover as a mechanism for maintaining the proteome.

Protein repair systems are limited and therefore proteins damaged by stresses associated with aging (i.e. reactive oxygen species, advanced glycation end-products) accumulate over time and contribute to cellular, organ and organism dysfunction. Protein synthesis is a primary mechanism for maintaining quality control and proteome fidelity (Charmpilas *et al.* 2015), and a commonly held belief is that bulk protein synthesis rates decline with aging (Rattan, 2010). Accumulated protein damage and resultant cellular dysfunction is the ultimate consequence of limited protein repair systems and slowed protein synthesis (Hipkiss, 2006; Tavernarakis, 2008). Reciprocally, when relatively high rates of protein turnover are maintained despite advancing age, damaged proteins are more quickly degraded and replaced, maintaining proteome fidelity (Fig. 1). Given that normal aging is associated with a slowing of protein synthesis, it is interesting that long-lived models also appear to have slower protein synthesis throughout the lifespan (Tavernarakis, 2008; Kapahi, 2010; Kapahi *et al.* 2010; Price *et al.* 2012; Dai *et al.* 2014; Karunadharma *et al.* 2015). The observation that both aging and slowed-aging have apparent decreases in protein synthesis is a paradox that has largely escaped the notice of researchers in protein metabolism and aging.

To help understand why both aging and slowed aging are associated with decreased protein turnover, robust approaches for assessing protein turnover must be used. The commonly used measurement of protein content does not provide this insight because both increases and decreases in protein turnover can occur without a change in protein content (Miller & Hamilton, 2012; Miller *et al.* 2014). Therefore, to capture changes in the important process of turnover, stable isotopically labelled amino acids are frequently used to provide a relatively acute measurement of protein synthesis. However, even these measurements require caution since a short labelling period can bias the measured synthesis rates toward rapidly turning over or abundant proteins (Miller *et al.* 2015). Therefore, we use a robust and insightful approach to measure rates of protein synthesis with a stable isotope of water, deuterium oxide (D_2O).

The use of D_2O allows for measurement of synthetic rates over prolonged periods of time in free living animal and human subjects provides information about the synthesis of both slowly and rapidly synthesized proteins of diverse abundances (Miller *et al.* 2015). Another major strength to using D_2O is that rates of DNA synthesis can be measured simultaneously with the rates of protein synthesis (details of calculations available in Neese *et al.* 2002). Through the simultaneous assessment of protein and DNA it has become clear to us that cell proliferation, as measured by DNA synthesis, is an important outcome when considering the slowed-aging effects of protein turnover. When cells proliferate (increased DNA synthesis) protein mass is doubled (increased protein synthesis) in the growth phases (G_1 and G_2), so that two daughter cells have the full complement of genetic material and protein machinery

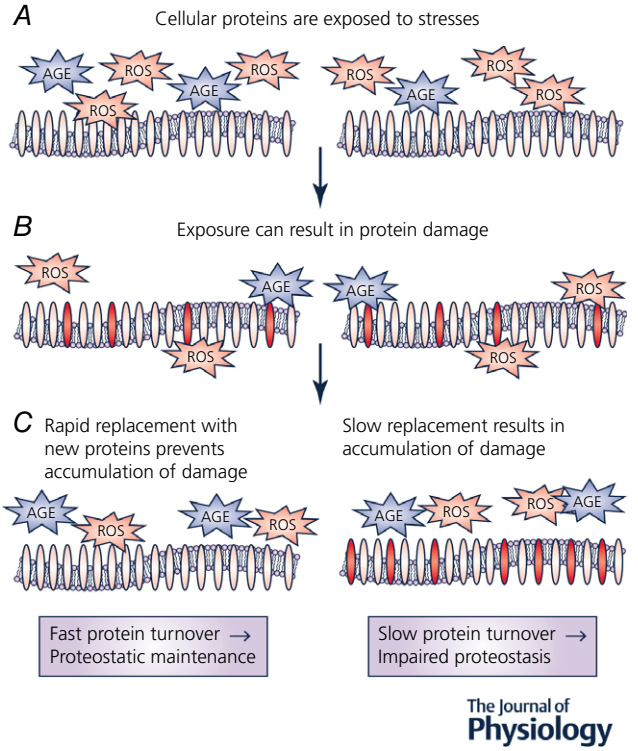

Figure 1. Protein turnover is an important proteostatic mechanism
A, cells are exposed to a variety of stresses. Stresses that may be more unrelenting with increasing age include reactive oxygen species (ROS) and advanced glycation endproducts (AGE). *B*, prolonged exposure to these stresses can result in damage to proteins (and lipids and DNA), represented here as red ovals. *C*, when protein turnover is maintained at relatively high rates, damaged proteins are quickly degraded and replaced with newly synthesized proteins, preventing accumulation of damaged proteins despite continued exposure to stresses (left). However, when protein turnover rates are slow, it is more likely that protein damage will accumulate (right). Therefore, maintaining rates of protein turnover represents an important mechanism for supporting proteome fidelity.

(Grebien *et al.* 2005). In post-mitotic cells like skeletal muscle fibres, growth is accompanied by DNA synthesis in a supportive cell and donation of that nuclear material from supportive cells to the post-mitotic cell (Collins *et al.* 2005) to minimize changes to the cytosolic volume to DNA ratio (Allen *et al.* 1999). In both cell types, the synthesis of new DNA is accompanied by the synthesis of new proteins. In contrast, when new proteins are synthesized for cellular remodelling or replacement of damaged proteins there is not a corresponding increase in DNA synthesis. Thus, when D_2O is used to label newly synthesized proteins during growth/proliferation, the ratio of protein synthesis to DNA synthesis essentially stays the same, whereas during maintenance or remodelling, the ratio of protein synthesis to DNA synthesis increases (Fig. 2; Miller *et al.* 2014). The simultaneous assessment of protein and DNA synthesis has unexpectedly provided great insight into the paradoxical observation that both aged and long-lived models have slowed protein turnover.

In 1977, Kirkwood hypothesized that in the wild predation results in evolutionary changes that favours the allocation of energetic resources toward rapid reproduction, which comes at the expense of maintaining somatic cells (Kirkwood, 1977). With this trade-off,

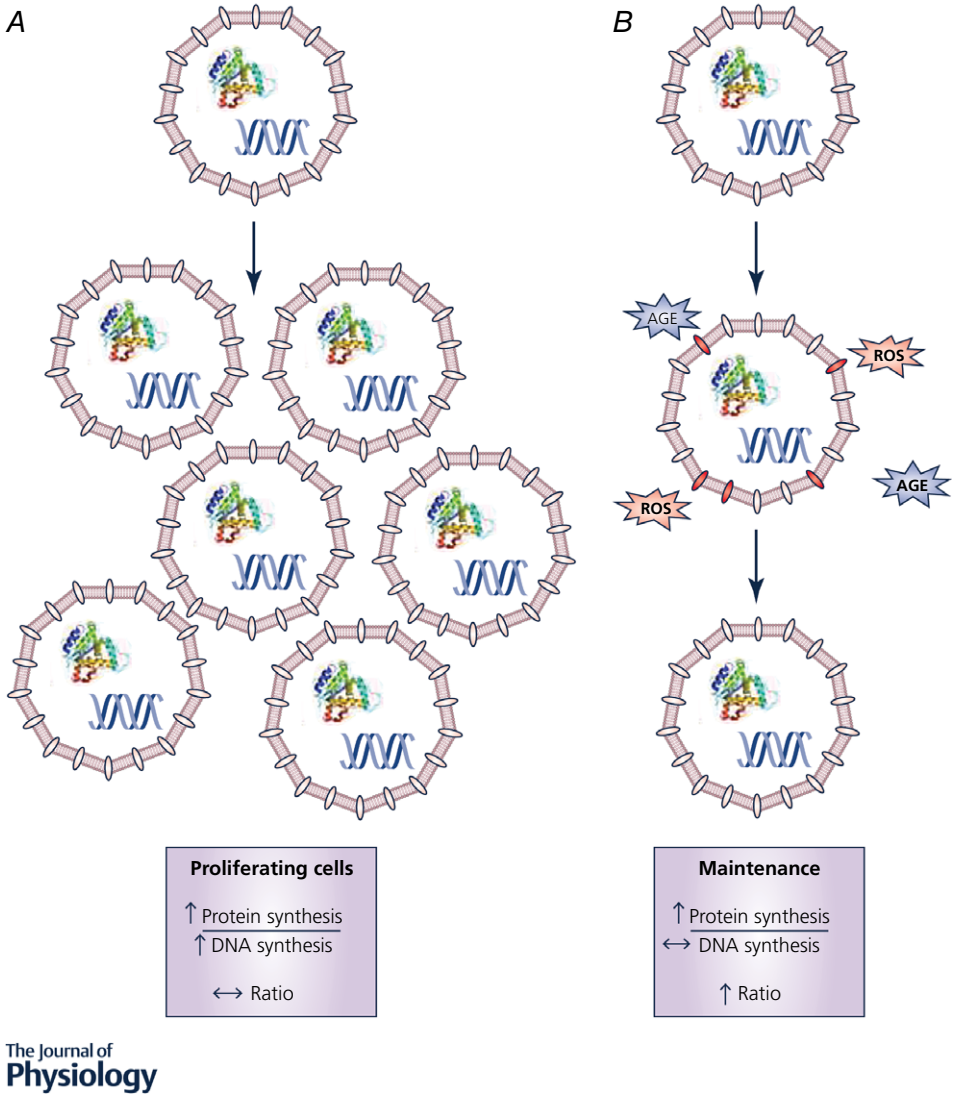

Figure 2. Considering cell proliferation influences the interpretation of protein synthesis
A, in proliferating cells (growth), measuring protein synthesis alone would lead to the conclusion that protein synthesis rates are increased. If protein and DNA synthesis are measured simultaneously, the rates of both protein and DNA synthesis are increased. Expressing rates of protein synthesis relative to DNA synthesis (ratio) leads to the conclusion that newly synthesized proteins are primarily being allocated to new cells and less so to maintaining existing cell structures. *B*, in contrast, when rates of protein synthesis are increased without much of a change in rates of DNA synthesis (proliferation), the ratio of protein:DNA synthesis rates is greater and the conclusion is that newly synthesized proteins are primarily being allocated to maintaining/replacing proteins in existing cells.

low energetic investment in maintaining somatic cells in favour of growth and reproduction results in accumulated protein damage and contributes to the aging process. However, relaxation of predation results in low energetic investment in growth and reproduction and is directed toward somatic maintenance in part through improved proteostasis. This concept has been supported by laboratory models that restrict energetic investment in reproduction and thus increase lifespan (Partridge *et al.* 2005). Simultaneous measurement of protein and DNA synthesis provides insight into the proteostatic mechanisms that give rise to somatic maintenance.

As mentioned, in multiple long-lived models it appears that protein synthesis is decreased compared to controls (Tavernarakis, 2008; Kapahi, 2010; Kapahi *et al.* 2010; Price *et al.* 2012; Dai *et al.* 2014; Karunadharma *et al.* 2015), and by this measurement alone, the conclusion would be that decreased protein synthesis gives rise to slowed aging. Although the fact that protein synthesis decreases is indisputable, the conclusion does not consider what cellular processes contributed to that observed decrease. When DNA synthesis is measured simultaneously with protein synthesis, it is apparent that cell proliferation is slower in long-lived models (Miller *et al.* 2012; Drake *et al.* 2013, 2014, 2015). Further, DNA synthesis decreases to a greater degree than protein synthesis thus indicating that a greater proportion of protein synthesis is directed toward maintaining existing cellular structures (somatic maintenance) and less toward proliferation (growth). Therefore, considering protein synthesis in the context of cell proliferation provides mechanistic insight into the directing of energetic resources toward somatic maintenance and proteostasis and away from cell growth and proliferation. Further, these assessments provide insight into the conundrum of why there is an apparent decrease in protein synthesis in long-lived models since although overall protein synthesis is decreased, it is largely due to decreased proliferation concurrent with increased proteostatic mechanisms.

Maintenance of mitochondrial protein synthesis as a proteostatic mechanism in models of increased lifespan/healthspan

The mitochondrial reticulum is an important determinant of cellular energetics, and is now also known to participate in direct communication with other sub-cellular organelles (Rose *et al.* 2016; Filadi *et al.* 2017) and carry out important protein quality control activities such as the mitochondrial unfolded protein response (Carreras-Sureda *et al.* 2017). Mitochondria are also an important site for production of reactive oxygen species important for signalling beneficial adaptations (Gonzalez-Freire *et al.* 2015; Gomez-Cabrera *et al.* 2016), but are also capable of contributing to macromolecular damage and dysfunction associated with aging and age-related chronic diseases (Hohn *et al.* 2016). It is widely accepted that maintaining mitochondrial function contributes to metabolic health, while deterioration of mitochondrial function is at minimum a characteristic of, but more likely a contributor to, aging (Lanza & Nair, 2009; Gonzalez-Freire *et al.* 2015) and age-associated chronic diseases including insulin resistance/type II diabetes (Di Meo *et al.* 2017) and cardiovascular diseases (Paneni *et al.* 2017).

Mitochondrial adaptation to changes in cellular energetic demands and energetic stresses, such as occur with calorie restriction, involves rapid and highly dynamic remodelling involving fission, fusion and turnover of proteins that are encoded by both nuclear and mitochondrial genes. Therefore, approaches for assessing mitochondrial protein turnover must be capable of capturing these dynamics. As an example, we use data from studies examining mitochondrial biogenesis during caloric restriction for which there are discrepant findings. Increased mitochondrial biogenesis is reported in calorie-restricted mice (Nisoli *et al.* 2005), rats (Lopez-Lluch *et al.* 2006) and flies (Zid *et al.* 2009), while others report no change in mitochondrial biogenesis in calorie-restricted rats (Hancock *et al.* 2011) and mice (Lanza *et al.* 2012). However, if one considers DNA synthesis, it appears that caloric restriction also decreases cellular proliferation (Miller *et al.* 2012), and when the ratio of protein synthesis to DNA is considered it is apparent that synthesis to maintain mitochondrial proteostasis actually increases while protein synthesis directed toward proliferation decreases (Miller *et al.* 2014).

Since our study in calorically restricted mice, we have made similar assessments in multiple long-lived models (Drake *et al.* 2013, 2014, 2015; Miller *et al.* 2013, 2014). In each of these models, we found that the ratio of mitochondrial protein synthesis to DNA synthesis is greater in the long-lived model compared to controls. Therefore, by considering protein synthesis and DNA synthesis together, the apparent paradox between why pro-aging and slowed aging both appear to have decreased rates of protein synthesis becomes explainable. In the case of aging, the decrease in protein synthesis likely indicates a decrease in somatic maintenance. In the case of slowed aging, the overall rate of protein synthesis decreases, but this is due to a decrease in the amount of protein synthesis dedicated toward growth and proliferation and an increased amount of protein synthesis dedicated to proteostatic mechanisms for the purposes of somatic maintenance.

Conclusions and a look forward

One of the primary conclusions from this symposium is that protein turnover is a key proteostatic mechanism.

Additionally, when assessing rates of protein synthesis, it is important to also consider rates of cell proliferation to provide insight about how newly synthesized proteins are being allocated – either to proliferating cells or to maintenance/replacement of existing structures. Using this approach with murine models of slowed aging led to the conclusion that mitochondrial proteostatic maintenance is a characteristic shared among these slowed aging models. Taking proliferation into account may explain the paradoxical findings that aging itself and slowed aging interventions can both seem to be associated with slower rates of protein synthesis.

Looking forward, there are a number of aspects of protein turnover as a mechanism of proteostasis maintenance that remain incompletely understood. For example, while it seems clear that turnover of mitochondrial proteins is a cellular priority for slowed aging, the mechanisms by which this selective translation occurs are unclear. Identifying the specific proteins that are selectively translated could also provide critical information for identifying targets for slowed aging interventions. While slower rates of tissue DNA synthesis have emerged as a consistent characteristic of slowed aging, whole tissues are complex and comprise many cell types. Identifying the specific cell types that have slower proliferation rates and understanding the phenotype of these more slowly proliferating cells could also help focus efforts to develop novel slowed aging interventions.

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Additional information

Competing interests

The authors have no competing interests or conflicts of interest to disclose.

Author contributions

Both authors participated in writing the manuscript and both authors approved the final manuscript. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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