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Using comparative biology to understand how aging affects mitochondrial metabolism

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ABSTRACT

Lifespan varies considerably among even closely related species, as exemplified by rodents and primates. Despite these disparities in lifespan, most studies have focused on intra-specific aging pathologies, primarily within a select few systems. While mice have provided much insight into aging biology, it is unclear if such a short-lived species lack defences against senescence that may have evolved in related longevous species. Many age-related diseases have been linked to mitochondrial dysfunction that are measured by decreased energy generation, structural damage to cellular components, and even cell death. Post translational modifications (PTMs) orchestrate many of the pathways associated with cellular metabolism, and are thought to be a key regulator in biological senescence. We propose hyperacylation as one such modification that may be implicated in numerous mitochondrial impairments affecting energy metabolism. Keywords: Comparative biology, Sirtuin 3, Acylation, Mitochondria, Aging

1. Introduction

The evolutionary path to longevity can act through a number of ecologically imposed restraints thrust upon a species, causing senescence to occur at drastically different rates. Cellular senescence was initially defined by Hayflick (1965) to describe the arrest of cellular proliferation. However, in its broader context, senescence can refer to the organismal degradation that occurs after maturation (Campisi and d'Adda di Fagagna, 2007), and can be used interchangeably with aging. Lifespan is a remarkably diverse trait that evolved independently numerous times: species of the subphylum Vertebrata range from just a few weeks in the case of coral reef pygmy goby (*Eviota sigillata*) (Depczynski and Bellwood, 2005) to over 200 years in rougheye rockfish (*Sebastes aleutianus*; Cailliet et al., 2001) and bowhead whale (*Balaena mysticetus*; Tacutu et al., 2013). Recently, radiocarbon labelling revealed a lifespan of at least 272 years in the Greenland shark (*Somniosus microcephalus*), making it the most recent champion of vertebrate longevity (Nielsen et al., 2016). Within the order Rodentia, lifespan can range from three to four years in mice (*Mus musculus*), to as long as 30 years in captive naked mole rats (*Heterocephalus glaber)* (Austad, 2009). Understanding the mechanisms involved in this broad range in lifespan across vertebrates, and even within species will provide valuable insight into human aging and its related diseases. By broadening the phylogenetic range using a comparative approach instead of focusing on one "model" species, investigations in aging research can elucidate unique mechanisms involved in adaptations of lifespan and senescence. Comparative biologists rely on two key approaches to shed light on a wide range of biochemical and physiological problems, including mechanisms of aging. The first approach, and perhaps most famous, embraces the use of the August Krogh Principle, which states, "For a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied (Krogh, 1929; see Krebs, 1975)". In other words, these animals may have evolved a phenotype that mimics a human condition, which can be exploited to solve a particular problem. The classical example of this approach is the use of the large and accessible axon from squid (*Loligo spp.*) to develop voltage clamping techniques, which made possible the first measurements of membrane action potentials (Hodgkin and Huxley, 1952). Natural selection has solved the problem of severe hypoxia and reperfusion injury in a number of species, such as the crucian carp (*Carassius carassius*), which can survive months of anoxia all the while maintaining acid-base homeostasis and withstanding reperfusion injury upon reoxygenation (Nilsson, 1990; Kowaltowski et al., 2009). The second key approach employed in comparative biology, which is often used in conjunction with the "extreme" organism identified in the first approach,

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requires a phylogenetic examination of natural variation that occurs among species (Hochachka and Somero, 2002). Doing so requires the identification of a putative adaptation that may not be correlated to the evolution of a continuous trait, and that considers the phylogenetic relationship of the species in question (Garland et al., 1992; Somero, 2011). Implementing phylogenetic contrasts acknowledges that individual species are not independent data points. Recent advancements in phylogenetic analyses, such as those involved in DNA sequencing, and the rapid expansion of the eukaryotic phylogenetic tree have broadened the appeal of using interspecies comparisons to an ever-growing bestiary from which to select.

Aging and comparative metabolic research have complemented each other for as many years as comparative biological research has existed. More than 100 years ago, Rubner (1908), compared energy metabolism against lifespan between horses, cows, humans, dogs, cats and guinea pigs, and noted an equivalent energy expenditure per gram tissue in all species (see Speakman, 2005 for review). The study behind mitochondrial mechanisms of aging have much to gain by utilizing this approach instead of limiting itself to the more commonly utilized study organisms for aging. Undoubtedly, mice (in particular, C57BL/6) have supported an impressive amount of research to the aging field. However, there are drawbacks to limiting the study of aging processes to an animal with such a short lifespan compared to other closely related rodents. Mechanisms that contribute to the longevity of naked mole rats may not exist or be as readily apparent in mice (Austad, 2009). Although the convenience of executing an aging study rapidly within the short lifespan of an organism has proven fruitful (Alper et al., 2015), the frontiers of aging research would be best served by building upon this work with longer-lived organisms to contrast against more convenient, short-lived relatives.

The aim of this review is to highlight select recent discoveries in mitochondrial metabolism and aging, and illustrate how the comparative approach can be used to effectively expand our understanding of the evolution and mechanistic underpinnings of aging. To date, there is a paucity of literature utilizing comparative tools to investigate the relationship between mitochondrial function and longevity. Examples are provided from fields of research that have elegantly applied comparative physiology and biochemistry to address questions that apply to aging, including hypoxia and oxidative stress, thermal stress, and hibernation, among others. Recently, much of the comparative research in aging has been led by the genomics field (see Gorbunova et al., 2014 for review), which has clearly shown the relationships in longevity and transcription. However, biochemical and physiological aspects of mitochondrial metabolism need to be applied to aging research as a means to rigorously test current existing hypotheses in aging and to generate new ones. We provide a post-translational modification hypothesis of aging, which posits that hyperacylation of mitochondrial proteins contributes to mitochondrial dysfunctions associated with aging and is linked to species lifespan. The investigation of evolved mechanisms responsible for both short and long-lived phenotypes can provide insights into human senescence, and may directly inform the development of novel therapeutic interventions.

1.1. Concepts of aging

In the broadest sense, aging is a functional decline over time affecting most living organisms. More specifically, aging is a multifactorial process that results in the gradual damage in a number of cellular systems. In their review, López-Otín et al. (2013) identified nine of these factors as tentative hallmarks of aging, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intracellular communication. Mitochondrial dysfunction is responsible for a very unique type of cellular senescence, possibly initiated by the decline of the NAD^+:NADH ratio (Stein and Imai, 2012; Wiley et al., 2016), which may explain common aging phenotypes conserved through many species.

But how or why did aging evolve in the first place? The evolutionary theory of aging, as originally established by Haldane (1941); Medawar (1952), and Williams (1957), suggests that the forces of natural selection decrease with age (see Fabian and Flatt, 2011; Gorbunova et al., 2014; for review). This inability to impose a selective pressure is supported by the mutation accumulation hypothesis. That is, deleterious mutations accumulated later in life occurring after reproductive age will not be passed on to future generations. Most organisms in the natural world are exposed to predators, competition and other impediments to longevity, and therefore die well before mutation-based diseases manifest themselves. In contrast, species such as the burrowing naked mole rate face limited predation and may have thus developed the molecular mechanisms to live longer (Gorbunova et al., 2014). Furthering the mutation accumulation hypothesis, Williams (1957) proposed that pleiotropic effects from alleles at later ages compared to younger animals may accelerate the aging process. That is to say, a mutation or genetic variance at a younger age may be beneficial at younger ages, but harmful at later stages of life (Flatt and Promislow, 2007). Recently, these concepts of evolutionary aging moved beyond the boundaries of genetic inheritance and have been applied to functional mitochondrial aging research (Sun et al., 2016). Antagonistic pleiotropy has been observed in several paradoxical examples of decreasing mitochondrial efficiency associated with benefits at younger ages. For example, the deletion of the nuclear-encoded mitochondrial enzyme superoxide dismutase 2 (Sod2) in mice, which limits the proliferation of keratinocytes, accelerated epidermal wound closure at younger ages. However, at older ages, the decrease in the stem cell pool induced by the mitochondrial damage via Sod2 deletion delayed wound closure in older mice (Velarde et al., 2015).

1.2. Concepts of mitochondrial metabolism

Given its primary role in energy production, mitochondria have been implicated in many theories of aging (Fig. 1). Mitochondria are an ancient and highly conserved organelle, thought to have been derived from proteobacterial origins (Andersson et al., 1998, 2003). The endosymbiotic hypothesis gained widespread support upon the discovery of mitochondrial DNA, present in all eukaryotes (Nass and Nass, 1963; Margulis, 1970). This complex organelle serves as the hub of aerobic respiration, in addition to a number of other signalling and metabolic processes. Mitochondria vary immensely in size, however, they are typically represented in a “bean” shape of approximately 3–4 μm in length. Different tissues will contain different quantities of mitochondria, ranging from as low as a few hundred up to several thousand per cell.

A two membrane system functionally divides the mitochondria between an inner and outer mitochondrial membrane. Between the inner and outer membranes exists an intermembrane space, and within the inner membrane is a space defined as the mitochondrial matrix. Inner membrane composed as cristae contain the components of the electron transport system, where food intake is exploited to create energy, via the coupling of respiration to the oxidation of carbon compounds. This concept was elegantly described in the revolutionary paper by Mitchell (1961) which would not achieve widespread appeal for another 15 years after its conception. A key element of the chemiosmotic principle is the unidirectional translocation of protons across the inner