August 19, 2016 – DRAFT

A Multidisciplinary Approach to Explain Biological Aging and Longevity

Brett N. Augsburger
Ph. 1-334-524-8851
Email: bna0003@auburn.edu

Contents

Abstract

Arguably the last major discovery in the field of aging research occurred over 50 years ago: Hayflick's discovery of cellular senescence and the existence of a replication counter. Despite this breakthrough and the multitude of theories proposed since then, little progress has been made towards reaching a consensus on why organisms age or why they live as long as they do. In this paper, a multidisciplinary approach is taken in an attempt to understand the root causes of aging and derive a theory of aging with fewer anomalies than existing theories. Nonequilibrium thermodynamics may play a previously unappreciated role in determining longevity by dictating the dynamics of degradation within biomolecular ensembles and the inevitability of information loss. The proposed model offers explanations for aging-related observations that are considered paradoxical within the current paradigms. This framework questions the role of declining selective pressure as the primary driver of aging, and implies a fatal flaw in the disposable soma theory that may be responsible for a number of misconceptions that have impeded progress in the field. In summary, unifying pertinent concepts from diverse disciplines leads to a theory of aging with fewer anomalies, and may be useful in predicting outcomes of experimental attempts to modulate the aging phenotype.

1 Introduction

Scientists have proposed many theories to explain why organisms age. Concepts from evolutionary theory, genetics, biochemistry, and cellular and molecular biology are most often used as the basis of these theories. Despite the fact that these efforts have resulted in a multitude of theories, each with serious anomalies, the focus over the last half century has remained in these areas—more fundamental physical law has been under or incorrectly applied, or simply ignored altogether. Notwithstanding the fact that deterioration is implicated nearly universally in the aging process, the connection to nonequilibrium thermodynamics and entropy production has not been firmly established and is infrequently mentioned. There have been a few notable exceptions; for example, Hayflick contends that entropy alone is sufficient to explain biological aging (Hayflick, 2000; 2004; 2007b; 2007a).

The second law of thermodynamics (hereafter abbreviated to "second law") stipulates that all energy, regardless of form, has a propensity to transition from a localized state to one that is more spread out—i.e. dispersed in space—at a rate determined by the ability of contributing external factors to counteract this tendency. In any system that is not at thermodynamic equilibrium, this tendency will result in entropy production by means of irreversible processes. A nonequilibrium system will continue to produce entropy indefinitely until equilibrium is reached, resulting in a transition from a higher concentration of molecular bond energy to a lower bond energy concentration (Demirel, 2014).

It has been argued that the second law only relates to closed systems and that since organisms are open systems the second law does not apply (Mitteldorf, 2010). This is false—the second law is universally applicable and always satisfied (Kondepudi and Prigogine, 2014). According to modern nonequilibrium thermodynamics, the second law describes the tendency for internal entropy to be produced by any system that is not in equilibrium. Clearly, organisms are not in thermodynamic equilibrium and therefore all living organisms continuously produce internal entropy. Despite existing in a nonequilibrium state, individual organisms resist the decay towards equilibrium, which eventually results in death, long enough to allow them to mature and reproduce. The term "longevity", as used in this paper, refers to the average length of time that individuals of a group live under ideal conditions. For different species, this can vary from hours to centuries.

Organisms combat entropy increases by exchanging heat and other forms of energy with their surroundings and importing/exporting entropy in the form of metabolites and catabolites. Some scientists have suggested that any entropy increase in an organism can always be counteracted without repercussions by simply expending energy; from this, some have concluded that there is no thermodynamic stipulation for aging to occur and no role for thermodynamics in explaining aging. This is a rather obvious *non sequitur*—yet this notion has been perpetuated in the aging literature, both explicitly and implicitly (Kirkwood, 1999; Mitteldorf, 2010; Trindade et al., 2013). I will demonstrate why this inference is a logical fallacy and how it neglects to consider critical effects of thermodynamic phenomena within an organism—particularly the influence of internal entropy production on the flow of biomolecular-encoded information over time and the dynamics of degradation within biomolecular ensembles.

2 Nonequilibrium Thermodynamics Stipulates Biomolecular Damage in Living Organisms

Thermodynamic equilibrium is characterized by the total absence of thermodynamic potentials within a system and the minimization of free energy. Energy remains highly concentrated in living organisms due to the chemical bonds holding biomolecules together. For this reason, considerable thermodynamic potentials exist—thus organisms are not in thermodynamic equilibrium. The resulting thermodynamic fluxes (thermal, chemical, diffusive, mechanical, electrical, etc.) contain significant spatial and temporal heterogeneity

at both the mesoscopic and macroscopic levels, providing a multitude of opportunities for biomolecular interactions to produce transitions to undesirable structural states. Examples include: mechanical force-based unfolding of proteins; unzipping or shearing of nucleic acids; protein folding alterations and improper protein associations due to crowding (Zhou, 2010); DNA hydrolysis, oxidation, and methylation reactions (Lindahl, 1993); denaturation of DNA and protein from excessive temperature; and disruption of hydrophobic interactions leading to altered protein structure. It should be apparent that while it is beneficial for an organism to minimize the probabilities of these occurrences, it is impossible to reduce them to zero.

As the atomic arrangement of biomolecules fails to maximize free energy[1], the second law stipulates that irreversible processes driving the system in the direction of equilibrium will occur and impose insults on biomolecular structure. Organisms counteract this phenomenon and establish an ordered structure with low entropy by utilizing energy and matter exchanged with the environment to produce negative entropy. In terms of preservation of overall biomolecular[2] integrity, an organism is a near steady-state nonequilibrium system—at least if considered over a snapshot-in-time that is short compared to total lifespan.

2.1 A Model System for Analyzing Thermodynamically Derived Biomolecular Degradation

To demonstrate the degradative effects of thermodynamic chemical forces on biomolecules, we will consider a system consisting of a fixed volume of cytosol. The analysis will focus on a single type of biomolecule; this could be any expressed protein, synthesized lipid or other biomolecule produced by the organism's cellular machinery. It is assumed for now that the biomolecule of interest is a protein. The temperature and pressure of the system are in equilibrium with the surroundings and equivalent to physiological values. The concentrations of all molecules aside from the protein of interest are held constant by chemiostats. The system remains in thermomechanical equilibrium at all times. Chemical reactions not involving the protein of interest are inhibited (reaction rates and affinities are zeroed), as are all biomolecular repair and replacement mechanisms. At time t_0, every molecule of the protein of interest is in a state where it is maximally capable of performing its intrinsic biological function.

In accordance with the second law, the described system will produce internal entropy and transition irreversibly from an initial state at time t_0 through a very large number of nonequilibrium states until all chemical reaction affinities are reduced to zero. Since the system as defined has no means of counteracting internal entropy production, the second law requires that the only stable or steady state is the chemical equilibrium state. Although some reaction affinities may be low, even the most improbably reactions must have nonzero reaction rates. During the progression towards chemical equilibrium, the protein can exist in a very large number of alternative internal states representing various degradative arrangements. The transitions between internal states can be characterized by reactions of the form

$$\sum_{\alpha=1}^{N_a} a_\alpha^{nm} A_\alpha + n \rightleftharpoons m + \sum_{\beta=1}^{N_b} b_\beta^{nm} B_\beta \qquad (1)$$

where n and m are the initial and new protein internal states, a_α^{nm} and b_β^{nm} are the number of molecules of reactant (A_α) or product (B_β) involved in the reaction, and N_a and N_b are the number of different reactants and products involved. Until the system reaches equilibrium, these reactions will take place and will produce internal entropy. The rate of internal entropy production $d_i S/dt$ at any time t up until equilibrium can be expressed as

$$\frac{d_i S}{dt} = \sum_{j=1}^{r} \sum_{i=1}^{k} \left(\frac{-v_i^{(j)} \mu_i}{T} \right) \frac{d\varepsilon_j}{dt} > 0 \qquad (2)$$

where k is the number of chemical species involved in a particular reaction, r is the total number of reactions taking place in the system, $v_i^{(j)}$ are the stoichiometric coefficients, μ_i are the chemical potentials, and $d\varepsilon_j/dt$ represents the reaction velocity at time t. At thermodynamic equilibrium, both the reaction velocity and the reaction affinity $A_r = \sum_{i=1}^{k} \left(-v_i^{(j)} \mu_i / T \right)$ will be zero. This represents a state where all of the examined biomolecule have fully degraded and internal entropy production has ceased.

[1] Not to be confused with free energy minimization during protein folding and the conformational changes of other biomolecules, which occur very quickly by comparison. In these examples, the free energy minimization only involves conformation options for a given atomic structure that can be transitioned to within a very short time period. Even in its lowest free-energy conformation, any given biomolecule will still possess considerable excess energy—largely stored in the bonds between its atoms—compared to an equilibrium state where energy dispersion is maximal.

[2] Unless otherwise noted, the biomolecules referred to are the proteins, carbohydrates, lipids, nucleic acids, and other macromolecules that define the structure of an organism and facilitate function.

The total entropy increase in the system at any time t is

$$dS_{sys,t} = \int_{t_0}^{t}\left(\frac{d_eS}{dt}+\frac{d_iS}{dt}\right) = \frac{Q_{tot}}{T} + \int_{t_0}^{t}\sum_{j=1}^{r}\sum_{i=1}^{k}\left(\frac{-\nu_i^{(j)}\mu_i}{T}\right)\frac{d\varepsilon_j}{dt} \qquad (3)$$

Here, d_eS/dt represents the rate of entropy gain/loss in the system due to the exchange of energy with the surroundings (heat flowing into or out of the system). Q_{tot} represents the total heat that has been transferred to/from the system between time t_0 and time t. Utilizing the change in system entropy $dS_{sys,t}$ for any time t and the increase in system entropy corresponding to the equilibrium condition $dS_{sys,max}$, we will define a new parameter termed "degradation state" to represent the degree to which the biomolecule under examination has degraded

$$D(t) = \frac{dS_{sys,t}}{dS_{sys,max}} \qquad (4)$$

A D of 1 corresponds to full degradation, while a value of 0 is representative of a pool of fully intact biomolecules.

Of course, degradative internal entropy production in a living organism is not limited to chemical reactions but also includes contributions from heat, mass, and momentum transfer as well as electrical, magnetic, diffusion and other effects. Modern nonequilibrium thermodynamic theory (and the second law in particular) can be utilized to similarly model each of these factors, establishing an arrow of time stipulating that the future is distinguishable from the past by an ever-increasing quantity of total internal entropy produced.

2.2 Preservation of Steady-state Nonequilibrium within a Biomolecular System

For homeostasis—i.e. steady-state—to be preserved and to avoid transitioning to an equilibrium state with maximum disorder, degradation must be combatted by biological mechanisms capable of producing sufficient negative entropy to fully offset the internal entropy being produced, such that the total system entropy is unchanged:

$$\dot{S}_{sys} = \frac{d_eS}{dt} + \frac{d_iS}{dt} = 0 \qquad (5)$$

Since the second law stipulates that $d_iS/dt > 0$, in order to maintain a steady state

$$\frac{d_eS}{dt} = -\frac{d_iS}{dt} < 0 \qquad (6)$$

Suppose that a replacement mechanism is incorporated which replaces $\dot{N}_{rc}$ moles s^{-1} of degraded biomolecules with newly expressed or fully repaired biomolecules. A steady state is maintained if

$$D_{rep}\frac{-dS_{sys,max}}{N_{tot}}\dot{N}_{rc} = -\frac{d_iS}{dt} \qquad (7)$$

where D_{rep} represents the average degradation state of a replaced biomolecule and N_{tot} is the total number of the biomolecule of interest in moles. Eq. *(7)* can be rearranged to solve for replacement rate. (The rate of internal entropy production will be denoted by $\dot{S}_i$ instead of $\frac{d_iS}{dt}$ for purposes of clarity.)

$$\dot{N}_{rc} = \dot{S}_i N_{tot}\left(D_{rep} * dS_{sys,max}\right)^{-1} \qquad (8)$$

The degradation state D of a biomolecular pool specifies the level of degradation of the average biomolecule but it does not indicate how well biomolecules perform at that degradation state. A new term, "biomolecular performance" P, will be used to quantify the relative ability of a biomolecule to perform its intrinsic biological function(s). A value for P of 1 indicates that the average biomolecule in an ensemble is able to perform 100% of its intrinsic biological function(s), or in other words, the ensemble will perform as if all biomolecules were in ideal condition. A P of 0 denotes that the average biomolecule is unable to perform any of its intrinsic biological function. Ultimately, we would like to express biomolecular replacement rate $\dot{N}_{rc}$ as a function of biomolecular performance. Several more relationships must be defined before this is possible.

2.3 Relating Biomolecular Performance to Degradation State

Biomolecular insults are inevitable occurrences; for this reason, biomolecules might be expected to retain the ability to perform their intrinsic biological function even when some level of damage is present. If a biomolecule did not have this capability, only a very small percentage of biomolecules within a pool would be functional at any given time.

Many small singular insults to a biomolecule may have little to no effect on biomolecular performance (although certainly some singular insults can render a biomolecule nonfunctional or significantly compromised). As the number of insults incurred by a biomolecular pool begins to accumulate, biomolecular performance must decrease at a rate that increases with further degradation. As the degradation state continues to increase, an inflection point will eventually be reached where the rate of decrease in P has achieved a maximum and further increases in degradation state result in increasingly lower rates of decrease in P. The described relationship between biomolecular performance and degradation state is approximated by a logistic curve. This can be represented as

$$P(D) = \left[1 + e^{k(D - D_{P50})}\right]^{-1} \qquad (9)$$

Parameter D_{P50} specifies the biomolecular degradation state value that corresponds to a biomolecular performance of 0.5. In other words D_{P50} is a way to signify how much degradation a biomolecular ensemble can incur before losing half its performance. D_{P50} can be thought of as a measure of biomolecular durability. The parameter k specifies the steepness of the curve, or the relative ability of a biomolecule to resist decreases in performance with increasing degradation; for this reason, k can be viewed as a measure of biomolecular resiliency (lower values indicate increased resiliency). Fig. 1 illustrates some hypothetical graphs of P as a function of D for various values of k and D_{P50}.

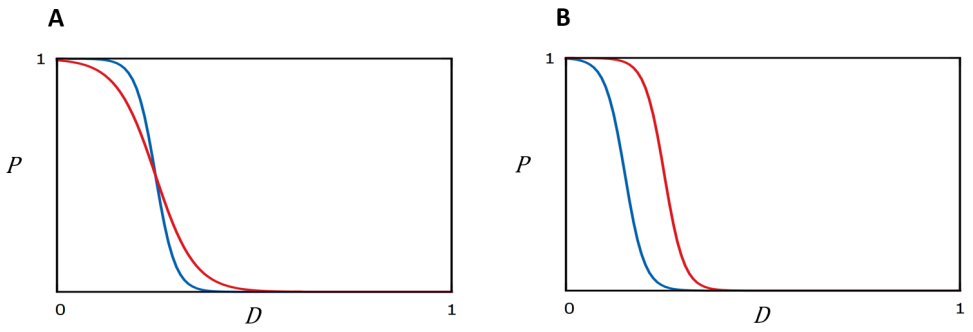

Fig. 1. Hypothetical biomolecular performance - degradation state curves that demonstrate the result of modulating different parameters from Eq. (9). (a) D_{P50} = 0.25, k = 40 (blue), k = 20 (red). (b) D_{P50} = 0.15 (blue), D_{P50} = 0.25 (red), k = 40.

2.4 Incorporating Biomolecular Repair and Replacement Mechanisms

Next, we will derive a means to express the average degradation state of a biomolecule undergoing turnover in terms of the biomolecular performance of the ensemble. For this purpose, it will be assumed that the biomolecular repair/replacement mechanisms are able to distinguish between the performance state of individual molecules and that the average biomolecular performance of a repaired/replaced biomolecule is m% of the average biomolecular performance of the ensemble. By rearranging Eq. *(9)* and incorporating the m term we arrive at the desired expression

$$D_{rep}(P) = D_{P50} + k^{-1}\ln\left(\frac{100}{mP} - 1\right) \qquad (10)$$

Obviously, this relation does not perfectly describe the exact behavior of a cellular biomolecular repair and replacement strategy, but it is an adequate approximation for the purposes of the current discussion.

2.5 Relating Internal Entropy Production Rate to Degradation State

Lastly, to express biomolecular replacement rate in terms of biomolecular performance for a steady-state scenario, we require an expression for the rate of internal entropy production as a function of degradation state. Eq. *(2)* described the rate of internal entropy production in terms of chemical reaction affinities and velocities. We can approximate a transformation of this equation into one that is a function of degradation state by considering some aspects of the reactions occurring within the system. For the time being, we will disregard radical and other chain-type reactions. We will assume that there are a very large number of potential reactions and that reaction velocities are widely and relatively evenly dispersed. Reactions with high reaction velocities will tend to occur before those with lower reaction velocities. In other words, reactions with high reaction velocities will be more prevalent at low degradation states. As degradation state increases, reactions with lower reaction velocities will begin to represent a larger proportion of the internal entropy being produced. However, there will be fewer total reactions because reactions with higher reaction velocities will

have already completed. Therefore, as degradation state increases, the reaction velocity of the average reaction will decrease (reducing $\dot{S}_i$) and there will be fewer total possible reactions (further reducing $\dot{S}_i$). For this reason, $\dot{S}_i$ as a function of degradation state can be approximated by an exponential decay relation

$$\dot{S}_i(D) = \dot{S}_{max} e^{-rD} \qquad (11)$$

where $\dot{S}_{max}$ is the maximum rate of internal entropy production (corresponding to a D of zero) and r is the exponential decay constant. Actual values of r should always be greater than zero.

2.6 Governing Master Equation Relating Biomolecular Repair/Replacement to Performance

We have defined all the requisite relationships to express biomolecular replacement rate $\dot{N}_{rc}$ as a function of biomolecular performance. Combining Eqs. *(8)* thru *(11)*, and solving for $\dot{N}_{rc}$ yields

$$\dot{N}_{rc}(P) = \dot{S}_{max} e^{-r D_{P50}} (P^{-1} - 1)^{-\frac{r}{k}} \left(D_{P50} + k^{-1} \ln\left(\frac{100}{mP} - 1\right)\right)^{-1} \frac{N_{tot}}{dS_{sys,max}} \qquad (12)$$

Biomolecular replacement rate is of particular importance as it closely correlates with the rate of energetic resource consumption required to maintain a specific level of biomolecular performance. The performance of a given biomolecular ensemble must satisfy cellular/organismal requirements. Therefore, it is of interest to consider how biomolecular performance and replacement rate relate to each other and how other parameters may affect this relationship.

In the first hypothetical scenario, we consider the repercussions of modulating the exponential decay constant r (Fig. 2, Case A). Higher values of r equate to an increase in the rate of decay of internal entropy production with increasing degradation state (demonstrated in Fig. 2A.2 by plotting Eq. *(11)* for three different values of r). A particular performance value exists above and below which any change in replacement rate has a diminishing effect on biomolecular performance. This is illustrated by plotting the derivative of Eq. *(12)*, $\frac{d\dot{N}_{rc}}{dP}$ as a function of P, as shown in Fig. 2A.4. The minima in this graph represent the biomolecular performance values where the return on investment (ROI, in terms of rate of consumption of cellular energetic resources) towards biomolecular replacement rate is maximized. This demonstrates the presence of a tradeoff between biomolecular performance and cellular energetic resource ROI.

Next we will consider two variations of a biomolecule that share the same degradation state for a particular performance value but differ in resiliency (parameter k) due to differences in biomolecular structure. This is depicted in Fig. 2B.1 for a shared performance value of 0.95. $\dot{S}_i(D)$ is similar for both variations (Fig. 2B.2). Increasing biomolecular resiliency (decreasing k) allows the same biomolecular performance to be achieved with lower replacement rates (Fig. 2B.3). All else being equal, selective pressure should favor biomolecular configurations that maximize resiliency.

2.7 Incorporating Radical and Other Chain-type Reactions

For the last scenario, we will consider the impact of radical and other chain-type reactions. The presence of these reactions will impact $\dot{S}_i(D)$. At low degradation states, there will be relatively few chain-type reactions as reactive product is required to generate these reactions. As degradation state increases, more reactive product will be available, leading to an increase in the number of chain-type reactions and a corresponding increase in internal entropy production. As the amount of reactive product increases, the amount of available reactant will decrease. At some degradation state value, the amount of reactant remaining will have decreased to the point that the quantity of remaining available reactant limits the reaction rate. This will result in a maximal contribution to $\dot{S}_i$ at this condition and a continual decrease in the magnitude of the contribution to $\dot{S}_i$ for higher degradation states. If we assume that this switchover occurs at a degradation state of 0.5, we can roughly approximate this behavior with the relation

$$\dot{S}_{i,rad}(D) = \dot{S}_{max} h(D - D^2) \qquad (13)$$

where h is a scaling term. The total internal entropy production equation becomes

$$\dot{S}_{i,tot}(D) = \dot{S}_{max}(e^{-rD} + h(D - D^2)) \qquad (14)$$

Combining this equation with Eqs. *(8)* thru *(10)*, and solving for $\dot{N}_{rc}$ yields the new expression

$$\dot{N}_{rc}(P) = \dot{S}_{max}\left(e^{-rD_{P50}}(P^{-1}-1)^{-\frac{r}{k}} + h(D_{P50}+k^{-1}\ln(P^{-1}-1)-(D_{P50}+k^{-1}\ln(P^{-1}-1))^2)\right)\left(D_{P50}+k^{-1}\ln\left(\frac{100}{mP}-1\right)\right)^{-1}\frac{N_{tot}}{dS_{sys},max} \quad (15)$$

By plotting Eq. *(14)*, we can see the influence of chain-type reactions on total internal entropy production for a hypothetical scenario (Fig. 2C.2). Not surprisingly, per Eq. *(15)*, incorporating chain-type reactions results in increased replacement rates for all performance values (Fig. 2C.3).

Of note is how it is conceivable for a biomolecular pool to have considerable radical-induced damage when functioning at a degradation state corresponding to that which maximizes cellular energetic resource ROI. Moreover, it demonstrates that it is reasonable to expect additional radical-induced damage (i.e. an increased degradation state) in situations where energetic resource availability is limited and energetic resource ROI is prioritized over peak performance. In other words, biomolecular performance levels that maximize energetic resource ROI are likely to correspond with higher levels of biomolecular radical damage compared to the higher performance levels utilized by organisms with lower energetic resource availability restrictions and where maximal biomolecular performance is a higher priority.

2.8 The Naked Mole-rat Paradox – Part I

The naked mole-rat (*Heterocephalus glaber*) may be an example of this phenomenon in action. The naked mole-rat has a maximum lifespan (MLSP) of ~31 years while its similar-sized cousin, the house mouse (*Mus musculus*), has an MLSP of only ~4 years (Tacutu et al., 2012). Yet, the naked mole-rat exhibits significantly higher cellular oxidative stress compared to the house mouse (Andziak et al., 2005). Naked mole-rats also have higher levels of oxidative damage, including increased lipid peroxidation and total protein oxidation (Andziak and Buffenstein, 2006; Andziak et al., 2006). Examination of mitochondrial protein fractions from heart tissue found that mitochondrial proteins are also more damaged on average in naked mole-rats compared to mice (Andziak et al., 2006). Despite this, naked mole-rats do not have superior biochemical defenses and in fact, they do not possess an antioxidant assemblage that is any more effective or efficient than that of mice (Andziak et al., 2005).

Naked mole-rats live in a hypoxic environment and have extremely low metabolic rates for their size (McNab, 1966). It is considered a paradox that the naked mole-rat exhibits high levels of oxidative stress and protein damage while having such extreme longevity compared to similarly sized, closely related species. In fact, this may be predictable and straightforward to explain. The first piece of the puzzle is explaining why oxidative damage levels are substantially elevated in the naked mole-rat. The naked mole-rat's limited access to oxygen restricts the rate of cellular ATP production via oxidative phosphorylation, thereby requiring that energetic ROI for cellular processes be highly prioritized. As demonstrated, biomolecular performance levels that maximize energetic ROI are likely to correspond with higher loads of radical (oxidative) damage. Related species that are not as energetic resource-restricted, for example the house mouse, may function at higher biomolecular performance levels (to help maximize athletic ability, growth rate, etc.) which will correspond to lower levels of oxidative, and other, damage present in their biomolecular pools. Therefore, it should not be surprising that the naked mole-rat has elevated levels of oxidative damage, which is indicative of a higher biomolecular degradation state. The second part of solving the naked mole-rat paradox is explaining why their high biomolecular degradation states do not determine, nor adversely affect, MLSP. This is addressed in section 0.

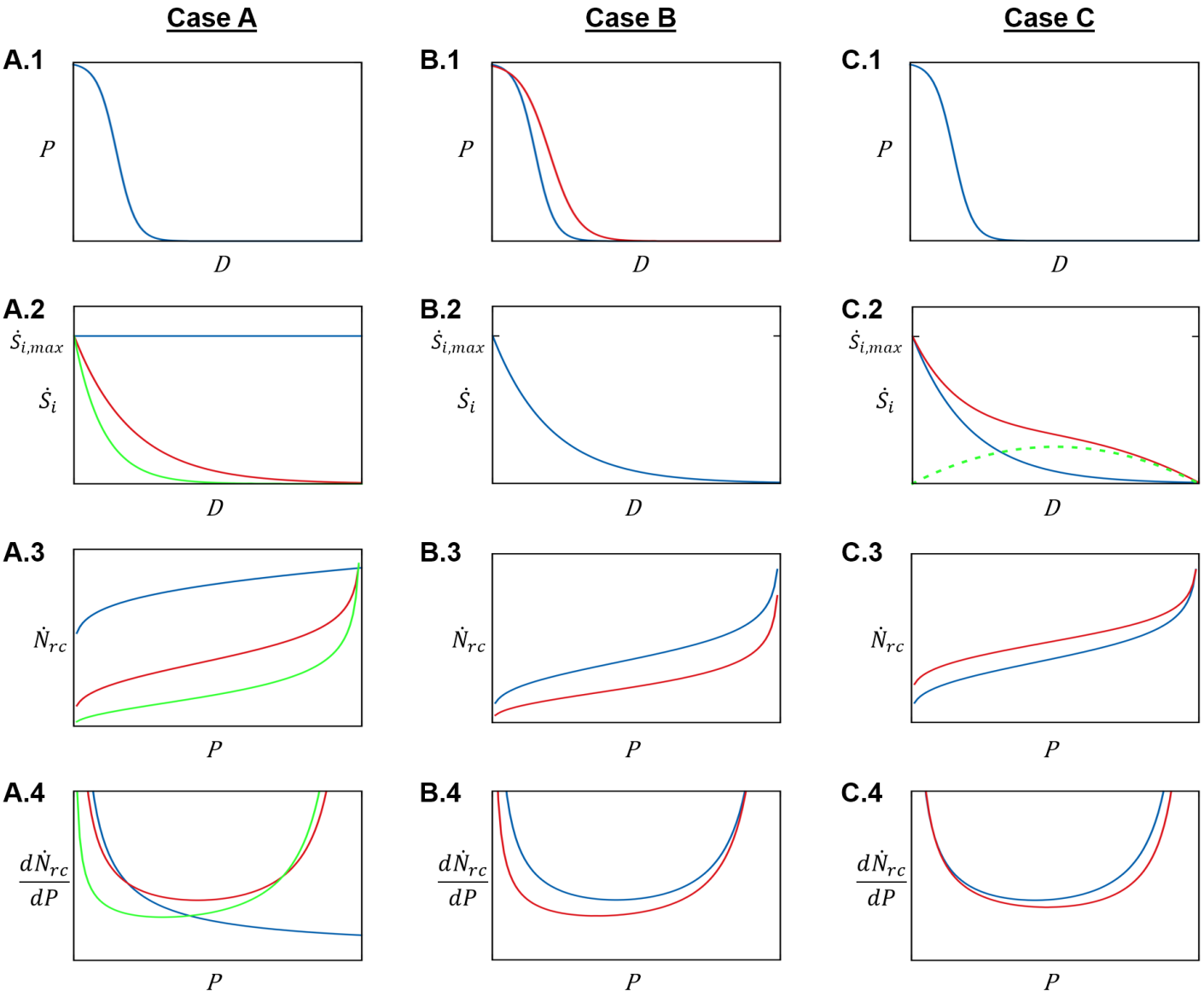

Fig. 2. Three hypothetical biomolecular repair/replacement scenarios demonstrating the relationships between biomolecular performance, degradation state, and repair/replacement rate by plotting Eqs. (9), (11), (12), (14), *and* (15). *Case A: Effects of altering parameter* r. D_{P50} = 0.15, k = 30, m = 10, r = 0 (blue), r = 5 (red), r = 10 (green). *Case B: Increasing biomolecular resiliency (lowering* k*) reduces the biomolecular replacement rate required to achieve a given performance. Both variations have the same degradation state at a performance value of 0.95.* D_{P50} = 0.15 (blue), D_{P50} = 0.199 (red, calculated), k = 30 (blue), k = 20 (red), m = 10, r = 5, P_{match} = 0.95. *Case C: Introduction of a radical reaction term into the internal entropy production rate equation.* D_{P50} = 0.15, k = 30, m = 10, r = 5, h = 1.0.

3 Managing Degradative Internal Entropy Production within an Organism

It should be apparent by now that it is not possible to achieve perfect fidelity in a biomolecular ensemble. As degradation state decreases, less and less negative entropy is produced by each biomolecular repair/replacement event; infinite resources would be required to attain perfect fidelity, i.e. a degradation state of zero. Degradative internal entropy production rate is predicted to decrease with increasing degradation state per Eq. *(11)*. The steady-state realized will be the state in which the rate of negative entropy production d_eS/dt from repair and replacement is equivalent in magnitude to the rate of degradative internal entropy production d_iS/dt.

Researchers have characterized many of the biological mechanisms utilized for producing negative entropy. These include biomolecular expression systems, molecular chaperones, degradation systems (proteasomes, lysosomes) and DNA repair enzymes, to name some of the most obvious. At the cellular level, stem cells and mitotic cell division, together with apoptosis, provide a means to

replace entire cells and, in some organisms, even tissues—thereby preserving (or at least attempting to preserve) the degradation state in these populations.

Biological factors that influence d_iS/dt are equally important in determining the degradation state. Reducing the rate of internal entropy production decreases the amount of negative entropy needed, and therefore the energetic investment required, to preserve homeostasis. The rate of internal entropy production is proportional to the sum of the contributions from all thermodynamic potentials acting on a biomolecular ensemble. This includes chemical reactions, heat, mass, momentum transfer, and other effects. The magnitudes of the thermodynamic potentials depends on the strengths of the respective "damage-inflicting" forces (which may vary significantly with time, particularly when a biomolecule is in an active state) and the ability of an organism's biomolecular structures to resist these forces.

3.1 Optimization of Biomolecular Structure

Biomolecular structural optimizations can modulate the effects of degradative thermodynamic potentials by resisting atomic rearrangements. Consider how hydroxyl radicals, which are capable of generating very strong chemical reaction potentials, may affect a protein. The amino acids cysteine and methionine are particularly vulnerable to oxidation reactions (Suto et al., 2006). Substituting another amino acid in place of a cysteine may help to protect a protein from aberrant structural modifications resulting from hydroxyl radical reactions. Alternatively, cysteine could be implemented in non-critical locations within a protein as a sacrificial means to scavenge free radicals and prevent damage to more critical domains. It should be considered, however, that a cysteine or methionine residue in a particular location could bestow an advantageous trait to a protein (improved catalytic activity, energy utilization, substrate specificity, etc.)—thus any benefits to inclusion must be weighed against the costs associated with the increased susceptibility to insult.

Other biomolecular structural optimizations that could help to reduce d_iS/dt include modifications that improve resistance to undesirable hydrophobic interactions and structural variations that resist temperature-induced denaturation. These biomolecular modifications could result in a deleterious increase in the physical size of the biomolecule or be otherwise disadvantageous, such as by limiting the rate of intrinsic biological function. Modifications to biomolecular structure could also affect the amount of energetic resources required for the production/replacement/repair of a biomolecule.

On the other hand, structural alterations that maximize a biomolecule's specific rate of work may reduce biomolecular durability/resiliency and increase d_iS/dt. An example of this is discussed in section 7.4, where it is demonstrated that the polyunsaturated fatty acid content levels of membrane lipids, which varies across species, likely represent evolved tradeoffs between the maximum specific rate of transmembrane protein work performed and internal entropy production rate.

3.2 Microenvironment Optimization

Microenvironmental conditions are also relevant to d_iS/dt within an organism since they define the magnitude of the degradative thermodynamic forces acting upon biomolecules. Temperature has a significant effect on reaction velocities and bond forces/energies. Although biomolecular conformation can change with temperature, lower temperature will generally improve molecular stability and reduce the rate of internal entropy production. On the other hand, higher temperatures will produce increased kinetic energy transfer during intermolecular collisions. This will increase the specific rate of biomolecular work that is achievable.

Other attributes of a microenvironment may have less obvious (and sometimes counterintuitive) ramifications. For example, conditions of higher oxidative stress will likely increase the magnitude of degradative thermodynamic forces. At first glance, this may seem purely undesirable from a biological standpoint. Yet, it was demonstrated earlier how arrangements that highly prioritize energetic resource ROI may exhibit elevated levels of oxidative stress.

Clearly, optimizing biomolecular structure and microenvironment are multifactorial compromises. Through evolution, relevant variables are "tested" iteratively and genotypes converge towards those that balance these factors in a way that maximizes species fitness.

3.3 Biomolecular Work Rate can Influence the Magnitude of Degradative Internal Entropy Production Rates

A transfer of energy occurs when a biomolecule performs its biological function, resulting in a brief period of time when the concentration of energy in close proximity to the biomolecule is elevated. This leads to higher thermodynamic potentials and increases d_iS/dt. All else being equal, a biomolecular pool that is inactive (not performing any intrinsic biological function) will exhibit lower

d_iS/dt than one in which biomolecules spend more time in the active state. d_iS/dt (which can be shortened to $\dot{S}_i$) can be approximated by

$$\dot{S}_i = (100 - p)\dot{S}_{i,static} + p\dot{S}_{i,active} \quad (16)$$

where $\dot{S}_{static}$ is the degradative internal entropy production rate of the system when biomolecules are not performing any intrinsic biological function and $\dot{S}_{active}$ is the rate when all biomolecules are actively performing their intrinsic biological functions. p represents the percentage of time the average biomolecule spends in the active state. It should be evident that conditions where biomolecules are spending more time in the active state (i.e. work rate is higher) will require increased biomolecular repair/replacement rates in order to maintain the same biomolecular degradation state.

3.4 Extrapolation of Degradation State Concepts to Larger Physical Scales

Although the model outlined in section 2 described a population of biomolecules, these same concepts are applicable across a wide range of physical scales. For example, organelles are repaired and replaced, and face damage due to degradative internal entropy production in much the same manner as the individual biomolecules that they are assembled from. Mitotic cell populations can also be thought of in this way, with individual cells utilized as the expressed (replicating) unit.

4 Preservation of DNA Molecular Information

Most types of biomolecules are replaced by expression of a genetic sequence or are the metabolic products of expressed biomolecules. The performance of these biomolecular pools can be preserved through replacement by the successful expression of the appropriate genetic sequence(s) or the relevant metabolic processes, and the removal or repair of any dysfunctional counterparts. With a given rate of turnover and assuming intact expression machinery, the preservation of biomolecular performance within a cell becomes dependent on: (1) the integrity of the genetic material responsible for biomolecular expression, and (2) the cell's ability to remove all dysfunctional biomolecules. While the last requirement should not be trivialized, this is a very attainable objective: that is to say, the specificity of degradation pathways can afford to err on the side of casting a wider net to help ensure that any dysfunctional biomolecule is eventually recognized since these biomolecules can be resynthesized anew. Indiscriminate purging of cellular content would eventually dispose of any unwanted products—it is much easier to discard in excess to rid of waste than it is to preserve ultimate integrity in a structure. For these reasons, integrity preservation in biomolecules that are not expressed, the genetic-encoding biomolecules—DNA, particularly warrants further scrutiny.

4.1 Decreases in Mutual Information of DNA Molecules

DNA molecules contain the information encoding for production of all other classes of biomolecules as well as the instructions for all cellular processes. They are unique among classes of biomolecules as they depend on their own integrity for replacement. Like all molecules, DNA is subject to damage due to internal entropy production and will incur an insult rate proportional to the damage-inflicting thermodynamic potentials of the microenvironment.

There are a number of ways that DNA damage can result in base alterations, cross-linking, strand breaks, and other modifications (De Bont and van Larebeke, 2004). Consider some of the possible outcomes when a double-stranded DNA molecule has suffered a single base excision:

1. The damage is properly repaired by endogenous base excision repair (BER) mechanisms
2. The damage is improperly repaired by BER mechanisms
3. Additional insults occur at the damage site before repair can take place
4. No repair or further damage occurs for a length of time

DNA replication takes place far from thermodynamic equilibrium. The accuracy of DNA polymerase is largely dependent on the differences in the free energy released or absorbed by the various possible base-pairing combinations of incoming nucleotides to template strand nucleotides (Arias-Gonzalez, 2012). Utilizing thermodynamic theory, researchers have estimated polymerase error rates and demonstrated them be non-zero (in alignment with empirical findings). Although BER often successfully repairs single-base excision damage (scenario 1)—restoring redundancy and preventing changes in stored information—there is always a possibility that a replication error will occur. Additionally, repair machinery must translocate to the site of the insult and perform the repair. This will not occur instantaneously. If further damage occurs at the site before repair takes place then information could be permanently lost.

Only a single level of redundancy is definite at all DNA base pairs—that provided by the pairing base on the opposite strand. Even an insult restricted to a single base will deplete this redundancy and can lead to a permanent change in DNA information. This does not

imply that insults that are more serious are not repairable. For example, double-stranded breaks can be repaired by homologous recombination in many cases, but there is no guarantee that a homologous site exists or that the repair will be successful.

Once a DNA molecule has suffered an insult, there is no means to guarantee restoration of redundancy. *As the second law stipulates that molecular insults are inevitable, the genetic data stored in DNA molecules must change with time—indefinite preservation of data is not possible. The concept of "perfect" DNA repair is flawed and unattainable.* This same inference has been drawn previously utilizing information theory: Yockey (1974) suggested that the noisy-channel coding theorem stipulates that, under ideal conditions, the stability of the genetic message can be such that the error is "arbitrarily small" but that the probability of error must always be non-zero.

Permanent losses in genetic data are typically discussed in terms of discrete mutations or mutation rate (Sniegowski et al., 2000). An alternative way to assess these losses is to use the concept of mutual information[3], which is a measure of the amount of information shared between two datasets. This provides a means to quantitate the amount of encoded data retained in, or transmitted between, DNA molecules over time. As genetic data must change with time, the mutual information of a discrete, non-replicating DNA molecule must also decrease with time; therefore, the rate of change in mutual information of DNA molecules will be negative and can be represented by

$$MIR_{DNA} = \frac{I_{DNA}}{t} < 0 \qquad (17)$$

I_{DNA} represents the amount of mutual information between the data stored in the DNA molecule at any initial time and after a period of time t has passed.

4.2 Applicability of the Degradation State Concept to DNA Molecular Ensembles

Synthesized biomolecules depend on the integrity of DNA for their correct expression. If the full integrity of DNA is preserved then, theoretically, negative entropy could be produced at a rate that maintains a steady level of performance in any expressed biomolecular pool (as discussed in section 2.2). Since DNA molecules rely on their own integrity for identical replacement, this biomolecular replacement scenario is not applicable to DNA molecules.

4.3 Considerations for Mutual DNA Information Preservation in Different Cell Types

For any sexually reproducing multicellular organism, the zygote contains the truest representation of the parentally derived genetic data anywhere in the individual and of any stage in life, i.e. the mutual DNA information between parent and offspring is maximal in the zygote. The informational integrity of an organism's DNA at any later point in life can be quantified by comparing to this baseline standard.

Consider how the requirements for preservation of mutual DNA information are likely to vary over the course of an individual multicellular organism's life and as a function of cell type. Somatic cellular function must remain at a sufficiently high level for some minimum amount of time to allow the organism to reproduce. Selective pressure for preservation of function begins to decrease as an individual ages past reproductive maturity (Hamilton, 1966; Medawar, 1952). The progeny of adult stem cells are the replenishment source for somatic cells; therefore, adult stem cells must retain more mutual DNA information, on average, than non-stem somatic cells for any given point in an individual's life.

Singular events that generate losses in mutual DNA information (i.e. mutations) most commonly have little or no effect on offspring fitness. Some mutations will result in decreases in fitness while only the rare insult produces increased fitness (Eyre-Walker and Keightley, 2007; Fisher, 1930). The distribution of these fitness effects can vary considerably between organisms. Evolutionary pressures must be sufficiently strong to select against "negative" mutations in order to prevent a loss of fitness.

The redundancy provided by diploidy/polyploidy, gene duplication, and functional overlap likely provides a degree of robustness that enables non-germ cells to tolerate a certain level of mutual DNA information loss with minimal performance impact on the individual (Medvedev, 1972; Plata and Vitkup, 2013; Riggs, 1994; Yockey, 1974). Similar levels of damage would be more detrimental in germ cells as they would propagate to all cells of the progeny. Therefore, the average mutual DNA information retained by germ cells must

[3] Although thermodynamics is useful for examining the causes of DNA molecular insults and assessing the magnitude of the damage-inducing potentials, concepts from information theory are more appropriate for analyzing DNA integrity quantitatively. To avoid confusing the fields, any use of the term entropy in this manuscript refers to thermodynamic entropy. Direct reference to Shannon entropy is avoided.

be greater than that of adult stem cells at the time of reproduction, which in turn must be greater than the mutual DNA information retained in non-stem somatic cells at the time of reproduction. This relation can be written

$$\bar{I}_{DNA}(zyg; som_{rep}) < \bar{I}_{DNA}(zyg; stem_{rep}) < \bar{I}_{DNA}(zyg; germ_{rep}) \quad (18)$$

where $\bar{I}_{DNA}(zyg; som_{rep})$ represents the average mutual information between the non-stem somatic cells of an individual at the time of reproduction and the same individual when it was a zygote, $\bar{I}_{DNA}(zyg; stem_{rep})$ is the average mutual information between adult stem cells and the zygote, and $\bar{I}_{DNA}(zyg; germ_{rep})$ is the average mutual information between germ cells and the zygote.

In agreement with Eq. *(18)*, organisms appear to come closest to fully preserving mutual DNA information in germ cells. This results from evolved strategies that place extraordinary emphasis on the preservation of both nuclear and mitochondrial genetic data in germ cells. The fidelity of mtDNA is effectively reset during oogenesis through a genetic bottlenecking process that selects for the healthiest mtDNA and eliminates less efficient, mutated mtDNA molecules (Lee et al., 2012; Wai et al., 2008). The nuclear DNA in germ cells is subject to very strict insult detection mechanisms (Bailly and Gartner, 2013; Hochwagen and Amon, 2006; Jaramillo-Lambert et al., 2010). Germ cells are more likely than somatic cells to undergo apoptosis when DNA damage is detected, rather than attempt to repair the damage (which often results in the loss of mutual DNA information). They are also sequestered in a protected microenvironment with various specialized cells whose sole purpose is to support and maintain the germ cells (Schulz et al., 2002).

Assessing the situation from a thermodynamics perspective suggests that the rate of mutual DNA information loss is minimized by keeping the thermodynamic potentials acting on the DNA molecules as low as possible. Primordial germ cells (gametogonia), as well as oocytes and spermatocytes, have relatively low rates of oxygen consumption (Brinster and Troike, 1979). Most adult stem cells are quiescent and frequently prioritize glycolysis over oxidative phosphorylation, leading to lower levels of free radicals and ROS (Rossi et al., 2008; Shyh-Chang et al., 2013; Suda et al., 2011; Tothova et al., 2007) and decreased mtDNA replication rates. This supports the notion that manipulation of thermodynamic potentials acting on DNA molecules, through modulation of cellular processes and microenvironmental conditions, is a realizable and effective means of reducing the rate of mutual DNA information loss in cells.

The amount of genetic information in the gametes that is common to the same individual when it was a zygote $I_{DNA}(zyg; gametes)$ is determined by not only inevitable germ cell mutual information losses throughout life but also losses due to genetic recombination during meiosis ΔMI_{recom}:

$$I_{DNA}(zyg; gametes) = I_{DNA}(zyg; germ_{rep}) - \Delta MI_{recom} \quad (19)$$

Absent effective evolutionary selection, the loss of mutual information between parent and offspring will lead to a decline in species fitness since advantageous mutations are rare. The proportion of progeny with low fitness must not be so excessive that evolution cannot successfully select for neutral and higher fitness offspring. Thus, a minimal limit $I_{DNA}(zyg; gametes_{min})$ is effectively placed on the mutual information of the progeny:

$$I_{DNA}(zyg; gametes) \geq I_{DNA}(zyg; gametes_{min}) \quad (20)$$

Germ cells must be maintained with adequate redundancy levels and a sufficiently stringent fidelity preservation strategy to satisfy Eq. *(20)*. In this way, mutual DNA information is largely preserved generation-to-generation.

There is a direct correlation between the lifetime risk of cancer in a tissue and the number of divisions of the stem cells maintaining that tissue (Tomasetti and Vogelstein, 2015). The strategies used to preserve stem cell integrity clearly do not achieve the fidelity preservation of germ cell strategies. Since the preservation of stem cell mutual DNA information requires dedicated niches with specialized microenvironments, there must be associated negative fitness costs to scaling these niches excessively—even though doing so may result in further reductions in the rate of mutual DNA information loss in the cell type in question. For this reason, an organism's stem cell niches must adequately support the respective target tissue over the lifespan of the individual, but not be so unnecessarily burdensome that they lower species fitness.

5 Establishing a Connection between Thermodynamic, Information, and Evolutionary Theory in Biological Aging

Modern nonequilibrium thermodynamic theory stipulates that all biomolecules will suffer degradative insults. Biological repair and replacement mechanisms cannot guarantee that mutual DNA information is preserved in individual cells: Cellular mutual DNA information must decrease with time. What are the repercussions of these losses for the individual and the species as a whole?

5.1 In the Individual

Most germline mutations are neutral or detrimental to fitness, with only the rare mutation being beneficial. It follows that mutations occurring in the somatic cells of an individual organism would exhibit this same pattern in regard to their contribution towards the viability of the individual. Therefore, without selection for only those changes that are neutral or beneficial to the individual, mutual DNA information loss in somatic cells will reduce individual viability with time, i.e. individual organisms will age.

Although natural selection is traditionally thought of as occurring at the level of the individual, a similar process takes place during the life and death cycles of the cells of a multicellular organism throughout an individual's life; single cells are the replicating unit in this scenario. For an individual multicellular organism containing cells undergoing mitosis, natural selection will occur on the cellular level and favor those cells that display the highest fitness. These configurations may not necessarily be the most beneficial to the individual as a whole. As natural selection will always be present at the level of the replicating unit (Baum et al., 2013; Szathmáry and Smith, 1995)—cells in this case—the individual must rely on imperfect innate biological mechanisms that attempt to select for only those configurations that do not reduce individual viability.

As there is no means for an organism to perform a comparative DNA sequence analysis, cells with undesirable base-sequence modifications are only detectable by phenotype. In the case of more severe damage, the cell is often able to detect the damage and initiate apoptosis (Zhou and Elledge, 2000). On the other hand, singular mutation events may exhibit very mild or no detectable undesirable phenotype; these cells are likely to avoid detection completely. For example, mutations whose effect is masked by redundancy are likely to have no detectable phenotype. A mutation may also occur in a region of the genome that is not currently active or relevant to the particular cell—there may be no immediate negative phenotype. This genomic region could become active later, at which time the mutation may have already spread to the cell's progeny.

Even in the ideal embodiment, the effects of multiple mutation events must eventually decrease individual viability; at some point, removing cells determined by biological mechanisms to be undesirable will no longer provide reprieve from losses in viability since cellular mutual DNA information will continue to decrease until all cells approach the detectable threshold of dysfunction. At this point, there would be no "good" configurations to select for to replace those cells determined to be undesirable, even if such cells could be detected with perfect accuracy.

Genetic redundancies are likely able to temporarily compensate for the loss of mutual DNA information in an individual—essentially delaying a detectable aging phenotype to at least the age of reproductive maturity (Fig. 3a). A second line of defense is provided by innate mechanisms that identify specific types of cellular dysfunction and eliminate cells displaying those phenotypes (Zhou and Elledge, 2000). Once the utility of these redundancies is expended and ever-increasing numbers of compromised cells circumvent innate detection mechanisms, the individual will no longer be able to avoid a loss in viability. This resulting dysfunction becomes progressively worse with time. *As no existing, or theoretical, biological means has been demonstrated or postulated to be capable of selecting only for those changes in cellular DNA information that are neutral or beneficial to the individual, it is inevitable that all individual organisms must eventually age if they live long enough.* The claim by Hamilton (1966) that senescence arises inevitably due to declining selection pressure with age at the level of the species, while not challenged here, is redundant.

Additionally, no mechanism can prevent natural selection from occurring at the level of the individual cell[4]. Therefore, cancer is also inevitable in any individual organism given sufficient time—despite the fact that cancer has yet to be detected in a small number of studied species. (The naked mole-rat has long been considered one such species. This was challenged in two recent articles that reported cancer in the naked mole-rat for the first time (Delaney et al., 2016; Taylor et al., 2016).)

5.2 In the Species

Due to gamete quality-enhancement mechanisms (Bailly and Gartner, 2013; Jaramillo-Lambert et al., 2010), the total mutual DNA information loss suffered between a gamete and the zygote that gave rise to the individual that produced the gamete will generally be less than the loss in somatic cells of an individual (at the age of reproductive maturity). By limiting the mutual DNA information loss in the gamete to a level low enough that selection for neutral and higher fitness offspring is possible, species fitness is preserved and can also increase. Natural selection is the only mechanism preventing inevitable mutual DNA information losses from generating mandatory reductions in species fitness.

[4] Or from selection occurring on a subcellular level amongst DNA-containing organelles (mitochondria and chloroplasts)

Since evolving mandates genomic changes, conditions where selective pressures are changing, or have recently changed, can lead to a rate of mutual DNA loss in the species that is greater than the average mutual DNA information loss between parent and offspring, $\overline{I}_{DNA}(zyg; gametes)$. Fluctuating selective pressures prevent the minimization of mutual DNA information loss.

Consider, however, what would happen if selective pressures were held constant. The rate of mutual DNA information loss will take some initial value as species fitness increases (Fig. 3c). Through the course of many generations, fewer configurations will be available that are capable of producing higher fitness than the current configuration. As a result, the rate of mutual DNA information loss eventually decreases and the rate of fitness increase is reduced. With fewer "positive" mutations available and selection tending to eliminate "negative" mutations, the rate of mutual DNA information loss in a species under static selective pressure could become less than $\overline{I}_{DNA}(zyg; gametes)$. Fitness would eventually approach a theoretical limit as the rate of mutual DNA information loss approaches zero (Fig. 3c).

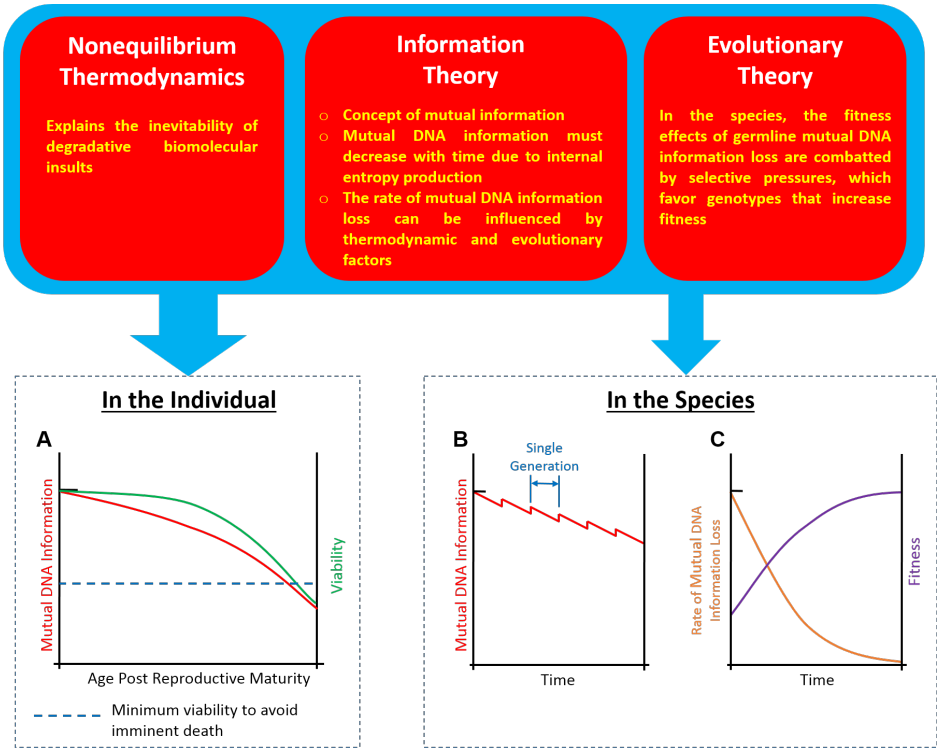

Fig. 3. The proposed connection between thermodynamics, information, and evolutionary theory in generating mandatory mutual DNA information losses in both the individual and the species. (a) Although a correlation between individual viability and somatic cell mutual DNA information loss is expected, genetic redundancies and other compensating mechanisms may attenuate reductions in individual viability due to mutual DNA information losses. (b) The average mutual DNA information in cells of all individuals of a species will decline as a generation ages. This loss can be largely reverted in subsequent generations by sequestering germ cells in conditions optimized for preservation of genetic data. (Generations are aligned for illustration purposes.) (c) In conditions of static selective pressure, the rate of mutual DNA information loss will decrease as fitness approaches a maximum value.

Since conditions of perfectly static selective pressure are not realizable and variations in selective pressures result in adaptive genetic changes, species mutual DNA information (as measured relative to a common ancestor) must trend downwards (Fig. 3b). This logic establishes a correspondence between the directionality of the second law and mutual DNA information loss in both individuals of a species and species themselves.

6 Examining Degradation Increases in Aging Organisms

Living organisms are highly ordered entities existing in thermodynamic nonequilibrium, leading to internal entropy production and ongoing molecular damage. Cellular mechanisms work towards counteracting this damage, coming close to establishing a steady state in terms of preservation of biomolecular integrity within a limited time window. Despite these efforts, the overall degradation state (i.e. the total entropy) of an individual organism increases with age.

6.1 Energetic Expenditures towards Biomolecular Repair and Replacement – a Paradox?

Proteins account for the majority of biomolecules within a cell. The rate of total protein synthesis has been empirically determined for a number of species (Table 1). Smaller organisms synthesize proteins at higher rates than larger species (Fig. 4a). Protein synthesis takes place at a rate sufficient to replace total body protein mass approximately every 5.3 days in mice, while a human requires 39.3 days. Of course, individual protein turnover rates can vary widely protein-to-protein, ranging from minutes to years (Hetzer, 2013).

Over the course of a lifetime, a long-living human will synthesize enough protein to replace total body protein mass 1137 times over (Table 1). Let us assume for the moment that degraded/dysfunctional proteins are "accumulating" as an individual grows older due to insufficient energy expenditure on repair and replacement, as suggested by the disposable soma theory of aging (Kirkwood, 1977;

Table 1. Protein Synthesis Rates, Number of Days to Turnover Total Body Protein Mass, and Number of Turnovers per Lifespan for Different Metazoan Species

Species	BW (kg)	Protein synthesis			Body Protein Composition (%)	Days to Replace Total Body Protein Mass	Maximum Lifespan (years)[10]	Turnovers Per Life
		g/day	g/kg BW per day	g/kg$^{0.75}$ BW per day				
Honey possum	0.010	0.280[1]	29.1	9.1	(17.0)†	6.9	2.0	106
Mouse, small	0.020	0.768[2]	38.4	14.4	20.3[7]	5.3	4.0	277
Rat	0.35	7.7[3]	22.0	16.9	20.8[7]	9.5	3.8	147
Rabbit	3.6	33.1[3]	9.2	12.7	19.4[7]	21.1	9.0	155
Cat (HP)	4.8	31.4[4]	6.5	9.7	21.8[7]	33.3	30.0	328
Dog	10.2	123.4[5]	12.1	21.6	22.1[7]	18.2	24.0	480
Sheep	63	351[3]	5.6	15.7	16.0[8]	28.7	22.8	290
Man	67	245[6]	3.7	10.5	14.4[9]	39.3	122.5	1137
Cow	575	1740[3]	3.0	14.8	22.5[7]	74.4	20.0	98

†Body protein concentration for honey possum not available, used 17.0% for "days to turnover" calculation. [1]Bradshaw and Bradshaw, 2009. [2]Garlick and Marshall, 1972. [3]Reeds and Harris, 1981. [4]Russell et al., 2003. [5]Everett et al., 1977. [6]Pacy et al., 1994. [7]Moulton, 1923. [8]Reid et al., 1968. [9]Mitchell et al., 1945. [10]Tacuta et al., 2012.

Kirkwood and Holliday, 1979; Kirkwood and Rose, 1991). Considering a worst-case scenario where all protein in an aged human is in need of replacement, it would only require an estimated 0.09% increase in daily resource investment in protein synthesis to offset the average daily increase in degraded protein. This translates to 0.23 calories per day[5]. With a daily dietary intake of 2500 calories, this is only 0.0092% of daily energy intake. Although this figure does not include the energetic repair and replacement costs for all classes of biomolecules, the total amount of protein dedicated to translation is 2-15 times greater than that dedicated to transcription and DNA maintenance (Liebermeister et al., 2014); protein synthesis represents a significant fraction of the total energy spent by an organism on biomolecular repair and replacement. In light of the very small additional investment needed to offset an increase in protein degradation, the disposable soma theory's claim that aging is caused by an energetic underinvestment in repair and maintenance that results in an accumulation of damage (Kirkwood, 1977; Kirkwood and Holliday, 1979; Kirkwood and Rose, 1991) is difficult to accept. In addition, organisms that turnover their proteins more frequently exhibit decreased longevity—not increased (Fig. 4b). Smaller organisms turnover protein at a faster rate than larger organisms (Fig. 4a) and have reduced longevity (Austad, 2005; Calder, 1984; de Magalhães et al., 2007).

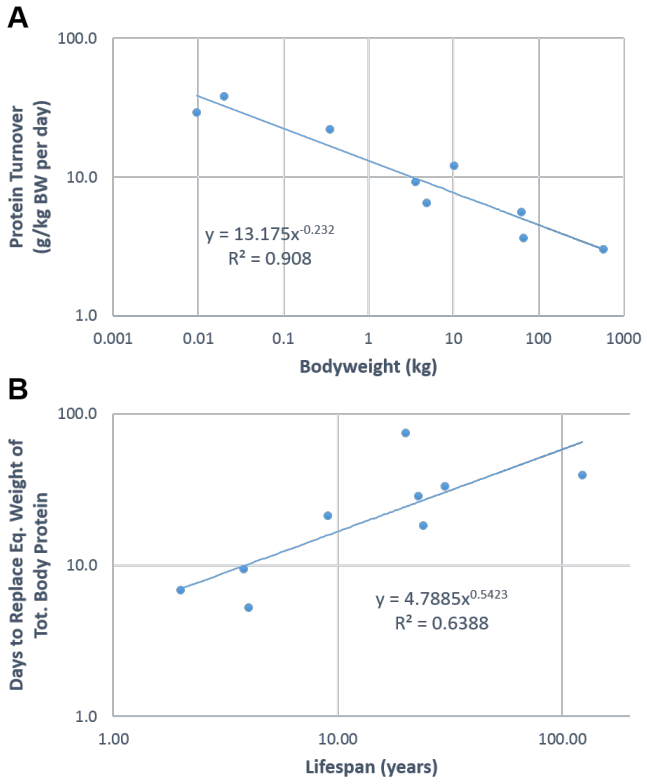

Fig. 4. (a) Protein turnover as a function of bodyweight for the species listed in Table 1 and (b) days to replace the equivalent weight of total body protein as a function of MLSP. Data from Table 1.

Caloric restriction extends longevity in some organisms. This also contradicts the disposable soma theory. Proponents of the disposable soma theory have attempted to explain this "paradox" by suggesting that caloric restriction generates a shift in resources away from reproduction and towards somatic

[5] Protein synthesis requires approximately 4.5 kJ of energy per gram of protein (Waterlow, 2006, p.170). Producing 245 g/day of protein (human rate) equates to roughly 1103 kJ or 264 Cal per day; 0.09% of this value is 0.23 Cals per day.

maintenance (Shanley and Kirkwood, 2000). While caloric restriction attenuates the age-associated reduction of protein synthesis in muscle tissue (Zangarelli et al., 2006), caloric restriction does not produce a direct increase in the rate of mixed protein synthesis (Miller et al., 2013) nor is mitochondrial protein synthesis significantly affected by caloric restriction in liver, heart, or skeletal muscle (Miller et al., 2011). Although it has been proposed that caloric restriction increases protein turnover (Tavernarakis and Driscoll, 2002), an examination of supporting data suggests that the decreased attenuation in protein synthesis with age resulting from extended caloric restriction was interpreted as an increase in protein turnover in direct response to caloric restriction—yet these are two distinct phenomena with very different implications.

A corollary of the disposable soma theory suggests that an increase in available energy would allow an organism to devote more resources to somatic maintenance and thus extend longevity. However, no studies exist demonstrating that increased caloric intake extends longevity—while it is well-known that obesity leads to decreased longevity and diabetes (Ahima, 2009). It is clear that energetic expenditures towards repair and replacement alone cannot explain the differences in longevity between species nor does it provide a solid rationale for why aging must occur in the first place.

6.2 Total Entropy Increases Slowly with Age in Comparison to Internal Entropy Production Rate

The high frequency at which the animals depicted in Table 1 replace their total body protein mass demonstrates that the rate of degradative internal entropy production d_iS/dt within an individual is much greater than the rate at which the total entropy of an individual organism increases with age dS_{age}/dt.

$$\frac{dS_{age}}{dt} \ll \frac{d_iS}{dt} \qquad (21)$$

Eq. *(21)* is intuitively evident when the speed at which biological material degrades at biologically relevant temperatures, even when conditions are sterile and optimized, is contrasted against the time that organisms with even moderate longevity are alive. Nevertheless, the global degradation state of an individual organism eventually worsens with time. The degradation process could be viewed as a progression through many discrete steady-state nonequilibrium conditions that ultimately result in a global degradation state that renders the individual nonviable. Why do organisms transition between these states and why is youthful homeostasis lost? DNA molecules face inevitable losses in mutual DNA information as an individual ages. This may explain why this transition occurs in DNA molecules—yet if they are the only class of biomolecule in an organism directly subject to inevitable, irreversible loss then the reasons why other classes of biomolecules reach elevated degradation states with age must be more complex.

6.3 Biomolecular Degradation - Accumulation versus Homeostatic Shifts

A closer look at the increased degradation exhibited in other classes of biomolecules provide some clues. Aging has been described as the accumulation of unrepaired damage. This implies that all of the biomolecular degradation in an aged individual results from lifelong accumulation. Perhaps this assessment is not entirely accurate.

As demonstrated earlier, a biomolecular ensemble can never be sustained at a degradation state of zero as this would require infinite resources; thus even in a youthful state of “homeostasis” organisms exhibit some degree of biomolecular degradation. “Misrepair” is not required in order to have degraded biomolecules as degradative internal entropy production is present in all living organisms. A reduction in biomolecular replacement/repair rate will increase degradation state and reduce average biomolecular performance. Should such a transition occur in an organism, a new degradation state would eventually be established at which time no further reductions in biomolecular performance should occur unless outside factors are at play.

This logic suggests that biomolecular replacement and repair rates alone cannot explain how an accumulation of damaged biomolecules would occur. Restoration of proteasome function in aged human dermal primary fibroblasts largely restores markers of protein aging to youthful levels (Hwang et al., 2007). This is analogous to a shift in the protein pool from a higher to a lower degradation state, and this demonstrates that the increase in degradation state occurring with age may be at least partially reversible. If the degraded protein was truly representative of accumulated, irreparable damage then upregulation of proteasomal function should not eliminate any damage. The fact that the condition is essentially reversible suggests that the increased biomolecular degradation found in aged individuals is not due to damage “accumulation” but is more likely attributable to reduced biomolecular turnover leading to a

565 corresponding shift in biomolecular degradation state[6]. Consistent with this notion, protein turnover does indeed significantly decline
566 during aging (Rattan, 1996; Richardson and Cheung, 1982; Ryazanov and Nefsky, 2001).

567 A distinction between *accumulated* dysfunctional biomolecules and a shift in biomolecular degradation state caused by reduced
568 turnover can be made by examining whether turnover is occurring. "Damage" that is actively and continuously turned over should not
569 be referred to as accumulated damage, even if the rate of turnover has decreased and the degradation state of the biomolecular pool
570 is high.

571 This raises further doubt over the disposable soma theory's assertion that aging is caused by an energetic underinvestment in repair
572 and maintenance resulting in an accumulation of damage (Kirkwood, 1977; Kirkwood and Holliday, 1979; Kirkwood and Rose, 1991).
573 The idea of an "energetic underinvestment" is a misnomer, as no amount of energetic investment will produce a perfect population
574 of biomolecules (i.e. a degradation state of zero). Increasing biomolecular turnover will reduce biomolecular degradation state but
575 energetic resource ROI will continually worsen as turnover rate is increased. An energetic underinvestment in repair and replacement
576 cannot explain why youthful homeostasis is lost—there must be a higher-level initiating cause.

577 The fact that biomolecular degradation state is determined in part by resource allocation towards repair and replacement, and
578 therefore must involve factors that affect fitness, suggests that species have evolved to function at biomolecular degradation states
579 that balance many factors including athletic performance, metabolic rate, physical size and others. This alone does not provide direct
580 insight into why organisms age. However, the concept of biomolecular degradation states is useful when considered together with
581 the inevitability of mutual DNA information loss in helping to explain why youthful homeostasis cannot be indefinitely preserved.

582 ### 6.4 Entropy-Driven Managed Deterioration – Basic Concepts

583 The energetic cost of repairing the degraded
584 biomolecules in an aged individual once is
585 small relative to the continuous investment
586 made to sustain viable biomolecular
587 degradation states. So why do biomolecular
588 degradation states increase in an aging
589 individual?

590 Fig. 5 depicts the basic interrelationships that
591 may explain the progression of the aging
592 phenotype in many metazoans. In this model,
593 the key top-level factor initiating the
594 transition from youthful homeostasis is
595 internal entropy production, which inevitably
596 generates losses in mutual DNA information
597 for both mitochondrial and nuclear DNA.
598 Mutual DNA information losses in
599 mitochondrial DNA (mtDNA) are ubiquitous in
600 aged-mammals (Wallace, 1999) and lead to
601 lower peak energy output (Yaniv et al., 2013).
602 This decline in mutual mtDNA information is
603 partially modulated by a controlled
604 deceleration in mitochondrial biogenesis
605 (Figge et al., 2012), which reduces the rate of

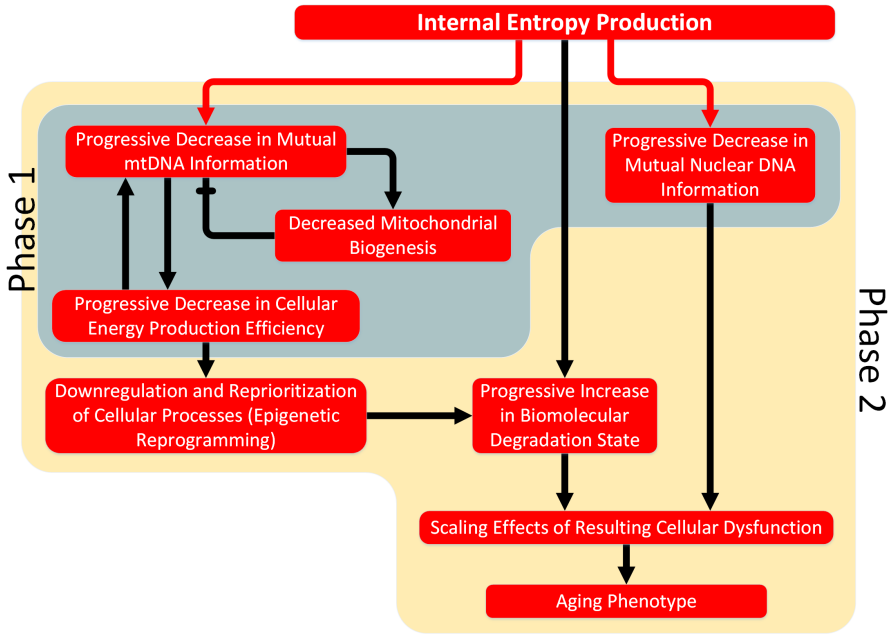

Fig. 5. The basic interrelationships between primary factors that may largely describe the progression of the aging phenotype in many metazoa. During 'Phase 1' of an individual's life, mutual DNA information loss has not reached levels sufficient to generate an aging phenotype. 'Phase 2' begins when dysfunction has progressed to the point that aspects of the aging phenotype begin to take hold.

[6] There are apparently a small number of biomolecules that can accumulate into dysfunctional products when an organism has aged; for example, advanced glycation end products (AGEs), amyloid beta and certain other aggregates (Verzijl et al., 2000). A global decline in biomolecular repair and replacement processes can produce biases leading to significant differences in repair and replacement rates between biomolecules. With infrequent turnover, the proportion of certain types of damaged product can expand, even when youthful turnover levels prevent accumulation. Superficially, this type of damage could be thought of as "accumulated". An example of this phenomenon was demonstrated by De Baets et al. (2011). There are no published data suggesting that this accumulation occurs under normal circumstances absent significantly decreased turnover such as that which occurs with advanced age. Many of the same protein species found to aggregate with age are produced, but concomitantly cleared, in younger individuals.

clonal expansion of degraded mtDNA and limits the exposure of mtDNA to the high thermodynamic stress conditions of replication events. The escalating deficit in cellular energy currency production equates to a progressively worsening inability to fund all cellular processes at youthful levels. This generates forced reductions in biomolecular turnover that increases biomolecular degradation state and lowers biomolecular performance—representative of a transition away from youthful homeostasis.

Losses in nuclear DNA fidelity produce a mosaic of stochastic cellular dysfunction that worsens with age (Bahar et al., 2006; Lodato et al., 2015). Together with the described mitochondrial dysfunction, this could largely explain age-linked cellular dysfunction and the overall aging phenotype of the organism. "Longevity optimization" genes may have evolved to attenuate the negative effects of mutual information losses in nuclear and mitochondrial DNA through reallocation of resources and physiological alterations. This model is discussed in more detail in section 8.

7 Longevity Determination

Leonard Hayflick has stated that aging is not driven by genes but by thermodynamics (Hayflick, 2004), while he has argued that the genome does, on the other hand, govern longevity. Additionally, Hayflick maintains that natural selection has led towards biomolecular arrangements that are capable of preserving fidelity until reproductive maturity, but that the survival value for arrangements exceeding this longevity is considerably diminished (Hayflick, 2007a).

If aging is driven by thermodynamics, as suggested by Hayflick and further supported here, then any and all factors that contribute towards resisting or promoting permanent thermodynamically-induced changes in any biocomponent subject to irreversible loss are implicated in longevity determination. This includes factors that directly or indirectly affect the magnitude of the thermodynamic stresses on these biostructures as well as factors that specify redundancy levels, which can offer varying degrees of protection from permanent information loss.

7.1 Investigating the Rate of Mutual DNA Information Loss in Individuals

The loss of mutual DNA information is inevitable in any individual given sufficient time. If such loss is paramount to aging, then a closer examination of the thermodynamics affecting DNA molecules is warranted and may assist in identifying primary longevity determinants.

Since DNA undergoing replication is significantly more likely to incur a mutation due to the impaired stability of single-stranded DNA (Frederico et al., 1990) and given the imperfect nature of DNA polymerases (Arias-Gonzalez, 2012), replicating and non-replicating conditions should be considered independently. Damage detection and repair systems correct or eliminate many DNA insults but a certain percentage avoid detection. Fig. 6 depicts a systems flow diagram of this arrangement. DNA actively being transcribed is also more susceptible to mutation (Kim and Jinks-Robertson, 2012), but it is generally believed that the vast majority of mutations arise from replication events or random DNA damage. For this reason, transcription is not considered as a separate state condition in this analysis.

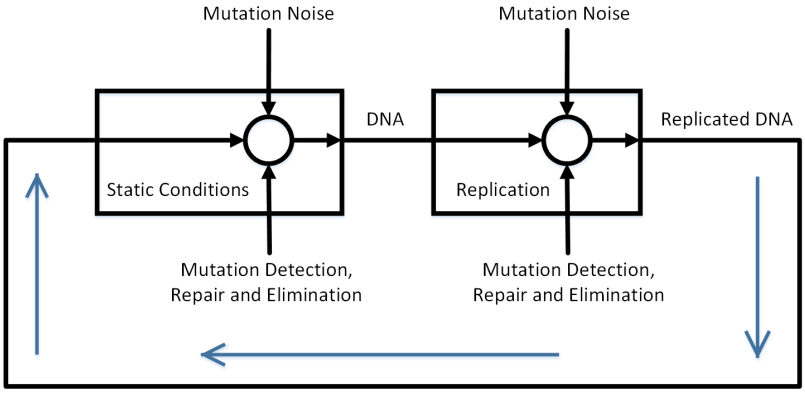

Fig. 6. A systems flow diagram of mutual information loss in a DNA ensemble within a living organism.

Assuming that the time spent in the replicative state is comparatively much less than the time in the static state, a general representation of the average rate of mutual DNA information loss takes the form

$$\overline{MIR}_{DNA} = -L_{DNA}\left(k_{rep}r_{rep}m_{rep} + k_{static}r_{mut,static}\right)(1 - p_{det}) \qquad (22)$$

where L_{DNA} is the length of the DNA molecule in base pairs, k_{rep} is the amount of mutual information lost in the average mutation event during replication and k_{static} is the same for static (non-replicating) conditions, r_{rep} is the DNA replication rate, m_{rep} is the length-specific incidence of mutation during replication, p_{det} is the probability that a mutation will be detected and eliminated by the cell, and $r_{mut,static}$ is the length-specific rate at which mutations occur in non-replicating conditions.

7.2 Preserving mtDNA Integrity

Most eukaryotic cells contain mitochondria, ranging from several hundred to thousands per cell. Each mitochondrion contains at least one copy of mtDNA. Compared to the nuclear genome, the mitochondrial genome is more susceptible to mutation (Larsson, 2010) and these mutations are more likely to cause dysfunction. The mitochondrial genome is replicated during mitobiogenesis, which is required for preservation of a healthy pool of mitochondria (i.e. a low degradation state). In any given cell, the mitochondrial pool will be maintained at relatively steady quantities by a combination of mitochondrial fusion, fission, mitophagy, and mitobiogenesis processes; this results in mtDNA replication rates that are very high compared to the rate at which nuclear DNA replicates. Due to the imperfect fidelity of replication with DNA polymerase (Zheng et al., 2006) and the vulnerability of the single-stranded mtDNA replicon (Frederico et al., 1990), each replication event involves a period of time where the possibility for a mutation is considerably higher than non-replicating conditions (Kennedy et al., 2013). The microenvironment within a mitochondrion is also particularly harsh compared to other cellular compartments due to the relatively high concentrations of ROS (Wallace, 1999), resulting in larger internal entropy-producing thermodynamic potentials and higher molecular insult rates. Furthermore, as the mitochondrial genome has evolved to be extremely compact, mitochondria are very susceptible to dysfunction resulting from single-base alterations.

The only known human mtDNA polymerase, DNA polymerase γ, is highly conserved across species as diverse as *Drosophila melanogaster* and *Saccharomyces cerevisiae* (Chan and Copeland, 2009), as are the GTPases implicated in mitochondrial fission and fusion (Ashrafi and Schwarz, 2012). Nuclear DNA repair pathways are also highly conserved (Gredilla et al., 2010); mitochondria possess many of the same repair mechanisms and share some of the nuclear DNA repair enzymes. PTEN-induced putative kinase protein 1 (PINK1) and the E3 ubiquitin ligase parkin regulate mitophagy in many metazoans and have homologs across species as diverse as humans and *Drosophila melanogaster* (Cookson, 2012). These similarities suggest that the length-specific frequency of a mutation event during mtDNA replication m_{rep} and the probability that a mutated mtDNA molecule is detected and eliminated or repaired p_{det} are comparable across a wide range of species.

In addition, since the molecular configuration of DNA is conserved, as are the potential reactions that can result in molecular modifications, it follows that the mutual information lost in the average mutation-causing event is constant; i.e. k_{rep} and k_{static} should be similar across species. This leaves the mtDNA replication rate r_{rep} and the static-condition mutation rate $r_{mut,static}$ as the likely primary factors from Eq. *(22)* responsible for any variation in the rate of mutual mtDNA information loss between species.

7.3 MtDNA Information Loss in Aged Organisms Primarily Results from Replication

MtDNA mutations increase in an age-dependent manner. High-sensitivity sequencing of human brain tissue from young and old individuals found that most mtDNA point mutations are transition mutations (Kennedy et al., 2011), consistent with replication errors. In addition, 90% of all age-related mutations in mtDNA from human colon are transitions (Greaves et al., 2012). The mtDNA mutation burden in aged *Drosophila melanogaster* is similar to vertebrate levels and also demonstrates a prevalence of transition mutations (Itsara et al., 2014). G:C to T:A transversions, which are typical of oxidative damage, only represented a small percentage of the mutations in these studies.

MtDNA mutation patterns display strand asymmetry consistent with spontaneous cytosine deamination on the lagging strand template during replication (Frederico et al., 1990) in both aged human brain (Kennedy et al., 2011) and aged somatic and germline cells of *Drosophila melanogaster* (Haag-Liautard et al., 2008; Itsara et al., 2014). Mitochondrial mutational spectra produced with purified human DNA polymerase γ accounted for 83% of the mutations found *in vivo* (Zheng et al., 2006). These data strongly suggest that: 1) the vast majority of mutations in mtDNA result from errors during replication; 2) the rate of mutual mtDNA information loss varies across species but total losses are similar at equivalent aged states; 3) oxidatively damaged mtDNA is repaired or eliminated with very high efficiency; and 4) oxidatively damaged mtDNA accounts for only a small percentage of mtDNA mutations occurring with age. These results are inconsistent with theories that implicate ROS levels and the resulting direct oxidative damage to DNA as a primary causative factor in aging.

A logical deduction from this is that mtDNA replication rate is higher in shorter-living animals. Unfortunately, the availability of data to support or refute this assertion is limited. Measuring the mtDNA turnover rate *in vivo* has historically proven difficult, although more recent techniques have overcome some of the issues (Collins et al., 2003). Primary cell cultures are required for deriving accurate mtDNA replication rates *in vitro*. Surprisingly, no studies quantitating mtDNA replication rates across a range of species have been published.

Mitobiogenesis is required to maintain mitochondrial component quality. Reducing mitobiogenesis excessively will compromise mitochondrial performance since less negative entropy is produced for counteracting degradative internal entropy production

(affecting mitochondrial components other than mtDNA), resulting in a shift to a higher degradation state. Since mitobiogenesis incorporates mtDNA replication, a decline in mitobiogenesis will reduce mtDNA replication rate r_{rep}. Thus, although reduced mitobiogenesis may negatively impact mitochondrial performance, it will lead to a lower rate of mutual mtDNA information loss per Eq. *(22)*.

On the other hand, with increased mitobiogenesis more negative entropy is available for offsetting degradative internal entropy production, effectively lowering mitochondrial degradation state. However, this will also raise the mtDNA replication rate $k_{rep,mtDNA}$ and generate increased exposure of mtDNA to the high thermodynamic-stress conditions experienced during replication—resulting in an increase in the rate of mutual mtDNA information loss.

Preservation of youthful mitochondrial homeostasis requires that the rate of negative entropy production from mitobiogenesis equals or exceeds the rate of degradative internal entropy produced within the mitochondrial network when in a youthful degradation state. If the rate of degradative internal entropy production within mitochondria varies between species, then the rate of mitobiogenesis required to preserve youthful homeostasis in mitochondrial components is also likely to vary. In addition, differences in the intrinsic mitochondrial degradation state between species could affect the rate of mitobiogenesis. This suggests that variations in the rate of mutual mtDNA information loss between species are likely due to differences in the rate of degradative internal entropy production within mitochondria and/or different mitochondrial degradation states.

7.4 A Closer Look at Mitochondrial Configurations and Membrane Composition

Since mitobiogenesis encompasses mtDNA replication—which accelerates losses in mutual mtDNA information—forfeiture of youthful mitochondrial homeostasis is not only inevitable but must occur after a period of time dictated, at least in part, by the rate of mitobiogenesis. How then might this rate differ by species, and why?

Examining cellular metabolic demands provides some clues. Across species, whole-organism basal metabolic rate scales allometrically with body mass: $BMR \propto M_b^f$ (Kleiber, 1932; Niklas, 1994; Peters, 1986).[7] Resting oxygen consumption expressed per unit body mass scales proportionally with $M_b^{-1/4}$. In other words, mass-specific BMR decreases by approximately 16% across species for every doubling of body mass. The inverse correlation between relative oxygen consumption and body mass has been verified in isolated hepatocytes from mammals (Porter and Brand, 1995) and birds (Else, 2004) as well as in mammalian liver slices (Couture and Hulbert, 1995). Porter and Brand (1995) found a 5.5-fold decrease in hepatocyte oxygen consumption for every 12,500-fold increase in body mass.

On average, cells from smaller species have increased oxygen consumption and ATP turnover rates compared to cells from larger organisms. As a result, cells from smaller species place greater energetic demands on their mitochondrial networks. Mitochondrial count correlates with mass-specific changes in tissue metabolic rate (Smith, 1956). However, the differences in mitochondrial number per cell cannot fully explain the variation in respiration rate with body mass (Porter and Brand, 1995).

Increasing the mitochondrial inner membrane surface area per unit volume of mitochondrial matrix allows for additional transmembrane-localized oxidative phosphorylation enzymatic machinery in the same volume of space. Organisms with higher ATP demands may benefit from increased membrane density. Indeed, a significant negative correlation has been found between mitochondrial inner membrane surface area per unit volume of matrix and body mass (Porter et al., 1996).

Mitochondrial membrane phospholipid composition also differs widely across species, particularly in fatty acid composition (Daum, 1985). Smaller mammals have mitochondrial membranes that are more polyunsaturated than larger mammals (Porter et al., 1996). Some light was shed on why membrane fatty acid composition varies allometry when the molecular activity of transmembrane proteins was examined in different membrane compositions. The cytoplasmic membrane-localized sodium pump (Na^+·K^+-ATPase) varies in molecular activity from approximately 8,000 ATP/min in mammals compared to 2,500 ATP/min in ectotherms (all data taken at 37°C) (Else et al., 1996). Cytoplasmic membrane crossover studies demonstrated that the activity of ectothermic sodium pumps increased significantly when transferred to mammalian membranes, while mammalian sodium pump activity was attenuated in ectothermic membranes (Else and Wu, 1999).

The higher sodium pump activities in endotherms were hypothesized to be due to influences from surrounding lipids, with polyunsaturated membranes promoting increased molecular activity compared to membranes with more monounsaturated

[7] The universal value of f is contentious. Scientists are largely divided into two camps: one arguing for 2/3 and the other for 3/4 (White and Seymour, 2005).

membranes. Hulbert and Else (1999) proposed a mechanism by which this may occur: The lateral diffusion coefficient of lipids within a membrane bilayer is greater than that of transmembrane proteins by two orders of magnitude (Storch and Kleinfeld, 1985). As such, membrane proteins are continuously colliding with membrane lipids. The kinetic energy exchanged during these collisions is believed to be critical in facilitating membrane protein function. The acyl chains of saturated and monounsaturated fatty acids are more flexible than polyunsaturated fatty acids. Therefore, collisions involving lipids containing polyunsaturated fatty acids transfer more energy to membrane proteins and result in higher protein activity than collisions with lipids with only highly saturated fats. Of the fatty acids found in membrane lipids, docosahexanoic acid (DHA or 22:6 n-3) contains the largest number of evenly spaced double bonds but is also particularly susceptible to peroxidation. DHA has been referred to as the "acme" of polyunsaturates and may serve as a membrane "energizer" (Hulbert and Else, 1999). Sodium pump molecular activity correlates with membrane DHA concentration in both ectotherms (Turner et al., 2005) and endotherms (Turner et al., 2003).

Peroxidation index (PI) is a measure of the susceptibility of membrane lipids to peroxidation and is closely tied to fatty acid unsaturation. The PI of mitochondrial phospholipids, predominantly driven by DHA content, negatively correlates with MLSP (Pamplona et al., 1998). Importantly, the same trend line holds for both mammals and birds (Hulbert et al., 2007). In addition, mitochondrial membrane remodeling resulting from various levels of caloric restriction in mice produced changes in PI and MLSP that fit the same trend line (Faulks et al., 2006; Hulbert, 2008).

In addition to the negative allometry of metabolic rate, body mass positively correlates with MLSP (Austad, 2005; Calder, 1984; de Magalhães et al., 2007). The discussed findings suggest that smaller organisms with reduced longevity may utilize membranes with more polyunsaturated membranes—largely dictated by DHA content—in order to increase the rate of work that can be performed by each transmembrane protein molecule and to satisfy functional requirements largely specified by recognized allometric relationships that characterize fitness optimization across species. A downside of polyunsaturated fatty acids is their susceptibility to oxidative damage and contribution towards increased free radical generation. In other words, polyunsaturated fatty acids are less resistant to molecular alterations resulting from the thermodynamic forces of their environment; the presence of higher levels of polyunsaturated fatty acids will lead to increased rates of degradative internal entropy production within mitochondria and will necessitate that mitobiogenesis rates be increased to maintain a given mitochondrial degradation state.

7.5 Identifying Longevity Determinants

Depicted in Fig. 7 is a concept explaining how a longevity determining effect could arise from the influence of membrane composition on biomolecular turnover rate, metabolism, and ultimately the rate of loss of mutual DNA information. I postulate that an organism's peak biological power density largely stipulates membrane composition and other defining characteristics of an organism. Here the term "peak biological power density" represents the maximum localized volume-specific rate of external work (power per unit volume) achievable within an organism. The cells or tissues where this potentiality exists may vary by species (for example, skeletal muscle in some organisms, neurons in others, etc.). "External work", in the context of peak biological power density, refers to the sum of the biomechanical, biochemical and bioelectrical work that is brought to bear on the immediate environment surrounding the localized region where this work originates. Examples include the mechanical work generated by myocytes, the chemical and electrical work produced by neurons, and the chemical work performed on metabolized products by hepatocytes. External work does not include work that is associated with housekeeping or "overhead" cellular processes such as biomolecular repair and replacement, maintenance of baseline membrane potentials or mitotic cell turnover.

To illustrate the sequence of interactions implicated in this theory, we will consider an arbitrary organism in which maximum fitness is achieved by a high level of peak biological power density compared to some reference organism (Fig. 7). This implies an increase in the maximum potentiality for external work rate per unit volume that is achievable by some cell, or group of cells, within the organism. To maximize the rate of work attainable from a given volume, the molecule-specific rate of work of the proteins must be as high as possible. One requirement for achieving this is to optimize the structure of the protein for peak work rate, **1** (bold numbers in this section refer to Fig. 7). This is likely to reduce biomolecular durability and resiliency (due to lower selective pressure on these parameters), and protein repair/replacement rate (turnover) may increase as a result. Secondly, the biomolecular performance of the protein pool should be maintained at a high level (i.e. degradation state should be low), **2**. In this way, the work contribution of the average protein molecule will be closer to the theoretical maximum. Maintaining low degradation states will increase the rate of protein turnover and lead to a less-than-optimal energetic ROI.

Higher peak biological power density implies the ability to perform work at an increased rate per unit volume somewhere within an organism. It does not mean that the maximum work rate is achieved at all times. On the other hand, if the maximum were never

789 approached then there would be no need to possess this ability in the first place. For these reasons, it is reasonable to expect that
790 high-output proteins are likely to perform work at a higher rate, on average, than proteins from lower peak biological power density
791 configurations, **3**. At a minimum, the cell must have the ability to provide sufficient energetic resources for these high-output proteins
792 even if peaks levels are only attained sporadically. This equates to a requirement for more usable energy per unit time (i.e. higher ATP
793 turnover)—i.e. increased metabolism.

794 To realize a maximal rate of work, high-output proteins must spend more time performing work and less time in a resting state. The
795 state of actively performing work involves the transfer of energy and conditions of higher thermodynamic potentials compared to the
796 non-working (resting) state. Thus, degradative internal entropy production is likely to be elevated with high-output proteins, resulting
797 in increased protein turnover and further contributing towards increased metabolism, **4**. This is consistent with the increased
798 metabolic rates found in smaller animals and the fact that smaller animals turnover protein at faster rates compared to larger species
799 (Fig. 4).

800 Transmembrane proteins have increased activity in membranes with lipids containing higher polyunsaturated fatty (DHA) content
801 (Else and Wu, 1999; Turner et al., 2003; 2005). Configurations that call for a high level of peak biological power density will likely
802 include these type of membranes, which are more susceptible to lipid peroxidation, **5**. This contributes towards an elevated rate of

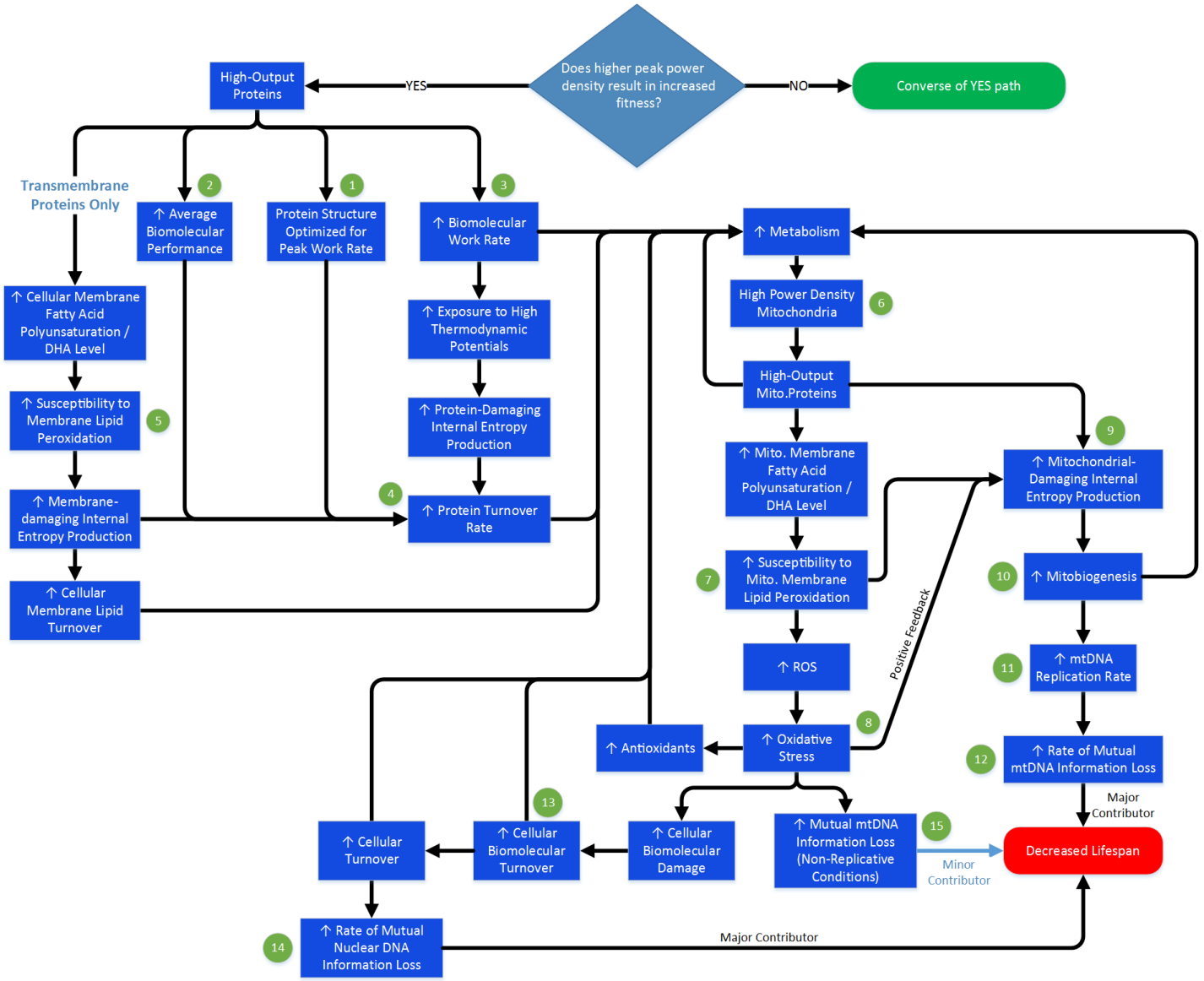

Fig. 7. A theoretical means by which an organism's peak biological power density may influence longevity. Bold numbers in section 7.5 text refer to this figure.

membrane-damaging internal entropy production that generates more frequent membrane lipid turnover and further increases in transmembrane protein turnover rate.

The aforementioned increased metabolic requirements generate a need for mitochondrial networks capable of satisfying these higher ATP turnover demands. This is realized with high power density mitochondria, **6**, the characteristics of which are outlined in Fig. 8. High-output mitochondrial proteins optimized for maximal ATP production are expected with these configurations. Similar to their cytoplasmic counterparts, high-output mitochondrial proteins are more susceptible to degradation and will further increase metabolism. Higher mitochondrial membrane polyunsaturated fatty acid (DHA) content will allow for increased peak ATP output through enhanced transmembrane protein activity but cause mitochondrial membranes to be more susceptible to peroxidation, **7**. Together with a positive feedback effect from elevated ROS and oxidative stress levels, **8**, this will increase mitochondrial-damaging internal entropy production, **9**. A higher rate of offsetting negative entropy production will be required in order to maintain mitochondrial quality and preserve youthful homeostasis. This need can only be realized through an upregulation of mitobiogenesis, which increases the mitochondrial membrane remodeling and protein turnover rate, **10**, but will coincide with a higher mtDNA replication rate, **11**, and an increased rate of mutual mtDNA information loss, **12**. As mtDNA integrity declines with age, the ability to produce usable energy will become compromised and worsen progressively. This will generate a downregulation of cellular processes which could largely be responsible for the aging phenotype.

Increased oxidative stress in high power-density mitochondrial configurations will likely elevate thermodynamic potentials in other parts of the cell, **13**. This and the other aforementioned contributors to increased biomolecular damage and turnover rates could be expected to increase the rate of cellular turnover. The rate of mutual nuclear DNA information loss will be heightened due to elevated replication rates, increasing the rate at which viable stem cells are depleted, **14**. Increased oxidative stress may also influence the rate of non-replicative mtDNA mutation, **15**. However, due to reasons already discussed, this contribution is probably small compared to the effects from the increased replication rate.

The loss of mutual DNA information in the individual is unavoidable. Notably, the logic established here describes how the rate of loss of mutual DNA information may be a function of an organism's peak biological power density requirements, at least in part. As this rate may be critical in determining the amount of time that passes before youthful homeostasis can no longer be sustained, a potential link is herein established between an organism's peak biological power density and longevity; by this token, peak biological power density could be thought of as a high-level longevity determinant.

7.6 The Naked Mole-rat Paradox – Part II

We can now propose a solution for the second half of the naked mole-rat paradox discussed earlier. Naked mole-rats live in very hypoxic environments and thus must function at extremely low metabolic rates. This necessitates low-output proteins, as high-output proteins have increased metabolic requirements for a number of reasons (Fig. 7). The situation is therefore the reverse of the high peak biological power density scenario previously discussed. Lower metabolism will lead to mitochondria better optimized for stability (Fig. 8). Consistent with this notion, naked mole-rats have 1/9th the content of DHA in their mitochondrial membranes compared to their similarly-sized cousin the house mouse (Mitchell et al., 2007). Decreased susceptibility to lipid peroxidation lowers the rate of damaging internal entropy production, mitobiogenesis, and mutual mtDNA information loss. Cellular turnover and the rate of mutual nuclear DNA information loss will also decrease. The lower rate of mutual DNA information loss increases the amount of time that passes before youthful homeostasis is lost due to transitions to higher degradation states. As a result, the naked mole-rat exhibits exceptional longevity for its size. The increased level of oxidative damage does not limit longevity as it is not the factor forcing a shift from youthful homeostasis but is merely indicative of the high degradation states that coincide with prioritizing energetic ROI for maximizing

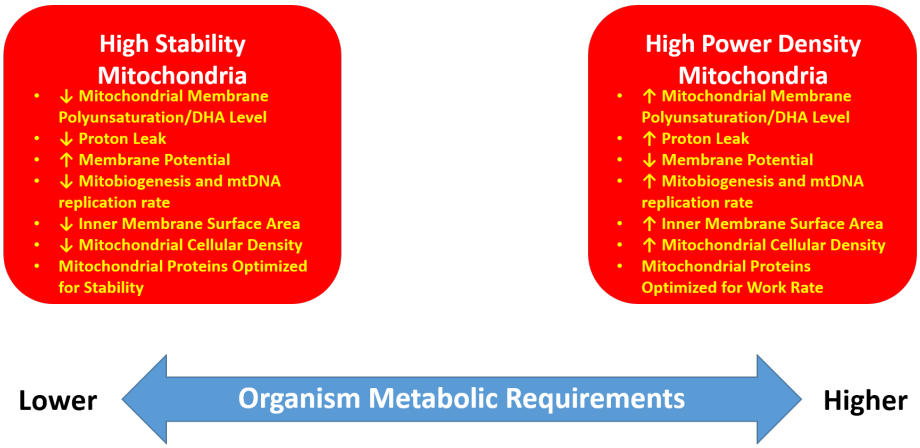

Fig. 8. The characteristics of high stability mitochondria compared to mitochondria optimized for peak biological power density. The requirements of the organism dictate where a particular species falls within the range of configurations between these two extremes.

852 evolutionary fitness in very hypoxic conditions. I postulate that the exceptional longevity of the naked mole-rat is primarily a byproduct
853 of the aforementioned requirement for extremely low metabolic rate as opposed to direct selective pressure for extreme longevity.

854 7.7 Allometric Relationships Describe Peak Biological Power Density Trends that Largely Predict Longevity

855 If peak biological power density is a primary longevity determinant, then how and why does this vary by species? Do variations in peak
856 biological power density align with allometric trends? Some answers to these questions may arise from examining how an organism's
857 mass-specific energetic cost of transport (COT) is driven by certain factors. COT is a measure of the quantity of metabolic energy
858 required to move one unit mass of an organism over one unit distance. In terrestrial animals, COT negatively correlates with body size
859 (Reilly et al., 2007; Strang and Steudel, 1990; Taylor et al., 1982). The reasons for the increased locomotor costs in smaller terrestrial
860 organisms have been discussed in detail elsewhere, including Reilly et al. (2007), and Kilbourne and Hoffman (2013). We will briefly
861 examine some of the more significant causes here. Although the mass-specific metabolic energy consumed per stride remains constant
862 across large and small mammals at the same stride frequency, larger animals require fewer strides to cover an equivalent distance;
863 this at least partly explains the reduction in COT with increasing body size (Heglund and Taylor, 1988; Heglund et al., 1982; Kram and
864 Taylor, 1990). The effect is compounded by the fact that larger mammals have disproportionately longer limbs (positive allometry)
865 (Pontzer, 2007).

866 In general, smaller animals cannot simply decrease their top speeds to offset the increased COT and preserve a low metabolic rate
867 since they must be able to achieve speeds sufficient to evade larger predators. This is demonstrated by the fact that, although top
868 speed does increase with body mass in mammals (Garland, 1982), the allometric scaling factor can only partially counteract the
869 increased COT in smaller mammals. In other words, the rate of mass-specific metabolic energy consumed by smaller mammals to
870 achieve their top speed is greater than that of larger mammals.

871 Posture can also significantly affect COT (Biewener, 1989). Smaller terrestrial animals tend to have limbs that are more abducted and
872 flexed during movement (Reilly et al., 2007). Larger animals have more upright postures, which confers a mechanical advantage to
873 anti-gravity muscles. This means that smaller mammals have increased muscular energetic demands for counteracting the flexing
874 moment of the ground reaction force. Larger animals are also able to benefit more from elastic storage since the capacity to store
875 energy in tendons positively correlates with tendon cross-sectional area (Bennett et al., 1986; Biewener and Blickhan, 1988; Biewener
876 et al., 1981). Pendular savings can reduce the metabolic cost of locomotion and become increasingly relevant as body size increases
877 in erect animals—but are insignificant in smaller crouched animals (Reilly et al., 2007).

878 For the above reasons, smaller terrestrial animals have higher peak metabolic rates in their skeletal muscles and supporting organs
879 (heart, lungs, etc.). As skeletal muscle is the major contributor to non-resting metabolism, it should not be surprising that field
880 metabolic rate (FMR) scales with negative allometry (Nagy, 2005). This also suggests that peak biological power density is likely to
881 positively correlate with skeletal muscle metabolism.

882 Surface area scales as a function of body mass per the relation $A \propto M_b^{2/3}$. The exponent in this case is less than one, signifying that
883 the mass-specific capacity for heat exchange decreases as body size increases. Since no thermodynamic process is 100% efficient, a
884 portion of the energy utilized for metabolism is converted to heat. The efficiency of the oxidative phosphorylation machinery in
885 mitochondria is highly optimized and not a function of body mass, as indicated by the fact that ATP turnover per unit of consumed
886 oxygen does not change with body mass in mammals (Porter and Brand, 1995). Therefore, in the absence of other limiters, the
887 maximum sustainable metabolic rate will be lower in larger organisms due to their reduced relative capacity to shed metabolic waste
888 heat. This translates to higher theoretical peak biological power densities in smaller organisms. A converse effect of the surface area
889 to mass ratio limits the minimum attainable body size of endothermic amniotes: maintenance of a constant body temperature, below
890 a particular body size for a given set of environmental living conditions, will require an increasing proportion of metabolic energy as
891 body size decreases.

892 Another factor likely further increases metabolic requirements in smaller animals and limits the minimum attainable body size. West
893 and colleagues argued that metabolic rate scaling is constrained by characteristics of the circulatory system (and other fractal
894 networks) that can be explained by principles from fluid dynamics (West et al., 1997; West and Brown, 2005). As vessels become
895 smaller, viscosity leads to greater energy dissipation due to increased damping. In addition, at some critical minimum size vessels are
896 no longer able to benefit from impedance matching, which can greatly reduce the energy lost due to reflections in larger vessel branch
897 points. These energy-consuming effects play an ever-increasing role as body size decreases and narrow vessels predominate.

Although the allometric relationships between BMR/FMR and M_b, and longevity and M_b, at the species level are well established, these describe only general trends. Clearly, the described allometric relationships are not imposing a strict, specific value on peak biological power density, metabolic rate, or longevity for a species or individual. Rather, they describe median values, which suggests that these parameters must evolve within upper and lower bounds that are a function of body size, and that the optimal compromise between peak biological power density, longevity and body size for a given species must also fit within these general constraints. Deviations from these trends are expected. For example, a species living in an environment with low predatory pressure may receive a fitness benefit from sacrificing peak athletic performance for increased longevity. Suppose that in this case, it is not necessary for the organism to function anywhere near the metabolic limit dictated by its capacity for heat exchange to ensure high rates of survival and fecundity over its lifespan—it receives more fitness benefit from maintaining a reasonable level of fecundity over an increased lifespan than from a marginal decrease in predation over a shorter lifespan. Another example of an expected significant deviation from the median are situations where organisms utilize a specific behavioral tactic or enhanced cognitive capabilities to increase their survival odds in lieu of maximizing peak biological power density. Humans are the ultimate embodiment of such a strategy.

These generalized allometric relationships do not apply to individuals within a species. For example, larger individuals in many species, such as dogs (Speakman et al., 2003) typically have shorter lives than smaller individuals. This may be in part because longevity determination has evolved, and is genetically engrained, at the species level. In other words, the genetic elements that specify peak biological power density, membrane composition, biomolecular turnover rates, stem cell reserve levels, and other factors that contribute towards resisting (or promoting) permanent thermodynamically induced changes in biocomponents subject to irreversible losses are mostly preset within the genome of a species and do not vary significantly as a function of body size. It is not surprising that significant deviations from the median body size would result in a compromised individual—and that this would include decreased longevity.

8 Longevity Optimization

Once mitochondrial dysfunction has progressed to the point that resource deficits prevent the funding of all cellular processes at youthful levels and/or genetic redundancies are no longer able to compensate for losses in nuclear DNA fidelity, an aged phenotype must begin to take shape. It is reasonable to expect that the optimal allocation of resources for preserving maximal survival and fecundity in an aged individual would be different from the configuration used in young adulthood when adequate resources are available to fund all cellular processes. Factors most critical to immediate survival are of highest priority to the individual. Therefore, a genotype optimized for an aging individual would increasingly deprioritize less vital processes and biocomponents as useable energetic resource availability decreases so that biocomponents that are more critical remain in states adequate to sustain life, and survival potential/fecundity are maximized. Eventually, a state is reached in which even vital factors cannot be adequately sustained and the individual's overall condition becomes unconducive to continued life.

Could such an anti-aging strategy exist in multicellular organisms? A large number of genetic elements regulating pathways apparently related to longevity have been identified (ENCODE Project Consortium et al., 2007). Many scientists believe that these pathways are responsible for causing aging and for modulating the rate of aging between species (Austad, 2009; Holliday, 2010; Kirkwood, 2005; Vijg and Campisi, 2008). A proposed complementary hypothesis is that longer-living species have evolved to contain superior mechanisms and/or biomolecules for retarding senescence; some scientists believe that incorporation of these changes into shorter-living organisms could lead to delayed senescence in these other organisms as well.

I submit here an alternative theory proposing that a major function of the putative aging pathways is to optimize the inevitable process of aging such that individual longevity and fecundity are maximized. Contained within these pathways, genetic elements that I term "longevity optimizers" work together to elicit a balanced response to the unavoidable progression towards increasing levels of irreversible biomolecular fidelity loss.

To my knowledge, this concept has not been formally proposed previously. There are several likely reasons for this. Firstly, scientists do not generally acknowledge that aging is inevitable, regardless of genotype. Many popular aging theories (e.g. antagonistic pleiotropy, mutation accumulation, and disposable soma) utilize evolutionary concepts to explain the existence of aging and do not consider fundamental physical law as relevant to the issue. These theories claim that aging is not unavoidable but rather that it exists because it projects beneficial effects on species fitness in other ways (antagonistic pleiotropy, disposable soma) or that there is insufficient evolutionary pressure to eradicate aging (mutation accumulation). If fundamental physical law does not mandate biological aging, then there is no need for mechanisms or strategies to resist it or to optimize it.

Here, I have provided rationale and evidence for why biological aging is an inevitable consequence of fundamental physical law that evolution cannot overcome. If this is the case, then it is reasonable to propose the existence of evolved mechanisms to resist and optimize an organism's susceptibility to these effects in order to maximize fitness.

A counterargument is that aging optimizations are unlikely to evolve because selective pressures begin to decrease for ages beyond reproductive maturity. However, as the potential for loss of mutual DNA information begins at conception—not at reproductive maturity—this phenomenon must be suitably combatted at all life stages. In order to maximize fitness, organisms require strategies for preventing the loss of mutual DNA information from reaching detrimental levels and to best handle the mutual DNA information loss that has occurred.

8.1 Selective Pressures Favor Genotypes that Attenuate Increases in Mortality and Losses in Fecundity Occurring After Reproductive Maturity

In the absence of compensating mechanisms, somatic mutations and other forms of irreversible degradation to a necessary biocomponent (biomolecule, cell, tissue, etc.) will nearly always have neutral or negative effects on mortality rate and fecundity. Therefore, the integrative effect of the systemic degradation occurring with age must eventually result in negative repercussions for the individual.

In any individual aging organism, biocomponents susceptible to irreversible fidelity loss will be the first to incur shifts from their youthful homeostatic states; for most organisms with at least moderate longevity, this is likely to be DNA molecules. The continual loss of mutual DNA information must eventually force shifts in the degradation state of other biocomponents. The magnitude of any deleterious impact on individual instantaneous mortality rate and fecundity due to an increase in the degradation state of a biocomponent will vary depending on the function of the biocomponent and the extent of the shift in degradation state. One such strategy for maximizing survival rate and fecundity in these conditions is to minimize the degradation state, or failure likelihood, of biocomponents most critical to these parameters. We will examine whether such a strategy would be evolutionarily favored.

Hamilton (1966) exploited the Euler-Lotka equation (Euler, 1767; Fisher, 1930; Lotka and Sharpe, 1911) to derive a measure of fitness r from age-specific survival and fecundity rates.

$$\int_0^\infty e^{-rx} l(x) m(x) dx = 1 \qquad (23)$$

Here $l(x)$ represents survival up to age x and $m(x)$ is fecundity at age x. Using a similar framework, Fisher (1930) introduced the concept of age-specific reproductive value $v(x)$ with the following relation

$$v(x) = \int_x^\infty e^{-r(y-x)} \frac{l(y)}{l(x)} m(y) dy \qquad (24)$$

Fisher (1930) described reproductive value as a measure of the contribution of individuals of age x to the future ancestry of a population and stated that (p.27) "the direct action of natural selection must be proportional to this contribution". In other words, genotypes that maximize reproductive value for a given age are evolutionarily favored over those that produce a lower $v(x)$. Fisher also discussed why reproductive value typically increases before reaching an apex and then declines with age.

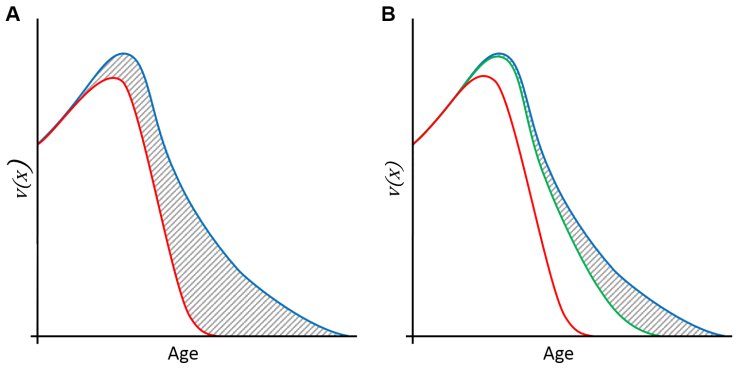

Fig. 9. Two scenarios of reproductive value curves for a hypothetical organism. (a) Optimization (blue curve) of the organismal response to irreversible losses in fidelity (as detailed in text) will provide a fitness advantage compared to a genotype lacking longevity optimizers (red curve), due to the increase in reproductive value depicted by the grey shaded region. (b) Variations on optimization. Three genotypes are illustrated: no longevity optimization (red curve), ideal longevity optimization (blue), and partial longevity optimization (green). All curves were modeled according to parameters described in the text and using Eqs. (23) and (24). Curves depict calculated trends.

Assume that in some hypothetical organism, $m(x)$ peaks near reproductive maturity before declining, and that mortality increases from this same point forward, accelerating the rate at which $l(x)$ decreases with age. We will also assume that these age-related declines are due to inevitable and irreversible fidelity losses. The red curve in Fig. 9a

depicts a typical plot of reproductive value as a function of age for this scenario (calculated using Eqs. *(23)* and *(24)*). This is representative of an organism lacking genetic elements for optimizing fecundity and survival in response to irreversible losses in fidelity. If the losses in $l(x)$ and $m(x)$ occurring after reproductive maturity are attenuated, peak reproductive value will increase and occur at a later age, and reproductive value will be maintained longer (Fig. 9a, blue curve). Due to the positive contribution to reproductive value, genes/genotypes that attenuate the described pattern of losses in $l(x)$ and/or $m(x)$ will be evolutionarily favored, provided they do not negatively influence early reproductive value.

8.2 Deterioration Management Strategies

It is illogical for an organism to have evolved such that fecundity or mortality is negatively affected (at ages where selective pressure is still above some minimal threshold) due to the disproportionate deterioration, or increased likelihood of failure, of one or a small number of vital biocomponents. Selection favors genotypes that avoid susceptibility to the catastrophic failure of a small number of weak links during aging.

I propose that cellular mechanisms and pathways have evolved to function in a progressive and dynamic manner to manage the irreversible, and inevitable, losses of fidelity afflicting an aging individual. Priority is placed on biocomponents most susceptible to degradation effects, and most critical to survival and fecundity. To illustrate this concept, I will describe two strategies: “managed deterioration” and “unmanaged deterioration”.

In unmanaged deterioration, the degradation of biocomponents occurs at a rate proportional to the biocomponent’s susceptibility to irreversible fidelity loss, or the direct and indirect effects of degradation present in other biocomponents (Fig. 10, left). Regardless of their importance to instantaneous mortality rate or fecundity, the most susceptible components reach failure levels first—leading to premature reductions in survival probability and fecundity—while other biocomponents could remain at relatively high performance levels (i.e. low degradation states).

In managed deterioration, longevity optimization genes modulate the deterioration rate of biocomponents so that those of similar importance degrade at comparable rates and/or reach their failure threshold at equivalent ages. Critical biocomponents are prioritized. No singular biocomponent is permitted to reach a degradation state that unduly compromises fecundity or survival probability—effectively increasing longevity and overall fitness (Fig. 10, right). This could be accomplished by several means, including:

1. Reallocating resources, at the cellular level and higher, as usable energetic resource availability declines to prioritize biocomponents most important for preserving reproductive value.
2. Adjusting microenvironmental conditions to decrease thermodynamic potentials on more vital biocomponents and thereby lowering the rate of damage-inflicting internal entropy production.
3. Reducing biocomponent turnover rates to delay the clonal expansion of irreversibly compromised biocomponents. (Effectively reduces the rate of mutual information loss.)
4. Altering physiology so that stresses on biocomponents that are more vital are reduced and maintained within functional limits.

One possible example of items (2) and (3) is the way by which controlled decreases in mitochondrial fusion and fission may attenuate mutual mtDNA information loss during aging. Mitochondrial fusion/fission rates are integral to mitophagy and mitobiogenesis (Twig et al., 2008; Youle and Narendra, 2011) and are therefore critical for preserving the quality (low degradation state) of mitochondrial components. Reducing fusion/fission compromises mitochondrial quality by increasing the load of ROS products and otherwise-damaged mitochondrial components. Mitochondria in aged organisms produce less usable energy (Yaniv et al., 2013), likely due to the combined effect of reduced mitochondrial fusion/fission and mutual mtDNA information loss that occurs with age. Figge et al. (2012) demonstrated that decelerating mitochondrial dynamics actually helps to preserve mitochondrial performance. Mutual mtDNA information losses are attenuated by reducing the exposure of mtDNA molecules to the high thermodynamic stress conditions encountered during replication and delaying the spread of parasitic mutated mtDNA molecules. This is evidently more critical to preserving mitochondrial performance than the tradeoff of increased degradation state in other mitochondrial biocomponents. In the context of the current discussion, the genes responsible for realizing this strategy are longevity optimizers.

Skeletal muscle mass decreases substantially with advanced age (Grounds, 1998). Scientists generally regard this loss as a purely undesirable physical manifestation of aging and view a safe and effective therapeutic intervention for reducing age-related skeletal muscle mass loss as being desirable and beneficial for the health of the elderly. For example, a substantial body of evidence suggests that the loss of function in satellite cells is a proximal cause of age-related muscle mass loss (Carlson and Conboy, 2007; Sousa-Victor

et al., 2014) and interventions have been proposed for "correcting" this deficiency (Carlson and Conboy, 2007; Dumont et al., 2015; García-Prat et al., 2013; Sousa-Victor et al., 2014; 2015).

I offer an alternative hypothesis for explaining this and perhaps other age-linked traits. Given that cardiac output declines significantly with age (Brandfonbrener et al., 1955) and is rooted in functional deficits at the cardiomyocyte level (Guo and Ren, 2006), a reduction of skeletal muscle mass will lower the stresses on an age-compromised heart by reducing the volume of blood in the body and decreasing the contractile forces required to circulate the blood. This raises the possibility that a decrease of skeletal muscle mass with age is a beneficial, evolved response—or at least a tolerated condition—which reduces cardiovascular stress and lowers the mortality risk of cardiac events. This is one example of how age-dependent physiological alterations could decrease the likelihood of failure of more critical biocomponents in light of inevitable losses in fidelity, as proposed in item (4) from the above list, and serve to extend longevity. To be clear, this hypothesis is not intended to explain extreme muscle wasting outside of normal age-related trends, which is undoubtedly a genuine pathological condition. In addition, there are certainly a number of undesirable aspects of age-related skeletal muscle dysfunction. The concept being put forth is the idea that age-dependent physiological alterations, even those that at first glance appear purely detrimental, may actually serve a purpose in establishing an optimally balanced configuration in the face of inevitable, and progressively increasing, fidelity loss.

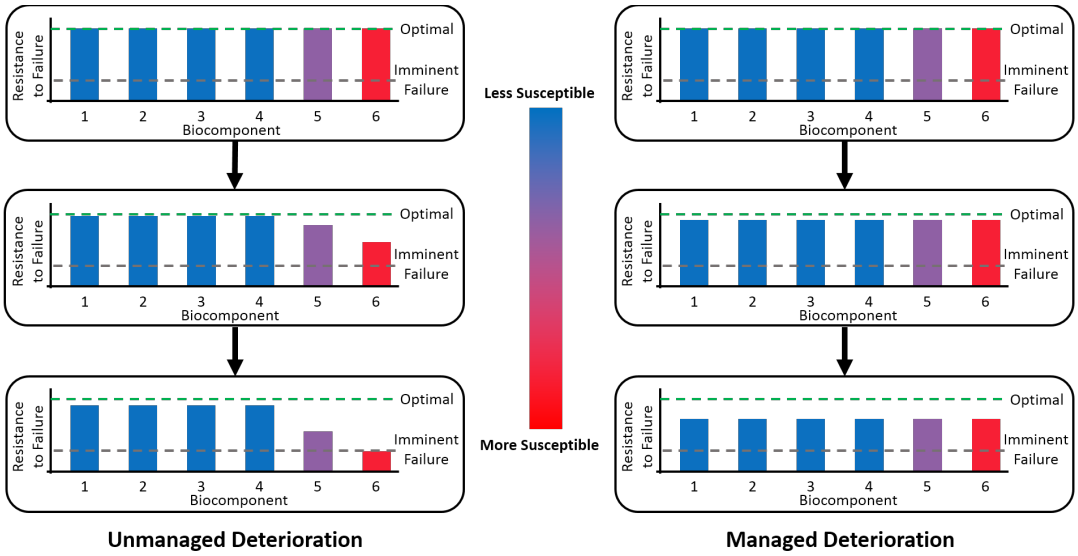

Fig. 10. Demonstration of unmanaged (left) and managed (right) deterioration strategies. A group of arbitrary biocomponents, equally vital to an organism's fecundity/survival, are depicted. Three ages are considered: Top—Young Adult, Center—Middle-Age, Bottom—Elderly. 'Red' indicates that a biocomponent is very susceptible to irreversible fidelity loss (which could be due to direct and/or indirect effects), while 'blue' signifies that a biocomponent has very little or no susceptibility to irreversible fidelity loss. The vertical axis depicts how resistant a biocomponent is to failing, given its current degradation state. In unmanaged deterioration, biocomponents will approach imminent failure at a rate proportional to their susceptibility to the effects of irreversible fidelity loss. Biocomponents most susceptible to irreversible fidelity loss will reach failure levels first and the organism will die prematurely. With managed deterioration, longevity optimization genes produce adjustments in the aging individual which partially offset decreases in resistance to failure of the most vital and susceptible biocomponents (details in main text). By not allowing any one vital factor to reach the imminent failure state at an earlier age than others, managed deterioration strategies may enhance longevity and increase fecundity.

It is prohibitively difficult to prove that the altered age-dependent expression of one gene represents an evolutionarily established tradeoff with some other gene(s) that extends longevity, as suggested by item (1). Beyond the evolutionary argument for their existence, there is other evidence suggesting that mechanisms of this type may exist—specifically, features of the proteomic, gene expression, and epigenetic signatures in aging individuals.

Gene expression signatures display a characteristic age-associated pattern of changes in specific genes in mice, rats and humans which is consistent across multiple tissue types (de Magalhães et al., 2009). One example is lysosomal genes, which are overexpressed with age. Decreased protein turnover could lead to a greater load of proteins that have degraded to the extent that they cannot be processed by proteasomes and must undergo lysosomal degradation. Although the energetic resources dedicated to increased lysosomal expression could have been allocated to lessening the severity of the general reduction in protein turnover, it may be that

age-dependent lysosomal overexpression optimizes the overall protein degradation state based on energetic resource availability and represents the best compromise for maximizing reproductive value.

DNA methylation expression patterns change in line with chronological age in humans (Bell et al., 2012; Bocklandt et al., 2011; Boks et al., 2009; Christensen et al., 2009; Christiansen et al., 2015; Florath et al., 2013; Garagnani et al., 2012; Gentilini et al., 2012; Hannum et al., 2013; Heyn et al., 2012; Horvath, 2013; McClay et al., 2013; Rakyan et al., 2010). Predictors can reliably estimate the age of human cells from any human tissue type based on epigenomic DNA methylation profiles (Hannum et al., 2013; Horvath, 2013). This supports the notion that age-related epigenetic signatures do not simply represent accumulated regulatory dysfunction, but that at least some component of this signature represents a progressive and dynamic response to aging.

8.3 Longevity Optimization Strategies from Early Adulthood May Serve as Templates for Those Used in Later Life

The theory advanced here proposes that selective pressures have led to the evolution of genetic optimizations that attenuate the rate of loss of mutual DNA information (and other irreversible fidelity losses) and the detrimental effects of these losses in aging individuals. There can be little doubt that in the face of inevitable, irreversible and progressive fidelity loss, a diverse array of intermediate configurations would be required to realize optimal aging during all stages of life. As compromised biomolecules reach non-trivial levels even during early adulthood (Ben-Zvi et al., 2009; Greaves et al., 2014), it is reasonable to propose that longevity optimizers have evolved to incorporate complex modulatory strategies to ensure optimal adjustments to the corresponding overall state of an aging individual.

This suggests that evolved, early adult-life longevity optimization pathways could serve as the basis for at least some of a late-life longevity optimization strategy. It may be largely through the extrapolation of these early adult-life mechanisms that the maximal lifespan of an organism can extend well beyond the age of peak reproductive value, particularly in species such as humans where older individuals have the benefit of protected environments. The use of pre-existing genes and pathways as a basis for later-life optimizations may also explain how genetic elements could evolve to a highly optimized state for relatively advanced ages, even though selective pressure decreases with age (Hamilton, 1966; Medawar, 1952; Williams, 1957). If genes and pathways for early adult-life longevity optimization were already present within an organism's genome, the extension of these strategies for late-life longevity optimization may require considerably less selective pressure.

Longevity optimization in all organisms is less than ideal. Due to declining selective pressure with age, it is likely to take an extremely long time for late-life longevity optimizations to evolve to approach the maximal longevity extension potential. Incorporating longevity optimization genes capable of maximally attenuating losses in fecundity, and increases in mortality, preserves late-life reproductive value (Fig. 9b, blue curve) compared to a genotype that lacks longevity optimization (red curve). Now suppose that longevity optimization is close to ideal for ages near peak reproductive value but becomes progressively less so as age increases and selective pressure decreases. The reproductive value curve for this scenario (Fig. 9b, green curve) is between the two described extremes. This last curve may be representative of the evolved state of the typical metazoan. The gray shaded region represents the "intervention potential"—the maximal gains in reproductive value attainable by further genetic longevity optimizations or through artificial manipulation of individuals (i.e. drugs and therapies, excluding therapies that restore fidelity in biocomponents subject to irreversible loss). Although beyond the scope of the current discussion, by examining statistics of proportionate mortality by pathological condition and other population data, it may be possible to calculate the ideal longevity optimization curve for a particular organism (Fig. 9b, blue curve).

8.4 Entropy-Driven Managed Deterioration in Further Detail

It is theorized here that metazoans have evolved to make compensatory adjustments as individuals age so as to minimize the deleterious effects of thermodynamic phenomena on reproductive value—resulting in survival, for the moment, but nonetheless unable to avoid an ever-increasing negative phenotype. These longevity optimizers may protect the biocomponents of an organism at all levels (biomolecules, cells, tissues, and organs) that are most critical to immediate survival and fecundity by sacrificing other aspects of health, leading to a diverse "spread the misery" phenotype. In essence, the diversity of the biocomponents affected during aging and the relatively high degree of conservation of the aging phenotype across taxa may be largely manifestations of these compromises. A more detailed depiction of this theory links further aspects of the aging process (Fig. 11).

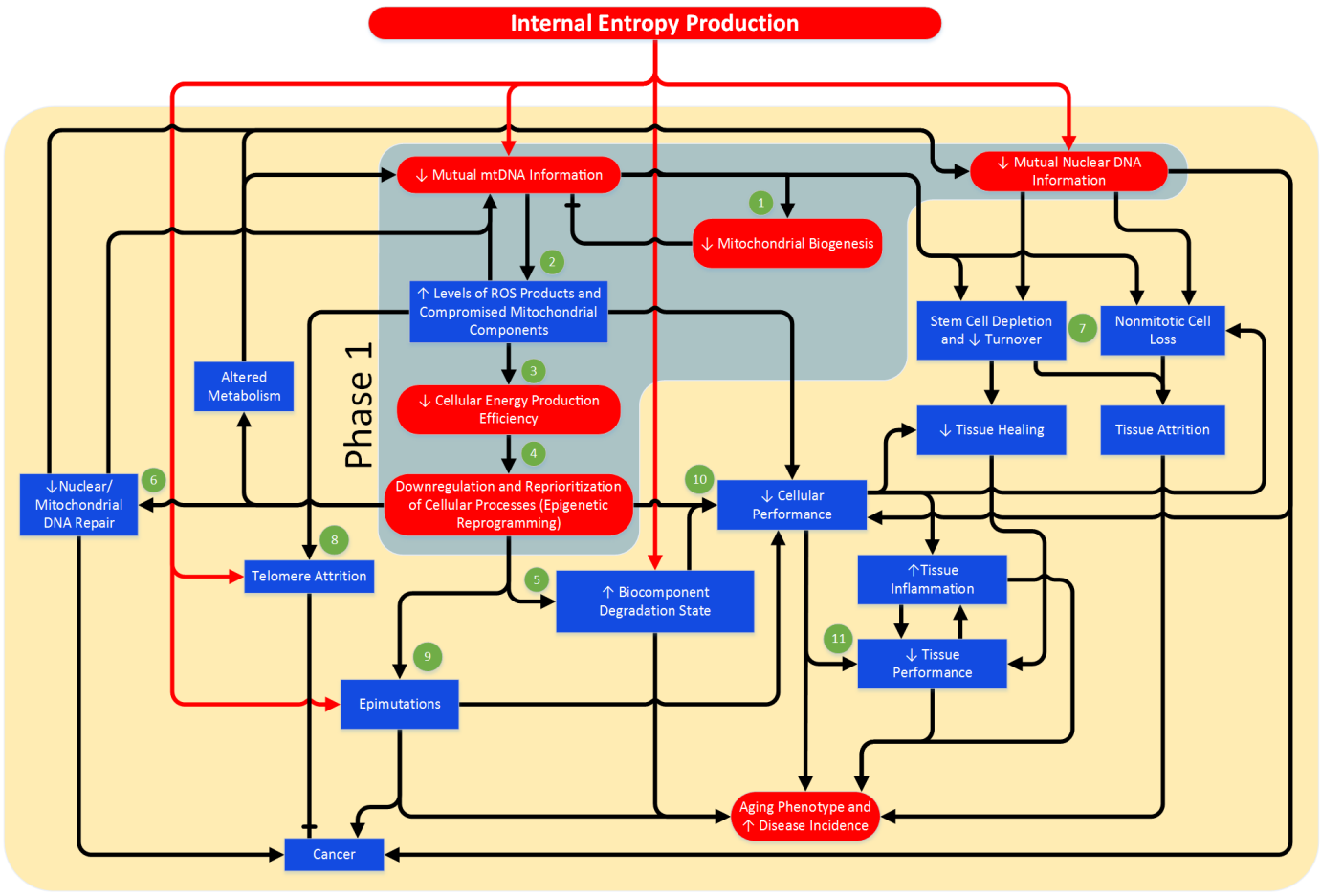

Fig. 11. A more detailed look at the higher-level interactions implicated in this theory during the progression of the aging phenotype. The red lines highlight where the degradative effects of internal entropy production are exerted. Mutual mtDNA information loss drives a deceleration in mitochondrial fusion/fission, ***1****—mitigating, but unable to prevent, further losses. Combined with reduced fusion/fission rates, losses in mutual mtDNA information lead to elevated levels of mitochondrial ROS products and compromised mitochondrial components (i.e. increased degradation state),* ***2,*** *which reduces peak cellular energy (ATP) output (Yaniv et al., 2013),* ***3****. The effects of this are partially tempered by an evolved response that includes resource allocation and physiological alterations,* ***4****, and is largely signified by the epigenetic state of a cell. These age-dependent epigenetic signatures should not be confused with epimutations, where distribution is mostly random (Heyn et al., 2012). Mandatory energy conservation reduces the cell's ability to preserve youthful biocomponent degradation states,* ***5****. Nuclear and mitochondrial DNA integrity is gradually further compromised as repair processes are downregulated,* ***6****. Losses in mutual nuclear DNA information contribute to increased cell-to-cell stochasticity in gene expression (Bahar et al., 2006) and clonal mosaicism (Lodato et al., 2015), causing average cellular performance to decrease. The loss of mutual DNA information will also decrease stem cell viability and consume stem cell reserves, in addition to generating losses in the number and viability of nonmitotic somatic cells,* ***7****. Dysfunctional telomeres can activate the DNA damage response pathway, engaging tumor protein p53 and leading to promotion of apoptosis or replicative senescence (Deng et al., 2008). Telomere attrition is upregulated in aged cells (Passos et al., 2007). This is an evolved mechanism, distinct from the length reduction that occurs during replication, believed to partially offset the increased likelihood of developing cancerous mutations in age-compromised cells (Campisi, 2005),* ***8****. This adaptive response involves the preferential degradation of telomeric DNA in conditions of increased mitochondrial superoxide production (Passos et al., 2007; Petersen et al., 1998; Zglinicki, 2002), as occurs with aging. Epigenome maintenance is downregulated in aged mammals (Cencioni et al., 2013), resulting in an increased number of unrepaired spontaneous epigenome mutations (Chambers et al., 2007),* ***9****. This, combined with escalating mutual DNA information losses and the downregulation of DNA damage repair mechanisms (Beerman et al., 2014; Zhang et al., 2010), contributes to an ever-increasing risk of developing cancer (Hansen et al., 2011), as seen with advancing age (American Cancer Society, 2013). Inevitably, the result of cellular component-level degradation is compromised cellular performance,* ***10,*** *and a concomitant loss in the performance of macro structures: tissues,* ***11****, organs and, ultimately, reduced viability of the organism itself.*

9 Connecting the Dots

9.1 Differentiating between Longevity Determinants and Longevity Optimizers

It is proposed here that two groups of factors contribute to the intrinsic longevity of a species: 1) longevity determinants and 2) longevity optimizers. It is important to differentiate between these distinct, but occasionally overlapping, groups. Longevity determinants are defined as factors that directly or indirectly influence the basal rate of loss of fidelity in any biocomponent (biomolecule, organ, tissue, etc.) susceptible to irreversible fidelity loss (such as DNA). The genetic arrangements that ultimately determine an organism's basal longevity are driven by fundamental physical law and evolutionary factors, and are further contingent on the exact environment and environmental interaction factors in which the species exists (Fig. 12). Any genetic element specifying a phenotypic characteristic that influences the basal rate of aging is a longevity determinant, as are the phenotypic characteristics themselves. Macro-level characteristics that may be longevity determinants include peak biological power density, physical size, athletic ability, and metabolic rate. At the micro-level, longevity determinants may include stem cell niche size, membrane composition, biomolecular degradation state, biomolecular durability/resiliency, and the degree of intramolecular genetic redundancy. Environmental determinants of species basal longevity include temperature/climate, resource availability (food, oxygen, etc.), predation pressure and other factors that mandate tradeoffs between fecundity/mortality and longevity. Survival strategies, and behavior in general, can also influence basal longevity by providing competitive advantages that result in reduced negative repercussions associated with characteristics that serve a role in longevity determination. The subdivision of longevity determinants into genotypic, phenotypic, and environmental elements allows for a clearer depiction of the interplay between the drivers and these different factors.

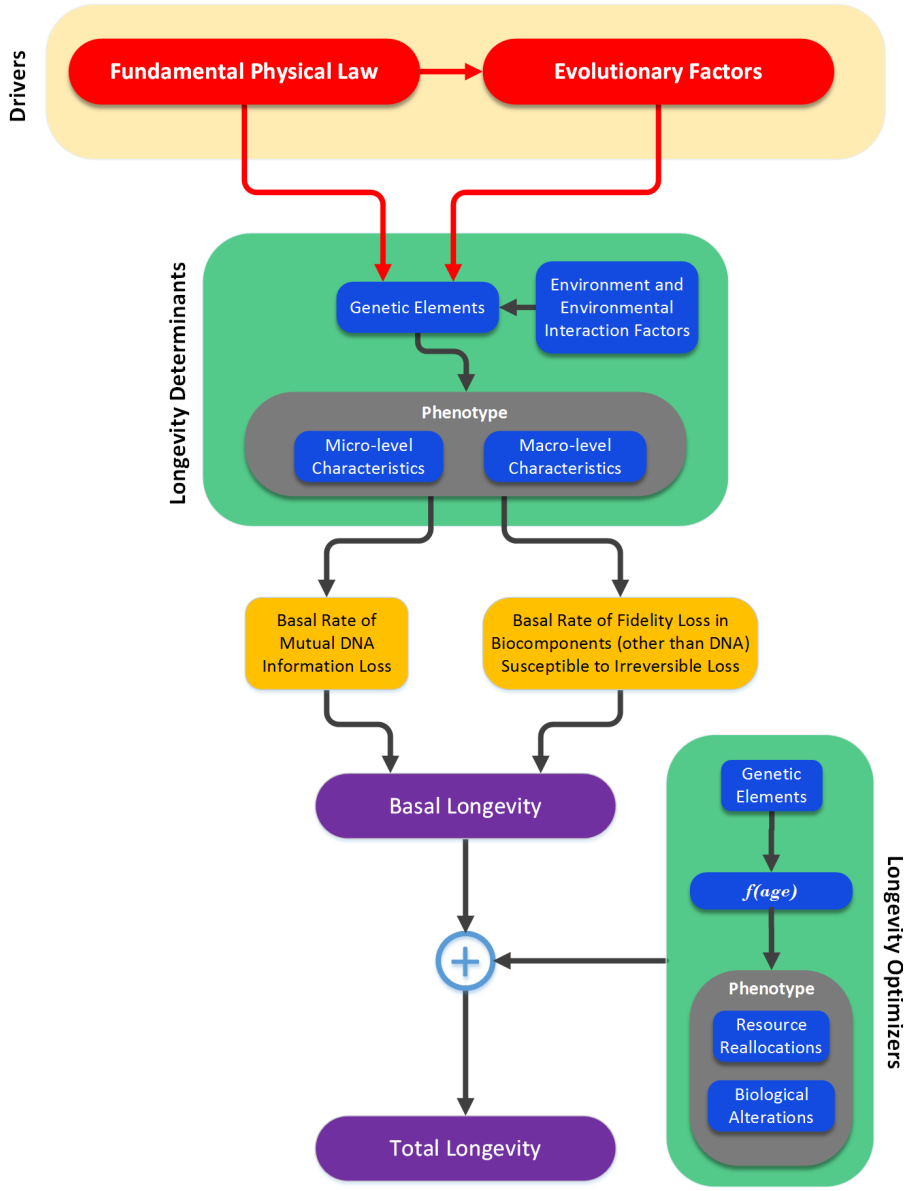

Fig. 12. The proposed relationship between longevity determinants, the root causes of aging (explained by fundamental physical law and evolutionary theory), longevity optimizers, basal longevity and total longevity in multicellular organisms.

In contrast to a longevity determinant, a longevity optimizer is any genetic element that increases basal longevity by contributing towards an effect that generally becomes progressively more dominant with age and that delays the severity, or rate of progression, of the aged phenotype. Longevity optimizers reallocate resources and alter physiology so that the overall state of the organism maximizes instantaneous survival rate and fecundity at all ages. In summary, longevity determinants define an organism's basal longevity while longevity optimizers seek to further maximize longevity through dynamic adjustments during the aging process that ultimately serve to balance the aging phenotype.

9.2 The Number of Genetic Elements Serving as Longevity Determinants/Optimizers Likely Positively Correlates with Organismal Complexity

Complex organisms are likely to have more cell and tissue types and to have increased specialization in these structures. Cellular interactions are more numerous and more sophisticated. Signaling pathways and their associated biomolecules, though often highly conserved, may utilize additional component derivatives to increase complexity. Due to this sophistication, organisms that are more complex will tend to have an increased number of opportunities for problems to occur. Complex organisms require additional "protective" mechanisms to temper this increase in vulnerability, further contributing to increased organismal complexity.

To minimize the severity of the aging phenotype, mechanisms must exist that alter resource allocation and that make other physiological adjustments. These mechanisms must provide a variable and dynamic response, in accordance with an organism's current state of degradation. In organisms with a greater number of biocomponents and potential interactions, additional corrective factors are required to manage these elements and provide an ideal configuration during all phases of life. This logic suggests that the number of longevity determinants and optimizers positively correlates with organismal complexity. On the other hand, this could help to explain how the longevity of simple organisms can benefit significantly from manipulation of only one or a few longevity determinants (Kenyon et al., 1993; Kenyon, 2010) while similar manipulations in organisms of greater complexity imparts far more modest longevity benefits (Blüher et al., 2003; Selman et al., 2008; Tatar et al., 2001).

9.3 The Rigidity of Species Longevity

Selective pressures have led to highly optimized physiology, based on compromises between factors affecting fitness (e.g. peak biological power density, physical size, longevity, etc.). Consider the potential implications of lowering the mass-specific metabolic rate of a mouse to $1/8^{th}$ its normal rate (approximately that of a human). The physiology of a murine heart is appropriate for the level of performance of the individual murine cardiomyocytes that it is comprised of and for the demands of a mouse body. Reducing the metabolic rate by such a large amount would likely mandate a loss in the peak biological power density of cardiomyocytes. These cardiomyocytes would be less able to generate the contractile forces necessary to counter the energy dissipation inherent to the murine circulatory system and hence blood circulation may be insufficient for sustaining life. This energy dissipation factor can only be reduced by making fundamental changes to the configuration of the vasculature. Yet, even with a configuration optimized for efficiency over performance, viable metabolic rates will be constrained to those values capable of satisfying certain physical requirements. Governing models derived from principles of fluid dynamics have been proposed (West et al., 1997; West and Brown, 2005) that provide an example of this type of phenomenon. Although the circulatory system is perhaps the easiest example to conceptualize, there are many other potential negative physiological implications of manipulating singular longevity determinants such as metabolism. A number of other tissues may be similarly compromised in this scenario, such as the liver and brain.

This logic offers an additional, and perhaps a more compelling, reason why metabolic manipulations found to increase longevity in simple model organisms do not translate well to organisms that are more complex. While such modifications are well tolerated by organisms such as *Caenorhabditis elegans* (Kenyon et al., 1993; Kenyon, 2010), which evidently remain viable at greatly reduced metabolic rates, comparable changes to metabolic rate in mammals are likely to render the organism nonviable. It should be noted that, even in *Caenorhabditis elegans*, reducing metabolic rate via genetic manipulation likely lowers fitness. This fitness cost would explain why *Caenorhabditis elegans* has not evolved to incorporate such changes in their genome: the tradeoff between increased longevity and factors that affect fitness is not sufficient to select for these changes. For these reasons, I hypothesize that longevity exhibits a degree of rigidity that increases with organismal complexity and that it is also bound by physiological limits explained by fundamental physics.

10 Summary and Conclusions

Although evolutionary theory predicts that senescence will arise inevitably due to declining selective pressure with age (Hamilton, 1966), fundamental physics stipulates that senescence is inevitable even in the absence of declining selective pressure. Blanket acceptance of declining selective pressure as the *singular* cause of aging is a logical fallacy (converse error)—yet many of the more popular aging theories are grounded in declining selective pressure as the sole root cause. If both declining selective pressure and physics separately stipulate aging, then explaining species longevity requires delineating the respective contributions of each.

10.1 Physics Helps to Explain Longevity Trends

It is an interesting observation that organisms with reduced longevity expend more energy on biomolecular repair and replacement. Although this is the precise opposite of what the disposable soma theory of aging would predict, it is in fact quite expected and the logic is straightforward when physics is considered. The model that I have described in this paper predicts that internal entropy

production will be higher in organisms with shorter longevity due to increased peak biological power density, and that this will necessitate a greater rate of negative entropy production (represented by upregulated biomolecular repair and replacement). I further described how one consequence of the increased metabolic activity needed to achieve this is that higher thermodynamic stresses are placed on DNA molecules (largely through increased replication rates)—resulting in an increase in the rate of loss of mutual DNA information and reduced longevity.

This logic offers an explanation for the negative correlation between metabolic rate and longevity, and for the allometry of longevity. These are two of the most long established and irrefutable species longevity trends. Yet remarkably, scientists have conveniently ignored these relationships in recent years. No theory incorporating declining selective pressure as the primary cause of aging has been able to explain these observations.

10.2 Physics Helps to Explain the Hayflick Limit

Leonard Hayflick demonstrated that primary cells undergo a limited number of doublings before senescing (Hayflick, 1965; Hayflick and Moorhead, 1961), an observation later dubbed the "Hayflick limit". Here I have explained why physics stipulates the loss of mutual DNA information and how the degree of loss may be largely a function of replication count. The presence of a replication counter to quantify these losses arises logically from this argument, as does the presence of a replication limit for disposing of cells that have exceeded some threshold of loss. Aging theories based on declining selective pressure are unable to explain why the Hayflick limit exists.

10.3 The Degradation State Concept Resolves Previously Unexplained Longevity Paradoxes

Physics clarifies why it is impossible to sustain a biomolecular ensemble in a perfect state of fidelity (since infinite resources are required). The degradation state concept introduced in this article provides a means to assess the relative condition of an ensemble. A prediction of the resulting model is that organisms that place extreme priority on maximizing energetic ROI are likely to operate at higher degradation states (i.e. with apparently more degradation). It also predicts that organisms with higher peak biological power densities will have lower degradation states at a given life stage, and vice versa. Both of these ideas are counterintuitive, but they help to explain observations previously considered paradoxical and which could not be explained by declining selective pressure.

Furthermore, when the inevitability of irreversible fidelity loss in a subset of biocomponents is considered along with the concepts of degradation state and managed deterioration, an explanation emerges as to how and why the overall aging phenotype may develop, including how an organism transitions between new homeostatic states as various changes occur during aging.

For the above reasons, it is reasonable to propose that stipulations resulting from fundamental physical law are more critical to longevity determination than declining selective pressure. On the other hand, declining selective pressure may be highly relevant for explaining why longevity optimization is less than ideal.

10.4 Drawing Incorrect Presumptions from Manipulation of the Aging Phenotype

Typically, the putative "aging pathways/genes"[8] have been branded as such because they were found to contain genetic elements which, when altered, modulated longevity in some model organism (often of low complexity, e.g. fruit fly or nematode) or produced a distinct effect on a characteristic(s) typically associated with the aging phenotype. However, if fundamental physical law implies that organisms of greater complexity are bound by more stringent physiological restrictions, then such manipulations may never deliver substantial longevity benefits to more complex organisms. Furthermore, if managed deterioration, as described here, is a true component of the aging process, then manipulations intended to address specific characteristics of the general aging phenotype might be expected to carry overall negative repercussions for the individual—regardless of whether or not they "correct" some negative aspect of the aging phenotype.

Observations of singular connections between genetic elements and particular phenotypes demonstrate only that the gene is responsible for modulating those characteristics—it should not imply that the gene is responsible for aging, nor does it necessarily reveal anything about the aging process. This thinking is quite a departure from the current mainstream approach, which focuses on establishing direct relationships between particular genes and their observable effects on the aging phenotype—leading to incorrect presumptions regarding the culpability of a particular factor as a root-level longevity determinant or "cause" of aging. For these

[8] As I subscribe to the belief that aging is a chance-driven catabolic process rather than a genetically engrained behavior (Hayflick, 2007a; 2007b), I view the terms "aging pathways" and "aging genes" as misnomers. I use these terms here only to refer to current literature. "Longevity determinants" and "longevity optimizers" are more appropriate terms for these factors, and this is used when referring to concepts discussed in this paper.

reasons, I believe that the current catalog of putative aging pathways/genes represents, at best, a grossly incomplete set of the factors truly relevant to longevity determination.

10.5 Charting a New Course

Aging is the greatest risk factor for severe pathology. Yet, most "aging research" is focused on age-associated pathologies or metabolic manipulations in lower life forms, rather than the fundamental biology of aging (Hayflick, 2000; 2007a). Because of this, and despite the fact that a number of pathways influencing longevity have been identified, one can argue that scientists today know little more about the true reasons for aging than was known 50 years ago. This is exemplified by the lack of any consensus theory of aging. Even worse, despite the ever-growing litany of serious anomalies challenging common aging theories, the scientific community remains complacent. The multitude of aging theories, the discontinuities between them, and the failure of the scientific community to agree on the root causes of aging, while disappointing, represents a clear opportunity to revisit this problem with a multidisciplinary and somewhat radical approach.

The theoretical framework discussed in this paper utilizes concepts from physics, information theory, as well as evolutionary theory to explain aging. I believe that the theoretical framework arising from this approach has fewer anomalies than existing singularly focused theories. While the theory put forth here is well supported by the findings of others in diverse fields, it is admittedly not devoid of speculative components. Additional data, such as delineating the species differences in mitobiogenesis rates, would bring better clarity to important questions that remain and would be very helpful in refining the argument presented here.

The idea that aging can be manipulated is alluring. Nevertheless, difficult problems are only solved by addressing their root causes. In the absence of a central theoretical framework for biological aging, it is hard to predict whether a particular strategy or approach for treating an age-associated pathology, or for increasing healthspan or longevity, has a chance of succeeding in humans. The theoretical framework outlined in this paper provides an explanation as to why the longevity benefits seen in simple organisms through manipulations of putative aging pathways/genes are not realized in more complex organisms. If accurate, this highlights the naivety of longevity extension efforts to identify and manipulate genes and molecular pathways that could substantially increase human longevity without compromising health or performance, and the futility of attempting to use simple model organisms such as *Caenorhabditis elegans* as the vehicle for such efforts. On the other hand, the capacity for these approaches to extend healthspan in higher organisms (the so-called "intervention potential"), though limited, may be rather straightforward to estimate.

The theoretical framework discussed here identifies fundamental physical law and evolutionary theory as potential root causes of aging. Of course, we cannot manipulate the laws of physics or fabricate exceptions to evolutionary theory, but it may be possible to resolve their most direct downstream negative repercussions on individual viability—irreversible fidelity loss[9] in susceptible biocomponents—and effectively retard the aging process, reduce susceptibly to age-associated pathologies, or even restore the individual to a more youthful state. For example, while mutual DNA information loss may be inevitable, the complete original genetic sequence of an aged individual can be recreated through the collective sequencing of a population of cells and overlapping the acquired sequences to arrive at the consensus sequence (i.e. high-throughput sequencing). Hypothetically, it should be possible to artificially produce youthful cells by sorting extant cells according to degree of mutual DNA information loss, correcting any genetic errors, resetting stemness or differentiation state if required, and propagating the cells. Reintroducing these cells into an aged individual could produce some of the aforementioned benefits. Although realizing an intervention such as this has a number of technical hurdles, the potential payoffs could be far greater than the indiscriminate approaches currently prioritized.

[9] It is important to note that "irreversible fidelity loss", in the context of this discussion, refers to losses that cannot be rectified by any existing, or theoretical, *biological* process. This does not mean to imply that such losses cannot be resolved through artificial means.

REFERENCES

Ahima, R.S. (2009). Connecting obesity, aging and diabetes. Nat Med *15*, 996–997.

American Cancer Society (2013). Cancer Facts and Figures 2013 (Atlanta: American Cancer Society).

Andziak, B., and Buffenstein, R. (2006). Disparate patterns of age-related changes in lipid peroxidation in long-lived naked mole-rats and shorter-lived mice. Aging Cell *5*, 525–532.

Andziak, B., O'Connor, T.P., Qi, W., DeWaal, E.M., Pierce, A., Chaudhuri, A.R., Van Remmen, H., and Buffenstein, R. (2006). High oxidative damage levels in the longest-living rodent, the naked mole-rat. Aging Cell *5*, 463–471.

Andziak, B., O'Connor, T.P., and Buffenstein, R. (2005). Antioxidants do not explain the disparate longevity between mice and the longest-living rodent, the naked mole-rat. Mech Ageing Dev *126*, 1206–1212.

Arias-Gonzalez, J.R. (2012). Entropy involved in fidelity of DNA replication. PLoS ONE *7*, e42272.

Ashrafi, G., and Schwarz, T.L. (2012). The pathways of mitophagy for quality control and clearance of mitochondria. Cell Death Differ *20*, 31–42.

Austad, S.N. (2005). Diverse aging rates in metazoans: targets for functional genomics. Mech Ageing Dev *126*, 43–49.

Austad, S.N. (2009). Comparative biology of aging. J Gerontol a Biol Sci Med Sci *64*, 199–201.

Bahar, R., Hartmann, C.H., Rodriguez, K.A., Denny, A.D., Busuttil, R.A., Dollé, M.E.T., Calder, R.B., Chisholm, G.B., Pollock, B.H., Klein, C.A., et al. (2006). Increased cell-to-cell variation in gene expression in ageing mouse heart. Nature *441*, 1011–1014.

Bailly, A., and Gartner, A. (2013). Germ cell apoptosis and DNA damage responses. Adv Exp Med Biol *757*, 249–276.

Baum, D.A., Futuyma, D.J., Hoekstra, H.E., Lenski, R.E., Moore, A.J., Peichel, C.L., Schluter, D., and Whitlock, M.C. (2013). The Princeton Guide to Evolution (Princeton University Press).

Beerman, I., Seita, J., Inlay, M.A., Weissman, I.L., and Rossi, D.J. (2014). Quiescent Hematopoietic Stem Cells Accumulate DNA Damage during Aging that Is Repaired upon Entry into Cell Cycle. Stem Cell *15*, 37–50.

Bell, J.T., Tsai, P.-C., Yang, T.-P., Pidsley, R., Nisbet, J., Glass, D., Mangino, M., Zhai, G., Zhang, F., Valdes, A., et al. (2012). Epigenome-wide scans identify differentially methylated regions for age and age-related phenotypes in a healthy ageing population. PLoS Genetics *8*, e1002629.

Ben-Zvi, A., Miller, E.A., and Morimoto, R.I. (2009). Collapse of proteostasis represents an early molecular event in Caenorhabditis elegans aging. Proc Natl Acad Sci USA *106*, 14914–14919.

Bennett, M.B., Ker, R.F., Dimery, N.J., and Alexander, R. (1986). Mechanical properties of various mammalian tendons. J Zool (London) *209*, 537–548.

Biewener, A.A. (1989). Scaling body support in mammals: limb posture and muscle mechanics. Science *245*, 45–48.

Biewener, A.A., and Blickhan, R. (1988). Kangaroo rat locomotion: design for elastic energy storage or acceleration? J Exp Biol *140*, 243–255.

Biewener, A., Alexander, R.M., and Heglund, N.C. (1981). Elastic energy storage in the hopping of kangaroo rats (Dipodomys spectabilis). J Zool (London) *195*, 369–383.

Blüher, M., Kahn, B.B., and Kahn, C.R. (2003). Extended Longevity in Mice Lacking the Insulin Receptor in Adipose Tissue. Science *299*, 572–574.

Bocklandt, S., Lin, W., Sehl, M.E., Sánchez, F.J., Sinsheimer, J.S., Horvath, S., and Vilain, E. (2011). Epigenetic Predictor of Age. PLoS ONE *6*, e14821.

Boks, M.P., Derks, E.M., Weisenberger, D.J., Strengman, E., Janson, E., Sommer, I.E., Kahn, R.S., and Ophoff, R.A. (2009). The relationship of DNA methylation with age, gender and genotype in twins and healthy controls. PLoS ONE *4*, e6767.

Bradshaw, S.D., and Bradshaw, F.J. (2009). Measurement of the rate of protein turnover and synthesis in the marsupial Honey possum (Tarsipes rostratus). J Comp Physiol B *179*, 183–192.

Brandfonbrener, M., Landowne, M., and Shock, N.W. (1955). Changes in Cardiac Output with Age. Circulation *12*, 557.

Brinster, R.L., and Troike, D.E. (1979). Requirements for blastocyst development in vitro. J Anim Sci *49 Suppl 2*, 26–34.

Calder, W.A. (1984). Size, Function, and Life History (Harvard University Press).

Campisi, J. (2005). Senescent Cells, Tumor Suppression, and Organismal Aging: Good Citizens, Bad Neighbors. Cell *120*, 513–522.

Carlson, M.E., and Conboy, I.M. (2007). Loss of stem cell regenerative capacity within aged niches. Aging Cell *6*, 371–382.

Chambers, S.M., Shaw, C.A., Gatza, C., Fisk, C.J., Donehower, L.A., and Goodell, M.A. (2007). Aging Hematopoietic Stem Cells Decline in Function and Exhibit Epigenetic Dysregulation. PLoS Biol *5*, e201.

Chan, S.S.L., and Copeland, W.C. (2009). DNA polymerase gamma and mitochondrial disease: Understanding the consequence of *POLG* mutations. Biochim Biophys Acta *1787*, 312–319.

Christensen, B.C., Houseman, E.A., Marsit, C.J., Zheng, S., Wrensch, M.R., Wiemels, J.L., Nelson, H.H., Karagas, M.R., Padbury, J.F., Bueno, R., et al. (2009). Aging and environmental exposures alter tissue-specific DNA methylation dependent upon CpG island context. PLoS Genetics *5*, e1000602.

Christiansen, L., Lenart, A., Tan, Q., Vaupel, J.W., Aviv, A., McGue, M., and Christensen, K. (2015). DNA methylation age is associated with mortality in a longitudinal Danish twin study. Aging Cell *15*, 149–154.

Collins, M.L., Eng, S., Hoh, R., and Hellerstein, M.K. (2003). Measurement of mitochondrial DNA synthesis in vivo using a stable isotope-mass spectrometric technique. J Appl Physiol *94*, 2203–2211.

Cookson, M.R. (2012). Parkinsonism due to mutations in PINK1, parkin, and DJ-1 and oxidative stress and mitochondrial pathways. Cold Spring Harb Perspect Med *2*, a009415.

Couture, P., and Hulbert, A.J. (1995). Membrane fatty acid composition of tissues is related to body mass of mammals. J Membr Biol *148*, 27–39.

Daum, G. (1985). Lipids of mitochondria. Biochemica Et Biophysica Acta *822*, 1–42.

De Baets, G., Reumers, J., Delgado Blanco, J., Dopazo, J., Schymkowitz, J., and Rousseau, F. (2011). An Evolutionary Trade-Off between Protein Turnover Rate and Protein Aggregation Favors a Higher Aggregation Propensity in Fast Degrading Proteins. PLoS Comp Biol *7*, e1002090.

De Bont, R., and van Larebeke, N. (2004). Endogenous DNA damage in humans: a review of quantitative data. Mutagenesis *19*, 169–185.

Delaney, M.A., Ward, J.M., Walsh, T.F., Chinnadurai, S.K., Kerns, K., Kinsel, M.J., and Treuting, P.M. (2016). Initial Case Reports of Cancer in Naked Mole-rats (Heterocephalus glaber). Vet Pathol *53*, 691–696.

Demirel, Y. (2014). Nonequilibrium thermodynamics: transport and rate processes in physical, chemical and biological systems (Elsevier).

Deng, Y., Chan, S.S., and Chang, S. (2008). Telomere dysfunction and tumour suppression: the senescence connection. Nat Rev Cancer *8*, 450–458.

Dumont, N.A., Wang, Y.X., and Rudnicki, M.A. (2015). Intrinsic and extrinsic mechanisms regulating satellite cell function. Development *142*, 1572–1581.

ENCODE Project Consortium, Birney, E., Stamatoyannopoulos, J.A., Dutta, A., Guigó, R., Gingeras, T.R., Margulies, E.H., Weng, Z., Snyder, M., Dermitzakis, E.T., et al. (2007). Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature *447*, 799–816.

Else, P.L. (2004). Respiration rate of hepatocytes varies with body mass in birds. J Exp Biol *207*, 2305–2311.

Else, P.L., and Wu, B.J. (1999). What role for membranes in determining the higher sodium pump molecular activity of mammals compared to ectotherms? J Comp Physiol B, Biochem Syst Environ Physiol *169*, 296–302.

Else, P.L., Windmill, D.J., and Markus, V. (1996). Molecular activity of sodium pumps in endotherms and ectotherms. Am J Physiol *271*, R1287–1294.

Euler, L. (1767). Recherches générales sur la mortalité et la multiplication du genre humain. In Memoires De L'academie Des Sciences De Berlin, pp. 144–164.

Everett, A.W., Taylor, R.R., and Sparrow, M.P. (1977). Protein synthesis during right-ventricular hypertrophy after pulmonary-artery stenosis in the dog. Biochem J *166*, 315–321.

Eyre-Walker, A., and Keightley, P.D. (2007). The distribution of fitness effects of new mutations. Nat Rev Genet *8*, 610–618.

Faulks, S.C., Turner, N., Else, P.L., and Hulbert, A.J. (2006). Calorie restriction in mice: effects on body composition, daily activity, metabolic rate, mitochondrial reactive oxygen species production, and membrane fatty acid composition. J Gerontol a Biol Sci Med Sci *61*, 781–794.

Figge, M.T., Reichert, A.S., Meyer-Hermann, M., and Osiewacz, H.D. (2012). Deceleration of Fusion–Fission Cycles Improves Mitochondrial Quality Control during Aging. PLoS Comp Biol *8*, e1002576.

Fisher, R.A. (1930). The genetical theory of natural selection. (Oxford).

Florath, I., Butterbach, K., Müller, H., Bewerunge-Hudler, M., and Brenner, H. (2013). Cross-sectional and longitudinal changes in DNA methylation with age: an epigenome-wide analysis revealing over 60 novel age-associated CpG sites. Hum Mol Gen *23*, 1186–1201.

Frederico, L.A., Kunkel, T.A., and Shaw, B.R. (1990). A sensitive genetic assay for the detection of cytosine deamination: determination of rate constants and the activation energy. Biochemistry *29*, 2532–2537.

Garagnani, P., Bacalini, M.G., Pirazzini, C., Gori, D., Giuliani, C., Mari, D., Di Blasio, A.M., Gentilini, D., Vitale, G., Collino, S., et al. (2012). Methylation of ELOVL2 gene as a new epigenetic marker of age. Aging Cell *11*, 1132–1134.

García-Prat, L., Sousa-Victor, P., and Muñoz-Cánoves, P. (2013). Functional dysregulation of stem cells during aging: a focus on skeletal muscle stem cells. FEBS J *280*, 4051–4062.

Garland, T. (1982). The relation between maximal running speed and body mass in terrestrial mammals. J Zool, Lond *199*, 157–170.

Garlick, P.J., and Marshall, I. (1972). A technique for measuring brain protein synthesis. J Neurochem *19*, 577–583.

Gentilini, D., Mari, D., Castaldi, D., Remondini, D., Ogliari, G., Ostan, R., Bucci, L., Sirchia, S.M., Tabano, S., Cavagnini, F., et al. (2012). Role of epigenetics in human aging and longevity: genome-wide DNA methylation profile in centenarians and centenarians' offspring. Age (Dordr) *35*, 1961–1973.

Greaves, L.C., Elson, J.L., Nooteboom, M., Grady, J.P., Taylor, G.A., Taylor, R.W., Mathers, J.C., Kirkwood, T.B.L., and Turnbull, D.M. (2012). Comparison of mitochondrial mutation spectra in ageing human colonic epithelium and disease: absence of evidence for purifying selection in somatic mitochondrial DNA point mutations. PLoS Genetics *8*, e1003082.

Greaves, L.C., Nooteboom, M., Elson, J.L., Tuppen, H.A.L., Taylor, G.A., Commane, D.M., Arasaradnam, R.P., Khrapko, K., Taylor, R.W., Kirkwood, T.B.L., et al. (2014). Clonal expansion of early to mid-life mitochondrial DNA point mutations drives mitochondrial dysfunction during human ageing. PLoS Genetics *10*, e1004620.

Gredilla, R., Garm, C., Holm, R., Bohr, V.A., and Stevnsner, T. (2010). Differential age-related changes in mitochondrial DNA repair activities in mouse brain regions. Neurobiol Aging *31*, 993–1002.

Grounds, M.D. (1998). Age-associated changes in the response of skeletal muscle cells to exercise and regeneration. Ann N Y Acad Sci *854*, 78–91.

Guo, K.K., and Ren, J. (2006). Cardiac overexpression of alcohol dehydrogenase (ADH) alleviates aging-associated cardiomyocyte contractile dysfunction: role of intracellular Ca2+ cycling proteins. Aging Cell *5*, 259–265.

Haag-Liautard, C., Coffey, N., Houle, D., Lynch, M., Charlesworth, B., and Keightley, P.D. (2008). Direct estimation of the mitochondrial DNA mutation rate in Drosophila melanogaster. PLoS Biol *6*, e204.

Hamilton, W.D. (1966). The moulding of senescence by natural selection. J Theor Biol *12*, 12–45.

Hannum, G., Guinney, J., Zhao, L., Zhang, L., Hughes, G., Sadda, S., Klotzle, B., Bibikova, M., Fan, J.-B., Gao, Y., et al. (2013). Genome-wide methylation profiles reveal quantitative views of human aging rates. Mol Cell *49*, 359–367.

Hansen, K.D., Timp, W., Bravo, H.C., Sabunciyan, S., Langmead, B., McDonald, O.G., Wen, B., Wu, H., Liu, Y., Diep, D., et al. (2011). Increased methylation variation in epigenetic domains across cancer types. Nat Genet *43*, 768–775.

Hayflick, L. (1965). The limited in vitro lifetime of human diploid cell strains. Exp Cell Res *37*, 614–636.

Hayflick, L. (2000). The future of ageing. Nature *408*, 267–269.

Hayflick, L. (2004). “Anti-aging” is an oxymoron. J Gerontol a Biol Sci Med Sci *59*, B573–578.

Hayflick, L. (2007a). Entropy explains aging, genetic determinism explains longevity, and undefined terminology explains misunderstanding both. PLoS Genetics *3*, e220.

Hayflick, L. (2007b). Biological Aging Is No Longer an Unsolved Problem. Ann N Y Acad Sci *1100*, 1–13.

Hayflick, L., and Moorhead, P.S. (1961). The serial cultivation of human diploid cell strains. Exp Cell Res *25*, 585.

Heglund, N.C., and Taylor, C.R. (1988). Speed, stride frequency and energy cost per stride: how do they change with body size and gait? J Exp Biol *138*, 301–318.

Heglund, N.C., Fedak, M.A., Taylor, C.R., and Cavagna, G.A. (1982). Energetics and mechanics of terrestrial locomotion. IV. Total mechanical energy changes as a function of speed and body size in birds and mammals. J Exp Biol *97*, 57–66.

Hetzer, M.W. (2013). Protein homeostasis: live long, won't prosper. Nat Rev Mol Cell Biol *14*, 55–61.

Heyn, H., Li, N., Ferreira, H.J., Moran, S., Pisano, D.G., Gomez, A., Diez, J., Sanchez-Mut, J.V., Setien, F., Carmona, F.J., et al. (2012). Distinct DNA methylomes of newborns and centenarians. Proc Natl Acad Sci USA *109*, 10522–10527.

Hochwagen, A., and Amon, A. (2006). Checking Your Breaks: Surveillance Mechanisms of Meiotic Recombination. Curr Biol *16*, R217–R228.

Holliday, R. (2010). Aging and the decline in health. Health *02*, 615–619.

Horvath, S. (2013). DNA methylation age of human tissues and cell types. Genome Biol *14*, R115.

Hulbert, A.J. (2008). The links between membrane composition, metabolic rate and lifespan. Comp Biochem Physiol, Part a Mol Integr Physiol *150*, 196–203.

Hulbert, A.J., and Else, P.L. (1999). Membranes as possible pacemakers of metabolism. J Theor Biol *199*, 257–274.

Hulbert, A.J., Pamplona, R., Buffenstein, R., and Buttemer, W.A. (2007). Life and death: metabolic rate, membrane composition, and life span of animals. Physiol Rev *87*, 1175–1213.

Hwang, J.S., Hwang, J.S., Chang, I., and Kim, S. (2007). Age-associated decrease in proteasome content and activities in human dermal fibroblasts: restoration of normal level of proteasome subunits reduces aging markers in fibroblasts from elderly persons. J Gerontol a Biol Sci Med Sci *62*, 490–499.

Itsara, L.S., Kennedy, S.R., Fox, E.J., Yu, S., Hewitt, J.J., Sanchez-Contreras, M., Cardozo-Pelaez, F., and Pallanck, L.J. (2014). Oxidative stress is not a major contributor to somatic mitochondrial DNA mutations. PLoS Genetics *10*, e1003974.

Jaramillo-Lambert, A., Harigaya, Y., Vitt, J., Villeneuve, A., and Engebrecht, J. (2010). Meiotic Errors Activate Checkpoints that Improve Gamete Quality without Triggering Apoptosis in Male Germ Cells. Curr Biol *20*, 2078–2089.

Kennedy, S.R., Loeb, L.A., and Herr, A.J. (2011). Somatic mutations in aging, cancer and neurodegeneration. Mech Ageing Dev *133*, 118–126.

Kennedy, S.R., Salk, J.J., Schmitt, M.W., and Loeb, L.A. (2013). Ultra-Sensitive Sequencing Reveals an Age-Related Increase in Somatic Mitochondrial Mutations That Are Inconsistent with Oxidative Damage. PLoS Genetics *9*, e1003794.

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A C. elegans mutant that lives twice as long as wild type. Nature *366*, 461–464.

Kenyon, C.J. (2010). The genetics of ageing. Nature *464*, 504–512.

Kilbourne, B.M., and Hoffman, L.C. (2013). Scale Effects between Body Size and Limb Design in Quadrupedal Mammals. PLoS ONE *8*, e78392.

Kim, N., and Jinks-Robertson, S. (2012). Transcription as a source of genome instability. Nat Rev Genet *13*, 204–214.

Kirkwood, T.B. (1977). Evolution of ageing. Nature *270*, 301–304.

Kirkwood, T.B., and Holliday, R. (1979). The evolution of ageing and longevity. Proc R Soc Lond, B, Biol Sci *205*, 531–546.

Kirkwood, T.B., and Rose, M.R. (1991). Evolution of senescence: late survival sacrificed for reproduction. Philos Trans R Soc Lond, B, Biol Sci *332*, 15–24.

Kirkwood, T.B.L. (2005). Understanding the Odd Science of Aging. Cell *120*, 437–447.

Kirkwood, T. (1999). Time of Our Lives : The Science of Human Aging (Oxford University Press).

Kleiber, M. (1932). Body size and metabolism. Hilgardia *6*, 315–353.

Kondepudi, D., and Prigogine, I. (2014). Modern Thermodynamics (John Wiley & Sons).

Kram, R., and Taylor, C.R. (1990). Energetics of running: a new perspective. Nature *346*, 265–267.

Larsson, N.-G. (2010). Somatic Mitochondrial DNA Mutations in Mammalian Aging. Annu Rev Biochem *79*, 683–706.

Lee, H.-S., Ma, H., Juanes, R.C., Tachibana, M., Sparman, M., Woodward, J., Ramsey, C., Xu, J., Kang, E.-J., Amato, P., et al. (2012). Rapid mitochondrial DNA segregation in primate preimplantation embryos precedes somatic and germline bottleneck. Cell Rep *1*, 506–515.

Liebermeister, W., Noor, E., Flamholz, A., Davidi, D., Bernhardt, J., and Milo, R. (2014). Visual account of protein investment in cellular functions. Proc Natl Acad Sci USA *111*, 8488–8493.

Lindahl, T. (1993). Instability and decay of the primary structure of DNA. Nature *362*, 709–715.

Lodato, M.A., Woodworth, M.B., Lee, S., Evrony, G.D., Mehta, B.K., Karger, A., Lee, S., Chittenden, T.W., D'Gama, A.M., Cai, X., et al. (2015). Somatic mutation in single human neurons tracks developmental and transcriptional history. Science *350*, 94–98.

Lotka, A.J., and Sharpe, F.R. (1911). A problem in age distribution. Philos Mag *21*, 435–438.

McClay, J.L., Aberg, K.A., Clark, S.L., Nerella, S., Kumar, G., Xie, L.Y., Hudson, A.D., Harada, A., Hultman, C.M., Magnusson, P.K.E., et al. (2013). A methylome-wide study of aging using massively parallel sequencing of the methyl-CpG-enriched genomic fraction from blood in over 700 subjects. Hum Mol Gen *23*, 1175–1185.

McNab, B.K. (1966). The metabolism of fossorial rodents: a study of convergence. Ecology 712–733.

Medawar, P.B. (1952). An unsolved problem of biology.

Medvedev, Z.A. (1972). Possible role of repeated nucleotide sequences in DNA in the evolution of life spans of differentiated cells. Nature *237*, 453–454.

Miller, B.F., Robinson, M.M., Bruss, M.D., Hellerstein, M., and Hamilton, K.L. (2011). A comprehensive assessment of mitochondrial protein synthesis and cellular proliferation with age and caloric restriction. Aging Cell *11*, 150–161.

Miller, B.F., Robinson, M.M., Reuland, D.J., Drake, J.C., Peelor, F.F., Bruss, M.D., Hellerstein, M.K., and Hamilton, K.L. (2013). Calorie restriction does not increase short-term or long-term protein synthesis. J Gerontol a Biol Sci Med Sci *68*, 530–538.

Mitchell, H.H., Hamilton, T.S., Steggerda, F.R., and Bean, H.W. (1945). The chemical composition of the adult human body and its bearing on the biochemistry of growth. J Biol Chem *168*, 625–637.

Mitchell, T.W., Buffenstein, R., and Hulbert, A.J. (2007). Membrane phospholipid composition may contribute to exceptional longevity of the naked mole-rat (Heterocephalus glaber): a comparative study using shotgun lipidomics. Exp Gerontol *42*, 1053–1062.

Mitteldorf, J. (2010). Aging is not a process of wear and tear. Rejuvenation Res *13*, 322–326.

Moulton, C.R. (1923). Age and chemical development in mammals. J Biol Chem *57*, 79–97.

Nagy, K.A. (2005). Field metabolic rate and body size. J Exp Biol *208*, 1621–1625.

Niklas, K.J. (1994). Plant Allometry (University of Chicago Press).

Pacy, P.J., Price, G.M., Halliday, D., and Quevedo, M.R. (1994). Nitrogen homoeostasis in man: the diurnal responses of protein synthesis and degradation and amino acid oxidation to diets with increasing protein intakes. Clin Sci *86*, 103–118.

Pamplona, R., Portero-Otín, M., Riba, D., Ruiz, C., Prat, J., Bellmunt, M.J., and Barja, G. (1998). Mitochondrial membrane peroxidizability index is inversely related to maximum life span in mammals. J Lipid Res *39*, 1989–1994.

Passos, J.F., Saretzki, G., Ahmed, S., Nelson, G., Richter, T., Peters, H., Wappler, I., Birket, M.J., Harold, G., Schaeuble, K., et al. (2007). Mitochondrial Dysfunction Accounts for the Stochastic Heterogeneity in Telomere-Dependent Senescence. PLoS Biol *5*, e110.

Peters, R.H. (1986). The Ecological Implications of Body Size (Cambridge University Press).

Petersen, S., Saretzki, G., and Zglinicki, von, T. (1998). Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. Exp Cell Res *239*, 152–160.

Plata, G., and Vitkup, D. (2013). Genetic robustness and functional evolution of gene duplicates. Nucleic Acids Res *42*, 2405–2414.

Pontzer, H. (2007). Effective limb length and the scaling of locomotor cost in terrestrial animals. J Exp Biol *210*, 1752–1761.

Porter, R.K., and Brand, M.D. (1995). Causes of differences in respiration rate of hepatocytes from mammals of different body mass. Am J Physiol *269*, R1213–1224.

Porter, R.K., Hulbert, A.J., and Brand, M.D. (1996). Allometry of mitochondrial proton leak: influence of membrane surface area and fatty acid composition. Am J Physiol *271*, R1550–1560.

Rakyan, V.K., Down, T.A., Maslau, S., Andrew, T., Yang, T.-P., Beyan, H., Whittaker, P., McCann, O.T., Finer, S., Valdes, A.M., et al. (2010). Human aging-associated DNA hypermethylation occurs preferentially at bivalent chromatin domains. Genome Res *20*, 434–439.

Rattan, S.I.S. (1996). Synthesis, modifications, and turnover of proteins during aging. Exp Gerontol *31*, 33.

Reeds, P.J., and Harris, C.I. (1981). Protein turnover in animals: man in his context. In Nitrogen Metabolism in Man, (London: Applied Science), pp. 391–408.

Reid, J.T., Bensadoun, A., and Bull, L.S. (1968). Some peculiarities in the body composition of animals. In Body Composition in Animals and Man.

Reilly, S.M., McElroy, E.J., and Biknevicius, A.R. (2007). Posture, gait and the ecological relevance of locomotor costs and energy-saving mechanisms in tetrapods. Zoology (Jena) *110*, 271–289.

Richardson, A., and Cheung, H.T. (1982). Current concepts: I. The relationship between age-related changes in gene expression, protein turnover, and the responsiveness of an organism to stimuli. Life Sci *31*, 605–613.

Riggs, J.E. (1994). Carcinogenesis, genetic instability and genomic entropy: insight derived from malignant brain tumor age specific mortality rate dynamics. J Theor Biol *170*, 331–338.

Rossi, D.J., Jamieson, C.H.M., and Weissman, I.L. (2008). Stems Cells and the Pathways to Aging and Cancer. Cell *132*, 681–696.

Russell, K., Lobley, G.E., and Millward, D.J. (2003). Whole-body protein turnover of a carnivore, *Felis silvestris catus*. Br J Nutr *89*, 29–37.

Ryazanov, A.G., and Nefsky, B.S. (2001). Protein turnover plays a key role in aging. Mech Ageing Dev *123*, 207–213.

Schulz, C., Wood, C.G., Jones, D.L., Tazuke, S.I., and Fuller, M.T. (2002). Signaling from germ cells mediated by the rhomboid homolog stet organizes encapsulation by somatic support cells. Development *129*, 4523–4534.

Selman, C., Lingard, S., Choudhury, A.I., Batterham, R.L., Claret, M., Clements, M., Ramadani, F., Okkenhaug, K., Schuster, E., Blanc, E., et al. (2008). Evidence for lifespan extension and delayed age-related biomarkers in insulin receptor substrate 1 null mice. FASEB J *22*, 807–818.

Shanley, D.P., and Kirkwood, T.B. (2000). Calorie restriction and aging: a life-history analysis. Evolution *54*, 740–750.

Shyh-Chang, N., Daley, G.Q., and Cantley, L.C. (2013). Stem cell metabolism in tissue development and aging. Development *140*, 2535–2547.

Smith, R.E. (1956). Quantitative relations between liver mitochondria metabolism and total body weight in mammals. Ann N Y Acad Sci *62*, 405.

Sniegowski, P.D., Gerrish, P.J., Johnson, T., and Shaver, A. (2000). The evolution of mutation rates: separating causes from consequences. Bioessays *22*, 1057–1066.

Sousa-Victor, P., García-Prat, L., Serrano, A.L., Perdiguero, E., and Muñoz-Cánoves, P. (2015). Muscle stem cell aging: regulation and rejuvenation. Trends Endocrinol Metab *26*, 287–296.

Sousa-Victor, P., Gutarra, S., García-Prat, L., Rodriguez-Ubreva, J., Ortet, L., Ruiz-Bonilla, V., Jardí, M., Ballestar, E., González, S., Serrano, A.L., et al. (2014). Geriatric muscle stem cells switch reversible quiescence into senescence. Nature *506*, 316–321.

Speakman, J.R., Van Acker, A., and Harper, E.J. (2003). Age-related changes in the metabolism and body composition of three dog breeds and their relationship to life expectancy. Aging Cell *2*, 265.

Storch, J., and Kleinfeld, A.M. (1985). The lipid structure of biological membranes. Trends Biochem Sci *10*, 418.

Strang, K.T., and Steudel, K. (1990). Explaining the scaling of transport costs: the role of stride frequency and stride length. J Zool (London) *221*, 343–358.

Suda, T., Takubo, K., and Semenza, G.L. (2011). Metabolic regulation of hematopoietic stem cells in the hypoxic niche. Cell Stem Cell *9*, 298–310.

Suto, D., Ikeda, Y., Fujii, J., and Ohba, Y. (2006). Structural analysis of amino acids, oxidized by reactive oxygen species and an antibody against N-formylkynurenine. J Clin Biochem Nutr *38*, 107–111.

Szathmáry, E., and Smith, J.M. (1995). The major evolutionary transitions. Nature *374*, 227–232.

Tacutu, R., Craig, T., Budovsky, A., Wuttke, D., Lehmann, G., Taranukha, D., Costa, J., Fraifeld, V.E., and de Magalhães, J.P. (2012). Human Ageing Genomic Resources: integrated databases and tools for the biology and genetics of ageing. Nucleic Acids Res *41*, D1027–1033.

Tatar, M., Kopelman, A., Epstein, D., Tu, M.P., Yin, C.M., and Garofalo, R.S. (2001). A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science *292*, 107–110.

Tavernarakis, N., and Driscoll, M. (2002). Caloric restriction and lifespan: a role for protein turnover? Mech Ageing Dev *123*, 215–229.

Taylor, C.R., Heglund, N.C., and Maloiy, G.M. (1982). Energetics and mechanics of terrestrial locomotion. I. Metabolic energy consumption as a function of speed and body size in birds and mammals. J Exp Biol *97*, 1–21.

Taylor, K.R., Milone, N.A., and Rodriguez, C.E. (2016). Four Cases of Spontaneous Neoplasia in the Naked Mole-Rat (Heterocephalus glaber), A Putative Cancer-Resistant Species. J Gerontol a Biol Sci Med Sci.

Tomasetti, C., and Vogelstein, B. (2015). Cancer etiology. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. Science *347*, 78–81.

Tothova, Z., Kollipara, R., Huntly, B.J., Lee, B.H., Castrillon, D.H., Cullen, D.E., McDowell, E.P., Lazo-Kallanian, S., Williams, I.R., Sears, C., et al. (2007). FoxOs Are Critical Mediators of Hematopoietic Stem Cell Resistance to Physiologic Oxidative Stress. Cell *128*, 325–339.

Trindade, L.S., Aigaki, T., Peixoto, A.A., Balduino, A., Mânica da Cruz, I.B., and Heddle, J.G. (2013). A novel classification system for evolutionary aging theories. Front Genet *4*, 25.

Turner, N., Else, P.L., and Hulbert, A.J. (2003). Docosahexaenoic acid (DHA) content of membranes determines molecular activity of the sodium pump: implications for disease states and metabolism. Naturwissenschaften *90*, 521–523.

Turner, N., Hulbert, A.J., and Else, P.L. (2005). Sodium pump molecular activity and membrane lipid composition in two disparate ectotherms, and comparison with endotherms. J Comp Physiol B *175*, 77–85.

Twig, G., Elorza, A., Molina, A.J.A., Mohamed, H., Wikstrom, J.D., Walzer, G., Stiles, L., Haigh, S.E., Katz, S., Las, G., et al. (2008). Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J *27*, 433–446.

Verzijl, N., DeGroot, J., Thorpe, S.R., Bank, R.A., Shaw, J.N., Lyons, T.J., Bijlsma, J.W.J., Lafeber, F.P.J.G., Baynes, J.W., and TeKoppele, J.M. (2000). Effect of Collagen Turnover on the Accumulation of Advanced Glycation End Products. J Biol Chem *275*, 39027–39031.

Vijg, J., and Campisi, J. (2008). Puzzles, promises and a cure for ageing. Nature *454*, 1065–1071.

Wai, T., Teoli, D., and Shoubridge, E.A. (2008). The mitochondrial DNA genetic bottleneck results from replication of a subpopulation of genomes. Nat Genet *40*, 1484–1488.

Wallace, D.C. (1999). Mitochondrial Diseases in Man and Mouse. Science *283*, 1482–1488.

Waterlow, J.C. (2006). Protein turnover (Oxfordshire: CABI).

West, G.B., Brown, J.H., and Enquist, B.J. (1997). A general model for the origin of allometric scaling laws in biology. Science *276*, 122–126.

West, G.B., and Brown, J.H. (2005). The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. J Exp Biol *208*, 1575–1592.

White, C.R., and Seymour, R.S. (2005). Allometric scaling of mammalian metabolism. J Exp Biol *208*, 1611–1619.

1585 Williams, G.C. (1957). Pleiotropy, Natural Selection, and the Evolution of Senescence. Evolution *11*, 398–411.

1586 Yaniv, Y., Juhaszova, M., and Sollott, S.J. (2013). Age-related changes of myocardial ATPsupply and demand mechanisms. Trends
1587 Endocrinol Metab *24*, 495–505.

1588 Yockey, H.P. (1974). An application of information theory to the Central Dogma and the Sequence Hypothesis. J Theor Biol *46*, 369–
1589 406.

1590 Youle, R.J., and Narendra, D.P. (2011). Mechanisms of mitophagy. Nat Rev Mol Cell Biol *12*, 9–14.

1591 Zangarelli, A., Chanseaume, E., Morio, B., Brugère, C., Mosoni, L., Rousset, P., Giraudet, C., Patrac, V., Gachon, P., Boirie, Y., et al.
1592 (2006). Synergistic effects of caloric restriction with maintained protein intake on skeletal muscle performance in 21-month-old rats:
1593 a mitochondria-mediated pathway. FASEB J *20*, 2439–2450.

1594 Zglinicki, von, T. (2002). Oxidative stress shortens telomeres. Trends Biochem Sci *27*, 339–344.

1595 Zhang, Y., Zhang, L., Zhang, L., Bai, J., Ge, H., and Liu, P. (2010). Expression changes in DNA repair enzymes and mitochondrial DNA
1596 damage in aging rat lens. Mol Vis *16*, 1754–1763.

1597 Zheng, W., Khrapko, K., Coller, H.A., Thilly, W.G., and Copeland, W.C. (2006). Origins of human mitochondrial point mutations as
1598 DNA polymerase γ-mediated errors. Mutat Res *599*, 11–20.

1599 Zhou, B.B., and Elledge, S.J. (2000). The DNA damage response: putting checkpoints in perspective. Nature *408*, 433–439.

1600 Zhou, H.-X. (2010). Rate theories for biologists. Quart Rev Biophys *43*, 219–293.

1601 de Magalhães, J.P., Costa, J., and Church, G.M. (2007). An analysis of the relationship between metabolism, developmental
1602 schedules, and longevity using phylogenetic independent contrasts. J Gerontol a Biol Sci Med Sci *62*, 149–160.

1603 de Magalhães, J.P., Curado, J., and Church, G.M. (2009). Meta-analysis of age-related gene expression profiles identifies common
1604 signatures of aging. Bioinformatics *25*, 875–881.