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A Multidisciplinary Approach to Explain Biological Aging and Longevity

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1   *Abstract*

2   Scientists have been unable to reach a consensus on why organisms age and why they live as long as they do. Here, a multidisciplinary

3   approach was taken in an attempt to understand the root causes of aging. Nonequilibrium thermodynamics may play a previously

4   unappreciated role in determining longevity by governing the dynamics of degradation and renewal within biomolecular ensembles

5   and dictating the inevitability of fidelity loss. The proposed model offers explanations for species longevity trends that have been

6   previously unexplained and for aging-related observations that are considered paradoxical within current paradigms—for example,

7   the elevated damage levels found even in youth within many long-lived species, such as the naked mole-rat. This framework questions

8   whether declining selective pressure is the primary driver of aging, and challenges major tenets of the disposable soma theory.

9   Unifying pertinent principles from diverse disciplines leads to a theoretical framework of biological aging with fewer anomalies, and

10   may be useful in predicting outcomes of experimental attempts to modulate the aging phenotype.

1   Introduction

12   Many theories to explain why organisms age have been proposed. Concepts from evolutionary theory, genetics, biochemistry, and

13   cellular and molecular biology are most often used as the basis of these theories. Despite the fact that these efforts have resulted in

14   a multitude of theories, each with serious anomalies, the focus over the last half century has remained in these areas—more

15   fundamental physical law has been under or incorrectly applied, or simply ignored altogether. Notwithstanding the fact that

16   deterioration is implicated nearly universally in the aging process, the connection to nonequilibrium thermodynamics and entropy

17   production has not been firmly established and is infrequently mentioned. There have been a few notable exceptions; for example,

18   Hayflick contends that entropy alone is sufficient to explain biological aging (Hayflick 2000; Hayflick 2004; Hayflick 2007b; Hayflick

19   2007a).

20   The second law of thermodynamics (hereafter abbreviated to "second law") stipulates that all energy, regardless of form, has a

21   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

22   ability of contributing external factors to counteract this tendency. Entropy is a measure of this "spreading out" of energy. In any

23   system not in thermodynamic equilibrium, the second law stipulates that entropy will be produced by means of irreversible processes.

24   This entropy is referred to as "internal entropy". A nonequilibrium system will continue to produce internal entropy indefinitely until

25   equilibrium is reached, resulting in a transition from a higher concentration of molecular bond energy to a lower bond energy

26   concentration (Demirel 2014).

27   It has been argued that the second law only relates to isolated systems and that since organisms are open systems the second law

28   does not apply (Mitteldorf 2010). This is false—the second law is universally applicable and always satisfied (Kondepudi and Prigogine

29   2014). According to modern nonequilibrium thermodynamics, the second law describes the tendency for internal entropy to be

30   produced by any system that is not in equilibrium. Clearly, organisms are not in thermodynamic equilibrium and therefore all living

31   organisms continuously produce internal entropy. Despite existing in a nonequilibrium state, individual organisms resist the decay

32   towards equilibrium, which eventually results in death, long enough to allow them to mature and reproduce. The term "longevity", as

33   used here, refers to the average length of time that individuals of a group live under ideal conditions (which can range between hours

34   to centuries, depending on the species).

35   Organisms combat entropy increases resulting from internal entropy production by exchanging heat and other forms of energy with

36   their surroundings and importing/exporting negative or positive entropy in the form of metabolites and catabolites. Some scientists

37   have suggested that, using these means, any entropy increase in an organism can always be counteracted without permanent negative

38   repercussions to the individual; from this, some have concluded that there is no thermodynamic stipulation for aging to occur and no

39   role for thermodynamics in explaining aging. This is a rather obvious *non sequitur*—yet this notion has been perpetuated in the aging

40   literature, both explicitly and implicitly (Kirkwood 1999; Mitteldorf 2010; Trindade et al. 2013). Here it is demonstrated why this

41   inference is a logical fallacy and how it neglects to consider critical effects of thermodynamic phenomena within an organism—

42   particularly the influence of internal entropy production on the flow of biomolecular-encoded information over time and the dynamics

43   of degradation and renewal within biomolecular ensembles.

2   Nonequilibrium Thermodynamics Stipulates Biomolecular Damage

Thermodynamic equilibrium is characterized by the total absence of thermodynamic potentials within a system and the minimization of free energy. As the atomic arrangement of biomolecules within an organism fails to maximize free energy[1]—energy remains highly concentrated due to the chemical bonds holding biomolecules together—the second law stipulates that irreversible processes driving the system in the direction of equilibrium must occur and impose insults on biomolecular structure. The resulting thermodynamic fluxes (thermal, chemical, diffusive, mechanical, electrical, etc.) within an organism contain significant spatial and temporal heterogeneity at both the mesoscopic and macroscopic levels, providing a multitude of opportunities for biomolecular interactions leading to undesirable structural states. Examples include: mechanical force-based unfolding of proteins; unzipping or shearing of nucleic acids; protein folding alterations and undesirable 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 all else being equal it is beneficial for an organism to minimize the probabilities of these occurrences, it is impossible to reduce them to zero. Organisms counteract this degradation by producing negative entropy. In terms of preservation of overall biomolecular[2] integrity, an organism could be viewed as 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

A model system is defined below to demonstrate the degradative effects of thermodynamic chemical forces on biomolecules. The analysis focuses on a single type of biomolecule existing in a fixed volume of cytosol. The biomolecule of interest could be any expressed protein, synthesized lipid or other biomolecule produced by the organism's cellular machinery. For this analysis, the temperature and pressure of the system are in equilibrium with the surroundings and equivalent to physiological values. The concentrations of all molecules apart from the biomolecule of interest are held constant by chemiostats. The system remains in thermomechanical equilibrium at all times. Chemical reactions not involving the biomolecule of interest are inhibited (reaction rates and affinities are zeroed), as are all biomolecular repair and replacement mechanisms. At time t_0, the biomolecule of interest is in a state where all of its constituents are 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 biomolecule 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

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

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 occur and produce internal entropy. The rate of internal entropy production d_iS/dt at any time t up until equilibrium can be expressed as

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

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.

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[1] Not to be confused with free energy minimization during protein folding and the conformational changes of other biomolecules, which occurs very quickly by comparison. In these examples, the free energy minimization involves conformation options for a given atomic structure that can be transitioned to within a 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.

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80 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

81 represents a state where all of the examined biomolecule have fully degraded and internal entropy production has ceased.

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

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

(3)

83 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

84 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.

85 Utilizing the change in system entropy $dS_{sys,t}$ for any time t and the increase in system entropy corresponding to the equilibrium

86 condition $dS_{sys,max}$, we define a new parameter termed "degradation state" D to represent the degree to which the biomolecule

87 under examination has degraded

$$D = \frac{dS_{sys,t}}{dS_{sys,max}}$$

(4)

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

89 Of course, degradative internal entropy production within a living organism is not limited to chemical reactions but also includes

90 contributions from heat, mass, and momentum transfer as well as electrical, magnetic, diffusion and other effects. Modern

91 nonequilibrium thermodynamic theory (and the second law in particular) can be utilized to similarly model each of these factors,

92 establishing an arrow of time stipulating that the future is distinguishable from the past by an ever-increasing quantity of total internal

93 entropy produced.

2.2   Preservation of Steady State Nonequilibrium within a Biomolecular System

95 For homeostasis—i.e. steady state—to be preserved and to avoid transitioning to an equilibrium state with maximum disorder,

96 degradation must be combatted by biological mechanisms capable of producing sufficient negative entropy to fully offset the internal

97 entropy produced, such that the total system entropy is unchanged:

$$\dot{S}_{sys} = \frac{d_eS}{dt} + \frac{d_iS}{dt} = 0$$

(5)

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

$$\frac{d_eS}{dt} = -\frac{d_iS}{dt} < 0$$

(6)

99 Suppose that a renewal mechanism is incorporated which exchanges $\dot{N}_{rc}$ moles s^{-1} of degraded biomolecules with newly expressed or

100 fully repaired biomolecules. A steady state is maintained if

$$\overline{D}_{ren}\frac{-dS_{B,max}}{N_{tot}}\dot{N}_{rc} = -\frac{d_iS_B}{dt}$$

(7)

101 where $\overline{D}_{ren}$ represents the average degradation state of a renewed biomolecule and N_{tot} is the total quantity of the biomolecule of

102 interest in moles. (The rate of internal entropy production will be denoted by $\dot{S}_i$ instead of d_iS_B/dt hereafter for purposes of clarity.

103 Also, note that S_B and $S_{B,max}$ refer to the fraction of the entropy associated with the total population of the biomolecule being

104 analyzed.) Eq. (7) can be rearranged to solve for renewal rate during steady state.

$$\dot{N}_{rc} = \dot{S}_i N_{tot}\left(\overline{D}_{ren} * dS_{B,max}\right)^{-1}$$

(8)

105 The degradation state D of a biomolecular ensemble specifies the level of degradation of the average biomolecule but it does not

106 indicate how well biomolecules perform at that degradation state. The term "biomolecular performance" P will be used to quantify

107 the relative ability of a biomolecular ensemble to perform its intrinsic biological function(s). A value for P of 1 indicates that the

108 average biomolecule in an ensemble is able to perform 100% of its intrinsic biological function(s), or in other words, the ensemble will

109 perform as if all biomolecules were in ideal condition. A P of 0 denotes that the average biomolecule is degraded to the extent that it

110 is unable to perform any of its intrinsic biological function.

5

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 likely be functional at any given time. It is hypothesized here that the falloff in P with increasing D could be approximated for many biomolecules by a logistic function. The following 4-parameter logistic function describing the relationship between P and D produced an acceptable fit to the empirical data examined here:

$$P(D) = C\left[1 + \left(k\left(D - D_{off}\right)\right)^{\delta}\right]^{-1} \tag{9}$$

where C, k, D_{off}, and δ are the fitting parameters.

2.4   Biomolecular Work Rate is a Primary Determinant of the Rate of Degradative Internal Entropy Production

A transfer of energy occurs when a biomolecule performs its biological function, resulting in a period of time when the concentration of energy in close proximity to the biomolecule is significantly elevated compared to the resting (or nonworking) state. This generates higher thermodynamic potentials in the participating biomolecules and therefore elevates $\dot{S}_i$—i.e. the rate of damage increases transiently during biological processes. This work-related damage is one and the same as that attributed to the inherent imperfectness of all biological processes, as discussed by Gladyshev (2013; 2014; 2016). Since it is impossible for a biological process to occur without a transfer of energy and hence increased thermodynamic potentials, no biological process can be perfect and stochastic deleterious biomolecular insults will inevitably occur at a higher rate while biological processes are in progress. All else being equal, a biomolecular pool that is inactive (not performing any intrinsic biological functions) will exhibit lower $\dot{S}_i$ than one in which biomolecules spend more time in the active (working) state.

2.5   Relating Internal Entropy Production Rate to Degradation State

The fraction of time that a biomolecule performs doing work compared to when the biomolecule is inactive is an important factor in determining the rate of internal entropy production. In addition, biomolecules existing at a lower degradation state, and thus exhibiting higher performance, are capable of performing more work within a given period of time and in doing so will transfer energy at a higher rate during a biological process, thereby leading to higher rates of damaging internal entropy production.

It is hypothesized here that, for a given biomolecule, the amount of internal entropy produced per unit of work produced is similar across a relatively large range of physiologically useful P/D values yet not without variance. The following equation summarizes this model of the rate of internal entropy production within a biomolecular ensemble resulting from work

$$\dot{S}_{i,W} = dS_{B,max}Kw_{dc}P \times \varphi(D) \tag{10}$$

w_{dc} is the work duty cycle (the relative amount of time biomolecules spend doing work, a value between 0 and 1) and K is a scaling factor. The function $\varphi(D)$ compensates for any non-linearities in internal entropy production rate across degradation state.

Internal entropy is also produced in the static (non-working) conditions. In the described model system, the magnitude of the thermodynamic forces on the biomolecules of interest during non-working conditions should remain essentially constant and be independent of P or D. The rate of internal entropy production during non-working conditions is represented here by $L \times \varphi(D)$, where L is a scaling constant for non-working conditions. The total internal entropy production is thus

$$\dot{S}_i = dS_{B,max}(L + Kw_{dc}P) \times \varphi(D) \tag{11}$$

From Eq. (4) we know that $\dot{S}_i = dS_{B,max}\dot{D}$. Combining this with the previous equation leads to an expression for the rate of change of the degradation state

$$\dot{D} = (L + Kw_{dc}P) \times \varphi(D) \tag{12}$$

144 For a system consisting of molecules with various degrees of deterioration, $\dot{D}$ can be determined by considering the number of
145 molecules existing at each represented equivalent[3] D and the corresponding equivalent $\dot{D}$ ($\dot{D}_D$, calculated using Eq. *(12)*)

$$\dot{D} = N_{tot}^{-1} \sum \dot{D}_D N_D \qquad \qquad (13)$$

2.6   Incorporating Biomolecular Repair and Replacement Mechanisms

147 Two different renewal scenarios will be considered. For both scenarios, it is assumed that the renewal process returns the
148 biomolecules to their ideal functional condition (equivalent D of 0). In the first and simplest scenario, biomolecules are chosen for
149 renewal at random; i.e. biomolecules in perfect condition (high equivalent D) have the same probability of being renewed as those in
150 poor condition (low equivalent D). In this case, $\overline{D}_{ren}$ is equal to D.

151 To reduce the resources directed towards renewal and to maximize the return on investment into renewal, the renewal machinery
152 should be able to distinguish more degraded biomolecules from those that are less degraded. In a second renewal scenario, this ability
153 is introduced. Here, the molecules chosen for renewal are those whose equivalent P places them in the lowest "m" fraction of all
154 molecules within the ensemble.

155 Ideally, any molecular damage that decreases P would be detectable by cellular machinery. In practice, this is not possible to achieve
156 with perfection. The only way to definitively confirm that a biomolecule is in ideal condition is to observe the biomolecule while it is
157 performing work, and verify that the magnitude and quality of the work performed are consistent with a biomolecule in ideal
158 condition; this requires faultless labelling and work-measurement systems which in turn would require their own such systems, etc.
159 Perhaps a more practical way of identifying biomolecular damage is by the direct recognition of molecular alterations with generic
160 signatures. For example, oxidative damage can readily be recognized with universal mechanisms. However, this is only one type of
161 damage and these mechanisms are inherently imperfect. As the number of possible molecular alterations that could affect
162 biomolecular equivalent P is essentially unlimited, recognizing every one of these alterations—and doing so with perfect precision—
163 is simply not possible.

2.7   Balanced Biomolecular Renewal and Degradation: Numerical Approximation of the Steady State Solution

165 A biomolecular ensemble undergoing continuous renewal and degradation will consist of biomolecules distributed across a range of
166 equivalent P / D values. As it is cumbersome to find a closed-form solution of this model, here an approximate solution is calculated
167 numerically using iteration. By combining Eqs. *(4)*, *(8)* and *(13)*, it can be shown that a steady state will be maintained within an
168 biomolecular ensemble (i.e. D will remain constant) when the following condition is satisfied

$$\left(\sum \dot{D}_D N_D \right) - \overline{D}_{ren} \dot{N}_{rc} = 0 \qquad \qquad (14)$$

169 That is to say that any positive contribution to D resulting from internal entropy production must be counterbalanced with an
170 equivalent negative contribution from renewal. The magnitude of the left-hand side of Eq. *(14)* is therefore a measure of solution
171 convergence.

172 In the numerical analysis undertaken here, a fixed number of biomolecules are distributed across a range of D values. The initial
173 distribution at $t = 0$ is not important. For all values of D other than 0 and for each time point $+\Delta t$, the number of molecules at each
174 equivalent D value represented, N_D, is calculated

$$N_D^{t+\Delta t} = N_D^{t} + \left(N_{D-\Delta D}^{t} \dot{D}_{D-\Delta D}^{t} - N_D^{t} \dot{D}_D^{t} - \dot{N}_{ren,D}^{t} \right) \Delta t \qquad \qquad (15)$$

175 where $\dot{N}_{ren,D}$ is the rate at which molecules of a particular equivalent D are renewed. This value is calculated according to the renewal
176 scenario (from section 2.6) that is specified and using the distribution of biomolecules across equivalent degradation states at time t.
177 $\dot{D}_D$ and $\dot{D}_{D-\Delta D}$ are calculated per Eq. *(12)* for each represented D.

178 For the present analyses, it is assumed that $\varphi(D) = 1$. This is done because (1) determining $\varphi(D)$ would require knowing the system
179 entropy for different P values and this is a difficult property to measure empirically, (2) it is reasonable to hypothesize that φ is
180 relatively constant within the utilized functional ranges found within organisms since any significant perturbations in $\varphi(D)$ would
181 indicate either an undesirable vulnerability or unnecessary stability for the sake of some other factor, and (3) the performance/renewal

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[3] As D and P are ensemble properties, when referring to individual molecules we will prepend the qualifier 'equivalent' to acknowledge this and to indicate that the referenced biomolecule's condition is analogous to the typical biomolecule within an ensemble at that given value.

182 rate insights that follow remain valid because D and $\dot{S}_i$ are intermediate variables in these calculations (the effects of φ will effectively
183 cancel out). D and $\dot{S}_i$ have been retained in the derivations because conceptually they help to convey a more thorough understanding
184 of the underlying physical phenomenon at play.

185 For the case where $D = 0$, the following is used in lieu of Eq. *(15)* (Note that all renewed molecules are initialized to $D = 0$.)

$$N_D{}^{t+\Delta t} = \left(\dot{N}_{rc} - N_D{}^t \dot{D}_D{}^t - \dot{N}_{ren,D}{}^t \right) \Delta t \qquad (16)$$

186 If the solution failed to converge to within a specified tolerance after a given number of iterations, the simulation was restarted using
187 altered parameters to increase precision (e.g. larger vector of D values, reduced Δt) at the expense of increased computational
188 resources. These steps were repeated until convergence was reached.

189 ## 2.8   Realizable Work Rate and Biological Power Density
190 The achievable rate of work per biomolecule $\dot{W}_{m,act}$ can be expressed as

$$\dot{W}_{m,act} = w_{dc} P \dot{W}_{m,max} \qquad (17)$$

191 where $\dot{W}_{m,max}$ is the maximum potential external work rate per molecule (corresponding to $P = 1$ and $w_{dc} = 1$).

192 "Biological power density", represented by σ, is defined as the volume-specific rate of external work (power per unit volume) realized
193 in a biomolecular ensemble. In this context, "external work" refers to the sum of the biomechanical, biochemical and
194 bioelectrical/electromagnetic work, representative of the intrinsic biological function of the biomolecule being analyzed, which is
195 brought to bear on the immediate environment. Examples include the mechanical work produced by myosins, and the chemical and
196 electrical work produced by cell surface receptors. Biological power density is given by

$$\sigma = \rho \dot{W}_{m,act} = \rho w_{dc} P \dot{W}_{m,max} \qquad (18)$$

197 where ρ is the volumetric density (number of biomolecules per unit volume).

198 ## 2.9   Demonstration of Model using Empirical Data
199 Given sufficient data describing the degradation characteristics of a biomolecule, the above model can be used to predict the following
200 steady state properties: (a) the distribution of biomolecules across equivalent P values for any renewal rate; (b) how P varies with
201 renewal rate; and (c) the renewal return on investment (ROI), calculated by dividing the work rate by renewal rate, as a function of P.
202 The degradation dynamics of the biomolecule under work conditions must be known; i.e. how P diminishes over time while the
203 biomolecules are actively performing work and no renewal is occurring. The degradation dynamics under non-working conditions are
204 required if static degradation is to be considered.

205 Several factors make fluorescent biomolecules a good choice for demonstrating this model. For one, the energy they use to produce
206 work comes from photons rather than a molecular energy source (e.g. ATP). Utilizing a readily available light source, a consistent and
207 controllable energetic flux can be provided externally without disturbing the molecular composition of the system under analysis. This
208 eliminates the need for chemiostats and the renewal/removal of molecular substrate. Since the work that is performed by fluorescent
209 molecules is also realized in the form of photons, measuring the amount of work performed is straightforward.

210 P will decrease as fluorescent molecules absorb and emit photons. This is mostly a result of photobleaching, the irreversible loss of
211 fluorescence due to photochemical modification of the fluorophore. Photobleaching is believed to be largely caused by interactions
212 between excited fluorophores and triplet oxygen, leading to irreversible chemical damage to the fluorophore (Diaspro et al. 2006).
213 The probability that a photon collision will result in excitation decreases with each subsequent photon absorbed. Since photobleaching
214 damage is sustained almost exclusively when the fluorophore is in the excited state, incidental damage from photon collisions that fail
215 to excite the fluorophore can be disregarded; i.e. provided that static degradation is slow compared to the rate of degradation from
216 work, we can assume that the vast majority of any degradation observed is a direct result of producing work and not due to off target
217 damage from the energy source.

218 All collisions between a photon and a fluorescent biomolecule will be considered work events, regardless of whether or not excitation
219 occurs (i.e. even if no work is produced). The work event period is defined as the average amount of time for photon absorption and

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220 emission to occur. w_{dc} can be precisely modulated by altering the intensity of the excitation source. The time to degrade under non-
221 renewing conditions from an initial P of 1 to any given P can be calculated

$$t_{deg}(P) = \int_0^{D(P)} \frac{dD}{\dot{D}}$$

(19)

222 Using this equation together with previously published photobleaching data (Shaner et al. 2004), fitting parameters for Eq. (9) were
223 determined for the fluorescent biomolecules eGFP, mRFP1, and mTangerine. Fig. 1A demonstrates the quality of the fit between the
224 actual and predicted P-t curves for the parameter values calculated. Since the illumination intensity for each fluorophore (normalized
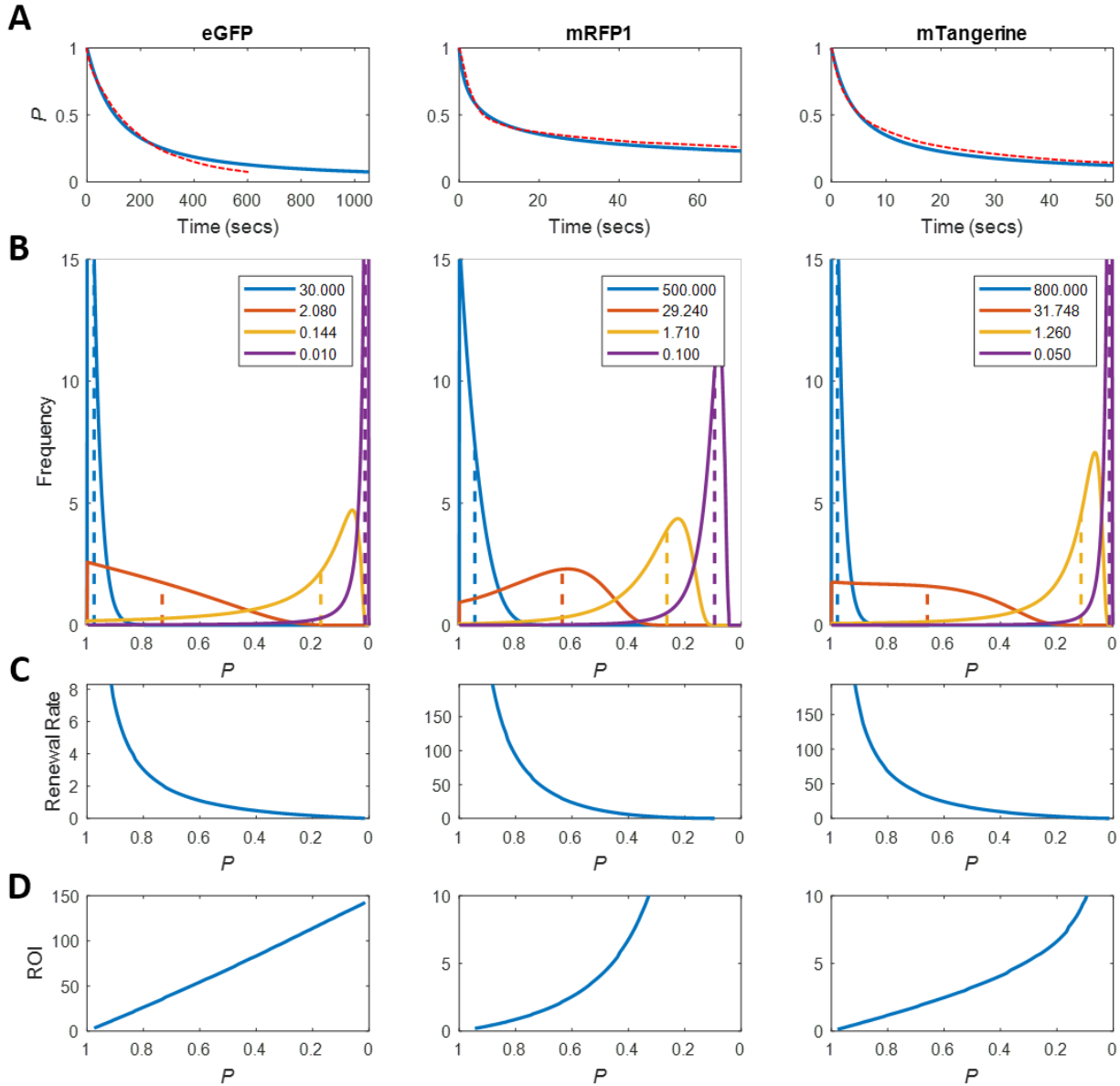
Fig. 1. Relating degradation, renewal and performance characteristics in eGFP, mRFP1, and mTangerine populations. (a) Loss of P with time in a non-renewing population of eGFP, mRFP1, and mTangerine under work-producing conditions. Fitted curves (solid blue lines) versus empirical data (dashed red lines) from Shaner et al. (2004). All curves were fit to Eq. (9). Fitting parameters: eGFP — $C = 2.7438$, $k = 0.1354$, $D_{off} = -7.3996$, $\delta = 350.2$; mRFP1 — $C = 2.5414$, $k = 837.3$, $D_{off} = -0.0025$, $\delta = 0.6$; mTangerine — $C = 1.3666$, $k = 38.2428$, $D_{off} = -0.0152$, $\delta = 1.85$. (b) Simulation of distribution of biomolecules across equivalent P values for different rates of random renewal. Each simulation was iterated through until the solution converged to steady state. Renewal rates are in % of total population replaced per second. e.g. 500% indicates that the typical molecule is renewed 5 times every second. Dashed vertical lines indicate P of the ensemble (i.e. average equivalent P). (c) Relationship between P (ensemble) and renewal rate under random renewal conditions. (d) Renewal ROI versus P under random renewal. ROI is in % of $\dot{W}_{m,max}$ / renewal rate. All simulations were performed with $N_{rc} = 1e9$.

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225 such that each molecule emitted 1,000 photons/s at time t = 0) remained constant throughout each experiment, w_{dc} can also be
226 considered constant across each degradation curve (although not necessarily the same between fluorophores). The initial rate of work
227 (photon emission) is considered to be representative of $P = 1$.

2.10   High P is Energetically Costly. Perfect Fidelity in a Biomolecular Ensemble is Impossible.

229 In order to maintain a specific P in any biomolecular ensemble, renewal must take place; otherwise, P will decrease with time. Random
230 renewal was simulated in eGFP, mRFP1, and mTangerine (using the fitting parameters found to approximate the deterioration
231 characteristics of each fluorescent biomolecule). Only work-related degradation was considered. For each renewal rate, the simulation
232 was allowed to iterate until convergence was achieved. It can be seen that the distribution of molecules across equivalent P values
233 varies with renewal rate and is also dependent on the degradation characteristics of the biomolecule (Fig. 1B).

234 The rates of renewal required to maintain a particular P are shown in Fig. 1C. A common trend is a dramatic increase in renewal rate
235 as P approaches 1, suggesting that achieving very high P requires an ever more costly investment in renewal. This trend is to be
236 expected in any biomolecular ensemble and is due to several factors. Firstly, since the average equivalent D of a replaced molecule is
237 lower when P is higher, the amount of negative entropy produced by each molecular renewal event will decrease with increasing P.
238 Second, for a static w_{dc}, the rate of internal entropy production increases with increasing P (as the amount of work produced
239 increases) which further contributes to an increased renewal rate for larger P values. Furthermore, there is always a non-zero
240 probability that some level of degradation will occur between the time a biomolecule is synthesized and the time it is renewed.
241 Maintaining a P of 1 within any ensemble is therefore impossible.

242 Renewal ROI trends downwards as P approaches 1 (Fig. 1D). In each case illustrated here, the lowest P values equated to the highest
243 renewal ROI. Biomolecules that initially degrade very rapidly but then transition to a much slower rate of degradation (e.g. mRFP1,
244 mTangerine) exhibit a more dramatic increase in renewal ROI with decreasing P than biomolecules with more linear degradation rates
245 (e.g. eGFP). Biomolecules that are considerably more stable at higher P values may exhibit a peak in their P-ROI curve, although ROI
246 will always decline as P approaches 1 (see Supp. Fig. 1 for a hypothetical example).

2.11   Effective Damage Recognition Mechanisms Increase Renewal ROI

248 To demonstrate the effects of biomolecular damage-recognition mechanisms, renewal selection was next biased towards
249 biomolecules exhibiting the lowest equivalent P. The parameter m specifies the lowest performing fraction of the population from
250 which the renewal machinery samples randomly upon each renewal event, ranked in terms of equivalent performance. For example,
251 an m of 0.30 indicates that those biomolecules with an equivalent P in the lowest 30% of all biomolecules constitute the renewal pool
252 of molecules while the remaining 70% of the population (the better performing molecules) will not be selected for renewal. Since
253 biomolecules degrade with time and renewed biomolecules begin life at a high equivalent P, a molecule can move between the two
254 pools over time.

255 By renewing the most degraded biomolecules, a typical renewal event will produce a larger amount of negative entropy compared to
256 a random renewal scenario. With damage recognition, a given P can be maintained with a lower rate of renewal (Fig. 2A). In addition,
257 renewal ROI will increase across the entire range of P, as demonstrated for all three fluorescent biomolecules (Fig. 2B).

258 Although renewal ROI improves with damage-recognition ability, the payoffs diminish considerably as m approaches zero. For
259 example, the renewal ROI with eGFP at $P = 0.80$ was 78.66% greater than random renewal when m was 0.20 (Table 1). Decreasing
260 m by a factor of twenty, to 0.01, improved the renewal ROI only marginally compared to $m = 0.20$ (82.30% versus 78.66%). At the
261 other end of the spectrum, a 19.64% increase in renewal ROI was realized with an m of only 0.80. These patterns were consistent
262 across all three biomolecules analyzed. This suggests that organisms do not need to invest in extremely precise damage-recognition
263 strategies to receive tangible benefits from damage-recognition capabilities; and furthermore, that extreme precision may not be
264 worth the associated extra costs.

10

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265

266
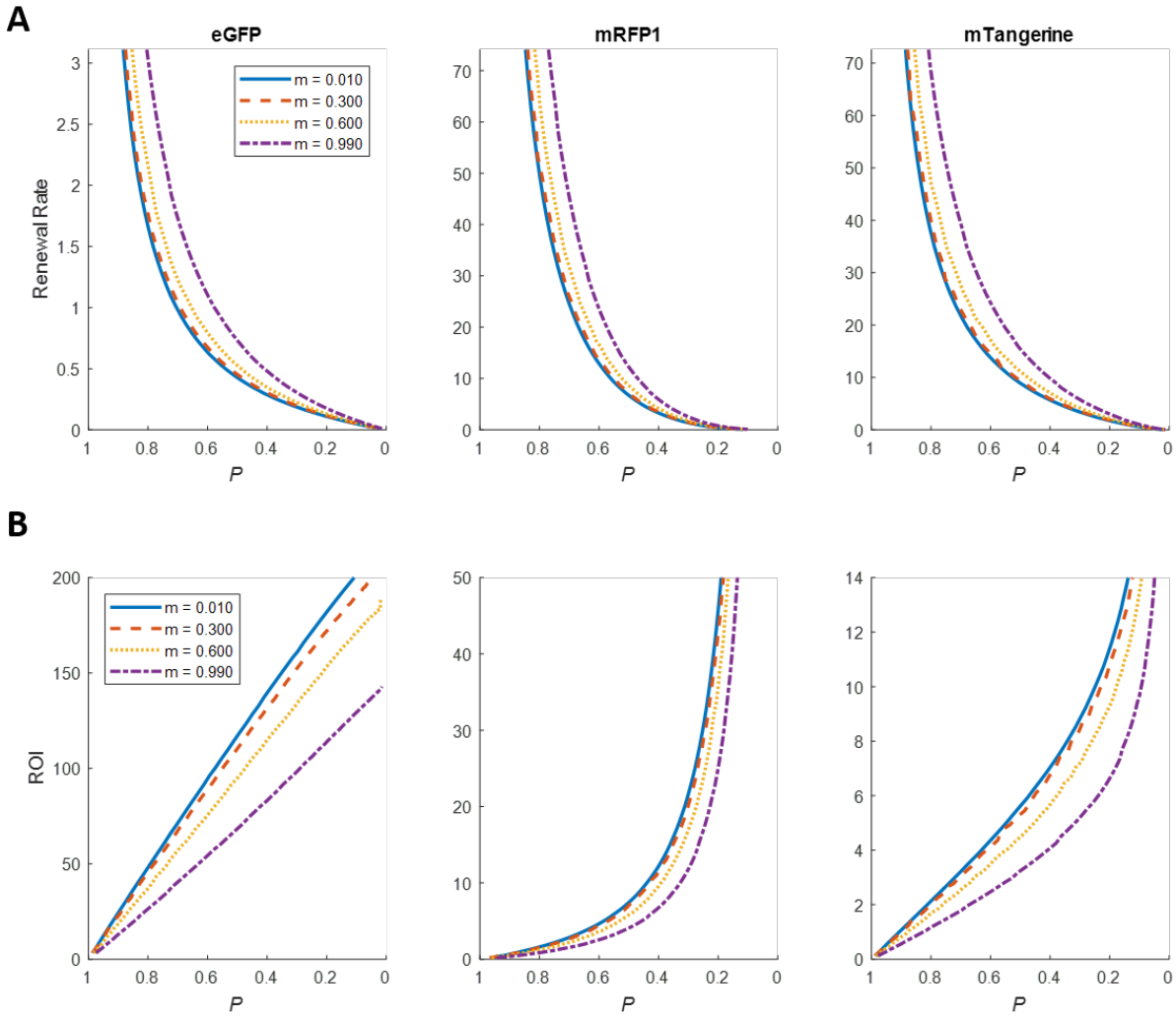
Fig. 2. The effect of varying simulation parameter 'm' on (a) renewal rate and (b) renewal ROI for eGFP, mRFP1 and mTangerine.

267

Table 1. Improvement in renewal ROI for different values of 'm' across a range of P values

	P										
% ROI↑	0.95	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.05
0.01	90.75	87.26	82.30	76.52	74.07	71.22	67.91	63.53	59.74	55.54	53.78
0.1	88.40	87.20	79.63	74.17	72.58	69.72	65.37	61.85	57.95	54.24	52.45
0.2	85.81	84.82	78.66	72.55	69.03	65.89	62.39	58.08	55.18	51.46	49.16
0.3	78.39	73.92	71.52	66.79	63.54	61.33	58.46	55.05	50.97	47.01	45.23
0.4	68.26	68.75	65.05	58.48	56.78	55.28	52.78	49.69	46.66	43.09	40.89
0.5	56.42	58.05	55.72	49.70	48.61	47.57	45.53	43.88	40.90	38.32	36.52
0.6	44.71	44.96	40.11	41.10	38.95	38.79	38.13	36.29	34.63	32.53	31.29
0.7	32.36	31.31	30.20	29.70	30.00	28.99	29.19	28.05	26.66	25.40	24.44
0.8	20.40	18.52	19.64	19.88	19.69	20.01	19.34	18.91	18.25	17.26	16.92
0.9	8.21	7.68	8.51	8.81	9.35	8.98	9.47	9.30	8.91	8.64	8.51

(Row labels on the left side labelled m)

Table values indicate the percent increase in renewal ROI compared to random renewal with eGFP.

3 Managing Degradative Internal Entropy Production within an Organism

3.1 Extrapolation of Degradation State Concepts to Larger Physical Scales

Although the outlined model was described for a population of biomolecules, a similar thermodynamics model could be applied across a wide range of physical scales. For example, organelles are renewed and face damage due to degradative internal entropy production in a conceptually similar fashion as do the individual biomolecules from which they are assembled. Mitotic cell populations may also be thought of in this way, with individual cells regarded as the renewed unit.

3.2 Structural Optimization

Many of the biological mechanisms utilized for producing negative entropy have been characterized. These include biomolecular expression systems, molecular chaperones, degradation systems (proteasomes, lysosomes) and DNA repair enzymes. 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 maintaining P in these populations for a period of time.

Reducing the rate of internal entropy production decreases the amount of negative entropy needed and, in turn, the energetic investment required to maintain 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 those forces.

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, might 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.) resulting in an increase in the biomolecule's $\dot{W}_{m,max}$. Thus, any benefits to inclusion must be weighed against the costs associated with the increased susceptibility to insult.

Other biomolecular structural optimizations that serve to reduce $\dot{S}_i$ include modifications that improve resistance to undesirable hydrophobic interactions, temperature-induced denaturation, and other alterations. A likely example of these optimizations can be found in the naked mole-rat, whose proteins are extremely resistant to urea-induced unfolding (Pérez et al. 2009).

Biomolecular modifications that optimize stability could result in a deleterious increase in the physical size of the biomolecule (limiting volumetric density) or be otherwise disadvantageous, such as by limiting the realizable rate of intrinsic biological function ($\dot{W}_{m,max}$). Modifications to biomolecular structure could also affect the amount of resources required for the production or renewal of a biomolecule.

On the other hand, structural alterations that maximize $\dot{W}_{m,max}$ may reduce biomolecular stability and increase $\dot{S}_i$. The polyunsaturated fatty acid content levels of membrane lipids, which varies across species, may represent the evolved configuration that balances biological power density and $\dot{S}_i$ according the to the specific requirements of a species. This is discussed in section 8.5.

3.3 Microenvironment Optimization

The magnitude of the degradative thermodynamic forces acting upon biomolecules is largely determined by microenvironmental conditions. 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 $\dot{S}_i$. On the other hand, higher temperatures will produce increased kinetic energy transfer during intermolecular collisions, which will increase $\dot{W}_{m,max}$.

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 should be considered that in situations where renewal ROI is highly prioritized such an increase may be acceptable if the ROI benefit from the increased D sufficiently overwhelms the downsides of an increase in $\dot{S}_i$ resulting from higher oxidative stress. Thus, in situations where an organism has evolved to place extreme priority on

314 renewal ROI (for example, due to limited availability of an energetic resource) and P is not as critical a factor, it should not be surprising
315 if the realized P is lower in these species. This will present as increased levels of degradation. Conversely, organisms that place lower
316 priority on renewal ROI are likely to function at a higher P and thus exhibit lower levels of degradation.

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

3.4   The Naked Mole-rat Paradox – Part I

321 Although oxidative damage is only one of many forms of biomolecular insult that can occur within a living organism, oxidative damage
322 levels are readily measurable and empirically derived values for various organisms have been published. The oxidative damage
323 patterns observed in the naked mole-rat (*Heterocephalus glaber*) are particularly interesting as they are not well explained by any
324 existing aging theory. The naked mole-rat has a maximum lifespan (MLSP) of ~31 years while its similar-sized cousin, the house mouse
325 (*Mus musculus*), has an MLSP of only ~4 years (Tacutu et al. 2012). Young naked mole-rats have significantly higher levels of oxidative
326 damage to proteins compared to physiologically age-matched mice (Andziak et al. 2006; Pérez et al. 2009). Furthermore, there is
327 nothing about the naked mole-rat's antioxidant assemblage that would suggest that their antioxidant defenses are any more effective
328 or efficient than that of mice (Andziak et al. 2005).

329 Naked mole-rats live in a hypoxic environment and have extremely low metabolic rates for their size (McNab 1966). It is considered a
330 paradox that the young naked mole-rat exhibits such high levels of oxidative damage while having extreme longevity compared to
331 similarly sized, closely related species. In fact, this may be predictable and straightforward to explain. The first part of the puzzle is
332 explaining why oxidative damage levels are substantially elevated in the naked mole-rat. The naked mole-rat's limited access to oxygen
333 restricts the rate of cellular ATP production via oxidative phosphorylation, thereby requiring that renewal ROI for cellular processes
334 be highly prioritized. As demonstrated earlier, the steady state conditions that maximize renewal ROI are likely to correspond with
335 increased levels of degradation. Related species that are not as energetic resource-restricted, for example the house mouse, may
336 function at higher Ps (to help maximize athletic ability, growth rate, etc.) which will correspond to lower levels of oxidative (and other)
337 damage—at least during youth. Therefore, the elevated levels of oxidative damage found in the young naked mole-rat is likely
338 indicative of the steady state balance between degradation and renewal that maximizes species fitness within the hypoxic constraints
339 of their environment. The second part of solving the naked mole-rat paradox is explaining why their high biomolecular degradation
340 states do not adversely affect MLSP. A possible explanation for this is detailed in section 8.7.

4   Thermodynamic Insights into Biological Aging

342 Youthful organisms are often perceived as existing in a state where biological structure is optimal and essentially perfect. Yet, it is not
343 possible to achieve perfect fidelity in any biomolecular ensemble—all biological systems will exhibit some level of damage, even in
344 youth. In addition, the functional state of biomolecules should not be viewed in binary terms "perfect" and "nonfunctional", since
345 there are many degrees of performance between these extremes. Rather, it is more accurate to view biomolecular pools as consisting
346 of a distribution of biomolecules across a range of performance/degradation states. As a biomolecule sustains damage, its ability to
347 perform its intrinsic biological function is compromised—but this will likely not manifest as an all or nothing scenario. For example,
348 biomolecular fluorescence decreases over time as the molecules sustain (predominantly oxidative) damage during the course of doing
349 work but, typically, many separate insults are required to completely eliminate the capacity for fluorescence emission.

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

351 Proteins account for the majority of biomolecules within a cell. The rate of total protein synthesis has been empirically determined for
352 a number of species (Table 2). Smaller organisms synthesize proteins at higher rates than larger species (Fig. 3A). In mice, protein
353 synthesis takes place at a rate sufficient to replace total body protein mass every ~5.3 days, while a human requires ~39.3 days. Of
354 course, renewal rates for individual proteins can vary widely protein-to-protein, ranging from minutes to years (Hetzer 2013).

355 Over the course of a lifetime, a long-living human will synthesize enough protein to replace total body protein mass 1137 times over
356 (Table 2). Let us assume for the moment that degraded/dysfunctional proteins are "accumulating" as an individual grows older due to
357 insufficient energy expenditure toward repair and replacement, as suggested by the disposable soma theory of aging (Kirkwood 1977;
358 Kirkwood and Holliday 1979; Kirkwood and Rose 1991). Considering a worst-case scenario where all protein in an aged human is in
359 need of replacement, it would only require an estimated 0.09% increase in daily resource investment in protein synthesis to offset the

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Table 2. Protein Synthesis Rates, Number of Days to Renew Total Body Protein Mass, and Number of Renewals per Lifespan for Different Metazoan Species

Species	BW (kg)	Protein synthesis			Body Protein Composition (%)	Days to Renew Total Body Protein Mass	Maximum Lifespan (years)[10]	Renewal 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 renew" 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.

360 average daily increase in degraded protein. This translates to 0.23 calories per day[4]. With a daily dietary intake of 2500 calories, this
361 is a mere 0.0092% of daily energy intake. Protein synthesis represents the majority of the total energy spent on biomolecular renewal,
362 and the total amount of protein dedicated to translation is 2-15 times greater than that dedicated to transcription and DNA
363 maintenance (Liebermeister et al. 2014). In light of the very small additional investment needed to offset an increase in protein
364 degradation, the disposable soma theory's claim that aging is caused by an energetic underinvestment in repair and maintenance that
365 results in an accumulation of damage (Kirkwood 1977; Kirkwood and Holliday 1979; Kirkwood and Rose 1991) is difficult to accept. In
366 addition, organisms that renew their proteins more frequently generally exhibit decreased longevity—not increased (Fig. 3B). Smaller
367 organisms renew protein at a faster rate than larger organisms (Fig. 3A) but have reduced longevity (Calder 1984; Austad 2005; de
368 Magalhães et al. 2007).

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

374 ### 4.2   Biomolecular Degradation and Aging - Accumulation versus Homeostatic Shifts
375 Aging has been described as the accumulation of unrepaired damage. This implies that the biomolecular degradation in an aged
376 individual results from lifelong accumulation. Perhaps this assessment is not entirely accurate.

377 Organisms exhibit biomolecular degradation even during youthful homeostasis. Reducing the rate of biomolecular renewal from the
378 youthful level (or any other level) will decrease P and increase the perceivable amount of degradation. Once steady state is
379 reestablished around a new renewal rate, a phenomenon that could be described as a "homeostatic shift", no further reductions in P
380 should occur—unless outside factors are at play.

381 Restoration of proteasome function in aged human dermal primary fibroblasts largely restores markers of protein aging to youthful
382 levels (Hwang et al. 2007). This is analogous to a shift in the protein pool from a lower to a higher P, and demonstrates that the
383 reduction in P occurring with age may be at least partially reversible. If the degraded protein was truly representative of accumulated,
384 irreparable damage then upregulation of proteasomal function should not have eliminated the damage. The fact that the condition is
385 essentially reversible suggests that the increased biomolecular degradation found in aged individuals is not due to damage

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[4] 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.

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"accumulation" but is more likely attributable to reduced biomolecular renewal leading to a corresponding reduction in P and thereby an increased degradation state[5]. Consistent with this notion, protein renewal does indeed significantly decline during aging (Richardson and Cheung 1982; Rattan 1996; Ryazanov and Nefsky 2001).

A distinction between *accumulated* dysfunctional biomolecules and a shift in biomolecular degradation state caused by reduced renewal can be made by determining whether renewal is actively occurring. Damage in molecules that are eventually renewed should not be referred to as "accumulated" damage, even if the rate of renewal has decreased and the degradation state of the biomolecular pool is high.

This raises further doubt over the disposable soma theory's assertion that aging is caused by an energetic underinvestment in repair and maintenance resulting in an accumulation of damage (Kirkwood 1977; Kirkwood and Holliday 1979; Kirkwood and Rose 1991). The idea of an "energetic underinvestment" is a misnomer, as no amount of energetic investment will produce a perfect population of biomolecules (i.e. a degradation state of zero). Increasing biomolecular renewal will reduce D but renewal ROI will continually worsen as renewal rate is increased. An energetic underinvestment in renewal cannot explain why youthful homeostasis is lost—there must be a higher-level initiating event. *Declining energetic expenditures towards renewal are merely another consequence of aging, not a root cause.* This begs the question of why renewal rates drop with age in the first place.

Fig. 3. (a) Protein turnover as a function of bodyweight for the species listed in Table 2 and (b) days to replace the equivalent weight of total body protein as a function of MLSP. Data from Table 1.

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

As highly ordered entities existing in thermodynamic nonequilibrium, living organisms are subject to internal entropy production leading to ongoing molecular damage. The high frequency at which the animals depicted in Table 2 replace their total body protein mass demonstrates that the rate of degradative internal entropy production $\dot{S}_{i,ind}$ within an individual organism is much greater than the rate at which the total structural entropy of the individual increases with age post reproductive maturity $\dot{S}_{tot,ind}$.

$$\dot{S}_{tot,ind} \ll \dot{S}_{i,ind} \qquad\qquad (20)$$

Eq. *(20)* 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 period of time that organisms—including those with only moderate longevity—are alive.

Cellular mechanisms work towards counteracting damage resulting from internal entropy production, coming close to establishing a steady state in terms of preservation of biomolecular integrity when observed over a limited time window. Despite the efforts of these renewal systems, the overall degradation state (i.e. the total structural entropy) of an individual organism increases with age post reproductive maturity. The aging process could be viewed as a progression through many discrete nonequilibrium steady states that eventually result in a global degradation state that renders the individual nonviable. Why then do organisms transition between these

---

[5] There are apparently some 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 renewal, the proportion of certain types of damaged product can expand, even when youthful renewal rates 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 renewal such as that which occurs with advanced age. The same proteins found to aggregate with age are produced, but concomitantly cleared, in younger individuals.

425 states and why is youthful homeostasis lost? The answer to this question may lie in how an organism combats internal entropy
426 production affecting nonrenewable structures, i.e. those subject to irreversible losses in fidelity.

5   Preservation of DNA Molecular Information

428 Most types of biomolecules are replaced by expression of a genetic sequence or are the metabolic products of expressed biomolecules.
429 The performance of these biomolecular pools can be preserved by the successful expression of the appropriate genetic sequence(s)
430 or the relevant metabolic processes, and the removal or repair of any dysfunctional counterparts. With a given rate of renewal and
431 assuming intact expression machinery, the preservation of biomolecular performance within a cell becomes dependent on: (1) the
432 fidelity of the genetic material responsible for biomolecular expression, and (2) the cell's ability to remove all dysfunctional
433 biomolecules. While the last requirement should not be trivialized, this is a very attainable objective: that is to say, the specificity of
434 degradation pathways can afford to err on the side of casting a wider net to help ensure that any dysfunctional biomolecule is
435 eventually recognized since these biomolecules can be resynthesized anew. Indiscriminate purging of cellular content would
436 eventually dispose of any unwanted products. For these reasons, fidelity preservation in biomolecules that are not expressed, the
437 genetic-encoding biomolecules, particularly warrants further scrutiny.

5.1   DNA Informational Fidelity Loss is Unavoidable

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

443 There are a number of ways that DNA damage can occur, resulting in base alterations, cross-linking, strand breaks, and other
444 modifications (De Bont and van Larebeke 2004). For example, consider some of the possible outcomes after a double-stranded DNA
445 molecule has suffered a single base excision:

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

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

457 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
458 restricted to a single base will deplete this redundancy and can lead to a permanent change in DNA information. This does not imply
459 that insults that are more serious are not repairable. For example, double-stranded breaks can be repaired by homologous
460 recombination in many cases, but there is no guarantee that a homologous site exists or that the repair will be successful.

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

467 Permanent losses in genetic fidelity are typically discussed in terms of discrete mutations or mutation rate (Sniegowski et al. 2000).
468 Perhaps a better way to assess these losses is to utilize the concept of mutual information[6], which is a measure of the amount of
469 information shared between two datasets. This provides a means to quantitate the amount of encoded data retained in, or transmitted
470 between, DNA molecules over time. As genetic data must change with time, the mutual information of a discrete, non-replicating DNA
471 molecule will decrease with time.

472 DNA insults do not only affect mutual information, they can also result in an increase (or decrease) of total information. For example,
473 insertions, duplications, and repeat expansion mutations increase the total amount of information. Although mutual information will
474 always trend downwards, it is important to recognize that the total amount of DNA information could increase, decrease, or stay the
475 same over time.

476 A means to quantify DNA informational fidelity Φ_{DNA} is to consider the ratio of mutual information shared between the two states
477 being compared I_{mutual} to the maximum of the total amount of information in the initial I_{init} and final I_{final} states.

$$\Phi_{DNA} = \frac{I_{mutual}}{\max\{I_{init}, I_{final}\}}$$

$(\,21\,)$

478 Only in the case where no original information has been lost and no new information gained will Φ_{DNA} be equal to its maximum
479 possible value of 1.

480 ## 5.2   Applicability of the Degradation State Concept to DNA Molecular Ensembles
481 Synthesized biomolecules depend on the fidelity of DNA for their correct expression. If the full fidelity of DNA is preserved then,
482 theoretically, negative entropy could be produced at a rate that maintains a steady P in any expressed biomolecular pool. Since DNA
483 molecules rely on their own fidelity for identical replacement, this biomolecular replacement scenario is not applicable to DNA
484 molecules.

485 ## 5.3   Considerations for DNA Informational Fidelity Preservation in Different Cell Types
486 For any sexually reproducing multicellular organism, the zygote contains the truest representation of the parentally derived genetic
487 data anywhere in the individual and of any stage in life, i.e. the DNA informational fidelity between parent and offspring is maximal in
488 the zygote. The informational fidelity of an organism's DNA at any later point in life can be quantified by comparing to this baseline
489 standard.

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

496 The redundancy provided by diploidy/polyploidy, gene duplication, and functional overlap provides a degree of robustness that
497 enables non-germ cells to tolerate some amount of DNA informational fidelity loss with minimal performance impact on the individual
498 (Medvedev 1972; Yockey 1974; Riggs 1994; Plata and Vitkup 2013). Similar levels of damage would be more detrimental in germ cells
499 as they would propagate to all cells of the progeny. Therefore, the average DNA informational fidelity of germ cells must be greater
500 than that of adult stem cells at the time of reproduction, which in turn must be greater than the DNA informational fidelity of non-
501 stem somatic cells at the time of reproduction. This relation can be written

$$\overline{\Phi}_{DNA}\big(zyg; som_{rep}\big) < \overline{\Phi}_{DNA}\big(zyg; stem_{rep}\big) < \overline{\Phi}_{DNA}\big(zyg; germ_{rep}\big)$$

$(\,22\,)$

502 where $\overline{\Phi}_{DNA}\big(zyg; som_{rep}\big)$ represents the average DNA informational fidelity of non-stem somatic cells of an individual at the time
503 of reproduction referenced to the same individual when it was a zygote, $\overline{\Phi}_{DNA}\big(zyg; stem_{rep}\big)$ is the average DNA informational

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[6] 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 fidelity 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.

504 fidelity of adult stem cells referenced to the zygote, and $\bar{\Phi}_{DNA}(zyg; germ_{rep})$ is the average DNA informational fidelity of germ cells
505 referenced to the zygote.

506 In agreement with Eq. *(22)*, organisms appear to come closest to fully preserving DNA informational fidelity in germ cells. This results
507 from evolved strategies that place extraordinary emphasis on the preservation of both nuclear and mitochondrial genetic data in germ
508 cells. The fidelity of mtDNA is largely restored during oogenesis through a genetic bottlenecking process that selects for the healthiest
509 mtDNA and eliminates mutated mtDNA molecules that compromise mitochondrial function (Wai et al. 2008; Lee et al. 2012). The
510 nuclear DNA in germ cells is subject to very strict insult detection mechanisms (Hochwagen and Amon 2006; Jaramillo-Lambert et al.
511 2010; Bailly and Gartner 2013). Germ cells are more likely than somatic cells to undergo apoptosis when DNA damage is detected,
512 rather than attempt to repair the damage (which often results in the loss of DNA informational fidelity). They are also sequestered in
513 a protected microenvironment with various specialized cells whose sole purpose is to support and maintain the germ cells (Schulz et
514 al. 2002).

515 Assessing the situation from a thermodynamics perspective suggests that the rate of DNA informational fidelity loss is minimized by
516 keeping the thermodynamic potentials acting on DNA molecules as low as possible to reduce the rate of molecular insult. Primordial
517 germ cells (gametogonia), as well as oocytes and spermatocytes, have relatively low rates of oxygen consumption (Brinster and Troike
518 1979). Most adult stem cells are quiescent and frequently prioritize glycolysis over oxidative phosphorylation, leading to lower levels
519 of free radicals and reactive oxygen species (ROS) (Tothova et al. 2007; Rossi et al. 2008; Suda et al. 2011; Shyh-Chang et al. 2013) and
520 decreased mtDNA replication rates. This supports the notion that manipulation of thermodynamic potentials acting on DNA molecules,
521 through modulation of cellular processes and microenvironmental conditions, is a realizable and effective means of reducing the rate
522 of DNA informational fidelity loss in cells.

523 The genetic similarity between gametes and the same individual when it was a zygotes $\Phi_{DNA}(zyg; gametes)$ is determined by not
524 only inevitable germ cell DNA informational fidelity losses throughout life but also losses due to genetic recombination during meiosis
525 $\Delta\Phi_{\text{recom}}$:

$$\Phi_{DNA}(zyg; gametes) = \Phi_{DNA}(zyg; germ_{rep}) - \Delta\Phi_{\text{recom}} \qquad (23)$$

526 Singular insult events that generate losses in DNA informational fidelity (i.e. mutations) most commonly have little or no effect on
527 offspring fitness. Some mutations will result in decreases in fitness while only the rare insult produces increased fitness (Fisher 1930;
528 Eyre-Walker and Keightley 2007). Absent effective evolutionary selection, genetic dissimilarities (resulting from the loss of mutual
529 DNA information between parent and offspring, and/or potential gain of new information) will lead to a decline in species fitness since
530 advantageous mutations are rare. The proportion of progeny with low fitness must not be so excessive that evolution cannot
531 successfully select for neutral and higher fitness offspring. For this reason, a minimal limit $\Phi_{DNA,min}(zyg; gametes)$ is effectively
532 placed on the DNA informational fidelity of the progeny as referenced to the parent:

$$\Phi_{DNA}(zyg; gametes) \geq \Phi_{DNA,min}(zyg; gametes) \qquad (24)$$

533 Germ cells must be maintained with adequate redundancy levels and a sufficiently stringent fidelity preservation strategy to satisfy
534 Eq. *(24)*. In this way, DNA informational fidelity is largely—but not perfectly—preserved generation-to-generation and the relatively
535 small fidelity losses that do occur can be eliminated from the gene pool if they negatively affect species fitness, or retained when they
536 increase species fitness.

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

544 ## 6   Establishing a Connection between Thermodynamic, Information, and Evolutionary Theory in Biological Aging

545 Modern nonequilibrium thermodynamic theory stipulates that all biomolecules will suffer degradative insults. Biological repair and
546 replacement mechanisms cannot guarantee that DNA informational fidelity is preserved in individual cells regardless of the level of
547 investment: Cellular DNA informational fidelity must decrease with time. Per Eq. *(21)*, this is sufficient to mandate a decrease in DNA

548 informational fidelity. What are the repercussions of these losses for an individual, species as a whole, and discrete genes within a
549 species genome?

6.1   In the Individual

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

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

562 As there is no means for an organism to perform comparative DNA sequence analyses, cells with undesirable base-sequence
563 modifications are only detectable by phenotype. In the case of more severe damage, cellular mechanisms enable detection of the
564 damage and initiation of apoptosis
565 (Zhou and Elledge 2000). On the other
566 hand, singular mutations may exhibit
567 very mild or no detectable undesirable
568 phenotype. For example, mutations
569 whose effects are masked by
570 redundancy are likely to have no
571 detectable phenotype. A mutation may
572 also occur in a region of the genome that
573 is currently inactive or irrelevant to the
574 particular cell—therefore there may be
575 no immediate negative phenotype. Such
576 a genomic region could become active
577 later, at which time the mutation may
578 have already spread to the cell's
579 progeny.

580 Even in the ideal embodiment, the
581 effects of multiple mutation events
582 must eventually decrease individual
583 viability; at some point, removing cells
584 determined by biological mechanisms to
585 be undesirable will no longer provide
586 reprieve from losses in viability since
587 cellular DNA informational fidelity will
588 continue to decrease until all cells
589 approach the detectable threshold of
590 dysfunction. At this point, there would
591 be no "good" configurations to select for
592 to replace those cells determined to be
593 undesirable, even if such cells could be
594 detected with perfect accuracy.

Nonequilibrium Thermodynamics

Stipulates the inevitability of degradative biomolecular insults

Information Theory

- Concept of mutual information
- I_{mutual}, and therefore Φ_{DNA}, must decrease with time due to S_i
- The rate of DNA informational fidelity loss $\dot{\Phi}_{DNA}$ is determined by thermodynamic and evolutionary factors

Evolutionary Theory

In the species, the fitness effects of germline Φ_{DNA} loss are combatted by selective pressures, which favor genotypes that increase fitness

In the Individual (A) — Φ_{DNA} / Viability vs Age Post Reproductive Maturity; Minimum viability to avoid imminent death

In the Species (B) — Φ_{DNA} vs Time; Single Generation

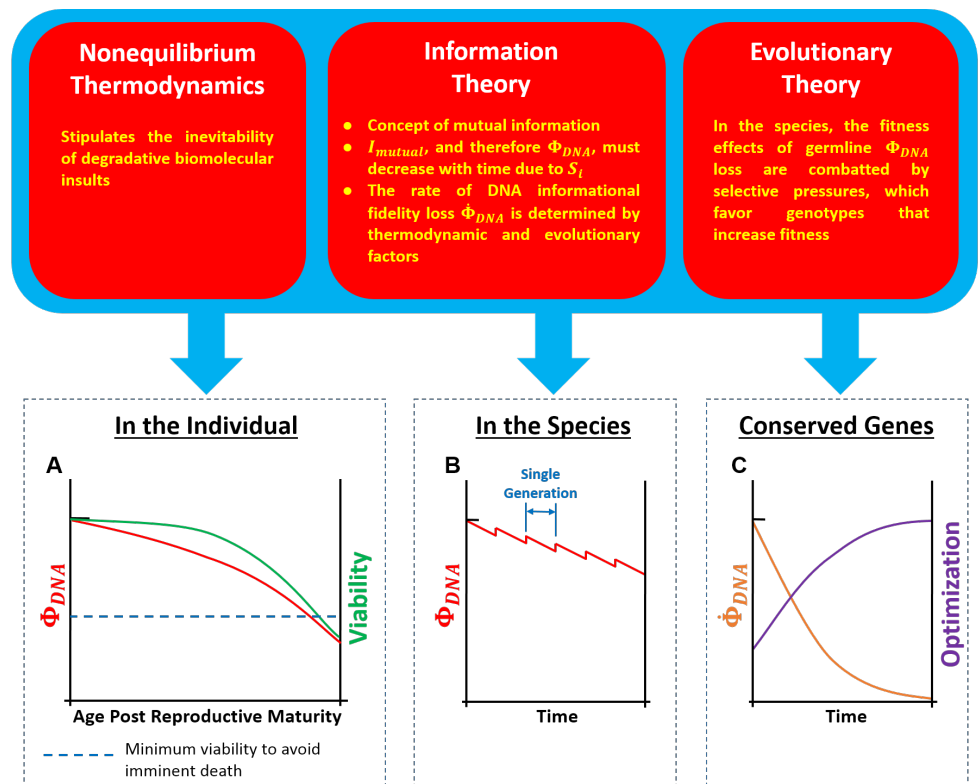**Conserved Genes** (C) — Φ_{DNA} / Optimization vs Time

Fig. 4. The proposed connection between thermodynamics, information, and evolutionary theory in generating mandatory DNA informational fidelity losses in individual organisms, the genomes of species, and individual genes within a species genome. (a) Although a correlation between individual viability and somatic cell DNA informational fidelity loss is expected, genetic redundancies and other compensating mechanisms may attenuate reductions in viability generated by DNA informational fidelity losses as individuals age. (b) The average DNA informational fidelity in cells of all individuals of a species, as measured against a common ancestor, will decline as the individual 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, such as may be approximated with highly conserved genes, the rate of DNA informational fidelity loss will decrease and approach zero as gene optimization approaches a maximal value.

Genetic redundancies are likely able to temporarily compensate for the loss of DNA informational fidelity in an individual—essentially delaying a detectable aging phenotype to at least the age of reproductive maturity (Fig. 4A). 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. The 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, the second law stipulates that all individual organisms must eventually age if they live long enough.* The claim by Hamilton (1966) that, at the species level, senescence arises inevitably due to declining selection pressure with age, while not challenged here, is redundant.

In addition, since there is no possible means to prevent natural selection from occurring at the level of the individual cell[7], the second law also stipulates that cancer is 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).

6.2   Within a Species

Due to gamete fidelity preservation mechanisms (Jaramillo-Lambert et al. 2010; Bailly and Gartner 2013), the loss in DNA informational fidelity suffered between a gamete and the zygote that gave rise to the individual that produced the gamete will typically be less than the average loss in somatic cells of the individual (at the age of reproductive maturity). By limiting the DNA informational fidelity loss in the gamete to a low enough level that selection for neutral and higher fitness offspring is possible, species fitness can be preserved and even increase—as can complexity. Natural selection is the only means to prevent inevitable DNA informational fidelity loss from generating mandatory reductions in species fitness. Since DNA insults can result in new information, the second law does not constrain complexity; through selection, complexity can increase as warranted to maximize fitness.

Fluctuations in species selective pressures result in adaptive genetic changes that drive species DNA informational fidelity (as measured relative to a common ancestor) downwards with time (Fig. 4B). Since the process of evolving mandates genomic changes, conditions where selective pressures are changing at a high rate can lead to DNA informational fidelity losses in the species that are greater than the loss predicted by $\overline{\Phi}_{DNA}(zyg; gametes)$.

6.3   Within Discrete Genes

A number of genes are highly conserved even amongst diverse species whose most recent common ancestor dates back hundreds of millions of years. If DNA informational fidelity loss is inevitable, how can the rate of DNA informational fidelity loss in these genomic sequences be so low?

With highly conserved genes, selective pressures remain very static and common between organisms. There are a number of reasons for this. Firstly, highly conserved genes typically have extremely specific functions that fulfill common needs across the range of species represented. Many of the genes with the most fundamental and specific functionality—e.g. ribosomes, tRNA, histones— also tend to be the most conserved (Isenbarger et al. 2008). Since an individual gene is far less complex than the organism it constitutes, there are considerably fewer ways for a highly conserved gene to achieve increased optimization to perform its intrinsic biological function. Thus with time these types of genomic sequences converge around a common solution that facilitates maximal fitness in a range of species. The second law remains very much active in these cases—i.e. DNA mutations still occur. However, selective pressures have a greater negating effect against DNA informational fidelity losses in these highly conserved genetic sequences.

Consider the evolution of a highly conserved genetic sequence (prior to species divergence) under conditions of static selective pressures. The rate of DNA informational fidelity loss $\dot{\Phi}_{DNA}$ will assume some initial value (Fig. 4C). Through the course of evolution, fewer configurations will be available that are more optimized than the current configuration. As a result, the rate of DNA informational fidelity loss decreases and the rate of optimization is also reduced. With fewer "positive" mutations available and selection tending to eliminate (i.e. revert) "negative" mutations, DNA informational fidelity losses are minimized. As the optimization of the genetic sequences approaches a theoretical maximum, the rate of DNA informational fidelity loss approaches zero. Essentially,

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

638 those losses produced as a consequence of the second law are effectively negated by a gain in DNA informational fidelity resulting
639 from selective pressures.

$$\Delta\Phi_{total} = -\Delta\Phi_{2nd\ law} + \Delta\Phi_{evol} \Longrightarrow 0 \qquad\qquad (25)$$

640 This can only arise when 1) selective pressures are essentially static and 2) a very long period of time has passed, and tends to obfuscate
641 the stipulated effects of the second law on highly conserved genetic sequences.

642 Compared to individual genes, many more factors come into play in defining selective pressures for an entire species. As it is incredibly
643 unlikely for all of these factors to remain stable for any length of time, conditions of perfectly static species selective pressure are not
644 realizable and the genomes of species, considered as a whole, do not approach this theoretical lower limit of DNA informational fidelity
645 loss. Therefore, the ramifications of the second law are more easily discerned when examining DNA informational fidelity losses in the
646 full genomes of species over time.

## 647   7   Entropy-Driven Managed Deterioration – Basic Concepts

648 Having identified one class of biological structure universally subject to mandatory and irreversible losses in fidelity, we return to our
649 previous question of why organisms undergo homeostatic shifts as they age.

650 Fig. 5 depicts the basic interrelationships that may explain the progression of the aging phenotype in many metazoans. In this model,
651 the key top-level factor initiating the transition from youthful homeostasis is internal entropy production, which inevitably generates
652 losses in DNA informational fidelity in both mitochondrial and nuclear DNA. Informational fidelity losses in mitochondrial DNA (mtDNA)
653 are ubiquitous in aged mammals (Wallace 1999) and lead to lower peak energy output (Yaniv et al. 2013). This decline in mtDNA
654 informational fidelity is partially modulated by a controlled deceleration in mitochondrial biogenesis (Figge et al. 2012), which reduces
655 the rate of clonal expansion of degraded mtDNA and limits the exposure of mtDNA to the high thermodynamic stress conditions of
656 replication events. The escalating deficit in cellular energy currency (ATP) production gives rise to a progressively worsening inability
657 to fund all cellular processes at youthful levels, and generates forced reductions in biomolecular turnover that increases biomolecular
658 degradation state and lowers biomolecular performance—representative of a transition away from youthful homeostasis.

659 Concurrent with these changes, increasing losses in nuclear DNA fidelity with age (Podolskiy et al. 2016) produce a mosaic of stochastic
660 cellular dysfunction that worsens over time (Bahar et al. 2006; Lodato et al. 2015). Together with the described mitochondrial
661 dysfunction, this could largely explain age-
662 linked cellular dysfunction and the overall
663 aging phenotype of the organism. "Longevity
664 optimization" genes may have evolved to
665 attenuate the negative effects of informational
666 fidelity losses in nuclear and mitochondrial
667 DNA through reallocation of resources and
668 physiological alterations. This model is
669 discussed in more detail in section 9.

670   8   Longevity Determination

671 Leonard Hayflick has stated that aging is not
672 driven by genes but by thermodynamics
673 (Hayflick 2004), while also arguing that the
674 genome does govern longevity. Additionally,
675 Hayflick maintains that natural selection has
676 led towards biomolecular arrangements that
677 are capable of preserving fidelity until
678 reproductive maturity, but that the survival
679 value for arrangements exceeding this
680 longevity is considerably diminished (Hayflick
681 2007a).
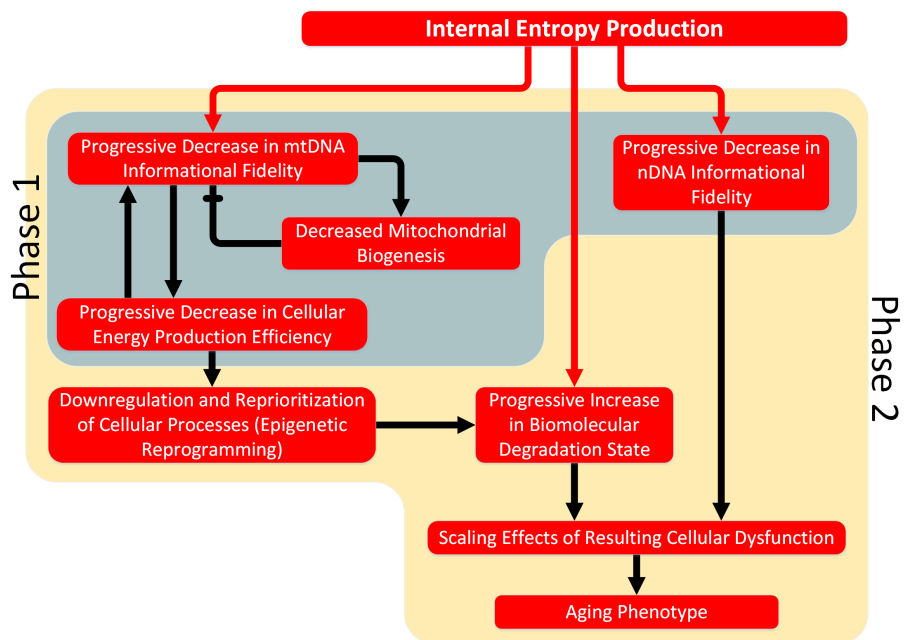
Fig. 5. The basic interrelationships between primary factors that may largely describe the progression of the aging phenotype in many metazoa. During 'Phase 1' of an individual's life, DNA informational fidelity losses have not reached levels sufficient to generate an aging phenotype. 'Phase 2' begins when dysfunction has progressed to the point that aspects of the aging phenotype begin to become apparent.

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

8.1 Investigating the Rate of DNA Informational Fidelity Loss in Individuals of a Species

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

691 Since DNA undergoing replication is significantly more
692 likely to incur a mutation due to the impaired stability of
693 single-stranded DNA (Frederico et al. 1990) and given the
694 imperfect nature of DNA polymerases (Arias-Gonzalez
695 2012), replicating and non-replicating conditions should
696 be considered independently. Although damage
697 detection and repair systems correct or eliminate many
698 DNA insults, a certain proportion avoids detection. Fig. 6
699 depicts a systems flow diagram of this arrangement. DNA
700 actively being transcribed is also more susceptible to
701 mutation (Kim and Jinks-Robertson 2012), but it is
702 generally believed that the vast majority of mutations
703 arise from replication events or random DNA damage.
704 For this reason, transcription is not considered as a
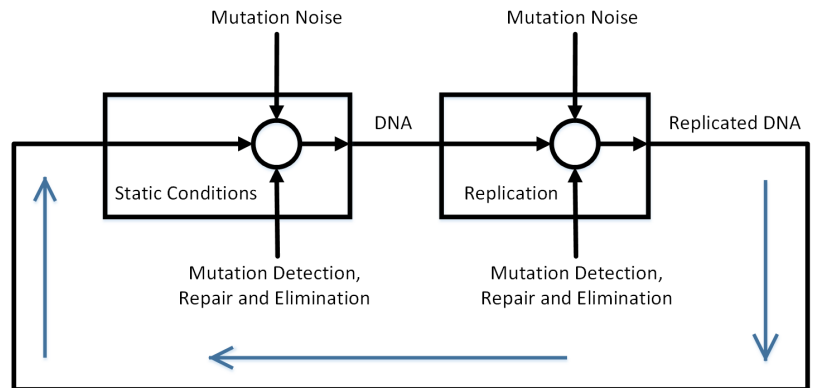705 separate state condition in this model.

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

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

$$\dot{I}_{lost/gained} = -L_{DNA}\left(\Delta I_{mut,rep} r_{rep} m_{rep} + \Delta I_{mut,static} r_{mut,static}\right)(1 - p_{det}) \qquad (26)$$

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

8.2 Preserving mtDNA Informational Fidelity

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

726 The only known human mtDNA polymerase, DNA polymerase γ, is highly conserved across species as diverse as *Drosophila*
727 *melanogaster* and *Saccharomyces cerevisiae* (Chan and Copeland 2009), as are the GTPases implicated in mitochondrial fission and

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

734 In addition, since the molecular configuration of DNA is conserved and the potential reactions that can result in molecular
735 modifications are accordingly similar, it follows that the mutual information lost or new information gained in the average mutation-
736 causing event is consistent; i.e. $\Delta I_{mut,rep}$ and $\Delta I_{mut,static}$ should be similar across species. This leaves the mtDNA replication rate r_{rep}
737 and the static-condition mutation rate $r_{mut,static}$ as the likely factors from Eq. *(26)* to be primarily responsible for any variation in the
738 rate of mtDNA informational fidelity loss between species.

8.3   MtDNA Informational Fidelity Loss in Aged Organisms Primarily Results from Replication

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

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

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

8.4   Higher Mitochondrial Performance Gives Rise to an Increased Rate of mtDNA Informational Fidelity Loss

761 As it facilitates mitochondrial renewal, mitobiogenesis is required to preserve the performance of individual mitochondrial
762 components and mitochondria as a whole. Reducing mitobiogenesis will compromise mitochondrial performance since less negative
763 entropy is produced for counteracting degradative internal entropy production (affecting mitochondrial components other than
764 mtDNA), resulting in a shift to a higher degradation state. As mitobiogenesis incorporates mtDNA replication, a decline in
765 mitobiogenesis will reduce mtDNA replication rate r_{rep}. Thus, although reduced mitobiogenesis may negatively impact mitochondrial
766 performance, it will lead to a lower rate of mtDNA informational fidelity loss per Eq. *(26)*.

767 On the other hand, with increased mitobiogenesis additional negative entropy is available for offsetting degradative internal entropy
768 production, effectively translating to a lower mitochondrial degradation state and higher performance. However, this will also raise
769 the mtDNA replication rate $k_{rep,mtDNA}$ and generate increased exposure of mtDNA to the high thermodynamic-stress conditions
770 experienced during replication—resulting in an increase in the rate of mtDNA informational fidelity loss.

771 Preservation of a mitochondrial degradation state representative of youthful homeostasis requires that the rate of negative entropy
772 production from mitobiogenesis equals or exceeds the rate of degradative internal entropy produced within mitochondria when
773 operating at a youthful degradation state (per Eq. *(7)*). If the mitochondrial performance requirements for a particular cell type are

774 greater in one species, then the rate of mitobiogenesis required to preserve youthful mitochondrial homeostasis within the cells of
775 that species will also be greater, as will the rate of mtDNA informational fidelity loss.

8.5   A Closer Look at Mitochondrial Configurations and Membrane Composition

777 Since mitobiogenesis encompasses mtDNA replication—which accelerates losses in mtDNA informational fidelity—forfeiture of
778 youthful mitochondrial homeostasis is not only inevitable but also temporally related to the rate of mitobiogenesis. How then might
779 this rate differ by species, and why?

780 Examining cellular metabolic demands provides some clues. Across species, whole-organism basal metabolic rate scales allometrically
781 with body mass: $BMR \propto M_b^f$ (Kleiber 1932; Peters 1986; Niklas 1994).[8] Resting oxygen consumption expressed per unit body mass
782 scales proportionally with $M_b^{-1/4}$. In other words, mass-specific BMR decreases by approximately 16% across species for every doubling
783 of body mass. The inverse correlation between relative oxygen consumption and body mass has been verified in isolated hepatocytes
784 from mammals (Porter and Brand 1995) and birds (Else 2004) as well as in mammalian liver slices (Couture and Hulbert 1995). Porter
785 and Brand (1995) found a 5.5-fold decrease in hepatocyte oxygen consumption for every 12,500-fold increase in body mass.

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

790 Increasing the mitochondrial inner membrane surface area per unit volume of mitochondrial matrix allows for additional
791 transmembrane-localized oxidative phosphorylation enzymatic machinery in the same volume of space (i.e. an increase in ρ). The
792 resulting increase in biological power density may help smaller organisms realize their higher ATP requirements. Indeed, a significant
793 negative correlation has been found between mitochondrial inner membrane surface area per unit volume of matrix and body mass
794 (Porter et al. 1996).

795 Mitochondrial membrane phospholipid composition also differs widely across species, particularly in fatty acid composition (Daum
796 1985). Smaller mammals have mitochondrial membranes that are more polyunsaturated than larger mammals (Porter et al. 1996).
797 Some light was shed on why membrane fatty acid composition varies allometrically when the molecular activity of transmembrane
798 proteins was examined in different membrane compositions. The cytoplasmic membrane-localized sodium pump (Na$^+$·K$^+$-ATPase)
799 varies in molecular activity from approximately 8,000 ATP/min in mammals compared to 2,500 ATP/min in ectotherms (all data taken
800 at 37°C) (Else et al. 1996). Cytoplasmic membrane crossover studies demonstrated that the work rate of ectothermic sodium pumps
801 increased significantly when transferred to mammalian membranes, while mammalian sodium pump activity was attenuated in
802 ectothermic membranes (Else and Wu 1999).

803 The higher sodium-pump work rates in endotherms were hypothesized to be due to influences from surrounding lipids, with
804 polyunsaturated membranes promoting increased molecular activity compared to membranes that are more monounsaturated.
805 Hulbert and Else (1999) proposed a mechanism by which this may occur: The lateral diffusion coefficient of lipids within a membrane
806 bilayer is greater than that of transmembrane proteins by two orders of magnitude (Storch and Kleinfeld 1985). As such, membrane
807 proteins are continuously colliding with membrane lipids. The kinetic energy exchanged during these collisions is believed to be critical
808 in facilitating membrane protein function. The acyl chains of saturated and monounsaturated fatty acids are more flexible than
809 polyunsaturated fatty acids. Therefore, collisions involving lipids containing polyunsaturated fatty acids transfer more energy to
810 membrane proteins and result in higher protein activity than collisions with lipids with only highly saturated fats. Of the fatty acids
811 found in membrane lipids, docosahexanoic acid (DHA or 22:6 n-3) contains the largest number of evenly spaced double bonds but is
812 also particularly susceptible to peroxidation. DHA has been referred to as the "acme" of polyunsaturates and may serve as a membrane
813 "energizer" (Hulbert and Else 1999), effectively increasing $\dot{W}_{m,max}$ (see section 2.8). Sodium pump molecular activity correlates with
814 membrane DHA concentration in both ectotherms (Turner et al. 2005) and endotherms (Turner et al. 2003).

815 Peroxidation index (PI) is a measure of the susceptibility of membrane lipids to peroxidation and is correlated to fatty acid
816 unsaturation. The PI of mitochondrial phospholipids, predominantly driven by DHA content, negatively correlates with MLSP
817 (Pamplona et al. 1998). Importantly, the same trend line holds for both mammals and birds (Hulbert et al. 2007). In addition,

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[8] 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).

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818 mitochondrial membrane remodeling resulting from various levels of caloric restriction in mice produced changes in PI and MLSP that
819 fit the same trend line (Faulks et al. 2006; Hulbert 2008).

820 In addition to the negative allometry of metabolic rate, body mass positively correlates with MLSP (Calder 1984; Austad 2005; de
821 Magalhães et al. 2007). The discussed findings suggest that smaller organisms with reduced longevity may utilize membranes with
822 more polyunsaturated membranes—largely dictated by DHA content—in order to increase the rate of work that can be performed by
823 each transmembrane protein molecule ($\dot{W}_{m,max}$) and to satisfy functional requirements largely specified by recognized allometric
824 relationships that characterize fitness optimization across species. A downside of polyunsaturated fatty acids is their susceptibility to
825 oxidative damage. In other words, polyunsaturated fatty acids are less resistant to molecular alterations resulting from the
826 thermodynamic forces of their environment; the presence of higher levels of polyunsaturated fatty acids will lead to increased rates
827 of degradative internal entropy production ($\dot{S}_i$) within mitochondria—even when mitochondria are functioning well below their
828 maximum work rate (i.e. w_{dc} is low). This will necessitate a higher rate of mitobiogenesis to maintain homeostasis at a given
829 mitochondrial degradation state.

8.6 Identifying Longevity Determinants

831 It is postulated here that peak biological power density σ_{peak} largely stipulates membrane composition and other defining
832 characteristics of an organism (e.g. biomolecular renewal rate, metabolism) that serve to largely determine longevity in many species
833 by defining the rate of loss of DNA informational fidelity in somatic cells of the individual. This concept is depicted in Fig. 7. Here the
834 term "peak biological power density" represents the maximum localized volume-specific rate of external work (power per unit volume)
835 achievable within an organism. The cells or tissues where this potentiality exists may vary by species (for example, skeletal muscle in
836 some organisms, neurons in others, etc.). "External work", in the context of peak biological power density, refers to the sum of the
837 biomechanical, biochemical and bioelectrical work that is brought to bear on the immediate environment surrounding the localized
838 region where this work originates. Examples include the mechanical work generated by myocytes, the chemical and electrical work
839 produced by neurons, and the chemical work performed on metabolized products by hepatocytes. External work does not include
840 work associated with housekeeping or "overhead" cellular processes such as biomolecular repair and replacement, maintenance of
841 baseline membrane potentials or mitotic cell turnover.

842 To illustrate the sequence of interactions implicated in this model, we will consider an arbitrary organism in which maximum fitness
843 is achieved by a high level of peak biological power density compared to some reference organism (Fig. 7). This implies an increase in
844 the maximum potentiality for external work rate per unit volume that is achievable by some cell, or group of cells, within the organism.
845 Per Eq. *(18)*, in order to realize the highest rate of work possible from a given volume several parameters should be optimized. For
846 one, P should be maintained at a high level, **1** (bold numbers in this section refer to Fig. 7). High P will increase renewal rate and
847 decrease renewal ROI (Fig. 1D). Secondly, biomolecule structure should be optimized for high peak work rate ($\dot{W}_{m,max}$), **2**. This will
848 likely correspond with a reduction in stability (due to lower selective pressure on this parameter), leading to an increase in $\dot{S}_i$ and
849 renewal rate. Thirdly, high-output biomolecules should spend more time performing work and less time in a resting state (i.e. $w_{dc} \rightarrow$
850 1), **3**. The state of actively performing work involves the transfer of energy and conditions of higher thermodynamic potentials
851 compared to the non-working (resting) state. This will also increase $\dot{S}_i$ and renewal rate. Finally, the biomolecule's volumetric density
852 should be as high as possible without negatively effecting the work rate per molecule, **4**. An increased renewal rate will be required
853 to maintain P in a larger biomolecular pool. The above factors all contribute towards increased metabolism and ATP requirements.
854 This is consistent with the increased metabolic rates found in smaller animals and the fact that smaller animals renew protein at faster
855 rates compared to larger species (Fig. 3).

856 Transmembrane proteins exhibit increased activity in membranes containing lipids with high polyunsaturated fatty (DHA) content
857 (Else and Wu 1999; Turner et al. 2003; Turner et al. 2005). Configurations that call for a high level of peak biological power density are
858 likely to use these types of membranes, which are also more susceptible to lipid peroxidation, **5**. This contributes towards an elevated
859 rate of membrane-damaging $\dot{S}_i$, more frequent membrane lipid renewal, and increased biomolecular renewal rate.

860 The aforementioned increased metabolic requirements generate a need for mitochondrial networks capable of satisfying these higher
861 ATP demands. This is realized with high power density mitochondria, **6**, the characteristics of which are outlined in Fig. 8. High-output
862 mitochondrial proteins optimized for maximal ATP production are expected with these configurations. Similar to their cytoplasmic
863 counterparts, high-output mitochondrial proteins will exhibit increased $\dot{S}_i$. Higher mitochondrial membrane polyunsaturated fatty acid
864 (DHA) content will increase peak ATP output through enhanced transmembrane protein activity but mitochondrial membranes will be
865 more susceptible to peroxidation, **7**. Together with a positive feedback effect from elevated ROS and oxidative stress levels, **8**, this will

25

866 further increase mitochondrial-damaging $\dot{S}_i$, **9**. A higher rate of offsetting negative entropy production will be required in order to
867 maintain mitochondrial quality and preserve youthful homeostasis. This need can only be realized through an upregulation of
868 mitobiogenesis, **10**, which increases mitochondrial renewal rate but will coincide with a higher mtDNA replication rate, **11**, and an
869 increased rate of mtDNA informational fidelity loss, **12**. As mtDNA informational fidelity declines with age, the ability to produce usable
870 energy is compromised and worsens progressively. This generates a downregulation of cellular processes which could largely be
871 responsible for the aging phenotype in many organisms.

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

878 The loss of DNA informational fidelity is unavoidable. Notably, the logic established here describes how the rate of loss of DNA
879 informational fidelity may be a function of an organism's peak biological power density requirements, at least in part. As this rate may
880 be critical in determining the amount of time that passes before youthful homeostasis can no longer be sustained, a potential link is
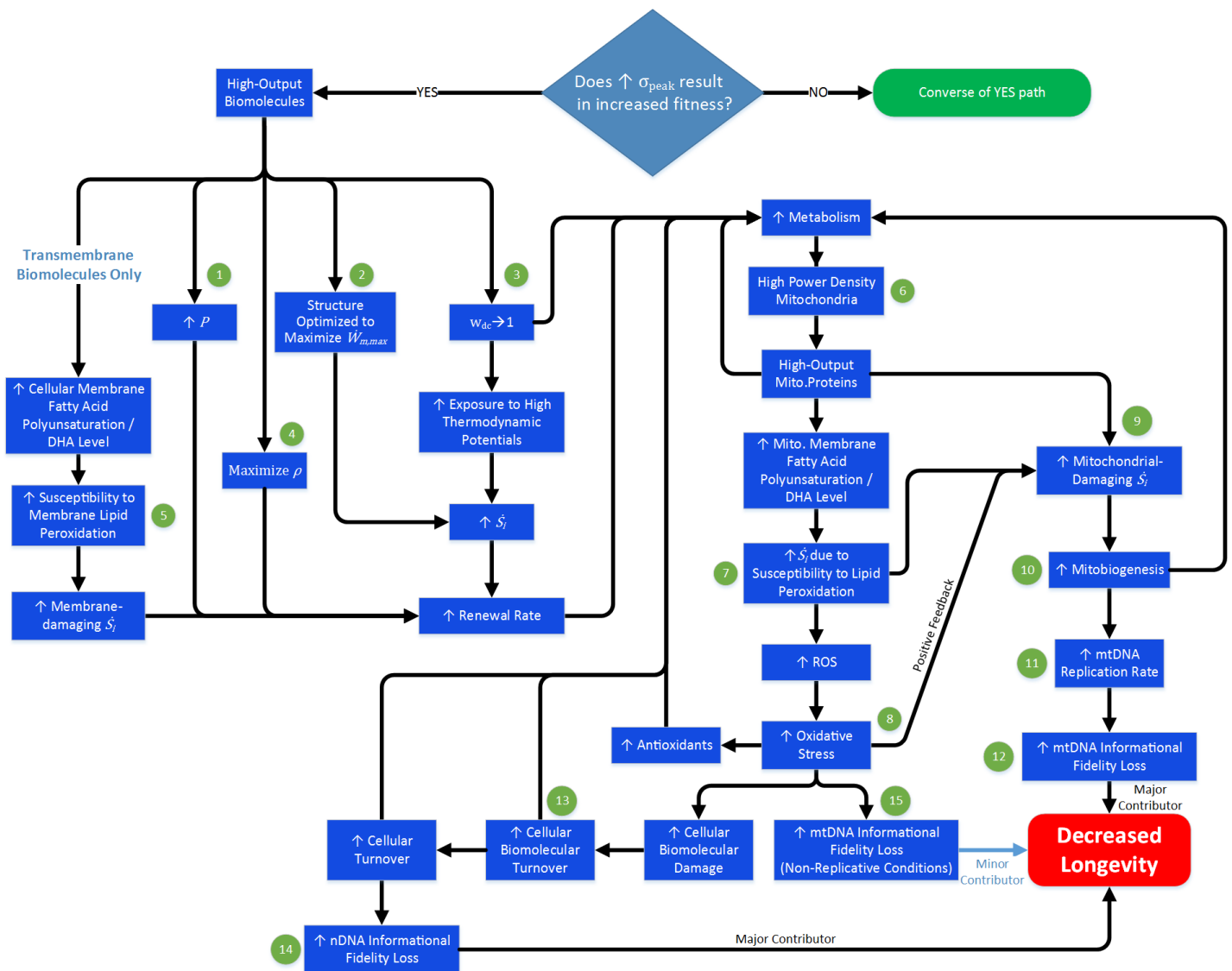

Fig. 7. A theoretical means by which an organism's peak biological power density may influence longevity. Bold numbers in section 8.6 text refer to this figure.

881 herein established between an organism's peak biological power density and longevity; by this token, peak biological power density
882 could be thought of as a high-level longevity determinant.

883 Although higher σ_{peak} implies the ability to perform work at an increased rate per unit volume somewhere within an organism, it does
884 not mean that $\dot{W}_{m,max}$ is achieved at all times or otherwise define w_{dc}. On the other hand, if this maximum work rate were never
885 approached then there would be no need to possess this ability in the first place. Thus, it is reasonable to expect that $\dot{W}_{m,act}$ in a
886 species with a higher σ_{peak} is greater, on average. At a minimum, the cell must have the ability to provide sufficient energetic resources
887 to satisfy σ_{peak} even if these levels are only attained sporadically. The attributes associated with this last requirement are likely to
888 increase $\dot{S}_i$, renewal rate and thereby DNA informational fidelity loss even in static (non-working) conditions. Thus, the potentiality
889 implied by an increased σ_{peak} may be sufficient to decrease longevity, even if $\dot{W}_{m,act}$ is lower on average compared to some other
890 species. This could manifest as species with basal metabolisms that are inconsistent with expected longevity trends.

891 ## 8.7   The Naked Mole-rat Paradox – Part II
892 We can now propose a solution for the second half of the naked mole-rat paradox discussed earlier. Naked mole-rats live in very
893 hypoxic environments and thus must function at extremely low metabolic rates. This necessitates low-output proteins, as high-output
894 proteins have increased metabolic requirements for a number of reasons (Fig. 7). The situation is therefore the reverse of the high
895 peak biological power density scenario previously discussed. Lower metabolism will lead to mitochondria better optimized for stability
896 (Fig. 8). Consistent with this notion, naked mole-rats have one fifth of the amount of DHA in their liver mitochondrial membranes
897 compared to their similarly-sized cousin the house mouse (Mitchell et al. 2007). Decreased susceptibility to lipid peroxidation lowers
898 the rate of damaging internal entropy production, mitobiogenesis, and mtDNA informational fidelity loss. Cellular turnover and the
899 rate of nuclear DNA informational fidelity loss will also decrease. The lower rate of DNA informational fidelity loss increases the amount

900 of time that passes before youthful
901 homeostasis is lost due to transitions to
902 higher degradation states. As a result, the
903 naked mole-rat exhibits exceptional longevity
904 for its size. The increased level of oxidative
905 damage does not limit longevity as it is not the
906 factor forcing a shift from youthful
907 homeostasis but is merely indicative of the
908 high degradation states that coincide with
909 prioritizing renewal ROI for maximizing
910 evolutionary fitness in very hypoxic
911 conditions. I postulate that the exceptional
912 longevity of the naked mole-rat is primarily a
913 byproduct of the aforementioned
914 requirement for extremely low metabolic rate
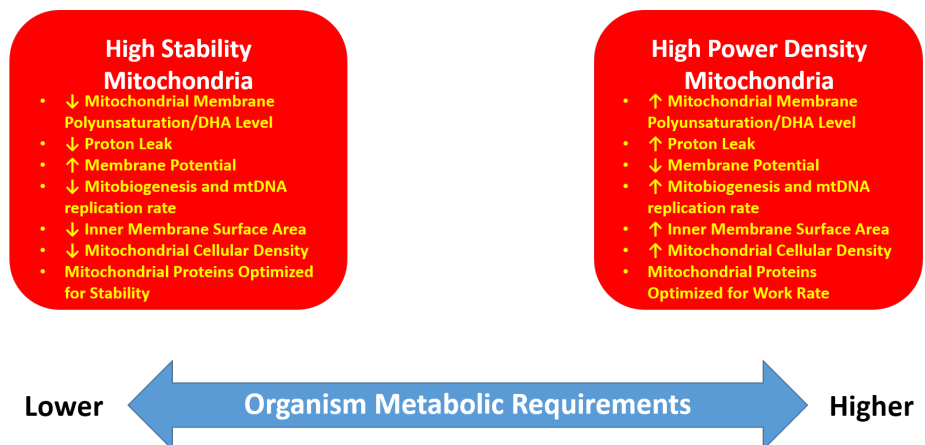915 as opposed to direct selective pressure for
916 extreme longevity.

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.

917 ## 8.8   Allometric Relationships Describe Peak Biological Power Density Trends that Largely Predict Longevity
918 If peak biological power density is a primary longevity determinant, then how and why does this vary by species? Do variations in peak
919 biological power density align with allometric trends? Some answers to these questions may arise from examining how an organism's
920 mass-specific energetic cost of transport (COT) is driven by certain factors. COT is a measure of the quantity of metabolic energy
921 required to move one unit mass of an organism over one unit distance. In terrestrial animals, COT negatively correlates with body size
922 (Taylor et al. 1982; Strang and Steudel 1990; Reilly et al. 2007). The reasons for the increased locomotor costs in smaller terrestrial
923 organisms have been discussed in detail elsewhere, including Reilly et al. (2007), and Kilbourne and Hoffman (2013). We will briefly
924 examine some of the more significant causes here. Although the mass-specific metabolic energy consumed per stride remains constant
925 across large and small mammals at the same stride frequency, larger animals require fewer strides to cover an equivalent distance;
926 this at least partly explains the reduction in COT with increasing body size (Heglund et al. 1982; Heglund and Taylor 1988; Kram and

27

Taylor 1990). The effect is compounded by the fact that larger mammals have disproportionately longer limbs (positive allometry) (Pontzer 2007).

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

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

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

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 the mass-specific capacity for heat exchange decreases as body size increases. Since no thermodynamic process is 100% efficient, a portion of the energy utilized for metabolism is converted to heat. The efficiency of the oxidative phosphorylation machinery in mitochondria is highly optimized and not a function of body mass, as indicated by the fact that ATP turnover per unit of consumed oxygen does not change with body mass in mammals (Porter and Brand 1995). Therefore, in the absence of other limiters, maximum sustainable mass-specific metabolic rate will be lower in larger organisms due to their reduced relative capacity to shed metabolic waste heat. In other words, smaller organisms are capable of functioning with higher peak biological power densities. A converse effect of the surface area to mass ratio limits the minimum attainable body size of endothermic amniotes: maintenance of a constant body temperature, below a particular body size for a given set of environmental living conditions, will require an increasing proportion of metabolic energy as body size decreases.

Another factor likely further increases metabolic requirements in smaller animals and limits the minimum attainable body size. West and colleagues argued that metabolic rate scaling is constrained by characteristics of the circulatory system (and other fractal networks) that can be explained by principles from fluid dynamics (West et al. 1997; West and Brown 2005). As vessels become smaller, viscosity leads to greater energy dissipation due to increased damping. In addition, at some critical minimum size vessels are no longer able to benefit from impedance matching, which can greatly reduce the energy lost due to reflections in larger vessel branch 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—but will also depend on a number of other factors. Thus, deviations from the 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 in order 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.

974 These generalized allometric relationships do not apply to individuals within a species. For example, larger individuals in many species,
975 such as dogs (Speakman et al. 2003) typically have shorter lives than smaller individuals. This may be in part because longevity
976 determination has evolved, and is genetically engrained, at the species level. In other words, the genetic elements that specify peak
977 biological power density, membrane composition, biomolecular turnover rates, stem cell reserve levels, and other factors that
978 contribute towards resisting (or promoting) permanent thermodynamically induced changes in biocomponents subject to irreversible
979 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
980 significant deviations from the median body size would result in a compromised individual—and that this would include decreased
981 longevity.

9   Longevity Optimization

983 Once mitochondrial dysfunction has progressed to the point that resource deficits prevent the funding of all cellular processes at
984 youthful levels and/or genetic redundancies are no longer able to compensate for losses in nuclear DNA fidelity, an aged phenotype
985 will begin to take shape. It is reasonable to expect that the optimal allocation of resources for preserving maximal survival and
986 fecundity in an aged individual would be different from the configuration used in young adulthood when adequate resources are
987 available to fund all cellular processes. At all life stages, those factors most critical to immediate survival are of highest priority to the
988 individual. Therefore, a genotype optimized for an aging individual would increasingly deprioritize less vital processes and
989 biocomponents as useable energetic resources become scarcer so that biocomponents that are more critical to immediate survival
990 are retained at degradation states sufficient to sustain life and survival potential/fecundity are maximized. As aging continues to
991 progress in the individual, eventually a state is reached where even vital factors cannot be adequately maintained; at this point the
992 individual's overall condition becomes unconducive to continued life.

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

999   I submit here an alternative hypothesis proposing that a major function of the putative aging pathways is to optimize the inevitable
1000 process of aging such that individual longevity and fecundity are maximized. Contained within these pathways, genetic elements that
1001 I term "longevity optimizers" work together to elicit a balanced response to the unavoidable progression towards increasing levels of
1002 irreversible biomolecular fidelity loss.

1003 To my knowledge, this concept has not been formally proposed previously. There are several likely reasons for this. Firstly, scientists
1004 do not generally acknowledge that aging is inevitable, regardless of genotype. Many popular aging theories (e.g. antagonistic
1005 pleiotropy, mutation accumulation, and disposable soma) utilize evolutionary concepts to explain the existence of aging and do not
1006 consider fundamental physical law as relevant to the issue. These theories claim that aging is not unavoidable but rather that it exists
1007 because it projects beneficial effects on species fitness in other ways (antagonistic pleiotropy, disposable soma) or that there is
1008 insufficient evolutionary pressure to eradicate aging (mutation accumulation). If fundamental physical law does not mandate biological
1009 aging, then there is no need for mechanisms or strategies to resist or optimize it.

1010 Here, I have provided rationale and evidence for why biological aging is an inevitable consequence of fundamental physical law that
1011 evolution cannot overcome. If this is the case, then it is reasonable to propose the existence of evolved mechanisms to resist and
1012 optimize an organism's susceptibility to these effects in order to maximize species fitness.

1013 A counterargument is that aging optimizations are unlikely to evolve because selective pressures begin to decrease for ages beyond
1014 reproductive maturity. However, as the potential for loss of DNA informational fidelity begins at conception—not at reproductive
1015 maturity—this phenomenon must be suitably combatted at all life stages. In order to maximize fitness, organisms require strategies
1016 for preventing the loss of DNA informational fidelity from reaching detrimental levels and to compensate for the DNA informational
1017 fidelity loss that has occurred at all stages of life.

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

In the absence of compensatory mechanisms, somatic mutations and other forms of irreversible degradation to an essential biocomponent (biomolecule, cell, tissue, structure, 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 tangible negative repercussions for the individual.

In any aging individual organism, biocomponents susceptible to irreversible fidelity loss will be the first to transition from their youthful homeostatic states; therefore, aging of the individual will initiate within these structures. Multicellular organisms with short lifespans, such as many insects, often contain critical structures that grow once and are not maintained. One example is the wings of a honeybee. A honeybee's wings are primarily constructed of dead cells that cannot be replenished. Thus, any damage sustained to the wings will remain throughout the honeybee's life. In contrast, although (in general) mammals lack the ability to repair damage resulting from major traumatic injury to critical structures, they are able to counteract everyday "wear and tear" for considerable lengths of time by way of renewal and repair at the cellular and tissue level.

In the case of organisms with short lifespans and susceptible critical structures, irreversible fidelity loss in such structures may be highly relevant to the initiation and progression of the aging phenotype of these organisms. On the other hand, most organisms with at least moderate longevity will have very few biocomponents that lack fundamental repair capacity. For this reason, in all but the shortest-living multicellular organisms, the susceptible biocomponent most likely to drive the aging process is DNA. The continual loss of DNA informational fidelity must eventually force shifts in the degradation state of other biocomponents.

The magnitude of any deleterious impact on individual instantaneous mortality rate and fecundity resulting from an increase in the degradation state of a biocomponent will depend 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, specifically of those 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; Lotka and Sharpe 1911; Fisher 1930) to derive a measure of fitness r from age-specific survival and fecundity rates.

$$\int_0^\infty e^{-rx} l(x) m(x) dx = 1 \qquad (27)$$

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

$$v(x) = \int_x^\infty e^{-r(y-x)} \frac{l(y)}{l(x)} m(y) dy \qquad (28)$$

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.

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. This age-related decline would be typical of the inevitable and irreversible fidelity losses described above. The red curve in Fig. 9A depicts a typical plot of reproductive value as a function of age for this scenario (calculated using Eqs. (27) and (28)). 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.

1058 ## 9.2   Deterioration Management Strategies

1059 It is illogical for an organism to have evolved such that
1060 fecundity or mortality is negatively affected (at ages
1061 where selective pressure is still above some minimal
1062 threshold) due to the disproportionate deterioration,
1063 or increased likelihood of failure, of one or a small
1064 number of vital biocomponents. Selection must favor
1065 genotypes that avoid susceptibility to the
1066 catastrophic failure of a small number of weak links
1067 during aging.

1068 I propose that cellular mechanisms and pathways
1069 have evolved to function in a progressive and
1070 dynamic manner to manage the irreversible, and
1071 inevitable, losses in fidelity afflicting an aging
1072 individual. Priority is placed on biocomponents most
1073 susceptible to degradation effects, and most critical
1074 to survival and fecundity. To illustrate this concept, I
1075 will describe two strategies: "managed deterioration"
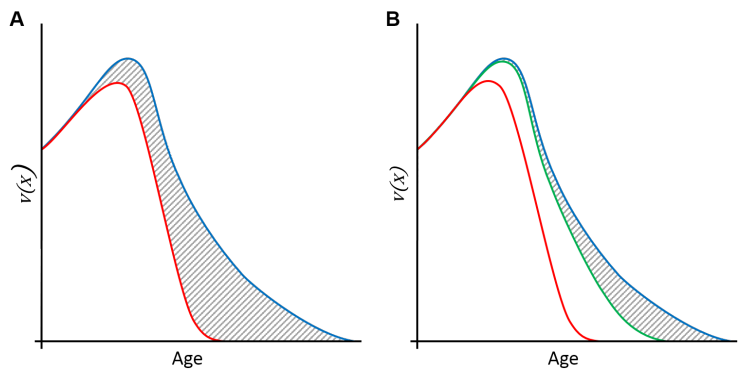1076 and "unmanaged deterioration".

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. (27) and (28). Curves depict calculated trends.

1077 In unmanaged deterioration, the degradation of biocomponents occurs at a rate proportional to the biocomponent's susceptibility to
1078 irreversible fidelity loss, or the direct and indirect effects of degradation present in other biocomponents (Fig. 10, left). Regardless of
1079 their importance to instantaneous mortality rate or fecundity, the most susceptible components reach failure levels first—leading to
1080 premature reductions in survival probability and fecundity—while other biocomponents could remain at relatively high performance
1081 levels (i.e. low degradation states).

1082 In managed deterioration, longevity optimization genes modulate the deterioration rate of biocomponents so that those of similar
1083 importance degrade at comparable rates and/or reach their failure threshold at equivalent ages. Critical biocomponents are
1084 prioritized. No singular biocomponent is permitted to reach a degradation state that unduly compromises fecundity or survival
1085 probability—effectively increasing longevity and overall fitness (Fig. 10, right). This could be accomplished by several means, including:

1086 1. Reallocating resources, at the cellular level and higher, as usable energetic resource availability declines to prioritize
1087   biocomponents most important to preserving reproductive value.
1088 2. Adjusting microenvironmental conditions to decrease thermodynamic potentials on more vital biocomponents and thereby
1089   lowering the rate of damage-inflicting internal entropy production.
1090 3. Reducing biocomponent turnover rates to delay the clonal expansion of irreversibly compromised biocomponents.
1091   (Effectively reduces the rate of fidelity loss.)
1092 4. Altering physiology so that stresses on biocomponents that are more vital are reduced to help maintain these biocomponents
1093   within their functional limits.

1094 One possible example of items (2) and (3) is the way by which controlled decreases in mitochondrial fusion and fission may attenuate
1095 mtDNA informational fidelity loss during aging. Mitochondrial fusion/fission rates are integral to mitophagy and mitobiogenesis (Twig
1096 et al. 2008; Youle and Narendra 2011) and are therefore critical for preserving the performance of mitochondrial components.
1097 Reducing fusion/fission compromises mitochondrial quality by increasing the load of ROS products and otherwise-damaged
1098 mitochondrial components. Mitochondria in aged organisms produce less usable energy (Yaniv et al. 2013), likely due to the combined
1099 effects of reduced mitochondrial fusion/fission and mtDNA informational fidelity loss that occurs with age. Figge et al. (2012)
1100 demonstrated that decelerating mitochondrial dynamics actually helps to preserve mitochondrial performance. MtDNA informational
1101 fidelity losses are attenuated by reducing the exposure of mtDNA molecules to the high thermodynamic stress conditions encountered
1102 during replication and delaying the spread of "parasitic" mutated mtDNA molecules. This is evidently more critical to preserving
1103 mitochondrial performance than the tradeoff of increased degradation state (reduced performance) in other mitochondrial
1104 biocomponents. In the context of the current discussion, the genes responsible for realizing this strategy are longevity optimizers.
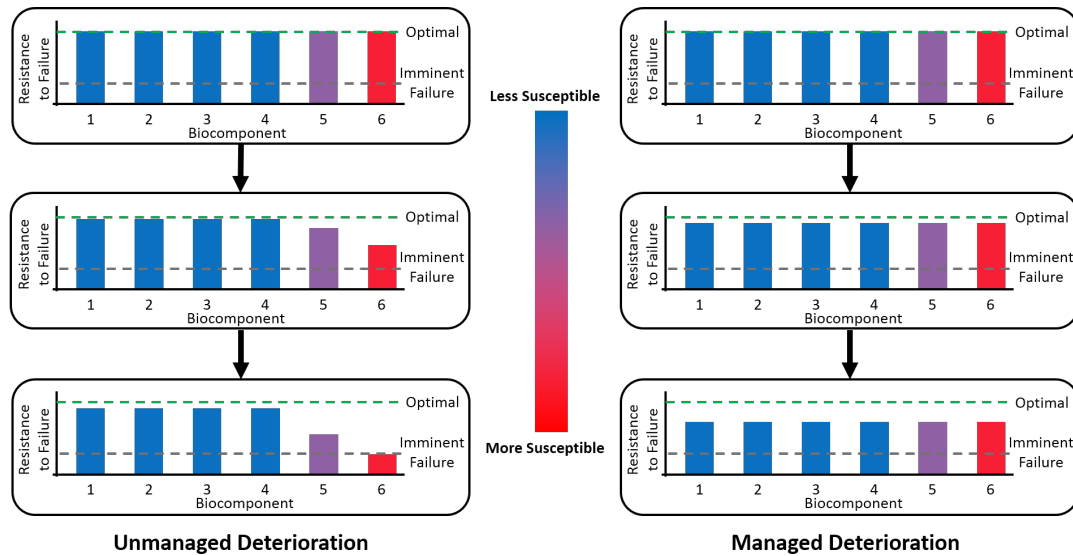
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Fig. 10. Demonstration of unmanaged (left) and managed (right) deterioration strategies. A group of arbitrary biocomponents, equally vital to an organism's fecundity/survival, are depicted. Three ages are considered: Top—Young Adult, Center—Middle-Age, 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.

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; García-Prat et al. 2013; Sousa-Victor et al. 2014; Sousa-Victor et al. 2015; Dumont et al. 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 put forth here is that age-dependent physiological alterations, even those that at first glance appear purely detrimental, may actually serve a purpose in establishing a balanced configuration in the face of inevitable, and progressively increasing, fidelity losses.

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). However, beyond the evolutionary argument for their existence, there is other evidence suggesting that mechanisms of this type might 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), demonstrating conservation of aging signatures across species.

For example, lysosomal genes are overexpressed with age. It is plausible that the decreased protein turnover exhibited in an aged individual could lead to a greater load of proteins that have degraded to the extent that they cannot be processed by proteasomes and therefore they 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 that this balance represents the best compromise for maximizing reproductive value.

DNA methylation patterns also change in line with chronological age in humans (Christensen et al. 2009; Boks et al. 2009; Rakyan et al. 2010; Bocklandt et al. 2011; Bell et al. 2012; Heyn et al. 2012; Gentilini et al. 2012; Garagnani et al. 2012; Horvath 2013; Hannum et al. 2013; McClay et al. 2013; Florath et al. 2013; Christiansen et al. 2015). Predictors can reliably estimate the age of human cells from any human tissue type based on epigenomic DNA methylation profiles (Horvath 2013; Hannum et al. 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.

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

The concept advanced here proposes that selective pressures have led to the evolution of genetic optimizations that attenuate the rate of loss of DNA informational fidelity (and irreversible fidelity losses in other susceptible biocomponents) 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 losses, 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 help establish a more balanced overall phenotype for each and every stage of life.

This suggests that evolved, early adult-life longevity optimization pathways could serve as a substantive basis for 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 is able to 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 advanced ages within a relatively short period of time, even though selective pressure decreases with age (Medawar 1952; Williams 1957; Hamilton 1966). 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 benefits would require considerably less selective pressure and could be realized within a fewer number of generations. Yet, since selective pressure declines with age, it would still likely take an extremely long time for late-life longevity optimizations to evolve in order to fully maximize longevity extension potential. For this reason, longevity optimization in all organisms is almost certainly not fully realized.

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 with increasing age and decreasing selective pressure. 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. 9, blue curve).

9.4   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

1176   and the relatively high degree of conservation of the aging phenotype across taxa may largely be manifestations of these compromises.

1177   A more detailed depiction of this model links further aspects of the aging process (Fig. 11).
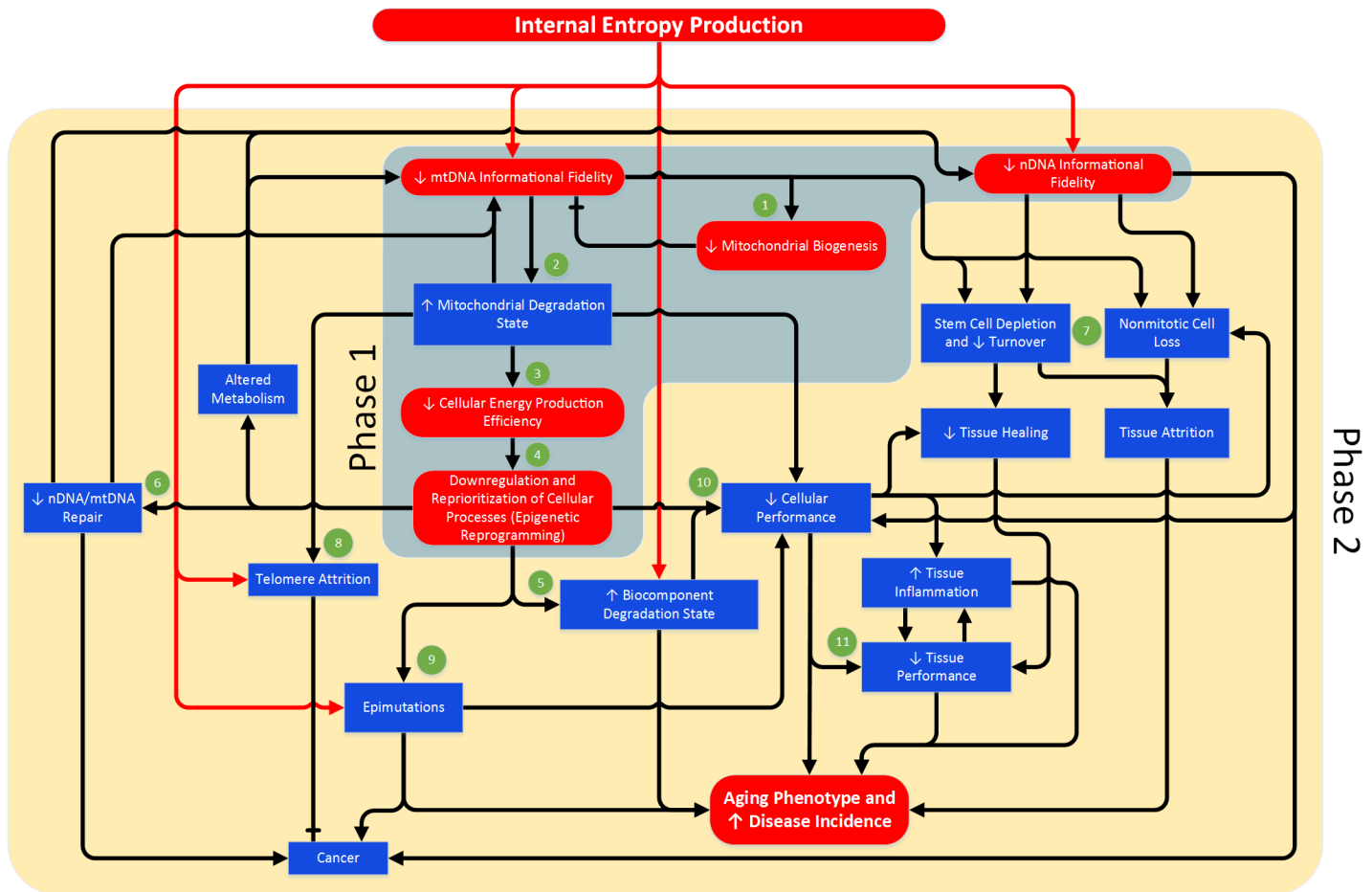

Fig. 11. A more detailed look at the higher-level interactions implicated in this theory during the progression of the aging phenotype. The red lines highlight where the degradative effects of internal entropy production are directly exerted. MtDNA informational fidelity loss drives a deceleration in mitochondrial fusion/fission, 1—mitigating, but unable to prevent, further losses. Combined with reduced fusion/fission rates, losses in mtDNA informational fidelity 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 changes in resource allocation as well as 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 fidelity is gradually further compromised as renewal processes are downregulated, 6. Increasing losses in nuclear DNA informational fidelity with age (Podolskiy et al., 2016) 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 DNA informational fidelity also decreases stem cell viability and consumes 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 DNA informational fidelity 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.

10   Connecting the Dots

10.1   Differentiating between Longevity Determinants and Longevity Optimizers

The model described here proposes 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 upon 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, 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.

In contrast to a longevity determinant, a longevity optimizer is any genetic element that increases 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. To summarize this model, 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.
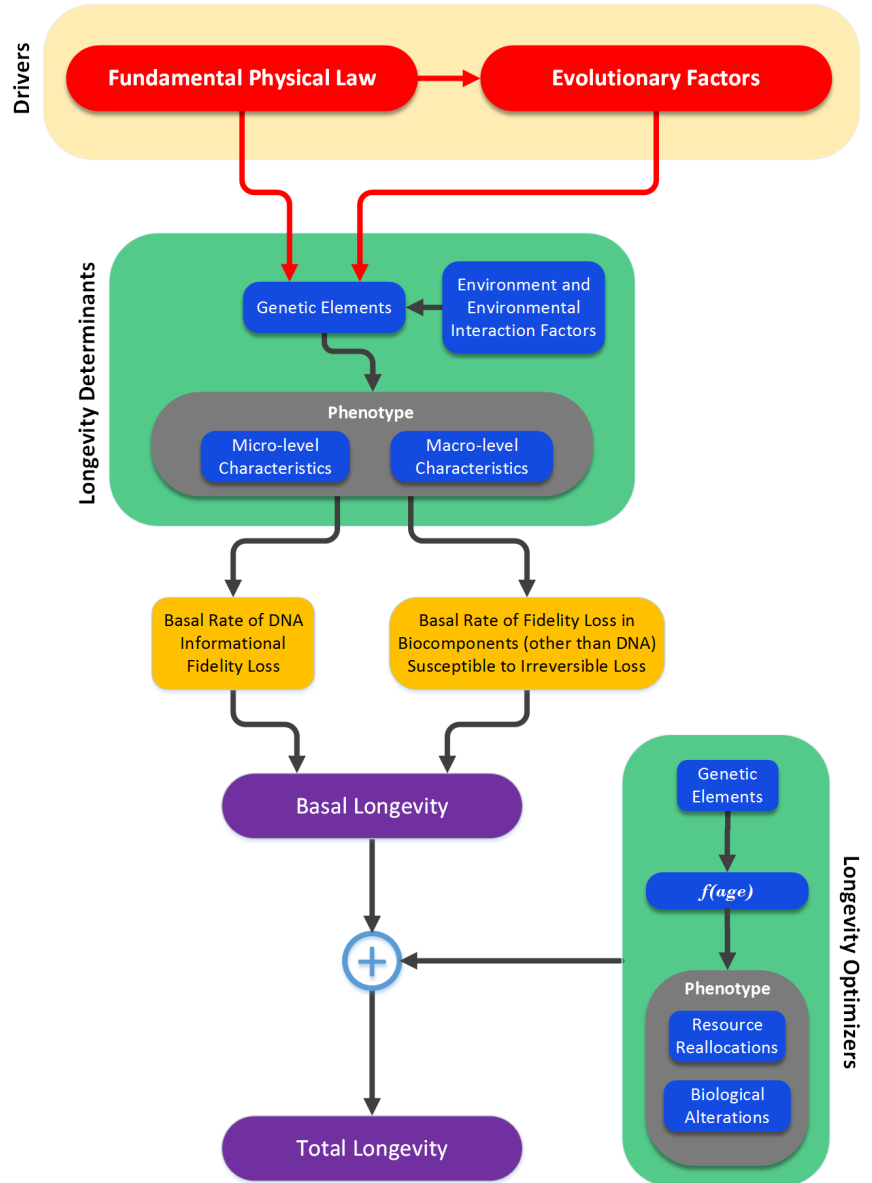

Fig. 12. The proposed relationship between longevity determinants, the root causes of aging (explained by fundamental physical law and evolutionary theory), longevity optimizers, basal longevity and total longevity of a multicellular species.

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

Complex organisms tend to have more cell and tissue types along with 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 have more 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 as the individual ages. 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 (Tatar et al. 2001; Blüher et al. 2003; Selman et al. 2008).

10.3 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 and the demands of a mouse body. While reducing the metabolic rate by such a large amount would reduce the thermodynamic stresses on cardiomyocytes, it would also mandate a loss in their biological power density. The cardiomyocytes would be less capable of generating the contractile forces necessary to counter the energy dissipation inherent to the murine circulatory system and hence blood circulation would 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 all 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, such as the liver and brain, might be similarly compromised in this type of scenario.

This logic offers an additional, more compelling reason why metabolic manipulations found to increase longevity in simple model organisms translate poorly 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 *C. elegans*, reducing metabolic rate via genetic manipulation undoubtedly lowers fitness. This fitness cost explains why *C. elegans* has not evolved to incorporate such changes in their genome: the tradeoff between increased longevity and other impacted factors that affect fitness is not sufficient to select for these changes. For these reasons, I postulate that longevity exhibits a degree of rigidity that increases with organismal complexity and is bound within physiological limits that are explained by fundamental physics.

11 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.

11.1 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 described here predicts that the rate of internal entropy production is

1273 greater in organisms with shorter longevity due to increased peak biological power density and that this necessitates upregulated
1274 biomolecular renewal to counteract. The result is an increase in the rate of irreversible fidelity loss in susceptible biocomponents.
1275 Particularly relevant for many species are the higher thermodynamic stresses placed on DNA molecules (largely through increased
1276 replication rates), which lead to an increased rate of loss of DNA informational fidelity and reduced longevity.

1277 This logic offers an explanation for the negative correlation between metabolic rate and longevity, and for the allometry of longevity,
1278 which are two of the most long established and irrefutable species longevity trends. Although exceptions to these relationships exist,
1279 the model described here also provides rationale for the existence of these exceptions. No theory incorporating declining selective
1280 pressure as the primary cause of aging has been able to explain these observations.

11.2 Physics Helps to Explain the Hayflick Limit

1282 Leonard Hayflick demonstrated that primary cells undergo a limited number of doublings before senescing (Hayflick and Moorhead
1283 1961; Hayflick 1965), an observation later dubbed the "Hayflick limit". Here it is explained how physics stipulates the loss of DNA
1284 informational fidelity and why the degree of loss may be largely a function of replication count. The presence of a replication counter
1285 to quantify these losses arises logically from this argument, as does the presence of a replication limit for disposing of cells that have
1286 exceeded some threshold of loss. Aging theories based on declining selective pressure are unable to explain why the Hayflick limit
1287 exists.

11.3 The Degradation State Concept Resolves Previously Unexplained Longevity Paradoxes

1289 Physics clarifies why it is impossible to sustain a biomolecular ensemble in a perfect state of fidelity (because infinite resources are
1290 required). The degradation state concept introduced here provides a means to quantify the relative condition of a biomolecular
1291 ensemble. A prediction of the described model is that organisms that place extreme priority on maximizing renewal ROI are likely to
1292 function at higher degradation states (i.e. with more apparent degradation). It also predicts that organisms with higher peak biological
1293 power densities will have lower degradation states at a given life stage, and vice versa. Both of these ideas are counterintuitive, but
1294 they help to explain observations previously considered paradoxical and could not be explained by declining selective pressure.

1295 Furthermore, when the inevitability of irreversible fidelity loss in a subset of biocomponents is considered along with the concepts of
1296 degradation state and managed deterioration, an explanation emerges as to how and why the overall aging phenotype may develop,
1297 including how an organism transitions between new homeostatic states as aging progresses and why many aspects of aging are
1298 conserved.

1299 For the above reasons, it is reasonable to propose that stipulations resulting from fundamental physical law are more critical to
1300 longevity determination than declining selective pressure. On the other hand, declining selective pressure may help to explain why
1301 longevity optimization is less than ideal.

11.4 Drawing Incorrect Presumptions from Manipulation of the Aging Phenotype

1303 Typically, the putative aging pathways/genes[9] have been branded as such because they were found to contain genetic elements which,
1304 when altered, modulated longevity in some model organism (often of low complexity, e.g. fruit fly or nematode) or produced a distinct
1305 effect on a characteristic(s) typically associated with the aging phenotype. However, fundamental physical law implies that organisms
1306 of greater complexity are bound by more stringent physiological restrictions; hence, such manipulations may never deliver substantial
1307 longevity benefits to organisms that are more complex. Furthermore, if managed deterioration, as described here, is an actual
1308 component of the aging process, then manipulations intended to address specific characteristics of the general aging phenotype will
1309 usually carry overall negative repercussions for the individual—regardless of whether or not they "correct" some negative aspect of
1310 the aging phenotype—because they disrupt the natural, evolved homeostatic balance that maximizes fecundity/survival at those later
1311 life stages.

1312 Observations of singular connections between genetic elements and particular phenotypes demonstrate only that the gene is
1313 responsible for modulating those characteristics—it should not imply that the gene is responsible for aging, nor does it necessarily
1314 reveal anything about the aging process. This thinking is quite a departure from the current mainstream approach, which focuses on
1315 establishing direct relationships between particular genes and their observable effects on the aging phenotype—leading to highly
1316 questionable presumptions regarding the culpability of a particular factor as a root-level longevity determinant or "cause" of aging.

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[9] 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.

1317 For these reasons, I believe that the current catalog of putative aging pathways/genes represents, at best, a grossly incomplete set of
1318 the factors truly relevant to longevity determination.

11.5 Charting a New Course

1320 Aging is the greatest risk factor for severe pathology. Yet, most aging research is focused on age-associated pathologies or metabolic
1321 manipulations in lower life forms, rather than the fundamental biology of aging (Hayflick 2000; Hayflick 2007a). Because of this, and
1322 despite the fact that a number of pathways influencing longevity have been identified, one can argue that scientists are no closer to a
1323 consensus theory of aging today than they were fifty years ago. Even worse, despite the ever-growing litany of serious anomalies
1324 challenging common aging theories, the scientific community remains complacent. The multitude of aging theories, the discontinuities
1325 between them, and the failure of the scientific community to agree on the root causes of aging, while disappointing, represents a clear
1326 opportunity to revisit this problem with a multidisciplinary and somewhat radical approach.

1327 The theoretical framework discussed in this paper utilizes concepts from physics, information theory, as well as evolutionary theory
1328 to explain why organisms age and why they live as long as they do. I believe that the theoretical framework arising from this approach
1329 has fewer anomalies than existing singularly focused theories. While the concepts put forth here are well supported by the findings of
1330 others in diverse fields, it is admittedly not devoid of speculative components. Additional data, such as delineating the species
1331 differences in mitobiogenesis rates, would bring better clarity to important questions that remain and would be very helpful in refining
1332 the arguments presented here.

1333 The idea that aging can be manipulated is alluring. Nevertheless, difficult problems are only solved by addressing their root causes. In
1334 the absence of a central theoretical framework for biological aging, it is hard to predict whether a particular strategy or approach for
1335 treating an age-associated pathology, or for increasing healthspan or longevity, has any chance of succeeding in humans. The
1336 theoretical framework outlined in this paper provides an explanation as to why the longevity benefits seen in simple organisms through
1337 manipulations of putative aging pathways/genes are not realized in organisms that are more complex and why they likely never will
1338 be. If accurate, this highlights the naivety of longevity extension efforts to identify and manipulate genes and molecular pathways that
1339 could substantially increase human longevity without compromising health or performance, and the futility of attempting to use
1340 simple model organisms such as *C. elegans* as the vehicle for such efforts. On the other hand, the capacity for these approaches to
1341 extend healthspan in higher organisms (the so-called "intervention potential"), though limited, may be rather straightforward to
1342 estimate.

1343 The theoretical framework discussed here identifies fundamental physical law and evolutionary theory as potential root causes of
1344 aging. Of course, we cannot manipulate the laws of physics or fabricate exceptions to evolutionary theory, but it may be possible to
1345 resolve their most direct downstream negative repercussions on individual viability—irreversible fidelity loss[10] in susceptible
1346 biocomponents—and effectively retard the aging process, reduce susceptibly to age-associated pathologies, or even restore the
1347 individual to a more youthful state. For example, while DNA informational fidelity loss may be inevitable, the complete original genetic
1348 sequence of an aged individual can be recreated through the collective sequencing of a population of cells from the individual.
1349 Hypothetically, it should be possible to artificially produce youthful cells by sorting extant cells according to degree of DNA
1350 informational fidelity loss, correcting any genetic errors, resetting stemness or differentiation state if required, followed by
1351 propagation and expansion *in vitro*. Reintroducing these cells into an aged individual could produce some of the aforementioned
1352 benefits. Although realizing an intervention such as this has a number of technical hurdles, the potential payoffs could be far greater
1353 than the indiscriminate approaches currently prioritized which have little to no chance of ever producing tangible results.

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[10] 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 imply that such losses cannot be resolved through artificial means.

1354   Glossary

1355

1356   **Biological power density:** The volume-specific rate of external biomechanical, biochemical and bioelectric work (power per unit
1357   volume) realizable in a specific cell(s), tissue, etc. *Peak* biological power density is the maximum localized value achievable within a
1358   particular organism.

1359   **Biomolecular performance:** A measure of the relative ability of a biomolecule to perform its intrinsic biological function.

1360   **Degradation state:** A parameter used to quantify the level of degradation existing within a biomolecular ensemble ("pool" of a given
1361   biomolecule).

1362   **Homeostatic shifts:** The concept that an individual organism transitions between different homeostatic setpoints as it ages and that
1363   these shifts are typified by increases in biomolecular degradation state, largely resulting from decreases in biomolecular turnover
1364   rates.

1365   **Internal entropy production:** The loss of order caused by the nonzero thermodynamic potentials that exist in all nonequilibrium
1366   systems. All forms of biomolecular damage result from the production of internal entropy.

1367   **Longevity determinant:** A factor that directly or indirectly influences the basal rate of loss of fidelity in any biocomponent susceptible
1368   to irreversible fidelity loss. Longevity determinants define an organism's basal longevity.

1369   **Longevity optimizer:** A genetic element that elicits an effect that generally becomes progressively more dominant with age and that
1370   delays the severity, or rate of progression, of the aged phenotype. Longevity optimizers increase longevity beyond the basal longevity
1371   specified by longevity determinants.

1372   **Managed deterioration:** The concept that longevity optimizers modulate the deterioration rate of biocomponents so that those of
1373   similar importance degrade at comparable rates and/or reach their failure threshold at equivalent ages. Managed deterioration is
1374   proposed as the main mechanism by which longevity optimizers realize their longevity extension effects.

1375   **Negative entropy:** Facilitates an increase in the order of a system. In organisms, negative entropy is generated by repair/replacement
1376   processes and biomolecular import. Utilized to combat internal entropy production and maintain biomolecular degradation state.

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1729 *Supplementary Data*

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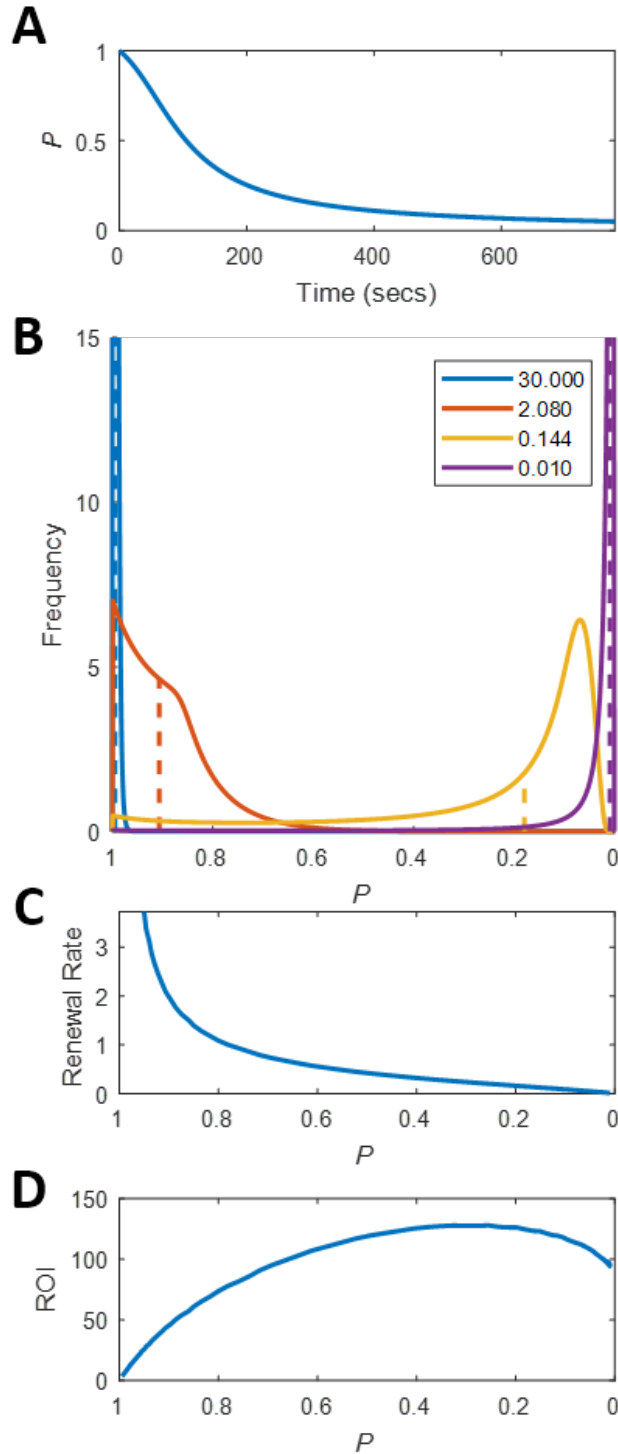
Supp. Fig. 1. Degradation, renewal and performance characteristics of a hypothetical biomolecule exhibiting excellent stability at high P values. (a) Loss of P with time in a non-renewing population. (b) Simulation of distribution of biomolecules across equivalent P values for different rates of random renewal. Renewal rates are in % of total population replaced per second. Dashed vertical lines indicate P of the ensemble (i.e. average equivalent P). (c) Relationship between P (ensemble) and renewal rate under random renewal conditions. (d) Renewal ROI versus P under random renewal. ROI is in % of $\dot{W}_{m,max}$ / renewal rate. Max ROI occurs at $P = 0.255$.