Systems Medicine 2021 BE333 Lecture Notes

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Lecture 7

Aging and the saturation of damage removal

We've just seen some basic facts about aging on the population level, such as the Gompertz law. We also discussed how different forms of molecular damage cause aging, in part through the accumulation of senescent cells. In this lecture we connect between the molecular and population levels. To do so, we build a conceptual framework to understand the stochastic processes of senescent cell accumulation and removal. Our payoff will be a first-principle explanation of the Gompertz law, of the variation in aging, and of the dynamics of anti-aging interventions.

Removing SnCs in mice slows age-related diseases and increases average lifespan

In 2016 an experiment by van Duersen et al (Baker et al., 2016) galvanized the aging field. It showed that accumulation of SnCs is causal for aging in mice. Continuous targeted elimination of whole-body SnCs increased mean lifespan by 25%, and attenuated the age-related deterioration of heart, kidney, and fat. The experiment has been repeated by many groups using different methods to remove SnCs. These methods include several families of drugs called **senolytics** that selectively kill SnCs in mice. Some of these drugs are toxic for humans, but improved drugs are under development. Senolytics delay cancer development and cause improvement in age-related diseases including diabetes, osteoarthritis, Alzheimer's and heart disease.

For a sense of the effects of SnC removal, see the picture of twin mice at age 2 years from van Duersen's lab (Fig 7.1). One had SnCs removed since the age of one year. It runs on the wheel, has shiny fur and overall better health. Its sibling, treated with mock injections, barely runs on the wheel. It looks like a typical 2-year-old mouse with a hunchback, cataract and fur loss (Fig 7.1 on the left).

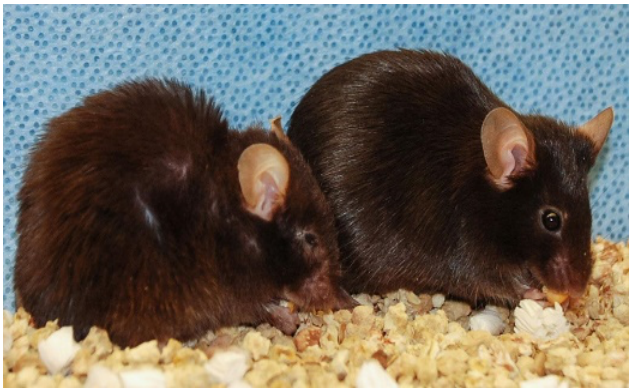

Figure 7.1

SnC are not the only cause of aging, as evidenced by the fact that these mice still age, get sick and die. But we will pretend that they are the only (or dominant) cause. We will also pretend that SnC are a single entity, even though they are heterogenous and tissue-specific. These simplifying assumptions will help us write a stochastic process that can explain many of the empirical obsrevations that we described in this lecture on the population features of aging.

In a nutshell, aging derives from our three laws:

- All cells come from cells (Stem cells produce differentiated cells)
- Cells mutate (Mutant stem cell number increases linearly with age, and they produce damaged/senescent cells)
- Biological processes saturate (damged/senescent cell removal eventually saturates eventually, and damaged/senescent cell levels rise sharply leading to inflammaging)

Natural selection is important here, as in the disposable soma theory of aging. Damage removal capacity is selected for the young, not the old, and hence does not increase with age. Later in the lecture we will make this even more general, to explain aging in invertebrates and even single celled organisms: damage-producing units that accumulate linearly with time, until they overwhelm the damage removal process which saturates.

Senescent cell dynamics show a nearly exponential rise with age and lengthening correlation times

We saw that **senescent cells** are an important accumulating factor that is causal for aging: removing senescent cells slows aging. In other experiments, adding them increases risk of death. It makes sense, then, to explore how the amount of senescent cells in the body, which we denote by X, varies with age in different individuals.

For simplicity, we will consider senescent cells as a single category, despite the fact that they are a name for many different cell states and cell types, accumulating in the different organs of the body. For organisms without senescent cells, such as *C. elegans* and fruit flies, we will think of X as a type of damage, such as protein damage, that is a primary cause for aging. More on that below.

To get a feeling for the dynamics of senescent cells, let's consider an experiment, by (Burd et al., 2013), who measured senescent cell abundance in 33 mice every 8 weeks for 80 weeks (Fig 7.2).

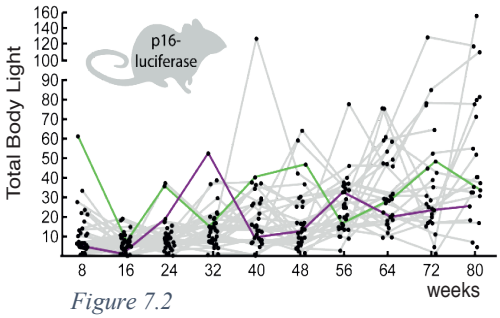

Figure 7.2

To measure whole-body senescent cell amounts, Burd et al used genetic engineering to produce mice that made photons in proportion to the number of senescent cells they have. In a nutshell, they used a gene from fireflies called **luciferase** that produces photons when it acts on a certain substrate. They introduced the luciferase gene into the mouse DNA and placed it under the control of a DNA element, called the p16 promoter, that is activated almost exclusively in senescent cells. Therefore, only the senescent cells in these mice make the protein luciferase.

When the substrate for luciferase is injected into the mouse, the mice produce light. Mice normally don't make photons, so that observing the light emitted from their special mice allowed Burd et al to estimate senescent cell abundance, X. The experiment has several limitations, such as stronger absorption of light from inner regions, some genetic disruption of the natural p16 system which enhanced the chance of cancer after 80 weeks, so that the experiment could not probe very old ages, and experimental noise. But the experiment serves as a good starting point.

Looking at total light emitted from these mice as a measurement of X, we see that X rises and falls across time and generally increases with age (Fig 7.3). The data suggests two timescales: fast timescale of fluctuations over weeks, and a slow timescale in which X rises on average over years (Fig 7.3). This fast-slow timescale separation will be useful for building our model.

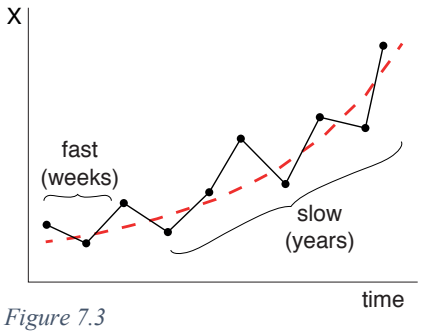

Figure 7.3

Analyzing the data provides four features:

(i) The *average X grows at an accelerating rate,* nearly-exponentially with age (Fig 7.4). Such nearly-exponential accumulation with age is also seen in senescent cells in human tissues.

(ii) The *variation in X between individuals grows with age* (Fig 7.5). Old mice span a larger range of X than young mice. Some old mice even have X levels similar to young mice (Fig 7.2). This variation grows, however, more slowly than the growth of average: the

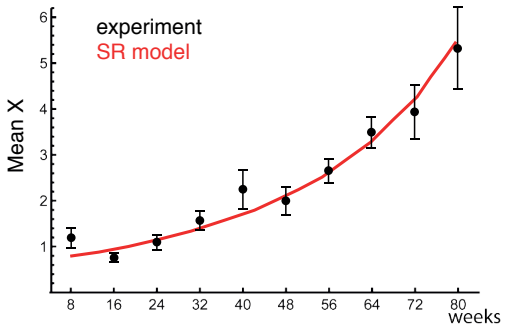

Figure 7.4

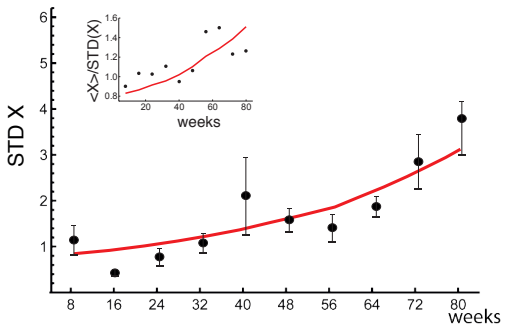

Figure 7.5

mean X divided by standard deviation grows roughly linearly with age $\frac{<X>}{std(X)} \sim \tau$ (Fig 7.5 inset).

(iii) Distributions of X among individuals at a given age are skewed to the right, so that there are more individuals with higher than average X than individuals with lower than average X (Fig 7.6). The skewness of these distributions gradually drops with age.

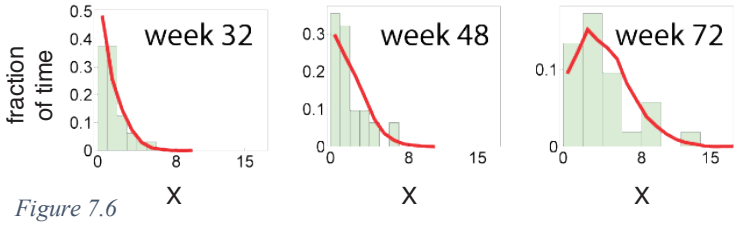

Figure 7.6

(iv) The correlation time of X increases with age. This means that a mouse that is higher or lower than average stays so for longer periods of time at old age than at young ages. (Fig 7.7). Thus, with age, the stochastic variation in X becomes more persistent.

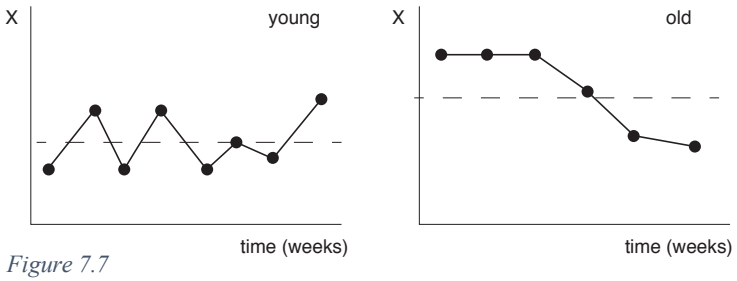

Figure 7.7

Interestingly, these features are shared with the human frailty index described in the last lecture, which also rises exponentially with age, shows widening variation (increasing standard deviation) with age that rises more slowly than the mean, and skewed distributions between individuals.

A model with increasing production and saturating removal can explain senescent-cell dynamics

These dynamical features of senescent cells can be explained by a simple model, called the **saturating removal (SR)** model, as discovered by Omer Karin in his PhD with me (Karin et al, 2019). Omer scanned a wide class of models, and found the essential features that a model needs in order to explain the senescent cells dynamics we just discussed.

The first important feature is to have **two timescales**, a fast and a slow timescale: X is produced and removed on a timescale that is much faster than the lifespan. This separation of timescales allows us to write an equation for the rate of change of X in which the parameters, such as production and removal rates, vary slowly and depend on age τ. The model also includes stochastic noise. Thus,

$$\frac{dX}{dt} = production - removal + noise$$

The model that best describes the data is biologically plausible. The production rate of X rises linearly with age, as $\eta\tau$. This aligns with the biological expectation, discussed in the previous lecture, that senescent cells arise from mutant stem cells S' that produce damaged differentiated cells D' that become senescent cells. The number of mutant stem cells rises linearly with age, as we saw in the previous lecture, and thus the production rate of senescent cells should also be linear with age:

$$production = \eta\tau$$

The removal of X is carried out by special garbage trucks, namely immune cells such as NK cells that kill senescent cells. The NK cells discover senescent cells by means of special marker proteins that senescent cells display on their surface. The NK cells then attach to the senescent cell, and inject toxic proteins to kill it. Mice without functioning NK cells show accelerated aging and large amounts of senescent cells. Other immune cells, including macrophages, also play a role by swallowing up the remains. Possibly other types of immune cells such as T cells also help to remove senescent cells.

If this removal process worked at a constant rate β per senescent cell, the probability unit time to remove each senescent cell would be constant with age. The removal term would thus be $-\beta X$. However, such a constant β does not match the data. It would result in a linear rise of X with age, as opposed to the nearly exponential rise observed. To see, the equation is $\frac{dX}{dt} = \eta\tau - \beta X$. We use τ for age and t for time to make sure that we understand that there are two timescales: a fast scale (days-weeks) in which damage reaches steady-state, and a slow timescale (years) over which production rate $\eta\tau$ changes. The steady-state solution, found by dX/dt=0, rises linearly with age $X_{st} = \eta\tau/\beta$.

Note that we can make such a steady-state solution thanks to separation of timescales. At a given age τ, the production and removal balance to determine X_st much faster than age changes. This steady-state level then tracks the much slower changes in age. It's like an equation for a child jumping- we can safely ignore the changes in the child's height during the jump.

Thus, it makes sense from the nearly exponential rise of X that *the removal rate per senescent cell should slow down with age*. Karin tested many mathematical ways for this reduction to occur. The simplest way to model this, which accounts for the four features mentioned above, is to assume that the **removal rate drops with the amount of senescent cells**. In other words, senescent cells inhibit their own removal. Such a drop could be due to several processes: immune cells that remove senescent cells could be down-regulated if they kill too often, or they can become inhibited by

factors that the senescent cells secrete. The drop in removal rate can also be simply due to a saturation effect, in which the removing cells become increasingly outnumbered by senescent cells as senescent cell numbers rise. Garbage trucks are overloaded. Indeed, NK cell numbers are about constant with age in humans.

To model such saturation, we use a Michaelis-Menten form (which is good both for inhibition due to secreted factors and for saturation by large numbers, see solved exercise 7.3)

$$removal = \frac{\beta X}{k + X}$$

Where β is the maximal senescent cells removal capacity, with units of senescent cells/time. It's the maximal capacity of the trucks. k is the concentration of X at which senescent cells inhibit half of their own removal rate. The removal rate *per senescent cell* thus drops with senescent cells amounts, $\frac{\beta}{k+X}$ (Fig 7.8)

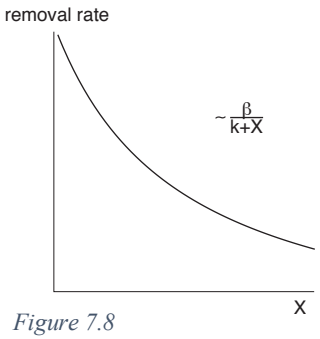

Figure 7.8

Combining production and removal, we obtain a model for the rate of change of X:

$$\frac{dX}{dt} = \eta\tau - \frac{\beta X}{X + \kappa} \qquad [1]$$

Where we use τ for age and t for time to make sure that we understand that there are two timescales: a fast scale (days-weeks) in which damage reaches steady-state, and a slow timescale (years) over which production rate $\eta\tau$ changes. Note that this model assumes that maximal removal capacity β does not decline with age. Adding such a decline, namely $\beta(\tau)$, generally leaves the conclusions the same. For simplicity we ignore this possibility.

Let's compute the steady-state X. On the fast timescale of weeks, the production rate $\eta\tau$ can be considered as constant. Setting $dX/dt = 0$ in Eq. 1 we find that the (quasi-) steady-state X of is

$$X_{st} \approx \frac{\kappa\eta\tau}{\beta - \eta\tau} [2]$$

Thus, X_{st} rises linearly with age at first. Then, the term on the bottom becomes closer and closer to zero, which is an explosion point. The rise in X thus accelerates and diverges at a critical age $\tau_c = \beta/\eta$ (Fig 7.9). In fact, this rise is almost indistinguishable from an exponential rise over the 5-fold range of the available experimental data (Fig 7.3, Fig 7.9, dashed line).

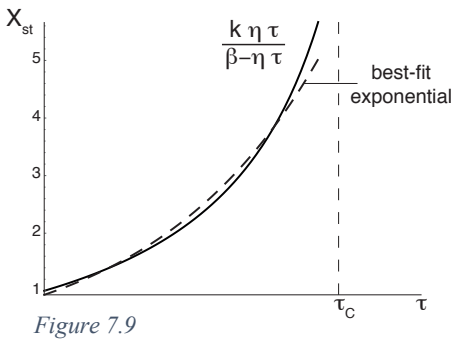

Figure 7.9

When X levels rise high enough, they reach levels not compatible with life. Thus, the critical age $\tau_c = \beta/\eta$ is a rough approximation for the mean lifespan. The lifespan is longer the bigger the repair capacity β, and longer the smaller the rate at which senescent cell production increases with age, η.

To get a graphic sense of why X accelerates with age, we can use a rate plot. We plot the production and removal terms in Eq 1. Removal is beta $\frac{\beta X}{k+X}$ which is a saturating curve (Fig 7.10). Note that removal rate *per cell* goes down with X as $\frac{\beta}{k+X}$, and the plot shows total removal rate, which is the removal rate per cell times X, and is therefore a rising and saturating curve.

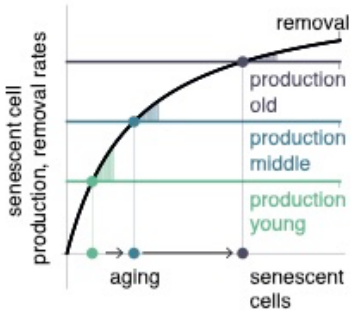

Figure 7.10

Production rate, represented by the colored horizontal lines, is low in young organisms and rises with age. The points to watch are where production equals removal. These are the steady-state points at each age. With age, the steady-state X accelerates to higher and higher levels (Fig 7.10) because of the saturating shape of the removal curve. When the production rises above the removal curve, which occurs when age goes beyond the critical age, the steady state points shifts to infinity, and X grows indefinitely.

Damage production rises with age, and saturates the repair capacity

Another way to understand this model is the parable of the garbage trucks. A young organism is like a small village that produces a small amount of garbage (senescent cells). The village has 100 garbage trucks, more than enough to clear the garbage. With age, the village becomes a big city producing a lot of garbage. Since we are not designed to be old, there are still 100 trucks. The trucks are overloaded, and garbage piles up in the streets. If there is a perturbation (infection, injury) and extra garbage is added, it stays for a long time. Once garbage is produced at a rate larger than the maximal capacity of the trucks, garbage piles up higher and higher.

Similarly, the body's immune cells that remove senescent cells get saturated or downregulated, and senescent cells pile up. The senescent cells cause inflammation and reduce stem cell renewal. The saturation of the immune cells also hampers their other tasks: fighting infection and cancer. Thus risks of illness and organ dysfunction rises with age.

Adding noise to the model explains the variation between individuals in senescent cell levels

So far, the model does not describe the fluctuations of X over time for each individual, nor the widening differences between individuals. To understand these stochastic features of the dynamics, we need to add **noise** to the model.

The simplest way to add noise is to add a white-noise term ξ with mean zero and a variance described by the parameter 2ϵ (the factor 2 is for convenience in the equations below). This noise describes fluctuations in production and removal due to internal or external reasons such as injury, infection and stress (cortisol). In fact, we don't currently know what the noise describes. White noise is a convenient way to wrap up our ignorance in a mathematical object that we can work with.

We thus arrive at the main model of this lecture, called the **saturated removal (SR) model**:

$$\frac{dX}{dt} = \eta\tau - \frac{\beta X}{X+\kappa} + \sqrt{2\epsilon}\xi \qquad [3]$$

We will use this model to understand the dynamics of senescent cells, and then to understand the origin of the Gompertz law. Let's begin with understanding the variation in X between individuals at a given age. To do so, we need to compute the distribution of X between individuals, P(X).

Solved example 1: compute the distribution of X at a given age

The distribution of X, denoted P(X), is the probability of having X senescent cells. To derive it, we use an approach analogous to free energy in statistical mechanics or in chemical kinetics. The temperature k_bT will be the analog of the noise amplitude ϵ in the SR model.

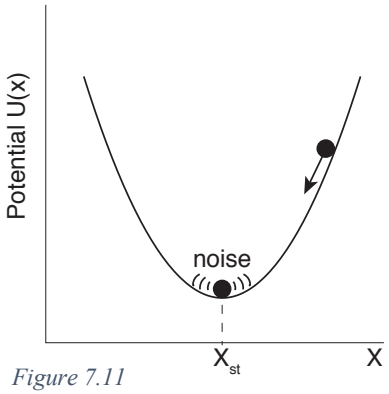

Figure 7.11

To calculate the distribution P(X), we use a general method that applies to any stochastic differential equation of the form: $\frac{dX}{dt} = v(X) + \sqrt{2\epsilon}\xi$. In the SR model, the 'velocity' v(x) equals production minus removal, namely $v(X) = \eta\tau - \beta X/(k+X)$. The idea is to rewrite the equation using a **potential** U(X), defined so that its slope is equal to minus the velocity: $\frac{dU}{dX} = -v(X)$.

The potential function can be imagined as a bowl of shape U(X) (Fig 7.11). The variable X is like a ball rolling in the bowl (Fig 7.11). The ball rolls down the sides of the bowl, with velocity -v(x)

that is equal to the slope of the bowl, dU/dX. The steeper the bowl, the faster the ball rolls. The bowl is coated with a thick goo (in the words of Storgatz's nice book on dynamical systems) and so the ball settles down at the minimum of the bowl without oscillating. At the minimum point the slope is zero, $dU/dX = 0$, and that is where X=X_{st}. The steeper the sides of bowl, the faster the ball returns to X_{st} if it is perturbed.

Let's now add noise. Noise jiggles the ball positon X so that it deviates from X_{st}. These jiggles cause a distribution of X values, P(X). Again, the steeper the bowl, the less the noise can move X away from X_{st}, and the narrower the distribution P(X).

The nice thing about the potential-function way of writing the equation is that we can easily compute the steady-state distribution. This distribution P(X) is given by the Boltzmann distribution, with ϵ playing the role of temperature:

$$P(X) \propto e^{-\frac{U(X)}{\epsilon}} \qquad [5]$$

An intuitive explanation is provided in solved exercise 7.1. The distribution P(X) is wider the shallower the bowl or the higher the 'temperature'.

For the SR model, the potential U(X) is

$$U(X) = (\beta - \eta\tau)X - \beta\kappa \log(\kappa + X) \qquad [6]$$

Which can be checked by taking the derivative $-dU/dX$ and verifying that it gives

$$\eta\tau - \beta \frac{X}{X+\kappa}.$$

We can safely assume that age τ is constant over the fast timescale needed to reach the steady-state distribution P(X), except at very old ages. Plotting U(X) shows that at young ages the bowl is steep, and therefore the distribution is localized around the mean (Fig 7.12). With age, the bowl becomes more and more shallow, because its right-hand slope drops as $-\eta\tau$. The mean position moves higher and higher. At the critical age, when $\eta\,\tau = \beta$, the bowl opens up and the steady-state goes to infinity.

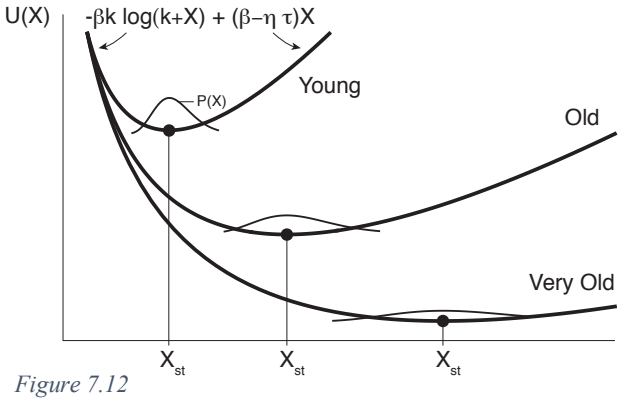

Figure 7.12

Plugging U(X) from Eq. 6 into the Boltzmann-like law of Eq 5 we obtain the distribution

$$P(X) \propto e^{-\frac{(\beta-\eta\tau)X}{\epsilon}}(\kappa + X)^{\frac{\beta\kappa}{\epsilon}} \qquad [6]$$

Which reaches a peak and then falls exponentially with X. This distribution of senescent cells in the SR model is skewed to the right, and quantitatively matches the skewed distributions observed in the mouse data (Fig 7.6, red lines).

This distribution, by the way, provides an estimate for the average X that takes noise into account,

$$\langle X \rangle \approx \frac{\kappa\eta\tau + \epsilon}{\beta - \eta\tau} \quad [7]$$

The mean X rises with age (Fig 7.4, red line). The standard deviation of X also rises with age and diverges at τ_c, as shown by calculating the standard deviation of P(X):

$$\sigma \approx \frac{\sqrt{\kappa\beta + \epsilon^2}}{\eta\tau - \beta} \quad [8]$$

This rise in standard deviation matches the observed rise with age of the standard-deviation of the light emitted from the mice of Burd et al (Fig 7.2). The SR model even captures the fact that variation rises more slowly than the mean – the phenomenon of reducing relative heterogeneity. The ratio between average and std (the inverse of the coefficient of variation) rises linearly with age observed as in the mouse senescent-cell data

$$\frac{< X >}{\sigma} \approx \frac{\kappa\eta\tau + \epsilon}{\sqrt{\kappa\beta + \epsilon^2}} \sim \tau.$$

The SR model also explains the increasing correlation times with age. At young ages, the bowl is steep. Thus, if X is away from Xst, it returns to Xst quickly (Fig 7.13). At old ages, in contrast, the bowl is almost completely flat. The trajectory of the 'ball' is dominated by noise, with very little restoring force coming from the steepness of the bowl (Fig 7.13). Hence individuals that stray away from X_{st} have a slower restoring force back to the mean, and stay away for longer times.

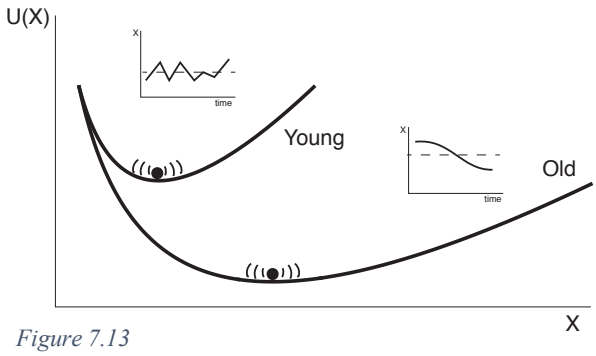

Figure 7.13

Such increasing correlation times have a general name in physics, "**critical slowing down**". They are a mark of an approaching phase transition. In our case, the phase transition is to infinite X, which is death. In the classical example of a phase transition, the boiling of water, large and slow fluctuations in density can be seen near the boiling point. In other areas of science, slowing down of fluctuations can be a warning sign of a big transition. Examples include climate

fluctuations before an ice age, or ecological fluctuations before a species extinction [Schaffer 2009].

The mouse data allows estimating all four model parameters, η, β, k and ϵ. The best fit parameters are approximately $\eta = 4\,10^{-4}\,days^2 \sim 0.15/year/day, \beta = 0.3/day, k = 1, \epsilon = 0.1$, in units where the average senescent cells in young mice is 1. The rough estimate of lifespan $\tau_c = \frac{\beta}{\eta} \sim 2\ years$ is about right for mice. These parameters give a concrete prediction for the half-life of a senescent cell. The half-life is about 5 days in young mice, and rises to about a month in old mice (25 days in 22 month old mice).

An experimental test shows that senescent cells are removed in days from young mice but in weeks from old mice

This prediction was interesting enough to test experimentally. We teamed up with Valery Krizhanovsky, a senescent cell researcher from our department, and his PhD student Amit Agrawal. The idea was to induce extra senescent cells in mice, and then to measure how quickly the senescent cell levels go back to steady state (Fig 7.14).

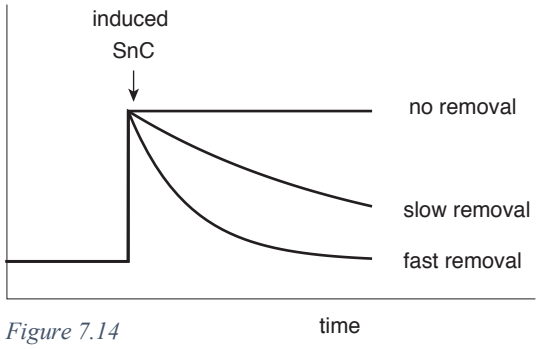

Figure 7.14

Krizhanovsky used a drug, called Bleomycin, that induces DNA damage which makes cells become senescent cells. The drug was introduced into the lungs of mice. The drug is cleared away within a day. Due to the DNA damage, after 5 days, the lungs are full of senescent cells. Then, mice were killed at various timepoints, and the amount of senescent cells in their lungs was measured; the lung was dissolved into single cells, which were stained with a die that labels senescent cells (called SA-beta-gal). The individual cells were photographed in a machine called an imaging flow-cytometer (Fig 7.15A), and the number of senescent epithelial lung cell were counted.

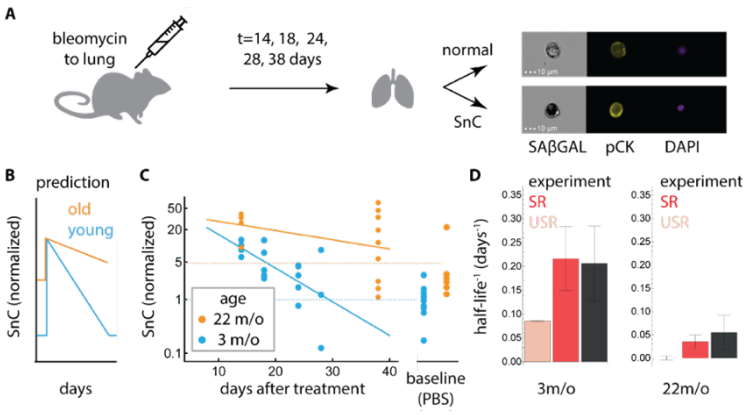

Figure 7.15

In young mice, the senescent cells half-life was 5 ± 1 days (Fig 7.15C). In old mice (22-month-old), removal was much slower, with an estimated half-life of about a month. Note the variation in senescent cells between the old mice. These measurements agree well with the predictions of the SR model (Fig 7.15D). The agreement is striking because the SR model was calibrated on the luciferase-mice, with a different marker for senescent cells (p16 versus SA-beta gal), and a different system (whole body versus lung). This agreement adds confidence in the prediction of the SR model that removal of senescent cells slows with age.

Mamamia/music ABBA

Mamamia, here we go again
My my senescent cells are growing
Mamamia, here we go again
My my removal rate is slowing
Oh cytokines are raging
Oh it must be inflammaging
My my, how can I reduce this load

Gompertz mortality is found naturally in the SR model

In the remainder of the lecture, we explore the implications of rapid senescent cells turnover and slowdown of removal for the question of variability in mortality. As we saw in the previous lecture, lifespan varies even in inbred organisms raised in the same conditions, demonstrating a non-genetic component to mortality. In many species, including mice and humans, risk of death rises exponentially with age, the Gompertz law, and decelerates at very old ages (Fig 7.16).

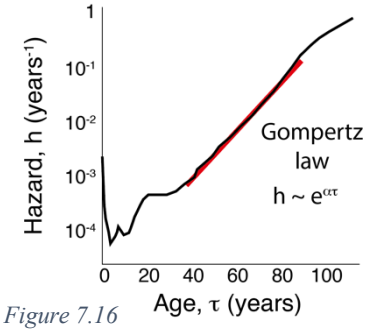

Figure 7.16

To connect senescent cells dynamics to mortality, we need to know the relationship between senescent cell abundance and the risk of death. The precise relationship is currently unknown. Clearly, senescent cells abundance is not the only cause for morbidity and mortality. It does, however, seems to be an important causal factor because removing senescent cells from mice increases mean lifespan, and adding senescent cells to mice increases risk of death and causes age-related decline.

Let's therefore explore the simple possibility that death can be modeled to occur when senescent cell abundance exceeds a threshold level X_C. The threshold represents a collapse of an

organ system or a tipping point such as sepsis (Figure 7.17). Thus, death is modelled as a **first-passage time process**, when senescent cells cross X_C. We use this threshold-crossing assumption to illustrate a way of thinking, because it provides analytically solvable results. Other dependencies between risk of death and senescent cells abundance, such as Hill-functions with various degrees of steepness, provide similar conclusions.

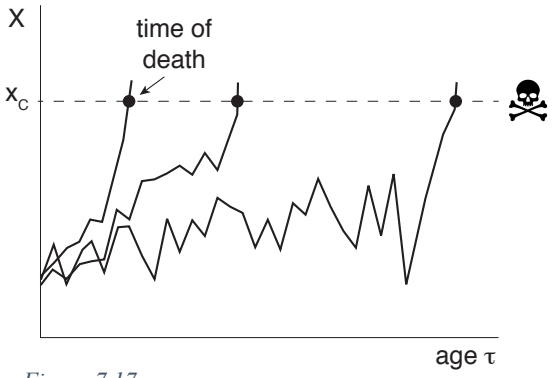

Figure 7.17

Solved exercise 2: Show that the SR model gives the Gompertz law of mortality.

To estimate the probability that X crosses the death-threshold X_c, we apply an approach which is analogous to the rate of a chemical reaction crossing an energy barrier ΔG. This rate is the Boltzmann factor $\exp\left(-\frac{\Delta G}{K_{bT}}\right)$. As always, in our case the noise amplitude ϵ plays the role of temperature k_bT, and the energy barrier is the difference between the potential U at X_c and at the steady-state value X_{st}, $\Delta G = U(X_c) - U(X_{st})$. Thus, the probability per unit time for X crossing X_c, namely the risk of death that we call the hazard, is

$$h \approx e^{-\frac{U(X_C)-U(X_{ST})}{\epsilon}}$$

This equation is called Kramer's equation in the field of stochastic processes. An intuitive explanation is that the ball in the bowl needs to climb a potential difference of $\Delta U = U(X_c) - U(X_{st})$ in order to fall off into the death region (Fig 7.18). It needs to climb using 'kicks' provided by the noise, each of size epsilon. Each noise kick can be either to the right or left. Since you need $\frac{\Delta U}{\epsilon}$ kicks, all in the right direction, the chance is exponentially small and goes as $e^{-\frac{\Delta U}{\epsilon}}$.

The potential U in our model is given by Eq.3. For the Gompertz law to hold, one needs the term $\frac{U(X_C)-U(X_{ST})}{\epsilon}$ to decrease linearly with age τ, so that $h \approx e^{\alpha\tau}$.

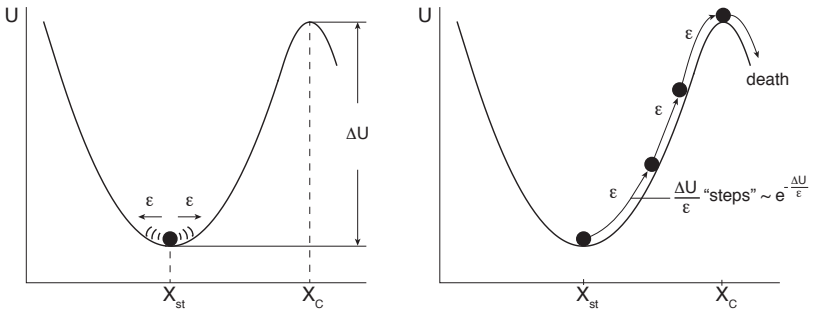

Figure 7.18

The exponent of the hazard rate in the SR model indeed shows the required linearity in time. This is the first factor with eta t in this complicated expression:

$$-\frac{U(X_C)-U(X_{ST})}{\epsilon}=\frac{(\kappa+X_C)\eta\tau-X_C\beta+\kappa\beta\cdot\mathrm{Log}\left[\frac{(\kappa+X_C)(\beta-\eta\tau)}{\kappa\beta}\right]}{\epsilon} \quad [8]$$

Which can be written, up to a prefactor that does not depend on age, as:

$$h(\tau)\approx(\beta-\eta\tau)^{\frac{\kappa\beta}{\epsilon}+1}e^{\frac{(\kappa+X_C)\eta\tau}{\epsilon}} \quad [9]$$

This is a big moment. The hazard rises exponentially with time as $e^{\alpha\tau}$ with an exponent α, called the **Gompertz ageing rate**, given by

$$\alpha=\frac{(\kappa+X_C)\eta}{\epsilon}.$$

The Gompertz ageing rate parameter (whose inverse yields the 8-year doubling time in humans) can thus be written in terms of molecular parameters.

Let's get an intuition for this expression. To see why the slope depends inversely on noise, for example, it helps to go to extreme limits. If there was no noise, epsilon=0, all individuals would die at the same time, a hazard slope with infinite slope. That's what you get from the formula if epsilon=0. If on the other hand noise is very large, epsilon->infinity, dynamics is dominated by a random-walk process. The levels of X go up and down randomly, with a constant probability per unit time of crossing Xc, in other words a flat hazard curve,alpha =0. And that's just what you get when you put in large epsilon. You can do the same with Xc- when it is high you get a very steep hazard curve with death mainly at old ages, and when it is low, a shallow curve with lots of death at young ages.

This solution also shows a deceleration in the rise of the hazard rate at very old ages (when $\eta t \approx \beta$), due to the prefactor $(\beta-\eta\tau)^{\frac{\kappa\beta}{\epsilon}+1}$ in Eq 9. This slowdown in hazard is observed in the empirical hazard curves. Note that this approximation begins to be inaccurate when $\eta t > \beta$, and simulations of the full SR model are needed to compute the hazard curve at old ages. Simulations show that rise of the hazard continues to slow with age.

The fact that this model provides the Gompertz law as a first passage time is special. Most other models do not show the Gompertz-law as a first passage time solution (Exercises).

The SR model thus analytically reproduces the Gompertz law, including the observed deceleration of mortality rates at old ages (Fig 7.19). It gives a good fit to the

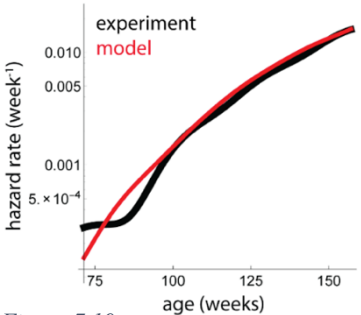

Figure 7.19

observed mouse mortality curve using parameters that agree with the experimental half-life measurements and longitudinal senescent cells data. The inferred threshold for death is $X_C = 17 \pm 2$, meaning that the threshold X_C is about 17 times larger than the mean senescent-cell level in young individuals. Thus, turnover of days in the young and weeks in the old provides senescent cells variation such that individuals cross the death threshold at different times, providing the observed mortality curves.

The SR model can address the use of drugs that eliminate senescent cells, known as senolytic drugs. To reduce toxicity concerns, it is important to take the drug at the lowest dose possible and as infrequently as possible. The model provides a rational basis for scheduling senolytic drug administrations. Specifically, treatment can start at old age, and be as infrequent as the senescent cells turnover time (~month in old mice) and still be effective.

Turnover of days in young and weeks in old can also explain the human Gompertz law

Let's use the results from the mouse data to study human mortality curves. In humans, mortality has a large non-heritable component (estimated at 80%) and hence we can assume that the parameters eta, beta k and epsilon are similar between people and that much of the variation is due to stochastic effects. A good description of human hazard curve, corrected for extrinsic mortality, is provided by the same parameters as in mice, except for a 60-fold slower increase in senescent-cell production parameter η with age (Figure 7.20). This slower increase in senescent cell production rate can be due to improved DNA maintenance and enhanced damage repair in humans compared to mice. It agrees with the observed slower rate of stem cell mutation accumulation in humans compared to mice.

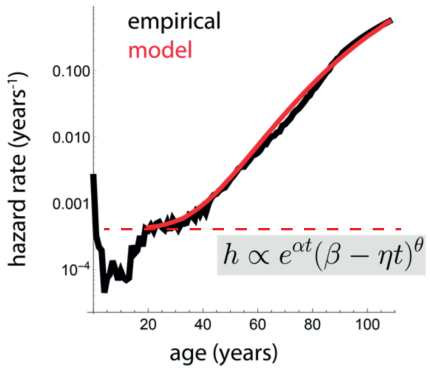

Figure 7.20

In fact, stem cell mutation accumulation rate is inverse to lifespan in mammals ranging from mice to dogs to horses (Fig. 7.21). It seems that the parameter η is the main way that evolution tunes the lifespan of different mammals, as in the mass-longevity triangle of the previous lecture. Indeed,

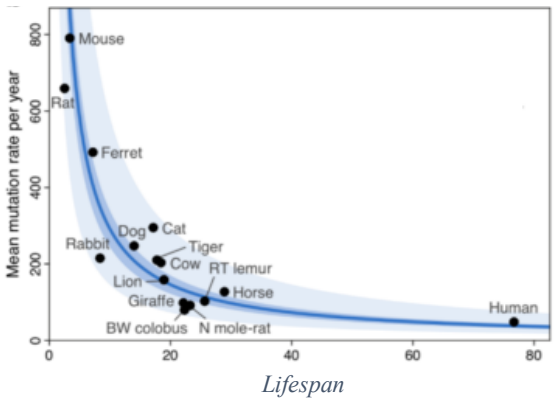

Figure 7.21 from Cagan, Stratton, Martincorena, 2021

long-lived animals such as elephants and naked mole rats have enhanced repair processes for DNA damage compared to mice.

Similar considerations can explain aging statistics in flies, worms and yeast

The SR model can be generalized beyond senescent cells. It should apply to any form of damage that whose production rises linearly with age and whose removal becomes saturated. We therefore explore the SR model to understand key experiments in model organisms such as the fruit fly *Drosophila melanogaster* and the worm (or more correctly the nematode) *C. elegans.*

The advantage of these model organisms is that interventions and mutations that affect lifespan can be studied with excellent statistics in lab conditions. Thus, let's consider X as a damage that is causal for aging, that accumulates with age and has SR-type dynamics, namely turnover that is much more rapid than the lifetime, rising production rate and self-slowing removal.

Even single-celled organisms show the Gompertz law under certain conditions. Examples include starved E. coli cells (Yang 2020) and yeast cells, as well as yeast cells with asymmetric replication called "budding". Let's therefore think of how the SR model can apply to a single cell. We need a toxic factor that accumulates in cells with time, an irremovable damage producing unit. One plausible physiological candidate is aggregates of unfolded proteins, which the cell is unable to remove. These aggregates grow in size is the cell, and cause secondary damage such as reactive oxygen species (ROS).

The amount of these aggregates, P(t), grows with time. Since the cell makes proteins at a constant rate, and each protein has a chance to be misfolded due to transcription/translation errors, one can assume that P rises at a constant rate, P(t)~a t. Fig 7.22 shows data on protein aggregates in worms (that live for about 2 weeks), showing that number of aggregate 'puncta' grows with age. It grows slower in a long lived mutant, daf-2.

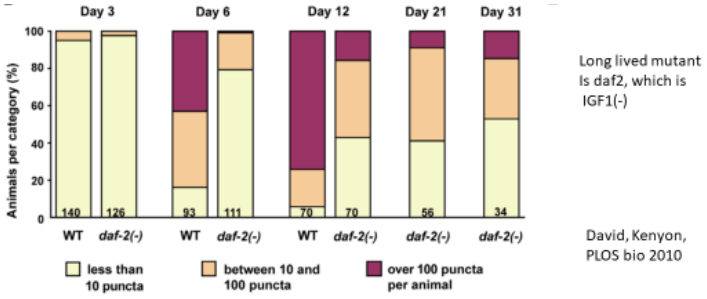

Figure 7.22

The aggregates are toxic to cells. They disrupt cellular functions and unleash reactive oxygen species (ROS) that damage cell components. Thus, the damage X in the cell, whatever its exact nature, is produced by P at rate b. Since P grows with time, damage production rate is thus $b\ P = a\ b\ t$. We can define the parameter η as η =a b. According to law 2, the removal of the damage saturates eventually, and we have the SR model, $dX/dt = \eta\ t - \beta\ X/(K + X) + noise$.

Thus the universal essence of the SR model is damage producing units that are irremovable and produced at a constant rate so that they rise linearly with time; these units cause damage X, and the removal of X saturates.

$$dP/dt = a$$

$$dX/dt = b\,P - \beta\,X/(+X) + noise$$

These aspects are likely to apply to different forms of damage and thus we continue to apply the SR model also to flies, worms and single cells.

Rapid shifts between hazard curves in Fruit flies

Work in *C. elegans* worms and *Drosophila* fruit flies provides constraints to test the SR model. For example, a classic experiment (Mair , Goymer, Pletcher, & Partridge, 2003) measured the effect of lifespan-extending interventions in fruit flies when applied at mid-adulthood.

Flies on lifespan extending diet or in low temperatures live longer. Their hazard curve is lower than flies in normal conditions. What happens when you shift in mid-life? Are the sins of the past forgiven? The answer depends on the intervention. Shifting to a better diet led to a rapid switch to the better survival curve, within a couple of days. The past was forgiven (Fig 7.23A).

In contrast, shifting temperature only changed the slope of the death curve, and the accumulated past hazard was not forgotten (Fig 7.23B).

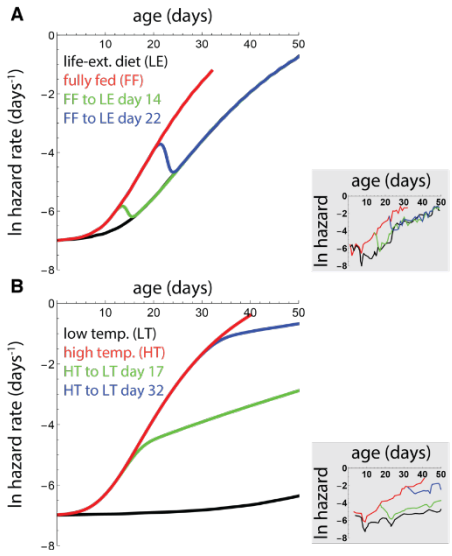

Figure 7.23

These results can be explained by the SR model due to the rapid turnover of damage X and the irremovable nature of damage producing units. [1]

The plummet to a lower curve seen in shifts of diet in Fig 7.23A can be explained if the diet lowers the amount of damage produced by each damage-producing unit P. For example, the life-extending diet expands the repair ability of cells, each P makes less damage X per unit time,

[1] A workable parameter set for flies is $\beta = 1\,hr^{-1}$, $\kappa = 1$, $\epsilon = 1\,hr^{-1}$and $\eta = 0.03 hr^{-1} day^{-1}$, $X_C = 15$. Flies on life-extending diet are fit by a lower slope $\eta = 0.02\,hr^{-1} day^{-1}$. This is just an example, the data is insufficient to pin down the parameters.

namely a lower value of the parameter b. Therefore, after the shift, the term eta t =b P(t) is lower, and the hazard curve shifts down. The time it takes to shift curves is the turnover time of the damage X, on the order of hours to days in flies. It is predicted that this 'transient' time should grow with age due to slowdown of removal, a prediction that remains to be tested.

In contrast, the slope change induced by temperature in Fig 7.23B can be explained by a reduced rate of production of new damage producing units. This is a reduction in the accumulation parameter a in the equation dP/dt=a. Thus, P keeps its accumulated number but is produced more slowly starting from the temperature shift at time t0. Thus, P(t)=a t0+ a' (t-t0), where a' is the reduced production rate. This results in the hazard curve shown in Fig 7.23B.

An analogous situation occurs when mammals are chronically exposed to conditions that cause mutations, such as chronic exposure to radiation or chemotherapy drugs. This raises the rate of production of mutant stem cells, our candidate for P in mammals, and to a rise in the slope of the hazard curve without forgetting the past hazard. A one-time strong irradiation or mutagenesis is predicted to cause a shift to a new, higher hazard curve.

Scaling of worm survival curves

A further test is whether the SR model can explain the scaling of survival curves for *C. elegans.* Many life-extending or life shortening genetic, environmental and diet perturbations change lifespan over an order of magnitude. The survival curves collapse on the same curve when age is scaled by mean lifespan (Figure 7.24), as discovered in an elegant experiment by (Stroustrup et al., 2016). Few things get physicists more excited than a good data collapse, where different curves fall on top of each other when normalized.

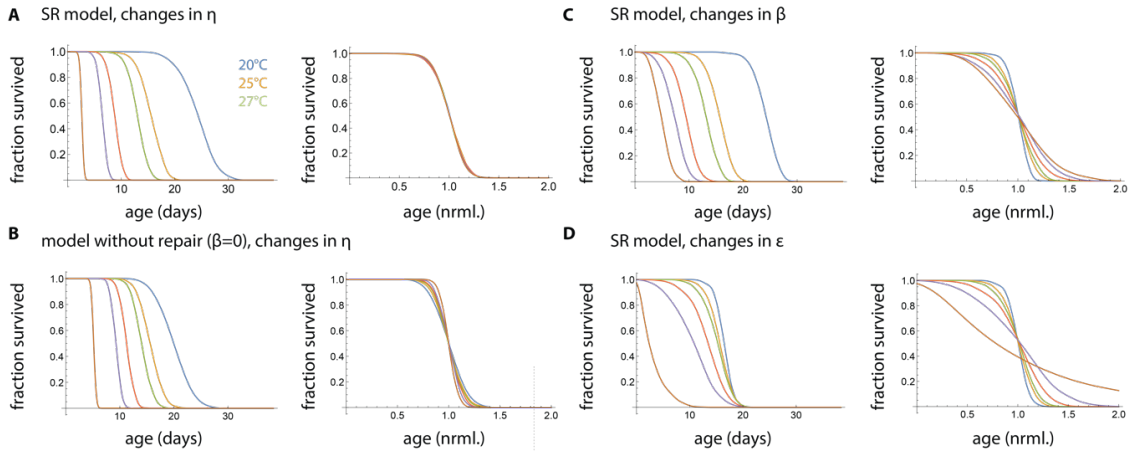

Figure 7.24

The SR model provides this **scaling** property to a very good approximation, for perturbations that affect the accumulation rate η (Figure 7.24 A). A good example for such an intervention is mutations that inhibit the IGF1 pathway, called daf-2 mutations in worms. This is one of the first life-span-extending mutations discovered, as you can read in the fascinating history by pioneer researcher Cynthia Kenyon [https://dx.doi.org/10.1098%2Frstb.2010.0276]. These mutations shift the entire survival curve to longer lifetimes, both median and maximal lifespan, and show scaling (Fig 7.25).

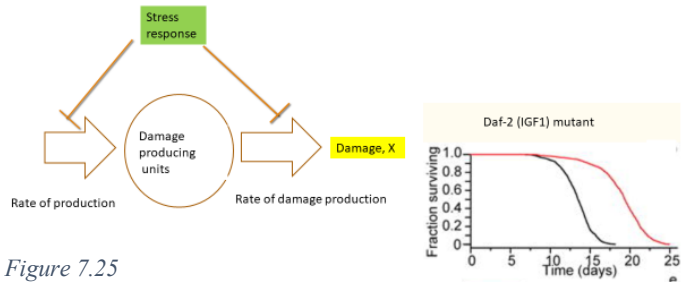

Figure 7.25

IGF1 pathway inhibition, across animals, makes the cells shift the balance from growth/reproduction to repair. They increase expression of **stress-response** pathways. Similar effects are found in caloric restriction. This may be an evolved response to starvation. Increased stress-response pathways repair damage and thus reduce the rate of damage formation per P, reducing η.

Interestingly, there is no scaling in the SR model when a perturbation affects other parameters, such as removal rate β or noise ϵ (Fig 7.24 B, D). This prediction that may apply to exceptional perturbations in which scaling is not found such as the *eat-2* and *nuo-6* mutations. In all cases, scaling cannot be found unless that model has rapid turnover.

We conclude that the SR model is a candidate explanation for scaling of survival curves in *C. elegans*.

Approaches to slow down aging and aging-related diseases:

Current medicine focuses on treating age-related diseases one at a time- diabetes, cancer, heart disease and so on. A different approach would be to deal with their shared risk factor - to slow the aging process, or more precisely to slow the rise of senescent cells (and other aging-related damage) (Fig. 7.26). This is the **Geroscience hypothesis**: slowing the core process of ageing will prevent and improve many age related diseases.

The conceptual framework we discussed points to two general strategies: reduce production rate η or increase removal capacity β.

Reducing production can be achieved by boosting cellular damage-repair systems. One way to achieve this is calorie restriction and other types of restricted feeding. Starvation seems to shift the balance from growth towards maintenance, and upregulate damage repair mechanisms in cells. A large effort is devoted to develop drugs that mimic calorie restriction by, for example, perturbing

the IGF1 pathway. One promising drug is metformin, used for treating diabetes since the 1920s. Metformin inhibits the IGF1 pathway, among several other effects such as lowering liver glucose production, and seems to tip the balance from growth (replenishing cell numbers in the adult) towards more repair. An encouraging sign is that people taking metformin have lower risks of cancer. A current effort is to convince the federal food and drug administration (FDA) to allow clinical trials for aging (currently only trials for a specific disease are allowed). Metformin is one suggested drug for such a trial, along with other inhibitors such as rapamycin.

Increasing the removal of senescent cells is also an attractive possibility. That is what **senolytic drugs** do. Senolytics remove senescent cells by exploiting the Achilles heals of senescent cells that are not found in most other cells. One such drug, for example, inhibits an anti-cell-death pathway called bcl2, exploiting the fact that this pathway helps the damaged senescent cells resist death to a greater extent than most other cells in the body. There are additional several families of senolytics. Some are entering clinical trials in humans for diseases such as idiopathic pulmonary fibrosis and osteoarthritis.

Another approach targets the factors that senescent cells secrete, such as pro inflammatory factors. Finally, immune-based strategies can potentially increase the number of garbage trucks, and hence the maximal removal capacity beta. In 2020, an immune approach to fight cancer cells was adapted to remove senescent cells in mice. In this approach, called CAR-T, T-cells are taken from the mouse and genetically engineered to express a T-cell receptor that recognizes a protein found only on the surface of senescent cells. These T-cells are re-introduced into the mice and kill senescent cells.

A sobering note for fantasies about immortality. Even if one removes senescent cells, the organism will still get sick and die eventually. For example, mutant stem cells produce damaged cells in all tissues, D'. Many of these damaged cells do not become senescent, but do have reduced function. As the fraction of damaged cells increases, the reduced function will eventually cause an organ system to fail. 80 year old individuals have on the order of 3000 mutations in each of their cells. Their organs like the skin, gut and lung are made of little local 'kingdoms' of cells, each from a different clone of stem cells, each kingdom with its individual random mutations. In about 10% of aged people, for example, all blood cells are made from one or a few stem cell clones in the bone marrow. Blood health depends on the luck of which mutations these stem cells have. Thus, although senescent cells are a major component, other damaged cells are likely to be important for aging.

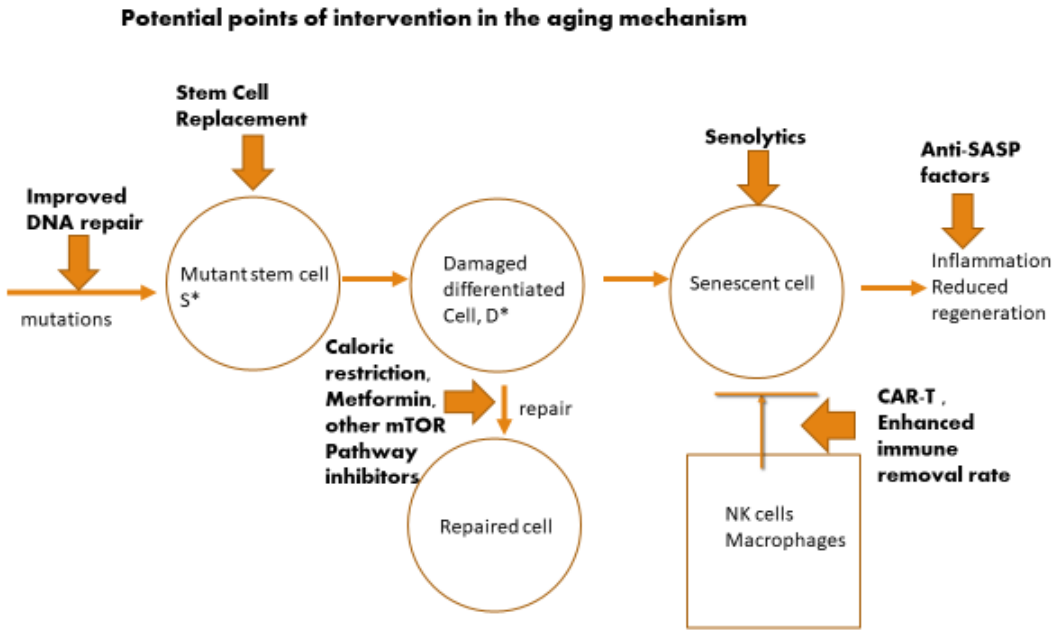

Figure 7.26

The research into removing senescent cells is accelerating over the past decade. As we have learned from past breakthroughs in biology, reality holds unexpected challenges, and initial promise usually doesn't fully materialize. We don't know if there ever will be a pill that will make you younger in terms of health. But there are so many avenues to try that it's likely that such a pill will help at least some people with some illnesses. These are exciting times.

For me, it's equally exciting that physiological laws can provide an equation which seems to capture many quantitative features that seem almost universal in aging. In the next lecture we will use it to understand age related diseases.

Property	SR Model
Dynamics	$\dot{X} = \eta t\ - \frac{\beta X}{X+\kappa} + \sqrt{2\epsilon}\xi_t$
Mean	$\langle X \rangle \approx \frac{\kappa\eta t + \epsilon}{\beta - \eta t}$
Standard deviation	$\sigma \approx \frac{\sqrt{\beta\kappa\epsilon + \epsilon^2}}{\beta - \eta t}$
Senescent cell half-life	$T_{1/2} \approx \frac{\log 2\ (\epsilon + \beta\kappa)}{\beta(\beta - \eta t)}$
Risk of death	$h(t) \approx \mathrm{H_0}(\beta - \eta t)^{1+\frac{\kappa\beta}{\epsilon}} \cdot e^{\frac{(\mathrm{X_C}+\kappa)\eta t}{\epsilon}}$

Exercises:

Solved exercise 7.1: Intuitive derivation of 'Boltzmann-like' form of the steady-state distribution:

Consider a stochastic process of the form $\frac{dX}{dt} = v(x) + \sqrt{2\epsilon}\xi$. The function $v(x)$ is called the velocity of x. In the SR model, we have a velocity equal to production minus removal: $v(x) = \eta t - \frac{\beta x}{k+x}$. Define the potential U(x) by $\frac{dU}{dx} = -v(x)$. Explain intuitively why, at steady-state, the probability distribution is $P(x) = P_0 \exp\left(-\frac{U(x)}{\epsilon}\right)$.

Solution: Consider a large number of particles moving along a one-dimensional pipe. They diffuse with diffusion coefficient ϵ and are also swept along the pipe by a velocity field $v(x)$. The particle density at steady-state is P(x). The flux at point x due to the velocity field is the velocity times the density: v(x)P(x). The flux due to diffusion can be found by Fick's law of diffusion, which shows a diffusive flux from high to low densities proportional to the gradient: $-\epsilon dP/dx$. At steady-state total flux is zero, so that the two fluxes must sum to zero: $v(x)P - \epsilon dP/dx = 0$. Thus, $\frac{dP}{dx} = \frac{v(x)P(x)}{\epsilon}$. The solution is $P(x) = P_0 \exp\left(-\frac{U(x)}{\epsilon}\right)$. Thus, at steady-state, in regions where velocity is large the density P(x) shows a steep opposing slope so that diffusion flux can balance velocity flux.

7.2. Survival and hazard functions:

(a) Show that hazard, $h(\tau)$, defined as the probability of death per unit time, is related to survival $S(\tau)$ as follows

$$h(\tau) = -\frac{1}{S}\frac{dS(\tau)}{d\tau} = -\frac{dlogS(\tau)}{d\tau}$$

(b) Show that $S(\tau) = e^{-\int h(\tau)d\tau}$

(c) What is the survival function S when the hazard follows the Gompertz-law? Plot this survival function.

(d) What is the survival function if hazard is constant $h(\tau) = h_0$?

(e) A tree has a hazard function that drops with age, $h(\tau) = \frac{a}{1+b\tau}$. What is the survival function? Plot and compare to d and c. What might be a biological cause of such a decreasing hazard function?

7.3 Removal **of Senescent cells based on saturating their own removal process:** Senescent cells are removed by immune cells such as NK cells, which we will denote by R. There are a total of R_T

removing cells in the body, and that this number does not change appreciably with age (as is indeed the case for NK cells in humans). The R cells meet Senescent cells, denoted X, at rate k_{on} to from a complex [R X] which can either fall apart at rate k_{off}, or end up killing the Senescent cells at rate v. Thus, R+X↔[RX]→R.

(a) Explain the following dynamic equation for the complex:

$$\frac{d[RX]}{dt} = k_{on} R\, X - (v + k_{off})[RX]$$

(b) Use the fact that R cells can be either free or in a complex, so that $R + [RX] = R_T$, to show that the removal rate of Senescent cells is

$$removal = \frac{\beta X}{k + X}$$

(c) What are the values of the maximal removal capacity β, and the half-way saturation point k? Explain intuitively.

7.4 No repair: Consider an accumulation process of damage with constant production and no removal

$$\frac{dX}{dt} = \eta + \sqrt{2\epsilon}\xi \ .$$

(a) What is the mean damage X as a function of age?

(b) What is the distribution P(X)?

(c) What is the hazard assuming that death occurs when X>Xc? Is there a Gompertz law?

7.5 Age-dependent reduction in repair capacity: Consider a process in which damage is produced at a constant rate η, and removal does not saturate. Removal rate per cell drops with age,

$$\frac{dX}{dt} = \eta + (\beta - \beta_1 \tau)X + \sqrt{2\epsilon}\xi \ .$$

(a) What is the mean damage X?

(b) What is the distribution P(X) at age ?

(c) What is the ratio of mean and standard deviation of X: $< X >/\sigma$?

(d) What is the hazard, assuming that death occurs when $X > Xc$? Is there a Gompertz law?

7.6 Deterministic model: Assume that the Gompertz law arises not from stochastic effects, but instead from individual differences, set a birth, in X production and removal parameters, in which each individual i has its own noise-free equation $\frac{dX}{dt} = \eta_i - \beta_i X$. Death is modelled when

X crosses threshold Xc. What distribution of production and removal parameters η_i, β_i can provide the Gompertz law? What features does this model not explain?

7.7 What is the effect on the hazard curve of the SR model of a change in each of the parameters β, η, ϵ, k? Plot examples of hazard curves to demonstrate your answer.

7.8 Senescent cell half-life: show that in the SR model, the half-life of a senescent cell is

$$t_{1/2} = \log(2)(k\beta + \epsilon)/\beta(\beta - \epsilon\tau)$$

7.9 Critical slowing down: Read (Scheffer et al., 2009).

(a) How does critical slowing down relate to the SR model?

(b) Suggest a phenomenon beyond those discussed in Scheffer which might show critical slowing down, and suggest an experiment or measurement to test this.

7.10 (Challenging question) General model: Damage is produced at rate $\eta(X, \tau)$ and removed at rate $\beta(X, \tau)$. The equation is $\frac{dX}{dt} = \eta(X, \tau) + \beta(X, \tau) + \sqrt{2\epsilon}\xi$

(a) What is the steady-state distribution at age tau?

(b) What is the risk of death as a function of age, modelled by first passage time of a threshold Xc?

(c) Under which conditions does risk of death go as the Gompertz law?

7.11 Strehler and Mildvan (1960) model for the Gompertz law. Strehler and Mildvan (STREHLER & MILDVAN, 1960) (SM) proposed a phenomenological process for the Gompertz law. Organisms are assumed to start with an initial survival capacity, termed *V*, declining linearly with age *x* as $V(x) = V_0(1 - Bx)$, where *B* indicates the fraction of vitality loss per unit time. Over life, animals experience random external challenges or insults with a mean frequency *K*. Challenges have random magnitudes, exponentially distributed with an average magnitude *D* that expresses the average deleteriousness of the environment. Death occurs when the magnitude of a challenge exceeds the remaining vitality. A detailed review of the SM theory can be found in Finkelstein (2012) (Finkelstein, 2012).

(a) Show that these assumptions produce the Gompertz law $h(\tau) = ae^{b\tau}$. Calculate a and b.

(b) What similarities and differences does this theory have with the SR model?

7.12 Stem cell therapy: Would adding young stem cells to an aged organism help to address aging, according to the conceptual picture in this lecture? What are your thought (100 words).

Reference:

This review brings evidence of senescent cells slowing their own removal:

https://www.sciencedirect.com/science/article/pii/S1568163721000271

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