Systems biology of Aging

Chapter 2 - What are the houses, garbage and trucks?

Uri Alon

We now explore the biological nature of houses, garbage and trucks in humans. This is fascinating for basic research and offers targets to one day slow the rate of aging.

Aging and the accumulation of molecular and cellular damage

Many many many things change in the body during aging. In this chapter we begin to dive into the molecular and cellular aspects of aging. Our goal is to identify the molecular and cellular processes that correspond to the houses, garbage and trucks, so we can go from specific molecular changes to the patterns of aging.

The consensus in aging research is that *aging is caused by accumulating damage to molecules and cells*. But what kind of damage?

Historically there have been many 'damage theories' of aging. They arose from experiments that show that damage rises with age - many varieties of damage including oxidative damage, protein damage, DNA damage, mutations, chemical modifications like glycation and cross-linking of fibers.

Two major theories that were dominant in the 20th century are now known to be incomplete. The first was the 'rate of living ' theory, where the faster metabolism is, the more damage is formed and the faster aging [https://en.wikipedia.org/wiki/Rate-of-living_theory] . The higher the flame the faster the candle burns out. But as mentioned in Lecture 1, some animals have fast metabolism and long life (bats are a good example).

Next came the oxidative stress theory of aging(Harman 1950s), where the driver is a product of metabolism called reactive oxygen species (ROS) that can damage proteins and DNA. It was dominant for several decades. Early findings were in accordance with this theory but slowly

evidence to the contrary started to accumulate. For example, inhibiting ROS (such as growth of yeast without oxygen or use of antioxidants in humans) does not slow down aging.

The search for a single driver of aging took decades and ended in frustration. Could it be a protein? Well, almost no protein in the body lasts long enough to be there for decades. and so on. Each subfield had its own candidate drivers. Fragmentation reigned.

Then, around 2013, the aging field developed a new paradigm called the hallmarks of aging (2013,2023). A hallmark is a kind of damage that ideally has three features (i) it rises with age (ii) increasing it speeds aging (iii) reducing it slows down aging in model organisms (point iii has only been demonstrated for a few of the hallmarks).

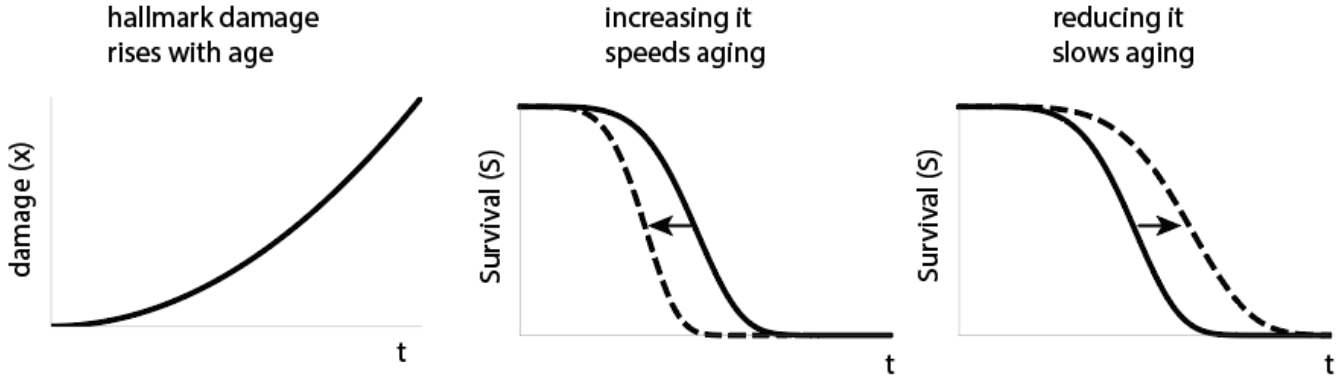

Figure 2.1

The hallmark concept is like a peace pipe. It helped to unite a fragmented field. If you work on one of the hallmarks, you are studying aging. You are one of us. The arrangement of the hallmarks in a circle , a shape that is smooth and inoffensive, suggests that they are all equally important. This goes with an assumption that aging emerges from complex interactions between numerous factors and there is no single driver of aging that is upstream of the rest [PMID: 24159899].

We all appreciate the importance of a united field. However, let's admit it, the hallmarks are a circular laundry list. It's worth exploring whether they can be understood more as a circuit with a flow from one to another. That approach- the quest for specific drivers (specific elements within each hallmark) that interact in specific ways and in a specific sequence to lead casually to the phenotypes of aging- guides me in this chapter and throughout the book where we will go into increasing detail.

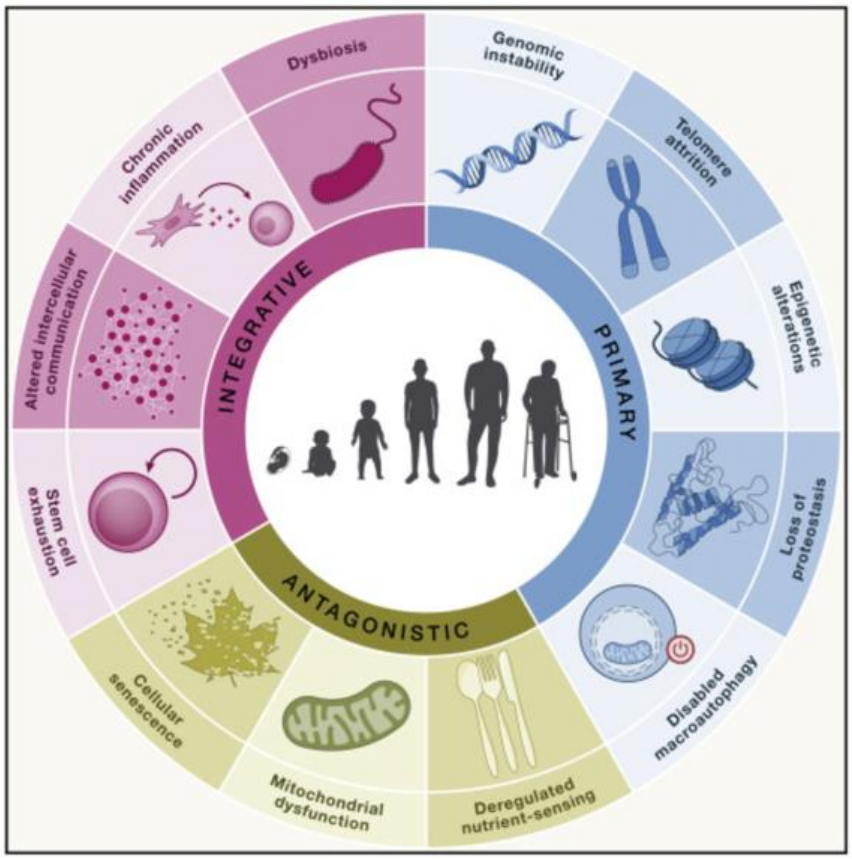

Figure 2.2 Hallmarks of aging- factors that rise with age, whose enhancement speeds aging and whose attenuation slows it down. Adapted from López-Otín et.al

None of these hallmarks have been connected to the Gompertz law or the other quantitative patterns of aging of lecture 1. Making this connection is one of our goals.

To do so, we will explore the possibility that, as in the garbage model, there is an upstream driver of aging (we will see that there are actually at least two drivers) shared by all aging individuals. These drivers cause the huge number of downstream molecular and cellular changes that occur with aging. They also cause the necessary conditions for diseases and functional decline with age.

The rising garbage strains physiology until something breaks to cause a disease. But which diseases occur and when they happen depends on the genetics and environment of the individual- making old people heterogeneous in terms of health.

Thus, we expect that unlike the diverse impairments of old age associated with genetics and environment, the upstream drivers will be shared and relatively homogenous between individuals.

Humans as a cake with three layers

We are a mixed readership of biologists and those new to biology including computer science/physics/chemistry people. So as we learn about the biological hallmarks of aging, we need to be gentle for people new to biology.

To begin let's look at a human being hierarchically as made of three levels of organization.

The top level are our organs. Organs are made of cells - cells are the middle level. Cells in turn are made of DNA and protein molecules, powered by mitochondria - the bottom level.

Hallmarks divide between these layers

Seven for the bottom level inside the cell - 3 for DNA, 2 for proteins, 2 for mitochondria.

Four for the top level of organs and systems.

And only one for the middle layer - senescent cells.

Thus the middle level of senescent cells is a bow tie- it is the funnel through which molecular errors translate to body-wide decline.

The three DNA hallmarks involve breaks, fraying ends, and misplaced marks that cause DNA to open

DNA is the long molecule that stores our genetic information. It carries genes that encode for proteins -proteins are the molecular machines that do the tasks of the cell. All this is powered by the mitochondria that produce ATP, the fuel for the protein machines.

DNA can break - leading to the hallmark called **genomic instability**. Most important for aging are double stranded breaks, where the DNA completely breaks- it needs to be rejoined otherwise the cell can't divide. In humans, individuals born with a rare mutation in certain DNA repair genes (recQ helicases) display accelerated aging- diseases such as Werner and Bloom syndromes with a combination of white hair, thin skin, and risk of cancer, diabetes, heart

disease and death at age 30-40 [https://en.wikipedia.org/wiki/Werner_syndrome].
https://doi.org/10.18632/aging.205537

The ends of DNA are especially vulnerable, called **telomeres**, and they can become shorter with each cell division, placing a limit on the number of times a cell can divide - **Telomere attrition**. Since Telomeres are abrupt ends of the DNA they can be erroneously sensed as a double stranded break, falsely igniting repair. So the telomeres are coated with proteins that prevent repair (the shelterin complex).

Telomere attrition can limit the number of times a cell can divide (and is thus an anti-cancer mechanism)- in the 1960s Hayflick found that cells from an old person placed in a dish divide only a few times, whereas those from a young person can divide many times but stop at the 'Hayflick limit' of 50-70 divisions. Telomeres cause problems even in cells that don't divide which as neurons - the telomeres can accumulate DNA damage that can not be easily repaired.

The third hallmark relates to the fact that DNA is wound up around protein balls called histones into a tight bundle called chromatin. Chromatin can be open or closed, and only the open regions can be easily accessed. Thus in a given cell every location on the DNA can be closed or open, as determined by special 'epigenetic' marks on the histones.

The open DNA can be read - this exposes the parts of DNA that encodes genes needed in the cell - for example muscle genes if it is a muscle cell, neuron genes if it is a brain cell. The closed DNA can not be read and carries genes that need to be silent - genes for muscle are silenced in a brain cell and vice versa.

This resolves the puzzle of how a single fertilized egg gives rise to all the organs and cell types in the body- the same DNA can give rise to muscle cells, brain cells and so on by arranging DNA to be open in just the right places in each cell type.

When this information becomes disrupted, namely when the histone marks are in the wrong place, DNA that should be closed becomes open - the hallmark called **epigenetic alterations**. This causes further troubles at the telomeres, since they are normally closed DNA. It can also awaken dormant viruses (retrotransposons) in our genome that are normally silenced by closed DNA. These retrotransposons (LINE1 elements) then jump, causing inflammation -
The cell is primed to detect such jumping DNA and react as if it is a virus. The transposin can integrate into other places in the DNA causing further mayhem.

All of these DNA hallmarks rise with age - genomes become more unstable, telomeres shorten and epigenetics alter.

-Find a bio person next to you, Pair and Share-

The two protein hallmarks have to do with protein aggregates and disrupted nutrient sensing that affects protein production and removal

The next two hallmarks are associated with damage to proteins. Each protein is a string of amino acids. This string is sticky and folds onto itself in a special 3D structure where it becomes a precise molecular machine.

If a protein becomes unfolded, it can stick to other proteins forming aggregates that gum up the cell. The cell prevents this using 'proteostasis' repair machinery - also made proteins of course- chaperones that refold proteins, proteases that degrade them, and autophagy ('self eating') to recycle them back to their amino acid building blocks. Protein unfolding and aggregates increases with age - the hallmark called **loss of proteostasis**.

The second protein-related hallmark regulates the rate of living according to nutrient sensing- the rate of making new proteins and breaking them down. This has to do with the balance between growth and repair mentioned in the first chapter. When cells have ample nutrients, and the right hormone signals, they grow and divide. But when nutrients are scarce the cells slow down the rate of making new proteins and focus their resources to repair and recycle damaged proteins.

This switch from growth to repair is governed by a central sensor called mTOR (a protein complex that does computations) whose input is nutrients (levels of amino acids and ATP) and whose output is rate of new protein production (ribosome translation rate) and expression of recycling machines (autophagy). Inhibiting mTOR reduces protein production and increases recycling when nutrients are low.

Interventions that inhibit mTOR can extend life by mimicking starvation and enhancing repair - leading to the hallmark called **deregulated nutrient sensing**. I mentioned before that caloric restriction can extend lifespan in model organisms. I also mentioned that reducing a hormone called IGF1, that instructs cells to grow, also extends lifespan. A classic TOR inhibitor is the drug rapamycin (TOR stands for Target Of Rapamycin). This drug extends life in organisms from worms to mice.

Mitochondrial hallmarks cause damage to the cell

Mitochondria were formed in a symbiosis about a billion years ago between a bacterium (the mitochondria) and another ancient cell. They produce ATP in reactions that cause oxidative damage (ROS), and have many other functions such as synthesizing building blocks for the cell.

With age there is a rise in **mitochondrial dysfunction**, causing oxidative damage, loss of ATP and triggering of immune-like responses inside the cell due to the mitochondrion's bacterial past. The cell attempts to recycle damaged mitochondria - and this process declines with age, the hallmark of **Disabled macroautophagy**.

-Pair and Share-

This bottom molecular level is all there is in single celled organisms like budding yeast. Yeast mother cells divide and make fresh bud cells - but the mother ages and dies after about 20 divisions. The mother keeps the damage and the new daughter cell starts off with essentially zero damage (except for daughters of very old
Mothers). The genes involved in yeast aging are often relevant even to mouse and human aging. Aging researchers have learned alot about aging of our human cells from the humble yeast. We will use yeast as a guide to find the drivers of aging in an upcoming lecture.

Garbage is the hallmark in the middle
Layer - zombie cells called senescent cells

Our proposed garbage, the driver of aging, is the accumulation of senescent cells. Senescent cells are damaged cells that enter a state where they stop dividing and secrete alarm signals.

When cells are damaged in any of the molecular hallmarks - namely protein, DNA or mitochondrial damage - and cannot repair themselves, they can become senescent cells **(**Fig 6.18).

Senescent cells serve an essential purpose in young organisms: they guide the healing of injury. When organisms are injured, cells sense that they have been damaged. If they keep dividing, they run the risk of becoming cancer cells. So the damaged cells often kill themselves by programmed cell death (apoptosis). However, if all injured cells kill themselves the tissue will have a hole. Therefore, many cells turn into a senescent cell state so that the tissue remains intact, and without the risk of cancer since the zombie cells don't divide.

Senescent cells are large metabolically active cells. They stop regeneration and healing around them (stopping stem cells). They also call the immune system screaming "remove me". To do so

they secrete signal molecules, collectively called the **senescence-associated secretory phenotype**, or **SASP** (Fig 2.3). The SASP includes signaling molecules that recruit the immune system to clear the senescent cells in an organized fashion. In other words, these signals cause inflammation. Once the senescent cells are removed, fresh new cells are made and healing proceeds.

Certain cells of the immune system are tasked with detecting and removing senescent cells, such as macrophages and NK cells. The NK cells and macrophages also have other important jobs such as removing virus-infected cells and cancer cells.
In addition to igniting inflammation, and slowing the rate of stem-cell

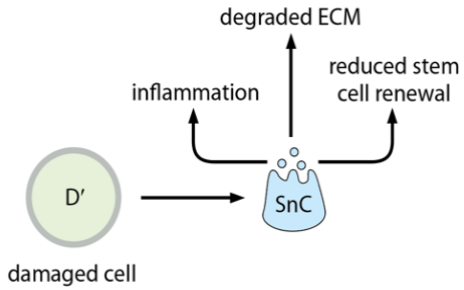

Figure 2.3: Damaged cells that are unable to repair themselves become senescent cells. Senescent cells secrete proteins called SASP that cause inflammation, degrade the extracellular matrix and reduce stem cell renewal.

renewal around the senescent cells, the SASP contains 'molecular scissors' that alter the extracellular matrix (ECM) around the cells, to allow the immune system to enter.

Thus, after an injury, senescent cells arise. They cause inflammation to call in the immune cells that remove them in an orderly process over several days to allow healing and regeneration. If the wound can't regenerate, it is filled in with scar tissue instead (fibrosis).

Senescent cells drive the top
Level hallmarks including inflammation and stem cell exhaustion

However, senescent cells have a dark side. This dark side arises because we are not designed to be old. The aging body becomes loaded with damaged and senescent cells, and therefore permeated with SASP.

It doesn't take a huge number of zombie cells to affect the entire body. We know that the entire body can be affected by secretions from only 1/10,000 of its cells because endocrine

organs like the thyroid or adrenal glands weigh only 10g, which is about 1/10,000 of the body's 100kg, but make enough hormones to affect every cell in the body. Thus, even if, say, only 0.1%-0.01% of the cells are damaged, their secreted inflammatory signals can affect the entire body, causing chronic inflammation.

Inflammation (without pathogens, called sterile Inflammation) is a hallmark of aging, sometimes called **inflammaging**. Inflammaging causes aches and pains, and drives age-related diseases. The SASP also slows stem cell renewal all over the body and alters the extracellular matrix. These effects increasingly lead to reduction in organ function.

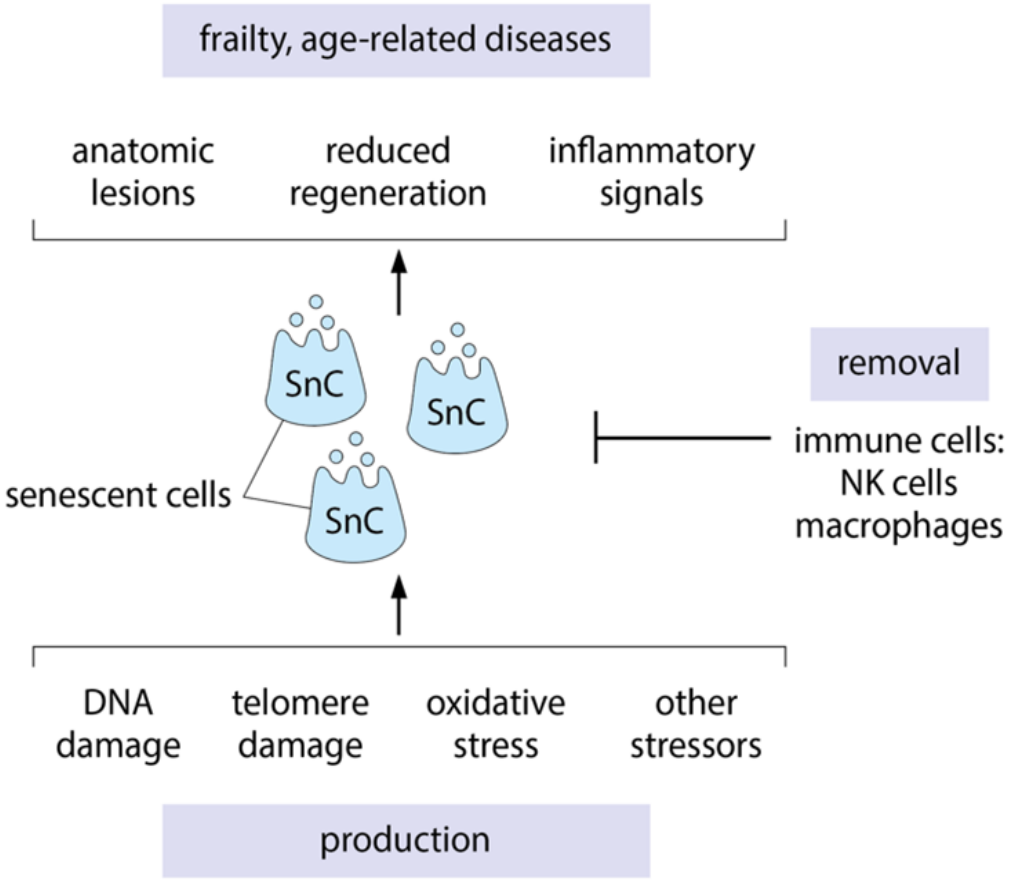

Figure 2.4: Almost all types of cellular damage can lead to senescent cells, which produce SASP proteins that lead to systemic effects that cause age-related diseases. Senescent cells are removed by immune cells including NK cells and macrophages.

Thus, senescent cell SASP contributes to the hallmarks at the top level - the layer of organs, circulation, hormones, immune system - the processes that orchestrate the different cells in the body. At this level we have the final four hallmarks.

As just mentioned, all aged individuals suffer from some degree of **chronic inflammation** - the body's attempt to repair damaged tissue. This differs from acute inflammation caused by pathogens like bacteria and viruses. Inflammation overrides normal functions, an emergency signal - it stops growth and normal function in order to prepare for attack.

Thus prolonged inflammation causes long term neglect and collateral damage. This leads to the concept of inflammaging. Inflammation is at the root of almost all age related diseases including the four horsemen cancer, diabetes, neurodegeneration and cardiovascular disease.

Thus, senescent cells sit at an interesting junction between the level of damage to cell components and the level of damage to organ systems (Fig 2.4). They unite the different molecular theories of aging, because virtually any form of cellular damage can push a cell to senescence - including ROS, DNA damage, shortened telomeres, epigenetic damage and so on. senescent cells in turn produce systemic effects that cause disease and physiological decline.

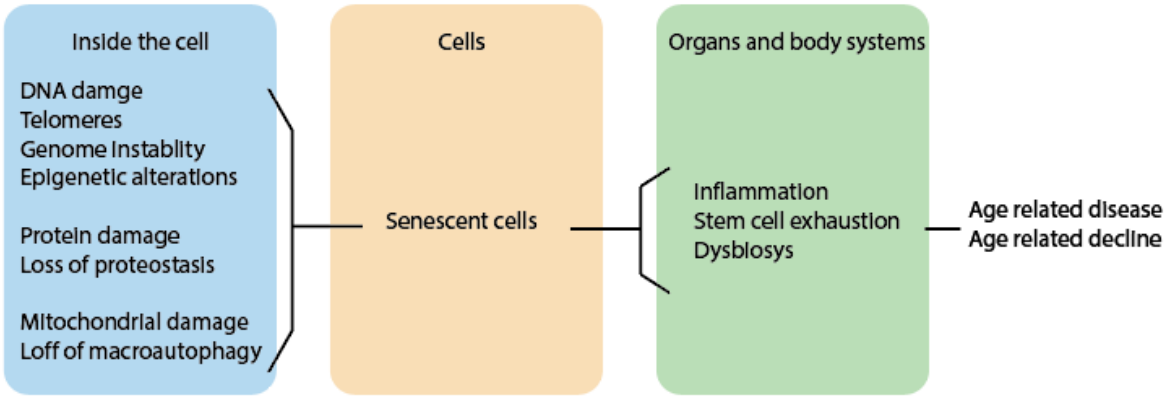

Figure 2.6

Senescent cells are a bridge from molecular damage to organs

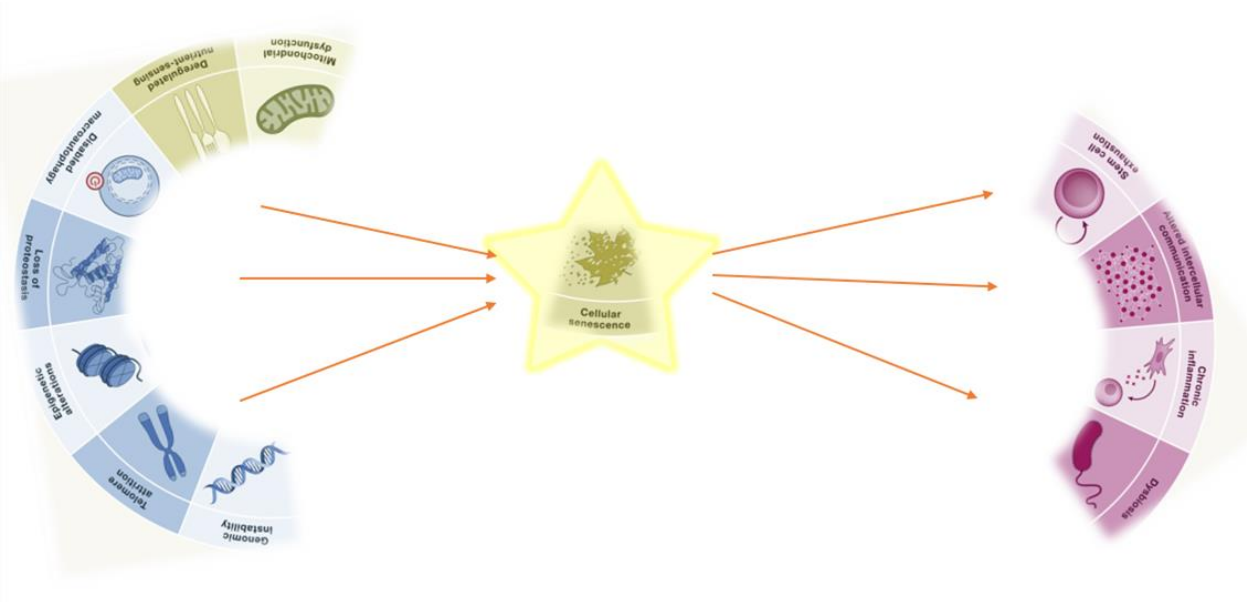

Figure 2.7

The houses are of two kinds- cells that don't divide, and altered stem cells.

If trucks are immune cells and garbage is senescent cells, what are the houses? What accumulating factor generates the senescent cells?
We can reason that houses must stay in the body for decades. They can't be a skin cell that's removed in a month or a gut cell that's removed in a few days.
There are at least two possibilities- cells that don't divide and last a lifetime like neurons.
Or stem cells that renew and stay in the body, generating the more ephemeral differentiated cells that make up our organs like skin, guts, blood and lung.

Stem cells are a key design feature of our body that renews many organs. To understand stem cells, let's consider the skin as an example. The top layer of the skin is made of dead cells that are removed within weeks. To make new skin cells, a deep skin layer called the basal layer of the epidermis houses skin stem cells, S (Fig 2.5).
These stem cells divide to make new stem cells, in a process called **stem cell renewal**. They also **differentiate** into skin cells, D. These differentiated skin cells divide a few times, a process called transit amplification. Each stem cell division gives rise to many differentiated cells due to transit amplification (for example if there are ten transit divisions, each stem cell division gives rise to

2^10=1024 differentiated cells). Thus slowly dividing stem cells can provide many new cells to the organ, without accumulating too many mutations.

The new skin cells rise in a column above the stem cells, until they reach the top layer of the skin, and are shed off. The stem cells continuously and slowly divide to replace the lost skin cells. They have enzymes that replenish their telomeres so they have no limit to their divisions in principle

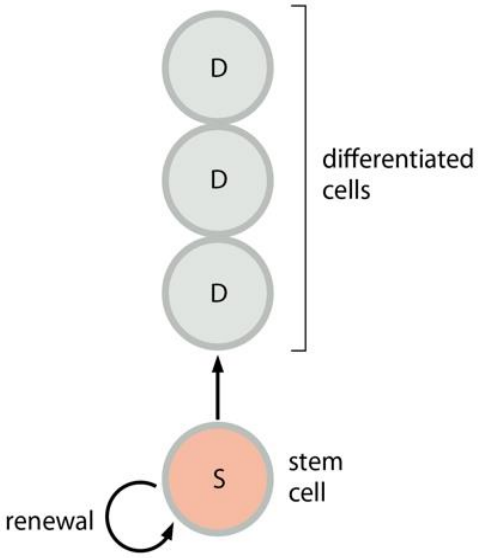

Figure 2.5: Stem cells S divide and differentiate into differentiated cells D. Stem cells often remain in the body for a lifetime, whereas differentiated cells in most tissues are removed in days to years.

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Many tissues have their own dedicated stem cells. Stem cells are found for example in the epithelial lining of the intestine, lung and skin. Stem cells in the bone marrow differentiate about once per month to produce the red and white blood cells.

Our proposed houses arise from stem cells and neurons. They account for aging in the body and in the brain.

We will go into more detail later.

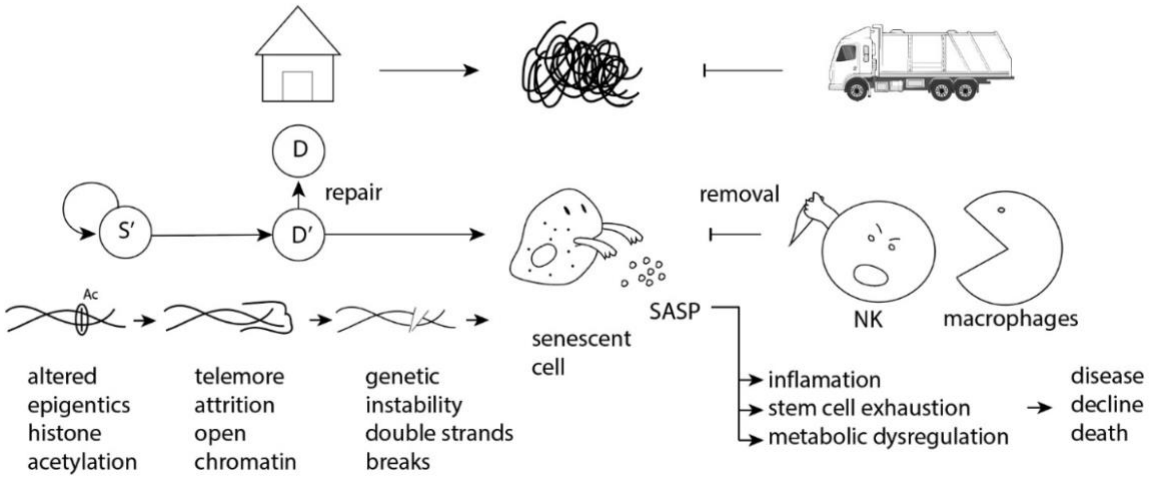
Stem cells accumulate epigenetic alterations

For now, we note that over time stem cells can become altered in which parts of their DNA are open and closed. This is governed by histone marks, which over time lose information.

Stem cells copy their histone marks to their progeny - this is part of their heritage, so that blood remains blood and skin remains skin. Altered stem cells have histone marks that are

harmless to the slowly dividing stem cell. But once inherited by their rapidly dividing progeny cells, the open DNA causes terrible problems- shortening of the DNA ends called telomere, unwanted structures that collide with the replication machinery to cause double stranded DNA breaks, and opening of normally silenced viruses (line1 elements) that jump and trigger the cells immune alarms (cGAS-sting) . All of this causes the damaged progeny cells to become senescent.

Thus altered stem cells are like zombie cell factories. Since the probability to become altered is low and alteration is stochastic, houses accumulate slowly but surely throughout life. They rise linearly with age. At old age they collectively make so many senescent cells that the immune trucks are overwhelmed, can't do their tasks of fighting cancer and infection, inflammation reigns -systemic aging.



The other type of houses are permanent cells like neurons. With age more and more neurons become damaged. Their driving damage is less related to DNA hallmarks which are more critical in dividing cells.

Instead neurons accumulate protein and mitochondrial hallmarks. Gunk called lipofusic accumulates inside their lysosomes, an acidic organelle in charge of recycling proteins and storing metabolites. lipofuscin, is an indigestible melange of fats and metals that gives aged cells a yellowish tinge. This causes disruption that leads to mitochondrial problems and protein aggregation. Neuroinflammation rises and brain problems can occur.

We will make this more precise when we delve into model organisms and what we learn from them, and when we talk about neurodegeneration in later chapters.

For now let's summarize that DNA hallmarks in stem cell houses drive senescent cell garbage removed by immune cell trucks. Protein and mitochondrial hallmarks drive damaged neurons which form another layer of houses garbage and trucks in the brain.

Our model is universal and should apply to other organisms besides humans. We now turn to the fascinating story of aging across the tree of life, where the model sheds light on different strategies of aging.