
REVIEW

Biological Clocks: Why We Need Them, Why We Cannot Trust Them, How They Might Be Improved

Josh Mitteldorf

Philadelphia, USA
aging.advice@gmail.com

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Abstract—Late in life, the body is at war with itself. There is a program of self-destruction (phenoptosis) implemented via epigenetic and other changes. I refer to these as type (1) epigenetic changes. But the body retains a deep instinct for survival, and other epigenetic changes unfold in response to a perception of accumulated damage (type (2)). In the past decade, epigenetic clocks have promised to accelerate the search for anti-aging interventions by permitting prompt, reliable, and convenient measurement of their effects on lifespan without having to wait for trial results on mortality and morbidity. However, extant clocks do not distinguish between type (1) and type (2). Reversing type (1) changes extends lifespan, but reversing type (2) shortens lifespan. This is why all extant epigenetic clocks may be misleading. Separation of type (1) and type (2) epigenetic changes will lead to more reliable clock algorithms, but this cannot be done with statistics alone. New experiments are proposed. Epigenetic changes are the means by which the body implements phenoptosis, but they do not embody a clock mechanism, so they cannot be the body's primary timekeeper. The timekeeping mechanism is not yet understood, though there are hints that it may be (partially) located in the hypothalamus. For the future, we expect that the most fundamental measurement of biological age will observe this clock directly, and the most profound anti-aging interventions will manipulate it.

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INTRODUCTION

“Biological clock” has three different meanings.

1. The body's circadian rhythm of wake/sleep cycles;
2. Time-keeping over the life cycle, controlling development, puberty, and (plausibly) aging;
3. (Since 2013) Computer algorithms for calculating biological age from epigenetic and other medical data.

In this manuscript, I shall refer to #1 as “circadian clocks”, #2 as “governing clocks”, and #3 as “algorithmic clocks”.

Mammalian circadian rhythms have been studied and located in the hypothalamus. The timing of development and gonadarche are governed by a mechanism which has not yet been elucidated. If, as most readers of this journal believe, the source of senescence can be traced to an extension of the developmental clock into

a mode of self-destruction (phenoptosis) late in life, then mechanistic understanding of this clock would be a breakthrough in anti-aging medicine. Prospective manipulation of this internal clock would be the true Philosopher's Stone.

Algorithmic aging clocks have been developed as a shortcut to evaluate anti-aging interventions without having to sample alternative mortality curves over decades. If the algorithms truly reflect the governing aging clock, then they can perform this function with ideal precision. But since the detailed mechanism of the governing clocks is yet unknown, algorithmic clocks are based on the next best thing – gene expression changes which are, presumably, the principal way in which the governing clocks affect the body globally.

A problem with this approach is that there are other gene expression changes that occur with age.

The best evidence is that the body is at war with itself late in life, and some of the observed changes in gene expression are direct expressions of the body's phenoptosis program, while other changes are actual defenses against that program. The body detects the (self-inflicted) damage, and deeply embedded programs are activated to repair that damage.

With age, the body is simultaneously activating a timed program of self-destruction and triggering a program of self-defense. It is crucial for any algorithmic clock to distinguish between these two, because a putative anti-aging intervention should be credited for reversing the former, *but debited for reversing the latter*. The two may be impossible to distinguish based on statistical analysis alone.

HISTORICAL CONTEXT OF ADAPTIVE AGING

In the twentieth century, evolutionary theory based on the "selfish gene" model of neo-Darwinism guided understanding of the cause and the mechanisms of aging in the animal kingdom. Aging has been regarded as a passive accumulation of damage, resulting from a selection shadow at late ages (accumulated mutations [1]) or as an unavoidable side-effect of selection pressure for maximal fertility (antagonistic pleiotropy [2]). Late in the century, a few visionary researchers had the courage to challenge these theoretical paradigms based on observation and experiment; there are aspects of the phenomenology of aging that defy predictions of theory based on accumulated mutations or antagonistic pleiotropy. The oldest and most robust intervention for extending lifespan is caloric restriction (CR), and the CR phenomenon does not fit well with either of the two classical theoretical models [3, 4]. Most obviously, life extension flies in the face of the popular disposable soma theory [5], which posits that aging is caused by a need to budget food-derived energy [6].

The earliest proponents of aging as an evolved adaptation were Libertini [7], Bowles [8], and Skulachev [9]. There is now an abundance of plausible, published models capable of explaining when and how natural selection might prefer a fixed lifespan to an indeterminate lifespan [10-16]. The conservative scientific community avoided discussion of this challenge to neo-Darwinian evolution, and the first suggestion of adaptive aging to appear in a high-profile Western journal was [17]. In my opinion, the most plausible and general models for evolution of aging are based on Gilpin's* population dynamics [15, 18] Others are based on dispersion [19], on shortening the generation time to increase the pace of evolutionary adaptation [20], and on keeping up with an evolving pathogen [7, 13, 16, 21].

THE SIGNIFICANCE OF METHYLATION CLOCKS

In the first decade of this century, many interventions were discovered that significantly extended median lifespan of laboratory animals. Maximum lifespan has been extended to a lesser extent in vertebrates. These included metformin [22], rapamycin [23], various short peptides [24], anti-inflammatories such as aspirin and curcumin [25], hormones such as vitamin D [26] and melatonin [27, 28], and mitochondrial modifiers [29, 30]. The question was ripe whether these measures would also extend lifespan in humans. But human lifespan studies are prohibitively impractical.

- The human lifespan is so long that significant results require decades and tens of thousands of study participants.
- This makes human studies expensive, as well as slow.
- People cannot be expected to adhere to any health regimen for decades at a time.
- It is unethical to ask control subjects to refrain from doing something that might extend their lives.

In 2013, Horvath [31] and Hannum [32] independently proposed a resolution to this dilemma. Their hypothesis was that there is a meaningful, measurable quality of a person or animal, dubbed "biological age", and that the effect of an intervention on biological age could be used as a proxy for its effect on life expectancy. The hypothesis sounds reasonable, and if true, then "biological clocks" are indeed a great boon to research in aging medicine. There are subtleties to the logic, however, which neither Hannum nor Horvath was eager to discuss in publications, possibly because of the conservatism of the evolutionary community, which I referred to above. I only know this because I was fortunate to be privy to Horvath's private thoughts on the matter. Their clocks only make sense for the intended use in the context of programmed phenoptosis, as I shall argue below.

The Horvath and Hannum clocks were based on methylation of cytosine, one of the four nucleotide bases in DNA. Methylation is one mechanism of epigenetic control, i.e., it is part of the way that the body expresses genes selectively, where and when they are needed. It is widely accepted that methylation (along with other mechanisms of epigenetic control) is part of the body's evolved, adaptive response. With age, the selectivity of methylation is blurred; this is called epigenetic drift. But there are also directional changes in methylation. Thousands of genes are turned off gradually, monotonically over a lifetime via hypermethylation, and more thousands are turned on via hypomethylation.

* Gilpin, M. (1975) Group Selection in Predator-Prey Communities, Princeton University Press, doi: 10.2307/j.ctvx5wbvr.

The consistency of these patterns compels the presumption that they are adaptive. But adaptive to what end? Why would senescence be associated with changes in gene expression?

1) If you believe in phenoptosis, then the directed changes in gene expression are means of self-destruction. Genes are turned on that increase inflammation, destroying arteries and neurons [33]. Apoptosis is up-regulated to the point where healthy muscle and brain cells are dying [34, 35]. Protective anti-oxidants, DNA repair, and autophagy are down-regulated [36]. If any intervention sets back the methylation clock, then there is less self-destruction, more repair and maintenance. We expect that the body will live longer.

2) If you believe the neo-Darwinian theory that the body cannot be purposely destroying itself, then aging is an accumulation of incidental damage at the cellular and molecular levels. If there are associated epigenetic changes, these cannot be causing the destruction, so they must be a response to the damage. Changes in gene expression as captured in the methylation clocks must be the body's effort to protect itself with increased immune function, increased autophagy, increased antioxidants, increased DNA repair. If any intervention sets back the methylation clock, then there is less repair and maintenance. We expect that setting the aging clock back to a younger age will actually decrease life expectancy. This insight is counter-intuitive, but, if correct, it changes the logic of methylation clocks.

Since 2013, there has been a kind of double-think in the world of anti-aging research. Most researchers, at least in public, continue to embrace perspective (2), even as they adopt methylation clocks to evaluate the interventions they develop.

I have been a leader in promoting perspective (1), and most readers of this journal might be sympathetic to the concept of phenoptosis. But in recent years, I have become convinced that epigenetic changes of both types (1) and (2) are taking place simultaneously as the body ages. The body is at war with itself. The self-destructive adaptations listed above are real: dialing down repair and maintenance, promoting systemic inflammation, apoptosis of healthy cells, derangement of the immune system. But the body retains its protective responses, and there are also changes in gene expression that ramp up the repair processes. All the present clocks include a mixture of (1) and (2); thus, we do not yet have a reliable metric for the efficacy of anti-aging technologies.

A methylation clock algorithm may correlate tightly with chronological age or even reflect expectancy of remaining life more accurately than chronological age, and still the same algorithm may give a deceptive assessment of a medical intervention. This can happen if the algorithm includes epigenetic markers (type (2))

for protective genes that are turned on in response to perceived damage. An intervention that modifies the epigenetics in such a way as to shut down the protective activity *without actually repairing the damage* will score as a lower age, even as, in reality, it decreases life expectancy.

To evaluate putative anti-aging interventions in reasonable time, we need aging clocks. But we cannot train these clocks on chronological age, or on measures of impaired health, or even on time to death. All these are likely to capture changes of both types (1) and (2). It is crucial to tease apart these two types of changes, but it is also difficult. It is a problem for students of metabolism, not just students of statistics.

Two questions will motivate the remaining portion of the present manuscript.

- What is the evidence that changes of types (1) and (2) are both components of all extant aging clocks?
- What are experimental methods by which we might separate the two, so that we can develop clocks based on (1) – (2) rather than (1) + (2)?

Two other questions will be addressed briefly below:

- What other issues with the current clocks might be ameliorated, independent of the central problem of distinguishing changes of types (1) and (2)?
- How does the body keep time? Changes of type (2) do not have to be on a chronological schedule, but changes of type (1) certainly do, and development is certainly programmed in part via programmed changes in gene expression. It is reasonable to assume that the body keeps time via some biochemical or bioelectric mechanism, and we expect that interventions that set back the body's "odometer" would be a royal road to rejuvenation, provided that a latent ability for robust repair persists into late life stages.

WHAT IS THE EVIDENCE THAT CHANGES OF TYPES (1) AND (2) ARE BOTH COMPONENTS OF ALL EXTANT AGING CLOCKS?

Some of the best-established interventions for extending lifespan do not affect the major algorithmic clocks, or do so modestly compared to what might be expected from their observed effects on lifespan. Rapamycin extends lifespan of male mice without affecting their methylation age in the Horvath rodent clock [37]. Participants in the CALERIE study who have adopted 25% CR diets showed no significant benefit according to either the GrimAge or PhenoAge clocks [38].

Conversely, Katcher's intravenous infusion of exosomes (E5) has a dramatic effect on the Horvath rodent/human clock, reducing epigenetic age by half

[39,40], but thus far seems to extend lifespan less consistently than the clock setback would imply [41]. The Conboys recently published a withering criticism of the utility of current methylation clocks, and of the machine learning algorithms from which they are created [42]. They report that clocks in common use do not respond as expected to known life-shortening conditions, such as Down Syndrome, inflammaging associated with arthritis, and Parkinson's Disease.

The GrimAge clock of Lu and Horvath [43] was trained on actual mortality data, using historic blood samples for which the future history of the donors was known. This was a major advance from previous clocks, based on chronological [31] age and on healthspan biomarkers [44]. But one element of the GrimAge development alerted me to the issue concerning type (2) changes, as described above.

Part of the training of GrimAge involved a methylation image of the subject's smoking history. Smoking is known to accelerate aging and shorten life expectancy. Certain patterns of methylation are associated with smoking, and are also valuable predictors of time until death. These were included in the GrimAge algorithm.

My assumption was that smoking decreases longevity by damaging tissue of the lungs, not by turning on the phenoptosis program. Therefore, if there are methylation changes associated with smoking, they are probably of type (2). In other words, the methylation signature of an "older" smoker is likely to include activation of more protective pathways than a "younger" smoker.

This is an important clue. The methylation profile of a smoker is useful in constructing a GrimAge clock, but it should be counted *in reverse*. Methylation changes associated with smoking are statistically associated with shorter lifespan, but mechanistically with protection. These changes should have been included in algorithmic clocks with *negative coefficients*, signaling a younger biological age. This was not how the GrimAge clock was constructed in fact. Methylation changes associated with smoking were included in the GrimAge clock with *positive coefficients*.

In general, the methylation image of smokers is an example of type (2). All type (2) changes should be counted with negative coefficients in methylation clocks, even though they are statistically associated with older ages and shorter remaining life expectancy.

WHAT ARE EXPERIMENTAL METHODS BY WHICH WE MIGHT DISTINGUISH, SO THAT WE CAN DEVELOP CLOCKS BASED ON (1) – (2) RATHER THAN (1) + (2)?

The story of GrimAge carries a message that suggests ways that methylation changes of types (1) and (2) might be teased apart in algorithmic clocks.

Present clocks do not distinguish between (1) and (2) so presumably the two types of methylation changes are combined in a way we might connote as (1) + (2). The goal would be to create a clock built on type (1) changes alone, or, more speculatively, penalize the clock for type (2) changes, so with the result that the algorithm measures (1) – (2) rather than (1) + (2).

The long-term goal would be to understand the metabolic consequences of each CpG change, separately and in combination, so that a clock could be constructed with full confidence that it scores beneficial and detrimental methylation changes appropriately.

Lacking this understanding in the interim, we might make progress toward distinguishing (1) and (2), by learning from the smoking example. One way to acquire a database of type (2) changes is that animal models might be injected with pro-inflammatory cytokines, and their epigenetic consequences mapped. The animals' immune systems might be challenged, or they might be subjected to laceration or small doses of radiation, again to chart the epigenetic response to compile a list of candidates for type (2) changes. These experiments could not ethically be performed on humans, however there are humans whose aging is accelerated by non-epigenetic factors, including alcohol and drug abuse. Such people might be tested as part of the quest for type (2) changes. People healing from physical and emotional trauma might also be presumed to have epigenomes modified in the direction of type (2).

Other examples of hormesis [45] may be useful. We might have most confidence in the epigenomes of people and animals subjected to caloric restriction [46]. Across the animal kingdom, CR is the most robust anti-aging strategy known at present, and we can be confident in subtracting CR-associated epigenetic changes from any algorithmic measure of biological age.

In addition to CR, there are dozens of interventions known experimentally to extend lifespan in rodents, including juvenile exosomes [47], rapamycin [48], certain peptides [24], vitamin D [26], NAC [49], SkQ [50], certain anti-inflammatories and angiotensin inhibitors. Recently, some of these have been tested for their effect on algorithmic clocks; in the future, the converse logic might be used to calibrate clocks. If an intervention is known to increase lifespan, then we may presume that epigenetic changes observed in response to that intervention are beneficial.

Some genes are known to be geropromoters, as, e.g., FAT10 [50], mTOR, and SIRT1 [51]. Other genes, e.g., FOXO [52], AMPK [53], and Klotho [54] are thought to be geroprotective. If methylation states can be found that affect the expression of these genes, they might be readily identified as types (1) or (2).

Before 2013, biological age was estimated with measures of performance and appearance: grip strength, gait speed, athletic endurance, memory, exhalation

volume (FEV), skin wrinkles, arterial inflammation, cartilage integrity. In the age of epigenetics, these physical characteristics retain their value as predictors of mortality, and a hybrid clock might be devised, combining physical and epigenetic factors.

A recent international collaboration [55] acknowledged that some epigenetic changes with age are “adaptive” while others cause “damage”, and proposed an innovative way to separate the two using genome-wide association (GWAS). Their method is to look across the genome for SNPs that are doubly associated, first with methylation at a particular CpG site, and second with a change in life expectancy (shorter or longer). On the presumption that the SNP is responsible for the methylation change and the methylation change is responsible for an effect on lifespan, they categorize particular CpG methylation sites as pro-aging or anti-aging. Some of these are sites change methylation status consistently through a lifetime, and these changes can be categorized presumptively as type (1) or type (2).

SENSITIVITY OF METHYLATION CLOCKS TO LAB ERROR

This is an avoidable problem with most of the extant methylation clocks. Methylation is measured on chips with microscopic dots containing antigen samples, each corresponding to a single CpG. It is easy for a few of these dots to be misread because of quirks in manufacturing or lab processing. Ideally, the clock algorithms should be based on many sites in such a way that democracy can minimize the importance of errors at any one site.

The Horvath clocks are each based on a linear combination of methylation levels (betas) at several hundred methylation sites. Coefficients are derived with an algorithm that optimizes accuracy and robustness. Since hundreds of components are summed, it would be reasonable to assume that a wild inaccuracy in any one of them would affect the readout only to the extent of a fraction of 1%. But this is not true. Roughly 60% of the coefficients are negative and 40% positive. There is cancellation between a large positive and a large negative sum, with the difference being more sensitive than necessary to potential anomalies that affect a single CpG. It is my experience that a single CpG out of 800,000 on the Illumina Epic chip can make a 10% difference in the computed age.

A simple solution to this dilemma would be to separate the sites that are hypermethylated with age from the hypomethylated sites, and run the same optimization procedure on the positive terms and the negative

terms separately*. This procedure creates two separate methylation clocks, one with only positive and one with only negative coefficients. The two clocks independently measure biological age, and each one has the desirable property that no one site can affect the output by more than 1%. An appropriately weighted average of the positive and negative outputs can be used as a best estimate of methylation age, avoiding the sensitivity to individual sites on the Illumina chip.

Levine has proposed a more elegant solution to this problem based on principal component analysis (PCA) [56]. She has reproduced the training algorithms of the major Horvath clocks, but instead of individual betas as primitives, she uses principal components. A principal component is a linear combination of thousands of betas that points in a direction of beta space defined by the shape of the statistical distribution in the training set [57]. Early analysis of this new approach [58] suggests that it is more robust to error but not yet as accurate as the older methods with betas for primitives, and that larger training sets could generate PCA clocks that are both more accurate and less error-prone.

IS THE GOVERNING CLOCK LINKED TO THE CIRCADIAN CLOCK?

The body’s circadian rhythm is controlled by the suprachiasmatic nucleus, located within the hypothalamus in the brain’s endocrine region [59]. Individual cells have their own timekeeping mechanism, and these are networked so as to create a consensus. The system is also subject to influence, particularly by light and by activity, which can reset the “time zone” without modifying the internal rate-generating circuits [60-62].

Circadian cycles can be affected with a small number of circulating hormones, whereas development and aging probably require timing and integration of an epigenetic network that is both complex and plastic in response to signaling from the metabolic and external environments. All the more reason to expect that a locus of a clock for aging and development might be situated in the neuroendocrine regions of the brain.

Cavadas [63, 64] has investigated the effects of neuropeptide Y (NPY), a short peptide deriving from the hypothalamus. She has collected evidence for a role of NPY in regulating aging at a systemic level [65]. Levels of NPY decline with age and in mice, NPY seems to be necessary for the life extension effects of CR [66].

Cavadas links six modes of aging to NPY levels:

- loss of proteostasis;
- stem cell exhaustion;

* Hypermethylated and hypomethylated should not be confused with type (1) and type (2). These ways of categorizing CpG sites are independent and cut across each other.

- altered intercellular communication;
- deregulated nutrient sensing;
- cellular senescence;
- mitochondrial dysfunction.

Though NPY may be a promising target for anti-aging therapies, it is probably not an upstream determinant of age because it is a neurotransmitter and not a transcription factor. FOXO and SIRT1 are transcription factors strongly linked to aging, but are not centrally sourced in the brain. Orexin and oxytocin derive from the hypothalamus and both have been linked to effects on aging [67]. Age-dependent increase in the pro-inflammatory signal NF- κ B seems to emanate directly from the hypothalamus [68].

Cai et al. have demonstrated that aging could be slowed in mice by inhibiting the inflammatory cytokine NF- κ B and the related cytokine IKK- β just in the hypothalamus. “In conclusion, the hypothalamus has a programmatic role in ageing development via immune–neuroendocrine integration...” They summarized findings from their own lab, suggesting that metabolic syndrome, glucose intolerance, weight gain and hypertension could all be exacerbated by signals from the inflamed hypothalamus. In agreement with Cavadas, they identified GnRH as one downstream target, and were able to delay aging simply by treatment with this one hormone. IKK- β is produced by microglial cells in the hypothalamus of old mice but not young mice. Genetically modified IKK- β knock-out mice developed normally but lived longer and retained youthful brain performance later in life [68].

Cai’s group identified micro-RNAs, secreted by the aging hypothalamus and circulating through the spinal fluid, that contribute to aging. A class of neuroendocrine stem cells from the third ventricle wall of the hypothalamus (nt-NSC’s) was identified as having a powerful programmatic effect on aging by secreting other micro-RNAs. Mice in which these stem cells were ablated had foreshortened life spans; old mice that were treated with implants of hypothalamic stem cells from younger mice were rejuvenated and lived 12% longer, despite the lateness of the intervention [69].

Transplanting a SCN from young hamsters into old hamsters cut their mortality rate by more than half, and extended their life expectancies by 4 months [70].

At Xiamen University, Leng et al. [71] have discovered that the decline of hypothalamic menin signaling with age is correlated with cognitive decline and possibly lifespan regulation in mice.

AGE-RELATED EPIGENETIC CHANGES IN DISPERSED CELLS

Effectiveness of the methylation clocks attests to a major role for methylation in the aging process;

but questions remain regarding the relationship of methylation in dispersed cells to the central regulation of aging.

– Is the dispersed methylation state of somatic cells an independent clock that determines the body’s age state, or is methylation an intermediate transmission of information about the body’s age, information that derives from a separate source, perhaps the suprachiasmatic nucleus itself?

– How much of the methylation change observed to take place with age is entropic “dysregulation”, and how much is directed?

The governing clock(s) that we wish to identify must have two conflicting properties. Of course, it must keep time reliably to trigger the phenotypes of growth, development, and then senescence on schedule. It must also be homeostatic. If the clock is perturbed, it must be able to find its way back to a remembered biological age. Homeostasis is a fundamental property of life. All biological systems tend to restore their state when deranged by the environment.

The need for homeostasis is a general property of biological systems. But how can a clock be homeostatic? If the body’s clock is knocked far off the biologically-determined age, where is the reference information from which it can be reset?

The only way that the clock can both keep time and restore itself after perturbation is with several independent time-keeping mechanisms which are continually exchanging information. This condition derives from theoretical considerations – a dangerous way to draw conclusions about biology. So, I am taking a chance to put forward this hypothesis: *there ought to be several independent time-keeping mechanisms in the bodies of complex organisms like the human animal, and there is continual cross-talk by which they are able to establish consensus, and reset the readout if one clock should differ substantially from the others.*

Evolutionary history has installed a fail-safe system in most higher animals to ensure that death happens on a (flexibly adaptive) schedule. The fast-acting force of individual selection seeks always to defeat the imperative of obligate death, and thus the death program is deeply embedded with alternative pathways so that it cannot easily be mutated away. This argument was made with different emphasis in George Williams’s influential antagonistic pleiotropy paper of 1957 [2].

We have identified a central clock in the neuroendocrine center of the brain, the suprachiasmatic nucleus, and we have found a probable second clock in the epigenetic state of dispersed cells around the body [72]. Horvath [73] has developed a pan-tissue methylation clock, and has measured differences in aging rates in different organs. Epigenetic changes in stem cells may deserve the status of a separate clock [74]. Telomere length in stem cells may constitute another clock [75];

a stem cell loses some of its telomere each time that it divides, and when telomeres become critically short, it becomes a senescent cell, secreting cytokines that not only cause systemic inflammation but also can trigger senescence in nearby cells. The immune system appears to have its own aging schedule [76]; and perhaps oxidative status of the dispersed mitochondria constitutes a sixth clock [77, 78]. A new possibility is raised by the research from Michael Levin's Tufts University laboratory [79]. Levin has demonstrated a role for electrical patterns in morphogenesis, healing, and regeneration. His model for cancer is not genetic; rather he has demonstrated that he can create tumors from normal genomes by disrupting their electrical connection to one another, and he can cure cancer in highly-mutated tumors without killing the cells, merely by restoring the cells' electrical connectivity [80]. Levin has speculated that electrical patterning is lost with age on the ground that many vertebrates are able to regenerate limbs or parts of limbs early in life, and gradually lose this capacity as they mature [81]. We should not be surprised to discover other independent clocks.

If my hypothesis is correct, then all these clocks are exchanging signals, cross-checking to establish a consensus age, and resetting accordingly. How do the various clocks exchange information? Given developments of the recent past [40, 82, 83], the obvious place to look is in exosomes carried in the blood [84]. This reasoning leads to the inference that exchanging young blood plasma for old ought to be a robust anti-aging strategy.

Of course, this work commenced nearly two decades ago. Experiments in heterochronic parabiosis [85, 86] have provided a proof of principle that rejuvenation through blood exchange is feasible. The Conboys have gone on to promote a perspective in which old age is established affirmatively by molecular species in the blood of old animals. Katcher and Wyss-Coray and Wagers have promoted the opposite perspective, that senescence is linked to a dearth of youthful signals in the blood of older animals. I think it likely that the most effective strategies for rejuvenation will involve both removal of pro-aging factors and addition of anti-aging factors to the blood plasma.

Wyss-Coray has championed therapeutic plasma exchange as a treatment for Alzheimer's disease [87]. Human umbilical plasma has been used to rejuvenate mice [88]. Wyss-Coray's company, Alkahest, has been acquired by the Spanish giant, Grifols, which has been a major player in plasmapheresis therapies. Research in the Conboy lab has centered on enhanced rate of healing in old tissues exposed to young plasma. Their results convince them that it is nothing in young plasma that makes the difference but rather the absence of inhibiting factors in old plasma. Simply removing plasma from an older animal and replacing it with saline solution plus albumin was shown to enhance rates of

healing [89]. In support of this strategy, blood donation is reported to extend life expectancy in humans [90]. To test this idea in therapeutic practice, the Conboys have allied with Dr. Dobri Kiproff to conduct a clinical trial of blood dilution [91]. Other American clinics experimenting with therapeutic plasma exchange include the Apeiron Center (Austin, TX) and Maxwell Clinic (Brentwood, TN). The experiments of Harold Katcher [39] have demonstrated clock rejuvenation and life extension in rats using young exosomes.

OTHER CLOCK IDEAS

If methylation is one of the body's methods of controlling gene expression, then gene expression itself might be a more direct measure of changes with age. This suggests a clock based on the proteome. Compared to a methylation clock a proteomic clock is one step closer to metabolism. Thus, it should be easier to separate type (1) from type (2) [92].

The technology of proteome measurement is not yet as well developed as methylation measurement. But the proteome is more easily understood in terms of its metabolic effects; thus, it should be possible to separate type (1) from type (2) changes based on known physiology. The first proteomic clock [92] is not yet as tightly correlated with age as the best methylation clocks, and it is far more expensive, but this is to be expected in the early stage of development.

The Conboy team has proposed a methylation clock that is not based on methylation values (betas) that change with age, but on dysregulation of methylation at CpGs that, on average, remain constant over a lifetime [42]. They find that the noise in these betas increases monotonically with age, where "noise" is defined as standard deviation in the absolute difference from the mean. It is not clear from their published description of their algorithm whether the standard deviation is computed within a population, and if so, how the algorithm could be applied to measure age of an individual.

LOOKING TO THE FUTURE

In the last decade, epigenetic clocks have opened the door to testing anti-aging interventions in humans on a short 1-2-year time scale. This is a major methodological breakthrough, but there are signs that existing clocks are not giving us a full and accurate report. I offer observations herein in service to the research effort in improving measurement of the body's age state and, potentially, modifying the signaling by which the body elicits coordinated, age-appropriate responses systemically.

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