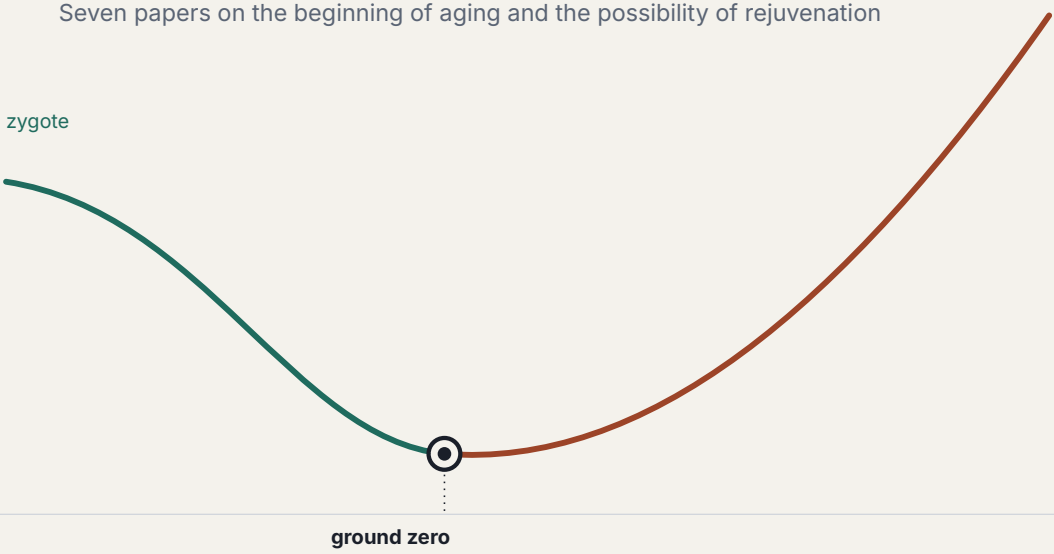
Entropy Lab · Life Science

The Ground Zero of Aging

When does aging begin?

Seven papers on the beginning of aging and the possibility of rejuvenation



Germ cells age. Yet each generation starts from the same place.
Somewhere in between, the clock must be wound back.
Seven papers went looking for that point.

The Ground Zero of Aging

Seven papers on the beginning of aging and the possibility of rejuvenation

The Ground Zero of Aging

When does aging begin? — Seven papers on the beginning of aging and the possibility of rejuvenation

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Compiled by Entropy Lab (Jeju, Republic of Korea)

This book reads seven papers on the ground zero hypothesis at the level of the primary literature and assembles them into a single argument. Every figure quoted in the text was checked against the original paper. Full citations and DOIs are collected at the back.

One of the seven (Zhang et al., 2022) is a preprint that has not been peer reviewed. The text says so each time it appears.

This is a science book. It contains no advice on the diagnosis or treatment of disease, or on the use of any drug.

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Prologue

The most important day of your life

The developmental biologist Lewis Wolpert liked to say that the truly important moment in your life is not birth, marriage or death, but gastrulation. For a long time this was read as a specialist's joke about how bodies get built. This book follows the possibility that it is also true of aging.

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When are you zero?

We count age from the day we were born, and so we quietly assume that birth is also the biological starting line. Look closely and the assumption gets strange.

The egg that made you was formed while your mother was herself a fetus. If you were conceived when she was thirty, that cell had been waiting for thirty years. Sperm are made a few months before they are used, but the stem cells that make them have been dividing since puberty. Neither parent contributes a freshly minted part.

Cells accumulate damage while they wait. Proteins get modified, the chemical marks on DNA drift out of register, metabolic byproducts pile up. That is the physical content of aging. So the question becomes awkward: how do two aged cells meet and produce a child who starts, every time, from about the same place?

There are only two kinds of answer. Either germ cells do not age, or the age is erased somewhere around fertilization.

> **Two old cells meet and a child starts from zero. Something happens in between.**

What this book covers

In 2020 Vadim Gladyshev at Harvard Medical School took the second answer and gave it a name. Somewhere in development there is a point at which biological age reaches its minimum, and it is there — not at conception, not at birth — that the life of the organism and its aging both begin. He called that point **ground zero**.

This book reads the seven papers that have appeared in the five years since. One built the hypothesis, four went looking for the curve in different species and at different resolutions, one tried to reproduce the reset in the lab, and one showed the most concrete molecular face the reset has yet been given.

Link		Role in the argument
02	Gladyshev 2021	the logical case — opinion, no data
01	Kerepesi 2021	first test in mouse data
04	Trapp 2021	a tool that reads one cell at a time
03	Kerepesi 2023	extension to human
05	Zhang 2022	extension to frog — unreviewed preprint
06	Gill 2022	implication 1 — doing it on purpose (MPTR)
07	Samaddar 2021	Implication 2 — molecular route of clearance

Figure 1: The seven papers share a question but do different jobs. One builds the logic, three test it across species, one builds the measuring tool, and two push the implications. The gap each link leaves becomes the task of the next.

The method of reading matters. Collect only the conclusions and you are left with the impression that several studies confirmed the same thing — an impression that is usually inaccurate. This book reads what each paper measured, what instrument the measurement depended on, and which limitations the authors conceded themselves.

Three rules for reading this book

One. Separate what was measured from what is being claimed. “The clock predicted a lower value” and “it was younger” are different sentences. Only the first is treated here as fact.

Two. Grade the evidence. Whether a result was peer reviewed, how many independent datasets support it, and whether anyone has reproduced it all change how much weight the same claim can carry.

Three. Do not erase the disagreements. Stitch the seven papers together and you get a tidy story, but a good deal of that tidiness comes from skipping the tension between them. The tension stays in the text.

These are not decoration. Nearly every common misreading in this field violates the first rule. “The embryo is youngest at E6.5” and “at E6.5 the epigenetic clock returned its lowest predicted value” are not the same sentence at different lengths. The first is a claim about what exists; the second is a report of a measurement. The data so far support only the second.

A note to the reader

This book assumes undergraduate biology. Terms like DNA methylation, transcription and developmental stage are explained when they first appear and not explained again. Anything unfamiliar is collected in the glossary at the back.

A fair amount of the book is about statistics and measurement, because that is where the real disputes live. Which clock was used, what tissue it was trained on, how many samples there were — these questions decide the fate of the conclusions. Skip them and what remains is a press release.

One last thing. One of the seven papers has not been peer reviewed. The text says so every time it appears. In science, “not yet reviewed” does not mean “wrong” — but it certainly does not mean “settled.”

Part I

The question

Germ cells age. Yet each generation starts from roughly the same place. For both sentences to be true, the clock has to be wound back somewhere in between. This part follows how that logical requirement became a hypothesis, and what the winding back actually looks like inside a cell.

Chapter 1

The myth of the ageless lineage

Since the nineteenth century biology has divided the body in two: the part that dies and the germ line that does not. The division is elegant and it has lasted. Measurements from the past twenty years say that half of it is false.

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Weismann's line

In the 1880s August Weismann reframed a question about heredity: why are acquired characteristics not inherited? His answer was structural. The cells of a multicellular organism fall into two lineages — the somatic cells that build the body and eventually die, and the germ line that is passed to the next generation. What happens in the body never reaches the germ line. So it is not inherited.

A corollary came along for the ride. Since the germ line is never broken across generations, it is in principle immortal. Textbooks usually stop there: the body ages and dies, the germ line does not.

The trouble is that "unbroken" and "ageless" are not the same claim.

The lineage continues, but the cell waits

Consider how long a human germ cell waits before it contributes to the next generation. Oocytes enter meiosis while the mother is still a fetus and then arrest. Decades pass before ovulation. During that time the cell is not dormant. It respires, makes protein, degrades protein.

Metabolism is not free. An active cell accumulates damage: modified long-lived proteins, methylation marks that have drifted out of place (these are called epimutations), oxidized lipids and metabolic byproducts.

Age-associated methylation patterns have in fact been reported in sperm — a father's age can be estimated from his sperm's methylation alone. That is direct evidence that time leaves a mark on germ cells.

Caveat · What exactly is aging?

"The germ line ages" can mean two different things: that the lineage degrades across generations, or that a germ cell accumulates damage while it is stored inside one individual. This book means the second. Supporting the first would require data spanning many generations, and none of the seven papers provides that.

So why is the child young?

Now the contradiction. If germ cells accumulate damage, children born to older parents should start from an older position. And across generations the starting line should drift steadily backwards. After a few million years our species should be born already old.

That has not happened. Parental age does measurably affect offspring, but not in a way that accumulates across generations. Each child starts from roughly the same place.

Logically there are three possible answers.

(a) Germ cells do not actually accumulate damage. — This conflicts with the methylation data.
(b) Damaged germ cells are filtered out. — Partly true. Most oocytes never ovulate, and early embryonic mortality is high. But selection alone struggles to explain "roughly the same place, every time."
(c) Somewhere around fertilization the damage is erased and age is wound back. — This is the answer the book follows.

Choosing (c) does not make (b) false. As Chapter 7 will show, data on developmental arrest in frogs indicate that selection is concentrated in a particular window. Filtering and resetting can happen in the same window.

Winding back has been observed

If "reversing age" sounds like fantasy, it is worth remembering that it is already routine lab work.

Since Shinya Yamanaka showed in 2006 that four transcription factors can return an adult cell to pluripotency, making induced pluripotent stem cells has become standard. Measure the epigenetic age of the result and it falls to near zero. An iPSC made from the skin of a sixty-year-old does not read sixty.

Complete reset, then, is real. What had been assumed is that it was an artificial laboratory manoeuvre. Gladyshev's question ran the other way: is nature not already doing the same thing every generation?

> **Going further · What "an iPSC reads zero" actually means**
>
> An epigenetic clock is a regression model that takes methylation values and returns an age. It was fitted on adult tissue, so feeding it a cell from outside that range is extrapolation. That is why negative ages appear. A negative age is not a biological state called "before birth"; it is what happens when a model extends a straight line past the data it was built on. Part IV returns to this.

What this chapter leaves you with

· Weismann’s unbroken lineage survives. His ageless cell does not.
· Germ cells metabolize while they wait, and accumulate damage. Sperm methylation age is the evidence.
· Yet the offspring’s starting point does not drift across generations.
· Therefore something must erase damage around fertilization. This is a logical requirement, not yet an observation.
· Complete reset is already demonstrated by iPSC reprogramming.

Chapter 2

Ground zero as a hypothesis

In 2020 Gladyshev gave the requirement from Chapter 1 a coordinate. There is a point in development where age is at its minimum, and that is where the life of the organism and its aging both begin. This chapter sets out exactly what the model claims — and what it does not.

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Paper 02 Evidence D

Gladyshev VN. The ground zero of organismal life and aging. *Trends in Molecular Medicine* 2021;27(1):11–19.
Opinion — no new experimental data

Asks When does aging begin, and how is rejuvenation possible?

Answers There is a minimum of biological age during development, and aging starts there

The D grade needs explaining first. There is no new data. That is not a defect, it is a genre: an opinion paper rearranges existing observations into testable propositions. The author himself writes that direct proof is currently lacking. In this book paper 02 is treated as a hypothesis, not a finding.

The model in five sentences

One. The zygote is not the minimum

The common view treats the zygote as the starting line. The model reverses this. A zygote carries the accumulated damage of both parents. Early development is therefore a clearing operation before it is a building operation. The author calls the stretch from conception to ground zero a "preparatory stage."

This fits a number of odd facts about early embryos. A zygote can split into twins. It can fuse with another embryo to form a chimera. For a while it runs on RNA and protein the mother packed in, not on its own genes. Telomeres lengthen rather than shorten. Epigenetic marks are erased. None of this looks like a finished organism beginning; it looks like something still being cleaned up.

Two. A minimum exists

There is a state of the epigenome — the "cleanest" naturally reachable one — at which organismal life and aging begin together. The author places it somewhere between blastocyst and pharyngula, most likely around gastrulation. Here he quotes Wolpert.

> **It is not birth, marriage or death, but gastrulation, which is truly the most important time in your life.**

Three. Aging and rejuvenation are not the same road run backwards
There is no Benjamin Button symmetry in which time simply reverses. Getting old and getting young again are different paths. The distinction matters later: the partial reprogramming of Chapter 10 reverses some markers of aging and not others.

Four. Development, rejuvenation and aging are three different things
Development is construction under genetic instruction. Rejuvenation is the removal of damage and the lowering of age. Aging is the byproduct of metabolism with no purpose behind it. The three overlap in time but they are not one process.

Blur the distinction and a familiar error follows: reading "undoing what was built" as "lowering age." Reverse a cell's differentiation and it becomes less specialized, but that alone does not remove damage.

Five. Ground zero could be pushed lower than nature puts it
Extend the clearing phase artificially, or reduce the mutational load by editing the genome, and an organism could in principle begin from a lower point than nature achieves. The author offers this as a thought experiment. Chapter 14 returns to it.

What gets reset, and what does not

The model has one important exception: the genome.

Methylation marks can be erased. Damaged proteins can be degraded. A mutation in the DNA sequence cannot be undone, because the cell holds no master copy to restore from.

So the author limits genome-level "rejuvenation" to two routes. One is purifying selection at the level of the species — individuals carrying harmful mutations reproduce less. The other is genome editing. The first is slow and brutal; the second is still hypothetical.

> This asymmetry recurs throughout the book. Rejuvenation is a problem about **the epigenome and about proteins**; the genome sits outside it. No paper here claims to have reversed a mutation.

The convergence argument — why gastrulation?

The author's reason for placing ground zero near gastrulation is not data but the convergence of several separate ideas. He argues that four different features land at the same point.

Phylotypic stage	the point at which embryos of different animals most resemble one another — the conserved waist of development
Minimum structural entropy	the point at which cellular organization is most ordered
Loss of regenerative capacity	the embryo-to-fetus transition, after which the whole can no longer be rebuilt
Peak embryonic mortality	the point at which deleterious alleles are filtered most strongly

If this is true, gastrulation is not merely a developmental stage but a singularity where several axes cross. It remains a hypothesis: no study has measured all four in a single species at once. The frog work in Chapter 7 comes closest.

Why the hypothesis is testable

A good hypothesis specifies how it could fail. This one makes three predictions.

Prediction 1. Measuring biological age through development should produce a U-shaped curve.
Prediction 2. The position of the minimum should be conserved across species.
Prediction 3. After the minimum, age should increase monotonically.

Testing these is the business of Part II. But testing them first requires deciding what "measuring biological age" means. That instrument is the epigenetic clock, and it gets its own interlude.

Caveat · The gap between model and measurement

All three predictions are statements about biological age. What actually gets measured is the output of a clock. Put an equals sign between the two and the test becomes circular. This problem shadows every chapter of Part II and is confronted directly in Part IV.

What this chapter leaves you with

· The ground zero model proposes a minimum of biological age in development, and aging starting there.
· From conception to that point, development is clearing before it is building.
· Development, rejuvenation and aging overlap but are three distinct processes.
· Genomic mutations are not subject to reset.
· The paper contains no data. It contains three testable predictions.

Chapter 3

Clearance

The ground zero model says damage is erased. That is an abstraction. In one kind of cell, though, the process can be watched minute by minute. A *C. elegans* oocyte receives a signal from sperm and clears its protein aggregates within twenty minutes.

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Paper 07 Evidence B

Samaddar M, Goudeau J, Sanchez M, Hall DH, Bohnert KA, Ingaramo M, Kenyon C. A genetic screen identifies new steps in oocyte maturation that enhance proteostasis in the immortal germ lineage. *eLife* 2021;10:e62653.
Genome-wide RNAi screen — peer reviewed

Asks How does the germ line clear protein damage each generation?

Answers A route runs from sperm signal to ER remodelling to lysosomal acidification to clearance

Why a worm

Caenorhabditis elegans is a one-millimetre nematode. It is transparent, so processes can be watched inside living cells; its generation time is three days; and its genes can be knocked down one at a time across the whole genome.

The system used here is more specific still. Instead of the usual hermaphrodite, the experiments use a mutant feminized worm. A hermaphrodite carries its own sperm, so an oocyte receives the maturation signal as soon as it forms. Block sperm production with a temperature-sensitive mutation and oocytes accumulate without ever receiving the signal. Mate such a worm to a male and the moment the signal arrives can be pinpointed.

The design, in other words, is an instrument for comparing **before and after a signal, minute by minute.**

The first observation: young oocytes have aggregates too

An intuition needs correcting first. It is natural to picture protein aggregates as rubbish that accumulates in an old cell.

But in age-matched young females, aggregates and ER patches are already present in the very first proximal oocyte to form. The aggregates are not the product of long waiting. They are the normal state of an oocyte that has not received a sperm signal.

That single observation changes the frame.

Immortality is not the absence of damage. It is a cycle in which damage is made and removed on schedule.

The screen: switching off 18,225 genes

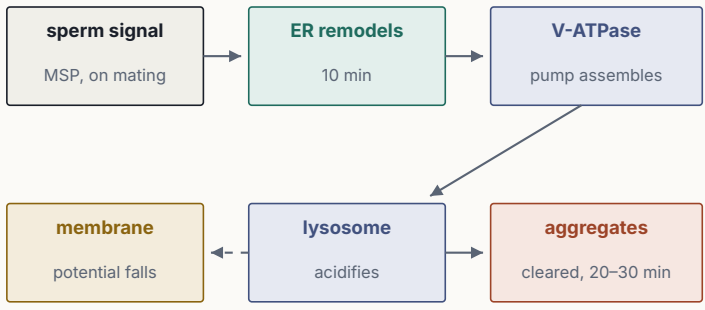
The team used an RNAi library of 18,225 clones covering roughly 87 per cent of worm genes, knocking down one gene at a time and asking whether aggregates failed to clear.

Primary screen	367 genes in which more than half the animals showed the aggregate phenotype
Replication	narrowed to 88 that reproduced in at least two of three runs
Specificity	81 confirmed after excluding male gonad defects and non-specific effects
Orthogonal check	53 of 55 reproduced with a different fluorescent reporter

That the result held with two independent reporters matters. It is not an artefact of one fluorescent protein.

The route: three organelles in series

Grouping the confirmed genes by function revealed a chain.



Dashed line mark links whose causal direction is not settled. The drop in membrane potential depends on acidification, but a persistently high potential has also been proposed as a checkpoint that blocks clearance.

Figure 2: How a *C. elegans* oocyte clears damaged protein just before fertilization. A sperm signal remodels the endoplasmic reticulum; the lysosomal proton pump is then assembled, the lysosome acidifies, and the acidified lysosome engulfs the aggregates. Mitochondria let the process through by lowering their membrane potential.

The ER moves first

In an oocyte that has not received the signal, the endoplasmic reticulum is clumped into bright patches that electron microscopy shows as stacked parallel sheets. After mating the structure is remodelled into a fine reticulum.

The timing is decisive. ER change begins about 10 minutes after mating; aggregate clearance happens at 20 to 30 minutes. **The cause precedes the effect.**

The lysosome is the switch

Here is the striking result: almost every hit from the screen blocked lysosomal acidification. The genes have wildly different functions, yet the phenotype converges on one place.

A lysosome needs an acidic interior for its degradative enzymes to work, and the acidity is produced by a proton pump called the V-ATPase. Every knockdown that blocked acidification also blocked the pump protein from gathering on the lysosomal surface.

The authors' model: as the ER is remodelled, one subunit of the pump moves to the lysosome and is completed there by docking with the rest. Only then does acidification begin, and only then does clearance start.

Mitochondria are the gate

There was one exception. Knocking down ATP synthase caused aggregates but did not block lysosomal acidification.

The first guess would be energy shortage. But knocking down the electron transport chain lowered oocyte ATP and reduced body size and brood size, and yet **produced no aggregates.** Clearance proceeds even when energy is low.

The remaining explanation is membrane potential. Blocking ATP synthase keeps the inner mitochondrial membrane potential high. The authors propose that a persistently high potential acts as a gate that prevents clearance. In normal oocytes the potential falls as the lysosome acidifies.

> **Going further · Separating energy from signal**
>
> This passage is worth studying as method. "It happened because ATP dropped" and "it happened because a signal changed" are easy to confuse. The team lowered ATP a different way — by blocking the electron transport chain — and asked whether the same phenotype appeared. It did not. That excludes the ATP explanation. When one manipulation changes several things at once, this is the standard way to isolate the cause.

What the paper does not establish

Caveat · Three reservations

No direct evidence for the mechanism. The central claim — that a pump subunit travels from ER to lysosome — has never been observed directly. Tagging the endogenous protein produced no visible signal in the germ line at all, so the experiments used an overexpressed version. The authors say immunogold electron microscopy will be needed.

No causation across generations. Nobody measured how many generations survive if clearance is blocked, or how the offspring fare. So the sentence "germline immortality involves proteostasis renewal" is not a conclusion this paper proved; it is the premise it is working towards.

Worm-specific biology. The sperm-derived signalling molecule, the temperature-sensitive feminization, the hermaphrodite sexual system — all are peculiar to this species. Whether the same route operates in mammalian oocytes is untested.

A note on vocabulary. The authors do not call their result "rejuvenation." They call it "proteostasis renewal," and that is the more accurate choice. This is not turning something old young again; it is removing damage on a cycle before it

accumulates. Placing it on the same level as the model in Chapter 2 is a category error.

Why the paper matters anyway

Three reasons.

First, it makes rejuvenation a concrete molecular event. Chapter 2 could only say that damage is erased. Here you can see which organelles do what, in what order.

Second, it moves the timing earlier. This clearance happens **before** fertilization, during oocyte maturation. That does not contradict placing ground zero at gastrulation, but it opens the possibility that the reset is a multi-stage process rather than a single point. Keep it in mind when Chapter 6 reports sperm reading younger than embryos.

Third, it connects to somatic cells. Knocking down the genes from the screen — the lysosomal pump genes in particular — accelerated age-related aggregation in somatic tissue as well. A mechanism found in the immortal lineage also operates in cells that age.

What this chapter leaves you with

· Protein aggregates are not the residue of an old oocyte. They are the normal state of one awaiting a signal.
· Sperm signal → ER remodelling (10 min) → lysosomal acidification → clearance (20–30 min).
· Mitochondria regulate the step through membrane potential, not through energy supply.
· The core mechanism rests on circumstantial evidence; generational causation is untested.
· This is renewal, not rejuvenation. The authors' own term is the better one.

Part II

The evidence

The hypothesis predicts one curve: age falls during development and then rises. Four papers went looking for that curve — in mice, in single cells, in humans, in frogs. Before reading them we look at the instrument. You cannot read a curve without knowing what drew it.

Interlude

What an epigenetic clock is

The whole of Part II rests on this instrument. What it takes as input, what it returns as output, and what is being assumed when that output is called an "age" — all of that is worth settling first.

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Methylation as a mark

A methyl group can be attached to a cytosine in DNA, most often where a cytosine is followed by a guanine — a site known as a CpG. The human genome contains roughly 28 million of them.

The mark does not change the sequence. An A does not become a T. What changes is how that stretch of DNA is read. A densely methylated promoter generally means a silenced gene.

One property matters here. Methylation patterns are copied to daughter cells through division, but not perfectly. They drift. And the drift has direction: some sites gain methylation with age, others lose it.

A clock is a regression model

In 2013 Steve Horvath used this property to build a model that predicts age. The principle is simple.

1. Measure methylation in tissue from people whose age is known.
2. Find the relationship between each CpG's value and chronological age.
3. Select a few hundred CpGs that predict age best and assign them weights.
4. For a new sample, read those sites and compute an age.

Horvath's multi-tissue clock uses 353 CpGs. Multiply 353 values by their weights, add them up, and an age comes out. Accuracy varies by tissue, but correlations above 0.9 are common.

One fact must be held onto here. **A clock is not a device that measures aging; it is a statistical model fitted to predict chronological age.** Which biological process moves the methylation is nowhere inside the model. The model learned a correlation.

So why trust it at all?

If it is merely a correlational model, why is it taken to be biologically meaningful? Three reasons.

First, the prediction error is small. For human multi-tissue clocks the median absolute error is a few years. Few molecular markers track the calendar that closely.

Second, the direction of the error means something. People whose predicted age exceeds their actual age tend to have higher mortality and disease risk. This is called epigenetic age acceleration, and it suggests the clock is reading something health-related rather than simply reading a calendar.

Third, the clock responds to interventions. Caloric restriction and growth hormone receptor knockout lower it. Reprogramming drops it sharply.

The clocks in this book

Several different clocks appear in Part II. It is worth keeping them apart.

Type	Trained on	Used in
Multi-tissue clock	bulk methylation across tissues	Ch. 4 (mouse)
rDNA clock	methylation in ribosomal DNA	Ch. 4 (mouse)
Single-cell clock (scAge)	bulk reference plus per-cell likelihood	Ch. 5
Intersection clock	refitted per sample on shared CpGs	Ch. 6
Amphibian array clock	adult skin, 1,068 conserved CpGs	Ch. 7
Transcriptomic clock	gene expression levels	Ch. 10

The list itself says something. Every time the field pointed the question at a new kind of sample, it needed a new instrument. No tool, no finding. And different tools give different numbers for the same cell.

Three traps to know in advance

These recur throughout Part II. Part IV handles them properly; here they are simply named.

Caveat · Trap 1 · Extrapolation

Nearly every clock in this book was trained on adult tissue. Applying one to an embryo means predicting outside the training range. A regression will happily return a value out there, but there is no way to check it, because no independent standard for "the embryo's true biological age" exists. Negative ages come from the same place.

Caveat · Trap 2 · Global methylation as a confounder

Methylation across the whole genome swings dramatically during development — it collapses after fertilization and rises again around implantation. A clock's output is a weighted sum of methylation values. When the overall level moves, the output moves with it. So the "age curve" seen in development may be a restatement of the global methylation wave.

Caveat · Trap 3 · Reference dependence

A clock takes the patterns of the tissue it was trained on as its yardstick. A liver clock reads liver cells well; a blood clock does not. Chapter 5 shows the same 19 cells scoring r = 0.952 under one clock and r = 0.363 under another. If so, what a clock measures may be similarity to a reference, not the age of a cell.

Why the field uses them anyway

The reason is simple: there is no alternative.

No independent method measures the age of an embryo. Telomere length increases during development but has poor resolution, protein damage is difficult to assay, and functional readouts are hard to apply to embryos. Methylation is close to the only marker that can read tens of thousands of sites from a tiny amount of material.

So every conclusion in this field is conditional. **If the epigenetic clock is a valid proxy for biological age**, then age falls and then rises during development. Strip the conditional clause and you have overstatement.

· An epigenetic clock is a regression model fitted to predict age from methylation values.
· Its error is small, the direction of its error tracks health, and it responds to lifespan interventions.
· But applying an adult-trained clock to an embryo is extrapolation.
· Global methylation waves and reference-tissue dependence can both confound the result.
· Every conclusion in Part II carries an implicit "if the clock is valid."

Chapter 4

Mouse — finding the U

In 2021 the ground zero hypothesis met data for the first time. Not one new experiment was performed. Existing clocks were applied to methylation data already in the public archives. A curve appeared.

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Paper 01 Evidence B+

Kerepesi C, Zhang B, Lee SG, Trapp A, Gladyshev VN. Epigenetic clocks reveal a rejuvenation event during embryogenesis followed by aging. *Science Advances* 2021;7(26):eabg6082.
Reanalysis of public data — peer reviewed

Asks Is ground zero real?

Answers In mouse embryos predicted age passes a minimum near E6.5–E7.5 and then rises

Design — testing a hypothesis with no new experiment

The strategy is unusual. No embryos were cultured and no methylation was newly measured. Sixteen datasets already deposited in public archives were assembled and existing clocks applied to them.

The trade-off is clear. It is cheap and fast, and it lets many laboratories' data be seen at once. But the quality and design of the data cannot be controlled, and conditions differ between datasets, producing batch effects. The paper's largest weakness comes from exactly there.

The data: five mouse early-embryo sets, one set covering late embryo to birth (139 samples), three human prenatal array datasets, and seven human pluripotent stem cell passaging datasets.

One new instrument was built: a clock based on methylation in ribosomal DNA. rDNA is present in many copies, so sequencing depth is high and age-associated CpGs are plentiful. The clock uses 355 CpGs and reached a correlation of 0.94 in independent testing.

Result one — age falls in early embryos

All five mouse early-embryo datasets pointed the same way. From zygote through blastocyst to E6.5–E7.5, predicted age fell consistently.

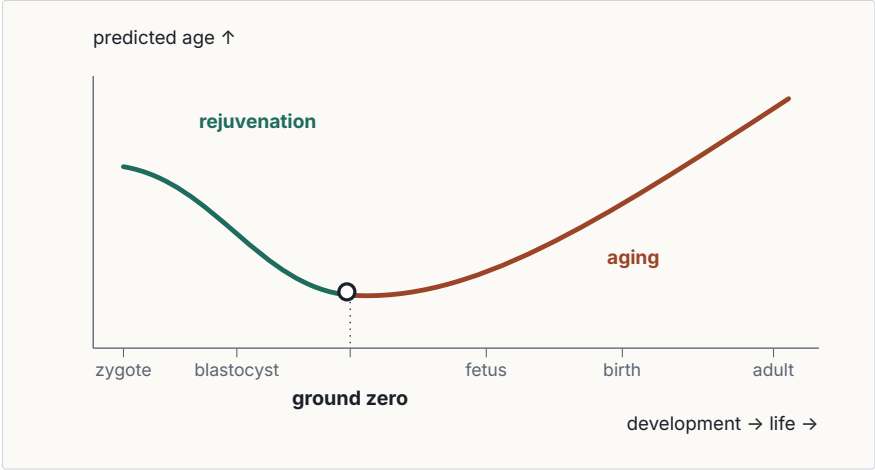

Figure 3: The characteristic shape of predicted epigenetic age during development. It starts at the zygote, passes a minimum around gastrulation, and rises for the rest of life. The vertical axis is what a clock predicts, not a directly measured "biological age." That distinction is the premise of this whole book.

This was not peculiar to the rDNA clock. Four genome-wide clocks gave the same direction. That the direction survives a change of instrument makes it less likely to be an artefact of one model.

Caveat · But how far did it fall?

The paper does not report the absolute magnitude of the decrease. Its figures rescale predicted age to the interval from 0 to 1 within each dataset. The rescaling removes differences of scale between datasets — and with them the very quantity we want, namely how many weeks or months were lost. The impression of a large fall comes from the shape of the plot, not from the unscaled numbers.

Result two — age rises from mid-gestation

In the only dataset containing both early and late embryos, E6.5 and E7.5 read significantly lower than E13.5.

A caveat attaches. The E13.5 samples are primordial germ cells — the direct precursors of sperm and egg. So this comparison is closer to "the germ lineage also has an age during development" than to "somatic cells begin to age." Those are different statements.

The real rising limb comes from another dataset. Across nine timepoints from E10.5 to birth, 139 samples, genome-wide clocks show consistent increase. The pattern holds within individual tissues.

Human prenatal data give a blurrier picture. Of 18 tissue-level comparisons, 5 showed significant increases, 9 non-significant increases, 4 non-significant decreases and none a significant decrease.

> **Going further · That 13 of 18 are non-significant**
>
> "Increase predominates" is an honest description — the direction clearly leans one way. But that 13 comparisons failed to reach significance is rarely mentioned in the retelling. It is mentioned here. With small samples, non-significant results are normal, and building a conclusion on direction alone in that state converts lack of power into confidence.

Result three — pluripotent cells do not age with passage

In human embryonic and induced pluripotent stem cell data, predicted ages were mostly below zero and did not rise past 100 passages. The datasets span passage 9 to 114 for ESCs and 4 to 69 for iPSCs.

Passaging is stressful for cells. The age did not move. The authors read this as evidence that cells corresponding to the early embryonic state do not age.

Another reading is available. The clock may simply have hit its floor near zero. Trained on adult tissue, the model may be unable to resolve differences among states far younger than that. This is called a floor effect.

A counterintuitive result — passaging makes cells younger

One experiment is worth dwelling on. Mouse embryonic stem cells were cultured with and without two inhibitors that support self-renewal, and ages at passage 0 and passage 5 compared.

Without the inhibitors, age after passaging was **lower.**

The authors' reading: incomplete self-renewal conditions push cells to keep attempting differentiation, and that attempt produces rejuvenation much as development does in vivo.

The result says two things at once. Rejuvenation can occur without any special manipulation — and **culture conditions move the clock.** The second is a warning.

Where ground zero sits

Splicing early and late data together produced a U shape in every case. The minimum fell at E7.5 for three of four clocks and at E10.5 for the fourth.

The published version added analyses. Separating genome-wide from rDNA clocks gave E6.5–E7.5 in four cases and E10.5 in two; a newly added mid-gestation dataset gave E6.5–E7.5 in five cases and E8.5 in one.

The final statement: the range is E4.5 to E10.5, with E6.5–E7.5 most likely. In the mouse that period corresponds roughly to gastrulation.

Comparison	Result	Caveat
E6.5/E7.5 vs earlier	age decreases	5 datasets, 5+ clocks
E6.5/E7.5 vs E13.5	significantly lower	E13.5 are germ cells; n = 2 each
E10.5 → birth	consistent increase	9 timepoints, 139 samples
Human prenatal, 18 tests	5 significant increases	0 significant decreases, 13 non-significant
Pluripotent cells >100 passages	no increase	possible floor effect
Ground zero	E6.5–E7.5 most likely	range E4.5–E10.5

The weakness the authors concede

The most candid passage in the paper is written by the authors themselves. Of the spliced figure they note that the number of overlapping clock CpGs differs between stages, producing a batch effect that actually shifted predicted ages.

Part of the rising limb of the U, in other words, may be the effect of changing datasets rather than biology. The authors rest their case on the fact that, even so, the minimum agrees in three of four clocks.

Caveat · When citing this paper

· Cite the *Science Advances* version, not the preprint. The published version adds two sections of mechanistic experiments and sharpens the claim about ground zero's position.
· Sample sizes are small. In one dataset there is a single sample per developmental stage, and one of the key comparisons is 2 against 2.
· Be careful with "conserved across species." The human data here span gestational weeks 9 to 23 and are already on the rising limb. Rejuvenation in humans is not claimed until the 2023 follow-up.

What this chapter leaves you with

· In mouse embryos predicted age reaches a minimum near E6.5–E7.5 and rises thereafter.
· That the direction survives a change of clock is the strongest part of the result.
· The absolute size of the fall is not reported; the figures show rescaled values.
· Culture conditions move the clock. Passaging made cells read younger in one experiment.
· The authors concede that part of the rising limb may be a batch effect.

Chapter 5

The age of a single cell

The unit of life is the cell. Yet every clock built since 2013 has measured the average of tens of thousands of them. The attempt to close that gap produced, unexpectedly, the firmest evidence the hypothesis has.

In this chapter

The problem — the unit of life and the unit of measurement differ 33
Two obstacles in single-cell data 33
The solution — drop the matrix, go to probability 34
Result — tissue age is recovered from one cell 35
Same cell, different clock, different age 35
A result to read carefully — muscle stem cells 36
The firmest result — lineage-selective rejuvenation 36
Why this matters 37
Limits 38
What this chapter leaves you with 38

Paper 04 Evidence B+

Trapp A, Kerepesi C, Gladyshev VN. Profiling epigenetic age in single cells. *Nature Aging* 2021;1:1189–1201.
Method development — peer reviewed

Asks Can age be measured in a single cell?

Answers A framework estimates one cell's age from a bulk reference and a likelihood calculation

The problem — the unit of life and the unit of measurement differ

The paper's opening states the problem precisely.

> **Individual cells are the units of life, yet all existing epigenetic clocks rely on measurements derived from bulk samples.**

Bulk measurement gives a good tissue average. It erases differences between cells. If some cells in a tissue age quickly and others slowly, an average cannot tell you.

The reason bulk was unavoidable is technical. Reading methylation requires bisulfite treatment, which fragments a great deal of DNA. Plenty of input material was needed.

Two obstacles in single-cell data

Technology advanced far enough to read methylation from one cell, but the resulting data do not have the shape existing clocks assume.

Sparsity

Only a fraction of CpGs are read in any one cell, and which ones get read is close to random. Every cell has a different set.

An existing clock demands values at 353 particular CpGs. If this cell has only 40 of them and that cell has a different 60, what do you do?

Binarization

In bulk, a methylation value is continuous between 0 and 1, because it is the proportion of cells methylated at that site. In one cell the site is either methylated or not. A value of 0.7 cannot exist.

The solution — drop the matrix, go to probability

The usual approach was to impute the missing values. Then the prediction depends on the imputation rule.

This paper takes a different route: it does not require a shared CpG matrix at all.

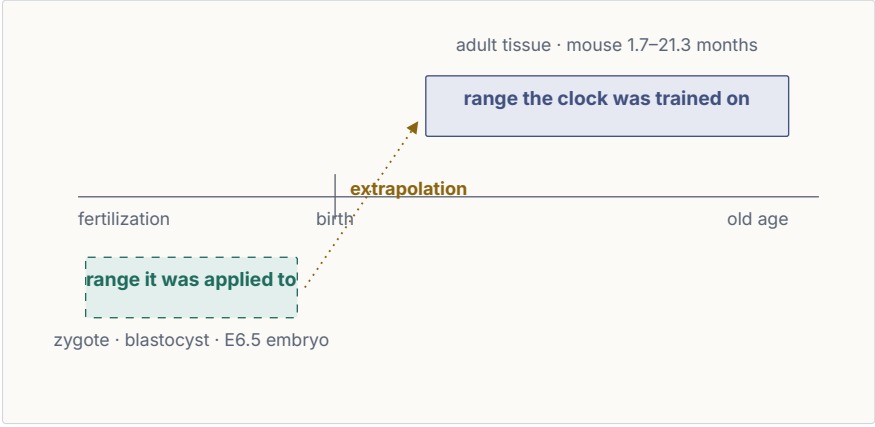

Figure 4: Epigenetic clocks are trained on adult tissue. Applying one to an embryo means reading values outside the training range. Because no independent ruler gives the embryo's "true age," there is no way to check whether the extrapolation is right. This is the structural weakness of the field.

Step 1. In the bulk reference, fit a straight line relating methylation to age at each CpG.
Step 2. Keep only the CpGs actually read in the target cell and take the top ones by correlation with age. Each cell therefore gets a different set.
Step 3. For a hypothesized age, read off from the line the probability that the site is methylated. If the bulk value is 0.7, a randomly chosen cell from that tissue has a 70 per cent chance of being methylated there.
Step 4. Multiply the probabilities across sites to build a likelihood over age, and take the most likely age.

Simple, but the framing is good: it asks which bulk age best explains this cell's binary pattern.

Result — tissue age is recovered from one cell

The cleanest result is in hepatocytes.

Cells	Clock	Correlation	Median absolute error
19 hepatocytes	liver clock	0.952	2.1 months
19 hepatocytes	multi-tissue clock	0.862	4.5 months
19 hepatocytes	blood clock	0.363 (not significant)	10 months
205 muscle stem cells	muscle clock	0.22	5.8 months
205 muscle stem cells	blood clock	0.088 (not significant)	12.1 months

A median absolute error of 2.1 months from a single cell compares well with bulk mouse clocks at 2 to 4 months. That is a genuinely impressive number.

The same table also contains the book's most important warning.

Same cell, different clock, different age

Nineteen hepatocytes scored by the liver clock give a correlation of 0.952. Scored by the blood clock they give 0.363, and significance disappears.

Same data, same cells, different reference.

The authors read this as a strength: the clock is tissue-specific, so tissue-specific aging trajectories can be followed.

Read the other way it says something else. What is being measured is not an age intrinsic to the cell but **how closely this cell's methylation pattern resembles bulk patterns of a given tissue at a given age.** For a cell type with no reference data, the method cannot in principle say anything about age.

Caveat · The two most important numbers in this book

0.952 and 0.363. They return in Chapter 12. They are the most direct evidence for the claim that "age" in this field is a position in a coordinate system rather than a measured quantity. No other paper gives so clean a controlled comparison.

A result to read carefully — muscle stem cells

Among 205 muscle stem cells, a group aged 1.5 months was compared with one aged 26 months. The real difference is about 24 months.

The detected difference in epigenetic age was described as "a slim but significant increase." No figure is given; a preceding study's phrase, "a few weeks," is quoted.

The median absolute error of the muscle clock is 5.8 months. The effect is an order of magnitude smaller than the measurement error.

In that state two possibilities cannot be separated. Muscle stem cells may genuinely age slowly, or the clock may be unable to detect their aging. The authors prefer the first, but the data do not compel the choice.

> **Going further · Reading small differences**
>
> "Significant" and "large" are different words. With a large enough sample, a tiny difference becomes statistically significant. 205 cells is not a small sample. The habit worth forming is to set the effect size beside the measurement error — which is why this chapter's table reports correlation and median error together.

The firmest result — lineage-selective rejuvenation

The paper's contribution to the ground zero hypothesis comes at the end.

495 cells from mouse gastrulating embryos were analysed, spanning E4.5 to E7.5. All three clocks showed predicted age falling monotonically across that interval.

So far this repeats Chapter 4 at single-cell resolution. The decisive part comes next.

Sorting the cells by lineage split the pattern.

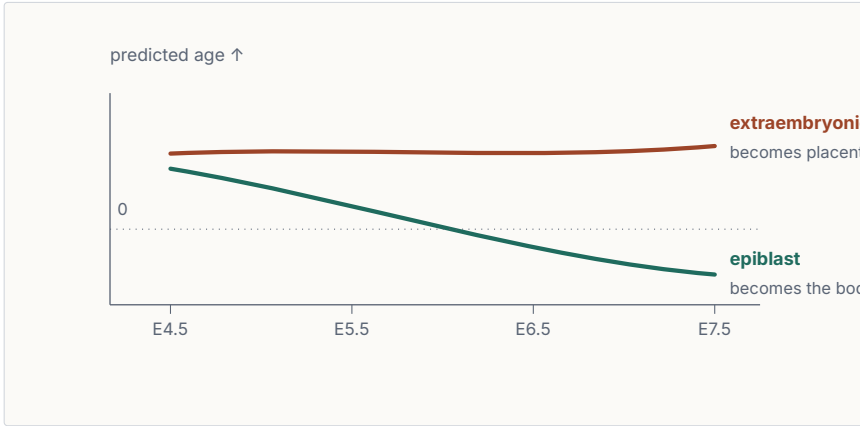

Figure 5: At single-cell resolution, rejuvenation does not happen in every cell of the embryo. Predicted age falls only in the lineage that will form the body; the lineage headed for the placenta holds a higher value at the same stage. This is hard to explain by global methylation alone.

Epiblast cells — those that will form the body of the embryo — fell strongly, to near zero or below. Extraembryonic ectoderm and visceral endoderm, which will build the placenta and associated structures, held significantly higher ages at the same stage.

Same embryo, same moment, different fate. Rejuvenation reached only one side.

The authors call this stratified rejuvenation: the natural reset is not uniform across the embryo but selective for the cells destined to become the organism.

Why this matters

Recall the second trap from the interlude: the objection that the developmental age curve is simply a restatement of the global methylation wave.

This paper is not immune to the charge. Predicted age correlates with mean global methylation at roughly −0.71 to −0.83.

But lineage selectivity is not easily handled by that explanation. If the global change happens across the whole embryo, why does age fall in only one lineage?

Lineage selectivity is the most direct material against the objection that rejuvenation is a byproduct of global demethylation. Within this book it is among the firmest support the hypothesis has. It is also a single analysis of a single dataset, with no independent replication.

Limits

Caveat · Four reservations

Parameters were set on the evaluation set. How many of the top CpGs to use was chosen after seeing benchmark results, and the benchmark used the very data on which performance is reported. The 0.952 may therefore be optimistic.

Coverage drives the result. Below about 500,000 CpGs per cell, predictions become unstable. Applying that filter cut muscle stem cells from 275 to 205 and embryonic cells from 758 to 495. If the dropouts are not random, the conclusion is distorted.

The independence assumption is untested. Probabilities are multiplied as if CpGs were independent. In reality neighbouring sites move together. The authors state the assumption explicitly.

Mouse only. The abstract says the approach was validated in mice. No application to human single-cell data appears in this paper.

What this chapter leaves you with

· A bulk reference plus a likelihood calculation can estimate the age of one cell.
· Median error of 2.1 months in hepatocytes, comparable to bulk clocks.
· The same cells score 0.952 and 0.363 under different clocks. A clock measures similarity to its reference.
· During gastrulation, rejuvenation is selective for the epiblast lineage.
· That selectivity is the strongest defence against the global methylation objection.

Chapter 6

Human — why it took two years

After the curve was found in mice, two years passed before the same question reached humans. Not because of any biological puzzle. Because there was no ruler for human embryonic data.

In this chapter

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The intersection clock — recut the ruler for each sample 40
Results 41
Sperm, an awkward result 41
Global methylation runs the other way 42
A chain of proxies 42
Another limit — the reported accuracy is not embryonic accuracy 43
What the paper contributes 43
What this chapter leaves you with 44

Paper 03 Evidence B–

Kerepesi C, Gladyshev VN. Intersection clock reveals a rejuvenation event during human embryogenesis. *Aging Cell* 2023;22(10):e13922.
Method plus analysis — peer reviewed (6-page short communication)

Asks Is there a rejuvenation event in human embryos too?

Answers Predicted age is flat before implantation and falls significantly afterwards

The technical barrier

The obstacle was data format.

Almost all human epigenetic clocks were built on methylation arrays — chips that read a fixed set of hundreds of thousands of sites at once. Arrays work well when there is plenty of material.

Human embryonic data do not exist in array form. An embryo has only a handful of cells, so it is read by sequencing. And sequencing data cover different sites in every study.

The authors put it plainly: although many human clocks exist based on methylation arrays, there was no aging clock based on human bisulfite sequencing.

So the paper had to do two jobs at once: build the tool and answer the question with it. That structure is both its strength and its weakness. Because the instrument is new, the reliability of the result cannot be assessed separately from the validity of the instrument.

The intersection clock — recut the ruler for each sample

The conventional fix was to impute missing values. This paper goes the other way.

For each test sample:

1. Find the CpGs shared between the training data and that sample.
2. Restrict both to that intersection.
3. Refit a clock on the restricted training data, producing five models by five-fold cross-validation.
4. Average the five predictions as that sample's epigenetic age.

The guarantee is clear: the test sample has no missing clock sites. No imputation is needed.

So is the cost. **Every sample gets a different clock.** Rather than measuring many samples with one ruler, a new ruler is cut for each sample.

The training data were 182 human peripheral blood samples: 56 individuals who had attempted suicide, 39 with major depressive disorder and 87 healthy controls.

Results

What is reported is direction and p value. Absolute mean predicted ages appear only in figures, not in the text.

Comparison	Result	p
Cleavage → blastocyst	no change	0.7378
Blastocyst → human ES cells	significant decrease	1.582×10^{-12}
Among pre-implantation stages	no change	—
Post-implantation (GW 6–11) vs pre-	significant decrease	—
Fibroblast → iPSC (control)	significant decrease	0.0136
Fibroblast → iPSC (patient)	significant decrease	2.679×10^{-5}
Primed vs naïve pluripotent cells	primed significantly lower	—

The key is the transition. Predicted age is flat through the pre-implantation stages and then drops at the next step. The authors connect this to a rejuvenation event in human embryos and place it near gastrulation.

Sperm, an awkward result

One secondary finding unsettles the story.

Sperm read **lower** than pre-implantation embryos — significantly so in two of three datasets. Metaphase II oocytes did not differ from pre-implantation embryos, but sperm read significantly lower than oocytes.

How should this be taken? It does not fit the simple picture in which rejuvenation begins in the embryo. It opens the possibility that part of the reset has already occurred in the germ cells.

Lay the worm result from Chapter 3 over it and the picture shifts further. The reset may not be an event at a point but a process spanning several stages around fertilization.

The authors themselves say it is not known whether male germ cells are rejuvenated during spermatogenesis.

Global methylation runs the other way

Another observation matters.

Across human development, global CpG methylation falls before implantation and rises afterwards. Predicted age does not change before implantation and falls afterwards.

The directions are exactly opposite.

The authors use this as evidence that the clock reads particular sites rather than the overall level — an answer to the second trap from the interlude.

Caveat · The same observation, inverted

The largest epigenetic event in human development is global demethylation. If a clock is blind to it, then what the clock is picking up becomes unclear: is it aging, or a programme specific to a developmental stage? The drop at the post-implantation stage may reflect not that cells became younger, but that those cells are simply unlike the training data.

A chain of proxies

This is the part to read most carefully.

The inside of a human embryo cannot be measured directly, so the inference passes through several layers of substitution.

To see epiblast → use human embryonic stem cells
To see post-implantation embryo → use liver and chorion from gestational weeks 6–11
Each step has literature support, but stacked together they considerably weaken the directness of the conclusion.

The gestational week 6–11 samples are a particular problem. Human gastrulation occurs about three weeks after fertilization. Liver and chorion at weeks 6–11 are already differentiated organ tissue, well past gastrulation.

So this paper's "near gastrulation" is an inference, not an observation. Chapter 8 returns to the point.

Another limit — the reported accuracy is not embryonic accuracy

Correlations and median errors appear in the figures, giving the impression of an accurate clock.

But those numbers come from cross-validation within the training data: 182 adult blood samples split and tested against themselves.

Since there is no ground truth for embryonic age, accuracy on embryos is unknown. The conclusion rests entirely on differences between stages in the model's output, and there is no guarantee that those differences are proportional to differences in true age.

> **Going further · The hidden cost of the intersection clock**
>
> Comparing mean values measured by different rulers is the paper's central analysis. In measurement theory this is a failure of measurement invariance. The authors stress the benefit — no missing values — and do not discuss the cost.

What the paper contributes

Three things.

First, it moves the question to humans. A phenomenon seen only in mice appears in human data in the same direction.

Second, it carries a methodological lesson. The two years between 2021 and 2023 were a problem of instruments, not of biology. In this field, discovery is a function of measurement.

Third, it adds information about the shape of the curve. Flat before implantation ($p = 0.7378$) and then a sharp fall ($p = 1.582 \times 10^{-12}$) suggests that rejuvenation is a staged transition rather than a gradual process.

What this chapter leaves you with

· There was no usable clock for human embryonic data. Half the work was building one.
· The intersection clock solves missing values but loses comparability between samples.
· Flat before implantation, falling after. The reset looks staged rather than gradual.
· Sperm read younger than embryos. Part of the reset may precede fertilization.
· "Near gastrulation" is inferred, not observed. The actual samples are organ tissue from weeks 6–11.

Chapter 7

Frog — five axes converge

Mice and humans are both mammals. For ground zero to be a vertebrate phenomenon it has to show up on a more distant branch. The frog study attempts that, and brings with it two axes that owe nothing to the clock. It has also not been peer reviewed.

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Five axes land on one moment 47
r-strategy and K-strategy 48
The convergence is not independent 48
The statistical fragility of the arrest argument 49
The most underrated risk — pooling 49
Extrapolation across species and tissue 50
What this chapter leaves you with 50

Paper 05 Evidence C+

Zhang B, Tarkhov AE, Ratzan W, Ying K, Moqri M, Poganik JR, Barre B, Trapp A, Zoller JA, Haghani A, Horvath S, Peshkin L, Gladyshev VN. Epigenetic profiling and incidence of disrupted development point to gastrulation as aging ground zero in *Xenopus laevis*. *bioRxiv* 2022.
Preprint — not peer reviewed

Asks At which developmental stage is ground zero?

Answers In frogs the minimum falls near gastrulation, and developmental arrest clusters there too

Caveat · Stated up front

This is a preprint posted to bioRxiv in August 2022, with "not certified by peer review" stamped on every page. Whether it was later published, and whether its numbers changed if so, could not be confirmed at the time of writing. No figure in this chapter should be read as settled science. It is included because the two independent axes it offers appear nowhere else among the seven papers.

The first amphibian clock

A frog clock had to be built first.

The mammalian methylation array carries 37,554 CpGs. Of those, 1,829 are conserved in the frog genome and 1,068 were usable.

Those 1,068 sites were used to train on 40 adult skin samples aged 2 to 19 years. The final clock uses 185 CpGs and achieved a mean absolute error of 1.82 years — about 10 per cent of the range.

Caveat · A small and skewed window

1,068 sites are a tiny fraction of frog genome methylation. They were selected for conservation, which biases them towards exons; the authors say so. They also report that measured frog methylation was strongly skewed towards hypermethylated regions. The clock operates through a narrow, tilted window.

Five axes land on one moment

The persuasive force of this paper comes from convergence rather than any single measurement. Five different quantities were measured, and all five point to the same moment.

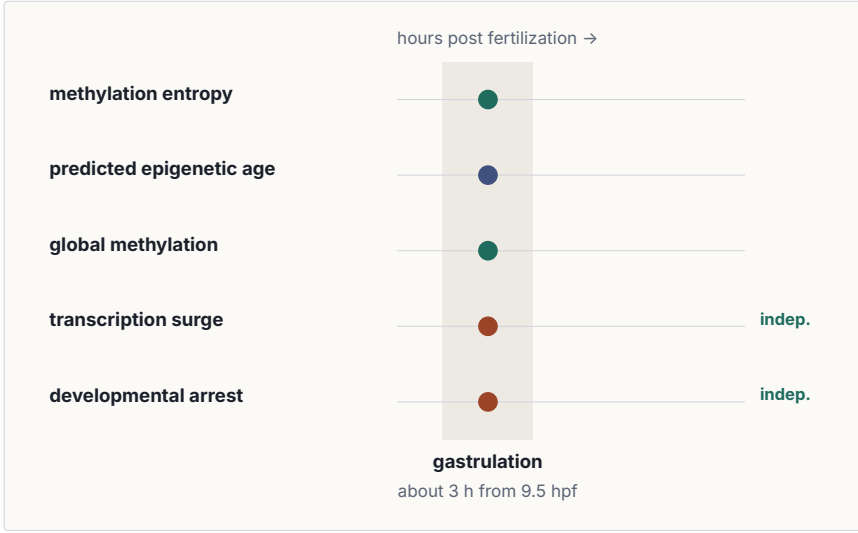

Figure 6: In frog embryos five measurements bend at nearly the same moment. But the top three are computed from the same 1,068 CpGs, so they are not fully independent. Only the transcriptome and the developmental arrest data are independent of the clock, and much of the argument rests on those two.

Axis 1 · predicted epigenetic age

Applying the adult skin clock to embryonic samples, predicted age dropped sharply to a minimum around 10 to 12 hours post fertilization, then rose very gradually. Gastrulation begins at about 9.5 hours and lasts around three hours.

Axis 2 · methylation entropy

The disorder of the methylation pattern also reached its minimum at 10 to 12 hours — the most ordered state.

Axis 3 · global methylation

Genome-wide methylation reaches a plateau at about 10 hours.

Axis 4 · the transcriptome

In public RNA sequencing data, mean expression was lowest just before gastrulation and rose sharply during it. Principal component analysis shows the first component peaking immediately before gastrulation and then collapsing.

The switch from transcriptional quiet to mass transcription coincides with gastrulation.

Axis 5 · developmental arrest

This is the most original part of the paper.

The team followed the individual trajectories of 6,457 embryos. Of the 5,546 that developed normally past the four-cell stage, 104 arrested and died within 24 hours of fertilization.

More than half of those 104 arrested around gastrulation. Aligning each embryo so that the start of gastrulation is time zero, 66 showed a serious abnormality within three hours of that start.

Three abnormalities were common: an aberrant asymmetric division just before gastrulation, failure to form the blastopore ring at its onset, and failure of the ring to constrict during it.

r-strategy and K-strategy

The most interesting part of the authors' interpretation is here.

Mammals are K-strategists: more than half of conceptions never reach maturity. Few offspring, heavy investment, early filtering.

Frogs are r-strategists. Many offspring, little investment. Mortality after the four-cell stage is only about 2 per cent.

And yet what little failure there is clusters at gastrulation. The authors' argument: this stage is not where failure happens but **where failure is not tolerated**. It is the most fundamental process there is — the body plan is set, germ line separates from soma, and the embryo's own transcription begins in earnest.

> **Gastrulation is not where failure occurs. It is where failure is not permitted.**

The convergence is not independent

If the persuasive force comes from convergence, the convergence itself needs checking.

There is a problem. Three of the five axes come from the same data.

Methylation entropy and global methylation are computed from 1,068 CpGs.
Predicted epigenetic age is computed from 185 of those same CpGs.
That the three bend at the same moment is in part the autocorrelation of one dataset.

The authors themselves report significant correlations among global methylation, entropy and epigenetic age.

Only two axes are genuinely independent: the transcriptome and developmental arrest. Neither uses methylation data. The contribution of this paper to the book rests mainly on those two.

The statistical fragility of the arrest argument

The most original axis is also the one to read most carefully.

Caveat · Four problems

Counts without normalization. Death is a cumulative event, yet the paper reports counts per interval rather than a hazard normalized by the population at risk. Gastrulation lasts about three hours, roughly 12.5 per cent of a 24-hour window. That 66 events fall there is striking, but without normalization "disproportionately high" cannot be quantified.

Conditional selection. Time zero is defined as the first observation of a blastopore ring fragment. Only embryos that reached gastrulation can be aligned that way. Embryos that died earlier are systematically excluded, which can tilt the distribution towards gastrulation.

Observer scoring. Arrest was judged by a "visible aberrant pattern" or absence of morphological change. There is room for subjectivity, and the authors concede the resolution limit of imaging every 15 minutes.

Small numbers. 104 events. Temperature and batch differences across seven trials are said merely to have been "mitigated" by normalization.

The most underrated risk — pooling

One sentence in the methods matters. Samples were pooled to approximately match total DNA, and the number of embryos per pool decreased as development proceeded.

So developmental stage and pool composition are systematically linked. Early stages pooled many embryos; later stages pooled few.

Why does that matter? Pooling many individuals averages away between-individual variation and makes the pattern look more "ordered." The entropy minimum could be a function of the pooling design rather than of biology.

In this book's view this is the most underrated methodological risk in the preprint.

Extrapolation across species and tissue

One last structural problem. The clock was trained on adult skin. It was applied to whole embryos. The time unit is hours, not years.

The age-related methylation pattern of a skin fibroblast and the methylation programme of a gastrulating whole embryo are entirely different biology. This is extrapolation far outside the training range, and the text offers no quantitative discussion of what unit or sign the embryonic predictions carry.

"Predicted age is low" is a claim about the relative shape of a curve, not about absolute values.

What this chapter leaves you with

· The first amphibian epigenetic clock: 185 CpGs, mean absolute error 1.82 years.
· Predicted age, methylation entropy, global methylation, transcription and developmental arrest all point near gastrulation.
· But the first three come from the same data. Only two axes are truly independent.
· The arrest argument has normalization and conditional-selection problems.
· Pooling design is confounded with stage. This is the most underrated risk.
· The paper has not been peer reviewed.

Part III

The reversal

If the curve is real, a conclusion follows. Aging does not begin with being old. It begins at the stage we were taught is the most important event of our lives. And the reset does not reach every cell.

Chapter 8

Gastrulation as a coordinate

Four papers point at the same period. Look closely and each points somewhere slightly different — and in the human case what is pointed at is an inference rather than an observation. This chapter puts that mismatch in plain view.

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Where the four papers point 53
The shape of the curve differs too 54
A point, a window, or a process? 55
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What gastrulation is

A zygote divides repeatedly into a hollow ball of cells. At that stage there is no up or down, no front or back, no inside or outside.

Gastrulation is the folding-in of one side of that ball to create layers. The fold produces three germ layers: ectoderm, mesoderm and endoderm. Skin and nervous system come from ectoderm; muscle, bone and heart from mesoderm; gut and lungs from endoderm.

Gastrulation is where the plan of the body is first written into space. That is why Wolpert ranked it above birth, marriage and death: if it goes wrong, everything after it goes wrong.

And it is not the only thing happening then.

· The three germ layers form and the body axes are fixed.
· The germ line separates from the somatic lineage.
· The embryo stops living on maternal RNA and protein and begins using its own genome in earnest.
· The capacity to rebuild the whole — embryonic plasticity — starts to disappear.

Part of the appeal of the ground zero hypothesis is that it adds one more line to this list: aging begins.

Where the four papers point

The difficulty is that they do not point at precisely the same place.

Paper	Point indicated	Nature of the evidence
01 (mouse)	E6.5–E7.5, range E4.5–E10.5	aggregated minima across clocks
04 (mouse, single cell)	decline across E4.5 → E7.5	an interval of decline, not a minimum
03 (human)	post-implantation, “near gastrulation”	inferred; samples are GW 6–11
05 (frog)	10–12 hours post fertilization	just after gastrulation begins

Mouse and frog actually measured material from the relevant period. The human work did not.

Caveat · Why the human "near gastrulation" is inferred

Human gastrulation occurs about three weeks after fertilization. The post-implantation samples used in paper 03 are liver and chorion from gestational weeks 6 to 11. Gestational age is counted from the last menstrual period, so that corresponds to roughly 4 to 9 weeks post fertilization. Either way it is well past gastrulation, and the material is already differentiated organ tissue.

What paper 03 observed is that values are flat before implantation and lower at some point afterwards. That the point is gastrulation comes from analogy with the mouse.

The shape of the curve differs too

There is a deeper difference.

In the mouse, predicted age **falls** from zygote to E6.5–E7.5. A descent is observed.

In the human, the pre-implantation stretch from zygote to blastocyst is **flat**, with a p value of 0.7378. No change. The fall appears at the next step.

Both are called a rejuvenation event, but the intervals in which it was observed are not the same. The descending limb in the mouse and the descending limb in the human are not developmentally the same place.

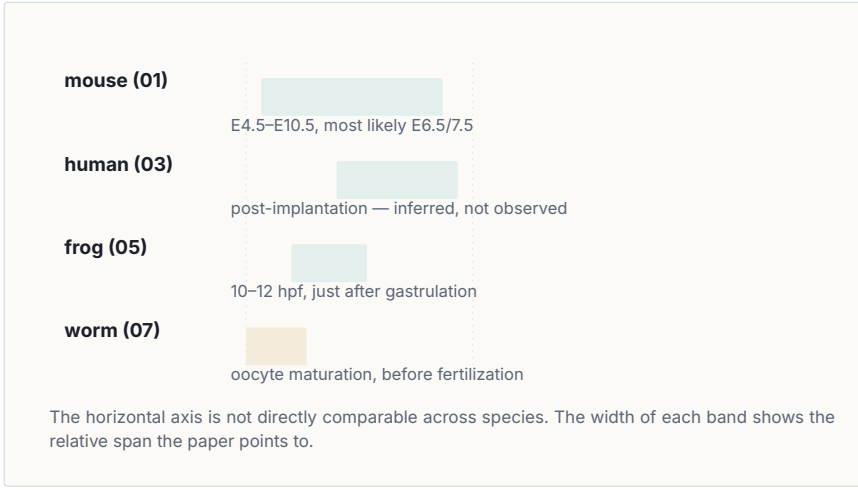

Figure 7: Each species points to a different minimum. That they do not converge on a single point is itself an argument for treating ground zero as a window rather than an instant. The paper that first proposed the model left room for "a period" as well.

A point, a window, or a process?

This raises a basic question. Is ground zero a point?

Lay the evidence side by side and "point" is hard to sustain.

02 (the model) left open from the start that ground zero might be a period rather than an instant.
01 (mouse) gives a range from E4.5 to E10.5, with four of six estimates at E6.5–E7.5.
04 (single cell) shows not a minimum but an interval of decline from E4.5 to E7.5.
05 (frog) names 10–12 hours but wavers in the text between "about 10 hours" and "around the gastrula stage."
07 (worm) shows clearance already underway before fertilization, during oocyte maturation.
03 (human) reports sperm reading younger than embryos.

Taken together the picture becomes this. Rejuvenation is less an event at a point than a process spanning several stages, from oocyte maturation to

gastrulation. And the end of that process — the turn from falling to rising — sits near gastrulation.

Ground zero is a window, not a point. That is the description the data support.

Why does the turn happen there?

Explanations remain at the level of hypothesis, but two are plausible.

Hypothesis one · transcription starts

Around gastrulation the embryo stops relying on what the mother packed in and begins running its own genome in earnest. The surge in transcription in the frog data is that event.

Once metabolism begins in earnest, byproducts begin too. Under the model's fourth proposition — aging as the purposeless byproduct of metabolism — it is natural that aging should start here.

Hypothesis two · plasticity is lost

Before this point the embryo can rebuild the whole. Remove some cells and the rest compensate. Split it and each half becomes a complete animal.

After gastrulation that capacity is gone. Each cell has a fate, and damage that occurs stays where it occurs.

Ground zero, on this reading, is the last moment at which damage can still be erased.

> **Going further · Are these testable?**
>
> Partly. One could delay or advance the onset of transcription pharmacologically and ask whether the minimum of the age curve moves with it. The difficulty is that interfering with transcription onset wrecks development itself, which makes the effects hard to separate. No such experiment has been reported.

A clue from the methylation enzymes

The published version of paper 01 added two sections of mechanistic experiments absent from the preprint. They are the closest anything in this literature gets to a mechanism, so they are worth noting.

E6.5 embryos lacking all three enzymes that remove methyl groups showed no difference in epigenetic age from controls. Embryos at E8.5 lacking the enzymes that add methyl groups showed increased age under five of six clocks.

The implication is that rejuvenation may depend not on **erasing** methyl marks but on **rewriting** them.

That runs against intuition. The natural picture is "wipe the marks to reset." The data suggest that wiping and then rewriting accurately is the operative part.

Caveat · Do not overstate this

Embryos lacking these enzymes are themselves developmentally delayed, so delay and apparent age increase are confounded. This is not "the cause of rejuvenation has been found." The authors are appropriately careful.

What this chapter leaves you with

· Gastrulation forms the germ layers, fixes the body axes and separates germ line from soma.
· All four papers point at this period, but not at precisely the same place.
· The human "near gastrulation" is an inference from the mouse, not an observation.
· The mouse shows a descent; the human pre-implantation stretch is flat. The curves differ in shape.
· The description the data support is a window, not a point.
· Rejuvenation may depend on rewriting methyl marks rather than erasing them.

Chapter 9

Who gets younger

If rejuvenation reached every cell of the embryo uniformly, it would probably be a byproduct of the developmental programme. The data say otherwise. Within one embryo some cells get younger and some do not. Where that line falls is the subject of this chapter.

In this chapter

One embryo, divided fates 59
Why this matters 59
Selection and reset share a window 60
Why that particular line? 61
Alternative readings 61
Two kinds of asymmetry 62
What this chapter leaves you with 62

One embryo, divided fates

Return to the result from Chapter 5.

Across 495 mouse embryonic cells from E4.5 to E7.5, mean predicted age fell. So far this repeats the bulk finding.

Sorting by lineage split the picture.

Lineage	What it becomes	Predicted age
Epiblast	the entire body of the embryo	falls strongly, to near zero or below
Extraembryonic ectoderm	part of the placenta	stays significantly higher
Visceral endoderm	yolk sac and related structures	stays significantly higher

Same moment, same embryo, same maternal environment. Only the cells destined to become the body got younger.

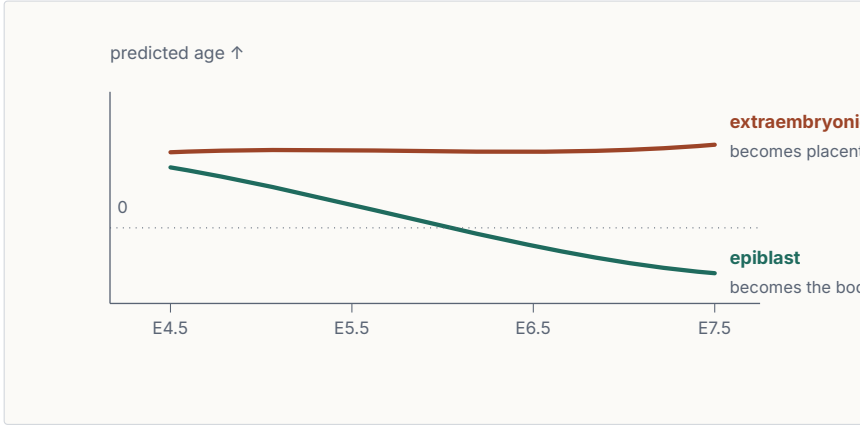

Figure 8: At single-cell resolution, rejuvenation does not happen in every cell of the embryo. Predicted age falls only in the lineage that will form the body; the lineage headed for the placenta holds a higher value at the same stage. This is hard to explain by global methylation alone.

Why this matters

Recall the second trap from the interlude: that the developmental age curve may be a restatement of the global methylation wave.

The objection is strong. Predicted age correlates with mean global methylation at roughly −0.71 to −0.83. Methylation swings dramatically through development, and a clock's output is a weighted sum of methylation values, so it would follow along.

Lineage selectivity is not handled by that explanation.

> Global demethylation and remethylation happen across the whole embryo.
> So why does age fall in only one lineage?
> "Because of global methylation" cannot answer that question.

This is why the result ranks among the firmest support the hypothesis has. It suggests the clock is reading something more specific than the shadow of a global level.

> **Caveat · What "firmest" means here**
>
> One dataset, one analysis, no independent replication. "Firmest" is a relative judgement within these seven papers, not a statement that the result is established. That is why the claim is graded B– in Chapter 13.

Selection and reset share a window

Move to the frog data and a different kind of selection appears.

Of 6,457 embryos followed, 104 arrested, more than half of them around gastrulation; 66 showed serious abnormalities within three hours of its onset.

Two things are happening in the same period.

> **Selection among cells.** Only the lineage that will form the body is rejuvenated. The rest are excluded.
> **Selection among individuals.** Embryos that cannot complete gastrulation are removed.

Recall the three answers from Chapter 1. Option (b), that damaged germ cells are filtered out, and option (c), that age is wound back, were never exclusive. Here is why. Filtering and resetting happen in the same window.

Why that particular line?

The epiblast becomes the body; the extraembryonic tissues become the placenta and yolk sac. The placenta is discarded at birth.

An economic explanation suggests itself. Rejuvenation costs something. There is no reason to spend it on tissue that will be thrown away in nine months.

Lay the worm result from Chapter 3 over this and the pattern sharpens. In the worm, too, clearance does not occur in every cell. It occurs in the proximal oocyte that received the maturation signal.

> **Rejuvenation is expensive. It is spent only on the cells that will carry the lineage forward.**

Plausible — but not demonstrated. None of the seven papers measured the metabolic cost of rejuvenation.

Alternative readings

Lineage selectivity also admits a less dramatic explanation.

It may be a difference of state

Epiblast and extraembryonic tissue are quite different cell states. Their methylation landscapes differ, their expressed genes differ, their division rates differ.

A clock is a function that reads a methylation pattern and returns an age. If two populations have different patterns, the outputs differ. Is that difference an age difference, or a difference of cell state?

The comparison of primed and naïve pluripotent cells in Chapter 6 carries the same problem. They are distinct cells grown in different conditions, and whether the clock difference is "rejuvenation" or "state" is unresolved.

It may be distance from the reference

Recall 0.952 and 0.363 from Chapter 5. What a clock measures is similarity to its reference.

Extraembryonic tissue resembles no adult tissue. The epiblast is the ancestor of every adult tissue. So a lower value for the epiblast may reflect distance from the reference rather than a difference in biological age.

Caveat · Can this objection be closed?

Not at present. Closing it would require a measure of age independent of methylation that can be applied to embryonic cells, and none exists. If someone measures protein damage or a functional readout lineage by lineage, the argument will move. For now the defensible statement is that lineage selectivity is not explained by global methylation.

Two kinds of asymmetry

Put this chapter together with Chapter 3 and a pattern appears. The way life stays young is not by repairing everything. It is by **discarding asymmetrically.**

Worm oocyte	aggregates are cleared in the oocyte that received the signal; those without it keep them
Mouse embryo	only the lineage that becomes the body is rejuvenated; the placental lineage is excluded
Frog embryo	individuals that cannot pass gastrulation are removed

The same structure at three scales. Nothing is saved wholesale. What will continue is selected; the rest is let go.

What this chapter leaves you with

· In mouse embryos rejuvenation is selective for the epiblast. Extraembryonic lineages are excluded.
· That selectivity is not explained by the global methylation objection. It is the firmest evidence here.
· Selection among individuals (developmental arrest) peaks in the same window. Filtering and resetting coincide.
· Whether the lineage difference in clock values is an age difference or a state difference remains open.
· At all three scales the strategy is the same: do not repair everything, select what continues.

Chapter 10

Imitating ground zero in the lab

Can what nature does every generation be reproduced in a dish? Leave the Yamanaka factors on to the end and a cell becomes pluripotent and loses its identity. What happens if you switch them off halfway? A 2022 study answered that question.

In this chapter

The three phases of reprogramming 64
MPTR — switching off in the middle 64
Results — they came back, and they were younger 65
But not everything came back 66
Longer is not better 66
Limits — how to read “30 years” 67
Why the paper matters anyway 68
What this chapter leaves you with 69

Paper 06 Evidence B

Gill D, Parry A, Santos F, Okkenhaug H, Todd CD, Hernando-Herraez I, Stubbs TM, Milagre I, Reik W. Multi-omic rejuvenation of human cells by maturation phase transient reprogramming. *eLife* 2022;11:e71624.
Cell culture study — peer reviewed

Asks Can somatic cells be made younger while keeping their identity?

Answers In cultured fibroblasts, transcriptomic and epigenetic age fell by roughly 30 years with identity restored

The three phases of reprogramming

Introduce the Yamanaka factors and reprogramming begins. In this system it takes about 50 days to reach full pluripotency.

The process has three phases. In the initiation phase the original identity genes switch off; in the maturation phase the cell passes through intermediate states; in the stabilization phase pluripotency is locked in.

The team asked where the age drop occurs. Tracking the whole process, DNA methylation age fell by about 20 years by day 10 and about 40 years by day 17.

Rejuvenation is concentrated in the maturation phase. So pass through that window and come back.

MPTR — switching off in the middle

The strategy is called maturation phase transient reprogramming.

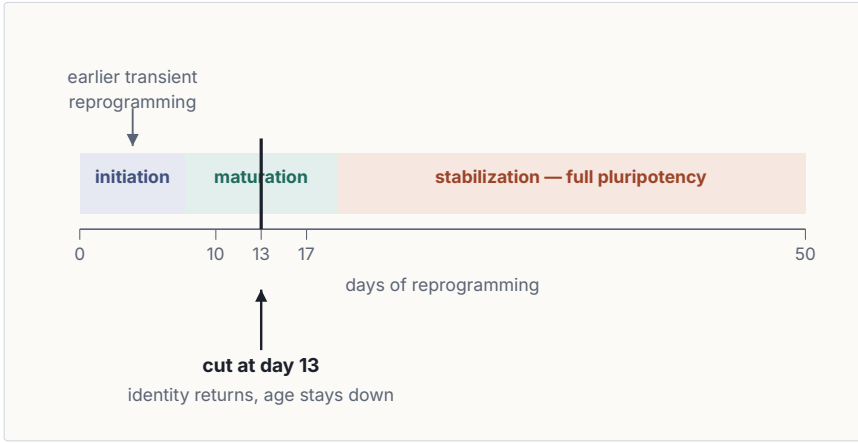

Figure 9: Full reprogramming takes about 50 days. MPTR cuts it off at day 13, in the middle of the maturation phase. The counterintuitive finding is that pushing longer did not make cells younger.

The method is simple. The Yamanaka factors are placed under doxycycline control, switched on for 10, 13, 15 or 17 days, then switched off. Cells are then cultured for four to five weeks to see what they become.

About 25 per cent of cells made the intermediate transition successfully and about 35 per cent failed. The donors were aged 38, 53 and 53.

Results — they came back, and they were younger

Identity was restored

Cells rounded up in the intermediate state and returned to fibroblast morphology after the factors were withdrawn. The roundness index after return did not differ significantly from before treatment.

In transcriptomic principal component analysis the intermediate state sits partway along the dedifferentiation trajectory and returns to the starting point afterwards. Fibroblast markers were retained; pluripotency markers rose transiently and were not expressed at the end.

Age came down

Three independent estimates converged.

Method	Estimated drop	Notes
PCA on age-associated genes	about 40 years	3,707 genes
New transcriptomic clock	about 30 years	mean absolute error 12.57 years
Second clock, refitted	about 20 years	29 genes
Horvath epigenetic clock	about 30 years	13-day treatment

This is where “about 30 years” comes from.

Some function recovered

Fibroblasts produce less collagen with age. After 13 days of treatment, collagen I and IV protein levels moved towards those of young donors. In a wound-healing assay the median migration speed improved.

But not everything came back

This is the most important passage in the paper.

> **Collagen** — at the mRNA level collagen I did not reach significance; at the protein level both improved.
> **Migration speed** — the median improved, but responses varied by sample. Some improved, some did not. And the response did not correlate with the transcriptomic or methylation clocks.
> **Telomeres** — unchanged, or slightly shorter.

The last item matters most. The clock says 30 years younger. The telomeres say nothing changed.

This is the experimental confirmation of the model’s third proposition from Chapter 2: aging and rejuvenation are not the same road run backwards. Aging proceeds along several axes at once, and rejuvenation reverses only some of them.

The clock being 30 years younger and the cell being 30 years younger are different sentences.

Longer is not better

One more counterintuitive result.

The extent of rejuvenation was not proportional to treatment length. By transcriptomic measures 10 or 13 days was best; by epigenetic measures 13 days was best. At 15 and 17 days the effect was smaller.

The authors call this a sweet spot, and suggest that longer reprogramming makes reversion harder and that cellular stress may let some cells "re-age."

The implication is that there is no simple dose-response relationship here. That is a real constraint on any therapeutic strategy built on this.

Limits — how to read "30 years"

Six criticisms must travel with any citation of this paper.

Caveat · The clock was refitted for this comparison

Existing transcriptomic clocks could not even predict the ages of the study's own control samples. So external data were imported, batch-corrected, and a new random forest clock trained. The problem is that the same external data were also used for the PCA that produced the "40 years" estimate. The measuring instrument and the comparison standard share a data lineage.

That the two refitted clocks give 30 and 20 years is itself a measure of the uncertainty. The authors concede that estimates range from 20 to 40 years.

Caveat · The error is 40 per cent of the effect

The transcriptomic clock has a mean absolute error of 12.57 years, and the claimed effect is 30 years. Treating "30 years" as a settled figure when the error is 40 per cent of it is not appropriate.

Caveat · Significance hangs on one outlier

The p value for the epigenetic clock result is given as 0.0238, with 0.257 in parentheses. The caption explains that these are the values excluding and including an outlier. Significance therefore turns on how one data point is handled.

Moreover "13 days is optimal" was chosen after comparing four conditions, with no multiple-comparison correction and no pre-registration. It should be treated as a hypothesis awaiting replication.

Caveat · This happened in a dish

There is no in vivo validation, no effect on lifespan or healthspan, no tissue-level functional recovery. Identity restoration was shown by population-level analysis and markers, not by lineage tracing that proves the returning cells were the original ones. Nothing excludes the possibility that cells which failed to reprogram had a proliferative advantage.

Caveat · Tumour risk remains

The authors are candid. They found no pluripotency signature in the transcriptome or methylome, but cannot exclude that a minority of cells retain pluripotent-like properties and could form teratomas on transplantation. The cassette used includes c-Myc, a known oncogene.

Caveat · Samples and interests

Three donors, two of them the same age. With no young donor given the same treatment, it is unknown whether the size of the effect scales with starting age.

One co-author is the chief executive and a shareholder of an epigenetic clock company; the corresponding author is a consultant and shareholder in another and is affiliated with a longevity company. Readers of a clock-based conclusion are entitled to know this.

Why the paper matters anyway

Rejuvenation can be separated from pluripotency. That is this paper's central contribution.

Until then, "reversing age" meant turning a cell into a pluripotent stem cell, which meant discarding its identity. A cell that is no longer a skin cell is of no use as a skin cell.

MPTR showed the two can be prised apart: there is a window in which the rejuvenation is gained and the pluripotency avoided.

The authors themselves connect the result to development. Citing paper 01's finding that epigenetic rejuvenation during embryogenesis coincides with de novo methylation of the genome, they suggest that part of the MPTR mechanism may overlap with the developmental one.

Set beside the methylation-enzyme results from Chapter 8, this opens the possibility that nature's reset and the laboratory's use the same molecular machinery. For now that is a similarity of phenomena, not a demonstrated identity of mechanism.

What this chapter leaves you with

· Switching the Yamanaka factors off during the maturation phase lowers age markers while identity returns.
· Three estimates converge on a range of roughly 20 to 40 years. "30" is the middle of it.
· Collagen recovered, migration partly, telomeres not at all. The axes behave differently.
· Longer treatment did not give more rejuvenation. There is a sweet spot.
· The clock was refitted for the comparison, and significance turns on one outlier.
· This is a proof of concept in a dish, with no in vivo test and no assessment of tumour risk.

Part IV

The limits

This part decides whether the book can be trusted. What exactly has been called “age” so far, and how much of the conclusion was manufactured by the instrument that produced it. A claim that collapses is better collapsed here than later.

Chapter 11

Five different ages

The word “age” has appeared many times so far. It has referred to at least five different things. A good share of the disputes in this field come from not keeping them apart.

In this chapter

One word, several objects 72
Rewriting the disputes in these terms 73
Why paper 06 is special 73
How to write a sentence 74
So what is biological age? 75
What this chapter leaves you with 75

One word, several objects

Lay the seven papers side by side and track what "age" refers to each time it appears, and five things emerge.

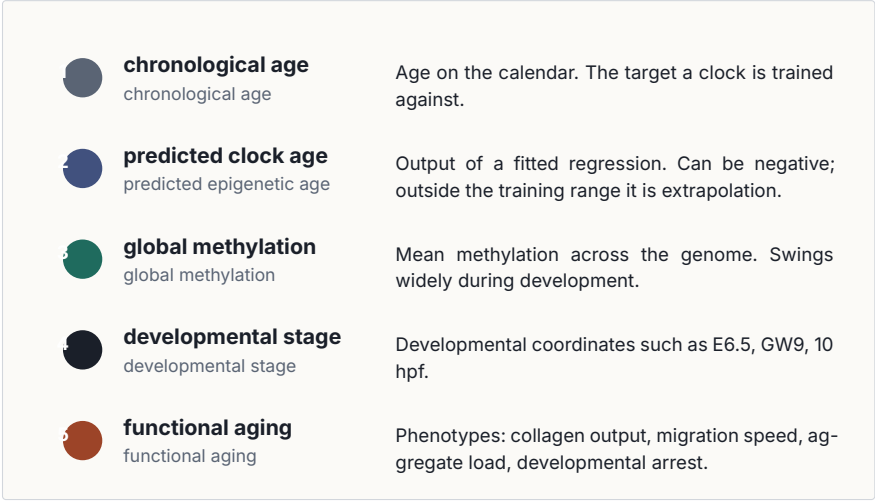

Figure 10: Most disputes in this field come from not separating five different things all called "age." Marking which one a sentence refers to is what separates a careful claim from an overstated one.

One · chronological age

Age on the calendar. Days post fertilization, years since birth. This is the target a clock is trained to hit.

It is the only one of the five that is unambiguous, which is why it serves as the reference point.

Two · predicted clock age

The output of a fitted regression. Put methylation values in, get a number out.

It can be negative. Outside the training range it is extrapolation. Different models give different values — 0.952 and 0.363 in Chapter 5 are the example.

Almost every "rejuvenation" result in this book concerns this second age.

Three · global methylation level

Mean methylation across the genome. It swings widely through development, collapsing after fertilization and rising again around implantation.

A clock's output is a weighted sum of methylation values, so when the overall level moves, the output moves. That is why two and three are hard to separate.

Four · developmental stage
Coordinates like E6.5, gestational week 9, 10 hours post fertilization. More a position than an age.

Five · functional aging
Collagen output, migration speed, aggregate load, incidence of developmental arrest. This is the closest to what we mean colloquially by "old."

Rewriting the disputes in these terms

Apply the distinction and the earlier arguments sort themselves out.

Paper	What it called "rejuvenation"	Which age
01 (mouse)	a fall in predicted clock values	2
03 (human)	a fall in predicted clock values	2
05 (frog)	a fall in predicted clock values	2
04 (single cell)	a fall in predicted clock values, split by lineage	2
06 (MPTR)	a fall in clock values plus partial functional recovery	2, partly 5
07 (worm)	removal of protein aggregates	5

The criticisms sort themselves out too.

"It is a byproduct of global methylation" — the claim that two is a proxy for three.
"It measures similarity to a reference" — the claim that two is not measuring biological age at all.
"It is a developmental programme, not aging" — the claim that changes in two are a function of four.

All three are demands not to read two as five.

Why paper 06 is special

Seen this way, it becomes clear why the MPTR study matters.

It is the only one that measured two and five in the same paper.

And they did not agree. The clock says 30 years younger; collagen recovered; migration recovered partly; telomeres did not move. On top of that, the degree of migration improvement did not correlate with clock values.

> **In the same cells, age two and age five give different answers. This is the most honest fact the field has.**

If two were a faithful proxy for five, the two should move together. That they do not means one of two things. Either the clock sees only part of aging, or rejuvenation reverses only part of aging.

At present both are probably true.

How to write a sentence

Keep the distinction and the sentences that can be written separate cleanly from those that cannot.

Defensible	To avoid
In mouse embryos the epigenetic clock returned its lowest predicted value at E6.5	The mouse was youngest at E6.5
In human embryonic data predicted values fell significantly at post-implantation stages	Human rejuvenation has been demonstrated
In cultured fibroblasts clock values were lowered by about 30 years	A treatment that reverses aging has been developed
A minimum was observed near gastrulation in frogs (preprint)	It has been confirmed in all vertebrates
In *C. elegans* an oocyte maturation signal triggers aggregate clearance	The mechanism of immortality has been found

The principle is single: separate **what was measured** from **what is being claimed**. Keep that separation and every sentence in the book is defensible.

> **Going further · Why this is not mere pedantry**
>
> Distinguishing the measured object from the inferred object is a practical matter, for two reasons.
>
> First, falsifiability changes. "The predicted value fell" can be confirmed or contradicted with another clock. "It got younger" offers no obvious handle.

> Second, the next experiment changes. Write the first sentence and you go on to ask whether other markers agree. Write the second and the question disappears. The telomere result in Chapter 10 exists because somebody asked the first question.

So what is biological age?

At this point a larger question appears. Which of the five is the real one?

The honest answer is that it has not been settled.

Biological age is a concept that depends on an operational definition. It can be defined by mortality risk, by functional decline, or by accumulated molecular damage. Each definition yields a different measurement, and each measurement a different conclusion.

Epigenetic clocks are used widely not because they define biological age but because they are easy to measure and correlate with mortality. Being a useful instrument and being a correct definition are different things.

Caveat · This book's position

This book does not answer the question of what biological age is. Instead it states what each paper measured and asks how far that measurement can carry. A surprising amount can be done without settling the definition.

What this chapter leaves you with

· "Age" refers to at least five things: chronological age, clock output, global methylation, developmental stage, functional aging.
· Nearly every rejuvenation result here concerns the second.
· The major criticisms all amount to: do not read the second as the fifth.
· The MPTR study measured both, and they disagreed.
· Biological age has no settled definition. Separating measurement from claim still allows the discussion to proceed.

Chapter 12

The instrument makes the conclusion

Five of the seven papers had to build a new clock. No tool, no finding. But what happens when the people who proposed a hypothesis build the instrument that tests it?

In this chapter

Five tools, five costs 77
Circularity — extrapolating past the training range 77
Reference dependence — the sharpest evidence 78
Global methylation as a confounder 79
Sample sizes 79
Choosing after the fact 80
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What this chapter leaves you with 81

Five tools, five costs

Looking back at Part II, each paper built an instrument to match the thing it wanted to measure. Each instrument required a new statistical device, and each device had a price.

Paper	Tool built	Price paid
01	rDNA clock	trained on adults, applied to embryos; batch effect conceded
04	single-cell clock	reference dependence; parameters set on the evaluation set
03	intersection clock	a different ruler per sample; comparability lost
05	amphibian array clock	a 1,068-CpG window; extrapolation across species and tissue
06	refitted transcriptomic clock	instrument and standard share a data lineage

The table is itself a conclusion. **Every "age" in this field is a position in a particular coordinate system.** Change the system and the value changes.

Circularity — extrapolating past the training range

Start with the structural problem.

Nearly every clock in this book was trained on adult tissue.

> 01 — trained on mouse adult tissues (1.7–21.3 months) → applied to zygotes, blastocysts, E6.5 embryos
> 03 — trained on 182 human adult blood samples → applied to human zygotes, blastocysts, ES cells
> 05 — trained on frog adult skin (2–19 years) → applied to whole embryos (5–31.5 hours post fertilization)
> 04 — trained on mouse adult bulk tissue → applied to single cells and gastrulating embryos

A regression returns values outside its training range; extending a line is trivial. Whether the value is right cannot be checked.

There is no independent standard for "the embryo's true biological age." When a clock reads low, there is no way to tell whether the state really is young or simply unlike adult tissue.

Negative ages come from here. scAge searches down to −20 months. A negative age is not a biological state called "before birth"; it is a line extended past its data.

> **Going further · Do the authors know?**
>
> They do. The published version of paper 01 adds a sentence acknowledging that clocks trained and validated on blood were used to analyse early embryos. Paper 04 notes negative predictions and out-of-range extrapolation in the text. Paper 05 avoids quantitative discussion of what its embryonic predictions mean.
>
> This is not a problem they are unaware of; it is a problem without an alternative. The point of the criticism is not that these people are wrong but that it is worth asking how far a conclusion obtained under these conditions extends.

Reference dependence — the sharpest evidence

Here again are the two most important numbers in the book.

> **The same 19 cells. 0.952 under a liver clock, 0.363 under a blood clock.**

Same data, same cells, different reference.

What makes the comparison special is that the control is perfect. Everything is held constant except the reference tissue, and the result flips from a strong significant correlation to no correlation at all.

Two readings follow.

(a) The clock measures an age intrinsic to the cell, and the blood clock is simply an inaccurate instrument for hepatocytes.
(b) The clock measures how closely this cell resembles bulk patterns of a given tissue at a given age, and hepatocytes resemble liver.

For (a) to hold, "an age intrinsic to the cell" would have to be independently defined. No such definition exists.

This book takes (b) to fit the present data better. That does not make clocks useless — measuring distance from a reference is a useful measurement. But calling that value an "age" is where the meaning slips.

Global methylation as a confounder

Three papers face the same charge.

01	the authors themselves propose that rejuvenation may be mediated by remethylation; the global remethylation window from E3.5 to E7.5 overlaps the observed rejuvenation window
04	predicted age correlates with mean global methylation at −0.71 to −0.83
05	entropy, global methylation and predicted age all derive from the same set of 1,068 CpGs

In all three cases the possibility that the "age curve" restates the global methylation wave cannot be excluded.

Only two pieces of evidence push back.

Lineage selectivity in 04. If the global change happens across the whole embryo, why does age fall only in the epiblast?
The independent axes in 05. The transcriptional surge and the clustering of developmental arrest use no methylation data.

The claim that rejuvenation is not a byproduct of global demethylation rests on these two. Both are single studies without independent replication.

Sample sizes

A quiet problem that matters.

One of the datasets in paper 01 has a single sample per developmental stage: one zygote, one two-cell, one eight-cell. One of the key comparisons is two against two.

The clock in paper 05 was trained on 40 adult skin samples and its performance reported by bootstrapping those same 40. The arrest analysis rests on 104 events.

Paper 06 used three donors, two of them the same age.

Caveat · What significance from a small sample means

Small samples make significance hard to obtain, so a significant result from one is often read as evidence of a large effect. But small samples also make chance extremes more likely to clear the threshold, and effect sizes obtained that way are systematically overestimated. This is called the winner's curse.

It is why this book reports n every time. A comparison with n = 2 and one with n = 205 do not carry the same weight at the same p value.

Choosing after the fact

Two papers show the pattern of comparing several conditions and reporting the best.

Paper 04 chose how many of the top CpGs to use after seeing benchmark results, and the benchmark used the data on which performance is reported. With no cross-validation, 0.952 may be optimistic.

Paper 06 compared 10, 13, 15 and 17 days and selected 13 as optimal, with no multiple-comparison correction and no pre-registration.

This is not misconduct. It is the normal course of exploratory research. But exploratory results should not be read as confirmatory ones. They are hypotheses awaiting replication.

So what survives?

Reading this chapter and concluding that nothing can be believed would be the wrong move. Things survive.

Consistency of direction. Different clocks, different species, different laboratories produced curves pointing the same way. If each tool is biased in its own fashion, the chance that the biases happen to agree is low.

Two axes independent of any clock. The frog transcriptional surge and the clustering of developmental arrest use no methylation data.

Lineage selectivity. A residual that the global methylation account does not handle.

What does not survive is equally clear: the precise location of ground zero, the absolute magnitude of the rejuvenation, direct observation in humans, and the ontological claim that this is the point of minimum biological age.

What this chapter leaves you with

· Five of the seven papers had to build new instruments, and each instrument cost something.
· Nearly every clock was trained on adults and applied to embryos, with no independent standard to check it.
· The same cells score 0.952 and 0.363 depending on the reference. A clock measures distance from its reference.
· Only two pieces of evidence hold off the global methylation confounder.
· Samples are small and selections were made after the fact. These are exploratory results.
· What survives is the consistency of direction. That is the real basis of the hypothesis today.

Chapter 13

How far can each claim go?

What is left after the criticisms of the last two chapters. Each claim gets a grade, with the basis for the grade and the strongest objection to it set alongside. This chapter is the book's balance sheet.

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How the grades work

Claims are graded A to D on four criteria.

Peer review — reviewed, preprint, or opinion without data.
Independent data — how many separate laboratories and datasets.
Reproducibility — has another group obtained the same result.
Size of the objection — does a live alternative explanation remain.

These grades are editorial judgements, not an official standard, and another assessor might grade differently. The reasoning beneath each grade is worth more than the letter.

Nine claims and their grades

No.	Claim	Basis	Grade
1	Predicted epigenetic age falls in early mouse embryos	01, 04	B+
2	Human embryos show the same fall	03	B−
3	Frogs show a minimum near gastrulation	05	C+
4	Ground zero exists and marks the onset of aging	02 claims it, 01·03·05 indirect	B−
5	Age can be measured in single cells	04	B+
6	Somatic cells can be made younger while keeping identity	06	B
7	Germline immortality depends on a clearance cycle	07	B
8	Rejuvenation is lineage-selective	04	B−
9	Pluripotent cells do not age with passage	01, 03	B

Claim by claim

Claim 1 · age falls in mouse embryos B+

The highest-graded claim in the book. Five datasets, five or more clocks pointing the same way, and the result repeated at single-cell resolution.

Caveat · Objections remaining

The structural problem of extrapolating an adult clock persists. The authors concede a batch effect where datasets were spliced. Sample sizes per stage are small. The absolute magnitude of the fall is never reported.

Claim 2 · humans show the same fall B–

Peer reviewed, with extremely small p values. The grade is low because of how the measurement is constructed.

Caveat · Objections remaining

A clock trained on 182 adult blood samples was applied to embryos. A different clock per sample means comparability between samples is not guaranteed. The post-implantation samples are differentiated organ tissue from gestational weeks 6–11, not epiblast. The reported accuracy is cross-validation within the training set, not accuracy on embryos.

Claim 3 · frogs show a minimum near gastrulation C+

A preprint cannot be graded above C. It is raised to C+ because it supplies two axes that owe nothing to the clock.

Caveat · Objections remaining

Not peer reviewed. A narrow, skewed 1,068-CpG window. Trained on adult skin, applied to whole embryos. Pooling design confounded with developmental stage. The arrest analysis lacks normalization and has a conditional-selection problem.

Claim 4 · ground zero exists and marks the onset of aging B–

The book's central claim, and the one to handle most carefully.

Directional evidence agrees across species. But the step from "a minimum exists" to "aging begins there" has no direct support. "Begins" implies that nothing was aging before, and showing that would require independently confirming that aging markers do not increase before the minimum.

Caveat · Objections remaining

The definition of ground zero is itself unsettled — point, interval or process differs between papers. Paper 02 has no data, and the support from 01, 03 and 05 is indirect.

Claim 5 · age can be measured in single cells B+

A methodological claim with a clear benchmark. A median absolute error of 2.1 months in hepatocytes is comparable to bulk clocks.

Caveat · Objections remaining

Cell types without reference data are out of reach. Low coverage destabilizes predictions, and the cells removed by the quality filter may not be a random subset. Parameters were set on the evaluation set. Mouse data only.

Claim 6 · somatic cells can be rejuvenated while keeping identity B

Multiple omics converge, and some functional readouts are included. But the conditions are many.

Caveat · Objections remaining

The clock was refitted for the comparison and estimates range from 20 to 40 years. The clock error is 40 per cent of the effect. Significance turns on how one outlier is handled. Three donors. No in vivo work. Telomeres did not recover.

Claim 7 · germline immortality depends on clearance B

A genome-wide screen is a strong design, and the result passed an orthogonal check.

Caveat · Objections remaining

The central mechanism — movement of a pump subunit — was never directly observed. Causation across generations was not measured. The biology is worm-specific. The authors themselves use “renewal” rather than “rejuvenation.”

Claim 8 · rejuvenation is lineage-selective B–

The most important defence against the global methylation objection. The grade is low for a simple reason.

Caveat · Objections remaining

One dataset, one analysis, no independent replication. Whether the lineage difference in clock values is an age difference or a cell-state difference is unresolved.

Claim 9 · pluripotent cells do not age with passage B

Consistently observed across datasets.

Caveat · Objections remaining

The clock may have hit its floor near zero. The same paper shows culture conditions moving the result. Platforms are mixed.

Six tensions worth keeping

Some disagreements do not fit into a grading table. They vanish when the papers are stitched together, but they are there in the data.

One. The shape of the curve differs by species. The mouse shows a descent; the human pre-implantation stretch is flat.

Two. Whether ground zero is a point, an interval or a process is unsettled. The data point towards a window.

Three. Every clock carries the same circularity: trained on adults, applied to embryos.

Four. The global methylation confounder cannot be fully excluded. Only two pieces of evidence hold it off.

Five. Whether artificial and natural rejuvenation share a mechanism is unproven. The resemblance is at the level of phenomena.

Six. "Rejuvenation" and "clearance" are not the same word. What paper 07 shows is removal before accumulation, not restoration after it.

The temptation to stitch

The hardest part of writing this book was refusing to produce a tidy story.

Splice 02 and 01 together and a handsome chain appears: the germ line ages, therefore rejuvenation is required, and rejuvenation is duly observed in embryos. Add 03 and 05 and you have cross-species universality; add 06 and you have application; add 07 and you have a molecular mechanism.

The chain is not false. But something is omitted at every joint.

> **Erase the tensions and the reading gets easier while the account gets further from the truth.**

The present state of science is rarely tidy. When it looks tidy, that is often a sign that nobody has looked closely enough yet.

What this chapter leaves you with

· Of nine claims: two B+, three B, three B−, one C+. There is no A.
· The firmest are the directional result in mouse embryos and the validity of single-cell measurement.
· The weakest is the frog result, which rests on a preprint.
· The central claim — that ground zero exists — is B−. The direction agrees; direct evidence is absent.
· Six tensions remain unresolved. Leaving them in place is the more accurate choice.

Part V

The implications

If ground zero is real and we can locate it, one question follows. Can it be moved. And should it be.

Chapter 14

A lower zero than nature's

If ground zero is real and can be located, one question follows. Can it be moved? This chapter sorts the possibilities into four routes, while making clear that none of them is something current technology can do.

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Four routes 90
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Starting from a thought experiment

Return to the fifth proposition of the model in Chapter 2. Extend the clearing phase artificially, or reduce the genome's mutational load, and an organism could begin from a lower point than nature achieves.

This is not a conclusion drawn from data. It is an inference that follows from the model, offered by its author as a thought experiment.

It is still worth taking seriously, because if the hypothesis holds, the goal of aging research changes: from repairing an old body to moving the starting line.

Four routes

One · extend the clearing phase

If ground zero is a window, holding the window open longer might deepen the reset.

There is one observation that bears on this: the mouse embryonic stem cell experiment from Chapter 4. Passaged without the inhibitors that support self-renewal, cells read younger afterwards. The authors interpreted this as cells repeatedly attempting differentiation and undergoing something like in vivo rejuvenation.

The implication is that culture conditions might be used to tune how deep the reset goes.

Caveat · But the same observation points elsewhere

The identical result can be read as "culture conditions move the clock." On that reading it demonstrates not controllable rejuvenation but unstable measurement. Separating the two readings requires confirmation with something other than a clock.

Two · select younger embryos

The authors of paper 01 mention using biologically younger embryos preferentially during in vitro fertilization.

The logic is simple: if embryos differ in clock value, choosing the lower one produces a child starting from a younger state.

There are many problems with the proposal.

No data. Nothing in that paper shows that differences in clock value between embryos relate to health or lifespan after birth. The connecting link is entirely absent.

The measurement is invasive. Reading an embryo's methylation requires removing cells. That is itself a risk.

We do not know what is being selected. As Chapter 12 showed, a clock value is closer to distance from a reference. Whether a difference between embryos reflects youth, developmental tempo or measurement noise cannot be distinguished.

The ethics are of a different kind. Selecting against a disease allele and selecting for "a better starting line" are not the same question.

This book treats the proposal as a normative extension unsupported by data. Cited at all, it should be labelled as the authors' outlook.

Three · imitate it with reprogramming

This is the MPTR work of Chapter 10 — the most concrete route and the most tested.

Strictly speaking, though, it does not move ground zero. It winds back a cell that is already old. It changes the current position, not the starting line.

The present state of play:

Established	clock values fall and some function recovers in cultured fibroblasts
Not established	in vivo effect, impact on lifespan or healthspan, tumour risk
Did not work	telomeres
Technical work remaining	replace the lentivirus with a non-integrating vector; remove the oncogene from the cassette

Four · edit the genome

As Chapter 2 set out, the genome is not subject to reset. Methylation marks can be erased; mutations cannot, because there is no master copy.

So the model offers genome editing as the route to genome-level "rejuvenation": correct harmful inherited mutations at the zygote stage and begin with a lower mutational load than nature provides.

This is less a question of current capability than a different kind of question altogether. Germline editing is prohibited in most jurisdictions, and the prohibition reflects social agreement rather than technical difficulty. This book does not enter that debate. It notes only that the ground zero model leads logically to this point.

What is missing

Put the four routes side by side and a shared gap appears.

> **The connecting link is absent.** None of the seven papers demonstrates a causal relationship between lowering a clock value and living longer in better health. In embryos, still less.

The gap matters because of the five ages in Chapter 11. What can be lowered is the second — a clock's predicted value. What is wanted is the fifth — functional aging. The evidence that the two travel together is not yet sufficient.

Chapter 10 illustrates the gap exactly. The clock fell by 30 years while telomeres did not move, and the improvement in migration speed did not correlate with clock values.

Why it is still worth asking

Most of this chapter ends in "not yet." There is still a reason to write it down.

The ground zero hypothesis changes the questions that aging research asks.

The older question	The ground zero question
How do we slow aging?	When does aging begin?
How do we repair old cells?	How does a cell reset itself?
How do we extend lifespan?	Where is the starting line?

The questions on the right are no easier to answer, but their answers would be more fundamental. And the strongest argument for the hypothesis is that nature is already doing the thing, every generation.

> **If something already works, understanding it beats building it from scratch.**

What this chapter leaves you with

· Four routes: extend the clearing phase, select embryos, reprogram, edit the genome.
· The embryo selection proposal is a normative extension unsupported by data.
· Reprogramming is the most concrete, but it moves the current position, not the starting line.
· The shared gap is one thing: no causal link between lowering a clock and living better for longer.
· Even so, the hypothesis changes the questions. That may be its largest contribution.

Chapter 15

When does an individual life begin?

The ground zero model contains one scientific claim and one philosophical claim attached to it. “There is a point of minimum biological age” and “an individual life begins there” are different kinds of sentence. This chapter separates them.

In this chapter

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What was measured, what was added 95
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One convergence worth noting 96
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What the two papers wrote

The abstract of paper 02 says that several processes converge on a single ground zero, a mid-embryonic state of lowest biological age where both organismal life and aging begin.

Paper 03 goes one step further in its discussion: as knowledge about ground zero develops, it may change our thinking about the beginning of human individual life.

The two sentences share a structure. They start from a measurement and arrive at a claim about what exists.

What was measured, what was added

Separate them.

Measured	Added
There is a point at which predicted clock values are lowest in development	That point is the minimum of biological age
Predicted values increase after that point	Aging begins at that point
That point appears to be near gastrulation	An individual life begins at that point

Each step from left to right contains an inference. The first is the problem of Chapters 11 and 12. The second depends on what "begins" is taken to mean. The third leaves biology altogether.

Why the third step is different in kind

"When does an individual life begin" has been argued over for a long time. Answers have included conception, implantation, the formation of the nervous system, and viability outside the womb.

The debate looks like a dispute about scientific facts, but it is not. Each position uses a different criterion: genetic individuality, connection to the mother, the possibility of consciousness, independent survival.

> Which criterion to adopt is not settled by observation. Observation can only tell you when a given criterion is met.

The ground zero hypothesis effectively proposes one more criterion: the moment the aging clock starts running.

Whether that criterion is better than the others is not a question biology can answer. And the authors of papers 02 and 03 know this. Both write the passage as an outlook rather than a conclusion.

Caveat · How this book handles the subject

This book takes no position on when an individual life begins, because it does not believe biological data can settle the question.

What it does instead is this: record that the authors wrote those sentences, and mark how far those sentences stand from the data.

One convergence worth noting

There is still something here worth pausing on.

Recall the convergence argument from Chapter 2. The author saw four things meeting near gastrulation: the stage at which embryos of different animals most resemble each other, the point of minimum structural entropy, the loss of the capacity to rebuild the whole, and the peak of embryonic mortality.

Add "the establishment of individuality" and the list grows.

Before gastrulation an embryo can split into twins. It can fuse with another embryo into a chimera. At that stage it is hard to call it a single individual: something that can become two, and two of which can become one, is an awkward candidate for "one."

After gastrulation that possibility is gone. The axes are fixed and each cell has a fate.

> **While one can become two and two can become one, it is not yet one.**

This observation is independent of the ground zero hypothesis. It is long-standing developmental biology. And it lands in the same period the hypothesis points to.

How to read the overlap

Two readings are available.

The deep reading

The establishment of individuality and the onset of aging are two faces of one event. While the whole can still be rebuilt, damage can still be erased. The moment each cell acquires a fate, damage begins to stay where it falls.

On this reading, becoming an individual and beginning to age are the same sentence. Becoming irreversible is the condition of becoming an individual.

The ordinary reading

Gastrulation is such a large transition in development that almost any marker you measure will change somewhere near it. Transcription starts, cells move, form changes, metabolism rises. Of course the methylation landscape changes too.

On this reading the overlap is not a meaningful convergence but the shadow of a big event.

> **Caveat · Current data cannot choose**
>
> Distinguishing the two would require manipulating aspects of gastrulation separately — shifting the onset of transcription alone, or delaying the loss of plasticity while leaving everything else intact. No such experiment has been reported, and performing one without destroying development is very hard.

This book finds the deep reading attractive, and notes that finding it attractive does not make it true.

The questions that remain

Collected, what is left after closing the book.

· Is the fall in predicted clock values during development a decrease in biological age, or a function of developmental stage?
· Is ground zero a point or a window? Does the window begin at oocyte maturation?
· Does lineage-selective rejuvenation replicate in other data?
· Is there a causal link between lowering a clock value and living better for longer?
· Do artificial and natural rejuvenation use the same molecular machinery?
· Are the establishment of individuality and the onset of aging two faces of one event?

The book answers none of them. It has tried to make the shape of the questions a little sharper.

What this chapter leaves you with

· The model contains a three-step inference from measurement to ontology.
· The last step — when an individual life begins — is not a question biology can answer.
· Even so, the establishment of individuality does overlap in time with ground zero.
· Whether that overlap is a deep convergence or the shadow of a large event cannot be decided on current data.

Epilogue

A curve not yet drawn

Reading the seven papers leaves you with one curve. That curve has not yet been drawn fully from data. What this book has done is mark where the drawing stops and the estimate begins.

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The part actually drawn 100
Two things this book tried to avoid 100
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The picture that remains

Compress the book into a single image and it looks like this.

A line starting at the zygote, descending to some point, turning there, and rising for the rest of life. The minimum sits near gastrulation. It never goes that low again.

The curve is appealing because it explains two things at once. Why each generation starts from the same place. And why the body ages ever after.

The part actually drawn

Mark which stretches of the curve were actually measured and the picture changes.

Segment	What was measured	Species
Descent	decline from E4.5 to E7.5; minimum at 10–12 hours	mouse, frog
Descent	flat before implantation, falling after	human (proxy samples)
Minimum	E6.5–E7.5 most likely; range E4.5–E10.5	mouse
Ascent	consistent increase from E10.5 to birth	mouse
Ascent	5 of 18 prenatal comparisons significantly increased	human

The table shows something. **No study has drawn a complete U in one species from one dataset.** The mouse U is spliced from separate datasets, and the authors concede a batch effect at the join.

In humans the ascending and descending limbs were observed in different studies, with different instruments, on different kinds of material.

> **There is one curve, but several hands drew it and several rulers measured it.**

Two things this book tried to avoid

One · a tidy story

Lay the seven papers end to end and a complete narrative appears: a hypothesis is proposed, confirmed in mice, extended to humans, resolved to single cells, broadened to another species, reproduced in the lab, and given a molecular mechanism.

The narrative is not false. But something is dropped at each joint — that the mouse and human descending limbs are not the same stretch, that the human "near gastrulation" is an inference, that the frog paper is a preprint, that nothing shows artificial and natural rejuvenation to share a mechanism.

Those omissions have been left in the text. It reads less smoothly and it is more accurate.

Two · making it look like something we can do now

Almost every experiment in this book was done in mice, frogs, worms or cultured cells.

None of it has been applied to humans. MPTR stops at fibroblasts in a dish, with no in vivo test, no lifespan data and no assessment of tumour risk. The embryo selection proposal is unsupported by data.

That is why the phrase "a technology that reverses aging" does not appear in this book.

So what is left?

What survives the criticism, restated.

Consistency of direction. Different clocks, different species, different laboratories produced curves pointing the same way. If each tool is biased in its own fashion, the chance that the biases agree is low.

Lineage selectivity. That only the cells destined to become the body are rejuvenated suggests this is not a byproduct of a global methylation change.

Two axes independent of any clock. The frog transcriptional surge and the clustering of developmental arrest use no methylation data.

A concrete molecular event. In a worm oocyte, aggregates are cleared twenty minutes after a signal arrives. It is the most direct evidence that rejuvenation is not an abstraction.

None of this proves the hypothesis. It is enough to make it worth taking seriously.

What is needed next

The gaps that recurred while writing.

One. An age curve measured continuously from zygote to adult, in one species, from one dataset.
Two. An embryonic age marker independent of methylation — protein damage, or a functional readout.
Three. Independent replication of lineage-selective rejuvenation.
Four. An intervention study linking a change in clock value causally to healthspan.
Five. Direct measurement of human embryonic tissue rather than proxies.

All five are technically hard, and the last carries heavy ethical constraints. The field will probably remain roughly where it is for some time.

Finally

This book began with the question of when we are zero.

The answer it can give now: probably not the day of birth, probably not conception either, and probably some interval around gastrulation. The size of that "probably" is still considerable.

Even so, that the question can be asked at all is a change. Instead of treating aging as something that happens because one is old, it has become possible to ask when it starts.

End with Wolpert's sentence again. That the most important moment in your life is not birth or marriage or death but gastrulation was a developmental biologist's joke about how bodies get built.

If these seven papers are right, it is not a joke.

Entropy Lab

Appendix A

The seven papers at a glance

Information scattered through the main text, collected in one place. What each paper asked and answered, what grade it received and why, on a single spread.

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Summary

No.	Paper and its job	Type	Grade
02	Gladyshev 2021, *Trends Mol Med* proposes the ground zero model	theory	D
01	Kerepesi et al. 2021, *Sci Adv* finds the U curve in mouse embryos	data	B+
03	Kerepesi & Gladyshev 2023, *Aging Cell* extends the question to humans	data and method	B−
04	Trapp et al. 2021, *Nat Aging* builds a framework to age a single cell	method	B+
05	Zhang et al. 2022, *bioRxiv* sees five axes converge in frogs	preprint	C+
06	Gill et al. 2022, *eLife* reproduces somatic rejuvenation in the lab	data	B
07	Samaddar et al. 2021, *eLife* maps the molecular route of germline clearance	genetics	B

Place in the argument

Link	What it does	What it leaves open
① logic	02 — the germ line ages, so rejuvenation is required; a minimum exists	no experimental data
② first test	01 — minimum at E6.5/E7.5 then rise, in mouse clocks	adult-clock extrapolation, batch effect, small n
③ human	03 — predicted age falls post-implantation in human embryos	proxy samples, a different clock per sample
④ resolution	04 — single-cell clock; discovery of lineage selectivity	reference dependence, mouse only
⑤ species	05 — minimum at gastrulation in frogs, plus arrest clustering	not reviewed, narrow CpG window, pooling confound
⑥ doing it	06 — somatic rejuvenation by MPTR	a dish, three donors, refitted clock
⑦ mechanism	07 — oocyte maturation signal triggers aggregate clearance	worm-specific, no generational causation

What can be said, and how

An expansion of the table in Chapter 11, written so it can be quoted directly.

Basis	Defensible sentence	To avoid
01, 04	In mouse embryos the epigenetic clock reached its lowest predicted value near E6.5/E7.5 and rose thereafter	The embryo is youngest at E6.5
03	In human embryonic data predicted values fell significantly at post-implantation stages	Rejuvenation in humans has been proven
05	A preprint reports a minimum near gastrulation in frogs	It has been confirmed across vertebrates
04	A statistical framework recovers tissue age from mouse single cells	Human single-cell age measurement is available
06	Clock age was lowered by about 30 years in cultured fibroblasts	A treatment that reverses aging exists
07	In *C. elegans* an oocyte maturation signal triggers aggregate clearance	The mechanism of human immortality is known
02 et al.	The ground zero model proposes that aging begins during development	The onset of aging has been established
04	In mouse single cells only the embryonic body lineage was rejuvenated	Rejuvenation is always selective

Key figures

Numbers from the text that are likely to be quoted.

Item	Value	Source
Most likely position of ground zero (mouse)	E6.5–E7.5 (range E4.5–E10.5)	01
Change across human pre-implantation	not significant (p = 0.7378)	03
Human blastocyst → ES cells	significant decrease ($p = 1.582 \times 10^{-12}$)	03
19 hepatocytes, liver clock	r = 0.952, median error 2.1 months	04

Same cells, blood clock	r = 0.363 (not significant)	04
Predicted age vs global methylation	r ≈ −0.71 to −0.83	04
Frog clock performance	185 CpGs, mean absolute error 1.82 years	05
Frog minimum	10–12 hours post fertilization	05
Embryos followed / arrested	104 of 5,546	05
Arrested within 3 h of gastrulation onset	66	05
MPTR rejuvenation	about 20–40 years, depending on estimate	06
MPTR transcriptomic clock error	mean absolute error 12.57 years	06
Worm aggregate clearance	20–30 minutes after mating	07
Worm RNAi screen	18,225 clones → 81 confirmed genes	07

Phrases this book does not use

· “aging was reversed” — some markers were reversed, not aging.
· “anti-aging technology” — no study here extended a lifespan.
· “has been proven” — no claim in this book earned an A.
· “the embryo is N years old” — a clock value is not an age.
· “in all animals” — what was measured is mouse, human (by proxy), frog and worm.

Glossary

Terms used in this book

Terms from the main text, in alphabetical order. The definitions are fitted to this book's context and are not complete scholarly definitions.

Bisulfite sequencing

A method that chemically converts unmethylated cytosines so that methylation state can be read from the sequence. The treatment fragments DNA, so large amounts were needed — which is why only bulk measurement was possible for a long time. Chapter 5.

Bootstrap

Resampling the same data repeatedly to fit many models and estimate uncertainty from their spread. It is not a substitute for an independent validation cohort. Chapter 7.

Epiblast

The population of cells in the peri-implantation embryo that will form the entire body. In mouse single-cell analysis only this lineage was selectively rejuvenated. Chapters 5 and 9.

Epigenetic age acceleration

How far a clock's predicted value exceeds actual age. It has been reported to track mortality and disease risk. Interlude.

Epigenetic clock

A regression model fitted to predict age from methylation values. It is not a device that measures aging but a statistical model that learned a correlation. Interlude.

Extraembryonic tissue

Lineages that form the placenta, yolk sac and related structures rather than the body itself. These were excluded from rejuvenation in mouse single-cell analysis. Chapters 5 and 9.

Extrapolation

A model returning predictions outside the range it was fitted on. Applying an adult-trained clock to an embryo is the case here, and it is why negative ages appear. Interlude, Chapter 12.

Gastrulation

The folding-in of one side of a hollow ball of cells to create the three germ layers. The body axes are fixed and the germ line separates from the soma. Chapter 8.

Global methylation

Average methylation across the genome. It swings widely during development, and the possibility that clock values are a proxy for this wave is the field's principal objection. Interlude, Chapter 12.

Intersection clock

An approach that refits a clock on the CpGs shared between the training data and each test sample. No imputation is needed, but comparability between samples is lost. Chapter 6.

Lysosome

An organelle holding degradative enzymes and maintaining an acidic interior. The proton pump responsible for that acidity is the V-ATPase. Chapter 3.

Methylation DNA methylation

Attachment of a methyl group to a cytosine in DNA. It does not alter the sequence but changes how the region is read. It occurs mainly at CpG sites. Interlude.

Methylation entropy

The disorder of a methylation pattern. Lower values mean a more ordered pattern. In the frog study it reached a minimum around gastrulation. Chapter 7.

Microautophagy

Direct engulfment of a portion of cytoplasm by a lysosome. Proposed as the clearance route in the worm, though the evidence is weak. Chapter 3.

MPTR maturation phase transient reprogramming

Switching the Yamanaka factors on only through the maturation phase of reprogramming and then off, with the aim of lowering age markers while identity is retained. Chapter 10.

Pluripotent stem cell

A cell able to differentiate into nearly any cell type. Those derived from embryos are embryonic stem cells; those made by reverting somatic cells are induced pluripotent stem cells.

Primordial germ cell PGC

The direct precursor of sperm and egg. Mouse E13.5 samples are of this type, and one of paper 01's key comparisons uses them. Chapter 4.

Protein aggregate

A clump of misfolded protein. In worm oocytes these are normally present until a maturation signal arrives. Chapter 3.

Proteostasis

The balanced maintenance of protein synthesis, folding and degradation. When the balance fails, aggregates accumulate. Chapter 3.

Proxy sample

Material used in place of something that cannot be measured directly — human embryonic stem cells standing in for epiblast, for instance. Each substitution may be defensible, but stacked they weaken the directness of a conclusion. Chapter 6.

Reference dependence

The property that a clock takes the patterns of its training tissue as a yardstick, so values change when it is applied elsewhere. The clearest case is the same hepatocytes scoring r = 0.952 and r = 0.363. Chapters 5 and 12.

scAge

A framework that estimates the age of a single cell from a bulk reference and a likelihood calculation. Its essential move is not requiring a shared CpG matrix. Chapter 5.

Telomere

The repetitive sequence protecting a chromosome end. It shortens with division and is used as an aging marker. It did not recover under MPTR. Chapter 10.

Weismann barrier

The nineteenth-century separation of somatic and germ lineages, implying that events in the body are not inherited. The continuity of the lineage survives; "germ cells do not age" does not. Chapter 1.

Winner's curse

The systematic overestimation of effect size among results that clear a significance threshold in small samples. Chapter 12.

Yamanaka factors

The four transcription factors that return a somatic cell to pluripotency. One of them, c-Myc, is also known as an oncogene. Chapter 10.

Chronology

The order in which this was built

The ground zero hypothesis was not completed in a single paper. Instruments came first, then the hypothesis, then the tests. Following the order shows how the field moved.

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Background

Year	Who	What
1880s	Weismann	separates somatic and germ lineages; the “ageless germ line” picture is formed
1986	Wolpert	leaves behind the line that gastrulation is the most important time in your life
2006	Yamanaka	returns somatic cells to pluripotency with four factors; complete reset becomes real
2013	Horvath	builds a multi-tissue clock predicting human age from 353 CpGs; the instrument exists

Hypothesis and test

Date	Paper	What
Sep 2020	02 Gladyshev	proposes the ground zero model; three testable predictions, no data
Mar 2021	01 Kerepesi et al.	preprint posted; reports a U curve in mouse embryos
Apr 2021	07 Samaddar et al.	maps the molecular route of aggregate clearance in worm oocytes
Jun 2021	01 published	*Science Advances* two sections of enzyme mechanism added
Nov 2021	04 Trapp et al.	publishes the single-cell clock; discovers lineage-selective rejuvenation
Apr 2022	06 Gill et al.	reports somatic rejuvenation by MPTR; separates rejuvenation from pluripotency
Aug 2022	05 Zhang et al.	frog preprint posted; presents the convergence of five axes
Oct 2023	03 Kerepesi & Gladyshev	moves the question to human embryos with the intersection clock

What the order tells you

Three things are worth reading out of this table.

Instruments come first

Without Horvath's clock in 2013 there is no hypothesis in 2020 and no test in 2021. And the two years between 2021 and 2023 are a gap created not by a biological puzzle but by the absence of a ruler for human embryonic data.

Every time the question was pointed at a new kind of sample, a new instrument was needed: scAge for single cells, the intersection clock for human sequencing data, an amphibian array clock for frogs.

The hypothesis arrives before the data

Paper 02 appeared in September 2020; the preprint of paper 01 in March 2021. Six months.

They come from the same research group. The people who proposed the hypothesis tested it. That is common in science and not in itself a problem, but the circularity discussed in Chapter 12 attaches here. It is also why independent replication is still thin.

Preprint time

Paper 01 was a preprint in March 2021 and published in June. In those three months two sections of mechanistic experiments were added and the claim about ground zero's position was sharpened.

Paper 05 was posted in August 2022, and whether it was subsequently published could not be confirmed while writing.

Two papers from the same group, two different fates. Worth remembering when reading any preprint.

This field is young. Its central hypothesis is less than five years old, and most of the key results come from one research group and its immediate orbit. It is early for independent replication to have accumulated. That is also the structural reason every grade in this book sits somewhere around B.

References

The papers this book read

Citations for the seven papers discussed in the text. Cautions for citing each are given beneath the entry.

In this chapter

The seven 115
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The seven

Gladyshev VN (2021). The ground zero of organismal life and aging. *Trends in Molecular Medicine 27(1):11–19.* doi:10.1016/j.molmed.2020.08.012
Paper 02 in the text. An opinion piece with no new experimental data. Cite it as a proposed model. PMID 32980264.

Kerepesi C, Zhang B, Lee SG, Trapp A, Gladyshev VN (2021). Epigenetic clocks reveal a rejuvenation event during embryogenesis followed by aging. *Science Advances 7(26):eabg6082.* doi:10.1126/sciadv.abg6082
Paper 01. Cite the published version. The preprint (bioRxiv 2021.03.11.435028) lacks two sections of mechanistic work. PMID 34172448.

Trapp A, Kerepesi C, Gladyshev VN (2021). Profiling epigenetic age in single cells. *Nature Aging 1(12):1189–1201.* doi:10.1038/s43587-021-00134-3
Paper 04. The host institution filed a related patent application with all authors listed as inventors. PMID 36211119.

Samaddar M, Goudeau J, Sanchez M, Hall DH, Bohnert KA, Ingaramo M, Kenyon C (2021). A genetic screen identifies new steps in oocyte maturation that enhance proteostasis in the immortal germ lineage. *eLife 10:e62653.* doi:10.7554/eLife.62653
Paper 07. The authors use "proteostasis renewal" rather than "rejuvenation." Preserve that distinction when citing.

Gill D, Parry A, Santos F, Okkenhaug H, Todd CD, Hernando-Herraez I, Stubbs TM, Milagre I, Reik W (2022). Multi-omic rejuvenation of human cells by maturation phase transient reprogramming. *eLife 11:e71624.* doi:10.7554/eLife.71624
Paper 06. Co-authors include the chief executive and shareholders of epigenetic clock companies. A proof of concept at the level of cultured cells, with no in vivo validation.

Zhang B, Tarkhov AE, Ratzan W, Ying K, Moqri M, Poganik JR, Barre B, Trapp A, Zoller JA, Haghani A, Horvath S, Peshkin L, Gladyshev VN (2022). Epigenetic profiling and incidence of disrupted development point to gastrulation as aging ground zero in Xenopus laevis. *bioRxiv.* doi:10.1101/2022.08.02.502559
Paper 05. A preprint posted 4 August 2022 and not peer reviewed. Any citation must say so.

Kerepesi C, Gladyshev VN (2023). Intersection clock reveals a rejuvenation event during human embryogenesis. *Aging Cell 22(10):e13922.* doi:10.1111/acel.13922
Paper 03. A six-page short communication; sample sizes and method details sit in the supplement. Code and predictions are public. PMID 37786333.

General rules for citing

Check the version. Paper 01 differs between preprint and published form. Paper 05 is still a preprint.

Separate measurement from claim. "The predicted value fell" and "it got younger" are different sentences. See the table in Appendix A.

State the species and the conditions. Mouse, human, frog or worm; in vivo or in a dish.

Check the interests. Papers 04 and 06 carry declared patent and commercial interests.

Other work mentioned in the text

Studies referred to as background. This book did not analyse them directly, and anyone citing them should consult the originals.

· Reprogramming of somatic cells by the Yamanaka factors (2006)
· Horvath's human multi-tissue epigenetic clock (2013)
· Reports of age-associated methylation patterns in sperm
· Reports of telomere lengthening on ESC passaging and extended lifespan in the resulting mice
· A report that an adult clock tracks development as early as 45 days post conception

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