

Sirtuins are Not Conserved Longevity Genes

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Abstract

It is central to biology that sequence conservation suggests functional conservation. Animal longevity is an emergent property of selected traits that integrates capacities to perform physical and mental functions after reproductive maturity. Though the yeast *SIR2* gene was nominated as a longevity gene based on extended replicative longevity of old mother cells, this is not a selected trait: *SIR2* is selected against in chronological aging and the direct targets of *SIR2* in replicative lifespan are not conserved. Though it would be difficult to imagine how a gene that advantages 1 in 5 million yeast cells could have anticipated causes of aging in animals, overexpression of *SIR2* homologs was tested in invertebrates for longevity. Because artifactual positive results were reported years before they were sorted out and because it was not known that *SIR2* functions as a pro-aging gene in yeast chronological aging and in flies subject to amino acid deprivation, a global pursuit of longevity phenotypes was driven by a mixture of framing bias, confirmation bias and hype. Review articles that propagate these biases are so rampant that few investigators have considered how weak the case ever was for sirtuins as longevity genes. Acknowledging that a few positive associations between sirtuins and longevity have been identified after thousands of person-years and billions of dollars of effort, we review the data and suggest rejection of the notions that sirtuins 1) have any specific connection to lifespan in animals and 2) are primary mediators of the beneficial effects of NAD repletion.

What Constitutes a Conserved Gene

Biology became a molecular science in the 20th century with the convergence of classical genetics, the central dogma of molecular biology, and biochemical advances¹. As I explain to my students, we understand that things are the way they are in biology on account of two rules:

Rule 1) Biology does not violate of any rule of chemistry or physics.

Rule 2) Biological traits are encoded by nucleic acids and passed down by mutation and selection.

Rule 1 compels us away from fantastical ideas like perpetual motion and time-independent processes. Rule 2 tells us that functions are constrained by inherited macromolecular sequences.

As a central principle of biology, Rule 2 allows us to define a conserved gene as a nucleotide or amino acid sequence under selective pressure to do some specific function. If we take the example of hexokinase, the function is phosphorylation of the 6 oxygen of glucose using ATP as the phosphoryl donor. If potential hexokinase sequences are identified on the basis of sequence similarity, we can test whether such sequences encode a hexokinase. Hexokinase sequences could drift into and be selected for additional or alternate functions. We'd consider them to be conserved hexokinase orthologs if they still fulfill the function of hexokinase but we'd call them paralogs if they are related by descent but are doing something else. A hexokinase might also have an emergent property such as promoting short or long lifespan in one particular life form. While one might postulate that this function is conserved throughout evolution, this thesis would have to hold up to experimental testing in order to be warranted. Indeed, we can expect that any enzyme family might have a conserved biochemical activity and distinct functional consequences revealed

by mutation in different forms of life that are not conserved. Both things can be true at the same time.

Sirtuins are Conserved as NAD-Dependent Protein Lysine Deacylases

The budding yeast *Saccharomyces cerevisiae* has haploid and diploid phases of its life cycle. Haploids come in two mating types, termed **a** and α , and are capable of undergoing a zygotic process to form **a**/ α diploids. Like haploids, diploids divide vegetatively, and they can also sporulate to form two **a** and two α haploids. Peculiarly, haploid yeast express **a** or α mating type information from the *MAT* locus but have cryptic copies of mating type genes on either side of *MAT* on chromosome 3. The system exists so that haploids can switch mating type in order to mate and form diploids. On account of the additional copies of mating type information, yeast has a system to keep the cryptic copies silenced². The *SIR2* gene is one of four genes required to keep cryptic mating type information silent (Fig. 1a)³⁻⁵. In other budding yeasts, such as *Candida albicans*, loss of *SIR2* leads to a high rate of phenotypic switching, potentially through a gene silencing mechanism⁶. In the fission yeast *Schizosaccharomyces pombe*, the *SIR2* homolog clearly has a conserved function in gene silencing⁷, suggesting that *SIR2*-homologous genes were conserved throughout disparate fungal lineages for gene silencing.

Biochemically, the Sir2 protein was shown to remove acetyl modifications of protein Lys sidechains in a manner that depends on nicotinamide adenine dinucleotide (NAD⁺)⁸⁻¹⁰. This activity has exhibited remarkably little drift across Sir2-homologous enzymes, which are termed sirtuins; nearly all of these enzymes remove an acetyl or other acyl modification of lysine in a manner that liberates nicotinamide (NAM) and which links the acyl group to the 2' and 3' oxygens on the leaving group ribose¹¹ (Fig. 1b). Clearly, this biochemical activity of sirtuins—found in bacteria, archaea, plants, animals and fungi¹²—has been highly conserved.

In sum, it is not controversial that sirtuins have an exceptionally well conserved biochemical function and that, in yeasts, they have a conserved function in gene silencing.

Two Models of Aging in *S. cerevisiae* Disprove the Notion that SIR2 was Conserved as the Mediator of the Longevity Benefit of Caloric Restriction

The nonsexual component of the *Saccharomyces cerevisiae* lifecycle is characterized by budding. Yeast cells replicate until they have used up essential inputs. Yeast lifespan can be characterized in two completely different ways termed replicative aging and chronological aging, both of which are extended by caloric restriction (CR)¹³. Despite the fact that CR extends lifespan in both models, *SIR2* advantages old mothers in the replicative aging model¹⁴ while disadvantaging all cells in the chronological aging model¹⁵. Thus, it cannot be said that *SIR2* is conserved as a mediator of the longevity benefit of CR even within *Saccharomyces cerevisiae*.

Existing cells are termed mothers while the buds they form are termed daughters. In the replicative lifespan assay, new daughters are arrayed on plates to serve as mothers. Every 90 minutes or so, laboratory workers remove the daughters to score how many times a single mother cell can produce daughters—this process continues for approximately 2 weeks until exhausted old mothers can no longer produce daughters (Fig. 2a).

It is essential to note that in a dividing culture, half of the cells are new daughters, who have never been mothers, and half of the mothers have only been mothers one time. Thus, old mothers are rare cells with a calculated frequency of $\frac{1}{2}^{(n+1)}$ where n is the number of times a mother cell has had daughters (Fig. 2b). The actual frequency of n -time mothers is, in fact, lower because mothers lose proficiency at forming daughters in their old age. In high glucose conditions, the average yeast mother lifespan is 21.2 generations, whereas in CR conditions, the average yeast mother lifespan is 26.2 generations¹⁶, meaning that CR allows a mother that comprises only 1 in 5 million cells ($\frac{1}{2}^{22.2} = 1/4.8 \times 10^6$) to divide

another 5 times. The longevity benefit of CR in this model depends on *SIR2*¹⁶ and has been attributed to accumulation of extrachromosomal ribosomal DNA circles (ERCs) in the yeast mother cells¹⁴. However, as a yeast culture can be regrown from any single cell, the exceedingly rare old mother cells are not under selective pressure to retain *SIR2* function. In addition, ERCs are not conserved as either a *SIR2* target or a cause of aging in other organisms and the centrality of *SIR2* as the mediator of the replicative longevity benefit of CR has been strongly questioned with respect to the effect of strain backgrounds¹⁷. Thus, the ability of *SIR2* to repress formation of ERCs can be defined as a nonconserved function of sirtuins that advantages rare old mother cells, allowing them a few additional cell divisions when grown in CR conditions.

The way Rule 2 works, of course, is that a trait must be under selective pressure to constrain macromolecular sequences and functions. Because a yeast culture can be regenerated from any one cell, there simply is no selective pressure on the *SIR2* gene in one highly dispensable cell out of 5 million to divide five more times.

While the replicative aging model provides no fitness advantage, the chronological aging model does provide clear selective advantages. In this model, a culture is grown to stationary phase and then plated over the next few days and weeks to score cell survival by the ability to form a colony. Unlike the replicative aging model in which the viability of the culture is unaffected by whether the oldest mothers continue to divide, chronological aging resembles every storage and survival condition that yeast has faced since it was first cultivated thousands of years ago. For yeast to be able to support the next batch of wine, bread or beer, it has to remain viable when it has run out of nutrients and stored. CR extends chronological lifespan but the presence of a wild-type *SIR2* gene shortens lifespan and greatly limits the lifespan extension that is experienced on CR¹⁵ (Fig. 2C).

Based on these data, it is logical to conclude that if yeast had a strong selection for lifespan, the *SIR2* gene would have been lost¹⁵ and thus, its presence in the yeast genome

suggests it has been conserved for other functions, potentially related to gene silencing^{3,4,6,7}. Given the fact that the replicative longevity phenotype only advantages 1 in 5 million cells¹⁶ via a mechanism and targets that are not conserved in the aging of other organisms¹⁴, it would be unreasonable to nominate *SIR2* as a conserved central controller of aging in animals. Unfortunately, the disadvantage conferred by *SIR2* in the evolutionarily selected type of aging was not described until 2005¹⁵ and this was long after dozens of review articles and pieces in the popular press had celebrated the fantastical idea that a yeast gene anticipated a limiting factor in animal aging. A small fraction of such reviews is here referenced¹⁸⁻²¹.

The Evolutionary Basis for Aging in Animals

Animals emerged from a last common eukaryotic ancestor approximately 600 M years ago—the emergence of animal-like sexual mating appears to have been earlier than this²². The complexity of heterotrophic and sexually dimorphic animal life demands that individuals are able to acquire food, avoid predation, develop to reproductive maturity, mate, and protect their offspring until their offspring are capable of all of these functions^{23,24}.

While animals that can only reproduce once are under no selective pressure to continue to live beyond their contribution to the gene pool, most animals evolved with the capacity to undergo multiple cycles of reproduction. From this capability, animal longevity appears to be an emergent property of all of the fitness traits that enable creatures to acquire resources, mate and promote the success of their offspring²⁴. What have been termed “Hamilton’s forces of natural selection”²⁵ make animals strong, clever, sex-appealing, fecund and protective of their young^{23,24}. To the degree that animals spread their genes by repeated cycles of mating and avoid predation and disease, they can also be long-lived with respect to the onset of their reproductive maturity. There are solid observations that the longest lived animals experience little predation, have a late onset of reproductive maturity,

and remain reproductively active throughout life^{26,27}. Human females are an important exception to this rule because they experience menopause at midlife: postreproductive female health is evidence for the important roles of mothers and grandmothers in the survival and reproductive success of young people²⁸.

Realizing that inbred male and female flies that are exposed to each other from early life are under little selective pressure for longevity, flies were kept as virgin males and females until late life in order to select for postponed senescence²⁹. Genomic analysis of the long lived flies that were obtained from these selections support the proposition that the genetic determinants of animal longevity are highly polygenic³⁰. Consistent with theory and experiment, analysis of the genomes of long-lived humans has not revealed strong monogenic longevity genes but rather complex webs of gene by environment and gene by gene interactions that allow some people to retain their faculties into a second century³¹.

In animal models, there have been a few notable discoveries of monogenic longevity mutants, which almost invariably map to loss of function alleles of genes under selection for vital processes such as growth and reproduction. For example, *Mth* encodes a developmentally essential G-protein coupled receptor (GPCR) in flies, deletion of one copy of which extends lifespan³² at the expense of neuromuscular function³³. Though the GPCR superfamily is conserved across all biological kingdoms, *Mth* is apparently under selective pressure for development and neuromuscular function and it does not have vertebrate orthologs³⁴.

The largest class of monogenic longevity mutants map to insulin-like growth factor (IGF-1) and growth hormone signaling pathways that are conserved between worms and mammals. These include the developmentally essential *daf-2* gene in worms^{35,36} and the genes associated with long-lived dwarf mice including those encoding and/or supporting production of growth hormone, IGF-1, prolactin, thyroid-stimulating hormone, and growth hormone receptor³⁷. Dwarf mice are stunted in growth, unable to compete for resources with

wild-type littermates, cold-sensitive and infertile. Thus, while in the laboratory, monogenic reduction in pituitary signaling clearly extends lifespan, it is equally clear that the genes were conserved to promote growth and development. Further, for reasons that are not understood but are almost certainly due to the many differences between free-living humans and laboratory mice, human dwarf syndromes linked to the same genes do not produce long-lived people³⁸.

In sum, because animal longevity integrates the function of all vital organ systems over time, it is highly polygenic. Monogenic regulators of mammalian longevity clearly exist, though the genes have been conserved for growth and development³⁸.

Highly Cited, Nonreproducible Experiments Established the Dogma that Invertebrate Sirtuins Extend Lifespan in Animals

2001 was a heady time in which the first assemblies of the human genome were published³⁹ and two-thirds of the Physiology Nobel Prize for discovery of key regulators of the cell cycle was awarded for work in yeast by Lee Hartwell and Paul Nurse⁴⁰. When the ERC-repressing mechanism for yeast replicative lifespan extension was published four years prior, the extremely low abundance of the target cell was clearly discussed¹⁴. However, this discussion was not included in more recent work, which claimed centrality for *SIR2* in mediating the longevity benefit of CR in yeast¹⁶. More thorough work refuting this had not yet been published¹⁷ and the selective disadvantage conferred by *SIR2* in chronological aging remained undiscovered¹⁵. It therefore must have seemed reasonable to test whether additional copies of the worm and fly *SIR2* orthologs might extend lifespan in invertebrate model organisms.

Indeed, it was reported in Nature that an extra copy of *sir-2.1* extended worm lifespan by “up to 50%”⁴¹. Accompanying this paper, David Gems opined “arguably, using budding yeast (*Saccharomyces cerevisiae*) to investigate the genetic determinants of ageing, and

consequently longevity, seems almost absurdly optimistic. Ageing yeast do not develop grey hair or poor eyesight, or start complaining about young people today, or have strokes. In fact, it is not even clear that they age at all, and when researchers talk of yeast 'lifespan', what they really mean is the number of times a yeast mother cell can reproduce by producing a bud⁴². After examining the data showing apparent worm lifespan extension by an extra copy of *sir-2*.¹⁴¹, Gems considered the result plausible and wrote that "it seems that some genetic determinants of longevity and ageing are conserved across animal groups"⁴². Shortly thereafter, overexpression of the apparent *Drosophila* ortholog of *SIR2* was reported to extend lifespan in flies by mediating the effect of CR⁴³.

Model building and storytelling are key parts of science. Proponents of the centrality of *SIR2* as a mediator of the longevity benefit of CR told a compelling story. If you ignored the follow-up work on the dispensability of *SIR2* for CR-extended longevity¹⁷ and did not consider the problem of the total dispensability of 1 in 5 million cells¹⁴, you could focus on the attractive idea that CR extends lifespan by virtue of beneficial stress that links metabolism to youthful gene expression¹⁶. Proponents of this model told us in multiple review articles that even though the targets of *SIR2* are not conserved from one organism to another, they are conserved for the purpose of longevity extension^{18-20,44}. With the apparent *SIR2* life extension results in worms⁴¹ and flies⁴³ that seemed to support *SIR2* homologs as dominantly acting longevity genes, the strain-specific effects in yeast seemed to be neutralized¹⁷.

Companies were formed, activators were sought, mouse models were created, review articles were written, grants were funded, and laboratories globally were mobilized to better understand human *SIRT1*, which was described as the *SIR2* ortholog among a "magnificent" set of seven sirtuins that would revolutionize human medicine⁴⁵. The amount of global hype around a yeast gene said to be conserved as a family of dominantly acting animal longevity genes is difficult to overstate. Indeed, this hype has permeated popular culture in books, podcasts and social media⁴⁶. Moreover, this author agrees with David

Gems that if a yeast gene were to have anticipated the limiting factors in animal aging, the unbridled enthusiasm about sirtuins would be warranted.

A decade after the initial worm result, Gems was the senior author of a paper written by investigators from seven different institutions, who wrote that “in *C. elegans*, outcrossing of a line with high level *sir-2.1* over-expression abrogated the longevity increase, but not *sir-2.1* over-expression. Instead, longevity co-segregated with a second-site mutation affecting sensory neurons. Outcrossing of a line with low copy number *sir-2.1* over-expression also abrogated longevity. A *Drosophila* strain with ubiquitous over-expression of dSir2 using the UAS-GAL4 system was long-lived relative to wild-type controls, as previously reported, but not relative to the appropriate transgenic controls, and nor was a new line with stronger over-expression of dSir2. These findings underscore the importance of controlling for genetic background and the mutagenic effects of transgene insertions in studies of genetic effects on lifespan. The life extending effect of dietary restriction on ageing in *Drosophila* has also been reported to be dSir2 dependent. We found that dietary restriction increased fly lifespan independently of dSir2⁴⁷.

There continued to be some back and forth with respect to overexpression of *SIR2* homologs in invertebrates. Acknowledging strain artifacts, the original proponents pared back and attempted to defend claims of lifespan extension in worms⁴⁸ and flies⁴⁹. After all the controls were carefully performed, it is now clear that the worm longevity effect of *sir-2.1* is not only strain-specific but also dependent on inclusion of thymidylate synthase inhibitor 5-fluorodeoxyuridine to block development of progeny⁵⁰. In flies, independent laboratories reported that loss of dSir2 does not shorten lifespan^{51,52} and that, in fact, deletion of one copy of this gene extends lifespan and greatly extends lifespan when flies are starved for amino acids⁵². There is not a general reproducibility problem with these assays because multiple methods of *daf-2* inhibition are reproducible as lifespan extenders in worms⁵³, whereas worm and fly Sir2 overexpressers are not and, in fact, dSir2 antagonizes healthy aging in the context of amino acid restriction⁵².

The problem we face is that the thesis that not just one but seven sirtuins function as dominantly acting longevity genes has been canonized in major lectures such as the Franklin H. Epstein Lecture in the New England Journal of Medicine⁵⁴. There are many hundreds of highly cited review articles on sirtuins and aging, which are premised on sirtuins conserved in yeast as longevity genes, sirtuins conserved in invertebrates in longevity genes, and sirtuins functioning as mammalian longevity genes. These reviews refer to each other for support and make little effort to question the underlying experimental support for the thesis. Indeed, the persistent influence of review articles and media pieces that are premised on overinterpreted or irreproducible data are such that this author was asked by dozens of scientists, multiple journal editors, and hundreds of laypeople to put together this critique.

The Story of Resveratrol as a Sirtuin Activator Constitutes a Deep Contamination of the Scientific Literature

Resveratrol is a polyphenolic compound found in peanuts and red wine that had already been the subject of a great deal of speculation prior to 2000⁵⁵. The French Paradox—a low incidence of coronary heart disease despite a high consumption of saturated fat—had been correlated with red wine consumption⁵⁶ so that people were motivated to identify health-promoting compounds in wine. Notably, there are countless potential explanations of the French Paradox, such that there is little likelihood that any single factor explains it and no limit to the stories that are created to support one's favorite cardioprotective food group or lifestyle⁵⁷.

Using a synthetic peptide substrate containing an aminomethylcoumarin reporter group, several polyphenols including resveratrol, fisetin and quercetin were reported to increase the activity of human Sirt1 (Fig. 3A). In the same paper, resveratrol was reported to extend the lifespan of yeast in the replicative aging model by functioning as a Sir2 activator

and CR mimetic⁵⁸. The investigators postulated the “xenohormetic” concept that fungal and animal sirtuins are activated by compounds produced when plants in their environment are stressed. The Nature paper was accompanied by a commentary entitled “Ageing: a toast to long life”⁵⁹ and coverage in Science entitled “Longevity research: In vino vitalis? Compounds activate life-extending genes”⁶⁰. Despite as many as 20 thought pieces on xenohormesis⁶¹⁻⁶³, the idea has never been tested (*i.e.*, would extracts of stressed versus nonstressed plants extend the life of insects?) but has entered global consciousness through a bestselling book and podcasts as though it were a scientifically established fact⁴⁶.

To publish a paper in a biochemistry specialist journal in which one identifies a putative enzyme activator or inhibitor, reviewers expect to see evidence that the small molecule-enzyme interaction is direct and is not artifactually related to the screening method. Such validating work was not apparently requested in the definition of resveratrol as a xenohormetic lifespan-extending Sirt1 activator⁵⁸. As the Sirt1 peptide substrate contained aminomethylcoumarin and also required trypsin to generate a signal, this was a clear failure of peer review. It was soon discovered that resveratrol does not activate Sirt1-dependent deacetylation of peptide substrates without the fluorescent reporter group^{64,65}. Further, in contrast to the initial report, resveratrol fails to extend lifespan of yeast or influence Sir2 activity *in vivo*¹⁷. Resveratrol was reported to extend lifespan in flies and worms by activating Sir2 homologs⁶⁶ but this effect could not be independently replicated⁶⁷.

How could this happen? Yeast replicative aging assays are done on plates that are kept for about two weeks time and are sensitive to humidity and other factors that are hard to control. If researchers are not careful, they could fool themselves into thinking that particular genetic or pharmacological interventions have an effect when the effects are driven by humidity or noise. Worse yet, researchers could throw out results that they cannot explain in order to construct a story in which all the pieces (representing noise) come together in a pleasing way. In their attempt to replicate the reported longevity-promoting effects of resveratrol in worms, the variability of the assays were noted⁶⁷—it is therefore critical that

data are not selected in order to be consistent with a story. It is also unfortunate that laboratory investigations are not generally performed in a blinded manner: laboratory personnel generally open-label their experiments and can be unduly influenced by their preconceived notion of what experiments are working versus what experiments can be discarded because they are not producing the expected results. Further, in large laboratories, there are cases in which more than one person is trying to obtain a result that the lab director has publicly predicted. This can lead to situations in which beautiful noise is selected and independent replication will fail.

Resveratrol is widely known to be a dirty drug with dozens of cellular targets and mechanisms⁶⁸. When resveratrol was added to a high fat diet, mice gained somewhat less weight, generally outperformed mice on the high fat diet, and had a higher density of mitochondria in liver⁶⁹. By Occam's razor, one might assume that resveratrol depressed fat absorption by any number of mechanisms, such that more fat was eliminated in the treated animals than the nontreated. However, the caloric content of fecal matter was not measured. The researchers did not conduct any direct or genetic test for Sirt1 activation but rather performed a western blot for the acetylation state of liver PGC1 α as a proxy for Sirt1 activity and reported that resveratrol-treated animals had a lower acetylation state than in the nontreated high fat animals⁶⁹. In fasting, PGC1 α deacetylation had been linked to Sirt1 activity and mitochondrial biogenesis⁷⁰ so it sort of made sense that Sirt1 might have acted on PGC1 α in the high fat-fed liver. However, we know that resveratrol acts through multiple mechanisms and the beneficial effect of resveratrol was only seen on high fat, in contrast to the expectations that Sirt1 would be more important in CR conditions. Moreover, there are dozens of factors that would influence the acetylation status of PGC1 α in liver that were not examined including determination of the percent of hepatocytes versus inflammatory cells, and determining the activity of all of the potential acetyltransferases and deacetylases that act on PGC1 α . Thus, irrespective of what global announcements claimed the paper showed,

the investigators surely did not provide any evidence that resveratrol causes Sirt1 to respond as though mice were in CR⁶⁹.

Nature accompanied the article with an piece entitled, “Let them eat cake.” The editorial read “You can have your cake and eat it. Fat, healthy and tipsy. Fountain of youth. These headlines and more greeted online publication of the article ‘Resveratrol improves health and survival of mice on a high-calorie diet’. What the paper does show is that consumption of resveratrol at doses achievable in humans (but not from red wine — the hundreds of bottles a day needed would have side effects) can reproduce many of the physiological effects of a low-calorie diet in mice, improving health and survival.” A selection of New York Times articles from this era were entitled “Fighting the effects of fat: Pass the pinot (Nov 1, 2006),” “Yes, Red Wine Holds Answer. Check Dosage (Nov 2, 2006),” “Aging Drugs: Hardest Test Is Still Ahead (Nov 7, 2006),” “An Age-Defying Quest (Red Wine Included) (Jul 8, 2007),” “Glaxo to buy Sirtris, Maker of a Drug Based on Red Wine (Apr 8, 2008),” “Hoping Two Drugs Carry a Side Effect: Longer Life (Jul 22, 2008),” and “Quest for a Long Life Gains Scientific Respect (Sep 28, 2009).”

As problematic as *SIRT1* was as a proposed dominant longevity gene, the pharmacology around Sirt1 activators became entirely indefensible. Resveratrol was known to be not a direct Sirt1 activator^{64,65} and the effect of resveratrol on high fat-fed mice had been attributed to Sirt1 without any genetic validation⁶⁹. A group at Sirtris and Harvard claimed direct Sirt1 activators with 1000 times the potency of resveratrol and biological activity in rodent models of diabetes⁷¹. Upon attempts to replicate the findings, none of these data were reproducible. In contrast, it was shown that SRT1720, SRT2183, SRT1460 and resveratrol do not activate Sirt1 with native peptide or full-length protein substrates but rather interact with the fluorophores used to assay sirtuins (Fig. 3), fail to lower blood glucose nor improve mitochondrial capacity in mice fed a high fat diet, and exhibit multiple off-target activities against diverse cellular targets⁷².

After GSK bought Sirtris for \$720 million and undertook a multibillion dollar program to develop the proposed SRT compounds, there was a powerful driving force to rescue this program, which appeared to be chasing a fluorophore artifact and off-target effects^{72,73}.

The proponents of resveratrol and SRT compounds as direct Sirt1 activators then published data claiming that the fluorogenic reporter interaction was mimetic of authentic aromatic interactions with Sirt1 substrates and further claimed that the ability of these compounds to activate Sirt1 depends on Glu230, an amino acid that is found in human, mouse and fly Sirt1 but—according to their own sequence alignment—not worm or yeast SIR2 homologs⁷⁴. This report is troubling because the same senior author claimed that resveratrol was identified as an activator of yeast Sir2⁵⁸ and worm *Sir-2.1*⁶⁶. How can the original papers be correct and supported by new data if the specific interaction between resveratrol and Sir2 homologs depends on an amino acid not found in these enzymes? The defense of the resveratrol-Sirt1 interaction moved the goalposts so far that the authors took away their own first score. Without Glu230, resveratrol should not be able to activate Sir2⁷⁴ but the senior author still claims that resveratrol activates Sir2 and, in his terms, gave the yeast cells the human equivalent of 50 extra years of life⁴⁶. At what point will co-authors or the journals ask for one or both of the conflicting resveratrol papers^{58,74} to be retracted? Note that at the time of this writing, this group's first misleading resveratrol Nature paper has >4500 citations⁵⁸ and their misleading mouse Nature paper has 5000 citations⁶⁹, making them not only the highest cited papers on resveratrol but apparently the highest cited original research publications in the entirety of the aging field, with thousands more citations than papers that have been foundationally important for replicable work that has produced deep insights into aging^{35,36}.

We know that resveratrol and SRT compounds have specific binding interactions with fluorophores^{64,65,72}, that the off-target effects of resveratrol are well documented^{68,72,75}, and that when pterostilbene, a more bioavailable derivative of resveratrol was given to humans, it

caused a dose-dependent increase in low density lipoprotein cholesterol⁷⁶, which is contrary to expectations based on hitting Sirt1.

It is the view of this author that there are overwhelming data to reject resveratrol and SRT compounds as specific activators of Sirt1 and to ignore the concept of xenohormesis as it was never anything more than a story.

***SIRT1* does Not Have the Properties Expected of a Mammalian Longevity Gene**

There are roughly 20,000 genes in mammalian gene sets. Following the identification of loss of function alleles of *daf-2* as conferring longevity in worms^{35,36}, loss of function alleles of several pituitary genes were shown to confer longevity in mice at the expense of size and fertility³⁷. In principle, dominant longevity genes³⁷ would be more exciting than loss of function alleles of growth genes because one could, in theory, generate mice of normal size and fertility that would be more fit for a longer time. Indeed, a popular book claims that sirtuin genes—identified as dominant longevity genes in yeast, conserved as such across all animals and activated by daily doses of resveratrol and nicotinamide mononucleotide (NMN)—make it such that we don't have to age⁴⁶.

Few scientists would have looked for longer-lived phenotypes in sirtuin overexpressing mice after seeing that invertebrate Sir2 overexpression doesn't extend lifespan in clean worm and fly backgrounds⁴⁷ or that loss of one copy of dSir2 extends lifespan in response to amino acid starvation⁵². However, mouse *Sirt1* transgenics were made after the initial nonreproducible invertebrate experiments were published^{41,43}.

The first *Sirt1* transgenic model failed to show lifespan extension but showed lower blood cholesterol and blood glucose⁷⁷. The paper was accompanied by a feature entitled "Is *Sirt1* a magic bullet for longevity?"⁷⁸. Two additional groups made mice transgenic for *Sirt1* and showed that they are protected against some of the metabolic effects of high fat diet,

also without extending lifespan^{79,80}. Interestingly, mediation of metabolic responses to fasting were specifically examined and found to be unaffected in these mice⁸⁰ but the paper was not written as a test of this foundational hypothesis of sirtuin research. In yet a fourth laboratory, *Sirt1* transgenic mice failed to show a longevity benefit but showed resistance to a liver carcinogenesis model driven in part by high fat diet⁸¹—this effect could potentially be due to the increased energy expenditure these mice exhibit on high fat^{79,80}.

In a fifth laboratory, multiple brain-specific *Sirt1* overexpression mouse lines were constructed. Line 10 extended lifespan by 11% while line 1 did not extend lifespan at all. Rather than publish this as a negative result, the authors claimed that *Sirt1* transgenic line 10 revealed the inner workings of Sirt1 by virtue of higher relative overexpression of *Sirt1* mRNA in particular brain regions. The methods for this were not sound. In both transgenic lines examined, *Sirt1* mRNA was more highly overexpressed in dorsomedial hypothalamus (DMH) and lateral hypothalamus (LH), which they argued were important. With an n of 3 mice and showing no statistics, the investigators calculated that the overexpression fold-change was greater in these two regions than in the arcuate, essentially arguing that *Sirt1* will extend lifespan if it is more highly overexpressed in DMH and HL than arcuate⁸². Brain transgenic lines 2 through 9 have not been discussed, nor has a hypothalamic overexpresser tested the hypothesis that this effect was driven by *Sirt1* rather than a transgene insertion site or other artifact.

With respect to knockouts, a first report indicated that *Sirt1* knockout mice do not increase their physical activity in response to CR⁸³ and another report claimed that Sirt1 is required for food anticipatory activity (FAA) by virtue of neuronal activities in the hypothalamus⁸⁴. However, when *Sirt1* knockout mice were reexamined, it became apparent that these mice are constitutionally inactive on *ad libitum* feeding, have a higher oxygen consumption rate than wild-types, and are hyperphagic yet smaller than wild-types⁸⁵. The lethargy of *Sirt1* knockout mice is a replicable result and, in fact, the activity difference is greater on high fat diet than on a standard chow diet, indicating that there is not a specific

deficit in CR-induced activity⁸⁶. The FAA-dependence on *Sirt1* could not be reproduced as neither whole body deletion, active-site substitution, forebrain deletion (*Camkll α* CRE), neuronal deletion (*Nestin* CRE), POMC neuron deletion (*Pomc* CRE) or tyrosine hydroxylase neuron deletion (*Th* CRE) of *Sirt1* eliminated FAA behavior⁸⁷. The nonreplicability of the hypothalamic effects of *Sirt1* is important because the same group which claimed that *Sirt1* is required for FAA⁸⁴ also claimed that specific ratios of *Sirt1* overexpression in hypothalamus versus arcuate are required to reveal a life-extending effect of this gene⁸².

In clinical research, best practices demand that hypotheses and primary endpoints are prespecified such that impartial observers will know when an intervention has failed to allow rejection of the null hypothesis. Indeed, the term HARKing was developed to warn scientists of the danger of forming Hypotheses After the Results are Known⁸⁸. *Sirt1* was supposed to extend lifespan and it didn't in four different laboratories^{77,79-81}. Then it was supposed to extend lifespan when overexpressed in brain. When it did in one but not other transgenic lines, the authors found ways, *post hoc*, to weave a story around relative overexpression levels in regions of the brain⁸² based on behavioral characterization⁸⁴ that also did not hold up to genetic analysis⁸⁷. While HARKing can sometimes explain discoveries and it is certainly legitimate to test hypotheses that emerge from unanticipated results, it is illegitimate to simply construct a story around noisy data that move the goalposts to where the ball has fallen.

Human SNP data are now extensive and allow for discovery of rare or common variants that are associated with increased lifespan. SNPs in *SIRT1* have been interrogated and have not been shown to be associated with increased human lifespan^{89,90}.

Carl Sagan taught us that extraordinary claims require extraordinary evidence.

The thesis that what *SIR2* is doing for a CR yeast mother cell is so fundamentally conserved that we would see lifespan extension across invertebrates and vertebrates^{18-20,44} is not supported. While the line 10 brain-specific overexpresser has a longer lifespan⁸², it is difficult

to reconcile this as strong positive support for the thesis because we have to stipulate that *SIR2* opposes longevity in the selected yeast model of CR-induced lifespan extension¹⁵, is dispensable in worms⁴⁷, opposes the benefit in flies⁵², is dispensable for FAA experienced in CR⁸⁷, and doesn't generally extend lifespan in mice^{77,79-81} unless the transgene is confined to the brain and then depends either on insertion site, relative level of expression in particular brain regions, or innumerable other confounding effects⁸². The obvious conclusion is that while *SIR2* represses ERC formation in old mother yeast cells, it would have been amazing if this nonselected trait were to anticipate and prevent aging in animals—and it doesn't.

Strangely, however, based on the lore from yeast, hyped predictions by industry, review articles and the popular press, negative results are rarely expressed as lack of support for *Sirt1* as a key mediator of the longevity benefit of CR. Indeed, the financial and intellectual investment in the idea that increased activity of the *Sirt1* gene would extend lifespan was so great that transgenics were made five times until this result could be scored positively^{77,79-82}. Even then, the underlying data document clear evidence of nonreproducibility and HARKing⁸². Given the reproducibility problems documented in yeast⁶⁴ and invertebrate^{47,67} aging assays and the bias toward publication of positive results⁹¹, one wonders how frequently any random transgene analyzed by five groups at multiple insertion sites would produce a report of increased lifespan.

Six Other Sirtuins Have Been Extensively Searched for Longevity Phenotypes

It is frequently observed in human nature that the framing of questions has an influence on the answers that are given⁹². *SIR2* was first defined as a gene that does gene silencing^{3,4} and could also have been simply characterized as a founding member of NAD⁺-dependent deacylases⁸⁻¹⁰ with substrates and biological functions in different organisms that are poorly conserved and not part of a single vital process.

Longevity is such an age-old concern, however, that since the identification of the role of *SIR2* in old yeast mothers¹⁴, this gene has been compellingly framed as a dominant longevity gene that could extend lifespan by making an organism feel the beneficial stress of CR. Even when the effect of *SIR2*-related genes is precisely against the expectations of longevity^{15,52} or when no strong result has been obtained, an abundance of research groups have been willing to commit time and treasure to find lifespan-extending effects of sirtuins. Three types of biases are at work, namely framing bias⁹², confirmation bias⁹³ and publication bias⁹¹.

The framing bias of sirtuin research is that review articles and the global lay literature defined sirtuins as longevity genes rather than genes conserved as protein lysine deacylases that were first identified as regulators of yeast gene silencing. This means that researchers would set out to find longevity phenotypes. Confirmation bias is that researchers who chose to work in this field thought these genes would positively confer longevity because that's what they thought the yeast replicative aging assay¹⁴ and countless reviews taught. Moreover, the framing bias was established long before yeast and fly genetics indicated the exact opposite^{15,52}. Imagine the research environment in which the first fungal *SIR2* longevity result was that *sir2* deletion produces an extraordinarily long lived yeast in CR¹⁵ and this has been conserved all the way to flies⁵². Framing and confirmation biases would then mobilize a community try to figure out why sirtuins are so bad for animal aging.

Positive results are easier to publish than negative results⁹¹. Many of the most positive-sounding longevity results were published in high profile journals despite a lack of rigor and were accompanied by breathless press coverage. In contrast, negative results are frequently abandoned by laboratories and, even when published, are frequently ignored in major review articles such that the general impression of working scientists can remain doggedly consistent with a false premise and a thoroughly failed thesis. At the time of this writing, for example, the Proceedings of the National Academia of Sciences paper claiming that dSir2 mediates the longevity benefit of CR has more than 1500 citations in Google

Scholar including hundreds of citations by highly cited review articles⁴³, while the Genome paper showing that loss of a copy of the dSir2 is required for lifespan extension in flies with amino acid restriction has only 13 citations and has yet to be cited by a major review article⁵².

The asymmetries between publication ease and rewards of positive versus negative data have been magnified by commercial and reputational interests that are deeply tied to tested and disproven theories. A prime example of this is the perseverance to find a mechanism by which resveratrol activates human Sirt1 even when the mechanism proposed⁷⁴ is fully inconsistent with the same author's claims that compound was discovered as a Sir2-stimulating yeast lifespan extender⁵⁸.

In the light of the global search for prolongevity functions of sirtuins, the set of potential mediators has been expanded. Though *SIRT1* was said to be the homolog of *SIR2*, it was proposed that all or any of the seven sirtuins could be dominant longevity genes^{18-20,44}. Here we consider the functions of *SIRT3* and *SIRT6*—interesting enzymes to be sure—but not conserved as longevity genes.

The mitochondrial deacetylase Sirt3 was claimed to be a specific activator of succinate dehydrogenase⁹⁴, long chain acyl coA dehydrogenase⁹⁵, superoxide dismutase⁹⁶ and other enzymes. This doesn't entirely make sense because deacetylases do not specify where acetyl groups are put on, and it was pointed out that the same lysines—generally found at enzyme active sites with perturbed pK_a values—are modified by multiple acyl groups by mass action in disparate conditions of metabolic stress^{97,98}. Seen in this light, Sirt3 is less a specific enzyme activator than a constitutive remover of acetyl modifications that are deposited due to the chemical reactivity of Lys residues with the partial positive charge on the carbonyl C of acetyl coA⁹⁷. *Sirt3* transgenic mice are not longer-lived but are resistant to inducers of mitochondrial stress such as doxorubicin that produce mitochondrial hyperacetylation⁹⁹. Remarkably, even before the *Sirt3* transgenic was investigated, a review

article on Sirt3 was entitled “Forever young: SIRT3, a shield against mitochondrial meltdown, aging, and neurodegeneration”¹⁰⁰.

Sirt6 knockout mice are small, lack subcutaneous fat, and are short-lived with a deficiency in DNA repair. The title of the paper termed this an “aging-like phenotype” but it looks more like a failure to thrive on top of low blood sugar, colitis and low circulating IGF-1¹⁰¹. *Sirt6* transgenic mice on a mixed background had a 10-15% lifespan extension in males but not females¹⁰². Though the male-specific longevity effect correlated with depressed IGF-1 circulation, the *Sirt6* knockout also had low IGF-1, such that the mechanism of lifespan extension was not clear¹⁰¹. The same group has continued to generate transgenics in additional mouse backgrounds and has now shown that on a C57BL/6J0laHsd mouse background, *Sirt6* overexpressers remain more active and have extended lifespan in both sexes with gene expression programs, depressed IGF-1 circulation and metabolic characteristics that are generally more youthful¹⁰³. In discussing the strain-specificity of these results, the authors noted that female mice circulate lower levels of IGF-1, such that if the mechanism of lifespan extension by *Sirt6* depends on depressed IGF-1, the effect of *Sirt6* would be dampened in strains and sexes with lower IGF-1¹⁰³. However, we know that *Sirt6* cannot be a negative regulator of IGF-1 circulation because *Sirt6* knockout mice have low circulating IGF-1¹⁰¹.

A consistent result from *Sirt6* knockouts and transgenics is that *Sirt6* is a positive regulator of gluconeogenesis, which appears to benefit older mice^{101,103}. However, one would expect the same mechanism to be contraindicated for humans because higher blood glucose tracks with better health in old mice and with worse health in people¹⁰⁴. Indeed, metformin has been contemplated as a longevity medicine¹⁰⁵ that works, in part, by depressing gluconeogenesis¹⁰⁶.

Those who are working on sirtuins with respect to aging are surely pleased if not relieved that some mouse strain backgrounds and circumstances have been found in

support of some aspects of the sirtuin-aging thesis. However, the results fall far short of support the overall thesis that sirtuins are conserved as longevity genes because the premise was already falsified in yeast and invertebrates. Moreover, if seven sirtuin genes were indeed conserved to promote animal lifespan, positive results would be penetrant across mouse strains and sexes, such as they are with inactivating mutations in growth hormone signaling³⁷. Here it is important to note that some of the dwarf mutant genes were identified in “mouse fancier” backgrounds before being crossed into standard laboratory strains¹⁰⁷. This caused no problem whatsoever because pituitary pathway genes clearly regulate aging in both sexes in all strains examined. It doesn’t matter which laboratory examines them and we don’t have to get into handwaving about insertion sites or make *post hoc* arguments about relative expression in beneficial versus nonbeneficial tissues.

In contrast, examination of mammalian sirtuin data reveal example after example in which framing bias, confirmation bias and publication bias perturbed the characterization of interesting results about physical activity, mitochondrial housekeeping, DNA repair and gluconeogenesis as though they primarily reflected insights into underlying processes of aging that are so fundamental they could have been anticipated by yeast cell biology.

In this researcher’s view, though the genes are interesting, there is no specific insight about aging that has emerged from the analysis of seven sirtuin genes in mammals. This researcher continues to read in reviews and seminar introductions that sirtuins mediate the longevity effects of CR but this is not true in yeast^{15,17}, not true in invertebrates^{47,52}, and not true in mice⁸⁷. Indeed, investigators might better understand the function of these genes if they were unshackled from the falsely premised framing bias that sirtuins are conserved longevity genes.

There is Little Evidence that the Benefits of Repletion of the NAD⁺ Metabolome is Primarily Mediated by Sirtuins

Shortly after discovery of the vitamin activity of nicotinamide riboside (NR)¹⁰⁸, we used the yeast system to show that NR increased cellular NAD⁺, Sir2 activity and replicative lifespan¹⁰⁹. We did not claim that provision of NR would extend human lifespan by virtue of increasing the activity of sirtuins. Though yeast replicative aging is an assay one can employ in the laboratory, it struck us as fantastical and a violation of Rule 2 that rare, old yeast mothers employed a nonselected genetic trait that would anticipate the causes of human aging.

During this era, the effect of resveratrol on high fat-fed mice had been described with the previously discussed lack of evidence of mediation by Sirt1⁶⁹. In the course of characterizing the mechanism of action of resveratrol in mice, it was discovered that AMP kinase is the apparent direct target, but a role for Sirt1 had not been discarded⁷⁵. Thus, when overfed mice were shown to have improved metabolism when fed NR¹¹⁰, the investigators did not probe the mechanism genetically but rather decided to blot for the acetylation state of nuclear transcription factor Foxo1 and mitochondrial Sod2. Seeing lower levels of acetylated Foxo1 and Sod2, they claimed that the beneficial effects of NR were mediated by Sirt1 and Sirt3¹¹⁰. As with the unsubstantiated claim that resveratrol activates Sirt1 to deacetylate PGC1 α ⁶⁹, the investigators did not look at what was causing these proteins to be acetylated, whether Sirt isozymes were really responsible for their deacetylation, or whether these modifications were important to the mechanism of action of NR.

Similarly, when mice were dosed with NMN, it was claimed that NMN suppressed age-associated body weight gain, enhanced energy metabolism, promoted physical activity and insulin sensitivity. No genetic evaluation was done of the mediators but the discussion speculated on activation of SIRT1, SIRT3, SIRT4, SIRT5 and SIRT6¹¹¹.

The redox functions of NAD coenzymes, NAD⁺, NADH, NADP⁺ and NADPH, are essential for fuel oxidation, oxidative phosphorylation, gluconeogenesis, ketogenesis, nucleotide, lipid and steroid synthesis, and detoxification of reactive oxygen species¹¹². These coenzymes function as the central catalysts of metabolism, the set of all of the processes that allow us to convert everything we eat into everything we are and everything we do.

For reasons that are hard to understand, the vital redox functions conferred by the NAD system have been nearly ignored throughout much of the sirtuin era. Perhaps because the sirtuin field embraced the fantastical idea that a yeast gene anticipated and could prevent the causes of aging in animals, speculative mechanisms related to NAD boosting often invoke the idea that supraphysiological levels of NAD⁺ would get sirtuins to levels of activity they were capable of in youth and/or in a leaner environment. These ideas persist without genetic testing.

Quantitative targeted NAD metabolomics¹¹³ has revealed that the NAD system is functionally disturbed by conditions of metabolic stress including fatty liver¹¹⁴, peripheral¹¹⁵ and central¹¹⁶ neurodegeneration, noise-induced hearing loss¹¹⁷, DNA damage¹¹⁸, heart failure¹¹⁹, postpartum¹²⁰, activation of specific oncogenes¹²¹, alcoholic liver disease¹²², interleukin 8 signaling¹²³, viral infection¹²⁴, mitochondrial disease¹²⁵, and inflammaging¹²⁶. Thus, when we have applied NR as a potential remedy, we have done so in the context that there is a functional deficit in key metabolites such as NAD⁺ or NADPH that are putting cells and tissues at risk of not meeting bioenergetic needs, not being able to repair DNA, not being able to detoxify reactive oxygen species, not being able to conduct anabolic processes, not being able to support the activity of a monoADPribosylating PARP family member that is specifically induced, etc. While many redox and repair mechanisms associated with repletion of the NAD system are pleiotropic, we urge researchers to use genetic and other sound analytical techniques to probe NAD repletion mechanisms rather

than just doing a western blot and weakly asserting SIRT-dependent mechanisms.

Reviewers and editors should expect more as well.

Conclusions

The NAD system features enzymes such as PARP1 that are intensely activated by the appearance of DNA damage¹¹⁸, monoADPribosylating PARPs that are strikingly induced by the innate immune system¹²⁴, SARM1 that is activated by accumulating NMN¹²⁷, CD38 that is activated by interleukin 8¹²³, and vital redox enzymes that are controlled by metabolites and electron fluxes. While sirtuins carry out interesting biochemical reactions and perform important functions for organisms, their degree of regulation is modest compared to most other enzymes that function in the NAD system. In addition, the degree of hype, framing and nonreproducibility in the sirtuin literature is persistently misleading. Dispassionate analysis of their functions does not support assignment as longevity enzymes or as principal mediators of the effects of NAD repletion.

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Competing interests

CB is chief scientific advisor of ChromaDex and Juvenis and a co-founder of Alphina Therapeutics.

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CB researched, wrote and edited this body of work.

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Fig. 1. Sir2 Enzymes are Gene Silencers in Fungi and are Conserved as NAD⁺-Dependent Protein Lysine Deacylases

a. In *S. cerevisiae*, the *SIR2* gene is required to silence genes in particular chromosomal locations. This function is to be conserved across divergent yeasts. **b.** The biochemical function of sirtuins is largely conserved. These enzymes deacylated protein lysine substrates in a manner that depends on NAD⁺ and which produces the deacylated protein lysine substrate, nicotinamide and acylated ADP-ribose.

Fig. 2. Sir2 favors a nonselected and disfavors a selected type of aging in budding yeast, both of which are extended by calorie restriction

a. In replicative aging, cells are arrayed on a petri dish for a two-week experiment in which daughter cells are removed every time the mother cell has replicated. The longevity benefit of CR—and the effect of *SIR2*—emerges after about 21.2 cell divisions¹⁶. **b.** In a yeast culture mothers who have divided 21.2 times constitute 1 in 4.8 million cells. Because a yeast culture can be regenerated from any cell, this is not a selected trait. **c.** Yeast have been recultured from the bottom of flasks, bottles and pottery for millenia: the ability to regrow a culture over an extended period of time after the original culture exhausted resources is a selected trait termed chronological lifespan. CR extends chronological lifespan in yeast and does so better with *sir2* deletion¹⁵.

Fig. 3. The fluorophore in peptide substrates used to screen for sirtuin activators interacts with resveratrol and other proposed activators

Sir2 was screened for activators using an acetylated peptide-methylcoumarinamide substrate in which the deacetylated product was incubated with trypsin to release aminomethylcoumarin, which is fluorogenic⁵⁸. Resveratrol and other activators interact with the reporter group^{64,65,72}.

References

- 1 Monod, J. *Chance and Necessity: Essay on the Natural Philosophy of Modern Biology*. (Vantage, 1971).
- 2 Haber, J. E. Mating-type genes and MAT switching in *Saccharomyces cerevisiae*. *Genetics* **191**, 33-64, doi:10.1534/genetics.111.134577 (2012).
- 3 Ivy, J. M., Klar, A. J. & Hicks, J. B. Cloning and characterization of four SIR genes of *Saccharomyces cerevisiae*. *Mol Cell Biol* **6**, 688-702, doi:10.1128/mcb.6.2.688-702.1986 (1986).
- 4 Rine, J. & Herskowitz, I. Four genes responsible for a position effect on expression from HML and HMR in *Saccharomyces cerevisiae*. *Genetics* **116**, 9-22 (1987).
- 5 Rine, J. *PhD Thesis*, University of Oregon, (1979).
- 6 Perez-Martin, J., Uria, J. A. & Johnson, A. D. Phenotypic switching in *Candida albicans* is controlled by a SIR2 gene. *EMBO J* **18**, 2580-2592, doi:10.1093/emboj/18.9.2580 (1999).
- 7 Freeman-Cook, L. L. *et al.* The *Schizosaccharomyces pombe* hst4(+) gene is a SIR2 homologue with silencing and centromeric functions. *Mol Biol Cell* **10**, 3171-3186, doi:10.1091/mbc.10.10.3171 (1999).
- 8 Landry, J. *et al.* The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc Natl Acad Sci U S A* **97**, 5807-5811 (2000).
- 9 Smith, J. S. *et al.* A phylogenetically conserved NAD⁺-dependent protein deacetylase activity in the Sir2 protein family. *Proc Natl Acad Sci U S A* **97**, 6658-6663 (2000).
- 10 Imai, S., Armstrong, C. M., Kaerberlein, M. & Guarente, L. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* **403**, 795-800 (2000).
- 11 Sauve, A. A. *et al.* Chemistry of gene silencing: the mechanism of NAD⁺-dependent deacetylation reactions. *Biochemistry* **40**, 15456-15463 (2001).
- 12 Bell, S. D., Botting, C. H., Wardleworth, B. N., Jackson, S. P. & White, M. F. The interaction of Alba, a conserved archaeal chromatin protein, with Sir2 and its regulation by acetylation. *Science* **296**, 148-151 (2002).
- 13 Denoth Lippuner, A., Julou, T. & Barral, Y. Budding yeast as a model organism to study the effects of age. *FEMS Microbiol Rev* **38**, 300-325, doi:10.1111/1574-6976.12060 (2014).
- 14 Sinclair, D. A. & Guarente, L. Extrachromosomal rDNA circles--a cause of aging in yeast. *Cell* **91**, 1033-1042 (1997).
- 15 Fabrizio, P. *et al.* Sir2 blocks extreme life-span extension. *Cell* **123**, 655-667 (2005).
- 16 Lin, S. J., Defossez, P. A. & Guarente, L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*. *Science* **289**, 2126-2128 (2000).
- 17 Kaerberlein, M., Kirkland, K. T., Fields, S. & Kennedy, B. K. Sir2-independent life span extension by calorie restriction in yeast. *PLoS Biol* **2**, E296 (2004).
- 18 Guarente, L. Sir2 links chromatin silencing, metabolism, and aging. *Genes Dev* **14**, 1021-1026 (2000).
- 19 Guarente, L. & Picard, F. Calorie restriction--the SIR2 connection. *Cell* **120**, 473-482, doi:10.1016/j.cell.2005.01.029 (2005).
- 20 Guarente, L. Calorie restriction and SIR2 genes--towards a mechanism. *Mech Ageing Dev* **126**, 923-928, doi:10.1016/j.mad.2005.03.013 (2005).

- 21 Wade, N. in *New York Times* A10 (2003).
- 22 Ros-Rocher, N., Perez-Posada, A., Leger, M. M. & Ruiz-Trillo, I. The origin of animals: an ancestral reconstruction of the unicellular-to-multicellular transition. *Open Biol* **11**, 200359, doi:10.1098/rsob.200359 (2021).
- 23 Rose, M. R. *Evolutionary Biology of Aging*. (Oxford University Press, 1994).
- 24 Brenner, C. Longevity Lessons. *Science* **377**, 718 (2022).
- 25 Rose, M. R., Rauser, C. L., Benford, G., Matos, M. & Mueller, L. D. Hamilton's forces of natural selection after forty years. *Evolution* **61**, 1265-1276, doi:10.1111/j.1558-5646.2007.00120.x (2007).
- 26 Johnson, A. A., Shokhirev, M. N. & Shoshitaishvili, B. Revamping the evolutionary theories of aging. *Ageing Res Rev* **55**, 100947, doi:10.1016/j.arr.2019.100947 (2019).
- 27 Gems, D. Evolution of sexually dimorphic longevity in humans. *Aging (Albany NY)* **6**, 84-91, doi:10.18632/aging.100640 (2014).
- 28 Pavard, S., CJ, E. M. & Heyer, E. Senescence of reproduction may explain adaptive menopause in humans: a test of the "mother" hypothesis. *Am J Phys Anthropol* **136**, 194-203, doi:10.1002/ajpa.20794 (2008).
- 29 Rose, M. R. Laboratory Evolution of Postponed Senescence in *Drosophila Melanogaster*. *Evolution* **38**, 1004-1010, doi:10.1111/j.1558-5646.1984.tb00370.x (1984).
- 30 Burke, M. K. *et al.* Genome-wide analysis of a long-term evolution experiment with *Drosophila*. *Nature* **467**, 587-590, doi:10.1038/nature09352 (2010).
- 31 Giuliani, C., Garagnani, P. & Franceschi, C. Genetics of Human Longevity Within an Eco-Evolutionary Nature-Nurture Framework. *Circ Res* **123**, 745-772, doi:10.1161/CIRCRESAHA.118.312562 (2018).
- 32 Lin, Y. J., Seroude, L. & Benzer, S. Extended life-span and stress resistance in the *Drosophila* mutant methuselah. *Science* **282**, 943-946, doi:10.1126/science.282.5390.943 (1998).
- 33 Song, W. *et al.* Presynaptic regulation of neurotransmission in *Drosophila* by the g protein-coupled receptor methuselah. *Neuron* **36**, 105-119, doi:10.1016/s0896-6273(02)00932-7 (2002).
- 34 de Mendoza, A., Jones, J. W. & Friedrich, M. Methuselah/Methuselah-like G protein-coupled receptors constitute an ancient metazoan gene family. *Sci Rep* **6**, 21801, doi:10.1038/srep21801 (2016).
- 35 Kimura, K. D., Tissenbaum, H. A., Liu, Y. & Ruvkun, G. *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* **277**, 942-946, doi:10.1126/science.277.5328.942 (1997).
- 36 Kenyon, C., Chang, J., Gensch, E., Rudner, A. & Tabtiang, R. A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461-464, doi:10.1038/366461a0 (1993).
- 37 Bartke, A. & Brown-Borg, H. Life extension in the dwarf mouse. *Curr Top Dev Biol* **63**, 189-225, doi:10.1016/S0070-2153(04)63006-7 (2004).
- 38 Bartke, A. Healthy aging: is smaller better? - a mini-review. *Gerontology* **58**, 337-343, doi:10.1159/000335166 (2012).
- 39 Venter, J. C. *et al.* The sequence of the human genome. *Science* **291**, 1304-1351, doi:10.1126/science.1058040 (2001).
- 40 Garber, K. Beyond the Nobel Prize: cell cycle research offers new view of cancer. *J Natl Cancer Inst* **93**, 1766-1768, doi:10.1093/jnci/93.23.1766 (2001).

- 41 Tissenbaum, H. A. & Guarente, L. Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. *Nature* **410**, 227-230 (2001).
- 42 Gems, D. Ageing. Yeast longevity gene goes public. *Nature* **410**, 154-155, doi:10.1038/35065765 (2001).
- 43 Rogina, B. & Helfand, S. L. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc Natl Acad Sci U S A* **101**, 15998-16003 (2004).
- 44 Chen, D. & Guarente, L. SIR2: a potential target for calorie restriction mimetics. *Trends Mol Med* **13**, 64-71, doi:10.1016/j.molmed.2006.12.004 (2007).
- 45 Dali-Youcef, N. *et al.* Sirtuins: the 'magnificent seven', function, metabolism and longevity. *Ann Med* **39**, 335-345, doi:10.1080/07853890701408194 (2007).
- 46 Sinclair, D. A. & LaPlante, M. D. *Lifespan: Why We Age—and Why We Don't Have To*. (Simon & Schuster, 2019).
- 47 Burnett, C. *et al.* Absence of effects of Sir2 overexpression on lifespan in *C. elegans* and *Drosophila*. *Nature* **477**, 482-485, doi:10.1038/nature10296 (2011).
- 48 Viswanathan, M. & Guarente, L. Regulation of *Caenorhabditis elegans* lifespan by sir-2.1 transgenes. *Nature* **477**, E1-2, doi:10.1038/nature10440 (2011).
- 49 Whitaker, R. *et al.* Increased expression of *Drosophila* Sir2 extends life span in a dose-dependent manner. *Aging (Albany NY)* **5**, 682-691, doi:10.18632/aging.100599 (2013).
- 50 Zhao, Y., Wang, H., Poole, R. J. & Gems, D. A fln-2 mutation affects lethal pathology and lifespan in *C. elegans*. *Nat Commun* **10**, 5087, doi:10.1038/s41467-019-13062-z (2019).
- 51 Newman, B. L., Lundblad, J. R., Chen, Y. & Smolik, S. M. A *Drosophila* homologue of Sir2 modifies position-effect variegation but does not affect life span. *Genetics* **162**, 1675-1685, doi:10.1093/genetics/162.4.1675 (2002).
- 52 Slade, J. D. & Staveley, B. E. Extended longevity and survivorship during amino-acid starvation in a *Drosophila* Sir2 mutant heterozygote. *Genome* **59**, 311-318, doi:10.1139/gen-2015-0213 (2016).
- 53 Venz, R., Pecek, T., Katic, I., Ciosk, R. & Ewald, C. Y. End-of-life targeted degradation of DAF-2 insulin/IGF-1 receptor promotes longevity free from growth-related pathologies. *Elife* **10**, doi:10.7554/eLife.71335 (2021).
- 54 Guarente, L. Franklin H. Epstein Lecture: Sirtuins, aging, and medicine. *N Engl J Med* **364**, 2235-2244, doi:10.1056/NEJMra1100831 (2011).
- 55 Fremont, L. Biological effects of resveratrol. *Life Sci* **66**, 663-673, doi:10.1016/s0024-3205(99)00410-5 (2000).
- 56 Renaud, S. & Gueguen, R. The French paradox and wine drinking. *Novartis Found Symp* **216**, 208-217; discussion 217-222, 152-208, doi:10.1002/9780470515549.ch13 (1998).
- 57 Ndlovu, T., van Jaarsveld, F. & Caleb, O. J. French and Mediterranean-style diets: Contradictions, misconceptions and scientific facts-A review. *Food Res Int* **116**, 840-858, doi:10.1016/j.foodres.2018.09.020 (2019).
- 58 Howitz, K. T. *et al.* Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* **425**, 191-196 (2003).
- 59 Finkel, T. Ageing: a toast to long life. *Nature* **425**, 132-133, doi:10.1038/425132a (2003).
- 60 Hall, S. S. Longevity research. In vino vitalis? Compounds activate life-extending genes. *Science* **301**, 1165, doi:10.1126/science.301.5637.1165 (2003).

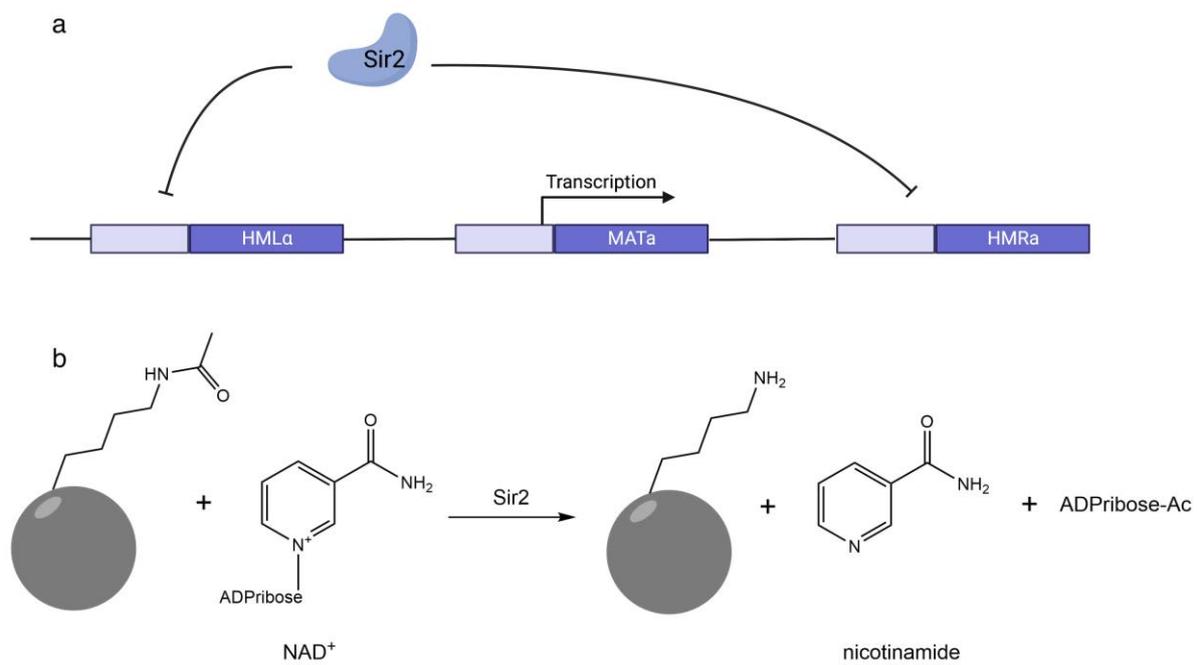
- 61 Lamming, D. W., Wood, J. G. & Sinclair, D. A. Small molecules that regulate lifespan: evidence for xenohormesis. *Mol Microbiol* **53**, 1003-1009, doi:10.1111/j.1365-2958.2004.04209.x (2004).
- 62 Howitz, K. T. & Sinclair, D. A. Xenohormesis: sensing the chemical cues of other species. *Cell* **133**, 387-391, doi:10.1016/j.cell.2008.04.019 (2008).
- 63 Baur, J. A. & Sinclair, D. A. What is Xenohormesis? *Am J Pharmacol Toxicol* **3**, 152-159, doi:10.3844/ajptsp.2008.152.159 (2008).
- 64 Kaeberlein, M. *et al.* Substrate-specific activation of sirtuins by resveratrol. *J Biol Chem* **280**, 17038-17045 (2005).
- 65 Borra, M. T., Smith, B. C. & Denu, J. M. Mechanism of human SIRT1 activation by resveratrol. *J Biol Chem* **280**, 17187-17195 (2005).
- 66 Wood, J. G. *et al.* Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* **430**, 686-689 (2004).
- 67 Bass, T. M., Weinkove, D., Houthoofd, K., Gems, D. & Partridge, L. Effects of resveratrol on lifespan in *Drosophila melanogaster* and *Caenorhabditis elegans*. *Mech Ageing Dev* **128**, 546-552, doi:10.1016/j.mad.2007.07.007 (2007).
- 68 Shaito, A. *et al.* Potential Adverse Effects of Resveratrol: A Literature Review. *Int J Mol Sci* **21**, doi:10.3390/ijms21062084 (2020).
- 69 Baur, J. A. *et al.* Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **444**, 337-342, doi:nature05354 [pii] 10.1038/nature05354 (2006).
- 70 Rodgers, J. T. *et al.* Nutrient control of glucose homeostasis through a complex of PGC-1 α and SIRT1. *Nature* **434**, 113-118 (2005).
- 71 Milne, J. C. *et al.* Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature* **450**, 712-716, doi:10.1038/nature06261 (2007).
- 72 Pacholec, M. *et al.* SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. *J Biol Chem* **285**, 8340-8351, doi:10.1074/jbc.M109.088682 (2010).
- 73 Schmidt, C. GSK/Sirttris compounds dogged by assay artifacts. *Nat Biotechnol* **28**, 185-186, doi:10.1038/nbt0310-185 (2010).
- 74 Hubbard, B. P. *et al.* Evidence for a common mechanism of SIRT1 regulation by allosteric activators. *Science* **339**, 1216-1219, doi:10.1126/science.1231097 (2013).
- 75 Canto, C. *et al.* Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle. *Cell Metab* **11**, 213-219, doi:10.1016/j.cmet.2010.02.006 (2010).
- 76 Brenner, C. & Boileau, A. C. Pterostilbene raises low density lipoprotein cholesterol in people. *Clin Nutr* **38**, 480-481, doi:10.1016/j.clnu.2018.10.007 (2019).
- 77 Bordone, L. *et al.* SIRT1 transgenic mice show phenotypes resembling calorie restriction. *Aging Cell* **6**, 759-767, doi:10.1111/j.1474-9726.2007.00335.x (2007).
- 78 Imai, S. Is Sirt1 a miracle bullet for longevity? *Aging Cell* **6**, 735-737, doi:10.1111/j.1474-9726.2007.00344.x (2007).
- 79 Pfluger, P. T., Herranz, D., Velasco-Miguel, S., Serrano, M. & Tschop, M. H. Sirt1 protects against high-fat diet-induced metabolic damage. *Proc Natl Acad Sci U S A* **105**, 9793-9798, doi:10.1073/pnas.0802917105 (2008).
- 80 Banks, A. S. *et al.* Sirt1 gain of function increases energy efficiency and prevents diabetes in mice. *Cell Metab* **8**, 333-341, doi:10.1016/j.cmet.2008.08.014 (2008).

- 81 Herranz, D. *et al.* Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer. *Nat Commun* **1**, 3, doi:10.1038/ncomms1001 (2010).
- 82 Satoh, A. *et al.* Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. *Cell Metab* **18**, 416-430, doi:10.1016/j.cmet.2013.07.013 (2013).
- 83 Chen, D., Steele, A. D., Lindquist, S. & Guarente, L. Increase in activity during calorie restriction requires Sirt1. *Science* **310**, 1641 (2005).
- 84 Satoh, A. *et al.* SIRT1 promotes the central adaptive response to diet restriction through activation of the dorsomedial and lateral nuclei of the hypothalamus. *J Neurosci* **30**, 10220-10232, doi:10.1523/JNEUROSCI.1385-10.2010 (2010).
- 85 Boily, G. *et al.* SirT1 regulates energy metabolism and response to caloric restriction in mice. *PLoS One* **3**, e1759, doi:10.1371/journal.pone.0001759 (2008).
- 86 Caron, A. Z. *et al.* The SIRT1 deacetylase protects mice against the symptoms of metabolic syndrome. *FASEB J* **28**, 1306-1316, doi:10.1096/fj.13-243568 (2014).
- 87 Assali, D. R. *et al.* Food anticipatory activity on a calorie-restricted diet is independent of Sirt1. *PLoS One* **13**, e0199586, doi:10.1371/journal.pone.0199586 (2018).
- 88 Kerr, N. L. HARKing: hypothesizing after the results are known. *Pers Soc Psychol Rev* **2**, 196-217, doi:10.1207/s15327957pspr0203_4 (1998).
- 89 Flachsbart, F. *et al.* Sirtuin 1 (SIRT1) sequence variation is not associated with exceptional human longevity. *Exp Gerontol* **41**, 98-102, doi:10.1016/j.exger.2005.09.008 (2006).
- 90 Kuningas, M., Putters, M., Westendorp, R. G., Slagboom, P. E. & van Heemst, D. SIRT1 gene, age-related diseases, and mortality: the Leiden 85-plus study. *J Gerontol A Biol Sci Med Sci* **62**, 960-965, doi:10.1093/gerona/62.9.960 (2007).
- 91 DeVito, N. J. & Goldacre, B. Catalogue of bias: publication bias. *BMJ Evid Based Med* **24**, 53-54, doi:10.1136/bmjebm-2018-111107 (2019).
- 92 Entman, R. M. Framing Bias: Media in the Distribution of Power. *J Communication* **57**, 163-173 (2007).
- 93 Del Vicario, M., Scala, A., Caldarelli, G., Stanley, H. E. & Quattrocioni, W. Modeling confirmation bias and polarization. *Sci Rep* **7**, 40391, doi:10.1038/srep40391 (2017).
- 94 Cimen, H. *et al.* Regulation of succinate dehydrogenase activity by SIRT3 in mammalian mitochondria. *Biochemistry* **49**, 304-311, doi:10.1021/bi901627u (2010).
- 95 Hirschey, M. D. *et al.* SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. *Nature* **464**, 121-125, doi:nature08778 [pii] 10.1038/nature08778 (2010).
- 96 Qiu, X., Brown, K., Hirschey, M. D., Verdin, E. & Chen, D. Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. *Cell Metab* **12**, 662-667, doi:S1550-4131(10)00407-9 [pii] 10.1016/j.cmet.2010.11.015 (2010).
- 97 Ghanta, S., Grossmann, R. E. & Brenner, C. Mitochondrial protein acetylation as a cell-intrinsic, evolutionary driver of fat storage: chemical and metabolic logic of acetyl-lysine modifications. *Crit Rev Biochem Mol Biol* **48**, 561-574, doi:10.3109/10409238.2013.838204 (2013).
- 98 Wagner, G. R. & Hirschey, M. D. Nonenzymatic protein acylation as a carbon stress regulated by sirtuin deacylases. *Mol Cell* **54**, 5-16, doi:10.1016/j.molcel.2014.03.027 (2014).

- 99 Pillai, V. B. *et al.* Sirt3 protects mitochondrial DNA damage and blocks the development of doxorubicin-induced cardiomyopathy in mice. *Am J Physiol Heart Circ Physiol* **310**, H962-972, doi:10.1152/ajpheart.00832.2015 (2016).
- 100 Kincaid, B. & Bossy-Wetzel, E. Forever young: SIRT3 a shield against mitochondrial meltdown, aging, and neurodegeneration. *Front Aging Neurosci* **5**, 48, doi:10.3389/fnagi.2013.00048 (2013).
- 101 Mostoslavsky, R. *et al.* Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* **124**, 315-329, doi:10.1016/j.cell.2005.11.044 (2006).
- 102 Kanfi, Y. *et al.* The sirtuin SIRT6 regulates lifespan in male mice. *Nature* **483**, 218-221, doi:10.1038/nature10815 (2012).
- 103 Roichman, A. *et al.* Restoration of energy homeostasis by SIRT6 extends healthy lifespan. *Nat Commun* **12**, 3208, doi:10.1038/s41467-021-23545-7 (2021).
- 104 Palliyaguru, D. L. *et al.* Fasting blood glucose as a predictor of mortality: Lost in translation. *Cell Metab* **33**, 2189-2200 e2183, doi:10.1016/j.cmet.2021.08.013 (2021).
- 105 Kulkarni, A. S., Gubbi, S. & Barzilai, N. Benefits of Metformin in Attenuating the Hallmarks of Aging. *Cell Metab* **32**, 15-30, doi:10.1016/j.cmet.2020.04.001 (2020).
- 106 Madiraju, A. K. *et al.* Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature* **510**, 542-546, doi:10.1038/nature13270 (2014).
- 107 Duquesnoy, R. J., Christensen, K., Pedersen, G. M. & Kemp, R. G. Development of Immunodeficiency of Pituitary Dwarf Mice. *Amer Zool* **15**, 167-174 (1975).
- 108 Bieganowski, P. & Brenner, C. Discoveries of nicotinamide riboside as a nutrient and conserved NRK genes establish a preiss-handler independent route to NAD⁺ in fungi and humans. *Cell* **117**, 495-502, doi:10.1016/S0092-8674(04)00416-7 (2004).
- 109 Belenky, P. *et al.* Nicotinamide Riboside Promotes Sir2 Silencing and Extends Lifespan via Nrk and Urh1/Pnp1/Meu1 Pathways to NAD⁺. *Cell* **129**, 473-484, doi:10.1016/j.cell.2007.03.024 (2007).
- 110 Canto, C. *et al.* The NAD(+) precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity. *Cell Metab* **15**, 838-847, doi:10.1016/j.cmet.2012.04.022 (2012).
- 111 Mills, K. F. *et al.* Long-Term Administration of Nicotinamide Mononucleotide Mitigates Age-Associated Physiological Decline in Mice. *Cell Metab* **24**, 795-806, doi:10.1016/j.cmet.2016.09.013 (2016).
- 112 Belenky, P., Bogan, K. L. & Brenner, C. NAD⁺ metabolism in health and disease. *Trends in Biochemical Sciences* **32**, 12-19, doi:10.1016/j.tibs.2006.11.006 (2007).
- 113 Trammell, S. A. & Brenner, C. Targeted, LCMS-based Metabolomics for Quantitative Measurement of NAD(+) Metabolites. *Comput Struct Biotechnol J* **4**, e201301012, doi:10.5936/csbj.201301012 (2013).
- 114 Trammell, S. A. *et al.* Nicotinamide Riboside Opposes Type 2 Diabetes and Neuropathy in Mice. *Sci Rep* **6**, 26933, doi:10.1038/srep26933 (2016).
- 115 Di Stefano, M. *et al.* A rise in NAD precursor nicotinamide mononucleotide (NMN) after injury promotes axon degeneration. *Cell Death Differ*, doi:10.1038/cdd.2014.164 (2014).
- 116 Vaur, P. *et al.* Nicotinamide riboside, a form of vitamin B3, protects against excitotoxicity-induced axonal degeneration. *FASEB J* **31**, 5440-5452, doi:10.1096/fj.201700221RR (2017).

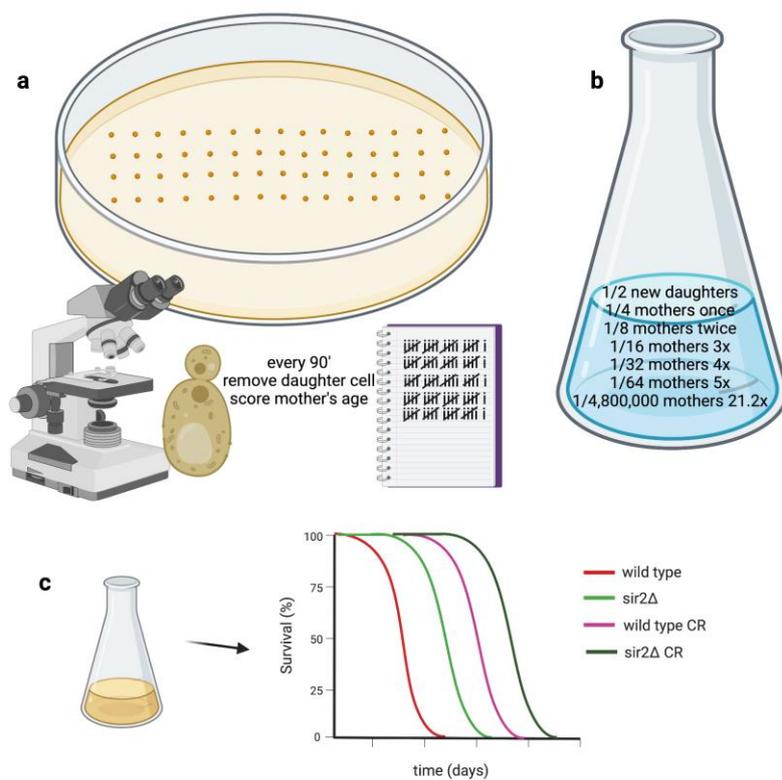
- 117 Brown, K. D. *et al.* Activation of SIRT3 by the NAD⁺ precursor nicotinamide riboside protects from noise-induced hearing loss. *Cell Metab* **20**, 1059-1068 (2014).
- 118 Murata, M. M. *et al.* NAD⁺ consumption by PARP1 in response to DNA damage triggers metabolic shift critical for damaged cell survival. *Mol Biol Cell* **30**, 2584-2597, doi:10.1091/mbc.E18-10-0650 (2019).
- 119 Diguët, N. *et al.* Nicotinamide Riboside Preserves Cardiac Function in a Mouse Model of Dilated Cardiomyopathy. *Circulation* **137**, 2256-2273, doi:10.1161/CIRCULATIONAHA.116.026099 (2018).
- 120 Ear, P. H. *et al.* Maternal Nicotinamide Riboside Enhances Postpartum Weight Loss, Juvenile Offspring Development, and Neurogenesis of Adult Offspring. *Cell Rep* **26**, 969-983 e964, doi:10.1016/j.celrep.2019.01.007 (2019).
- 121 Fons, N. R. *et al.* PPM1D mutations silence NAPRT gene expression and confer NAMPT inhibitor sensitivity in glioma. *Nat Commun* **10**, 3790, doi:10.1038/s41467-019-11732-6 (2019).
- 122 Parker, R., Schmidt, M. S., Cain, O., Gunson, B. & Brenner, C. The NAD Metabolome is Functionally Depressed in Patients Undergoing Liver Transplantation for Alcohol-related Liver Disease. *Hepatology Communications* **4**, in press (2020).
- 123 Nam, T. S. *et al.* Interleukin-8 drives CD38 to form NAADP from NADP(+) and NAAD in the endolysosomes to mobilize Ca(2+) and effect cell migration. *FASEB J* **34**, 12565-12576, doi:10.1096/fj.202001249R (2020).
- 124 Heer, C. D. *et al.* Coronavirus infection and PARP expression dysregulate the NAD metabolome: An actionable component of innate immunity. *J Biol Chem* **295**, 17986-17996, doi:10.1074/jbc.RA120.015138 (2020).
- 125 Pirinen, E. *et al.* Niacin Cures Systemic NAD(+) Deficiency and Improves Muscle Performance in Adult-Onset Mitochondrial Myopathy. *Cell Metab* **31**, 1078-1090 e1075, doi:10.1016/j.cmet.2020.04.008 (2020).
- 126 Covarrubias, A. J. *et al.* Senescent cells promote tissue NAD(+) decline during ageing via the activation of CD38(+) macrophages. *Nat Metab* **2**, 1265-1283, doi:10.1038/s42255-020-00305-3 (2020).
- 127 Zhao, Z. Y. *et al.* A Cell-Permeant Mimetic of NMN Activates SARM1 to Produce Cyclic ADP-Ribose and Induce Non-apoptotic Cell Death. *iScience* **15**, 452-466, doi:10.1016/j.isci.2019.05.001 (2019).

Figure 1



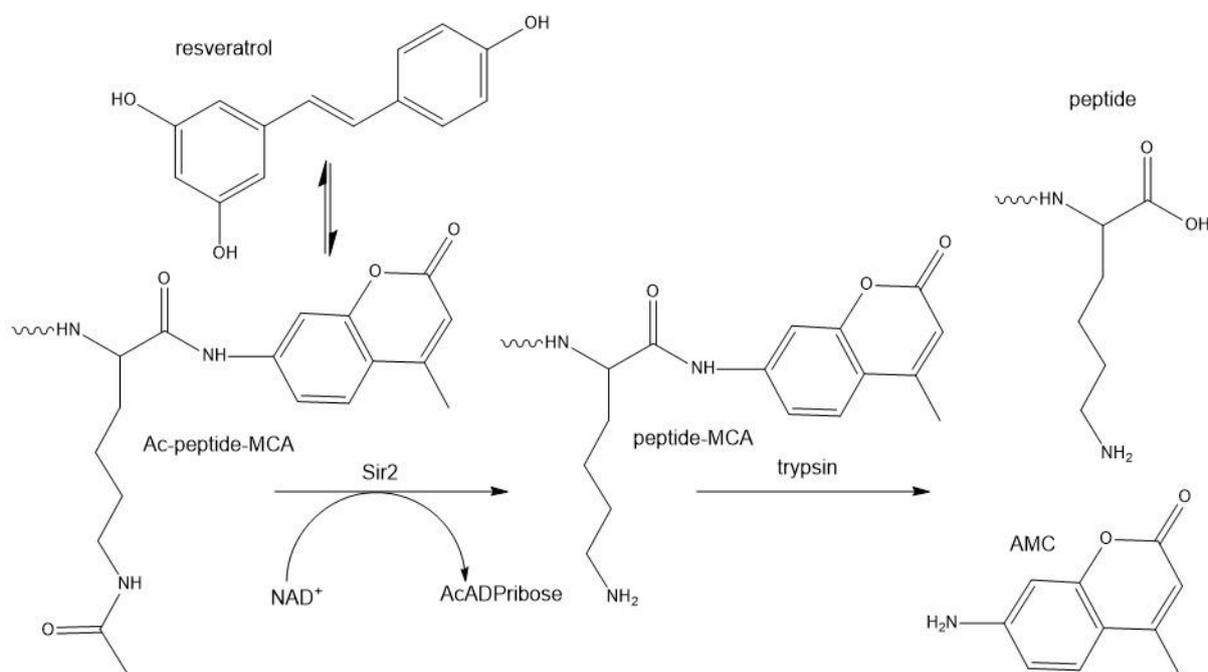
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Figure 2



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Figure 3



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