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Targeting Chromatin Aging - The Epigenetic Impact of Longevity-Associated Interventions

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"Targeting Chromatin <u>Aging</u> - The Epigenetic Impact of <u>Longevity-Associated</u> Interventions"

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0.0 Abstract

A rapidly growing body of evidence has shown that chromatin undergoes radical alterations as an organism ages, but how these changes relate to <u>aging</u> itself is an open question. It is likely that these processes contribute to genomic instability and loss of transcriptional fidelity, which in turn drives deleterious <u>age-related</u> phenotypes. Interventions associated with increased healthspan and longevity such as reduced insulin / IGF signalling (IIS), inhibition of mTOR and energy depletion resulting in SIRT1 / AMPK activation, all have beneficial effects which ameliorate multiple facets of <u>age-associated</u> decline. The impact of these interventions on the epigenome is less certain. In this review we highlight the potential of these interventions to act directly upon the epigenome and promote a youthful chromatin landscape, maintaining genetic and transcriptional memory throughout the lifecourse. We propose that this is a fundamental mechanism through which these interventions are able to curtail the incidence of <u>age-related</u> disease. By revisiting these well characterised interventions, we may be able to identify targetable effectors of chromatin function and use this knowledge to enhance healthspan and longevity in human populations through the measured application of dietary and small molecule interventions.

1.0 Introduction

Aging is generally defined as the increasing morbidity and mortality of an organism with time following the onset of sexual maturity. It is clear that even among multicellular organisms, aging is not universal (Gampe et al., 2015). Even in those organisms which do age there is a striking variation in longevity even between biologically similar organisms. The origins of this divergence are far from certain and are the focus of intense research. Thanks to pioneering work by Kenyon et al it is clear that the aging process is plastic, capable of being accelerated or attenuated by a range of small molecule dietary and genetic interventions (Kenyon, 2010). A clearer understanding of how the aging process can vary so wildly between species and within species, is a prerequisite for full application of this knowledge in the pursuit of improving public health.

1.1 Chromatin Degeneration is a Conserved Feature of Aging

The greatest barrier to health and longevity in any organism, is the maintenance of macromolecules critical to survival. Foremost is the DNA, in complex with histone and non-histone proteins collectively termed 'chromatin'. Chromatin and the mechanisms through which it modulates programs of gene expression, referred to as epigenetics, represents an attractive target for aging intervention as its down-stream effects are critical to all aspects of health and longevity. Disruption of epigenetic programs can therefore impact a huge spectrum of biological functions. By this logic it is hoped that through ensuring epigenetic stability, disease-free lifespan can be preserved.

During organismal aging the epigenome undergoes remodelling at both the histone and DNA level. Broadly speaking, it is believed that age-related chromatin degeneration is underscored by a

loss of heterochromatin at the regions it usually occupies from birth (Imai and Kitano, 1998; Villeponteau, 1997). A number of specific chromatin alterations define this process. Loss of trimethylation of histone 3 at lysine 9 (H3K9me3) and loss of histone protein 1 (HP1) are common features observed in invertebrates and humans. Flies with low HP1 are short lived and reconstitution of HP1 levels is sufficient to restore lifespan. Old flies show age-dependent loss of heterochromatin markers such as H3K9me2. In humans, lower abundance of both HP1 and H3K9me3 are observed in aged human fibroblasts and those suffering from the 'premature aging syndrome', Hutchinson-Gilford progeria (HGPS) (Larson et al., 2012; Scaffidi and Misteli, 2006). In the euchromatic regions of the genome, transcriptional dysregulation is accompanied by a loss of histone 3 lysine 36 trimethylation (H3K36me3) in invertebrates and yeast (Ni et al., 2012; Sen et al., 2015). On the level of DNA methylation, classic studies by Vanyushin et al showed that global cytosine methylation decreases globally with age in rats and mice, though more recent evidence of this phenomenon is lacking and no such trend has yet been well documented in humans (Vanyushin et al., 1973; Wilson et al., 1987). More recently it has been shown that global methylation levels do become more variable with age, in a phenomenon described as epigenetic drift (Egger et al., 2004). On a more local level, specific gene promoters display an age-dependant increase in methylation at CpG islands, associated with transcriptional repression (Waki et al., 2003).

1.2 Linking Chromatin and Longevity

Most successful longevity interventions are thought to act through metabolic effectors. Interventions such as calorie restriction and rapamycin generally work through inhibition of anabolic pathways, perhaps freeing the cell to invest resources into maintenance and stress resistance which functions to improve the health and longevity of the cell, tissue and ultimately the whole organism.

Chromatin represents a hub through which all manner of environmental queues are integrated and acted upon. The extent of cross-talk between the chromatin and the metabolome highlights the possibility that chromatin may represent a nexus upon which the proposed trade-off

between fecundity and longevity is decided, ultimately influencing the aging trajectories of the organism (Gut and Verdin, 2013).

The re-wiring of the cellular machinery towards a state conducive to longevity relies on epigenetic alterations to potentiate down-stream effects on gene expression. The fact that in some cases lifespan increases are preserved even after transient application and withdrawal of the intervention, or indeed are to one extent or another inherited by subsequent generations by epigenetic means, is evidence that epigenetic processes have a functional role in determining the rate of age-associated decline (Bitto et al., 2016; Greer et al., 2012).

Up to now most observations tying epigenetic processes to age-associated decline have been correlative. Interventions which impact the aging process will play a formative role in determining which chromatin alterations are drivers of aging, as opposed to being a result of aging itself. Accelerated aging models are replete with caveats which make them unsuitable for such a task, not least of all there is the so far unanswered question of how accurately they phenocopy physiological aging. Luckily there is a wealth of data to support the claim that interventions such as rapamycin, CR and reduced IIS directly impact true effectors of physiological aging in relatively 'natural' model contexts. The comparative study of epigenetic perturbations in young vs old organisms, with and without longevity-associated interventions, holds promise in delineating which epigenetic profiles are causative to the aging process and how they may be attenuated.

In this review, we will focus upon the direct impact of longevity interventions on two well characterised epigenetic read-outs, DNA methylation and histone post-translational modifications. We will propose models and mechanisms through which longevity may be preserved by ensuring epigenetic stability, as well as highlighting future approaches to apply these findings to enhance longevity and delineate epigenetic components of aging mechanisms. The subject of which epigenetic changes accompany the aging process will not be discussed further in this publication.

This topic has been the subject of a number of excellent reviews, to which the reader is directed in the following citations (Booth and Brunet, 2016; Brunet and Berger, 2014; Sen et al., 2016).

2.0 Longevity Interventions Impact Age-Associated Epigenetic Aberrations

Recent studies have focused on identification of age-associated chromatin marks, followed by mechanistic investigations usually through genetic modification to amplify or repress the marks in question and deduce its effects on known aging mechanisms. It is worth asking how these age-—associated changes respond in a long lived organism, to observe their effects in a physiological model of longevity. One would expect that should this epigenetic damage have a causative role in age-related decline, then such damage should be ameliorated or reversed upon application of interventions known to attenuate aging. This is indeed the case in a number of instances. (Table.1)

Organism	Mark	Change with Age	Phenotype	Intervention	Result	Citation
Rat	DNA methylation	Decrease	Hypomethylation of specific gene loci	CR	Methylation Increase at specific gene loci	(Hass et al., 1993)
Rat	DNA methylation	Increase / Decrease	Hyper/Hypomethylation of specific gene loci	CR	Attenuation of hyper/hypomethylation	(Kim et al., 2016)
Human	H3K4ac	Increase	Activation of P16	CR	H3K4ac reduced, suppression of P16	(Li and Tollefsbol, 2011)
Human	H3K9me3	Decrease	Activation of P16	CR	H3K9me3 increased, suppression of P16	(Li and Tollefsbol, 2011)
Mouse	DNA methylation	Decrease	Loss of global DNA methylation	Reduced growth hormone	Retention of global DNA methylation	(Armstrong et al., 2014)
Mouse	H3K27me3	Decrease	Loss in brain tissue	Rapamycin / CR	Retention of H3K27me3	(Gong et al., 2015)
Mouse	H3R2me2	Decrease	Loss in brain tissue	Rapamycin / CR	Retention of H3R2me2	(Gong et al., 2015)
Mouse	H3K79me3	Decrease	Loss in brain tissue	Rapamycin / CR	Retention of H3k79me3	(Gong et al., 2015)
Mouse	H4K20me3	Decrease	Loss in brain tissue	Rapamycin / CR	Retention of H4k20me3	(Gong et al., 2015)
Yeast	H3 Acetylation	Increase	Repression of autophagy, reduced lifespan	Spermidine	Reduced H3 acetylation, reversed phenotype	(Eisenberg et al., 2009)

Table.1 Effects of longevity-associated interventions on age-associated epigenetic alterations.

2.1 Diet and Calorie Restriction

Chromatin and the metabolome are inextricably linked through metabolic cofactors. Products of the TCA cycle, glycolysis and β-oxidation feed into chromatin modifying enzymes, altering their activity and tailoring epigenetic responses to the metabolic phenotype of the cell (Gut and Verdin, 2013). Calorie restriction (CR) is probably the best studied and most efficacious of the longevity promoting interventions. It is becoming increasingly clear that at least some of its effects are tied to epigenetic responses. Over ten years ago Hass et al demonstrated that pancreatic acinar cells isolated from CR rats were found to have more methylation present at specific gene loci, including well known proto-oncogenes such as Ras, when compared to an ad-libitum fed rats. Ex-vivo cultures of cells isolated from the CR mice were more resistant to oncogenic transformation. This suggested that CR may protect from age-associated decline by promoting maintenance methylation at tissue specific repressed genes, preventing spurious activation. This effect appeared to be mediated through an increase in the activity of maintenance methyltransferase DNMT1 (Hass et al., 1993).

Related observations around this time showed that age-related DNA methylation of the ER gene CpG island correlates with colon neoplasia (Issa et al., 1994). It is now clear that a wide variety of genes including tumor suppressors become progressively more methylated with age, more than likely contributing to the age-related incidence of diseases such as cancer (So et al., 2006; Waki et al., 2003). It seems clear that the age-dependent loss of the cell type specific transcriptional regulation and genomic integrity afforded to cells by their genome methylation profile is a causative factor in age-related decline (Rakyan et al., 2010; Teschendorff and Menon, 2010). That these changes may be attenuated by CR is an exciting prospect.

The more recent application of next-generation sequencing technologies to the question of DNA methylation has yielded more promising results. Short term CR in 25-month-old rats seemed to attenuate age-dependant methylation alterations in the promoters of genes associated with a large

number of age-associated degenerative phenotypes including cancer diabetes and inflammation (Kim et al., 2016). However, this study is not without its limitations. In the future studies using unbiased whole-genome approaches at a single base-pair resolution, to map changes in the methylation landscape in response to age and longevity interventions such as CR, will need to be performed. These will reveal the precise magnitude and location of these alterations, as well as their potential to be attenuated by interventions. More work needs to be done to delineate the effects of age-related methylation events and investigate whether their reversal by CR is a potential causal mechanism in CRs longevity promoting effects.

CRs effects on the epigenome are not limited to DNA methylation. CR seems to increase the activity of histone deacetylases (HDACs), particularly SIRT1, which during CR is localized to age-associated genes such as p16. This contributes to their repression through H3 hypoacetylation particularly at lysine 4, with an associated increase in H3K9me3 at the p16 promoter. This effect increases the replicative lifespan of human fibroblasts in vitro (Li and Tollefsbol, 2011).

The evidence seems to point towards epigenetic reprogramming playing an important role in shaping the mammalian cell's gene expression program toward a state conducive to longer life and reduced disease incidence.

2.2 Reduced Growth Hormone Signalling

The Ames dwarf mouse is a long lived mouse strain, living almost 50% longer than matched wild-type mice. They are characterised by a disabling mutation in the Prop1 gene, resulting in reduced circulating growth hormone, IGF1, prolactin and thyroid stimulating hormone by virtue of an underdeveloped pituitary gland. Similarly to CR mice, Ames dwarf mice have been found to have lower protein expression of DNMT1 and higher expression of DNMT3a (Armstrong et al., 2014), compared to normal age matched counterparts, suggesting a role for DNA methylation in the Ames

dwarf's maintenance of health and longevity. Global analysis of Ames dwarf DNA methylation suggested that the dwarf mouse may maintain DNA methylation better throughout life-course than its wildtype counterparts (Armstrong et al., 2014).

It is worth considering how the DNA methylome of Ames dwarf mice might be linked to its metabolome. For example, Ames dwarf mice exhibit elevated glycine N-methyltransferase (GNMT) (an enzyme that converts S-adenosyl-methionine (SAM) to S-adenosyl-homocysteine and sarcosine), and correspondingly lower levels of SAM, a substrate for DNA methyl transferases (Uthus and Brown-Borg, 2003, 2006). Thus, elevated GNMT in Ames dwarf mice might depress the typical age-associated methylation of CpG islands (Rakyan et al., 2010; Teschendorff and Menon, 2010), perhaps contributing to delayed cancer incidence in these mice (Ikeno et al., 2003).

2.3 Rapamycin and TOR inhibition

The mammalian target of rapamycin (mTOR) is a master metabolic switch governing resource allocation in the cell. Its activation by growth factors and bountiful amino acid availability drives cell anabolism through activation of protein translation through signalling to the S6 ribosomal protein. It's repression under conditions of nutrient deprivation or rapamycin treatment, drives cellular catabolism, de-repressing autophagic programs and shutting down protein synthesis to free up cytosolic resources. mTOR inhibition has been broadly implicated as a longevity assurance mechanism (Johnson et al., 2013). Rapamycin, probably the most well characterised means by which to inhibit mTOR, has been repeatedly demonstrated to increase lifespan and improve healthspan in a range of models (Wilkinson et al., 2012).

With mTOR occupying such a vital node in metabolic resource allocation, it seems likely that these effects would be propagated through epigenetic means. Indeed, recent in-vivo evidence suggests that the longevity effects associated with rapamycin persist after transient application and withdrawal in middle aged mice, with the length of longevity gain being proportional to the amount of time spent on rapamycin. This may suggest that some form of epigenetic reprogramming

underlies rapamycin's longevity enhancing effects and undoubtedly warrants further investigation (Bitto et al., 2016).

Evidence to this effect was observed in a recent study by Gong et al on the brain tissues of young and old mice, compared to those treated with longevity interventions including rapamycin. Brain tissues of young (6 month) and old (22 month) were isolated and analysed for a number of histone modifications. Seven modifications were found to be lost with age. Intriguingly, treatment with rapamycin for 3 months from 19 months of age reversed the loss of four of these histone marks H3K27me3, H3R2me2, H3K79me3 and H4K20me2 (Gong et al., 2015).

It is worth noting that Gong observed similar results in life-long CR treated mice, maintained at 70% of their recommended calorie intake. That both of these interventions show similar effects suggests shared mechanisms are at play, implicating mTOR inhibition in maintenance of histone modification profiles with age.

2.4 Histone Deacetylase Inhibitors

Histone acetylation is thought to be associated with gene activation through cumulative negation of histones' positive charge, causing disruption of the nucleosome on the chromatin and ultimately generation of a more open chromatin structure (Zentner and Henikoff, 2013). Altered histone acetylation profiles have been associated with aging phenotypes. Most convincingly, increased histone acetylation seems to correlate with reduced lifespan in yeast and drosophila. Inhibition of lysine acetyl transferases and acetyl-CoA synthases resulted in H3 and H4 hypoacetylation and increased lifespan (Eisenberg et al., 2014). These observations open up the possibility that by ameliorating global age-related histone hyperacetylation using small molecule and dietary intervention, we may be able to enhance health and longevity.

Spermidine is a naturally occurring polyamine that has been shown to counteract age-related hyperacetylation of histones related to age-dependant reduction in polyamine metabolism. Introduction of spermidine to aged yeast cells reversed hyperacetylation of histone H3 and activated gene programs associated with autophagy and cell stress resistance promoting longevity. Depletion of polyamines from growth media resulted in hyperacetylation and early death (Eisenberg et al., 2009).

The hyperacetylation paradigm runs counter to observations that histone deacetylase inhibitors can enhance healthspan and longevity (Krishnan et al., 2011; Zhao et al., 2005). It is becoming evident that histone acetylation plays a more nuanced role in gene regulation, with site specific changes in histone acetylation playing just as important a role as more global alterations (Peleg et al., 2016). It is notable that lifespan increases associated with treatment with histone deacetylase inhibitors (HDACi's) such as trichostatin A (TSA) and butyrate often show more targeted gene-specific effects (Tao et al., 2004; Walsh et al., 2015). There may be a role for both approaches in maximising health and longevity, whereby global reduction in histone acetylation can be paired with gene specific retention of histone acetylation to maintain optimal gene expression programs through the life course, by carefully tailored application of nutritional interventions in conjunction with HDAC inhibitors. Also worth considering, but beyond the scope of this review, are the diverse effects of HDACs and histone acetyltransferases (HATs) on none-histone substrates.

3.0 A Two-Tier Strategy for Targeting Age-related Chromatin Decline

Global and site-specific chromatin rearrangements accompany aging. Evidence is mounting that these changes have profound deleterious effects on cell homeostasis, gene expression programs and genomic integrity, which can drive the aging phenotype (López-otín et al., 2013).

The literature highlighted here is proof that these alterations can be attenuated through genetic, small molecule and dietary means and through doing so, we can ameliorate age-related decline. However discrepancies remain. Though we may broadly describe the landscapes of the aging epigenome as undergoing 'heterochromatin loss' or 'global hypomethylation', the true nature of age-related chromatin decline can only be satisfactorily described by the product of gross chromatin alterations such as these and the myriad of small site-specific often gene-level changes in specific histone modifications or DNA methylation events. Both tiers of chromatin organisation must be carefully considered and factored in to any future strategy designed to alleviate epigenetic dysfunction. We may pragmatically group interventions into two categories based on the scope of their action.

Interventions can be considered 'globally acting' if they have the potential to affect the availability of a large range of co-factors and may drive non-specific chromatin rearrangements or suppress age-associated global changes. Global shifts in metabolic cofactors brought about by calorie restriction or macronutrient deprivation may alter cellular availability of enzyme co-factors and substrates, such as s-adenosyl-methionine (SAM), acetyl-Co-A (Ac-CoA) and nicotinamide adenine dinucleotide (NAD), that will affect all enzymes which rely on these factors, many of which being controllers of chromatin methylation and acetylation events. Case in point being that a calorie restricted diet seems to attenuate global loss of DNA methylation, potentially by altering the co-factor abundance of alpha ketoglutarate (αKG) which drives demethylation through TET family demethylases, or SAM which is primary methyl donor for a wide range of methyltransferases (Gut and Verdin, 2013). Future studies may wish to dissect how these dietary interventions alter metabolism and what ramifications these changes have for the epigenome and maintenance of a youthful chromatin profile.

It is the case that alterations which are beneficial on a global scale, may be deleterious at the local level for certain genes. Targeting the age-associated trend towards global hypomethylation

may beneficially promote genome stability. However an unbiased approach promoting enhanced

global methylation may inadvertently promote hypermethylation of <u>tumor</u> suppressor genes or

longevity assurance genes, negating beneficial effects. A similar scenario can be envisioned for

histone acetylation and methylation. This <u>realization</u> makes it readily apparent that we require more

knowledge on which chromatin modifying enzymes regulate which genes at a local level and how

they do so.

When we reconcile these two tiers, we may rationally design nutritional and

pharmacological interventions which can buffer against deleterious changes to the global chromatin

landscape, whilst protecting longevity and health promoting gene circuitry through the rational

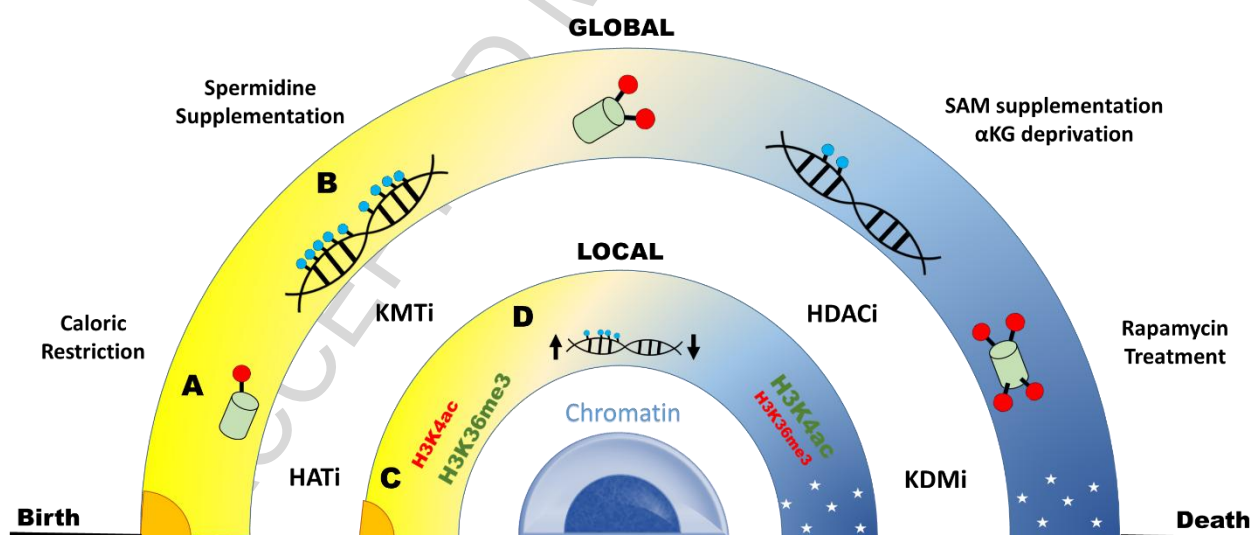application of more targeted interventions (Figure.1).

Fig.1 Visual representation of two-tier approach to ameliorating age-related chromatin decline. Global focusing on broad dietary and supplementary interventions to counteract **A** Histone hyperacetylation **B** DNA hypomethylation. Local focusing on rational design of targeted small molecule combination therapies to counteract **C** Gene-level alterations in histone PTM profiles **D** Gene-level alterations In DNA methylation profiles.

4.0 Concluding remarks

A small but important literature has demonstrated that longevity interventions attenuate

<u>age-associated</u> chromatin decline. The result should be cause for excitement and will hopefully

inspire more studies to answer our first fundamental questions in this regard. It will be interesting to see how much overlap exists between different longevity interventions and the epigenetic phenotypes they influence. Future studies may highlight the importance of shared chromatin alterations in the pathophysiology of age-related disease and could hint at shared effector pathways of known interventions. This is certainly a question worth pursuing, as it could open up new avenues by which we can leverage old and new interventions to model the epigenome to a state conducive to longevity.

The literature has demonstrated that through a case-control based study design, we can implicate new chromatin marks in age-related epigenetic progression (Fig.2). Gong et al., (2015) showed in their study that aged mice show a number of changes to their histone PTM profile compared to young mice, some of which were new to age-related cognitive decline, the phenotype in question. Further information was gleaned by the fact that some of these marks are reversed upon application of longevity interventions. This may help highlight which changes have a key mechanistic role in aging. Furthermore, both longevity interventions induced changes in post-translational histone profile, at sites which showed no change with age. The importance of these marks remains to be explored, they may represent passenger changes unrelated to aging, or more excitingly these marks may demonstrate their own longevity promoting roles.
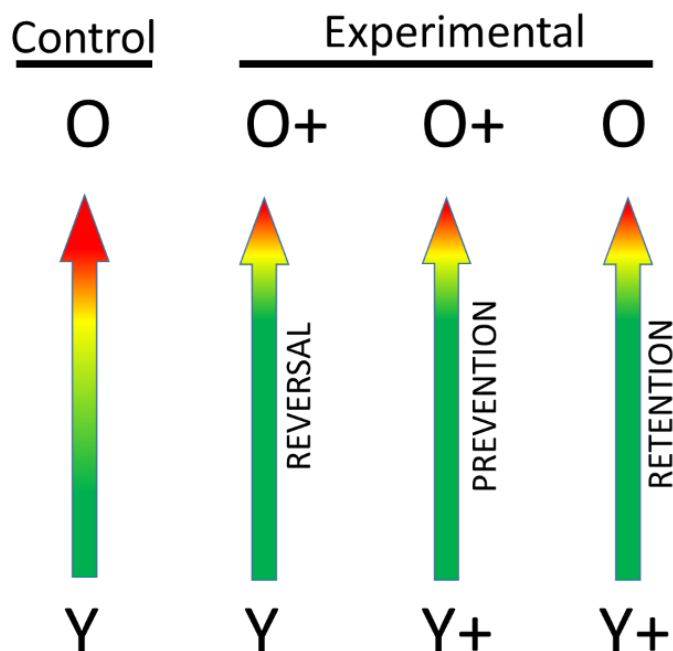
Fig.2 Schematic for study design to ascertain epigenetic effects of longevity interventions in aging models. All three experimental conditions can be compared to control condition or each other to gauge temporal impact on chromatin. Arrows depict passage of time with gradient indicative of declining health from green to red. Chromatin to be analysed at least once at 'young time point' and once at 'old' time point. From left to right: No intervention – Normal aging. Late life intervention – Reversal of age-related chromatin changes. Whole life intervention – Prevention of age-related chromatin changes. Early life intervention – Enhanced retention of youthful chromatin into late life.

Pursuit of these questions should be prioritized and will prove highly complementary to the correlative studies of age-related chromatin alterations. Then in the near future we may pose the most exciting question; by what magnitude can epigenetic interventions be leveraged to stave off aging and disease?

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Highlights

- Ageing is associated with gross alterations to chromatin and epigenetic profiles
- These epigenetic changes are associated with phenotypes of age related decline
- Interventions associated with longevity attenuate age related epigenetic change
- Drug and dietary interventions may ensure longevity through epigenetic stability