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The Epigenetics Behind Human Aging

Introduction:

Today, humans have made incredible leaps in the understanding of genomics, medicine, and genetics. Over the last several decades, our lifespan has increased dramatically thanks to advances in healthcare, sanitation, and drugs. We now are looking for more ways to increase our lifespan, but we have reached a bottleneck in our pursuit for the fountain of youth. Hospitals and powerful medications can only do so much in increasing the average lifespan; our new quest lies within our DNA and how our individual epigenomes can be modified through rejuvenation to increase health span and life span.

Why is this so important? How can the increase in life span affect us a species? Let us look to the stars and chart out our past. It was only a few decades ago that we managed to put a man on the moon. Now, we are looking for ways to travel to Mars, and eventually other solar systems in a few centuries. It is clear that these trips will take large amounts of time, and therefore might kill off settlers before they even arrive to their destination. Advances in cell rejuvenation could make space travel to distant planets possible, and can allow humans to reach their target with few complications. Longer lifespans will also allow us to contribute more to society and will let us to maximize the pursuit of knowledge from generation to generation. The overall wealth of knowledge in our species will rise dramatically, and new advances will be made in all aspects of life from technology to genomics. Even though it may seem like a distant reality, if we can keep our cells and tissues “youthful”, we will transform our species in revolutionary ways.

The Importance of Epigenetics:

How do stem cells differentiate when they have the same DNA sequence? How can identical twins with the same DNA develop different diseases later in life? How can one person have two different eye colors? All of these questions can be answered with epigenetics. Epigenetics is the study of cellular and physiological traits inherited by daughter cells that are not caused by a change in the DNA sequence. It is amazing to think that our bodies can change without ever altering a base of DNA, and this is why epigenetics is so important. This field can be directly correlated to the process of aging because it can explain why identical twins are similar at young ages, but different at older ages. It can explain why exactly a healthy diet leads to a longer lifespan and how smoking hurts other organs besides the lungs. In fact, epigenetics can single handedly show us why out lifespans are finite, and when we start to experience aging. By better

understanding how epigenetics affects our individual genomes, we will finally be able to pinpoint what causes aging and ways to slow the process.

Factors That Cause Aging:

Before I discuss the epigenetics behind aging, it is important to briefly explain what causes aging. As people age, they experience a slow, yet gradual decline in the functionality and structure of cells, tissues, organs, and bodily functions. Part of this is the result of an accumulation of damaged macromolecules, such as DNA, proteins, and lipids. These molecules are damaged by free radicals, and as humans age, the production of these free radicals increases. Furthermore, the Hayflick limit is another piece of the aging picture. Human cells can only replicate so many times; with each division, the telomeres associated with a cell's DNA will shorten and eventually reach a point where they are too short to continue division. Environmental factors play another large role in aging as they can cause somatic cell mutations. These mutations build up over time and cause "replicative aging" and "chronological aging" with cell division; this leads to potential diseases as well as an increased rate of aging. Furthermore, proteotoxicity occurs more often in aged organisms and produces aggregates in brain tissues, causing Alzheimer's and Huntington's. These are just several out of hundreds of factors that cause aging; it is clear that there are many components that can lead to aging, but one thing is certain. Aging cannot be cured, or stopped for that matter, but can likely be slowed. It is this slowing process that gives us hope in extending human longevity.

Lastly, I must establish the real issue with aging. Many believe that in order to prevent aging, we must delay the physical process within the cell. It is vital to understand that "extending life span is not equivalent to delaying aging" [1]. No matter what techniques are described in the rest of this paper, cells will continue to divide, and aging will exist. It is simply just a matter of reducing the rate at which "youthful cells" turn into "adult cells", or preventing youthful cells from transforming into adult cells at all. Another key concept to reducing aging is to extend lifespan. If we extend lifespan, we can live longer, and we can do this by extending our health span. A combination of diet and exercise has been proven to result in longer lifespan because those two activities increase health span. The key to "curing" aging is to increase our health span by genetically altering our epigenomes through novel advances in genetics.

Experiments and Treatments:

One treatment that is key to epigenetics and aging is called somatic cell nuclear transfer, or SCNT. Dr. John Gurdon showed that it was possible to take a differentiated nucleus from a mature somatic cell in a tadpole, and transfer it into enucleated *Xenopus* eggs, giving rise to mature and fertile male and female frogs [1]. This experiment showed that a somatic cell nucleus could be "reprogrammed by oocyte cytoplasm to allow the development of a new member of the species" [1]. Ultimately, the experiment proved that any signs of aging in somatic nucleus were reset upon transplantation. This set groundwork showing that the nuclei of adult somatic cells could be rejuvenated and

have pluripotency restored. SCNT was the process that led to the cloning of Dolly the sheep, and even though most of its breakthroughs are involved in the fields of stem cells and cloning, it has massive implications regarding aging. If we can discover what causes the nucleus to become reprogrammed, we can solve a big mystery in the molecular process of aging. Before we can extend our own lives, we must understand how to extend the life of our cells and tissues, which brings us to another treatment.

Along with SCNT, the process of creating induced pluripotent stem cells (iPSC's) has important results for aging. Differentiated adult cells from humans, "can be converted to pluripotent stem cells by the introduction of a small number of transcription factors such as Oct4, Sox2, and Klf4" [1]. This is another massive stride for aging, because if we can discover ways to reset normal adult cells to stem cells, we can theoretically replenish our bodies with brand new pluripotent cells every time our adult cells become too old to continue the replication process. The breakthrough of iPSC's and their implementation in humans "has provided an exceptional opportunity for personalized regenerative medicine, which would be particularly impactful for diseases of age"[4]. Of course, the challenge we now face with iPSC's is determining how far we should rejuvenate adult cells, for if we revert them back to a postnatal development state, we will be subjecting our bodies to cells that cannot perform the proper tasks. This issue is discussed later in the paper, but as of now, it remains a barrier to implementing this technique in experiments, as we do not know how to control the "aging clock" [1]. Along with SCNT, iPSC's show promising results for transforming old cells into new stem cells and can have incredible results if used properly in humans (Figure 2).

"One possible rejuvenating consequence of iPSC reprogramming is reactivation of the expression of telomerase, the enzyme responsible for maintaining telomere length and long-term self-renewal potential in embryonic stem cells. Genetic defects in components of the telomerase complex may prevent the restoration of telomere length and full telomerase activity during iPSC reprogramming in some conditions but not others" [1]. This is another treatment option that is still being researched today, but can have incredible implications in the near future. As mentioned previously, the Hayflick limit prevents a somatic human cell from dividing more than sixty times because the telomere shortens a tiny bit with every division. If we can discover a way to keep telomerase active in the cell, the telomeres will not shorten with division, and cell replication might be able to continue indefinitely until apoptosis occurs. This is just one method currently in design that might be key to extending the life of cells in humans, and ultimately, lifespan.

Another form of research and "treatment" for aging has been discovered in heterochronic parabiosis, which allows for rejuvenation without dedifferentiation (Figure 1). Heterochronic parabiosis involves pairing two separate circulatory systems (and organisms) together in a surgery. Afterwards, results about cell rejuvenation can be observed and analyzed. In the older mouse, "aged cells in muscle and liver adopted a more youthful functional phenotype, but the molecular signatures of aging were to restored to a more youthful state" [1]. Likewise, in the younger mouse, the cells developed a more aged molecular and functional state. This experiment is key in showing that the systematic environment of an organism is an incredibly powerful determinant of the age of cells [1]. "In addition to illuminating the influence of the systemic environment on cellular function, such heterochronic studies emphasize the

potential role of environmental factors in rejuvenating aged cells" [1]. This treatment, though not practical for humans, is "arguably one of the most intriguing aspects of the nongenetic control of aging" because it is the reversal of aging without genetically modifying the organism (epigenetics). Changes in aging affected by nongenetic factors are "relatively long lasting" and suggest that "certain epigenetic mechanisms, which can be relatively stable in nature, are a pivotal component of this regulation" [4].

In addition to all these methods, scientists have also found a family of proteins that "protects from several age-related diseases and extended the disease-free portion of life"[4]. The Sirtuin family of protein deacylases deacetylate histones, and their efficiency "is enhanced when the ratio between nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide dehydrogenase is high". This means that chromatin regulation is linked to dietary restriction and exercise; these two environmental interventions are key epigenetic factors that affect the span of human longevity. When DNA is damaged due to environmental factors, it was found that the Sirtuins proteins were relocalized, and this process possibly drives "epigenetic changes and genomic instability during aging"[4]. Even though this process is genetic and is correlated to genetic factors, such as DNA alteration, there are still epigenetic factors at play. The epigenetic factors are what cause damage to the DNA in the first place, and thereby affect protein regulation, ultimately leading to the onset of aging. If this study shows us anything regarding human longevity, it is clear that epigenetic modifications cause genetic modifications, which in turn alter aging. Yes, there might be genomic factors involved with longevity, but these factors are all induced by the epigenome; this concept is vital if we hope to slow aging processes.

Epigenetic Studies of Aging:

Now that we have discussed important advances, causes, treatments, and discoveries of the process of aging, I would like to discuss epigenetic studies that have been conducted regarding human longevity.

The study of twins is an exciting research process because both organisms have the same DNA and genetic content, but age differently. A study was conducted on monozygotic (MZ) twins to assess global an gene-specific methylation levels, "mainly in order to elucidate the phenotypic divergence of MZ over time with respect to their susceptibility to diseases or other phenotypes" [2]. It was found that early in life, younger twins are "epigenetically indistinguishable", but in later years, older twins exhibit major "tissue-specific differences in their overall content and genomic distribution of 5-methylcytosines" [2] which in turn affected both twins' gene-expression. This study suggests that environmental factors play an important role in the early stage of life when long-lasting epigenetic changes are established. The study also found that DNA-methylation stability is "genetically determined" which means that the epigenome is regulated by "genetic, stochastic, and/or systematic factors" [2]. It is important to look back at twin research and analyze the organisms'' differences either as genetic or environmental, as this will narrow down epigenetic factors for aging.

Because the epigenome can be modified by so many factors, it was found that DNA methylation patterns associated with aging are also altered by genes for tumor

suppression (*COX7A1*, *LOX*, *RUNX3*, *TIG1*, *p16INK4A*, *RASSF1*, *DUSP22*), metabolism (*ELOVL2*, *SLC38A4*, *SLC22A18*, *MGC3207*, *ECRG4*, *ATP13A4*, *AGPAT2*, *LEP*) development, and growth (*IGF2*, *cFos*) just to name a few. This suggests that DNA methylation is correlated with age-related pathological phenotypes, and why aging is highly associated with epigenetics. Environmental factors, nourishment, and life experiences, all affect aging because DNA methylation is regulated by these factors [2]. This is a massive leap regarding the process of aging and shows that human longevity is not directly associated with genomics alone. Scientists and researchers have been trying to discover genes associated with aging for decades, and even if they exist, they will have little importance in expanding human life. "We define epigenetics liberally as 'changes to the genome that do not involve changes in DNA sequence'" [4], and it has been shown through twins that the DNA sequence itself does not directly correlate to aging. If we assess the epigenetics of aging, including environmental factors, we will reach a hallmark in our understanding of the aging process.

Continuing on the talk of DNA methylation and epigenetics, changes in gene expression are linked to CpGs clusters of CpG repeats, located upstream of gene promoters. Epigenetic alteration of gene expression leads to tissue differentiation, and this can be a key finding to aging. Developmental animal studies have shown changes in methylation patterns in specific genes as the result of dietary intake "at certain crucial points of development" [3]. "Perinatal environment—especially excess fat in the maternal diet—seems to alter liver histone modification and predispose offspring to obesity in monkeys. Likewise, over feeding in the neonatal period in rats led to hyper-methylation of the pro-opiomelanocortin gene and a permanent disposition to obesity. Neonatal stress can also lead to longstanding methylation changes, which may influence ageing" [3]. This is a massive breakthrough for the epigenetics of aging, because now it has been shown that human longevity is not entirely regulated by just genes. Of course, certain diseases and defects present in different people will lead to different patterns in DNA methylation and different paths of aging, but the epigenetics is a strong indication of how the aging will occur. Cigarette smoking is another example where DNA methylation is affected. It was found in smokers that DNA methylation levels were reduced at multiple genomic loci, but this methylation could be partially restored if smokers stopped or even slowed smoking [6]. Environmental factors have shown over multiple studies to directly lead to the aging process in organisms, and this is a factor that simply cannot be ignored.

I already mentioned some novel treatments that can replace old cells with new ones and increase telomere length, but these are decades away from being implemented properly in humans. Beyond the genetics and pathways of aging, we still do not know what environmental changes contribute to epigenetic change. Possible sources of some carcinogens, such as asbestos and cigarette smoke, cause epigenetic alterations. Interestingly enough, these changes can be "mitigated by diets rich in leafy green vegetables and multivitamins" [3]. In addition to healthier diets, vitamin B12, folic acid, choline, and betaine are molecules considered as methyl group providers. If these are added into diets, there will be increased methylation, and therefore, the chance of demethylation occurring will drop dramatically. This in turn extends the disease-free portion of life and prevents aging from occurring in young individuals.

The Importance of ENCODE:

Another important piece of information to briefly discuss is ENCODE. ENCODE, or Encyclopedia of DNA Elements, is a public research project launched by the US National Human Genome Research Institute in September of 2003. This project is intended to expand upon the Human Genome Project by identifying all functional elements in the human genome. ENCODE is incredibly important in our quest for linking epigenetics to aging, because it contains data regarding gene expression and DNA methylation patterns across different tissues. After analyzing this data, we can correlate aging patterns to tissue functionality, and we will ultimately be able to track this functionality based on the regulation of our genes. Remembering that gene regulation is determined by DNA methylation, and that DNA methylation is determined by the environment, we come to a startling hypothesis. Our genome can regulate anything and everything in our body, but doesn't know how to do so until it receives instructions from our epigenome. A fantastic comparison can be made to computers, where the human genome is equivalent to the hardware of a computer, and the epigenome is equivalent to the software. Epigenetic modifications alter the genome, and in turn, the genome sends signals that either inhibit or encourage the aging process in humans. By mapping the human epigenome, we will finally be able to make connections between gene expression and chromatin regulation in histones; this is why ENCODE is the future to understand the processes of aging.

How Environmental Factors Affect Aging and Diseases:

"DNA methylation, which consists in the binding of a methyl group ($–CH_3$) to the carbon in position 5 of a cytosine moiety, appears to be frequently involved in the processes associated both with healthy and diseased aging, particularly in neurological and neurodegenerative diseases" [5]. As mentioned in the first section of this paper, there are a plethora of environmental factors that affect the way we age because they trigger DNA methylation in a cell. Many "complex diseases" associated with aging show DNA methylation alterations in cells, which in turn alter the way genes are expressed and how tissues function. "Increasing evidences suggest that environmental factors may contribute to neurodegeneration through the modulation of the epigenetic pattern resulting in alterations of gene expression programs" [5], and it is this increasing evidence that supports the link between epigenetics and aging (Figure 3).

Let us take a step back from the molecular aspects of aging and assess clear evidence between the link of epigenetics and aging. What about emergency room physicians? Why is it that the life expectancy of a person drops if he or she decides to become a doctor? It has been proven that doctors age more quickly than the average person, and this can be attributed directly to environmental factors, such as high stress situations, uneven sleeping patterns, poor diet, long shifts, and sporadic work hours. How about our president? Why do presidents age so quickly in the White House? We can see that only after a few months in office, the current president will age dramatically, from hair color to skin appearance. This too is caused by epigenetics as external factors,

stress being the main contributor, drastically dictate the aging process in these individuals. When we look at the Japanese population, we see an incredibly high average life expectancy. Why is this? Japanese diets are rich in fish and vegetables, which contain large amounts of vitamin B12. In addition, the elderly living in Japan live relatively stress free lives, and do not have to worry about finances, as their children support them. This outline of life is imbedded into Japanese culture, and can explain why the aging process has "slowed-down" in these individuals. We can analyze thousands of different lifestyles and cultures with this approach, and we will always come back to the same conclusion. The environment is by far one of the most important factors regarding human longevity.

Concerns and Drawbacks:

Despite the many advances and breakthroughs regarding adult cell rejuvenation, there are several inherent risks that accompany modification of the epigenome. One example lies within adult stem cells. When proliferative activity increases through rejuvenation methods, such as heterochronic parabiosis, there is an increased risk of developing "malignancies" in cells. This is because the cells have already acquired genomic mutations in their lifespans, but are then subjected to excessive "proliferative potential by this rejuvenating intervention" [1]. This issue leads to one of the largest challenges in cell rejuvenation, for it is not practical in humans, and even if it was, would still be associated with high risk factors. Heterochronic parabiosis might be beneficial in the study of the processes of cell rejuvenation, but cannot be implemented to slow down the aging process in humans.

Our goal is to therapeutically rejuvenate stem cells in humans. The challenge in this goal though is resetting "the aging clock back to the appropriate adult stage" [1]. When rejuvenating cells and tissues, we want to restore them to a "'young adult' state from an elderly state" without rewinding the aging clock to embryonic or postnatal developmental stages. Finding the correct process for this rejuvenation is still currently a barrier, because we do not yet possess the knowledge on how to control how far back we convert differentiated cells to iPSC's. Once we can control the exact transformation process, we will be able to use iPSC's much more efficiently in humans; these iPSC's can be personalized for different individuals, and have the potential to restore certain parts of the body.

Furthermore, questions remain regarding the "epigenetic mechanisms" involved in cellular rejuvenation. We do not yet have a full understanding of how long the rejuvenated phenotype will last after it is therapeutically treated. Researchers have discovered that iPSC's are stably induced through several cell transcription factors. The question now remains, "Will the affects of rejuvenated stem cells last several years, or several weeks?" Another question, regarding the number of transcription factors required to reach an induced pluripotent state, remains for scientists researching aging. It is known that "inhibiting single signaling pathways" can restore some youthfulness to cells, but we still do not know "the number of transcriptional regulars that need to be modified to result in full rejuvenation" [1]. One last interesting question lies in which type of cell the epigenome marks dominant. "Is the youthful state or the aged state dominant" in an

epigenome where both aged and rejuvenated cells exist? [1]. These questions are propagating advances in aging research and allowing scientists to better understand the processes behind rejuvenation methods such as somatic cell nuclear transfer, the formation of induced pluripotent stem cells, and heterochronic parabiosis.

Conclusion:

In conclusion, it is vital to study epigenetics and modifications in the epigenome if we are to better understand DNA methylation, and how changes within these patterns lead to aging. If we can find a way to use iPSC's along with a modified epigenome, we will discover how to slow the process of aging, along with what genes affect it, and how specifically the environment plays a role in methylation. By using epigenetics, we will never truly stop aging, but we will be able to slow the process, and that in turn will increase lifespan tremendously. Diet and exercise has always been the go to answer to live longer, but now, we are finally beginning to understand why these environmental factors are so important. Projects such as ENCODE are advancing out knowledge of how epigenetics affects the genome and how in turn this changes tissue functionality with age. Novel experimental diagnoses, such as heterochronic parabiosis and SCNT, have shown that cells can in fact be reprogrammed to erase previous signs of age. Other studies, such as the function of telomerase, are also advancing our knowledge of cell age and showing ways to make divisions occur over a longer period of time. In the future, cancers can be better diagnosed if we learn how to prevent demethylation in cells. In addition to this, once we learn exactly how genes become unregulated through DNA methylation, we will be able to use pharmacogenomics and other forms of treatment to personalize human longevity for single individuals. The future looks surprisingly bright for increasing human longevity, and even though we can never suppress aging, an increased understanding of epigenetics and methylation patterns will be the key to living longer.

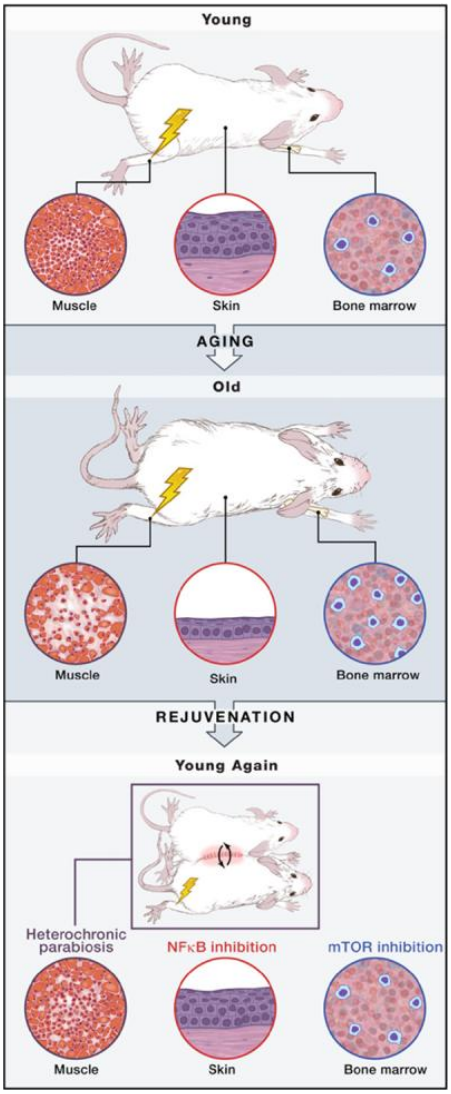

Figure 1
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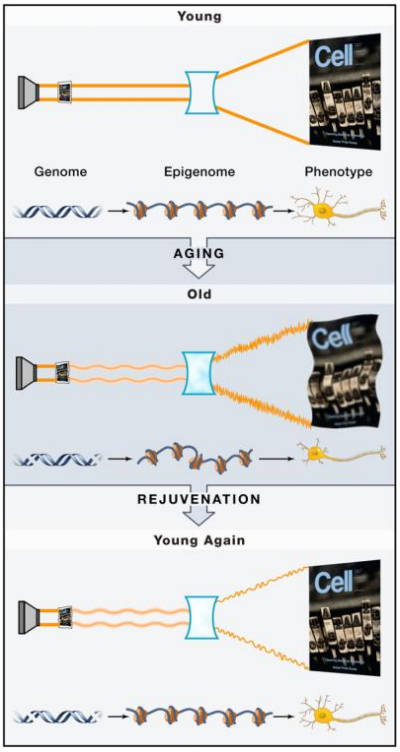

Figure 2
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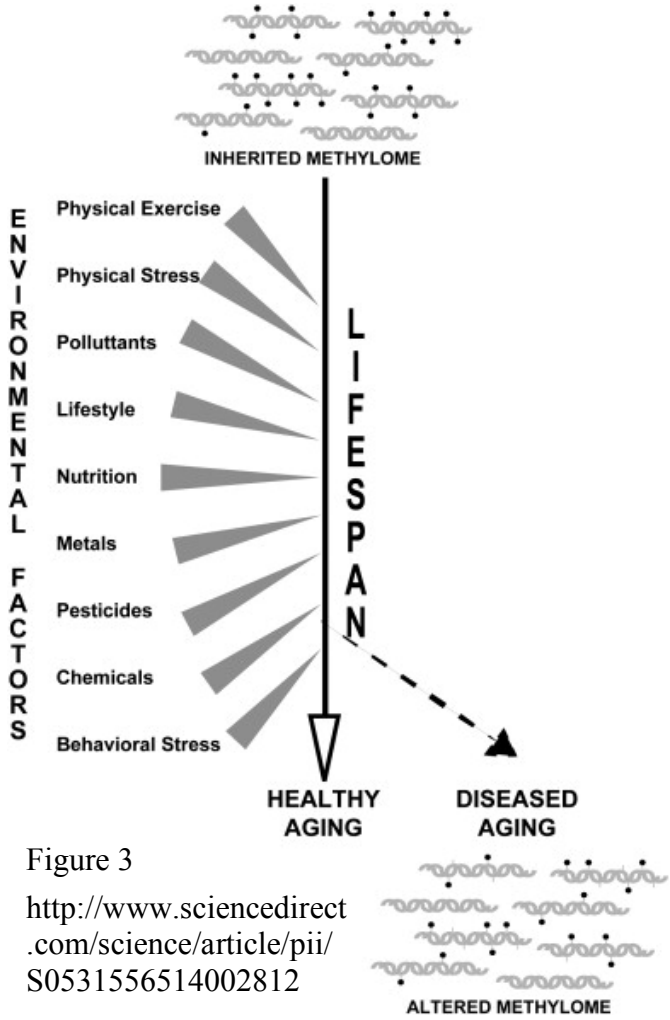

Figure 3
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Figure 4
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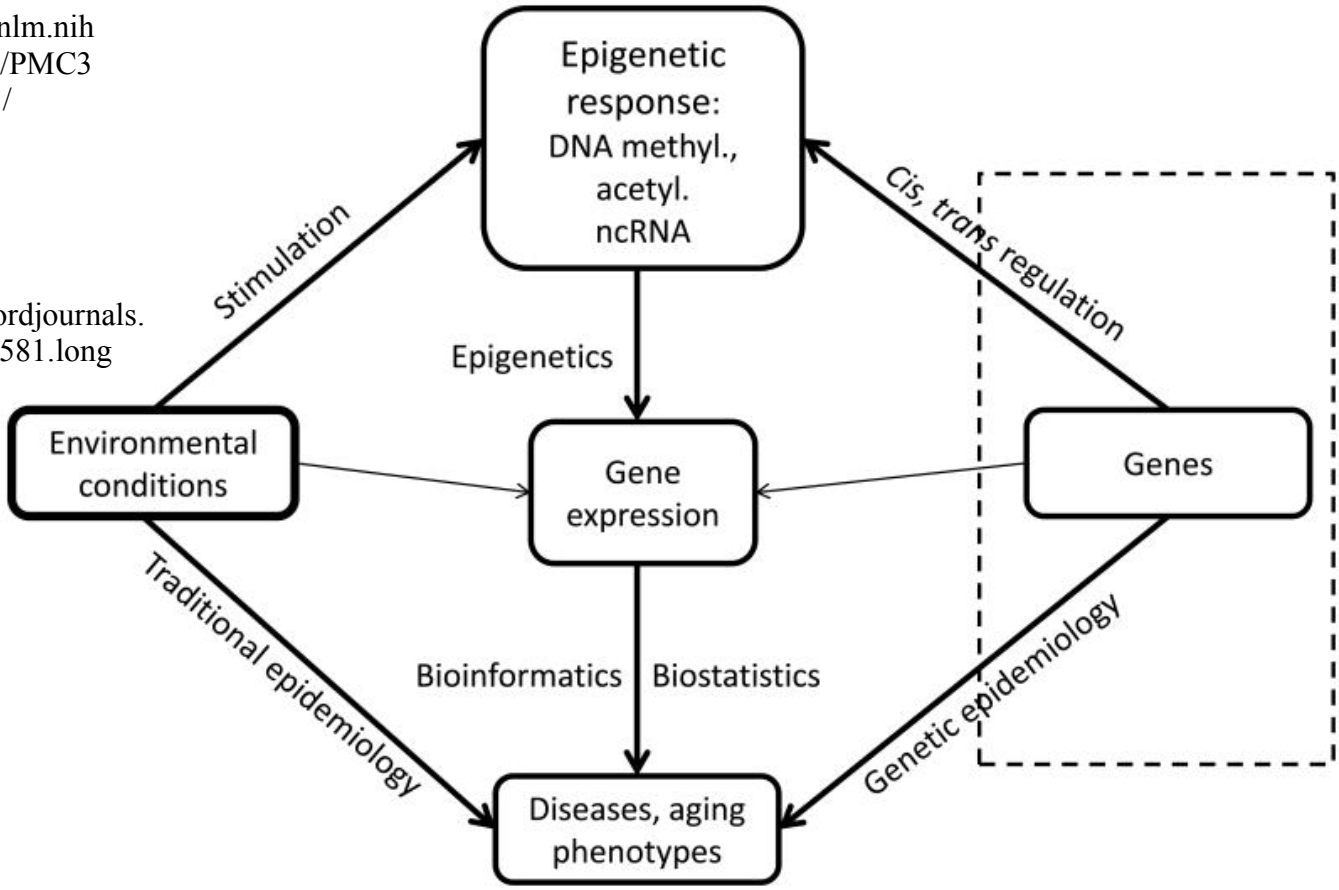

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