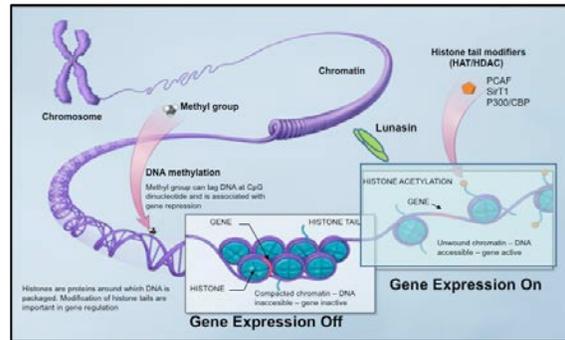
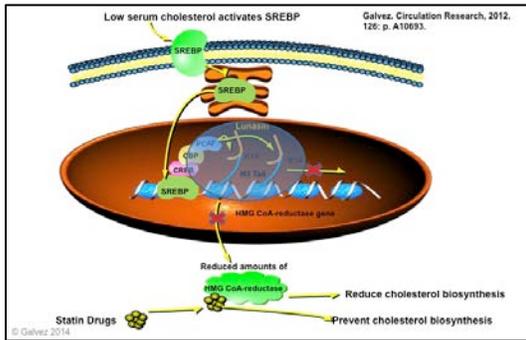


Lunasin

Epigenetic Revolution in Health and Nutrition

Alfredo F. Galvez, Ph.D.



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Introduction

The emerging role of epigenetics in gene regulation is causing a paradigm shift in healthcare and nutrition. The Lunasin peptide with its novel epigenetic mechanism of action provides an effective and nutritional alternative against non-communicative, degenerative chronic diseases. Lunasin's ability to turn on and off genes makes it a viable and potentially groundbreaking treatment option for chronic diseases, such as cancer and heart disease. But how and why does this peptide work and how can it be used in combination with diet and nutrition for optimal results? This report will outline the effects of Lunasin on the body, providing an overview of epigenetics and outlining the effects Lunasin has on the human body at the cellular and molecular level.

The science of Epigenetics

Epigenetics is changing the way we look at the role of genes in preventing and treating non-communicable degenerative chronic diseases. Though it is currently revolutionizing biology, many people including medical professionals, are not aware of the power of epigenetics. By extension, epigenetics will also change the way we view medicine and pharmaceutical drugs. It will also undoubtedly influence the role of nutrition and how nutrition plays in healthcare today and in the future.

The emerging role of dietary nutrients in modulating epigenetic mechanisms that lead to changes in gene expression is causing a paradigm shift in nutrition research. Lunasin is the first dietary example of an epigenetic factor with this novel mechanism of action. What makes Lunasin unique is that it actually binds to the histone tails. Histones serve as the "packaging material" for DNA and modifications to the histone tails by lunasin binding can cause changes in gene expression that eventually lead to favorable health outcomes.

The Accidental Discovery of Lunasin

Dr. Alfredo Galvez wasn't trying to discover Lunasin when he attempted to isolate the gene encoding methionine-rich proteins from soybeans at the University of California Berkeley, but he did. Along the way, it was discovered that the gene isolated actually encoded a signal peptide and two subunits. The small subunit, now known as Lunasin, was unique because it had an unusual acidic structure at its carboxyl end.

The storage parenchymal cells of these seeds are unique because they violate the main rule in cell division, which is that once DNA is replicated, division occurs. Cell division doesn't happen after DNA replication in storage parenchymal cells. As a result, these cells grow larger so they could store as many of the nutrients necessary to support the growth of the developing embryo into seedling.

Galvez discovered that Lunasin was only found in storage parenchymal cells and was also only found in the nuclei in those cells that were growing. When researchers sub-cloned the Lunasin gene in bacteria that inadvertently expressed the lunasin peptide, the resulting elongated bacterial cells were characterized by the absence of the segment that is associated with filamenting bacteria. Once this was discovered, researchers attempted to link its functional role in seed development with another hypothesis. It was suggested that the expression of Lunasin leads to those elongated bacterial cells and was linked to its functional role in seed development of enlarging storage parenchymal cells.

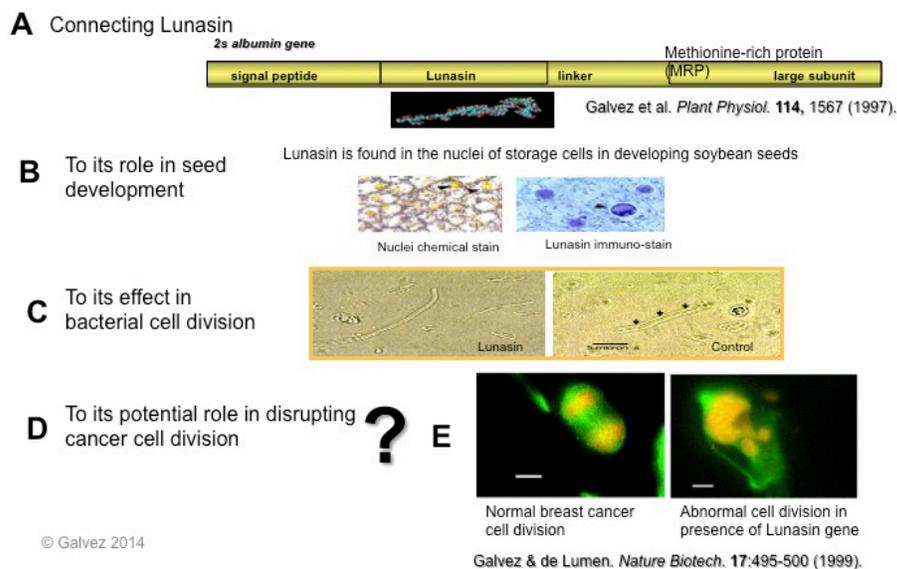


Figure 1

Based on Lunasin's involvement in cell division in seed storage parenchyma cells and bacterial cells, Galvez hypothesized that Lunasin might have the same effect on mammalian cancer cells. The theory was that the gene could potentially halt cancer cell division. And when the Lunasin gene was inserted in cancer cells, that's exactly what happened.

Under normal cell division conditions in breast cancer cells, there is a symmetric division of the chromosomes. But when the Lunasin gene is added to mammalian cells, abnormal cell division occurs. This dying cell cannot divide.¹

Research

Lunasin was shown to inhibit skin tumor formation in mice. Figure 2 is a collection of images from the first Lunasin animal experiment. During this experiment, the skin of mice was exposed to chemical carcinogens to produce tumors. But when Lunasin was added and a new batch of mice was exposed to the chemical carcinogen, 70 percent of the mice did not form tumors.

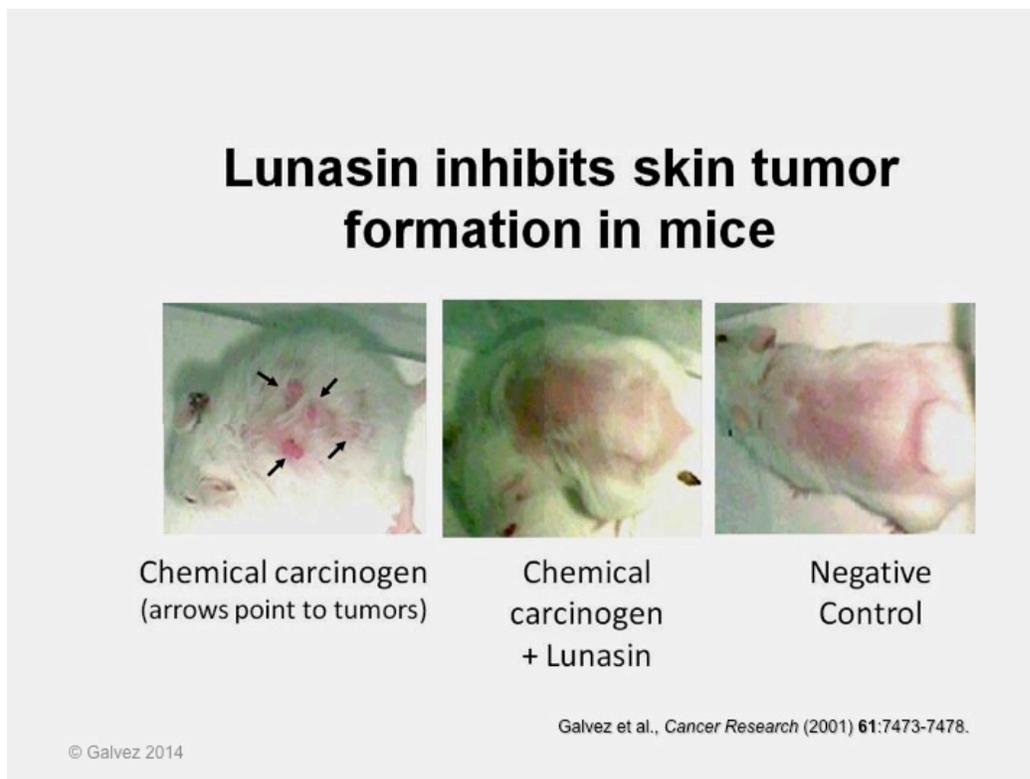


Figure 2

¹ Nature Biotech, 1999.

Researchers wanted to know what the mechanism of action was for Lunasin. One of the most unique things discovered about this peptide was that it could attach to what is called a green fluorescent protein. A green fluorescent tag was used to mark Lunasin in a research experiment. With the help of this visual aid, researchers saw that Lunasin actually infiltrated the mammalian cells. After one cycle of cell division, most of the Lunasin has bound to the chromatin.

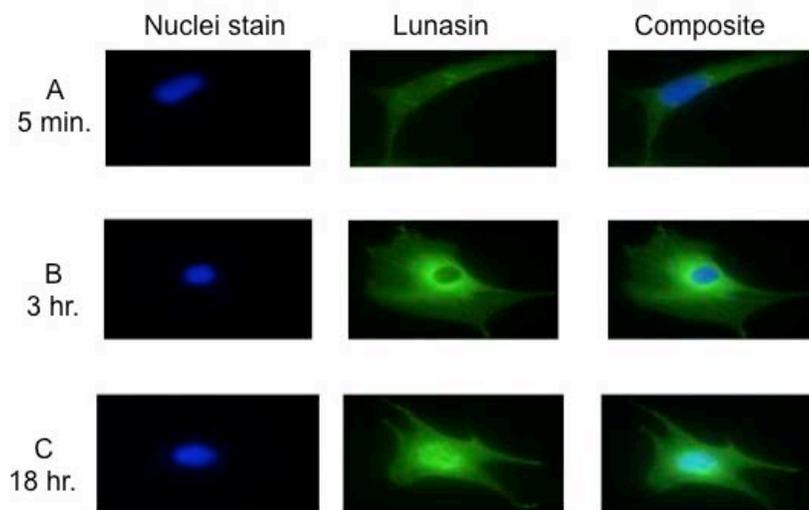


Figure 3: Lunasin infiltrates the cell and binds to chromatin in the nucleus

Interestingly, Lunasin is one of the rare plant proteins to have a functional RGD motif. This arginine glycine aspartic acid motif allows Lunasin to bind to the integrin receptors of mammalian cells and get inside those cells through endocytosis.. Lunasin also has an amino acid motif (or chromobinding motif) that is associated with binding to deacetylated or hypoacetylated chromatin. This chromobinding motif is also associated with heterochromatins found in humans, in drosophila, in yeast and in nemotodes.

Proteins with a chromo-domain bind specifically to condensed chromatin composed primarily of deacetylated histones. But researcher needed to know if Lunasin was just like those other proteins with chromobinding domain. Specifically, they needed to know if it would bind to deacetylated histones. An experiment was done to examine this question.

The cartoon diagram in Figure 4 represents the chromatin wrapped around histone molecules. The tails of the illustration correspond to the location of Lysine that is modified in the histone H3 and histone H4 tails. This amino acid sequence in histone H3 and H4 tails are the most conserved in nature. In fact, the histone H3 and H4 tails found in humans, mice, plants and even yeast are all essentially the same.

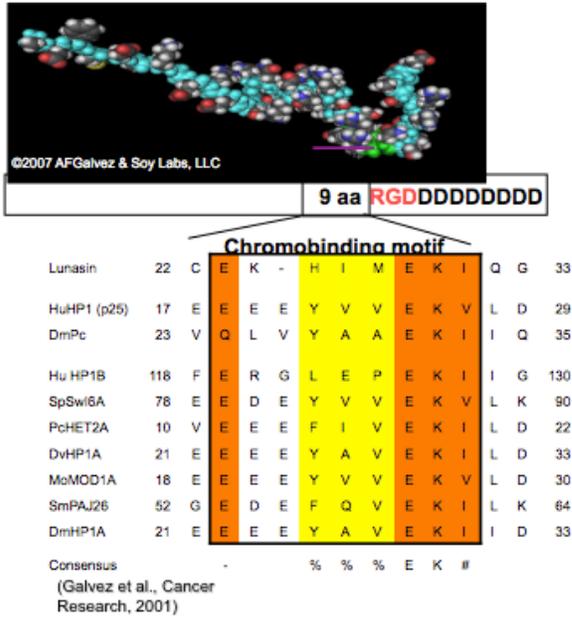
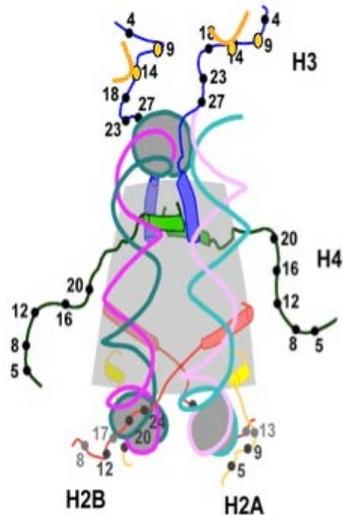


Figure 4

For the experiment, researchers conducted an in vitro assay, adding Lunasin into the assay. This demonstrated how much acetylation could occur in the histone tails. In this particular case, researchers concentrated on the histone H3 tails. Researchers looked at Lysine 9 and 14 as initial locations for the effects of Lunasin. Through a series of experiments, they discovered that Lunasin reduced the acetylation of Lysine 9 and 14.



Galvez. Circulation Research, 2012. 126: p. A10693.

Figure 5

Subsequent experiments looking at specific Lysine groups showed that Lunasin only binds to Lysine 14 histone H3.

Lunasin's epigenetic mechanism of action of binding to histone tail causes inhibition of histone acetylation. This mechanism of action has obvious effects inside the cell. Chromosomes normally have DNA attached to histone tails. There are two forms of histones: condensed and noncondensed chromatin. As mentioned earlier, Lunasin binds to histone H3 nonacetylated tails. When it does that, the PCAF histone acetylated enzymes cannot acetylate. This turns off gene expression. Turning off gene expression in this way does not involve the DNA sequence, instead it manipulates how the genes within those chromatins are being packaged, or being unwound or wound. Thus, condensed and uncondensed chromatin can be affected by the Lunasin peptide.

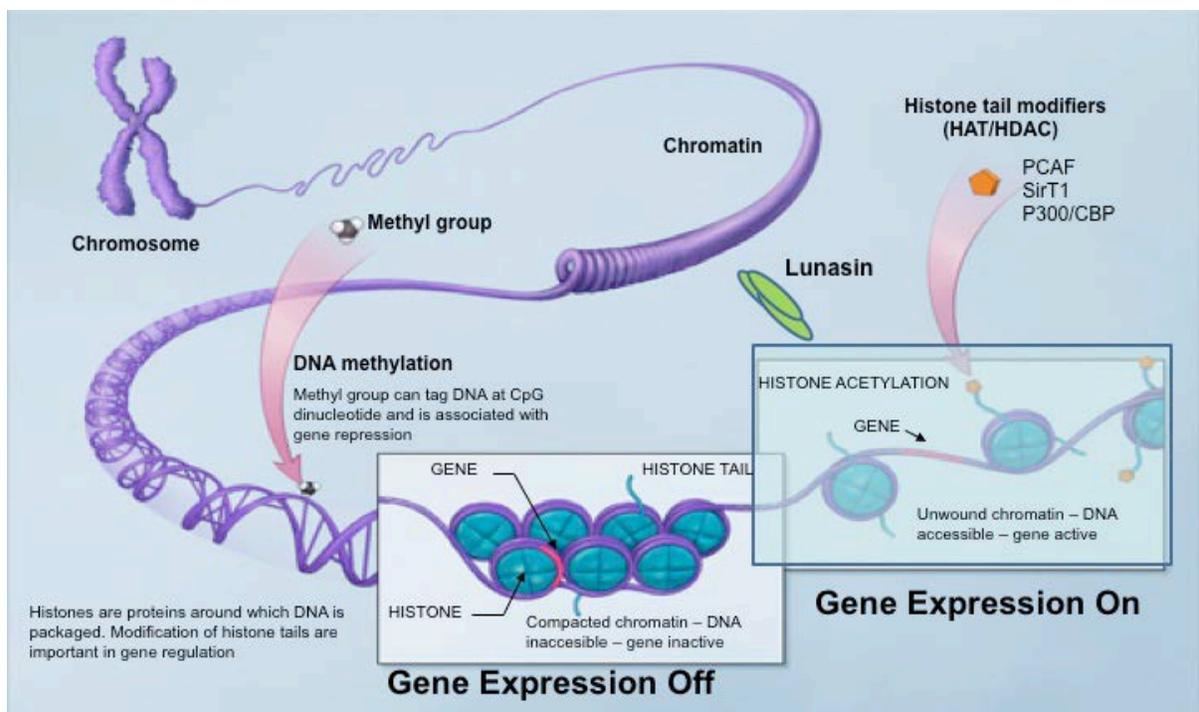
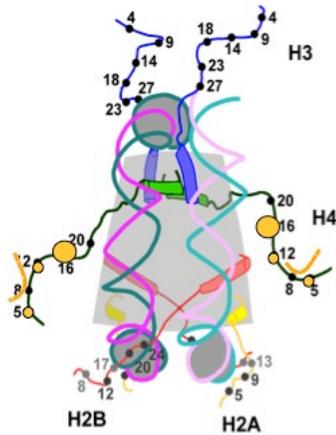


Figure 6

Lunasin has not been shown to affect Lysine 5 and Lysine 12; it only binds to acetylated Lysine 8.

Lunasin binds to deacetylated H4-Lys8 and increases H4-Lys16 acetylation



- Reduced Ac-Lys8 by Lunasin suggests histone binding/masking.
- Increased Ac-Lys16 by Lunasin suggests H4 tail conformational changes to allow p300 access to Lys16.

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Galvez et al., *Nutr Cancer*, 2011. 63(4): p. 623-36.

Figure 7

Lunasin was shown to reduce the acetylation of Lysine 8, suggesting histone binding and masking potential. The increased acetylation of Lysine 16 by Lunasin indicates that the H4 tail conformational changes allow p300 access to Lysine 16.

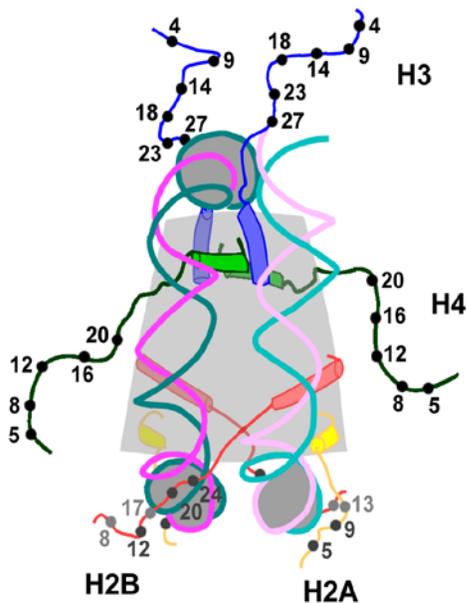


Figure 8

Researchers found that Lunasin upregulates gene expression by increasing H4-Lysine 16 acetylation. The reason this is significant is that loss of H4-Lys 16 acetylation is a common epigenetic hallmark of cancer.^{2, 3}

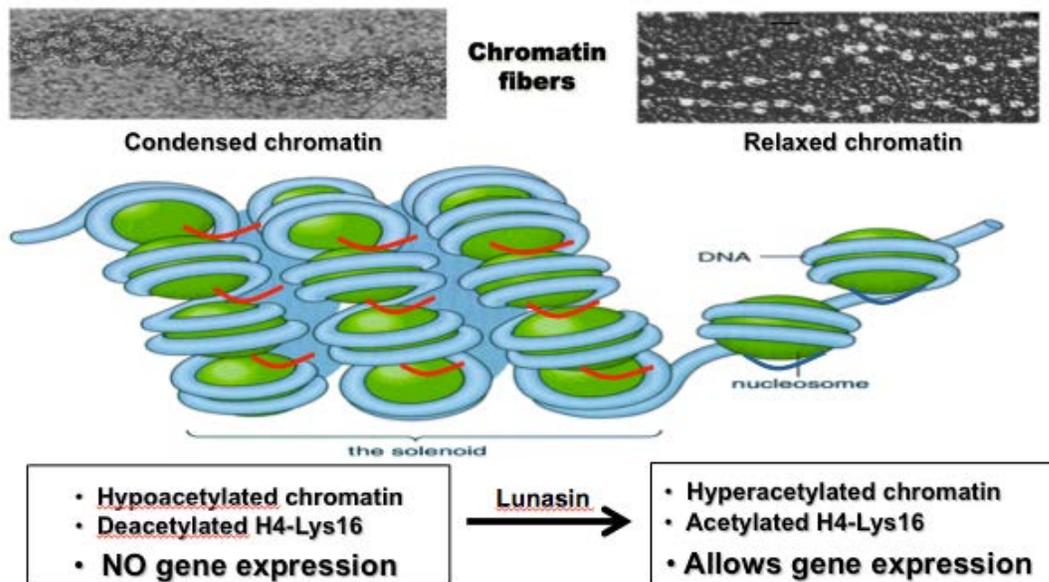


Figure 9

The Effects of Lunasin on Cancer

Ultimately, Galvez found that Lunasin inhibits the expression of genes involved in cell proliferation and tumor formation. Some of the genes that can be turned off include the Ras oncogene, which is found in 45 percent of all cancers. It also increases the expression of genes associated with tumor suppression, apoptosis, mitotic control and DNA repair.

² Galvez et al., Nutrition & Cancer, 2011. 63(4): p. 623-36.

³ Fraga et al., Nat Genet, 2005. 37(4): p. 391-400.

Lunasin as a dietary nutrient affects multiple genes in cancer pathways

Lunasin inhibits expression of genes involved in cell proliferation and tumor formation.

Lunasin increases expression of genes associated with tumor suppression, apoptosis, mitotic control and DNA repair.

Tamoxifen drug inhibits estrogen receptor (ER)

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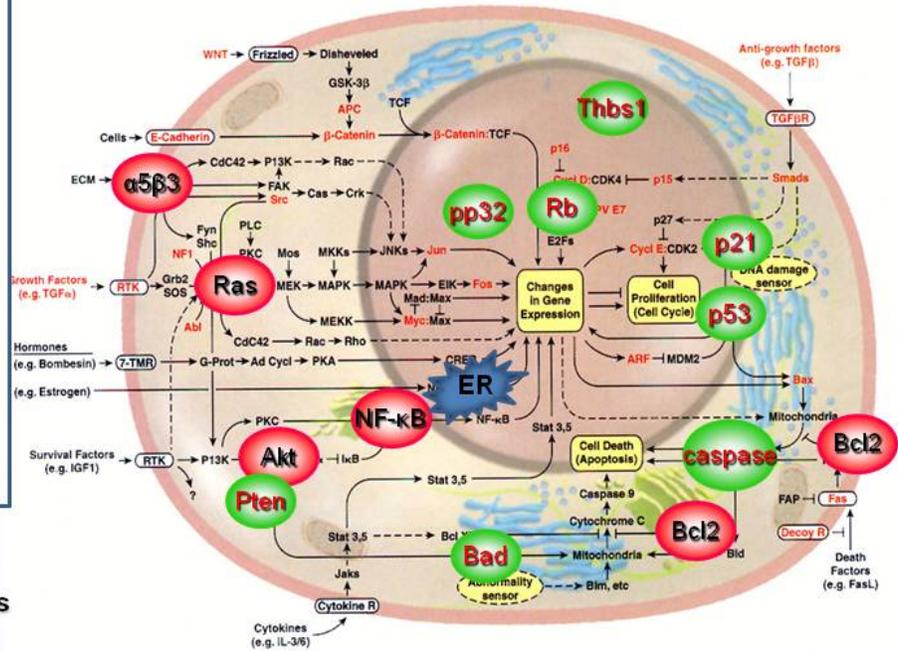


Figure 10

One of the main issues with cancer drugs is that they might inhibit one pathway, but cancer cells find another way around that pathway. These drugs only slow the path of the cancer, they do not stop it from progressing. That's precisely why so many cancer drugs have not been successful.

Looking at Lunasin on the molecular level, researchers have examined the effects of this peptide on gene expression. One notable study showed that Lunasin inhibits HMG-CoA reductase expression by inhibiting H3-Lysine 14 acetylation by PCAF. The HGM-CoA reductase gene is the rate-limiting enzyme involved with cholesterol biosynthesis. The mechanism to turn on HGM-CoA reductase when there are low cholesterol levels in the blood is through SREBP. SREBP activated in the endoplasmic reticulum when there is low serum cholesterol in the blood. It then enters the nucleus. This allows the cellular machinery to tell the cell to essentially turn on the HGM-CoA reductase gene. This also allows expression of the HGM-CoA reductase gene.

The addition of Lunasin showed a dramatic reduction of H3 acetylation in research experiments. As a result, there are reduced amounts of the HGM-CoA reductase mRNA and a 50 to 60 percent reduction of HGM-CoA reductase enzyme. Statin drugs are developed to specifically inactivate HGM-CoA reductase to prevent cholesterol biosynthesis, which is the most effective way of lowering cholesterol because it mimics the amount of cholesterol produced in the liver.

Figure 9 shows the PCAF acetylation of the histone H3 tail by Lunasin. This serves as the basis of the Lunasin bioactivity assay.. The main issue with most natural bioactive agents is that there is no measure of whether it's still bioactive or not when it is digested and enters the bloodstream.

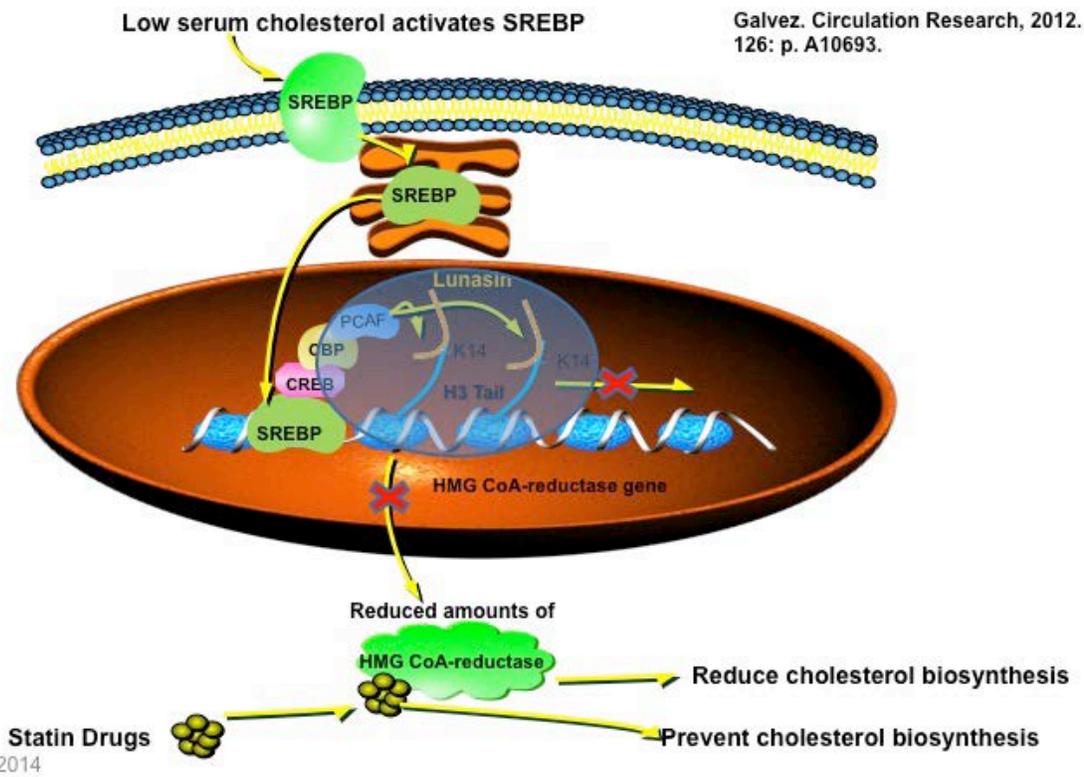
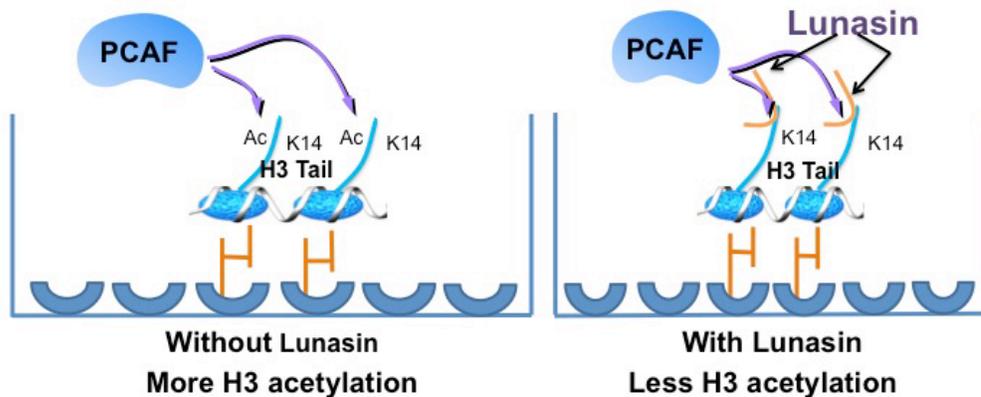


Figure 11

Lunasin Bioactivity Assay

As mentioned earlier, Lunasin expression binds to Lysine 14, preventing the acetylation of Lysine 14. Bioactivity is measured by the inhibition of H3 acetylation to determine the amounts of bioactive Lunasin.



Measure inhibition of H3 acetylation to determine the amount of bioactive lunasin

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Figure 12

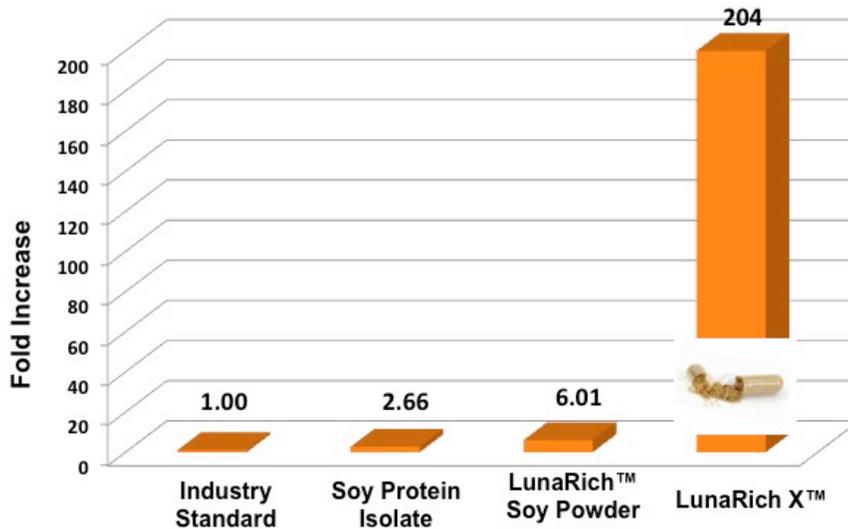
Researchers used the assay to determine the amount of Lunasin in soybean germplasm or soybean varieties. To do this, they selected the best varieties possible for extracting Lunasin. They first selected varieties with the highest quantities of Lunasin, and then determined which varieties had the most bioactive Lunasin after it was digested. By determining this, they were able to identify the best soybean varieties and develop manufacturing protocols to produce soy preparations with the most bioactive Lunasin.

The methods to produce soy protein actually contain the Lunasin peptide; but once it is eaten, it becomes inactivated. Researchers identified the best varieties of soybeans and came up with manufacturing protocols to produce LunaRich soy powder that has increased amount of bioactive Lunasin that was one to six times the levels found in the industry standard.

Because they had the Lunasin bioactivity assay, researchers were also able to conduct different extraction protocols on a lab scale, pilot scale and commercial scale. This led to the development of the LunaRich X formula. LunaRich X contains a more concentrated form of bioactive Lunasin and is protected when it is digested and even becomes more bioactive in

this process. It contains approximately 200 times more bioactive Lunasin than the industry standard.

Lunasin bioactivity of soy protein formulations



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Figure 13

The development of LunaRich X allowed researchers to conduct experiments to determine whether Lunasin is definitively the active ingredient in soy protein responsible for cholesterol lowering. This will be significant because a meta-analysis done in 1995, led to the approval of an FDA health claim for soy protein. This study showed that consuming 25 grams of soy protein compared to 25 grams of casein protein, or milk proteins led to a 12.5 percent reduction in LDL cholesterol.

The problem is that in 1999, when the FDA health claim was approved, soy protein producers started to look at ways to increase soy protein content in the soy protein preparations. When they did this, thinking the soy protein was the active ingredient, most of the Lunasin peptide gets inactivated when ingested. In 2006, another meta-analysis was done on all human clinical trials that were done after the FDA health claim was approved. Through this study it was discovered that the subject would now need to consume 50 grams of soy protein to get a 3 percent reduction in LDL cholesterol. This has to do with the reduction of bioactive Lunasin in these soy protein preparation.

Is lunasin the active nutrient in soy protein responsible for reducing LDL cholesterol?



**50 gm
Soy Protein**

➤ 3% reduction in
LDL cholesterol

**25 gm
Soy Protein**

➤ 12.5% reduction
in LDL cholesterol

**250 mg
LunaRich X™**

➤ 8.6% reduction
in LDL cholesterol

- **Lunasin is the active ingredient in soy protein responsible for lowering LDL cholesterol.**
- **Lunasin lowers LDL cholesterol by reducing cholesterol biosynthesis in the liver.**
- **Lunasin has similar mechanism of action as statin drugs.**

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Galvez. Circulation Research, 2012. 126: p. A10693.

Figure 14

Research trials on pigs with mutated LDL receptors have shown Lunasin's ability to lower cholesterol by reducing cholesterol biosynthesis in the liver. In this experiment, pigs were fed with casein protein. The casein protein was not replaced with soy protein. The pigs consumed about a kilogram of casein-based protein every day. They were also given 250 mg of LunaRich X. When this was done, researchers tracked an 8.6 percent reduction in LDL cholesterol. This was reported in the American Heart Association in 2012.

Currently, statin drugs are the only drugs that have been shown to lower cholesterol and reduce the risk of cardiovascular disease. Other cholesterol lowering bioactive agents lower cholesterol but they have not been shown to lower the risk of cardiovascular disease. Lunasin has a similar mechanism of action as statin drugs. Thus, not only does Lunasin lower cholesterol, it can also explain the reason why soy protein reduces the risk of cardiovascular disease.

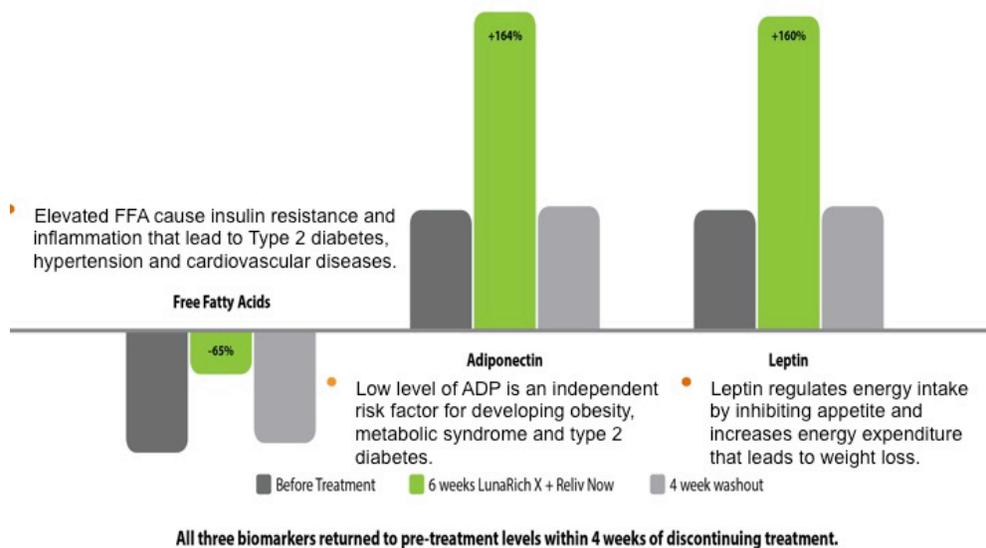
An additional experiment combined LunaRich X with Reliv Now. The goal was to see if there were any major health effects when supplemented with this combination. For this experiment,

researchers looked at five pigs and tested for biomarkers associated with health. A significant biomarker affected by LunaRich X and Reliv NOW was free fatty acid levels, which were reduced by 65 percent. This is significant because elevated free fatty acids cause insulin resistance and inflammation that can lead to Type 2 diabetes, hypertension and cardiovascular disease.

Additional experiments were conducted to determine how Lunasin was reducing free fatty acids. There are two fat hormones that are involved in free fatty acid metabolism . These include adiponectin and leptin. When looking at adiponectin levels, researchers found that these levels were increased by 64 percent. This might explain why free fatty acid was reduced. Having low levels of adiponectin is an independent risk factor for developing obesity, metabolic syndrome and Type 2 diabetes. Researchers looked specifically at leptin, the obesity gene that regulates energy intake by inhibiting the appetite and increasing energy expenditure that leads to weight loss. They found that the combination of LunaRich X and Reliv Now also increased leptin levels by 60 percent.

When LunaRich X and Reliv Now were removed from the pigs’ diet, in all cases free fatty acid levels increased up to baseline levels. With this removal, adiponectin and leptin also both went back down to baseline levels. This indicates that the treatment itself was causing the effect. In other words, it wasn’t a placebo effect. All biomarkers returned to pre-treatment levels within four weeks of discontinuing the treatment.

LunaRich X + Reliv Now Study

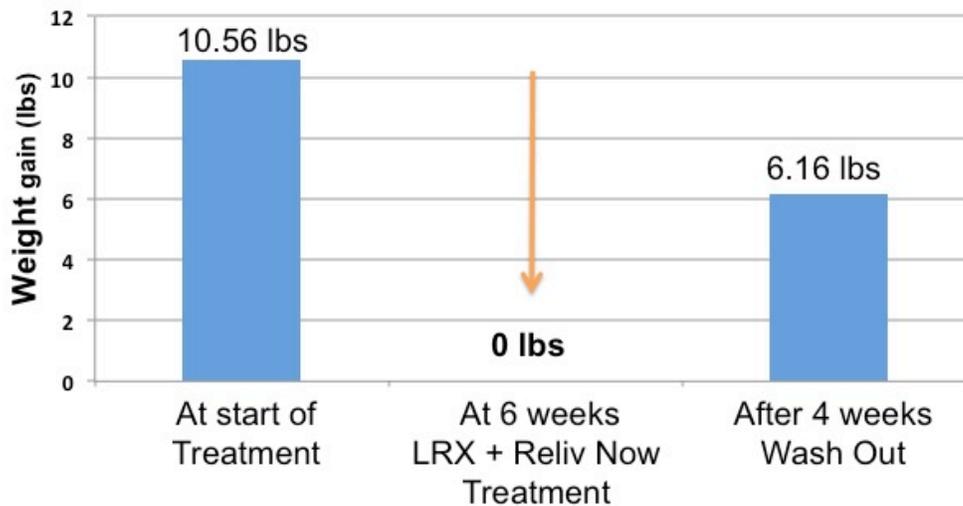


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Figure 15

This combination treatment also affected the pig's weight gain. At 6 weeks when there was maximal leptin and adiponectin levels, the pigs did not gain any weight, but started to gain weight again when the treatments were stopped.

LunaRich X + Reliv NOW reduce weight gain in obese pigs



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Figure 16

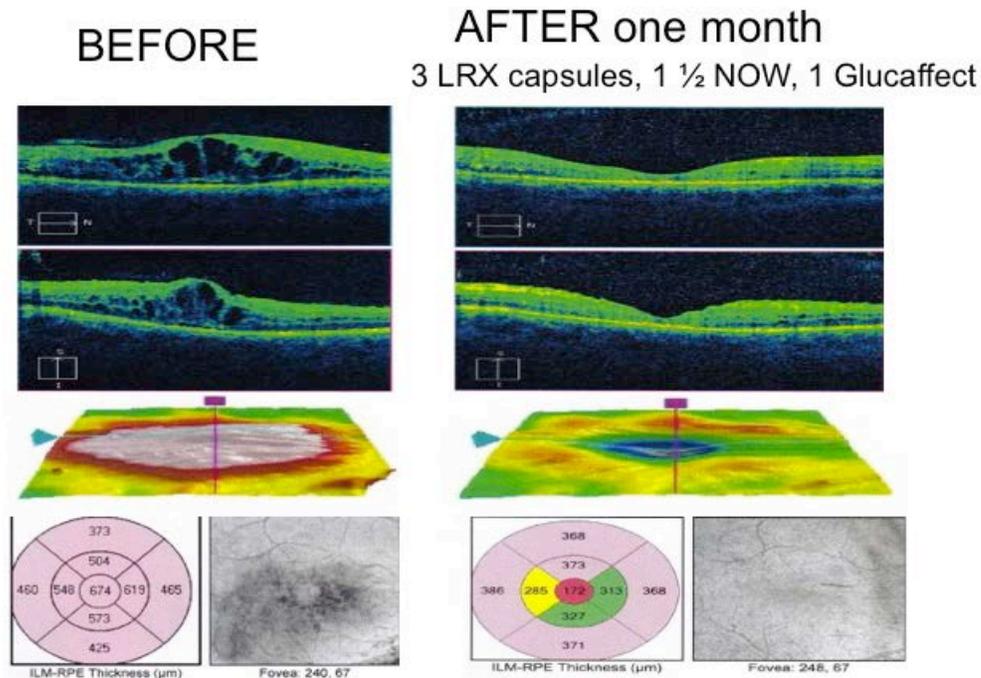
On the Market

LunaRich X was released to the public in January of 2013. Since then, many have reported health benefits. One of the most notable results have to do with diabetic edema. Typically, with diabetic edema, the macula is swollen because it is full of fluids. This often causes blindness. Figure 17 shows the outcomes of a particular diabetic patient. This 21 year-old had Type 1 diabetes, with diabetic edema that caused blindness. The swelling of the macula was thick. Standard treatments would use an injection of anti-angiogenesis drugs to address this issue. Cancer drugs like Avastin are typically injected directly into the macula to treat the

problem. But it can take from six months to more than a year of monthly injections to see any effects in the reduction of the macular edema.

In this case, Reliv Now, LunaRich X and Glucaffect, the supplement for diabetics, was administered. Within one month, the issue had cleared.

Retinal Scan of a Diabetic Patient



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Figure 17

Gene Disease Paradigm

When mutations occur, this can lead to dysfunctional biochemical signal transduction pathways, causing disease phenotypes. Drugs for cancer and other chronic diseases are developed specifically to counterbalance these defective gene products and pathways.

Gene –Disease Paradigm

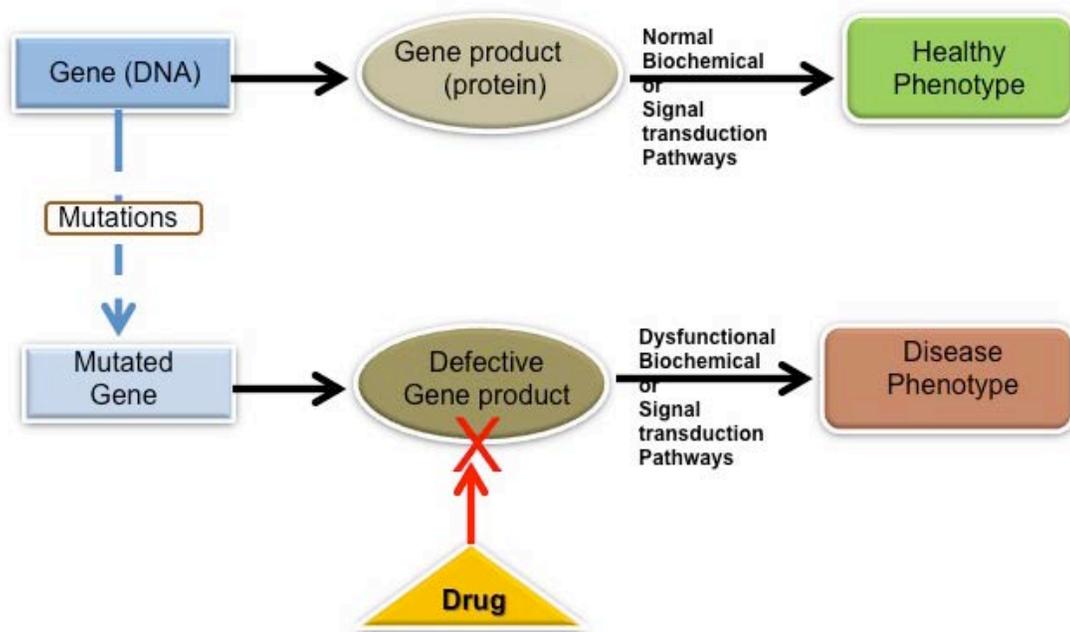


Figure 18

But there is an issue with this because genes associated with all biochemical pathways and signal transduction pathways in the body comprise only 2 percent of the human genome. So that means only 2 percent of the human genome encodes all the genes associated with those biochemical signal pathways that determine all of the metabolic pathways in humans. So what is that 98 percent of “junk DNA” doing in this particular case? This is where things become problematic. Research determined that about 80 percent of that junk DNA is associated with regulating the expression of genes. In other words, the complexity that makes humans human has to do mainly with the regulation of genes, not just on the genes themselves.

There are only 20,887 protein-coding genes in the human genome

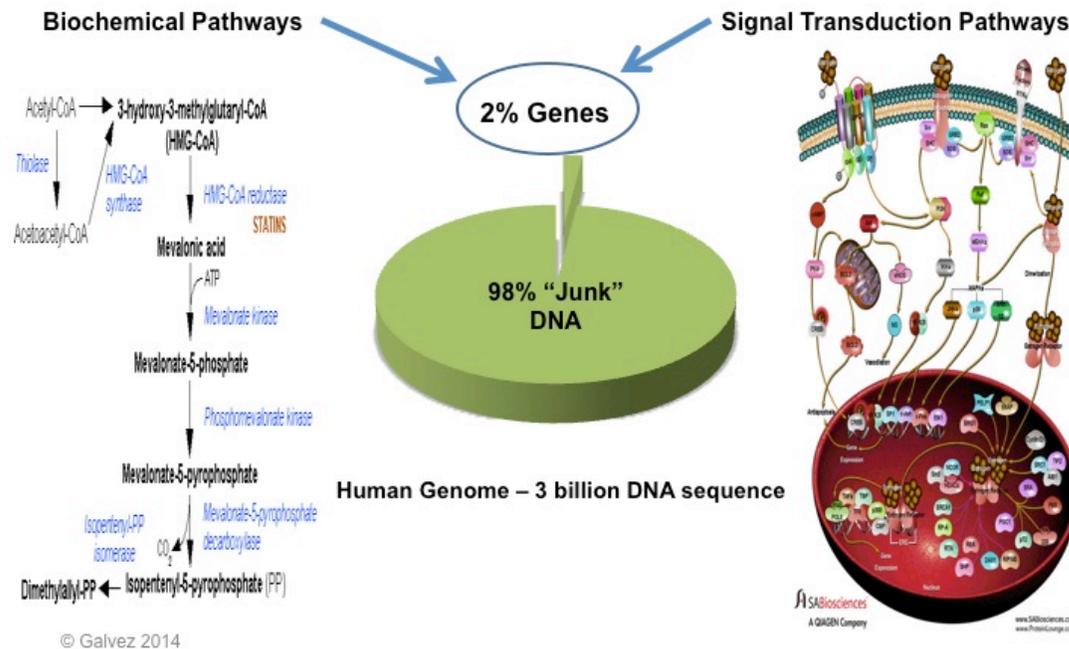


Figure 19

Diet affects the genome and DNA in many ways. It can lead to changes in gene regulatory and physiological pathways, impacting gene expression and ultimately influencing health outcomes. Diet can also affect the epigenome, a level of complexity involving the packaging of DNA, that determines which genes are turned on or off. The body has more than 300 different cell types, and each has a different epigenome. Recent studies have shown that in most chronic human diseases, changes to the epigenome happen first. In cases where driver mutations occur and cancer develops, these mutations may essentially just be a consequence of these epigenetic changes that allow these mutations to occur and proliferate.

The interaction between epigenetic mechanisms and gene regulatory pathways is another crucial element that needs to be addressed when studying effects of diet on health. This interaction is controlled by the so-called "junk DNA", which contains enhancers, transposons and non-coding RNAs, such as microRNAs and RNAi's, that can regulate expression of genes associated with metabolic and physiological pathways. Environmental factors like diet can also alter gene expression through its effects on the 'junk DNA'.

By looking at the genome, epigenome and the non-coding "junk DNA", instead of the genome alone, researchers hope to understand the overall effect of diet and environment on the human body as it leads to health outcomes.

Conclusion

Lunasin has a conserved and integral role in seed development in nature. It helps with the cell enlargement process that is necessary for developing seeds to store more nutrients for the developing embryo. It does this by binding to histone tails and preventing seed storage cell division after DNA replication. The fact that the histone tails in our bodies are the same as those in plants demonstrates how Lunasin could effectively integrate into our biology. And because the body has co-evolved with the Lunasin peptide, it is able to determine which genes to turn on and which genes to turn off in the human genome. Combining Lunasin with healthy environment and lifestyle choices has been shown to improve the effects of this peptide against non-communicative, degenerative chronic diseases. Its unique mechanism of action makes the Lunasin peptide unlike other cancer treatment options because it is able to fully integrate and adapt within the body. Though more long-term research is still being conducted on Lunasin, it is evident that this peptide could have a monumental impact on cancer and chronic disease treatment in the coming years.

Contributor

Dr. Alfredo Galvez is currently the chief scientific advisor for SL Tech, Inc., a subsidiary company of Reliv International. He is also a project scientist at the Center for Nutritional Genomics at UC Davis. Galvez discovered the Lunasin peptide in 1996 as a postdoctoral scientist at UC Berkeley and has since been instrumental in elucidating its novel mechanism of action and numerous health benefits. He has partnered with SL Tech, Inc., to develop the LunaRich soy powder and LunaRichX capsules that optimize the amount of bioactive Lunasin found in Reliv products.