Epigenetics – A New Way to Rejuvenate Skin

By Esther Belser

Editor's Note: It is not often that an author can clearly explain a highly complex phenomenon to the relatively untrained and uninitiated. Combined with a flair for following and understanding the rapid increase of information with time the author has produced a "Must-Read" article. It explains much to those involved in striving to learn the basics of Epigenetics and the opportunity this understanding provides for rapidly escalating our knowledge in seeking out the Holy Grail of "Beautiful-Skin-in-Our-Lifetimes".

Introduction

GENES

If you understand genes and the genetic code, you understand humans. This has been the consensus among scientists for a long time, although many questions still remain unanswered.

EPIGENETIC GENE REGULATION

Experts in the field of molecular biology are currently working on decrypting an equally important code to that embedded in knowledge of the inner working of genes – and that is the promising area of Epigenetic Gene Regulation.

We begin this journey with the observation that in nature, epigenetic processes are responsible for queen bee development.

Based on this capability of bees, a promising question yearns to be asked and answered for humans.

"How does epigenetics influence us and are we ourselves able to influence this second code with active cosmetic ingredients?"

To answer this question, an active ingredient that mimics royalactin, the "Queenmaker" protein in bees, was tested on its ability to change the epigenetic status of skin cells.

The Human Genome

26 June 2000 saw the publication of the first results of the Human Genome Project. The results were by no means complete, but researchers considered this event one of the biggest breakthroughs in scientific history. These findings were considered to hold the key to research on health and disease. However, disillusionment soon set in. Instead of the estimated 100,000 genes, only slightly more than 23,000 were found – some plants or even water flies had more to show. And it turned out we share nearly 98.9% of our genes with our close relatives the chimpanzees, who after all look and behave very differently to us.

Scientists had uncovered a code of 3.2 billion base pairs, but the mysteries of the human blueprint were far from being understood. Identical twins, for example, have identical DNA. Yet with increasing age, more and more differences appear between the siblings that cannot be explained by DNA mutations, but rather are founded in their different lifestyles.

The Epigenome: The Regulator for Gene Expression

It has since been discovered that genes are not the only players involved. Basically, a gene is a stretch of DNA that encodes the information – mostly for a protein – to be built. But not all proteins are produced in the same amount in every cell. This means that the expression, i.e. the reading of the genes and the subsequent production of the encoded protein, has to be controlled. There are different mechanisms that specifically regulate the genes. Genes can even be completely switched on and off again. Because there are processes that are superordinate to the genetic code, they were called 'epigenetic' (in Greek epi = over). Epigenetics has since become a completely new branch of biological research, and its discovery was honoured with a Nobel Prize in 2006 [1]. Many parallels have been found between epigenetic regulation and various diseases. These parallels, and their significant impact on human well being include, but are not limited to cancer, and new therapeutic approaches are being developed as a result.

Molecular Mechanisms of Epigenetics

Genes undergo epigenetic modification; that is, they are given chemical patterns, but the DNA sequence is not altered however. Up to now, the following three main mechanisms have been discovered (Figure 1):

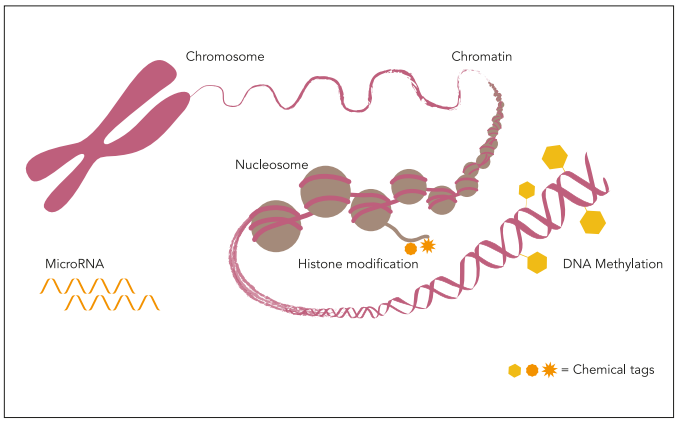

Figure 1: The epigenome orchestrates gene expression: the three main mechanisms of epigenetic modification of the DNA.

1. DNA methylation (chemical change in the bases cytosine and adenine in the DNA)
2. Histone modification (chemical change in the histones, the protein "core" around which the DNA is coiled)
3. Production of microRNA (very short chains of nucleotides, that influence gene regulation. Nucleotides are the basic structural units and building blocks for DNA)

Epigenetic imprinting is influenced by both development and ageing processes. When a human germ cell is fertilized, it first divides into two, then four, and then into hundreds and thousands of cells, all with an identical genetic make-up having the same DNA. More than 210 different cell types originate from these in humans. Each time, every individual cell type only uses the genes necessary for its nature-designated function. Most, but not all genes are silenced by epigenetic modifications. The butterfly caterpillar has the same basic genetic make-up as the butterfly that develops from it. The fact that it looks a lot more magnificent than its appearance during the larval stage, is solely due to epigenetic regulation which activates a completely new genetic program in its cells.

Epigenetically induced patterns in DNA can be passed onto daughter cells. For example, skin cells always produce skin cells with the same epigenetic activation program. As a result, – the organ, the largest in the human body, remains stable – and it can reliably fulfil its function.

Environmental Influences and the Epigenome

Our lifestyle also influences the manner in which the epigenome is regulated. Different genes are activated depending on the nutrition, physical activity, stress or air pollution we are exposed to. Environmental stress in a pregnant woman (e.g. smoking) can even affect up to three generations: the mother, the unborn child and, in case of a female foetus, the egg cells that are already present in that foetus.

Some epigenetic modifications are even still apparent years later in the DNA of the children, and are associated with various diseases such as diabetes and lung disease [2]. In connection with the determination of left or right handedness it has also recently been discovered that an asymmetric gene expression is apparent

in the spinal cord of fetuses as early as the 8th week in utero. It wasn't the genes themselves that were responsible for this, but the epigenetic processes caused by environmental influences. It is therefore assumed that, in addition to genetic predisposition, up to 75% of hand preference is also determined by epigenetic processes [3].

Unlike the genome, the epigenome is mostly flexible. Therefore, if we change our lifestyle positively for a longer period of time, we can reverse many negative imprintings.

Inheritance by the Next Generation

For a long time, it was thought that it was not possible to pass on epigenetic patterns onto the next generation. However, it was seen quite early on in plants that this generally is not the case. For example, back in 1740, Carl Linnaeus discovered specimens of *Linaria vulgaris* that had very different flowers despite an otherwise identical growth pattern that did not fit his classification system. They could even pass this feature on to their offspring! It took nearly 250 years for the secret to be revealed: the DNA of the plants was identical – the cause of the modification in the flowers was the heritable (epigenetic) methylation of the DNA.

And What About in Humans?

Transgenerational epigenetics was considered impossible for a long time in mammals, as when the germ cells (cells that gives rise to either eggs or sperm) mature, multiple DNA methylation patterns are deleted and completely rewritten.

Despite this, there is now evidence that some genes manage to escape this important decoding phase. For example, historic records from Sweden indicate that grandchildren of men who lived through famine before puberty suffer less frequently from heart disease or diabetes [5]. Or, there is the case of the pesticide methoxychlor, which has been banned for years, yet it is suspected that it is causing an increased incidence of kidney and ovarian diseases, as well as overweight in children and great-grandchildren [6].

Many scientists remain skeptical of heritance of epigenetic patterns in humans, however, since epidemiological studies are usually unclear and it is impossible to rule out all interference factors. Nevertheless, new results on male rats have been published in recent years, which have attracted wide interest. The animals had to sniff acetophenone, a sweet, almond-like aroma, for a few days, and were given a weak electric shock straight after. Ten days later the male rats were allowed to mate with non-conditioned female rats. The offspring showed a scared response to acetophenone and behaved more anxiously. Even the second generation behaved more nervously as soon as the smell of acetophenone was in the air [7].

Epigenetics and Cosmetics

For researchers and developers of active cosmetic ingredients, the relation between epigenetics and skin ageing is of course of particular interest. Unfortunately, too few studies on this have yet been carried out. However, it is clear that chronic exposure to air pollution, UV light and other external stress factors can cause long-term skin ageing. These factors modify the epigenetics of the skin cells and the cells continue to age even when the stress factors are no longer present. In its research, Mibelle Biochemistry was therefore on the lookout for active ingredients that normalized these epigenetic changes to counteract skin ageing. We found an interesting analogy in the development of the queen bees.

Queen and Worker Bees Share the Same DNA

Honeybees *(Apis mellifera)* live socially in colonies where they are organized into castes: workers (sterile females), queens (fertile females) and drones (males). Among the females, queens greatly differ from workers: they have a much larger body size – a ten times longer lifespan – and they behave in a completely different manner. Furthermore, queens are extremely fertile- while workers are functionally sterile. However, surprisingly, all of the honeybees share the same DNA. They are clones of each other.

Royalactin – the "Queenmaker" in Royal Jelly

How does it work? Honeybees modify their genetic code by consuming a special diet called royal jelly. This peculiar juice leaves chemical "markers" on the DNA. Thus, royal jelly is the epigenetic trigger that determines a honeybee larva's fate.

The mechanism of action and active ingredient of royal jelly, however, was a mystery until 2011 when a Japanese scientist discovered the "queenmaker" protein royalactin[8]. This 57 kDa protein is the one crucial factor that drives all the changes needed to make a queen bee. Interestingly, the effects of royalactin are not just limited to bees. Scientific studies have revealed a kind of "cell doping" effect in other insects. For example, royalactin increases longevity in fruit flies. In mammals, on the other hand, royalactin was reported to enhance proliferation of rat liver cells [9]. All of these effects are due to the putative interaction of royalactin with the epidermal growth factor (EGF) receptor [8]. Activation of the EGF signaling pathway not only triggers the development of a regular honey bee larva into a queen, but it also promotes tissue

regeneration and delays signs of senescence in human skin cells [10, 11]. And thus, a pathway to the long sought fountain of youth in skin appearance.

RoyalEpigen P5 – a pentapeptide mimicking the key function of the queenmaker protein

Inspired by epigenetic science, Mibelle Biochemistry developed RoyalEpigen P5 to delay the aging of skin. RoyalEpigen P5 is a biologically active pentapeptide that mimics the key function of royalactin. Interestingly, only fresh royal jelly is fully potent. This is due to the rapid degradation of royalactin after a short time of storage. Encapsulation of the peptide into a nano-structured lipid matrix ensures its delivery to the target skin layer without losing any activity.

Epigenetic Mechanism: RoyalEpigen P5 influences miRNA Expression

As mentioned previously, another one of the epigenetic mechanisms that can influence the expression of genes are the actions of microRNAs (miRNAs). By binding to the mRNA, miRNAs can block protein production being expressed from genes (Figure 2). Equally, when less miRNAs are present in a cell, this would lead to an unhindered protein production.

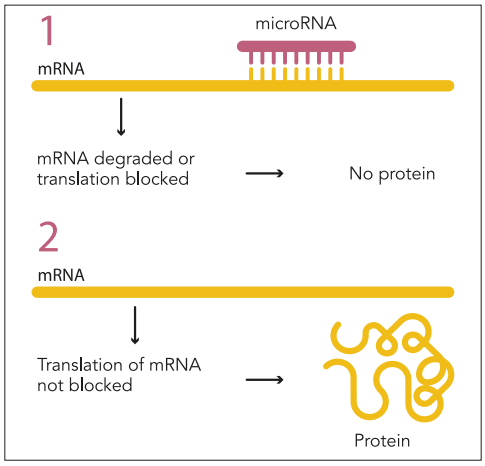

Figure 2: Epigenetic mechanism: miRNAs bind to the mRNA and block protein production / lead to degradation of the mRNA (1). Reduced miRNAs expression leads to an incread protein production (2).

To investigate whether RoyalEpigen P5 is able to induce epigenetic changes in human skin cells, expression of >1000 miRNAs was analyzed in aged fibroblasts either untreated or treated with 0.1 % RoyalEpigen P5. The seven most downregulated miRNAs were analyzed for their target mRNAs. Interestingly, these miRNAs target Collagen, two types of Keratins and a proliferation factor, which are all downregulated as skin ages. This means that RoyalEpigen P5 is reducing the miRNAs that would inhibit the production of these important proteins. Consequently, treatment with RoyalEpigen P5 leads to rejuvenation of fibroblasts through an epigenetic mechanism.

RoyalEpigen P5 Triggers Skin Cell's Regenerative Capacity

The regenerative potential for skin, of a cosmetic active, can be tested in an in vitro wound healing assay. In short, the basic step involves creating an "artificial wound" in a cell monolayer, capturing images of the preparation at the beginning and at regular intervals during cell migration, which eventually closes the wound. RoyalEpigen P5 shows the remarkable capacity to accelerate proliferation and migration of keratinocytes to efficiently close the lesion (Figure 3). The obtained images indicate a potent regenerative effect of the peptide, indeed very similar to the reference compound EGF (Epidermal Growth Factor, a polypeptide that speeds wound recovery).

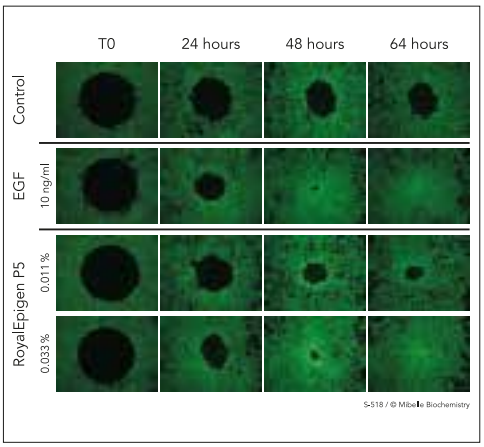

Figure 3: Wound healing assay. RoyalEpigen P5 accelerates proliferation and migration of keratinocytes to close the lesion. The effect is similar to the reference EGF.

Clinical Study

To test the rejuvenating effect of RoyalEpigen P5 in vivo, twenty volunteers (average age 49) with inhomogeneous skin tone participated in a placebo-controlled study. A cream containing 2% of RoyalEpigen P5 was applied twice a day for 28 days to the inner side of the forearm. The renewal time of the stratum corneum decreased by almost 3 days (Figure 4) resulting in a much smoother skin surface (+16%). A positive effect was observed in 100% of the volunteers.

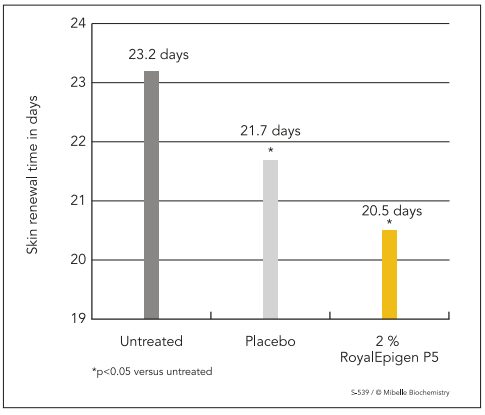

Figure 4: Improvement of skin renewal capacity.

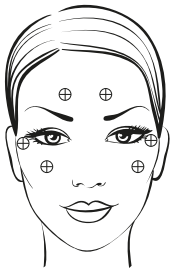

Figure 5: Evaluation of the skin homogeneity on six selected locations across the face.

Furthermore, RoyalEpigen P5 significantly improved skin homogeneity when it was applied on the face. The heterogeneity of their complexion was analyzed by measuring the skin lightness (L*) on six selected locations across the face (the forehead, temples and cheeks) using a chromameter (Figure 5). From there, the standard deviation of these six measurements was calculated. The higher the standard deviation, the higher was the heterogeneity of the complexion. Results showed that RoyalEpigen P5 led to a rapid decrease in standard deviation of the L* values measured on the six points in the face (Figure 6). RoyalEpigen P5 therefore increased the homogeneity of the skin by 6 % after 14 days and by 12.6 % after 28 days. Thus, by improving the uniformity of the complexion, RoyalEpigen P5 makes the skin more radiant. The increase in skin clarity was observed on 80 % of the volunteers.

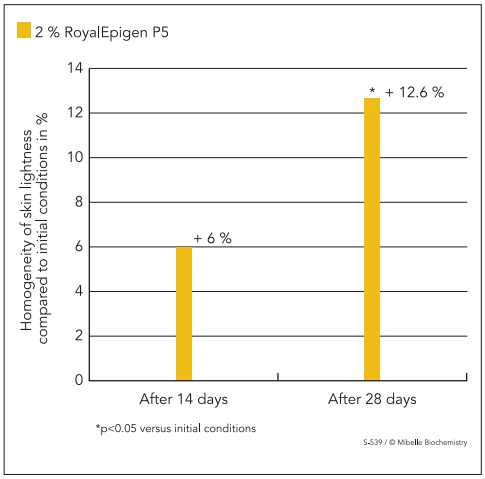

Figure 6: Increase in skin homogeneity.

Conclusion

The outside world can and does have a direct effect on the DNA of skin cells. RoyalEpigen P5 is based on the queen maker protein royalactin, the "magic factor" that converts a regular honeybee larva into a queen. We have shown in our testing that the five amino acid peptide modulates miRNA expression and accelerates keratinocyte proliferation. This capability provides aged human skin with a strong regenerative effect. RoyalEpigen P5 keeps the skin supple and gives it a smoother texture and more even appearance.

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