



The Power of Royalactin

Inspired by epigenetic science, Mibelle Biochemistry present RoyalEpigen P5 – a five amino acid peptide to rejuvenate the skin

For a long time it was believed that the way we look and act is predetermined in our genes that we inherit from our parents. This is only part of the truth. While our genes contain all the information that make us, the environment influences the way these genes are read and the information is interpreted. This regulation on top of genetics is called epigenetics. Epigenetics is the reason why a butterfly looks very different from a caterpillar although it always possesses the same genes: they are only switched on and off in a different pattern. In humans, the influence of epigenetics is best observed in identical twins. Although they share the same DNA sequence, differences in lifestyle and the environment such as smoking, diet and living in a polluted area, was shown to heavily impact their ageing process. This can lead to identical twins that appear to differ in age by many years.

Although the effects of epigenetics are obvious in nature, the exact mechanisms through which genes are regulated are still being unravelled in this relatively new

field. It is however known that epigenetic changes can be inherited from one cell to another – and in some cases even from one generation to another. This means that bad lifestyle choices could affect us for a long time even when the trigger for the epigenetic change is removed. The good news is that, as opposed to genetic mutations, epigenetic changes are reversible. Therefore, epigenetics is a highly interesting topic for cosmetic formulations which strive to protect and rejuvenate the skin.

RoyalEpigen P5 captures the power of the queenmaker protein royalactin

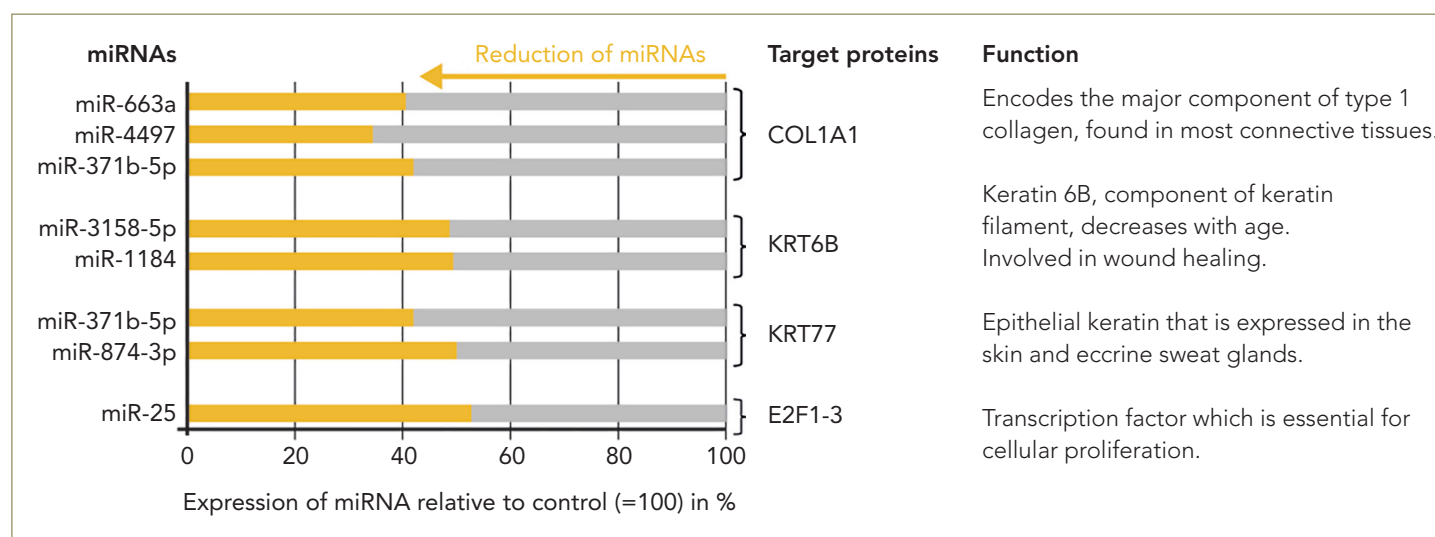
A well-studied example of how strong the epigenetic influence is can be found in bees. The queen bee and the workers in a hive are all genetically identical twins. However, the queen bee is larger, fertile, more resistant to environmental stresses and lives ten times longer than her worker sisters. The reason for this lies in the diet of the bees: the queen bee is exclusively fed royal jelly, which leads to these epigenetic changes. Recently, Japanese scientists discovered the

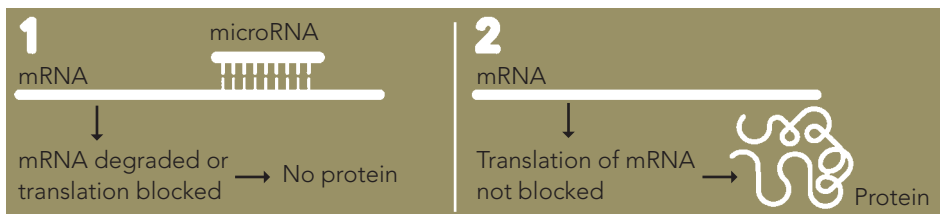
component responsible for queen bee development. It is a single protein called royalactin, which drives the epigenetic reprogramming of the queen bee. Interestingly, the effects of royalactin are not just limited to bees. Scientific studies have revealed that royalactin delays ageing and increases longevity in fruit flies and worms. In mammalian cells, royalactin was reported to enhance cell proliferation. This makes royalactin an ideal factor to promote rejuvenation of cells via epigenetic changes.

As the full protein royalactin is not stable in a formulation, Mibelle Biochemistry has developed RoyalEpigen P5, a five amino acid peptide that contains the conserved active sequence of royalactin. To ensure its stability in a formulation and enhance its delivery into the skin, the peptide was encapsulated in a soft sphere carrier system based on shea butter.

RoyalEpigen P5 influences miRNA expression

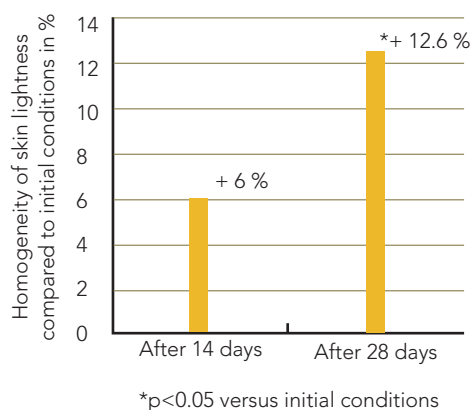
One epigenetic mechanism to influence the expression of genes without interfering





with the genetic code are micro RNAs (miRNAs). MiRNAs are short RNA pieces that are complementary to a part of a messenger RNA (mRNA) encoding for a specific protein. By binding to the mRNA, miRNAs can block protein production. Equally, downregulation of miRNAs would lead to upregulation of protein production. To investigate whether RoyalEpigen P5 is able to induce epigenetic changes in human skin cells, expression of >1000 miRNAs was analysed in aged fibroblasts either untreated or treated with 0.1 % RoyalEpigen P5 for 24h. The seven most downregulated miRNAs were analysed for their target mRNAs. Interestingly, these miRNAs target Collagen, two types of Keratins and a proliferation factor, which are all downregulated in aged skin. 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.

■ 2 % RoyalEpigen P5



RoyalEpigen P5 activates the cell's own cleaning system

Another mechanism that contributes to ageing of the skin is the decreasing activity of the proteasome. The proteasome is a cellular machinery which degrades damaged and oxidized proteins. In aged skin, this cell cleaning mechanism is reduced which leads to an accumulation of oxidized proteins that results in uneven skin and a dull complexion. An important factor labelling damaged proteins for degradation by the proteasome is called SKP1. Treatment of keratinocytes with RoyalEpigen P5 increases the gene expression of SKP1 in a dose-dependent manner. Therefore, RoyalEpigen P5 activates the protein turnover by stimulating the proteasome.

RoyalEpigen P5 improves cell proliferation and migration

To investigate the rejuvenating effect of RoyalEpigen P5 on a cellular level, several *in vitro* assays were performed with human keratinocytes. An *in vitro* wound healing assay with human keratinocytes demonstrated that treatment with 0.033 % RoyalEpigen P5 accelerated wound healing by one and a half days. Interestingly, this effect was comparable to that of the positive reference epidermal growth factor (EGF, 10 ng/ml).

To test whether RoyalEpigen P5 is also able to increase cell proliferation in aged cells, keratinocytes were grown in a special *in vitro* pro-ageing medium which enables skin cells to age in conditions that more closely reflect reality. Skin cells grown in this specific pro-ageing environment exhibited a reduced

proliferation and a shorter lifespan whilst remaining vital and maintaining a normal morphology. When these keratinocytes grown in the pro-ageing medium were additionally treated with 0.008 % RoyalEpigen P5, their proliferative capacity increased by 203 % after 3 weeks compared to an untreated control.

Taken together, RoyalEpigen P5 stimulates cell proliferation and migration and can even help to maintain an active cellular proliferation despite the skin ageing process.

Treatment with RoyalEpigen P5 increases skin smoothness and homogeneity

To test the rejuvenating effect of RoyalEpigen P5 *in vivo*, 20 women aged 40-60 applied a cream containing 2 % RoyalEpigen P5 and the corresponding placebo cream on each forearm twice daily. After 28 days, skin smoothness was increased by 16 %. A positive effect was visible in 100 % of the volunteers. Additionally, the skin homogeneity was measured before and after applying 2 % RoyalEpigen P5 on the face for 28 days. To measure skin tone evenness, the skin lightness L* was measured on six points of the face and the standard deviation of these six values was determined. The smaller the standard deviation, the more even the skin. RoyalEpigen P5 significantly improved skin homogeneity by almost 13 % after 28 days.

RoyalEpigen P5 is a biomimetic peptide that possesses the activity of royalactin, the component of royal jelly that drives epigenetic reprogramming in the queen bee. In human skin cells, RoyalEpigen P5 induces epigenetic mechanisms that lead to a smoother and more radiant skin for a youthful look.

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