HOW GENE EXPRESSION AFFECTS THE ANTIAGING PROCESS

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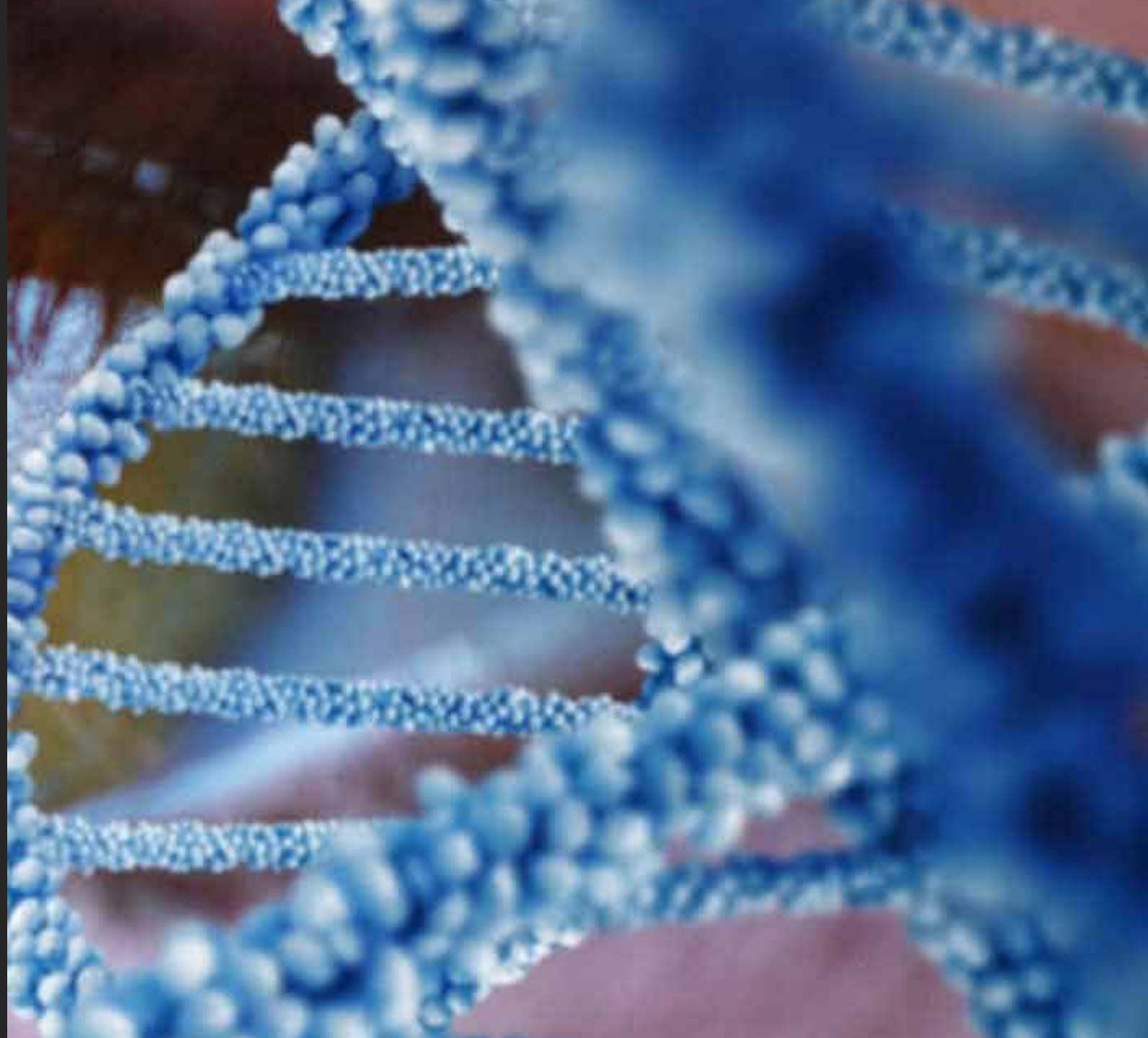
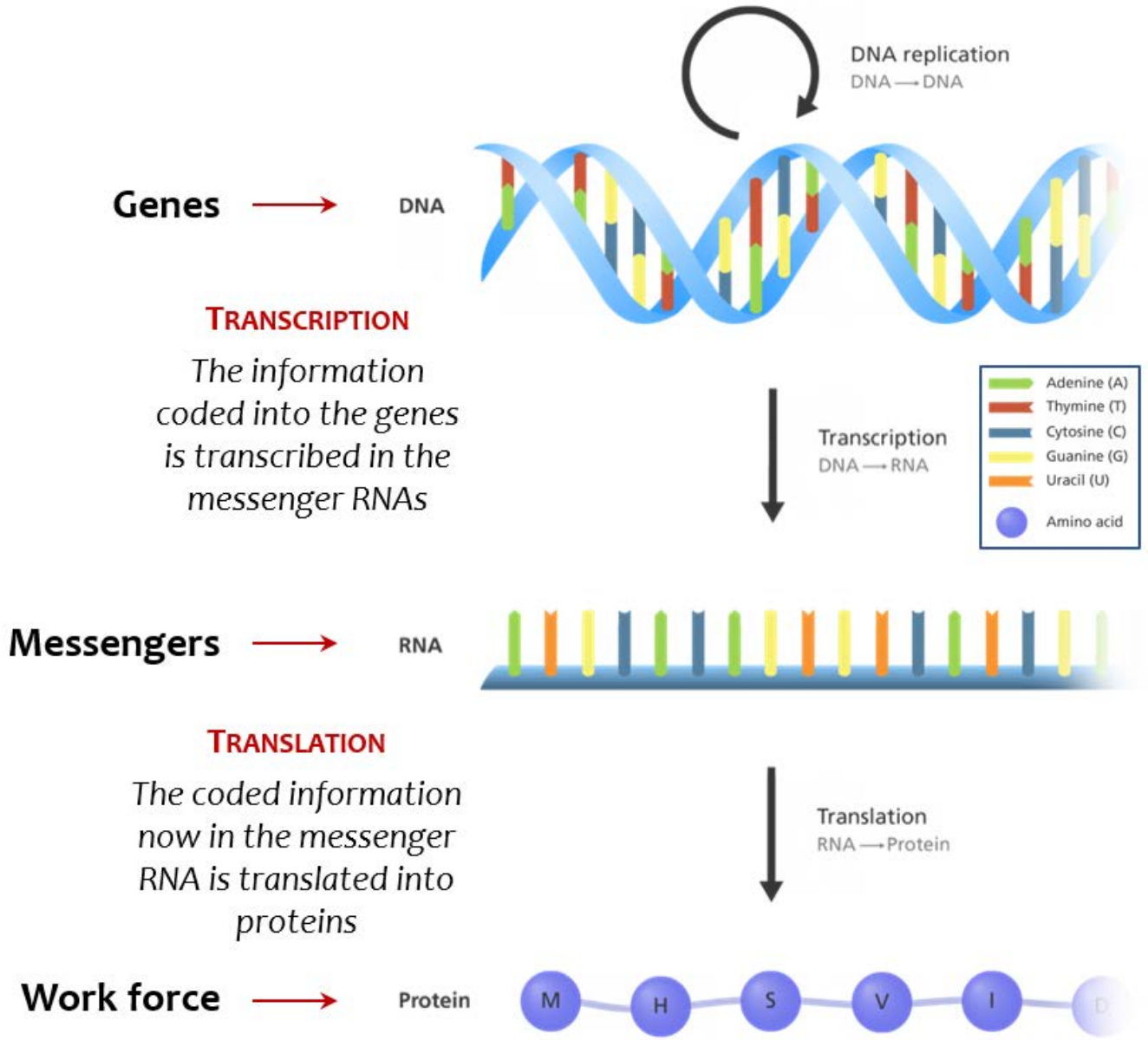


WHAT IS GENE EXPRESSION?

Gene expression is the process by which the **GENE INFO** OF THE DNA SEQUENCE is made into a FUNCTIONAL GENE product, such as **PROTEINS** or RNA.

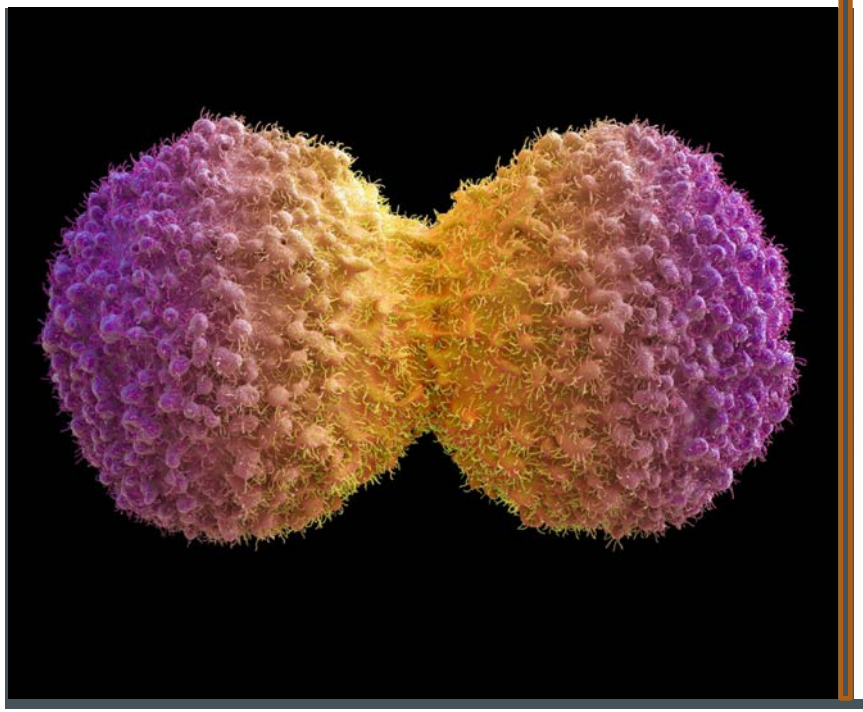


INDIVIDUALS WITH YOUTHFUL SKIN

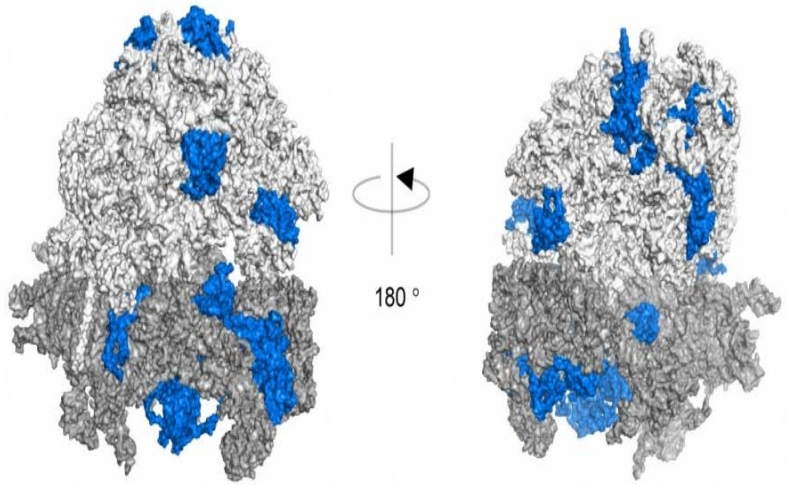
(WITHOUT BOTOX, FILLERS, LASERS, RADIOFREQUENCY, ETC)

THE YOUNGER LOOKING OLDER WOMEN HAD INCREASED ACTIVITY IN GENES ASSOCIATED WITH OTHER BASIC BIOLOGIC PROCESSES SUCH AS:

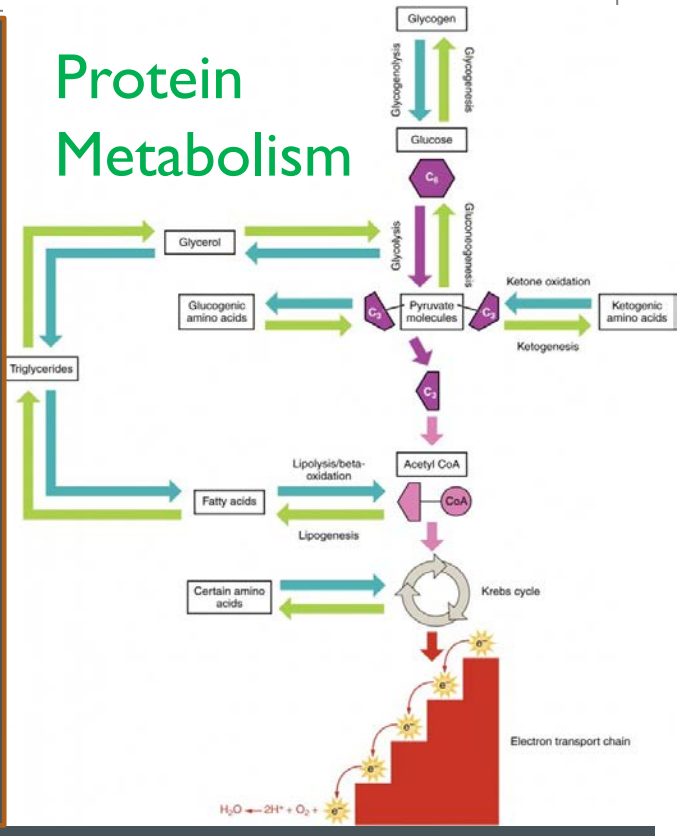
cell replication



response to oxidative stress

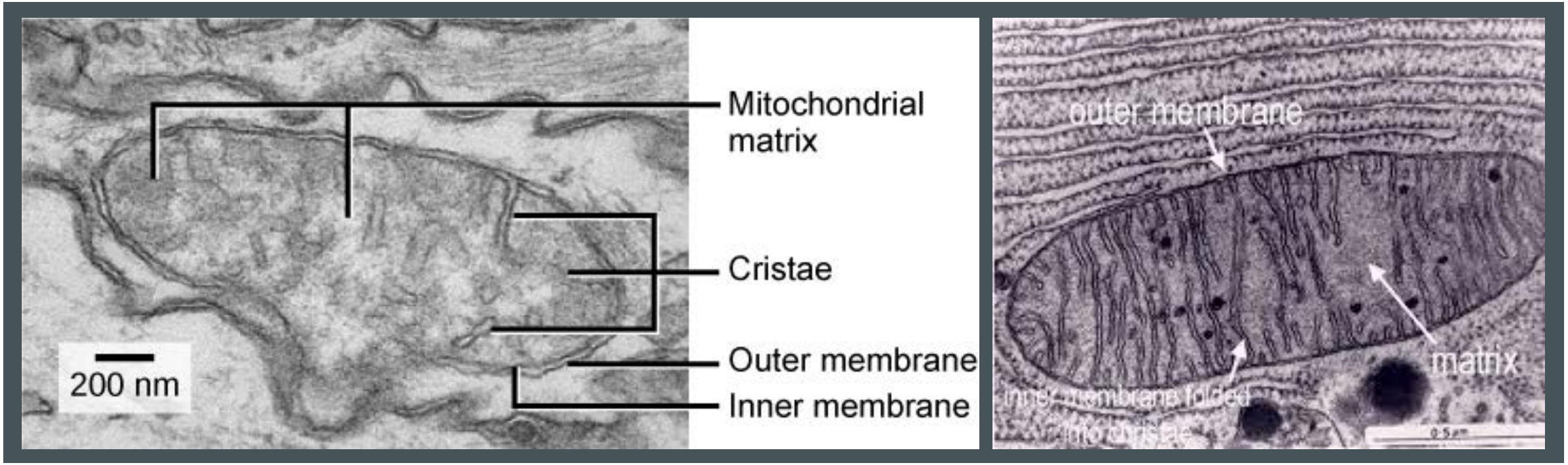


Protein Metabolism



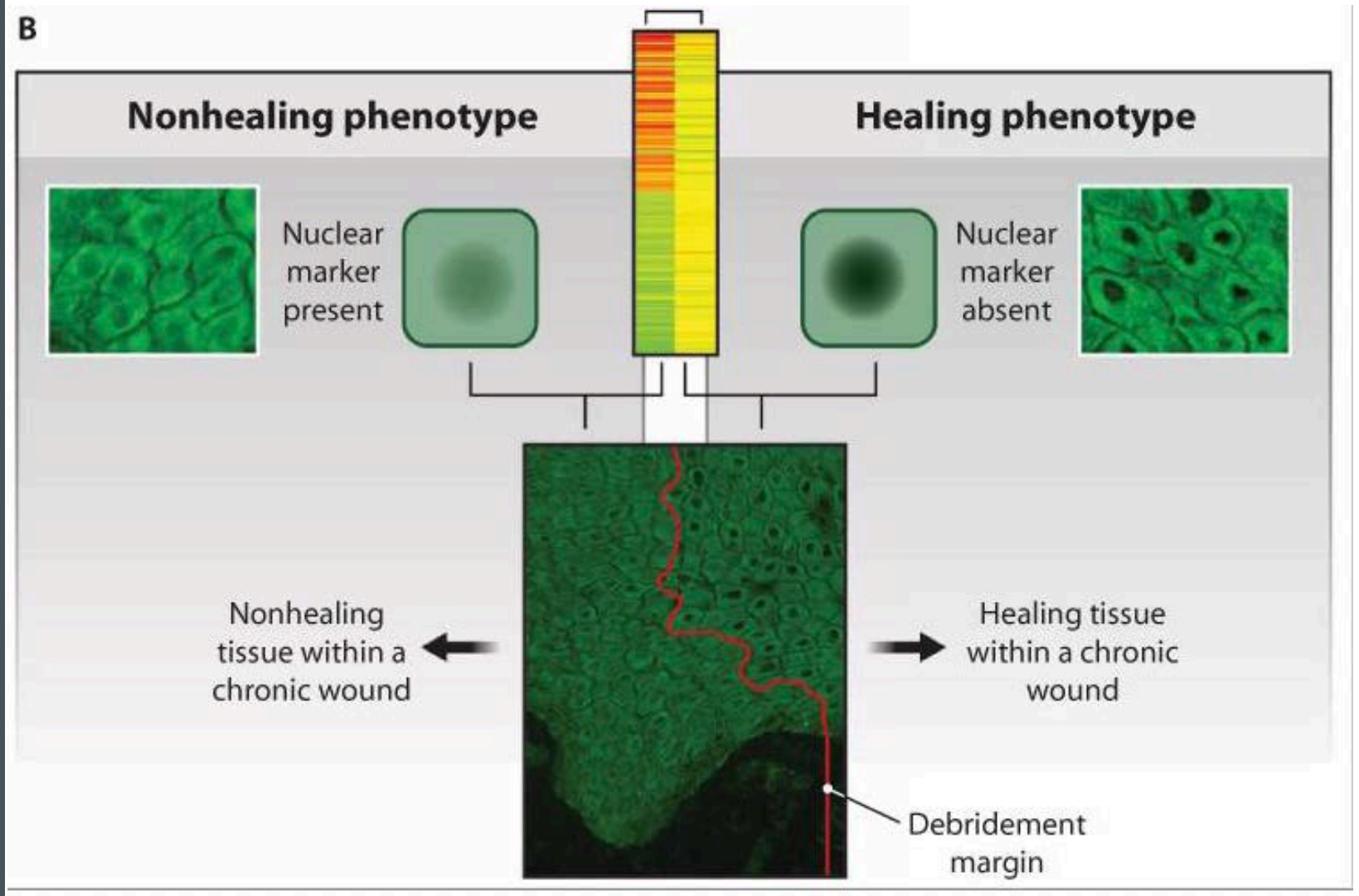
WOMEN WITH EXCEPTIONALLY YOUTHFUL-APPEARING FACIAL SKIN IN OLDER AGE GROUPS ALSO HAD HIGHER EXPRESSION GENES ASSOCIATED WITH:

- Mitochondrial structure
- Metabolism



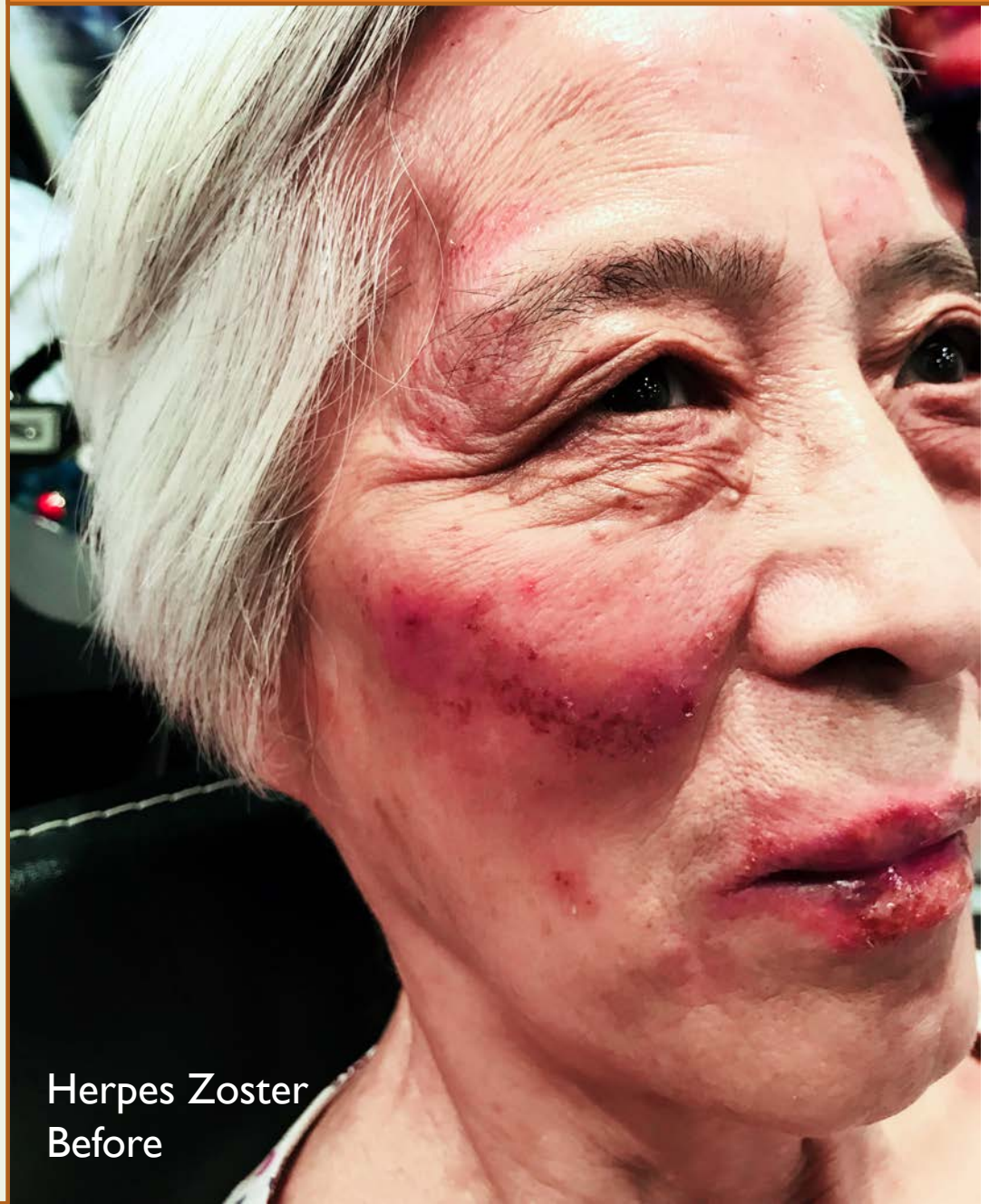
Women with exceptionally youthful-appearing facial skin in older age groups also had higher expression genes associated with:

- Overall epidermal structure
- Barrier function in their facial epidermal samples
- Dermal matrix production

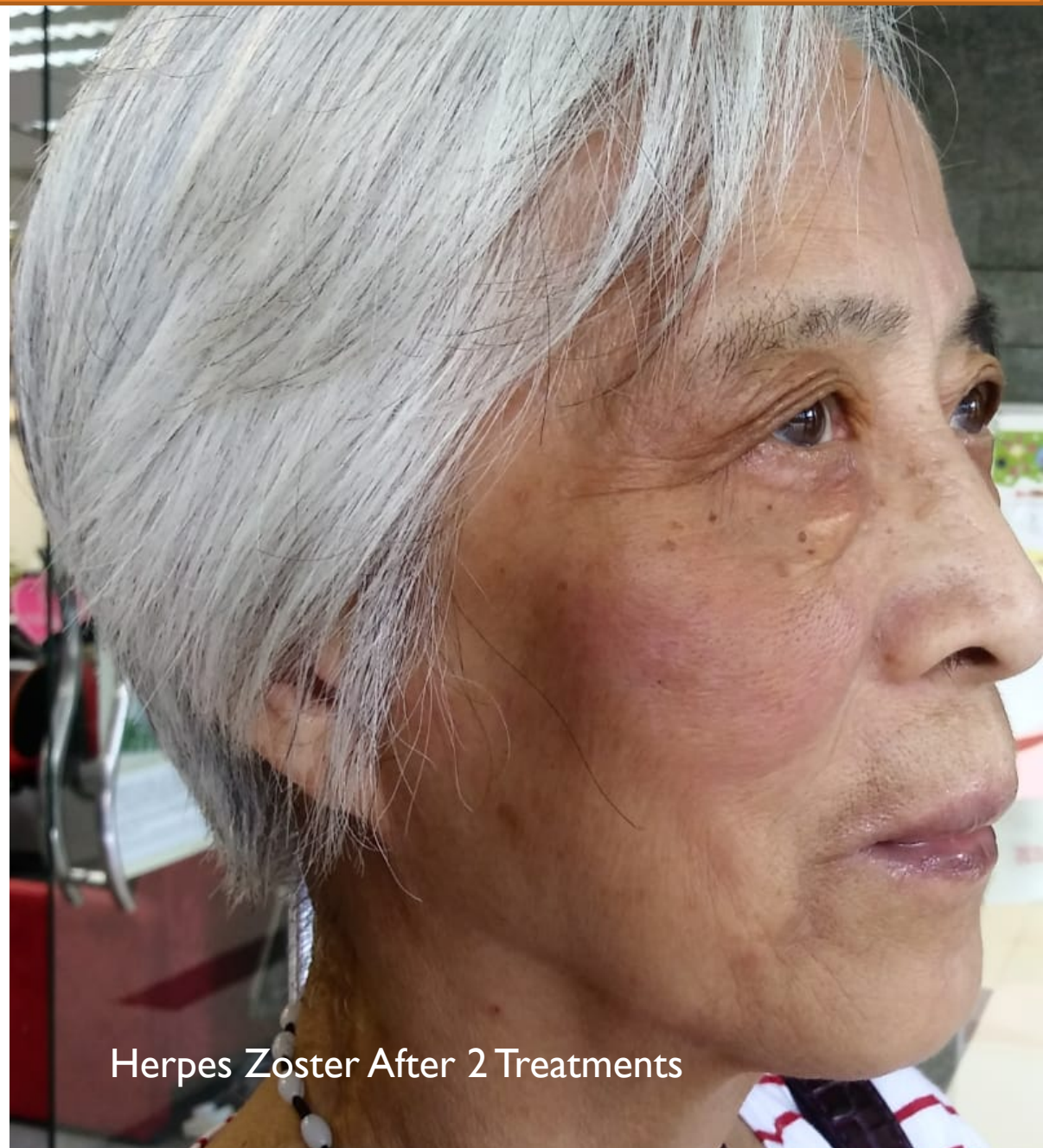


Signaling affecting wound healing – Sabine et al (2014)

Signaling Effects on Skin and Wound Repair – Clinical studies today



Herpes Zoster
Before

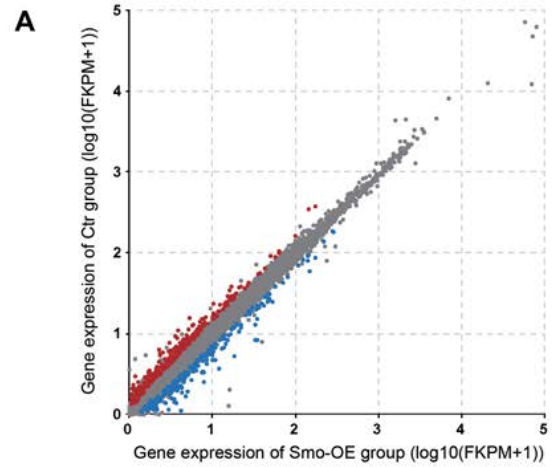


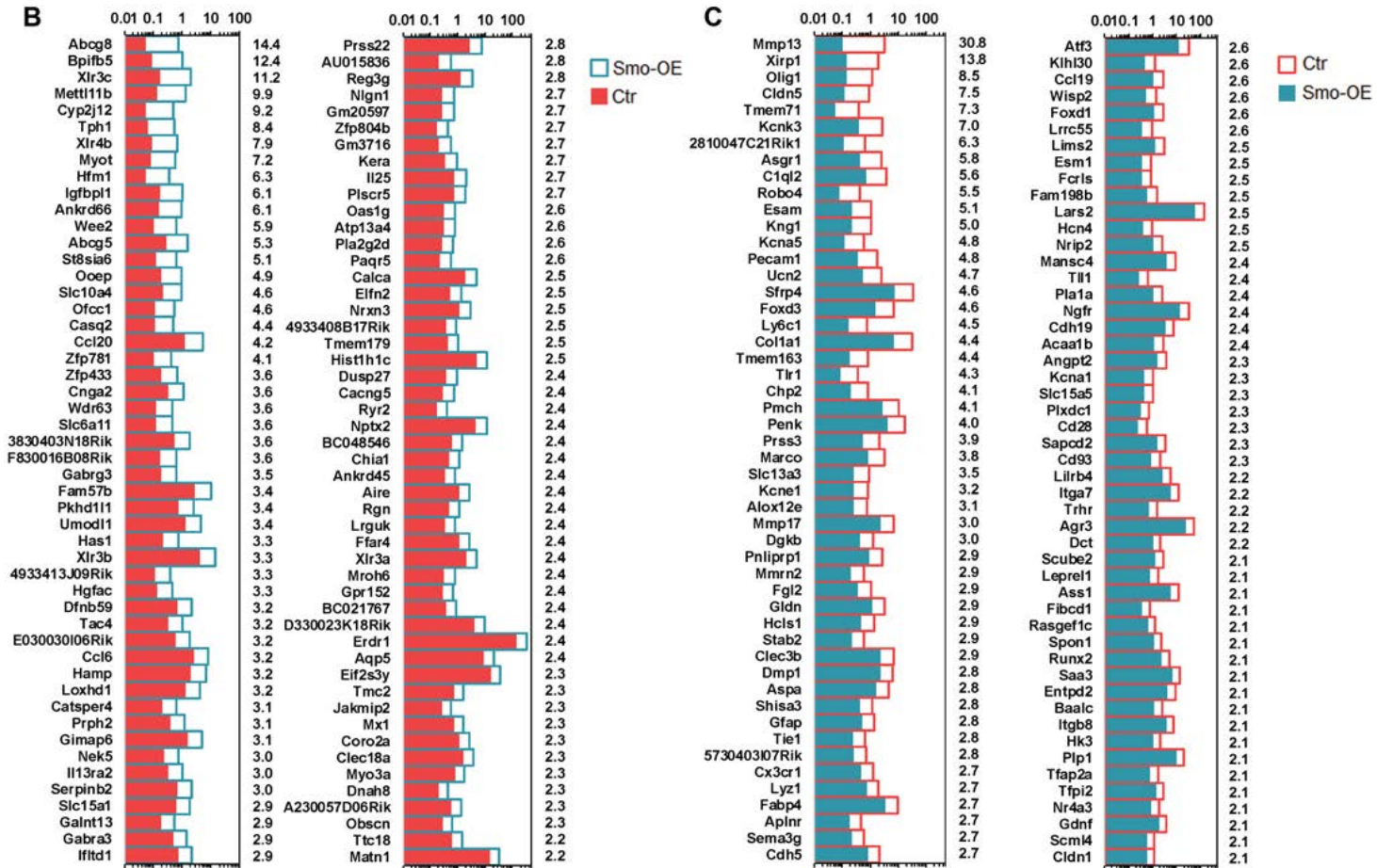
Herpes Zoster After 2 Treatments

Women with exceptionally youthful-appearing facial skin in older age groups also had higher expression genes associated with:

- Hair matrix production.

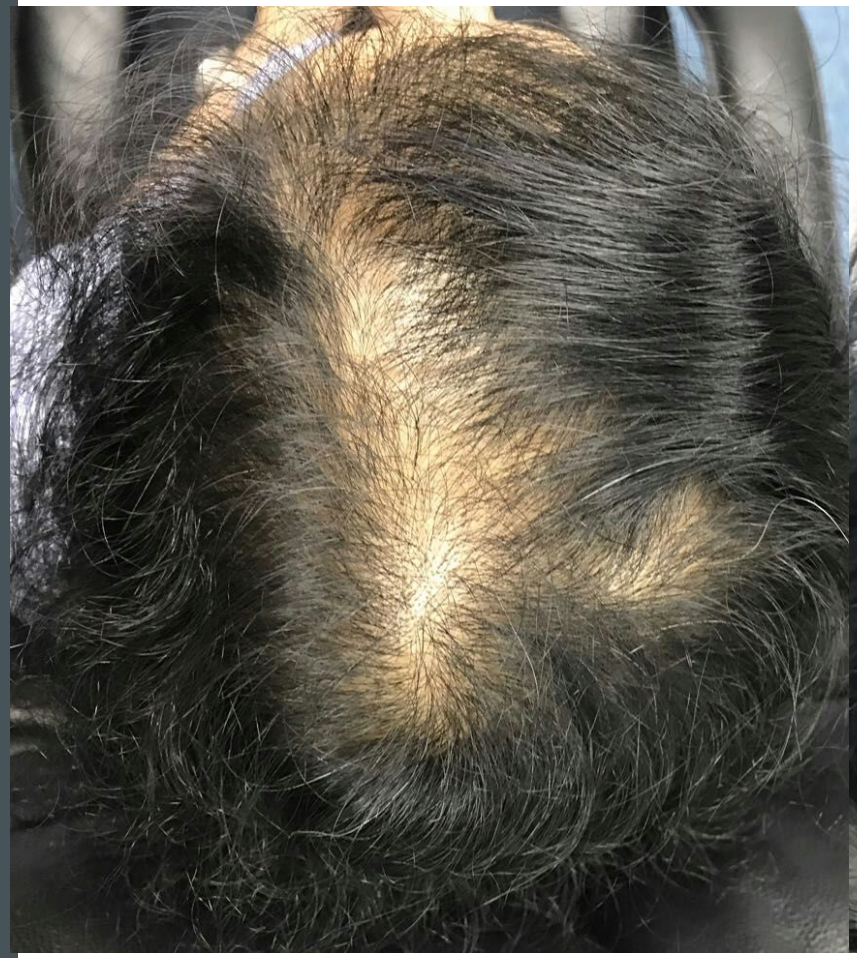
Hedgehog signaling on hair cell proliferation
Chuen et al. 2017

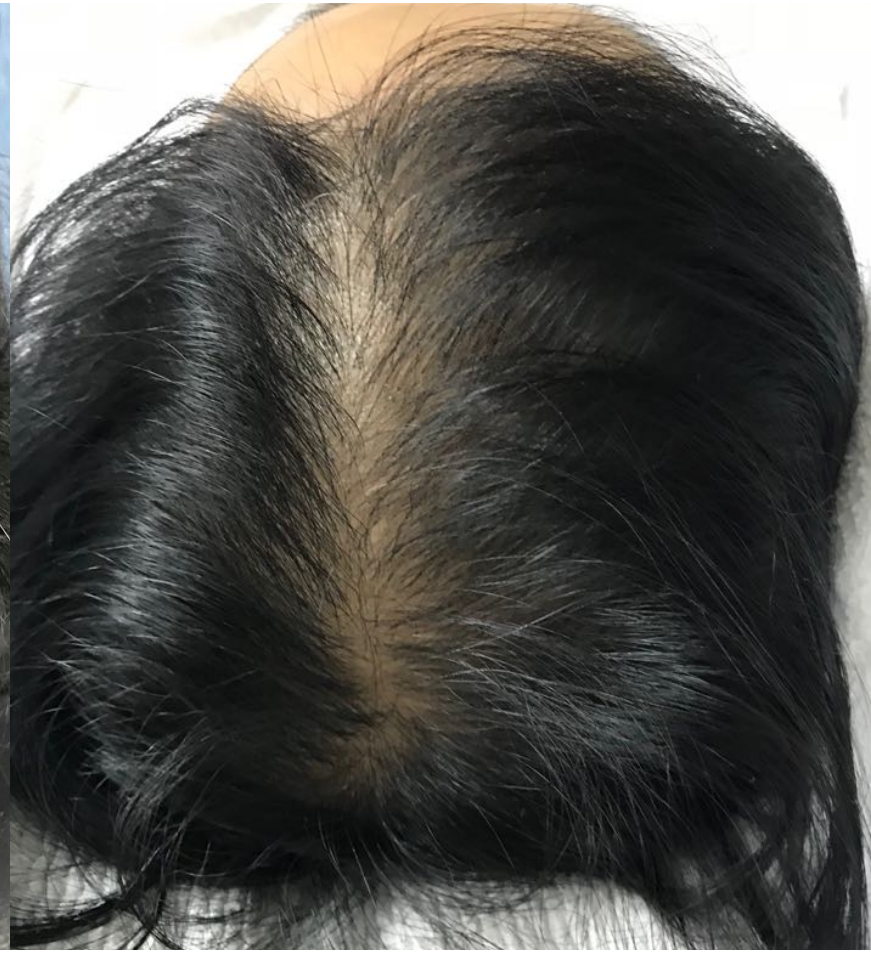


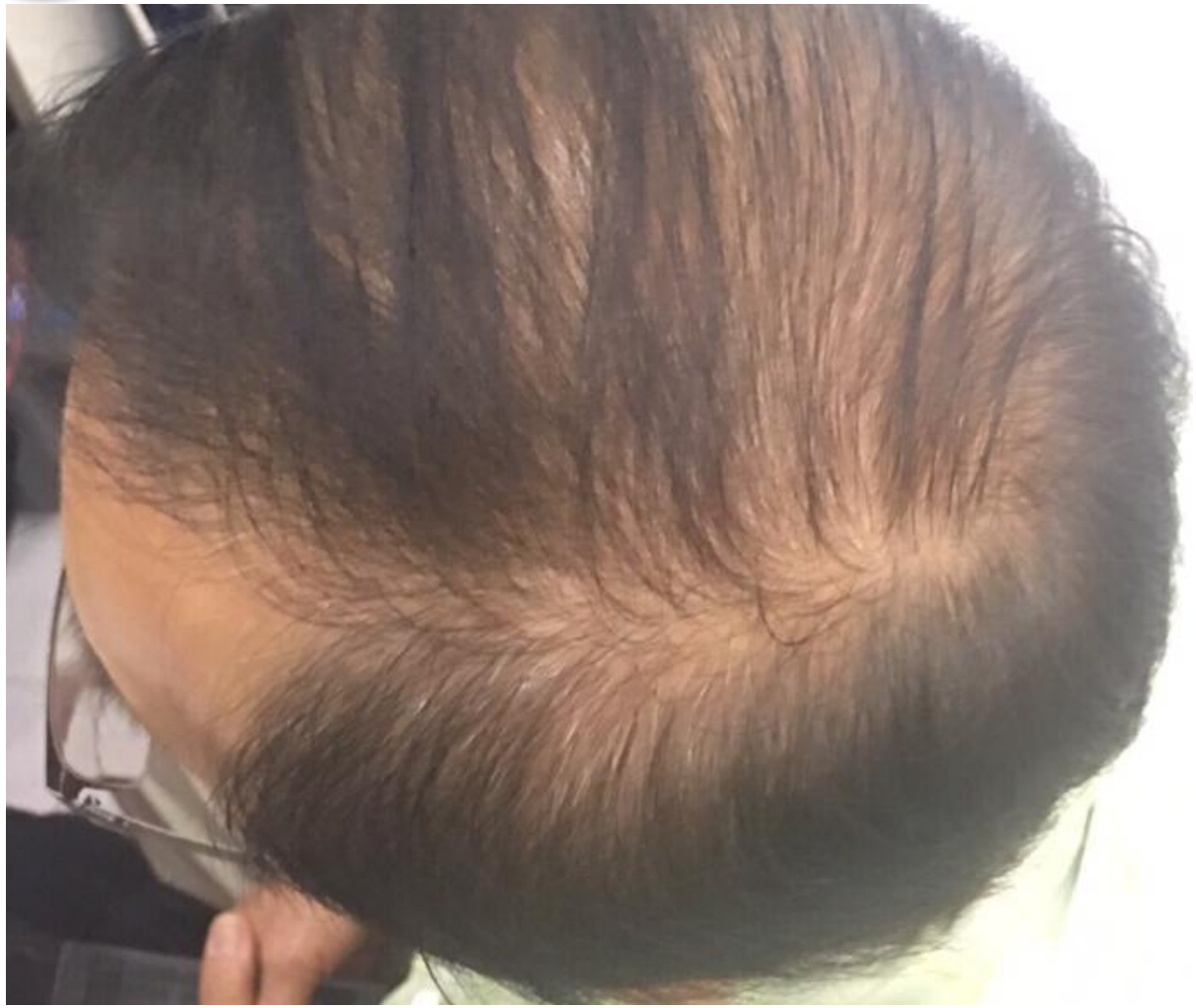


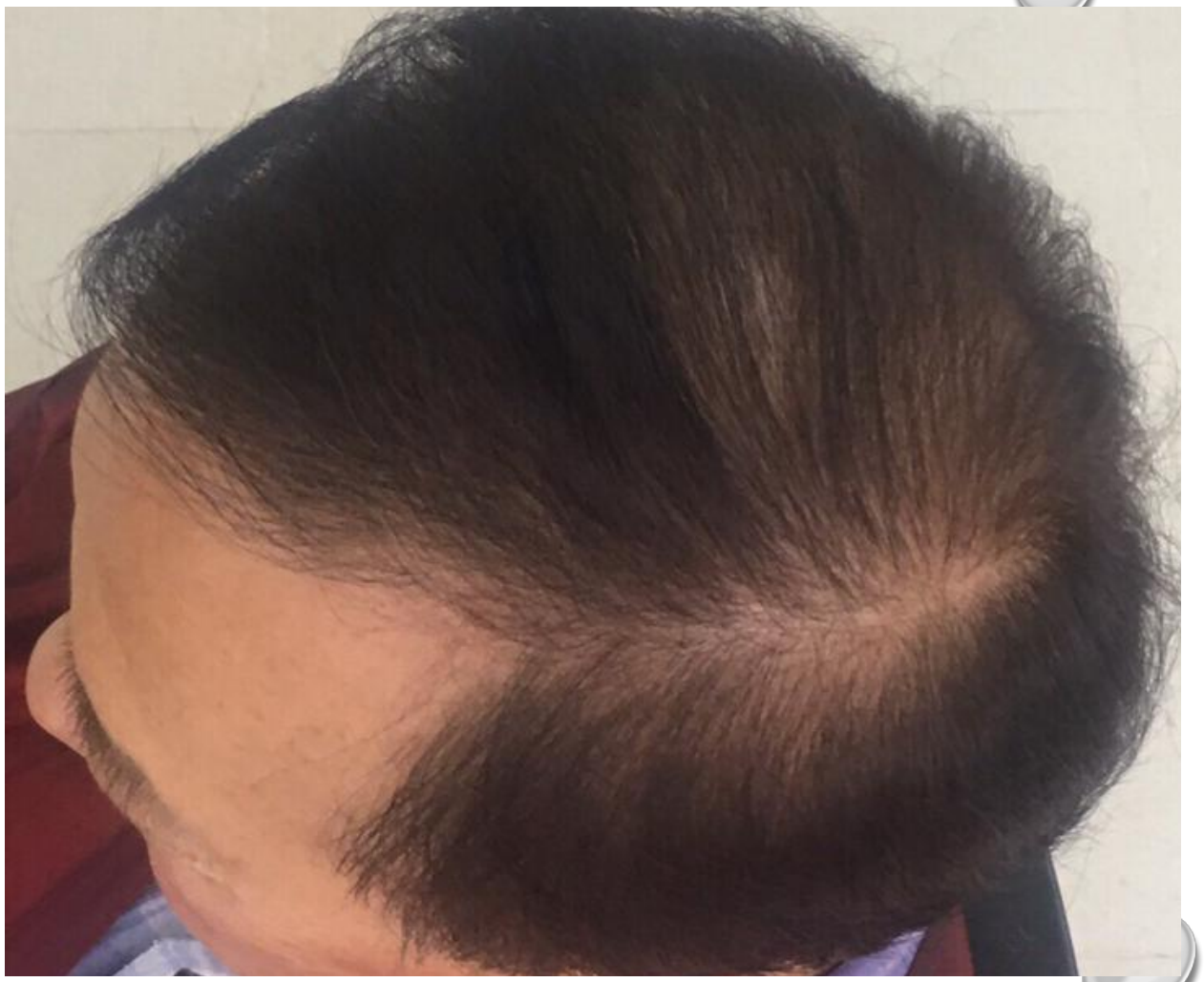
SIGNALING EFFECTS ON VISIBLE HAIR GROWTH (IN THE ABSENCE OF OTHER HAIR GROWING METHODS)

CLINICAL STUDIES TODAY









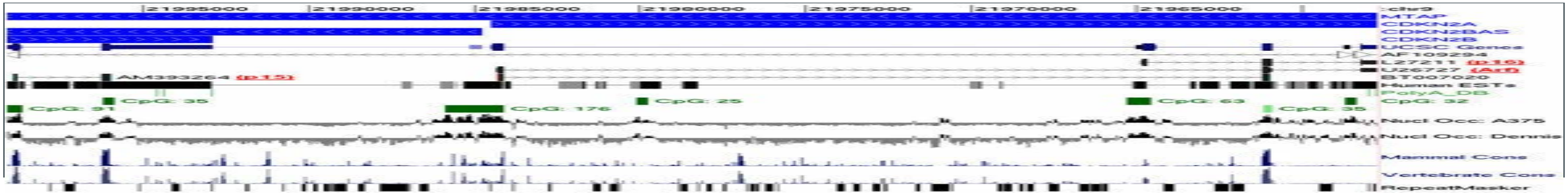
RATE OF EXPRESSION OF A GENE

THE RATE OF EXPRESSION OF A PARTICULAR GENE IS CONTROLLED BY

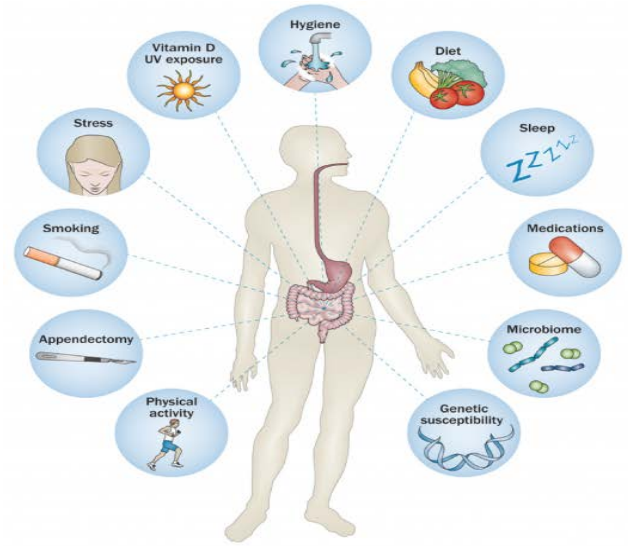
1. ITS LOCATION WITHIN NUCLEOSOMES

2. NUCLEOSOME DYNAMICS INVOLVE AN INTERPLAY OF:

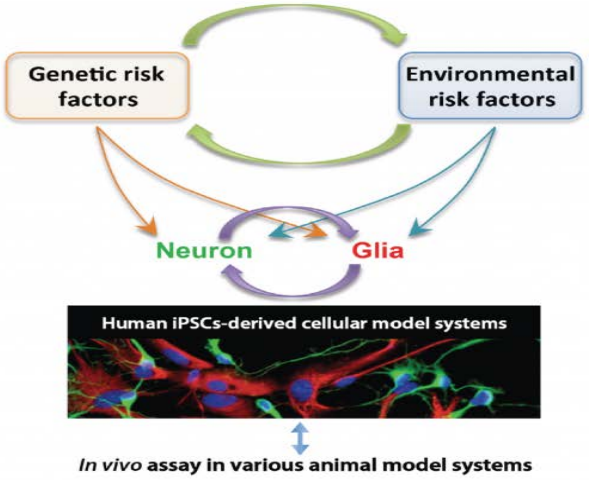
- HISTONE COMPOSITION
- HISTONE POST-TRANSLATIONAL MODIFICATION
- NUCLEOSOME OCCUPANCY AND POSITIONING WITHIN CHROMATIN
- CHROMATIN REMODELLERS
- CHAPERONES
- POLYMERASES

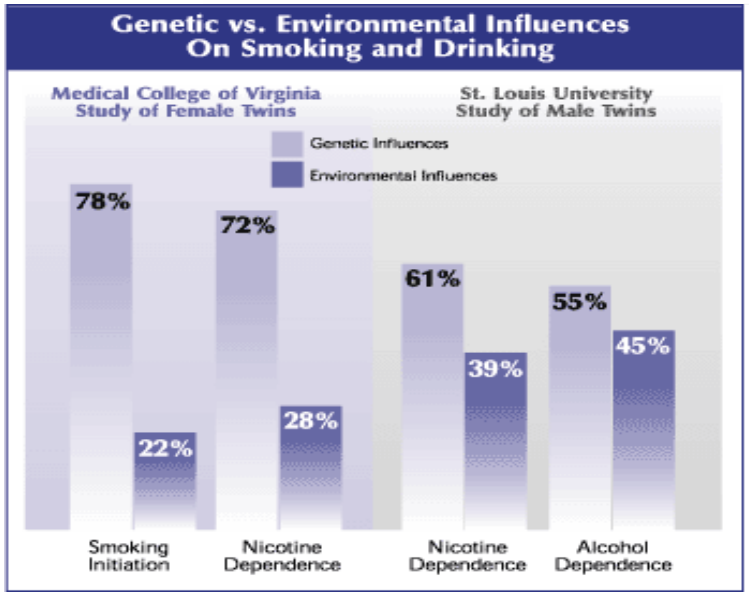


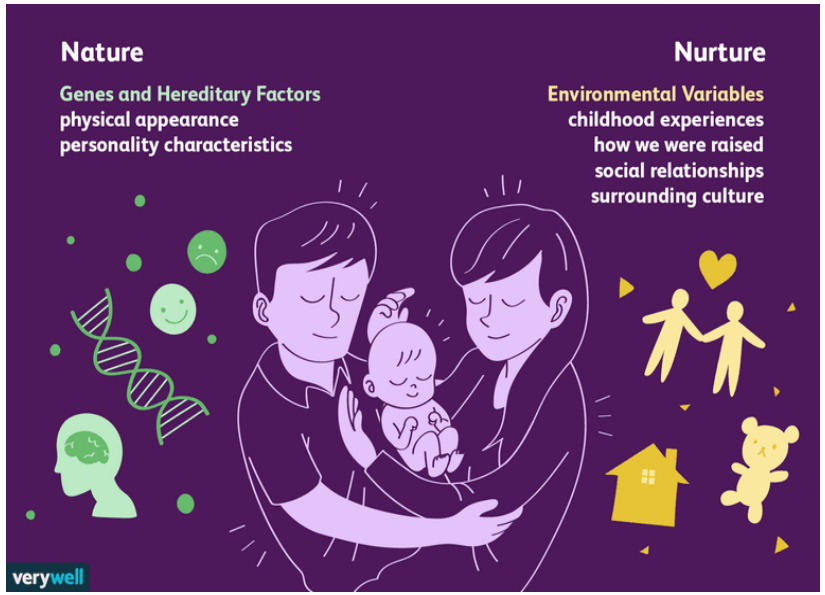
Virginie Orgogozo[1*], Baptiste Morizot[2] and Arnaud Martin[3]



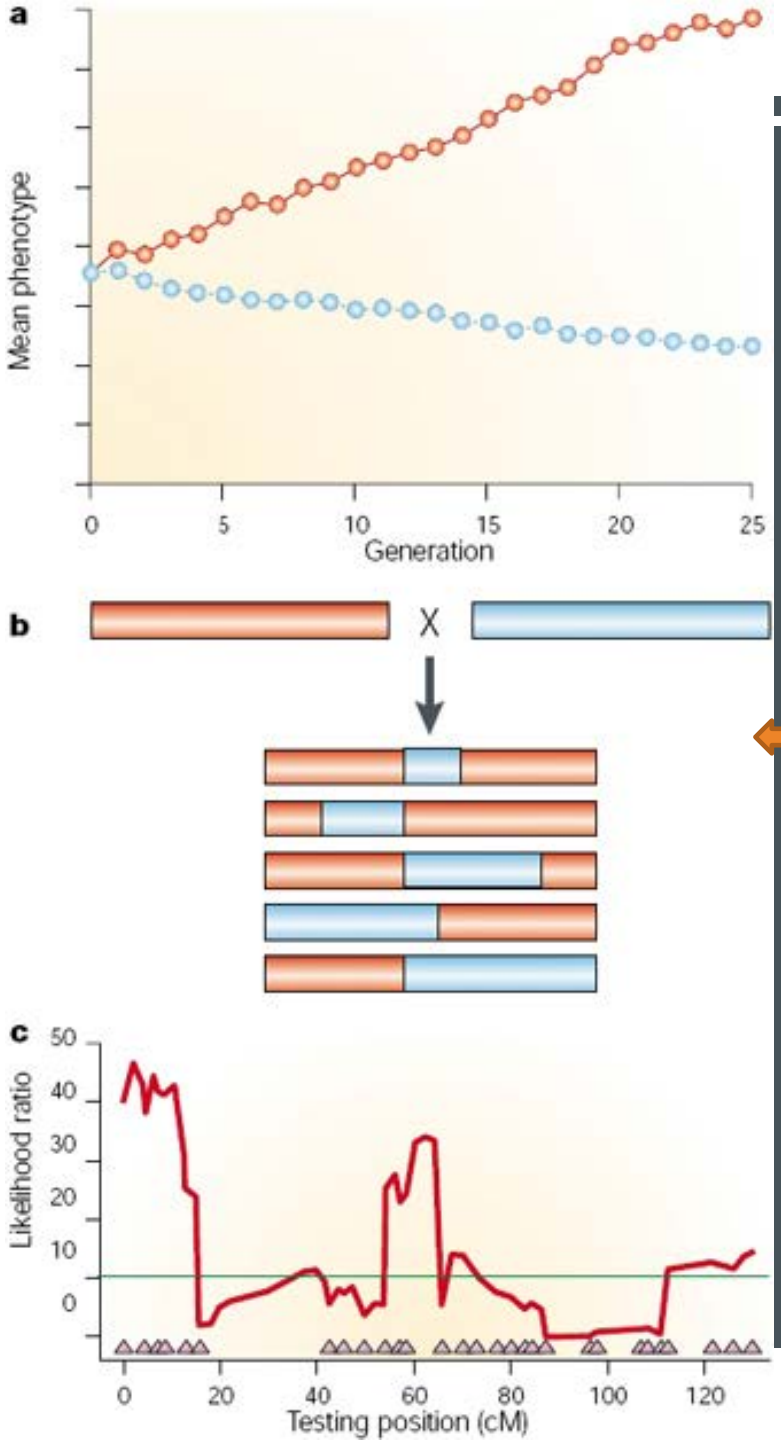
Sommer and Bäckhed, *Nat Rev Microbiol.* (2013)







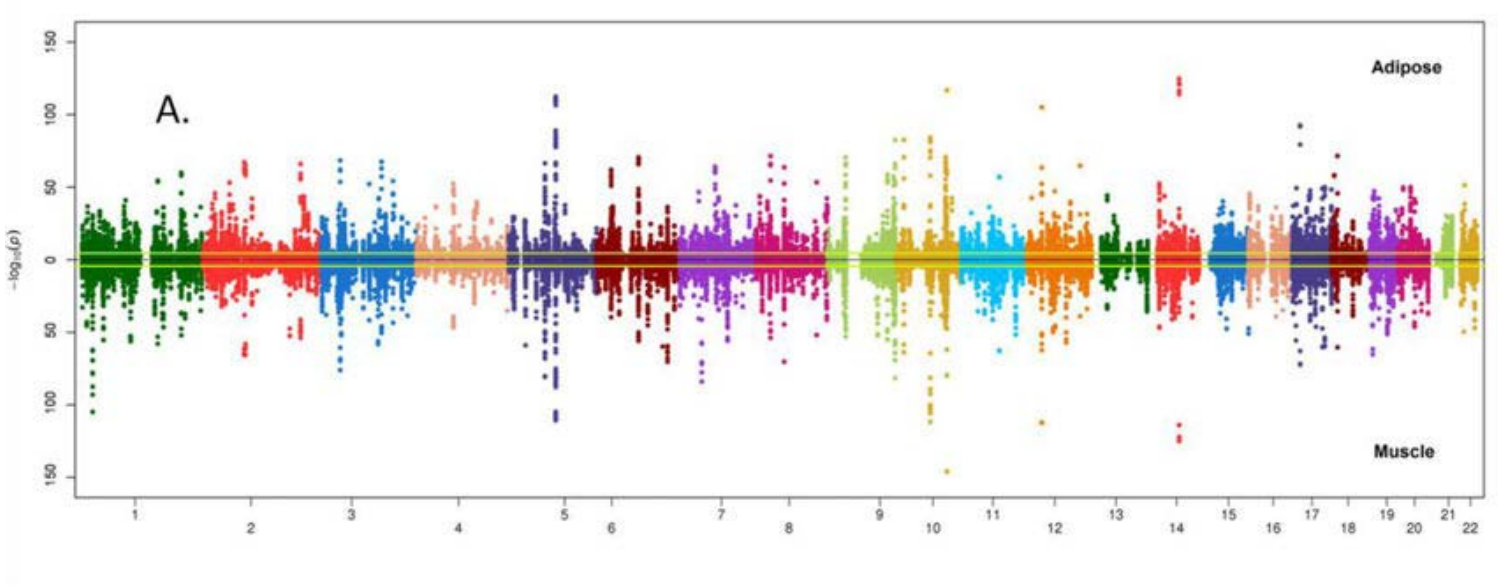
- Gene expression is regulated by genetic effects and environmental factors (Brem et al. 2002; Cheung et al. 2003; Morley et al. 2004; Grundberg et al. 2012).
- Multiple genes interact with multiple environmental variables to produce the phenotype. (Orgogozo et al, 2015)

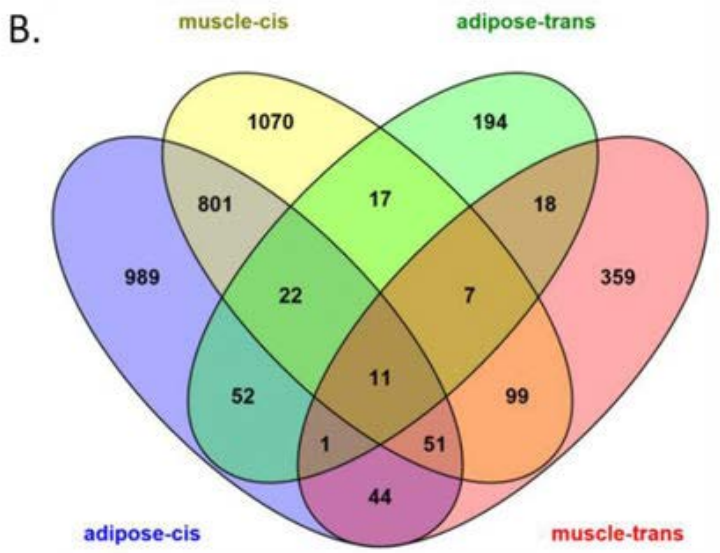


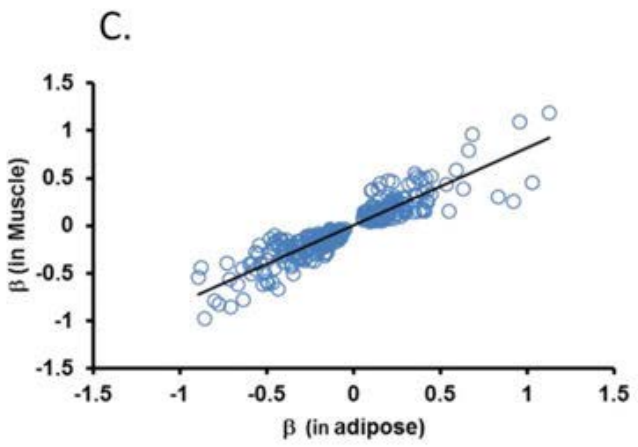
- **A large number of studies have investigated the expression of quantitative trait loci, (eQTLs) and discovered that most genes are affected by at least one eQTL in at least one tissue (Albert and Kruglyak 2015)**
- **Quantitative trait locus** (QTL) **analysis** is a

statistical method which links the phenotypic data (**trait** measurements) to genotypic data (usually molecular markers) to explain the genetic basis of variation underlying complex **traits** (Miles and Wayne, 2008).

- A **quantitative trait locus** (**QTL**) is a region of DNA associated with a particular trait
- This trait is ultimately determined by genes and their environment.
- QTLs may be on different chromosomes.
- The number of QTLs indicates the genetic architecture of a trait.



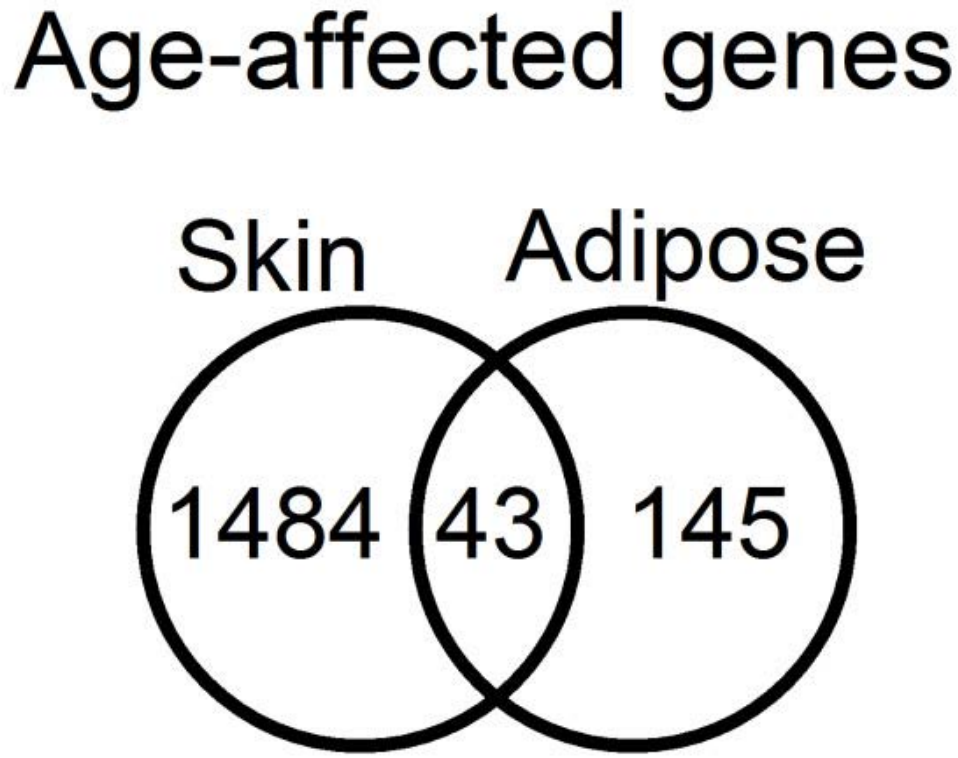




SKIN SHOWS THE MOST AGE-RELATED GENE EXPRESSION CHANGES OF ALL THE TISSUES INVESTIGATED

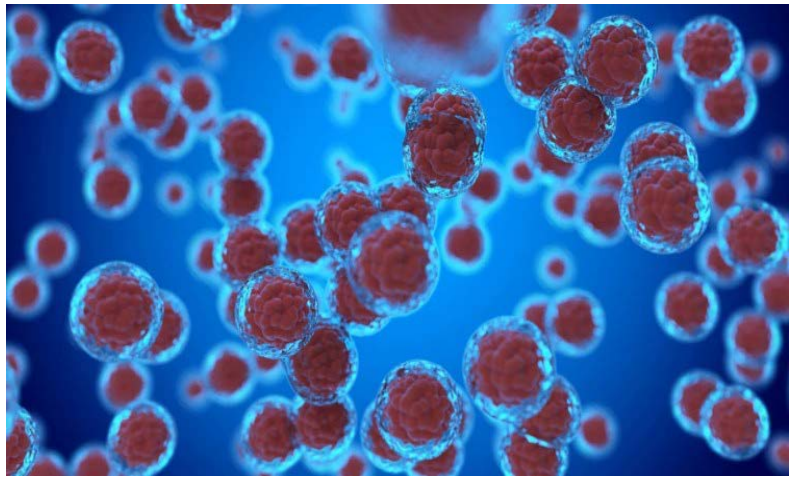
- Glass et al (2013) demonstrated that gene expression **changes with age in skin, adipose tissue, blood and brain**

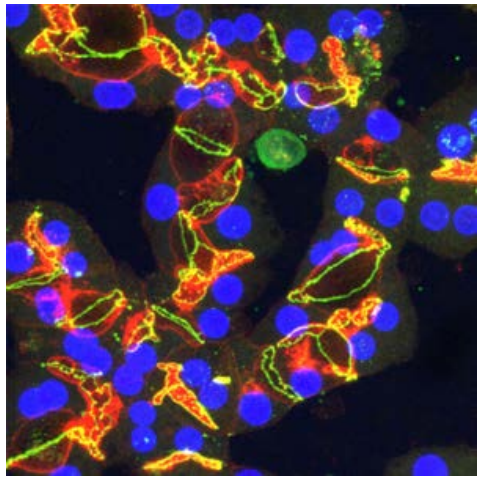
- Skin shows most age-related gene expression with genes involved in
    1. Fatty acid Metabolism
    2. Mitochondrial Activity
    3. Cancer
    4. DNA / RNA Splicing
- A significant proportion of age-related changes in gene expression appear to be tissue-specific with only a few genes sharing an age effect in expression across tissues.

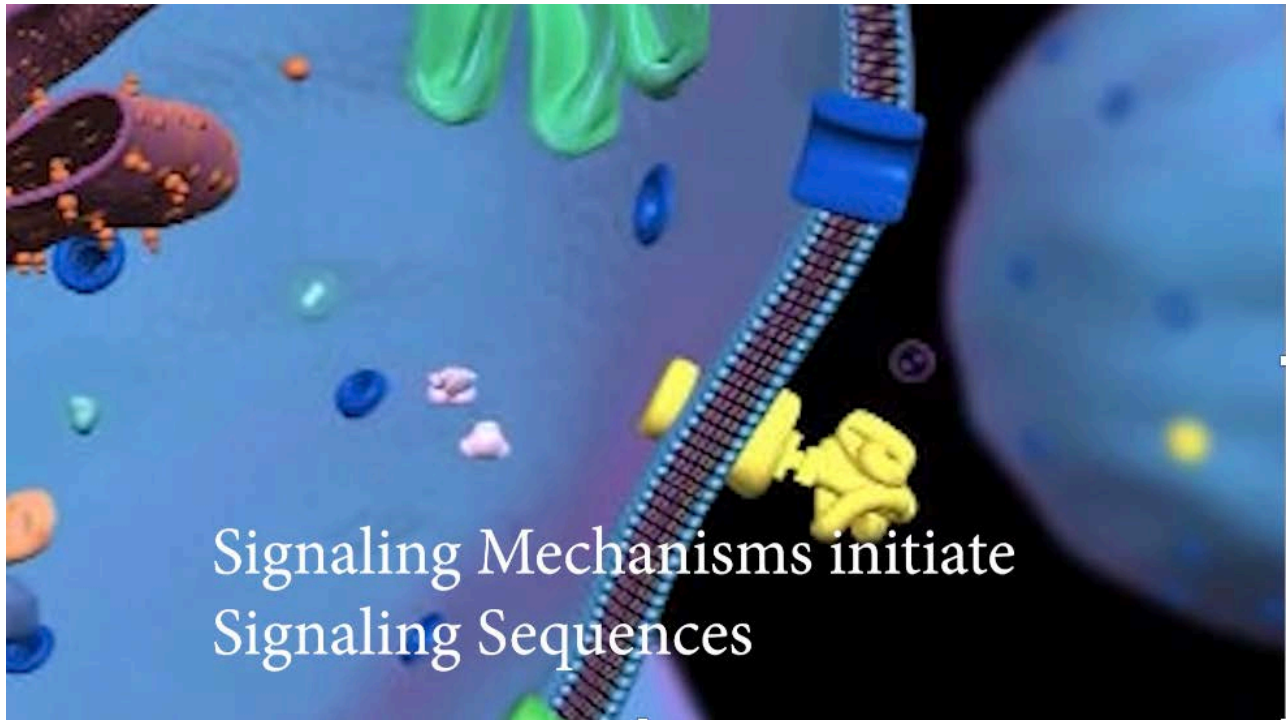


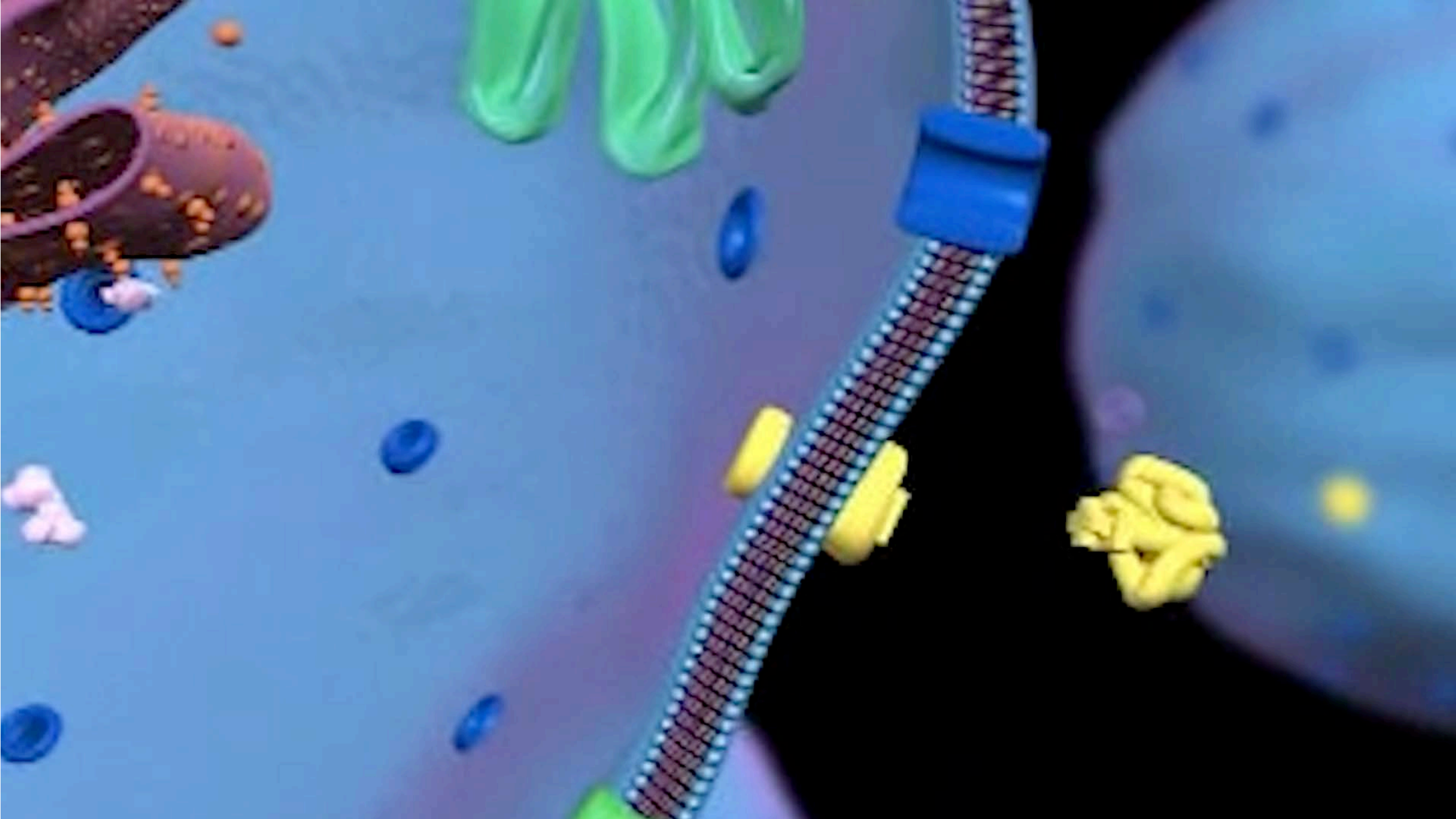
The 43 shared genes in skin and adipose tissue showed a single common identifiable pathway related to the stress response.

- Signalling pathways are the key biological mechanisms that transduce extracellular signals to affect transcription factor mediated gene regulation within cells.
- Cell signalling plays a key role within biological systems
  -- i.e. to relay extracellular signals in order to regulate intracellular gene expression.

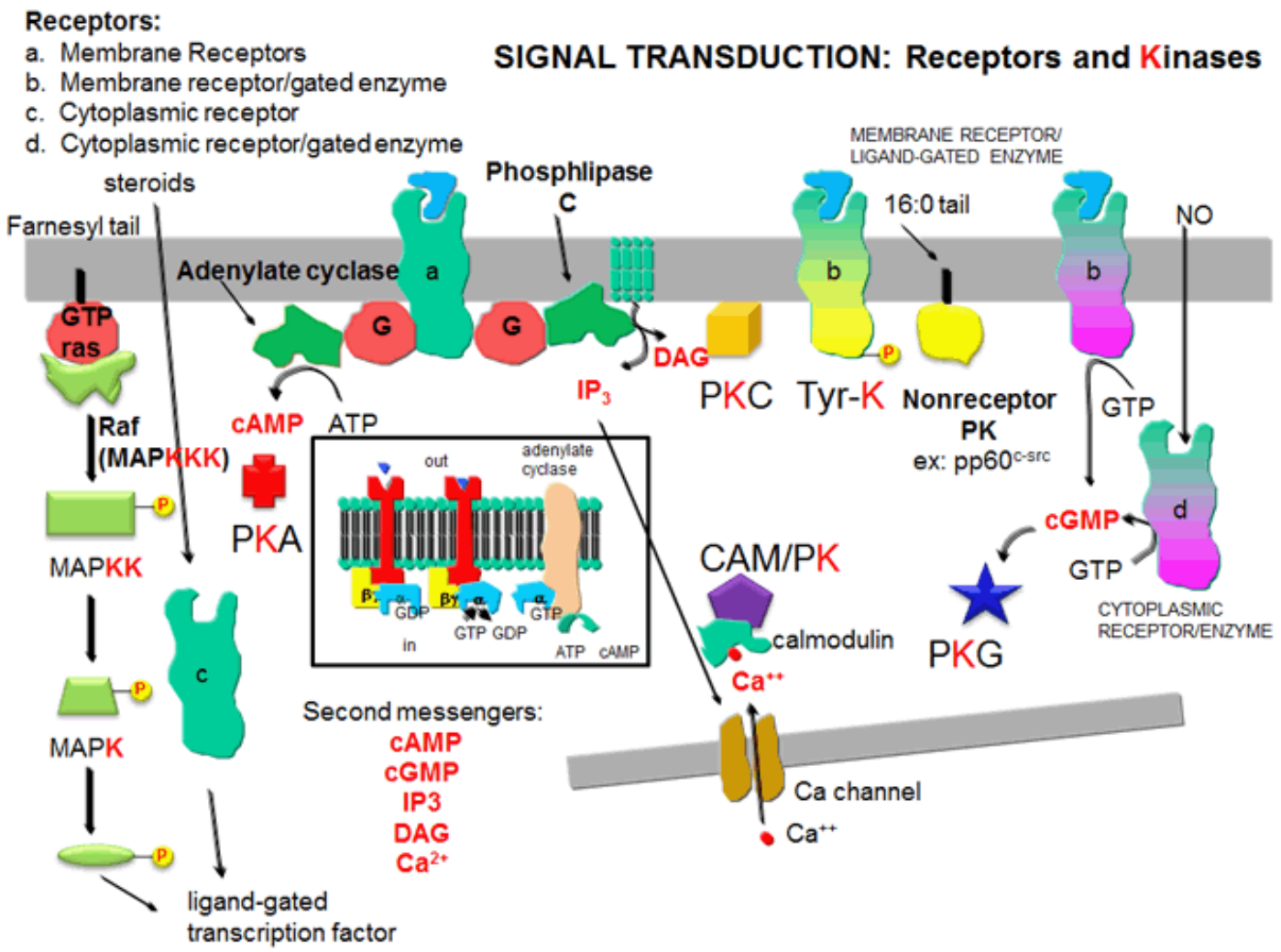




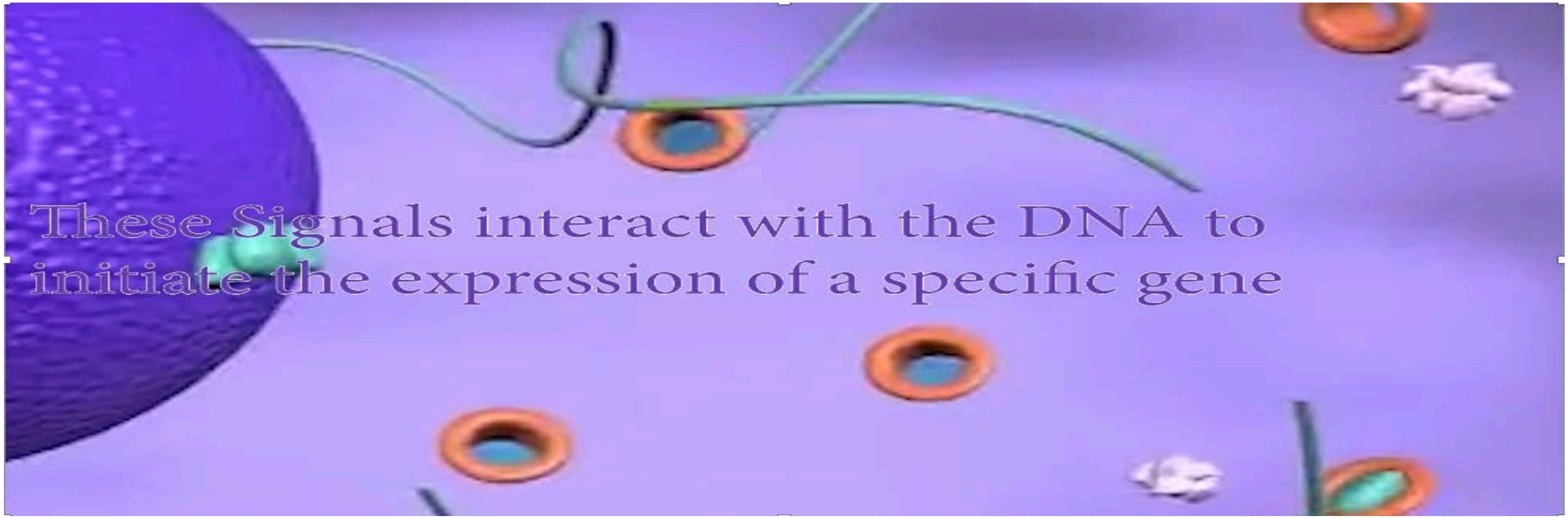




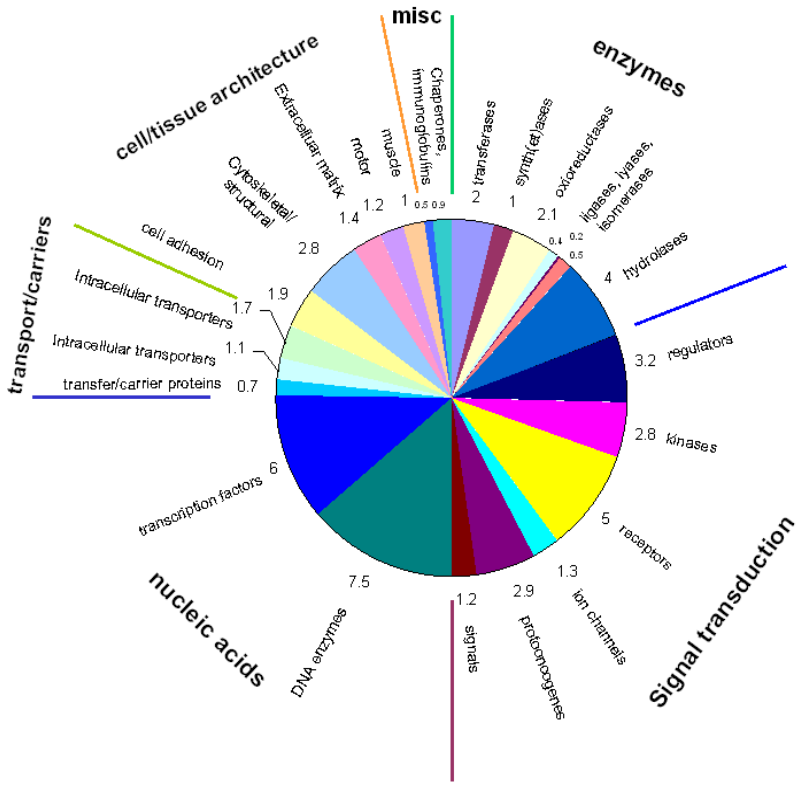
- SIGNAL TRANSDUCTION MAY BE INITIATED BY THE BINDING OF A LIGAND TO A MEMBRANE-BOUND RECEPTOR
- THIS TRIGGERS A CASCADE OF INTERCELLULAR SIGNALLING ACTIVITIES THROUGH MULTIPLE KINASES
- AND IMPACTS ON HOW TRANSCRIPTION FACTORS REGULATE DOWNSTREAM GENE EXPRESSION.

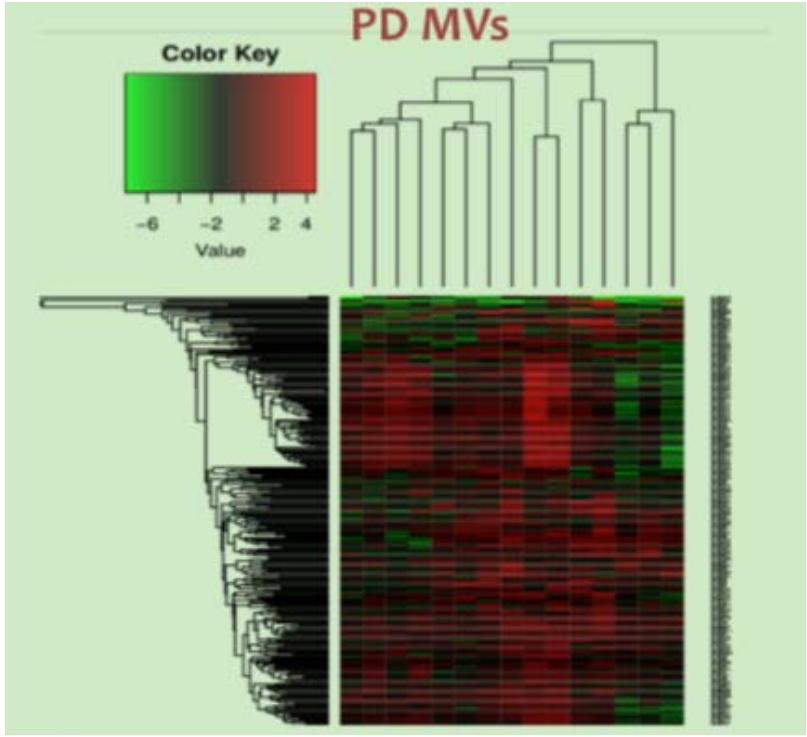


CELLS DETECT INPUT SIGNALLING MOLECULES USING RECEPTORS, PROTEINS USUALLY LOCATED ON THE CELL SURFACE WHICH TRANSMIT THE SIGNAL TO THE INTERIOR OF THE CELL THROUGH A SERIES OF DOWNSTREAM PROCESSES THAT TYPICALLY LEAD TO CHANGES IN GENE EXPRESSION, RESULTING IN AN APPROPRIATE OUTPUT RESPONSE TO THE INPUT.



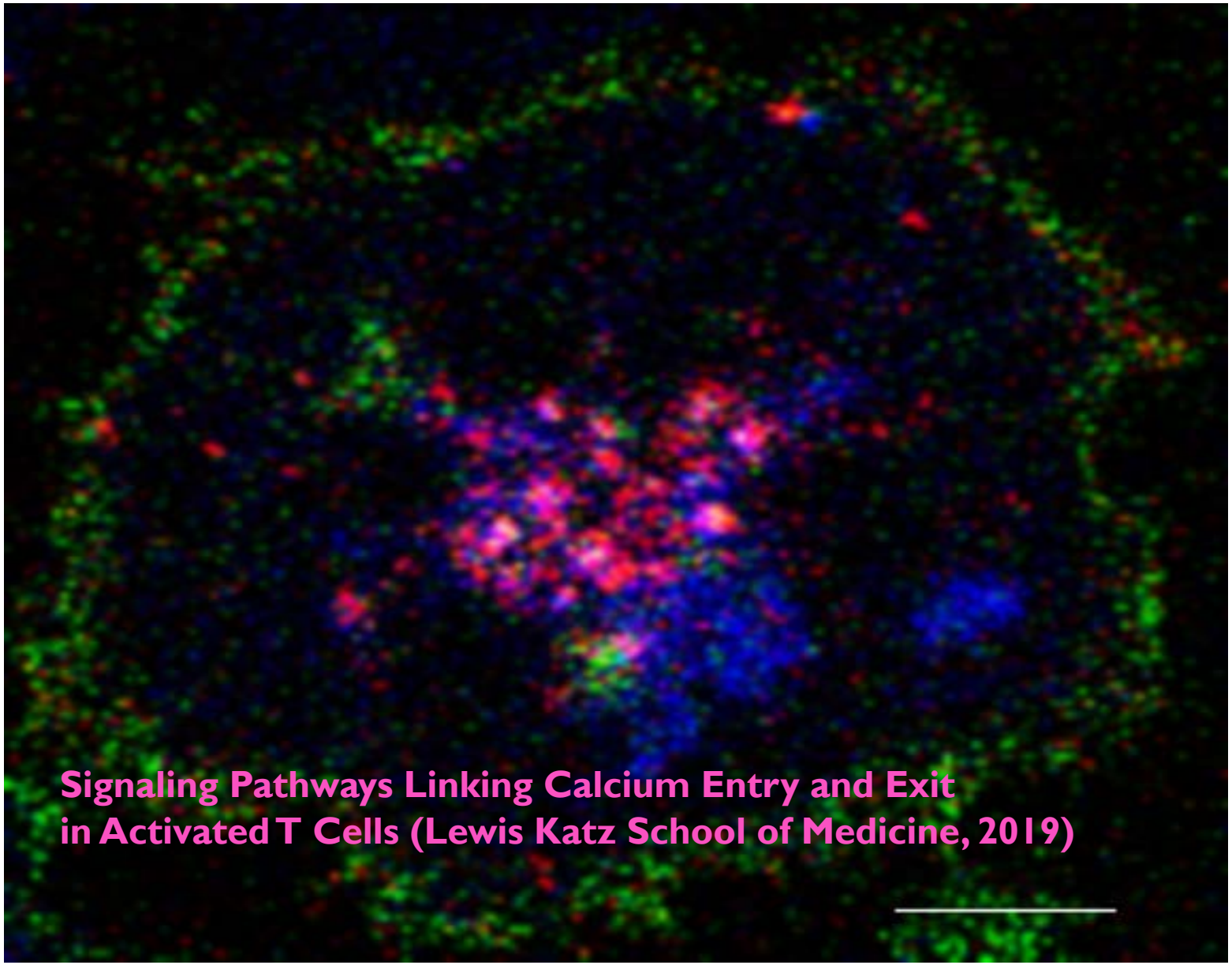
The coordinated activity of different signalling pathways within and between multiple cell types is the basis of many important biological processes, such as gene expression involved in human development, tissue repair and immunity.





PD MVs
AMPK signaling pathways
Calcium signaling pathway
Signaling pathways regulating pluripotency of stem cells

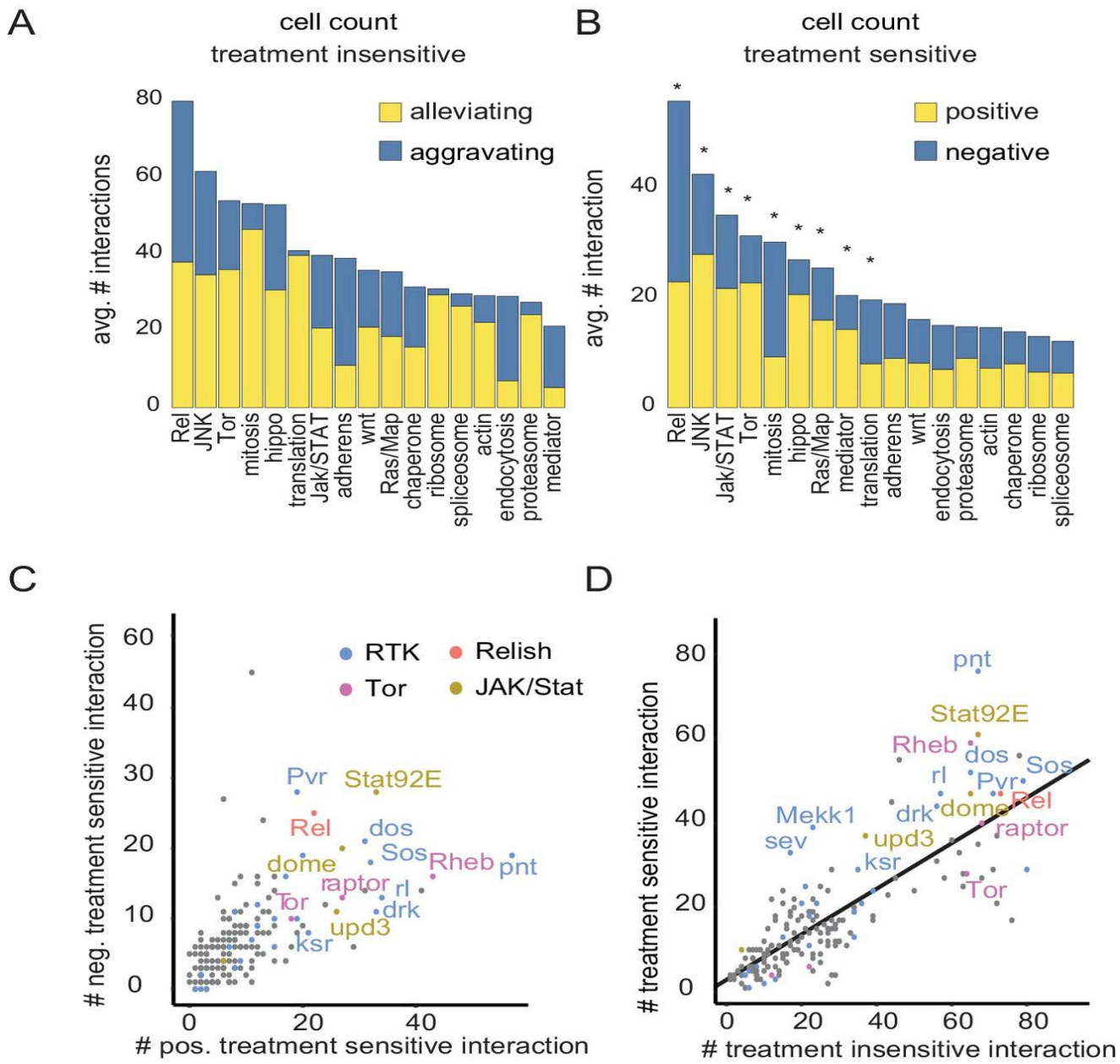
- The response of biological systems to changing environmental conditions and aging factors is a dynamic process.
- Activation of different signalling pathways can lead to numerous physiological or cellular responses, such as:
  * cell proliferation
  * cell death
  * cell differentiation
  * cell metabolism
- Signalling determines the aging process



Signaling Pathways Linking Calcium Entry and Exit in Activated T Cells (Lewis Katz School of Medicine, 2019)

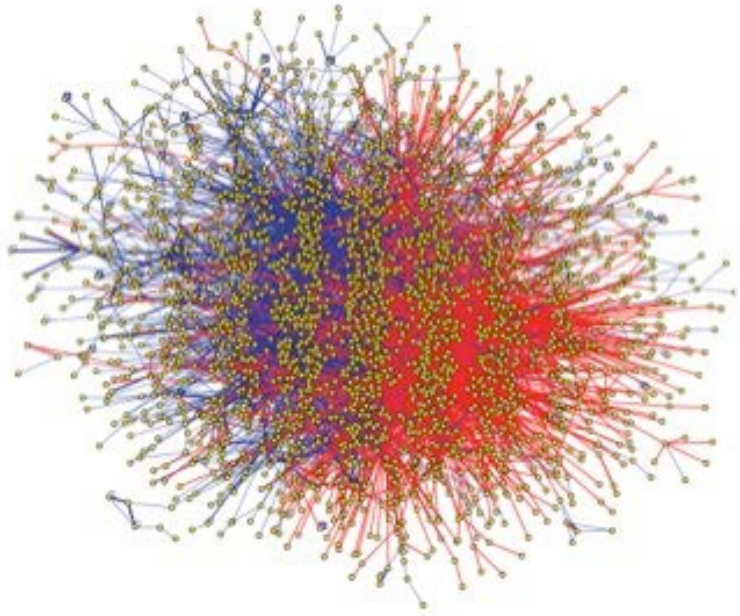
* A clear understanding of the activity / interaction between signalling pathways can help to design rational disease treatment &

* Tissue regeneration strategies for Anti-aging Medicine.



- We can identify the important signalling pathways of a cell by using gene expression and protein-protein interaction (PPI) data sets.
- Extensive, publicly available PPI data provide an opportunity to establish a general signalling pathway blueprint, to which cell type-specific gene expression data can be mapped
- We can then refine the general signalling pathway blueprint into a cell-type specific blueprint.

- A number of computational methods utilize PPI data along with gene expression data to uncover known signalling pathways.



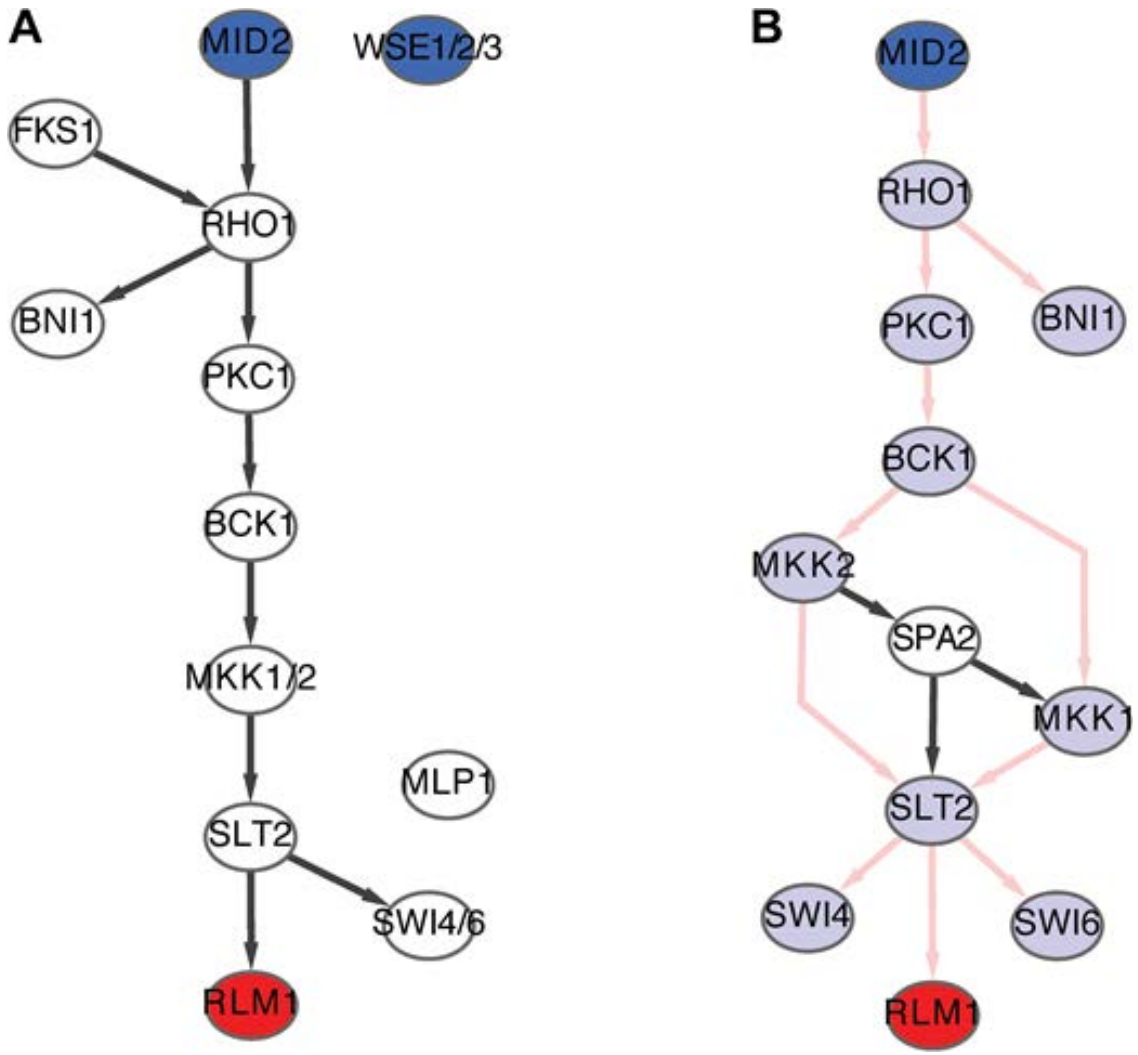
The **interactome** is the totality of PPIs that happen in a cell, an organism or a specific biological context.

Protein-protein interaction networks (PPIN) are mathematical representations of the physical contacts between proteins in the cell.

HYBRID INTELLIGENT APPROACH FOR IDENTIFYING DIRECTED SIGNALING PATHWAYS (HISP)

- A recently published method is called *HISP*
- It applies genetic algorithms relevant to:
  - selection
  - crossover
  - mutation
- It selects the topologies of resultant signalling pathways
- It uses gene knockout data to get directionality of the signalling pathways.

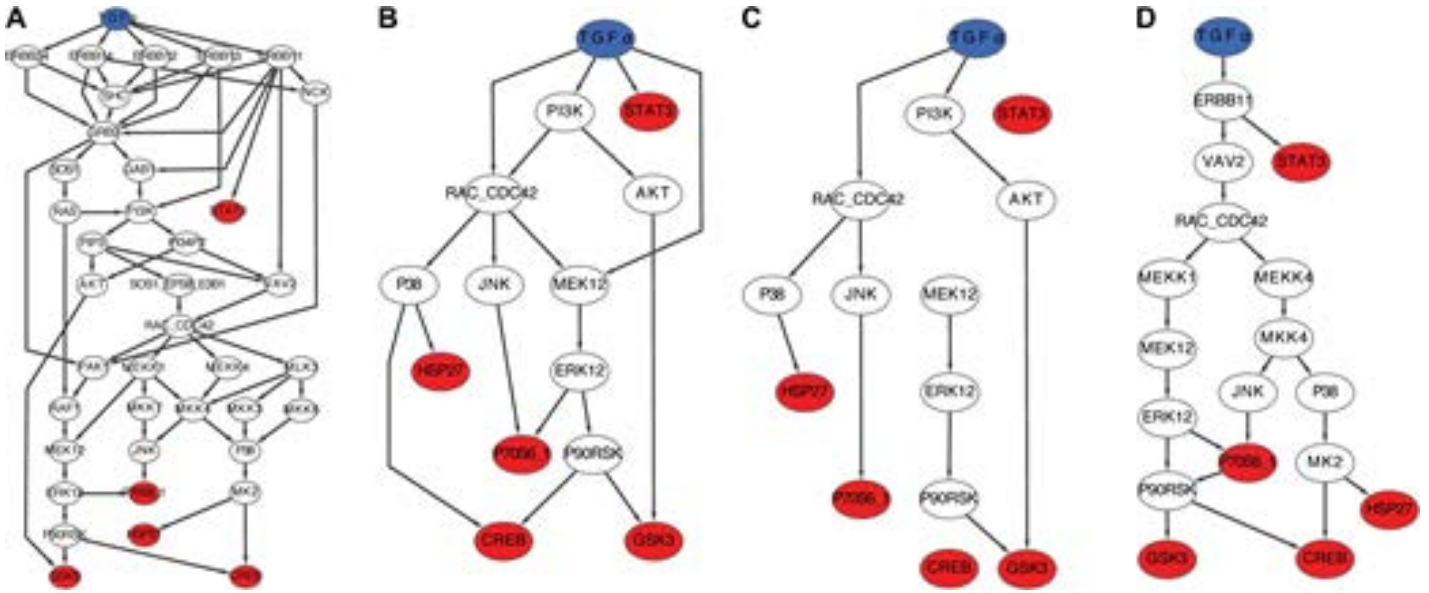
The signaling network of cell wall integrity



Dark blue points are starting points and the red ones are ending points.

INCREASING LEVELS OF COMPLEXITY

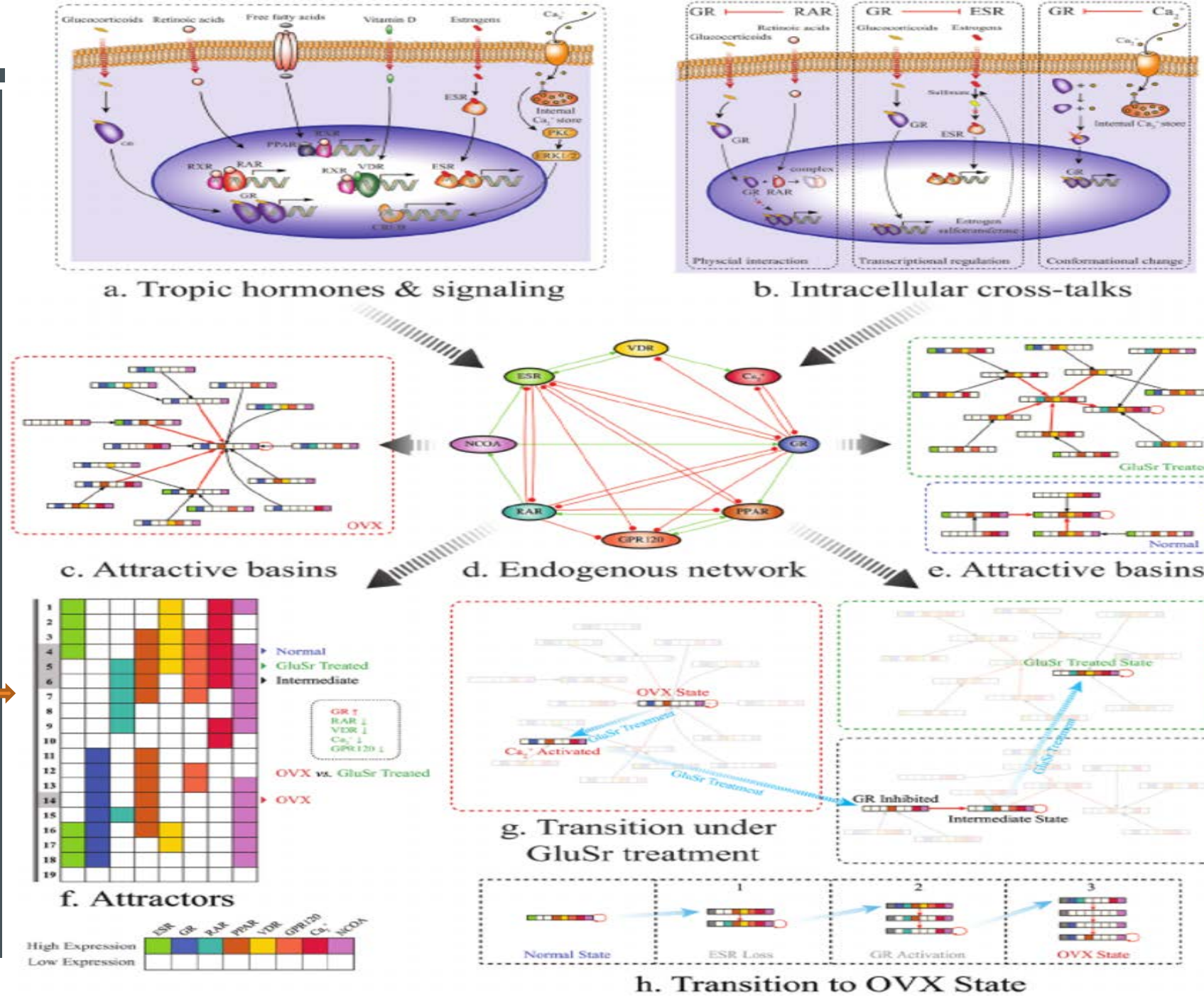
- EGFR/ErbB signaling pathway in human hepatocytes, HISP unveils a high-resolution signaling pathway, where many signaling interactions were missing by existing computational approaches.



EGFR/ErbB signaling pathway in human hepatocytes.

Signalling technology offers the most direct and comprehensive method in both understanding gene expression and in utilizing interactions between specific signalling pathways and gene expression as a successful interventions in several diseases as well as in Anti-aging Medicine.

Schematic diagrams of the proposed molecular mechanism for osteoporosis due to osteoblast function loss



OUR RESEARCH
LONDON UNIVERSITY RESEARCH (1990)

- Stimulation induced very rapid musclular hypertrophy associated with an increase of up to 250% in the RNA content of the muscles and an abrupt change in the species of RNA produced.
- Repression of the fast-type genes and activation of the slow-type genes.
- Fast-type IIB genes appeared to be the default genes, but that the skeletal slow genes were expressed as a response to overload

Biochemical Society Transactions

Stretch and force generation induce rapid hypertrophy and myosin isoform gene switching in adult skeletal muscle

Geoffrey Goldspink, Andrew Scutt, Jane Martindale, Thomas Jaenicke, Lucien Turay and Gerald-F. Gerlach
Unit of Molecular and Cellular Biology, The Royal Veterinary College, London University, Royal College Street, London NW1 0TU, U.K.

Summary
Using electrical stimulation to control force generation and limb immobilization to alter the degree of stretch, we have studied the role of mechanical activity in inducing hypertrophy and in determining fast and slow muscle fibre phenotype. Changes in gene expression were detected by analysing the RNA in hybridization studies employing cDNA probes specific for fast and slow myosin heavy chains and other genes. As a result of overload in the stretched position, the fast contracting tibialis anterior muscle in an adult rabbit is induced to synthesize much new protein and to grow by as much as 30% within a period as short as 4 days. This very rapid hypertrophy was found to be associated with an increase of up to 250% in the RNA content of the muscles and an abrupt change in the species of RNA produced. Both stretch alone and electrical stimulation alone caused repression of the fast-type genes and activation of the slow-type genes. It appears that the fast-type IIB genes are the default genes, but that the skeletal slow genes are expressed as a response to overload and stretch. These findings have implications as far as athletic training and rehabilitation are concerned.

Introduction
Muscle is a tissue in which gene expression is regulated to a large extent by mechanical signals. Mammalian muscle consists of populations of slow-contracting, oxidative fibres and fast-contracting fibres which are characterized by different protein isoforms. Therefore, post-natal growth and the differentiation into the fast type or the slow type of fibres must presumably involve the regulation of expression of different subsets of genes. Here we have focused on the expression of myosin heavy chain genes and their response to mechanical stimuli.

The intrinsic velocity of contraction ($V_{max.}$) of muscle fibres is related to the specific activity of their myosin ATPase [1]. Myosin is a double molecule that consists of two heavy chains each of about 220 kDa. The actin-attachment site and the ATPase site are located in the S1 region (head of the myosin molecule) of each heavy chain. Associated with the S1 fragment are smaller polypeptides called light chains which are believed to modulate the cross-bridge ATPase activity [2]. Subtypes of fast muscle fibre have been identified histochemically and these may exist because of different combinations of myosin heavy and light chains and different mitochondrial content. Slow fibres differ in several ways from fast fibres in that they have many more mitochondria, different cytoplasmic isoenzymes, as well as different isoforms of myofibrillar proteins. The isoforms of myosin have been shown to be the product of a multigene family and their expression is tightly regulated in a stage-specific and tissue-specific manner [3, 4]. Phenotypic expression of muscle genes is known to be influenced by thyroid hormone [5, 6] and altered patterns of innervation [7]. However, the influence of physical activity at the gene level was unclear. We have, therefore, studied changes in transcriptional levels of the fast and slow myosin heavy chain genes in response to stretch and force generation.

Abbreviation used: TA, tibialis anterior.

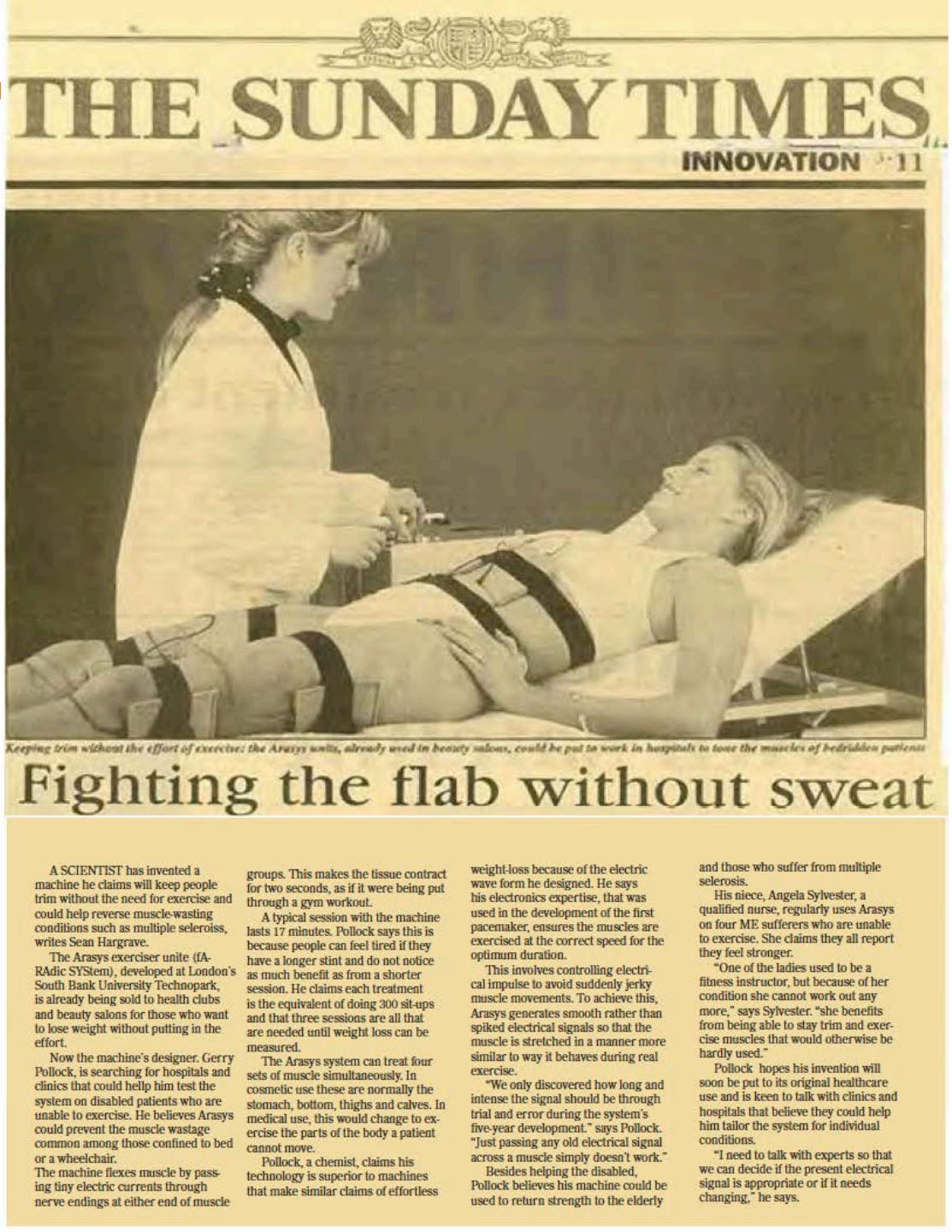
Methods

Stimulation and acute-stretch procedures
Tibialis anterior (TA) muscles in adult Netherland dwarf rabbits were stimulated using Teflon-coated stainless-steel electrode wires implanted into the popliteal fossa [8] under valium/Hypnorm anaesthesia. The electrode wires were externalized at the back of the neck and attached to a miniature stimulation circuit which was held in position by a small saddle fashioned out of an elastic bandage. Several circuit designs were used which generated biphasic pulses at frequencies ranging from 2 Hz continuous to 120 Hz intermittent. A 30 Hz intermittent circuit was designed to give the same number of pulses/min as a 2 Hz continuous, and a 120 Hz and 60 Hz intermittent circuit gave the same number of pulses/min as a 10 Hz continuous circuit. In this way, the hypothesis that it is the number of pulses delivered which determines muscle fibre phenotype could be tested. The pulse length was 0.1 ms and the pulse amplitude was adjustable from 1 to 3 V and each miniature stimulator was fitted with an on/off switch. Muscle

LONDON UNIVERSITY INVENTION BY THE CO-INVENTOR OF THE FIRST PACEMAKER

In 1994 the Sunday Times, UK published an article about Gerry Pollock's invention in London University which he built in London University after 17 years of empirical research. Several other news publications followed.

Since then all our research remains part of our IP and therefore proprietary



THE SUNDAY TIMES

INNOVATION 3·11

Keeping trim without the effort of exercise: the Arasys units, already used in beauty salons, could be put to work in hospitals to tone the muscles of bedridden patients

Fighting the flab without sweat

A SCIENTIST has invented a machine he claims will keep people trim without the need for exercise and could help reverse muscle-wasting conditions such as multiple seleroiss, writes Sean Hargrave.

The Arasys exerciser unite (fA-RAdic SYStem), developed at London's South Bank University Technopark, is already being sold to health clubs and beauty salons for those who want to lose weight without putting in the effort.

Now the machine's designer. Gerry Pollock, is searching for hospitals and clinics that could hellp him test the system on disabled patients who are unable to exercise. He believes Arasys could prevent the muscle wastage common among those confined to bed or a wheelchair.

The machine flexes muscle by passing tiny electric currents through nerve endings at either end of muscle groups. This makes the tissue contract for two seconds, as if it were being put through a gym workout.

A typical session with the machine lasts 17 minutes. Pollock says this is because people can feel tired if they have a longer stint and do not notice as much benefit as from a shorter session. He claims each treatment is the equivalent of doing 300 sit-ups and that three sessions are all that are needed until weight loss can be measured.

The Arasys system can treat four sets of muscle simultaneously. In cosmetic use these are normally the stomach, bottom, thighs and calves. In medical use, this would change to exercise the parts of the body a patient cannot move.

Pollock, a chemist, claims his technology is superior to machines that make similar claims of effortless weight-loss because of the electric wave form he designed. He says his electronics expertise, that was used in the development of the first pacemaker, ensures the muscles are exercised at the correct speed for the optimum duration.

This involves controlling electrical impulse to avoid suddenly jerky muscle movements. To achieve this, Arasys generates smooth rather than spiked electrical signals so that the muscle is stretched in a manner more similar to way it behaves during real exercise.

"We only discovered how long and intense the signal should be through trial and error during the system's five-year development." says Pollock. "Just passing any old electrical signal across a muscle simply doesn't work."

Besides helping the disabled, Pollock believes his machine could be used to return strength to the elderly and those who suffer from multiple selerosis.

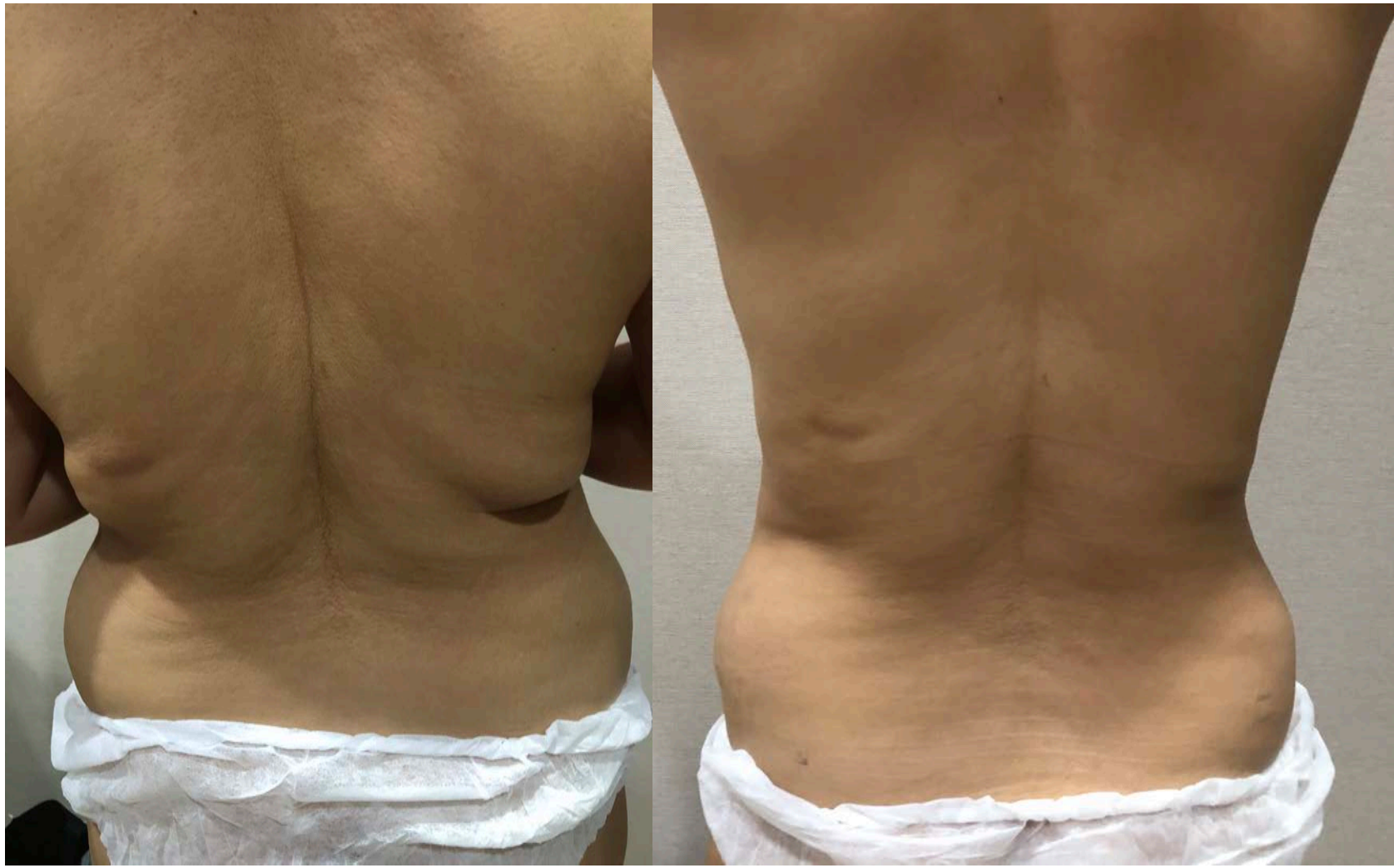
His niece, Angela Sylvester, a qualified nurse, regularly uses Arasys on four ME sufferers who are unable to exercise. She claims they all report they feel stronger.

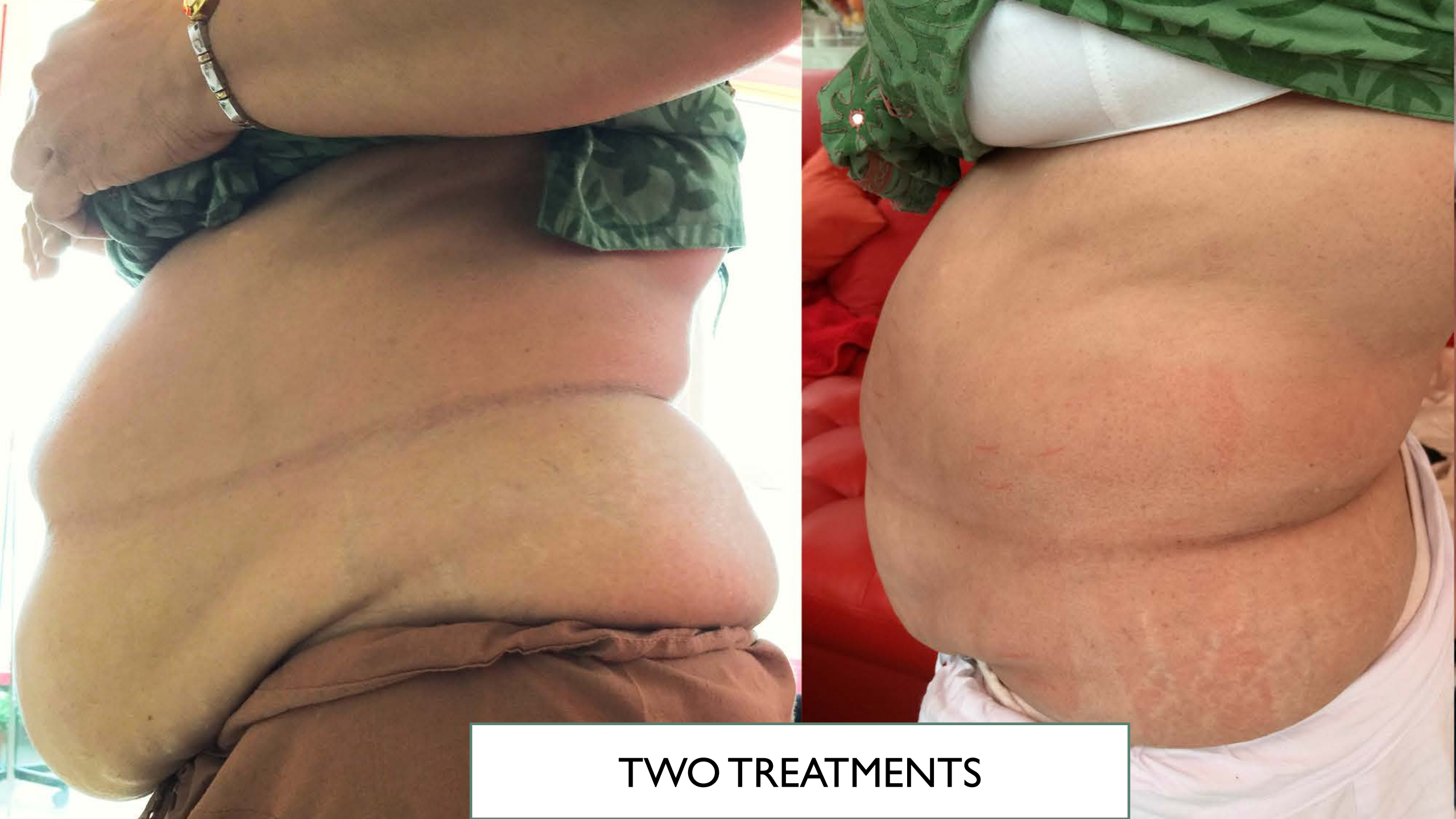
"One of the ladies used to be a fitness instructor, but because of her condition she cannot work out any more," says Sylvester. "she benefits from being able to stay trim and exercise muscles that would otherwise be hardly used."

Pollock hopes his invention will soon be put to its original healthcare use and is keen to talk with clinics and hospitals that believe they could help him tailor the system for individual conditions.

"I need to talk with experts so that we can decide if the present electrical signal is appropriate or if it needs changing," he says.

Results of this technology today after additional additional 25 years of research (a total of 44 years of combined research) offers visual body changes after 20-60 minutes





TWO TREATMENTS

GENE EXPRESSION YOUNG = OLD / YOUTHFUL APPEARANCE

The gene expression patterns from the women in the study who were younger appearing were similar to those in women who were actually younger in age.

Older women with Youthful Appearance had increased gene activity related to DNA Repair

American Academy of Dermatology 2017

SIGNALING TO REPAIR DNA

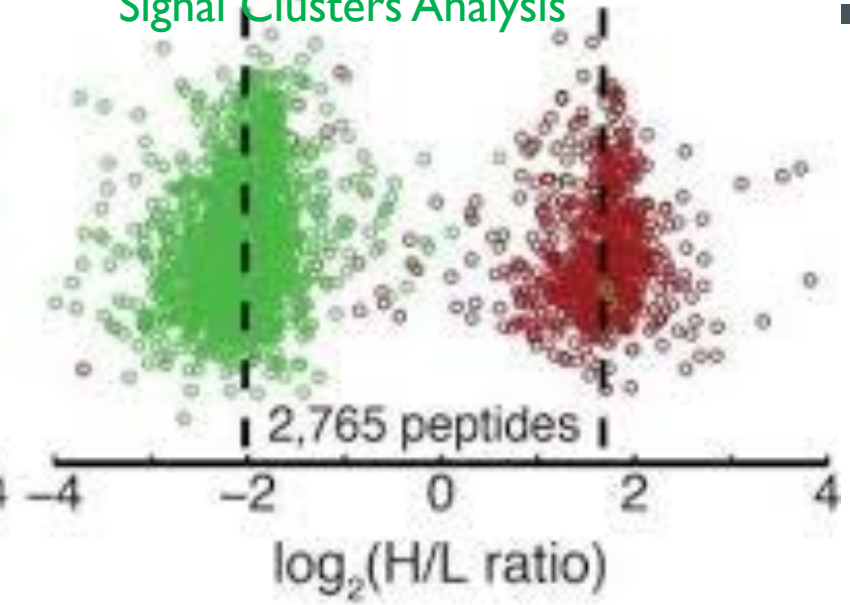
- Our current Research focuses on the investigation of biosignals and how timing affects their meaning within the biological network

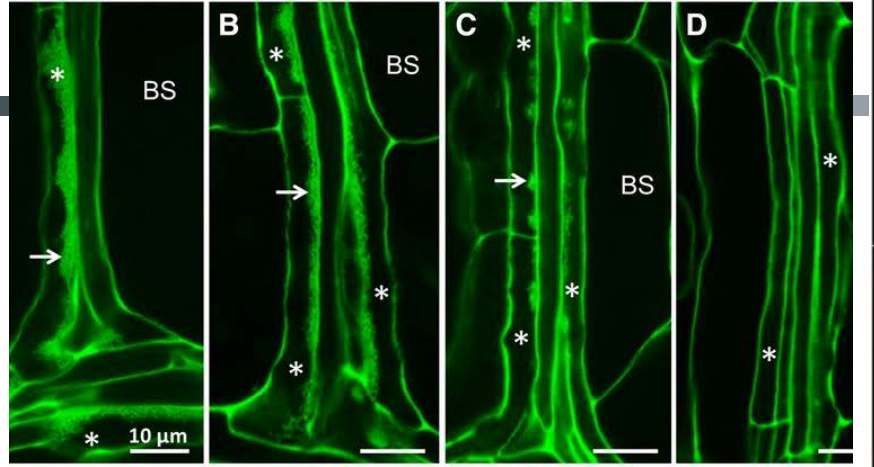
SIGNALING CAN REINSTATE FADED OR BROKEN BIO-SIGNALS BY EMITING HIGHLY BIORESONANT SIGNALS THAT CAN FUSE WITH BIOLOGICAL SIGNALS TO AMPLIFY FADED BIOLOGICAL SIGNALS

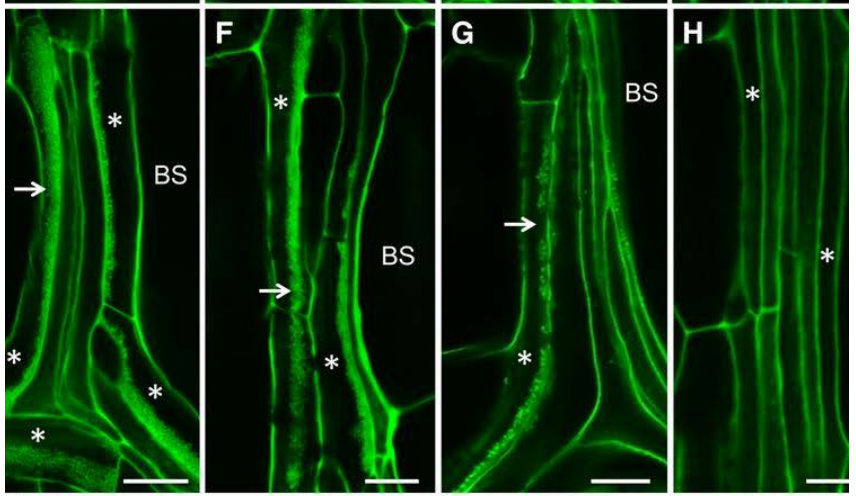
SIGNALING CAN REINSTATE FADED OR BROKEN BIO-SIGNALS BY EMITING HIGHLY BIORESONANT SIGNALS THAT CAN FUSE WITH BIOLOGICAL SIGNALS TO FILL IN THE GAPS OF BROKEN BIOLOGICAL SIGNALS THUS REINSTATING THEIR ORIGINAL MEANING

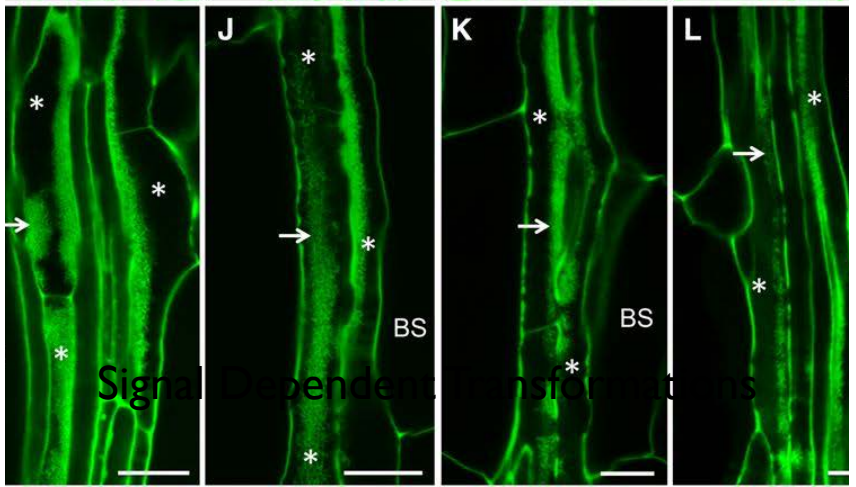
SIGNAL PROCESSING BLUEPRINTS

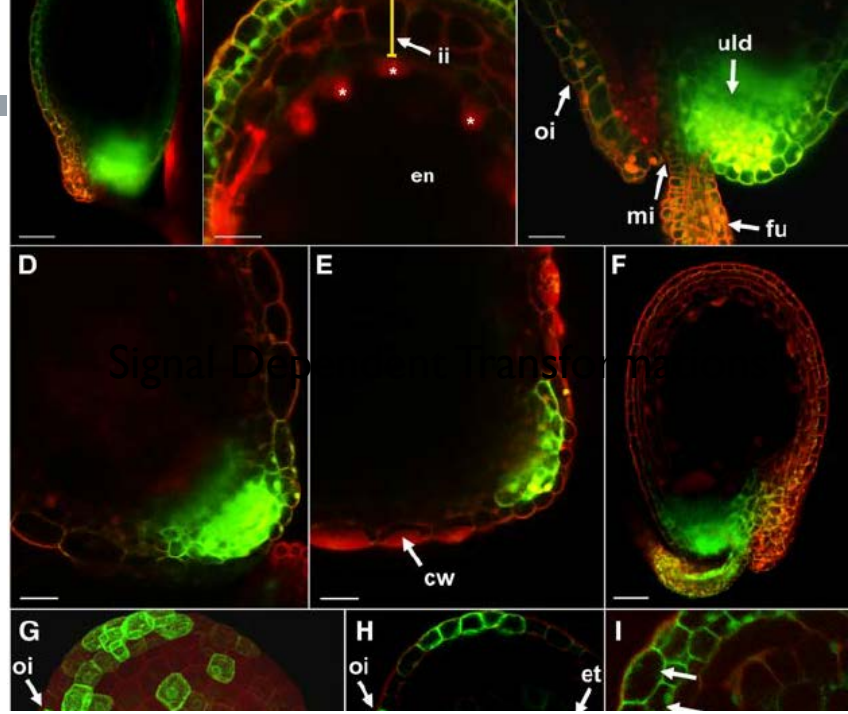
Signal Clusters Analysis









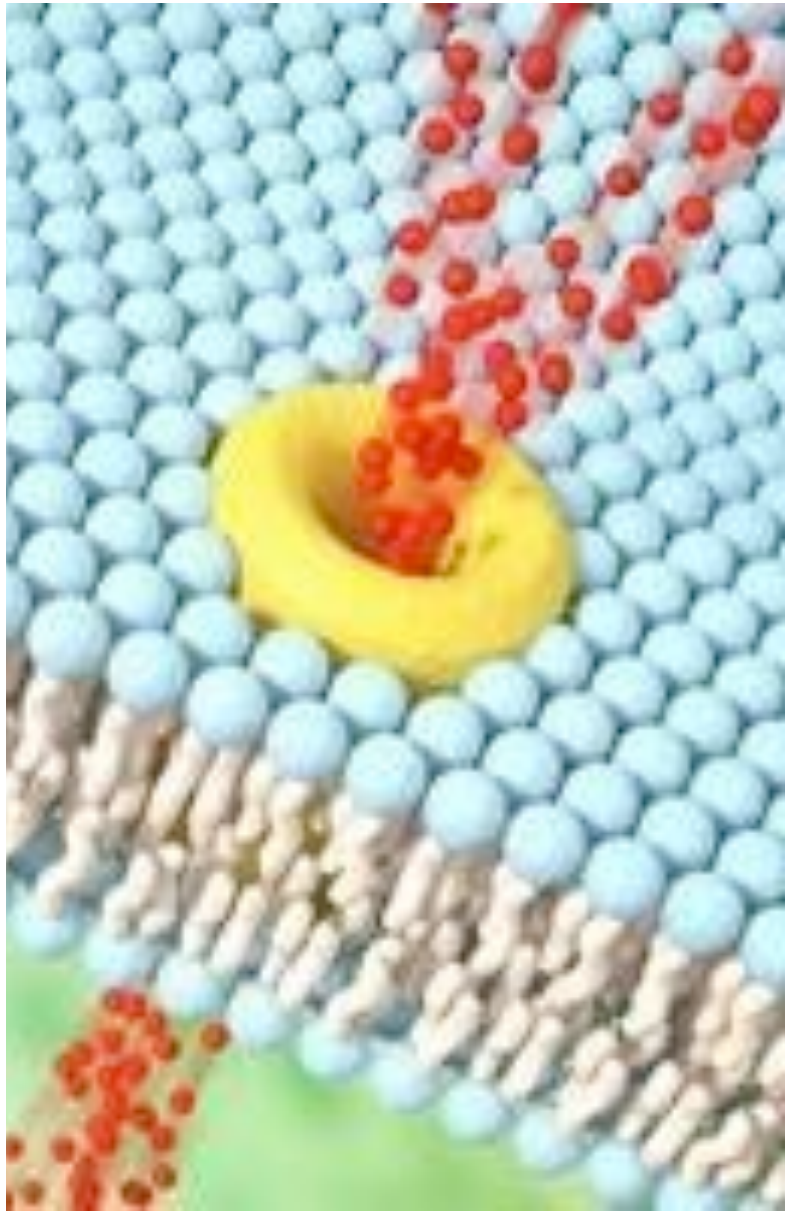


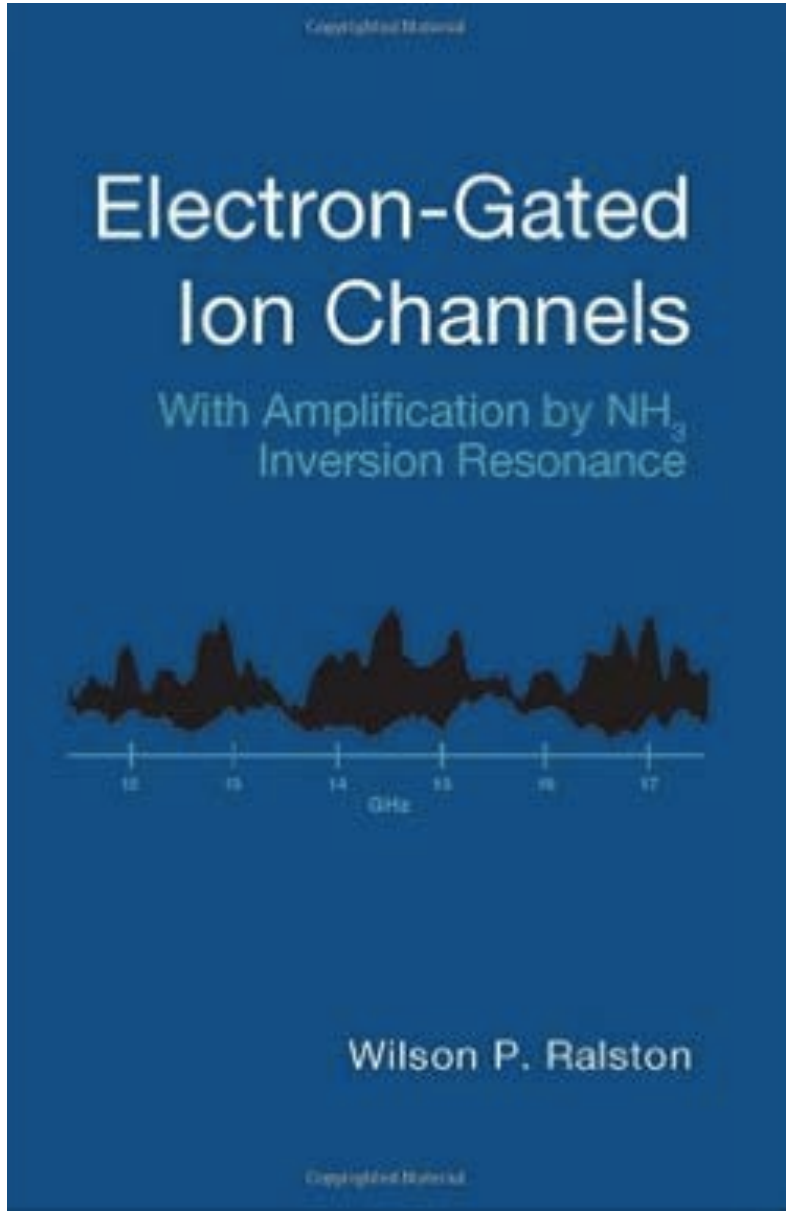
Signal Dependent Transformations

SIGNALS ARE COMPOSED BY COMPLEX WAVEFORM CONSTRUCTS

SIGNALS MUST BE DELIVERED AT ULTRA LOW ENERGIES (BELOW THERMAL NOISE)

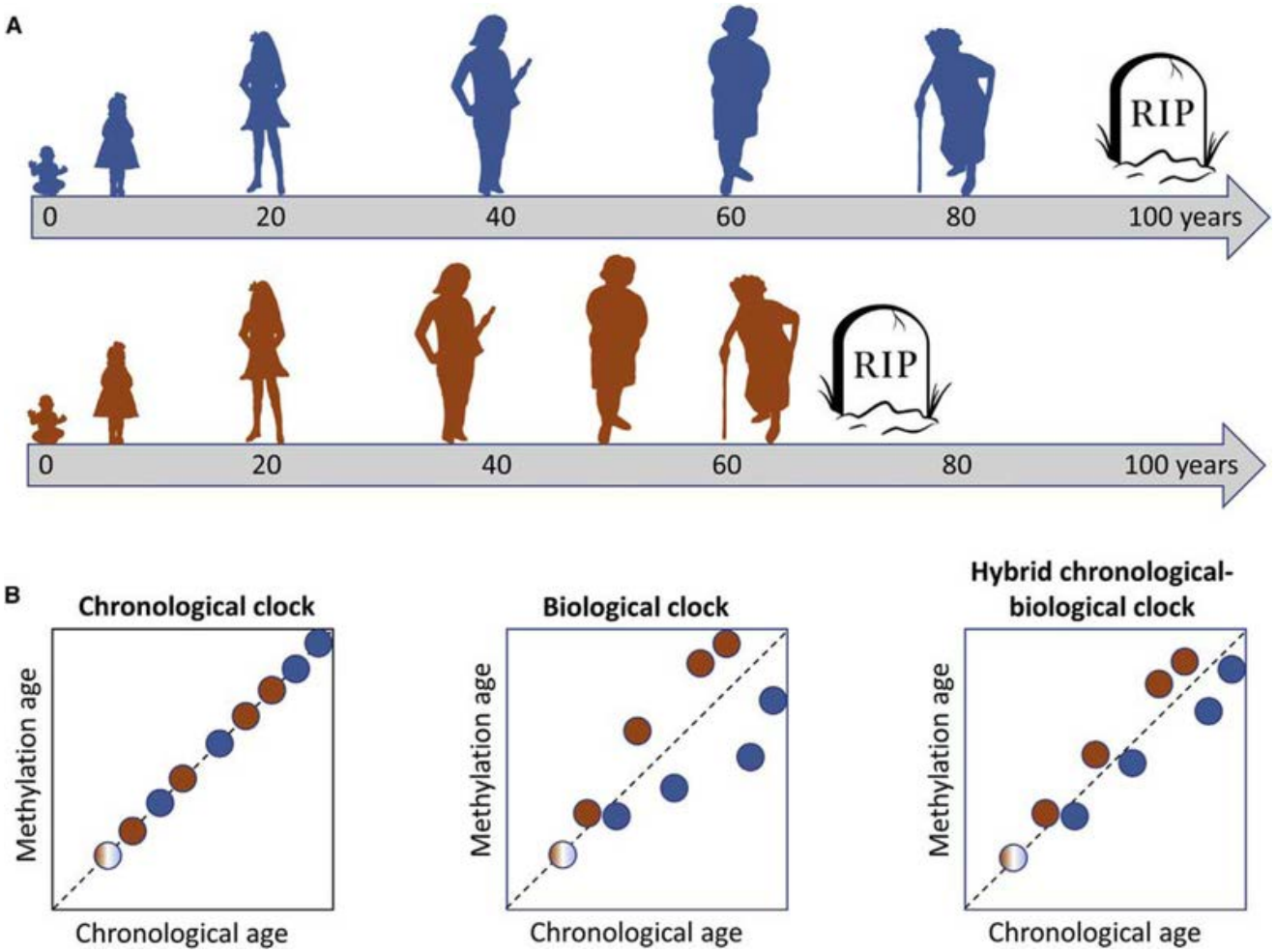
- **At very low energies in the nanorange electrons RESONATE & *amplify* the energy of Ion Channels by increasing or decreasing the height of the energy at the gating cavity in this Ion Channel**



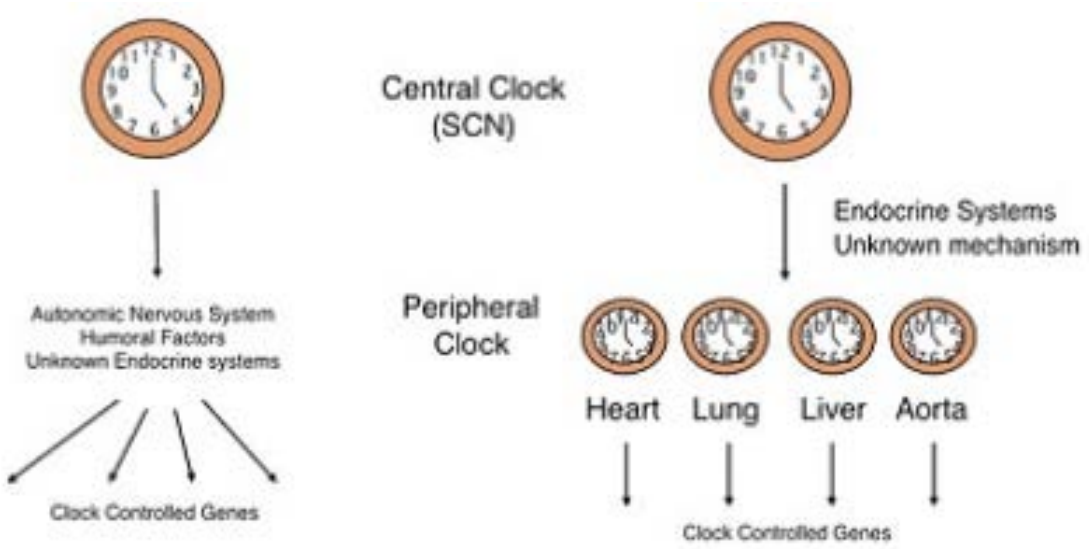


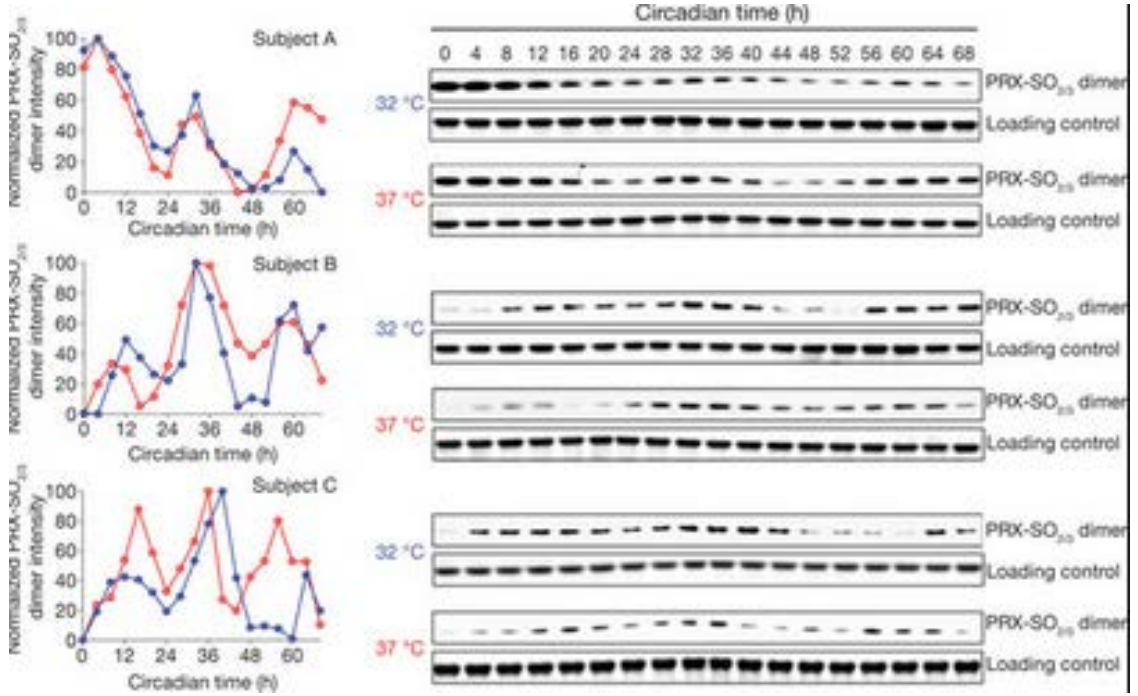
DNA METHYLATION CLOCKS IN AGING

- Two people, blue and red, born at the same time, will always share the same chronological age. But because of genetic, epigenetic & environmental factors / lifestyle choices, their functional decline / aging will be atdifferent rates.
- In early life, red and blue are assumed to have the same biological age.



Field et al, 2018

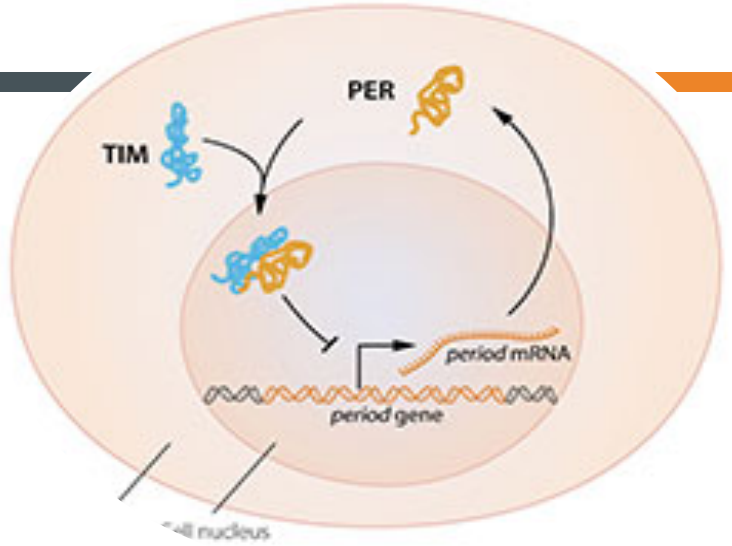




OUR CELLS HAVE A CIRCADIAN CLOCK

CLOCK-DEPENDENT REGULATION OF THE CELL CYCLE IS AN ESSENTIAL IMMUNE CONTROL MECHANISM.

- "**Every single cell** in your body is **controlled by its own circadian clock**. It helps every cell figure out when to use energy, when to rest, when to repair DNA, or to replicate DNA." *Salk Institute circadian researcher Satchin Panda*



CELLS ARE GOVERNED BY THEIR BIOLOGICAL CLOCKS IN ORDER FOR OPTIMUM COMMUNICATION TO TAKE PLACE BETWEEN ARTIFICIAL INTELLIGENCE (AI) BLUEPRINT SIGNALS AND NATURALLY OCCURING BIOLOGICAL SIGNALS, THE AI SIGNALS MUST BE DELIVERED WITHIN PRE-DEFINED VARIABLE TIMES THAT MAPS THE TIME SCHEDULE OF BIOLOGICAL SIGNALS. THEREFORE THE IREVIVE IS DESIGNED ON THE BASIS OF A MATRIX OF SIGNALS DELIVERED WITHIN A TIME MATRIX

The Nobel Prize in Physiology or Medicine 2017

Jeffrey C. Hall

Prize share: 1/3

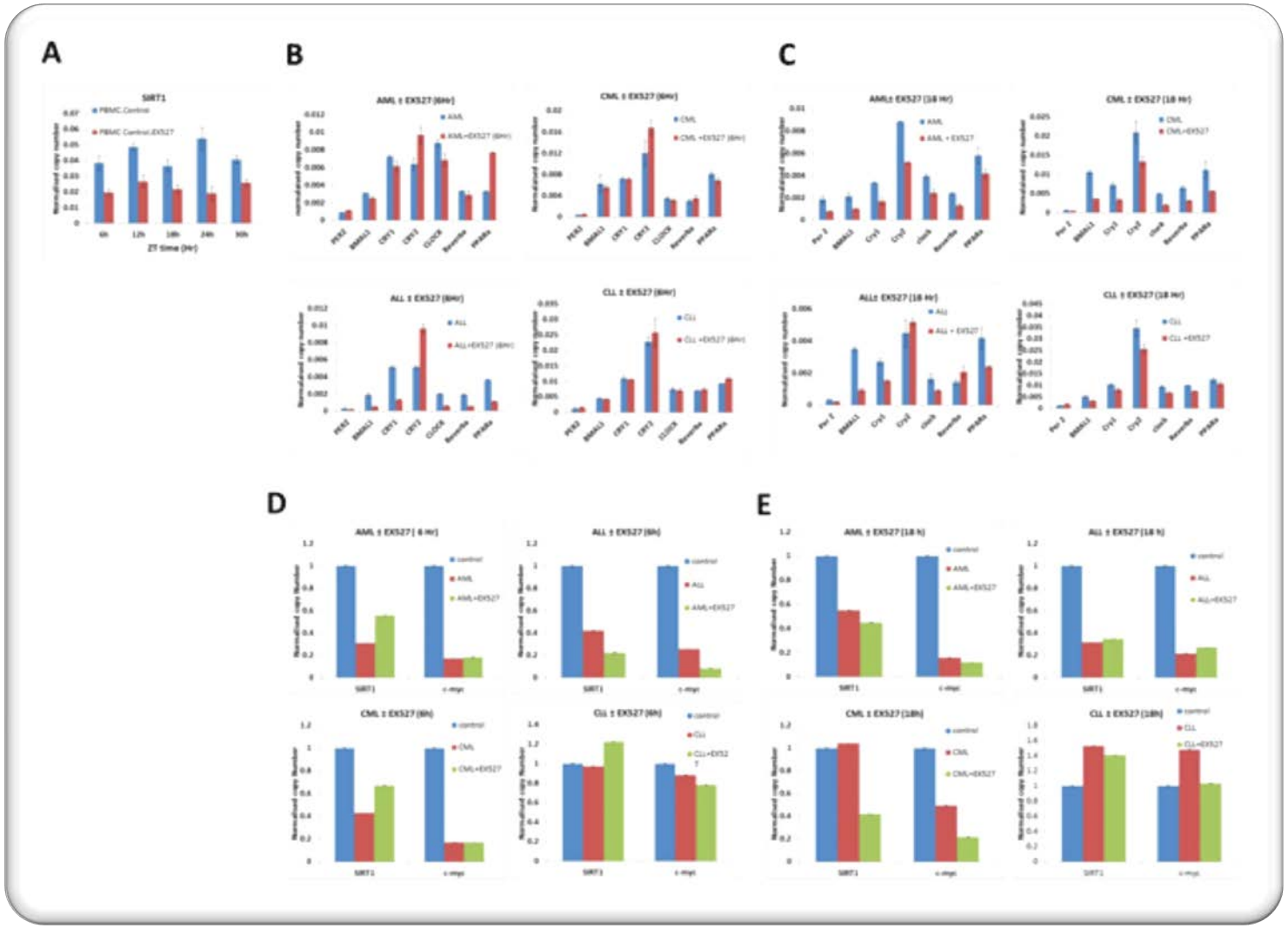
Michael Rosbash

Prize share: 1/3

Michael W. Young

Prize share: 1/3

The Nobel Prize in Physiology or Medicine 2017 was awarded jointly to Jeffrey C. Hall, Michael Rosbash and Michael W. Young "for their discoveries of molecular mechanisms controlling the circadian rhythm."

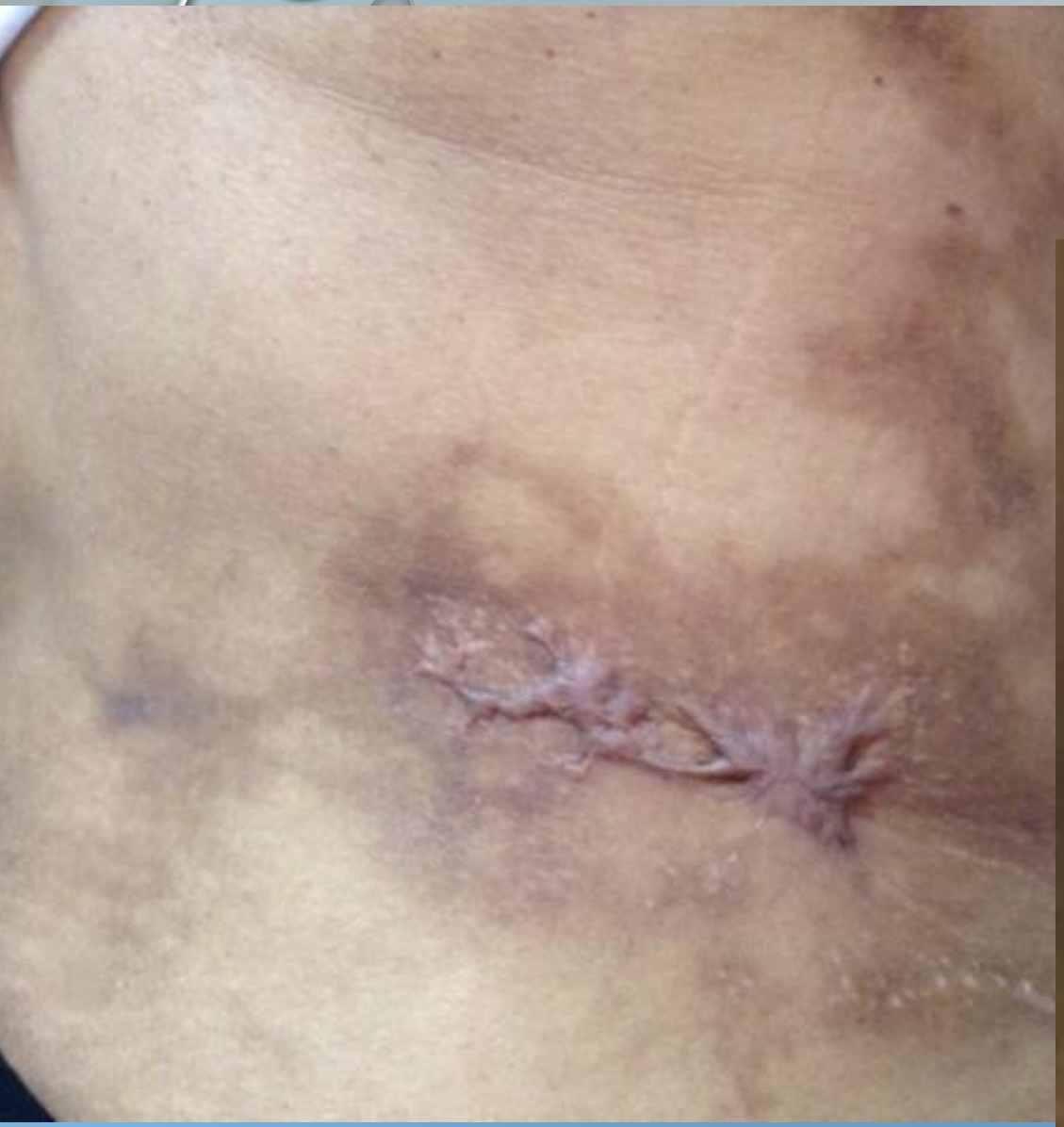


Circadian Genes in Leukemia

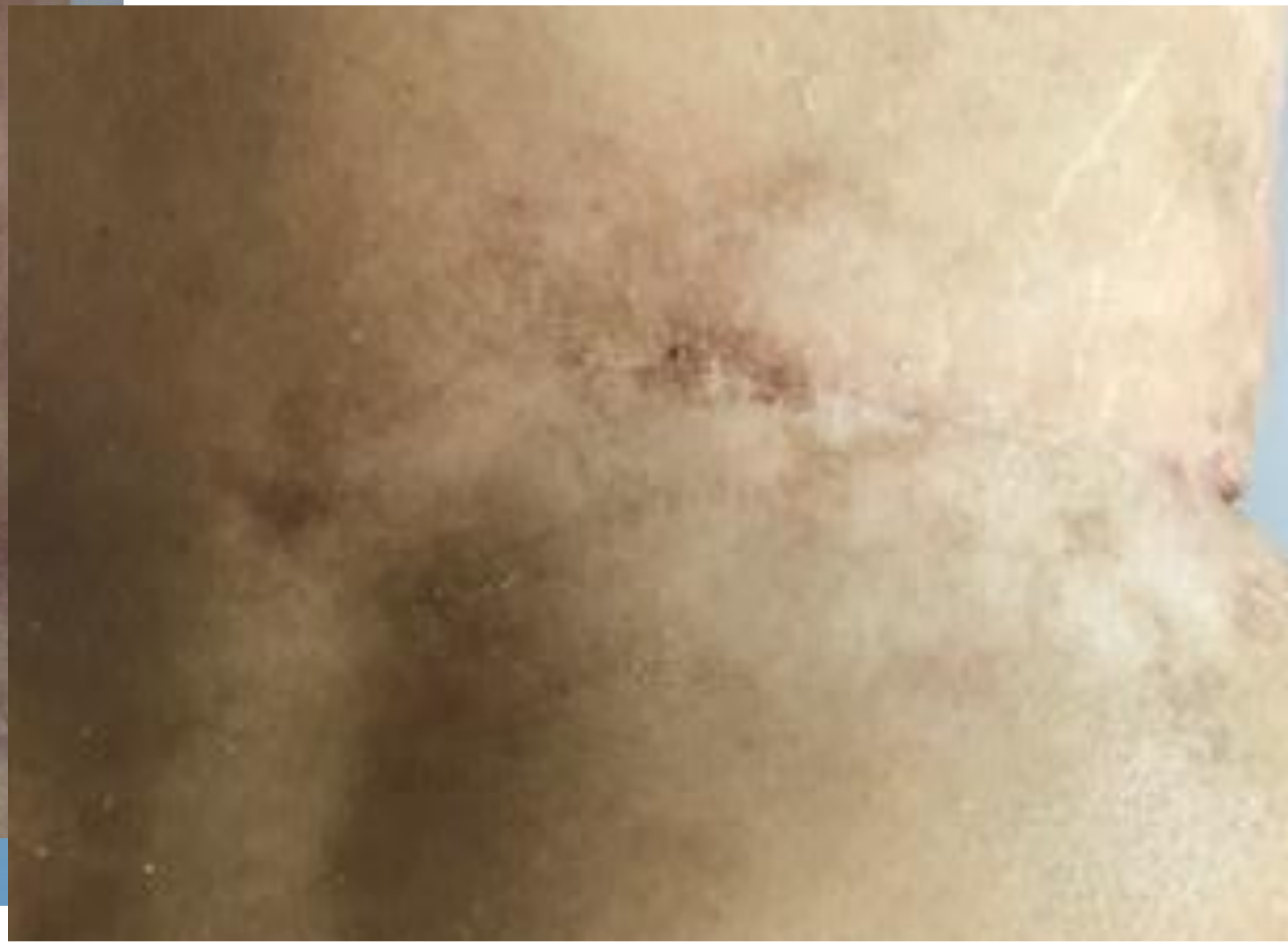
CLOCK GENES CAN DETERMINE SKIN DISORDERS, AGING AND DESEASE

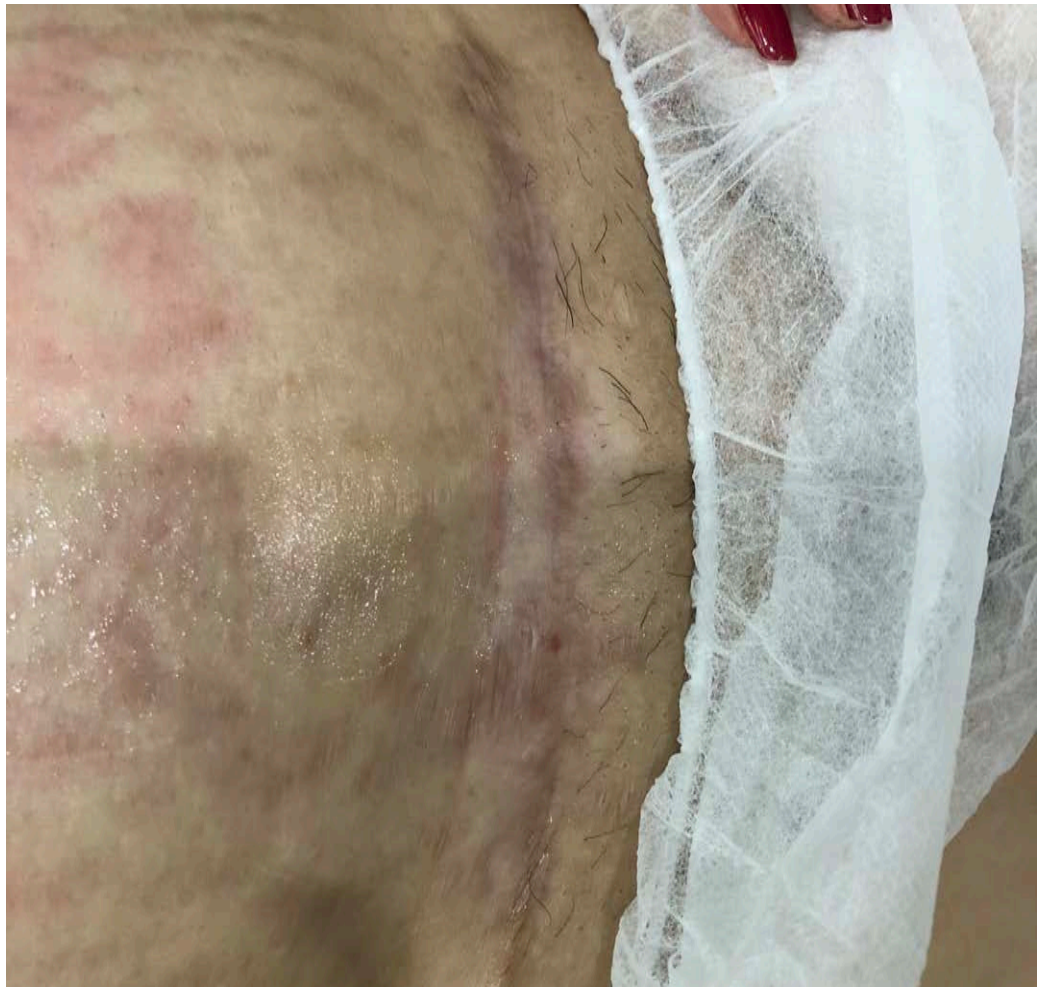
- At a cellular level we have a range of 'clock genes'
- Clock GENES influence cell activity.
- Clock genes preprogram proteins to guide cells when to use energy, when to rest, when to repair DNA, or to replicate DNA.

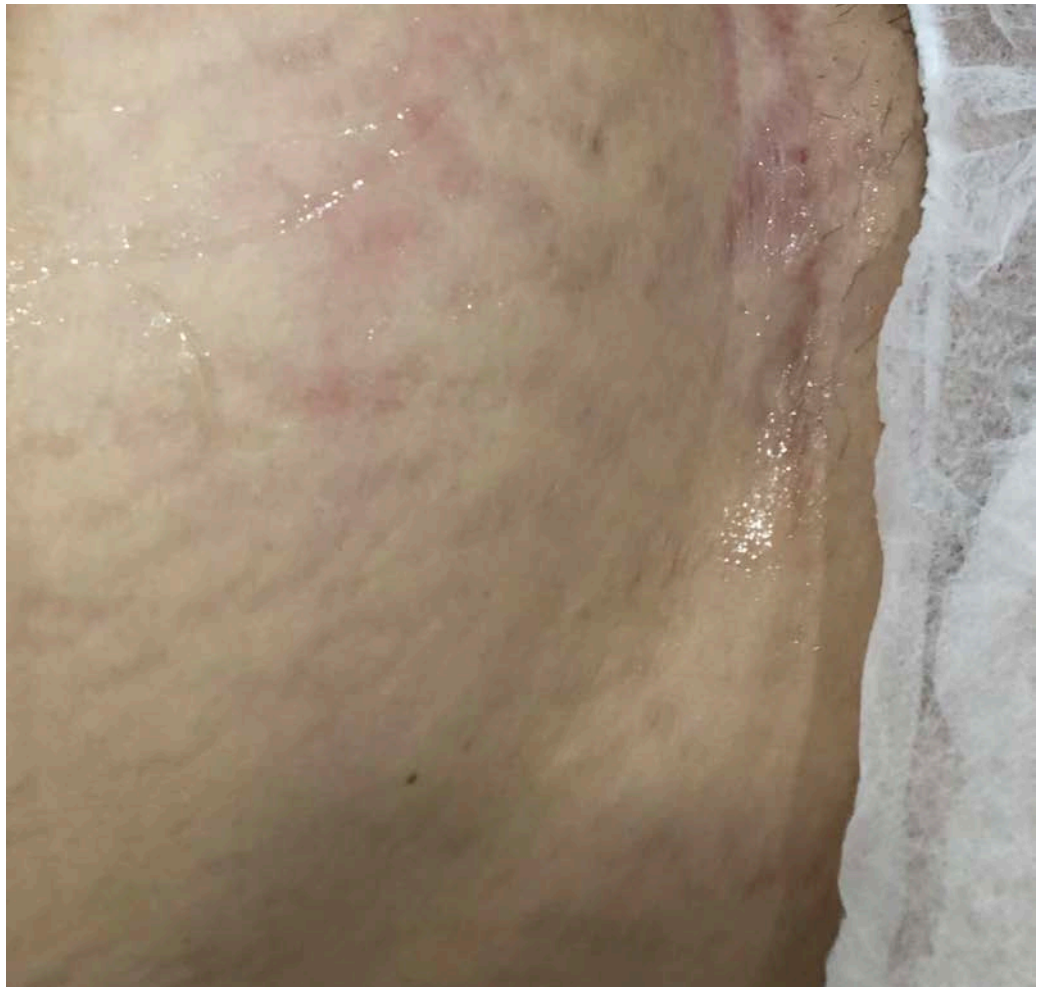
KELOID SCARS ARE THE RESULT OF DISREGULATION IN GENE CLOCKS



KELOID AFTER LIPOSUCTION WOUND TREATED WITH SIGNALING – 6 TREATMENTS

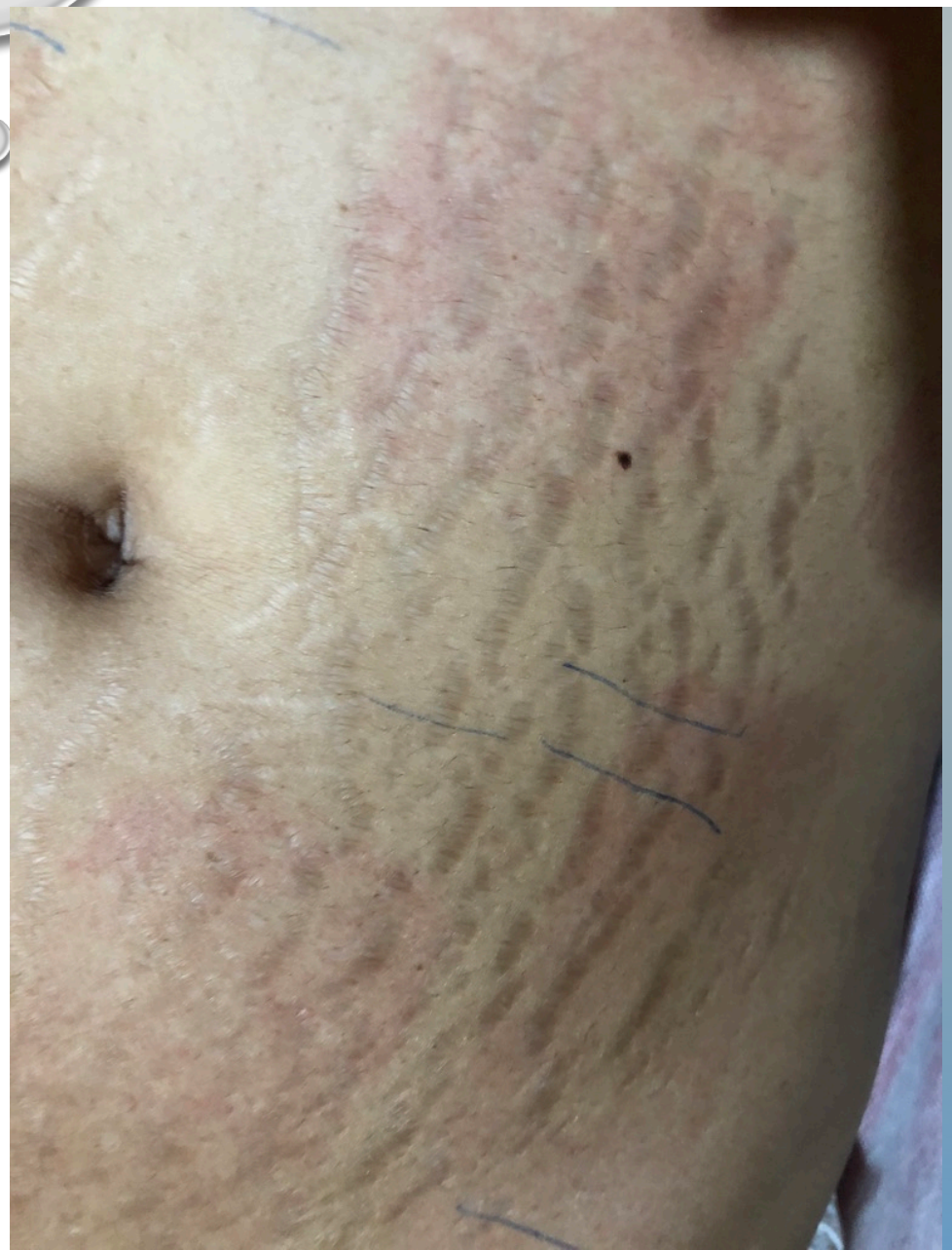


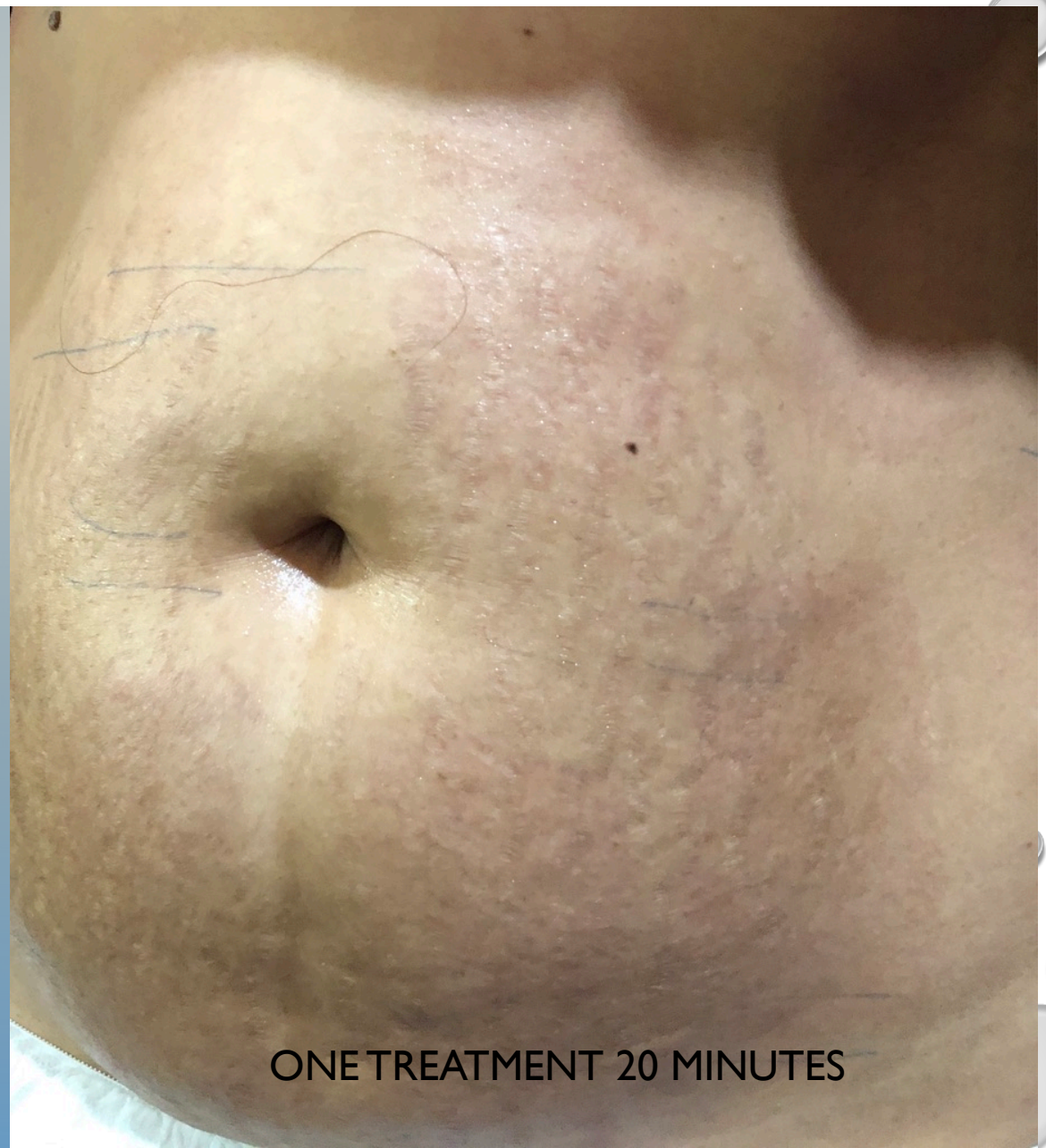




AGTER 20 MINUTES OF SIGNALING

THE SAME DISTURBANCE IN GENE CLOCKS IS INVOLVED IN STRETCHMARKS





ONE TREATMENT 20 MINUTES

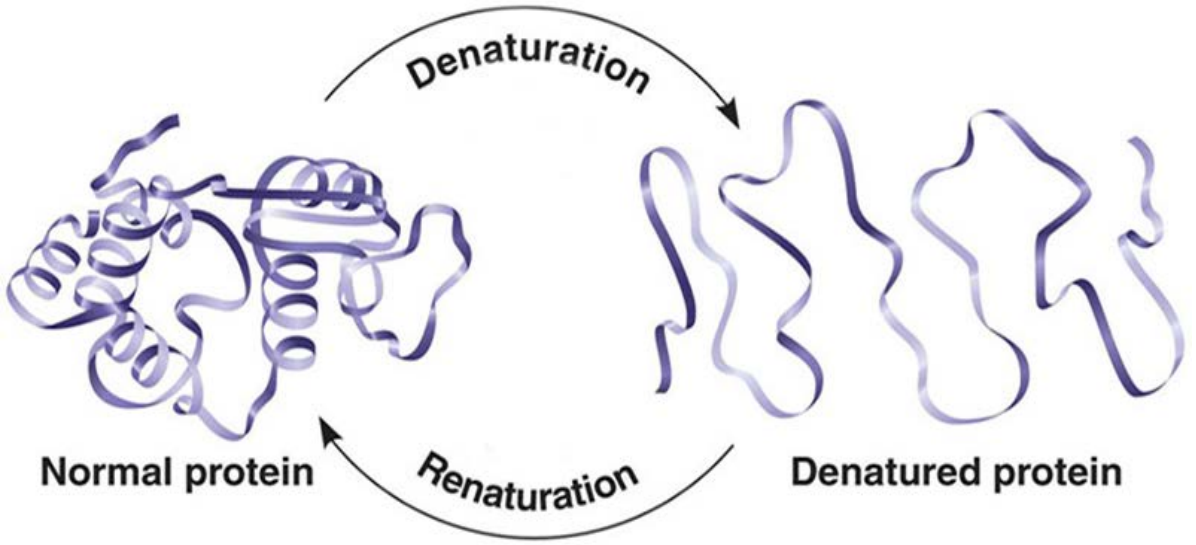
ANTI-AGING VIA PROTEIN RENATURATION / Protein Folding WITHIN THE PARAMETERS OF TIME WITH RESPECT TO CIRCADIAN CLOCKS

HOW TO TEST RESULTS ARE DUE TO PROTEIN FOLDING?

Second virial coefficient (SVC) measurements

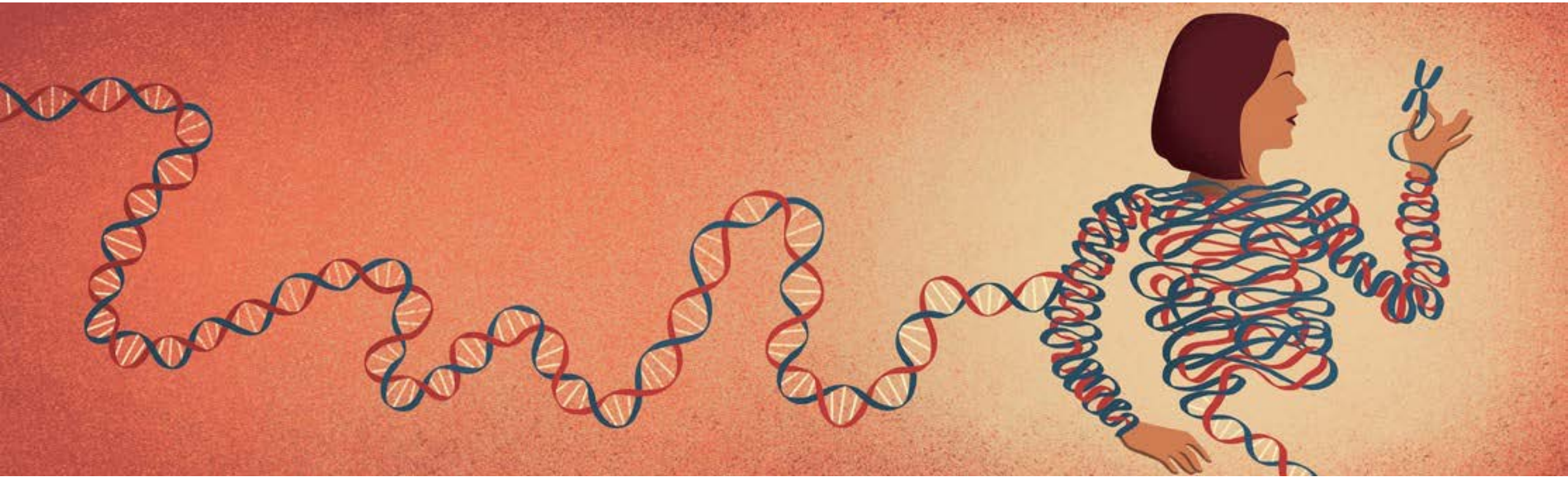
* -VE SVC is indicative of -VE protein–protein interactions. - **Protein aggregation INCREASES** during refolding Protein Refolding compromised

* +VE SVC indicates +VE protein–protein interactions
- **Protein aggregation DECREASES**
  Protein Refolding Successful



Jason G.S. Ho,[1] Anton P.J. Middelberg,[1] Paul Ramage,[2]Hans P. Kocher[2]Protein Sci.10.1110/ps.0233703

THANK YOU FOR YOUR KIND ATTENTION



Any questions please e-mail: science@iellios.com