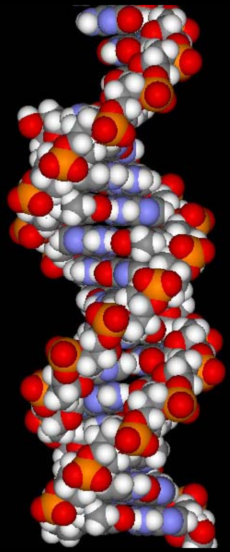
La tutela della salute nelle attività sportive e la lotta al doping



Biomarcatori genomici nel doping genetico

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Centro di eccellenza per lo studio del rischio genomico in patologie complesse multifattoriali
emiliano.giardina@uniroma2.it

Roma, 17 maggio 2012

3 times as expensive and 8 million times less capacity.

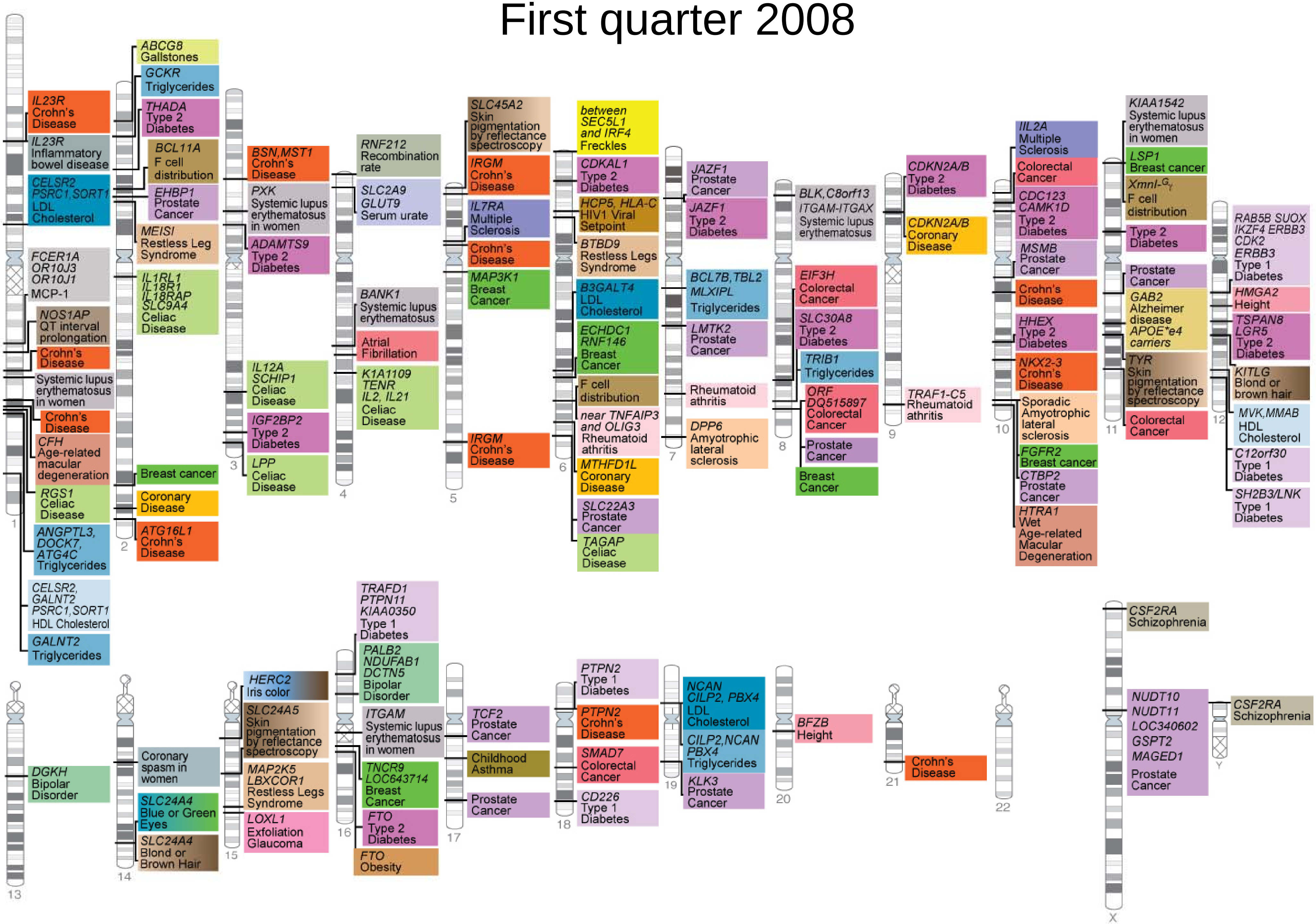
$795 in 1977
(=$2,800 in
current $

Recent advances of human genetics are driven by Accelerating Technology

- In 1997 it took about a day to genotype a one Single Nucleotide Polymorphism
  - Cost was ~$100
- Now in a matter of days one can genotype an individual at >2,000,000 sites
  - At a cost of < $500
    - Reduction in cost of >400,000 fold


TIME
THE FUNNIEST MAN IN AMERICA
What scientists have uncovered about HOW MEMORY WORKS and how to improve it
THE I.Q. GENE?
LIFE
WERE YOU BORN THAT WAY?
Personality, temperament, even life choices. New studies show it's mostly in your genes.
optimism aggress thrill-seekin anxiety shynes obesity obesity addict
TSUNAMI SCIENCE: ONE YEAR AFTER THE WAVE THAT ROCKED THE WORLD
SCIENTIFIC AMERICAN
Alternatives to Toxic Tests on Animals
JANUARY 2006
WWW.SCIAM.COM
Know Your DNA
Inexpensive gene readers will soon unlock the secrets in your personal double helix
The Hazy Origin of Brown Dwarf Stars
TIME
COUNTDOWN TO WA
SPECIAL REPORT
SOLVING THE MYSTERIES OF DNA
The 50th Anniversary: Reliving Watson and Crick's historic discovery
How gene science has changed our lives
Visions of the future
TIME
THE FUTURE OF MEDICINE
LIFE
The Secret of Life
2NeatBooks.com

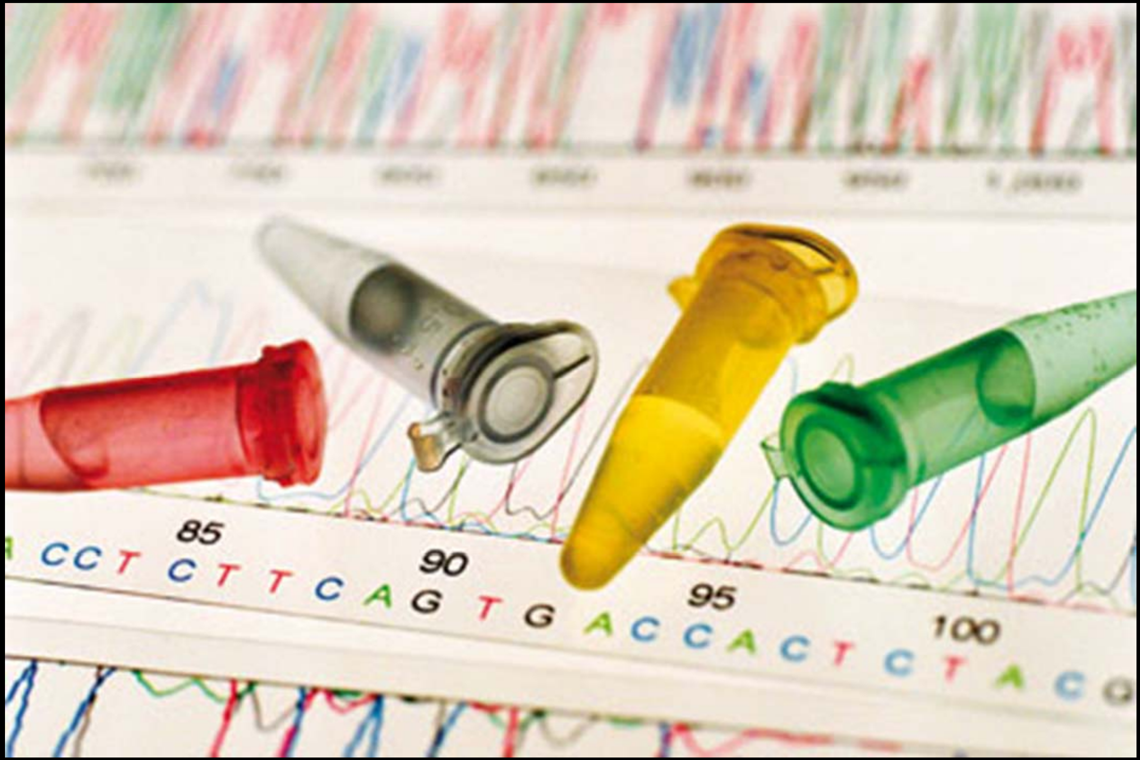
First quarter 2008

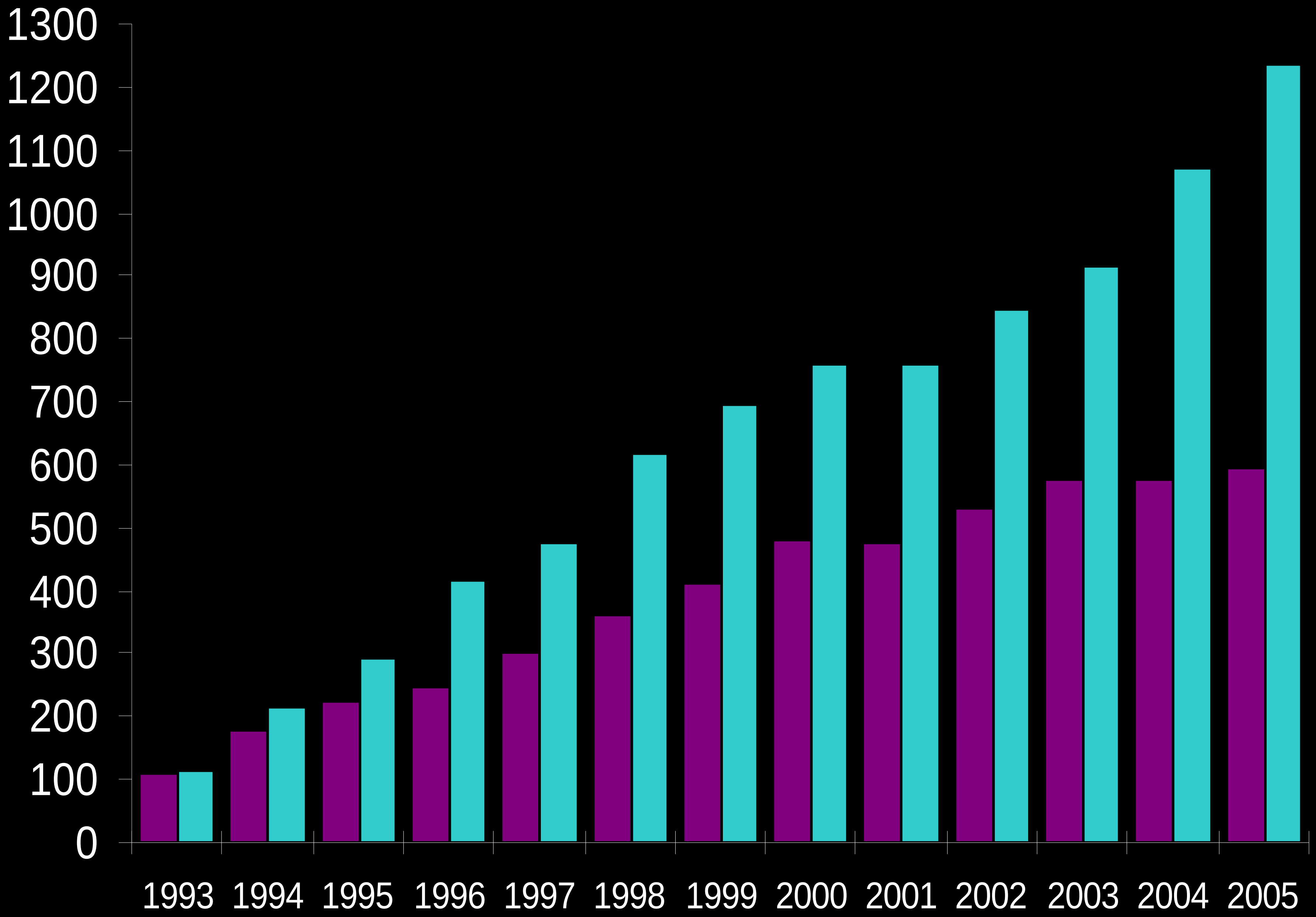


Manolio, Brooks, Collins, J. Clin. Invest., May 2008

What Can We Do With Such Technology?

- WGS is now a practical reality
  - The genes for virtually all “genetic”disorders will soon be elucidated
- Many Genome Wide Association Studies (GWAS) in common diseases disclosing an impressive number of susceptibility genes





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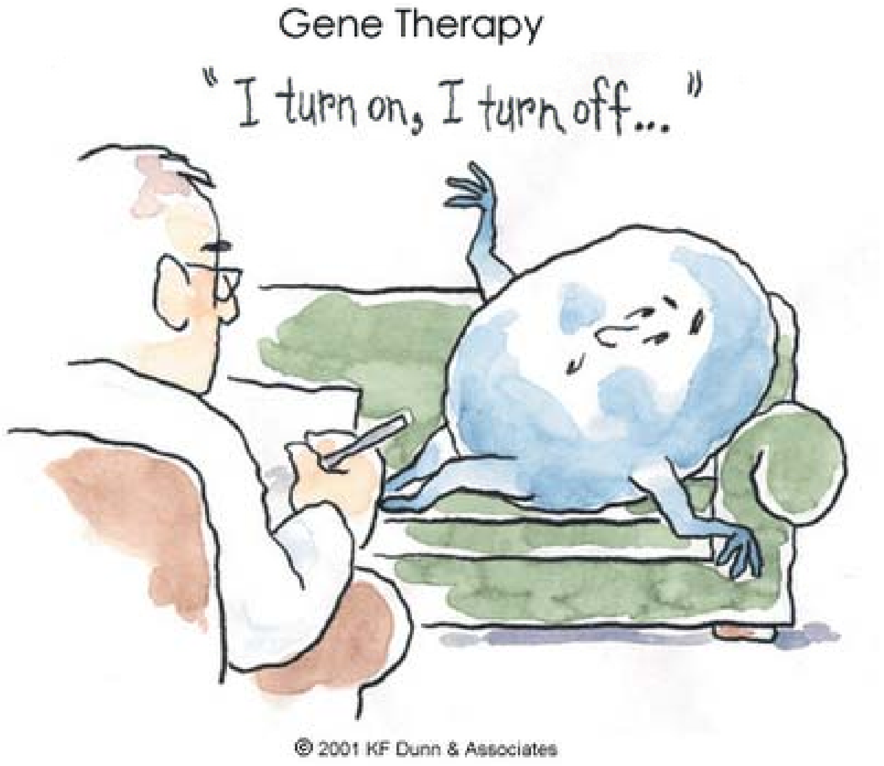
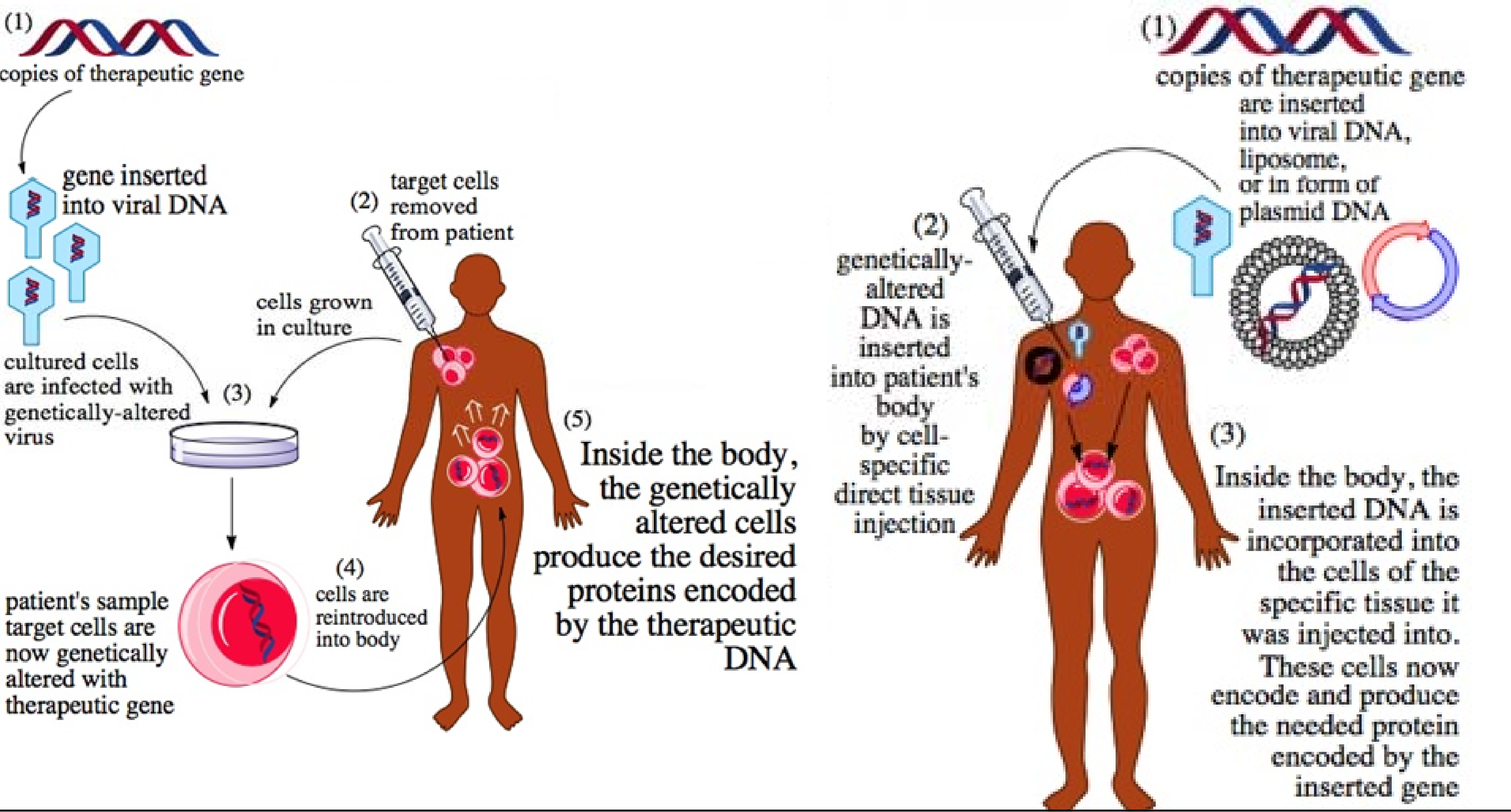
Definition

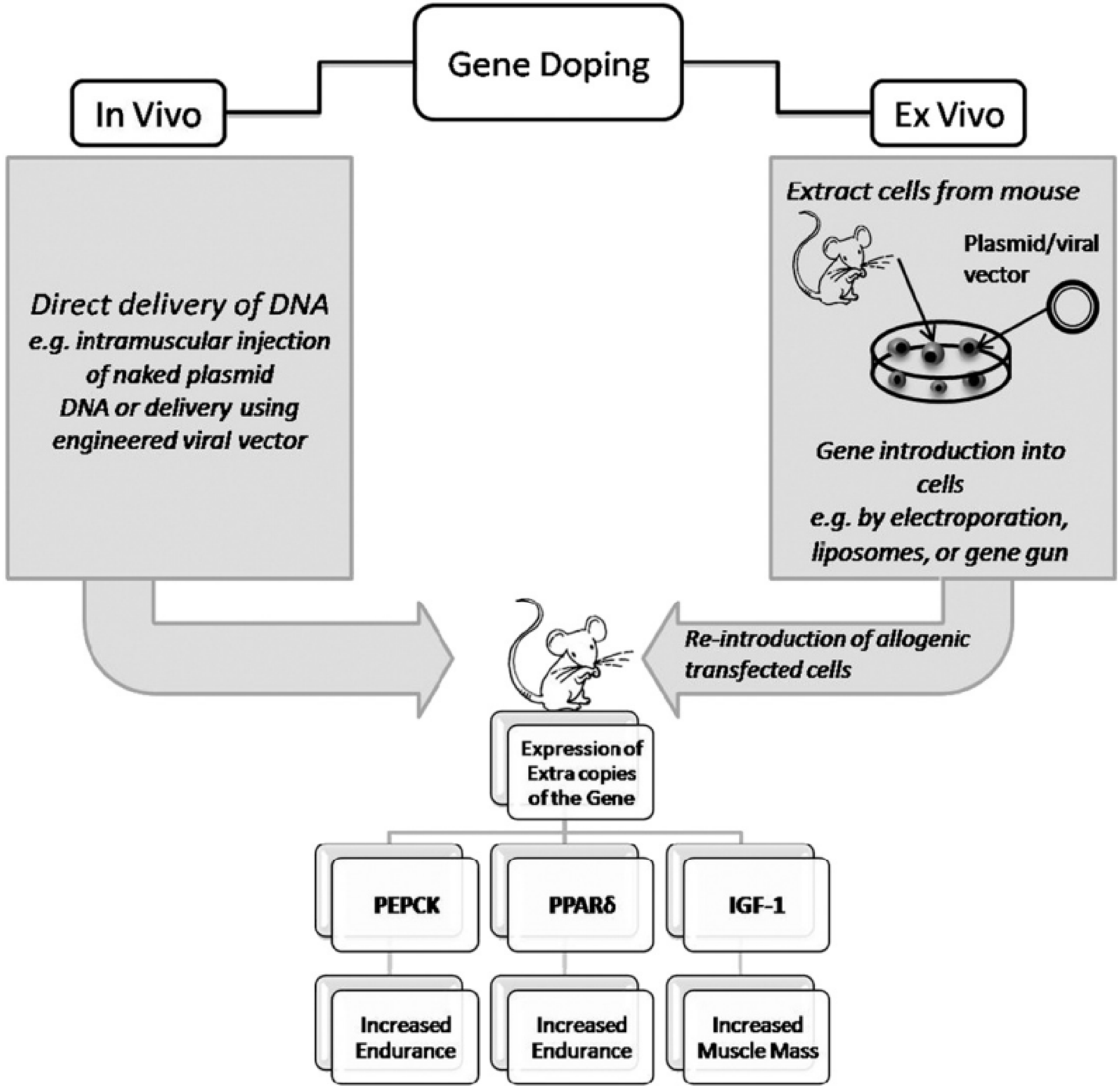
The “non-therapeutic use of cells, genes, genetic elements, or modulation of gene expression, having the capacity to enhance athletic performance”



Ex vivo / In vivo gene therapy

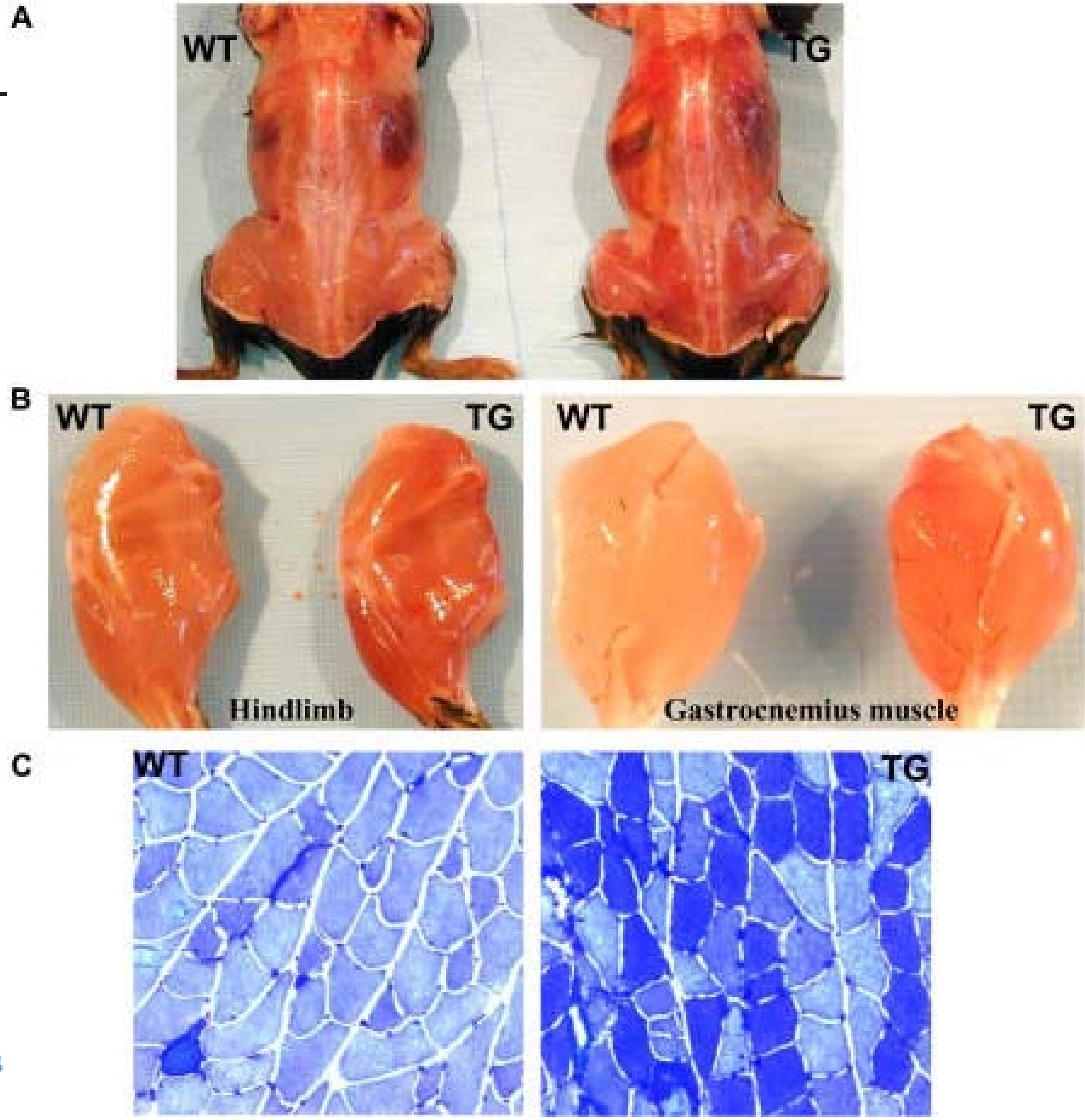


- Il doping genetico si basa sul presupposto teorico che è possibile produrre qualsiasi proteina in vivo, a patto che il suo gene sia noto e che sia possibile introdurlo nelle cellule dell'atleta.
- Sono ovviamente compresi ormoni ed enzimi regolatori del metabolismo. Le cellule dell’atleta sfruttano il gene introdotto per produrre la proteina ricombinante, che risulta del tutto simile alla sua controparte endogena.


Gene Doping
In Vivo
Ex Vivo
Direct delivery of DNA
e.g. intramuscular injection of naked plasmid DNA or delivery using engineered viral vector
Extract cells from mouse
Plasmid/viral vector
Gene introduction into cells
e.g. by electroporation, liposomes, or gene gun
Re-introduction of allogenic transfected cells
Expression of Extra copies of the Gene
PEPCK
PPARδ
IGF-1
Increased Endurance
Increased Endurance
Increased Muscle Mass

'Super mice' studies

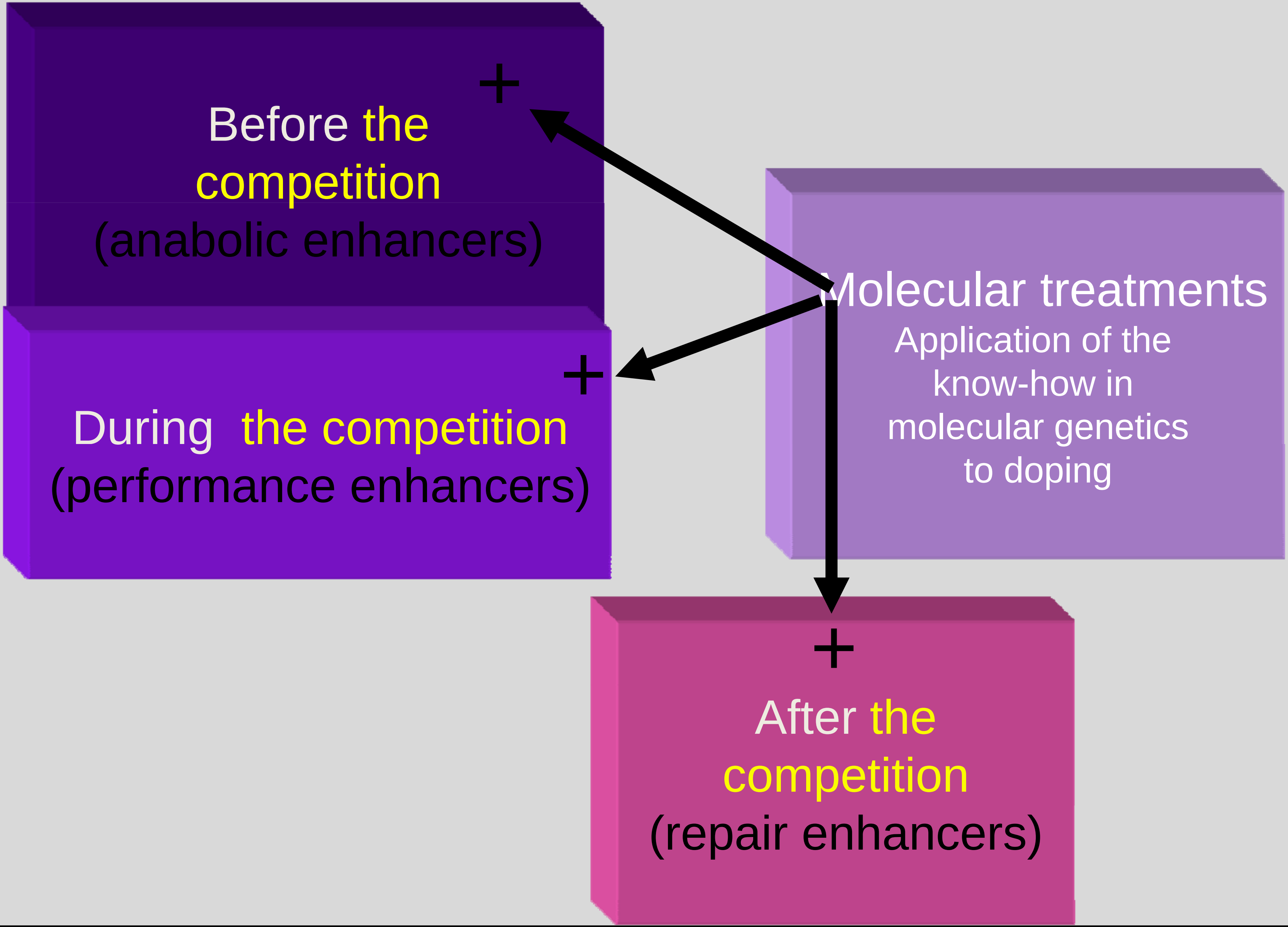
Target gene	Study	Findings
IGF-1	Gene insertion using an adeno-associated viral vector ($n=24$)	• 20%–30% increase in muscle strength and mass and an increase in endurance. • Contractile properties of flexor hallucis longus muscle were measured *in situ*.
PPAR δ	Gene insertion into mice zygotes ($n=4$, for each of the control and transgenic groups)	• Running time improved by 67% while the distance improved by 92% (as determined by running of mice on a treadmill, exhaustion endpoint was when the mice could not avoid repetitive electric shocks). • Resistance to obesity even in lack of exercise and on fat-rich diet.
PEPCK	Transgenic mouse line (9 transgenic mice, 10 controls)	• Extended life span relative to control animals • Mice up to an age of 2.5 years ran twice as fast as 6–12-month-old control animals • Transgenic mice ate 60% more than controls but had half the body weight and 10% body fat. • Transgenic mice ran a distance of at least 4.9 km as opposed to control which stopped at 0.2 km



Increased Oxidative Type I Fibers in the Transgenic Mice

Super-mices and blue cows

nature genetics



+
Before the competition
(anabolic enhancers)
+
During the competition
(performance enhancers)
Molecular treatments
Application of the know-how in molecular genetics to doping
+
After the competition
(repair enhancers)

Possibili applicazioni nel doping genetico

- *<u>ex vivo</u>*, hematopoietic tissue:
  pro hematopoietic (Epo receptor, oxygen transport...)

- *<u>in vivo</u>* local (example muscle):
  metabolic enhancers, growth factors,
  muscular fiber changers, cardio-modulators
  (glucose/oxygen, MGF, IGF-1, anti-myostatin, Epo)

- *<u>in vivo</u>* local (example joints):
  pain reducers, inflammation inhibitors, recovery and repair factors (anti-TNF, BMPs, ...)

- *<u>in vivo</u>* systemic:
  anabolic enhancers, endocrine factors, pain killers, vascular controllers,
  (hormone metabolising enzymes, proenkephalins, ...)

Rischi della terapia genica

- Immune response to vector
- immune response or long term side effects from new or foreign gene product
- General toxicity of viral vectors
- Adventitious contaminants in recombinant viruses
- Random integration in genome
  -> insertional mutagenesis (-> cancer risk)
- Contamination of germ line cells

Rischi del doping genetico

- I rischi associati al doping genetico possono essere raggruppati in due aree maggiori.

1. Rischi correlati alle procedure di terapia genica
2. Rischi correlati all’espressione incontrollata dei geni

- Gli adenovirus usati come vettori sono associati a morbidità in alcuni trial di terapia genica.

I rischi

Short -mid term

- Autoimmunity
- Hyperimmunity
- Toxic shock

Long term

- Fibrosis
- Cancer
- conventional side- effects of administered factors
- Inaccessibility to future gene therapy interventions (immunity to vectors)

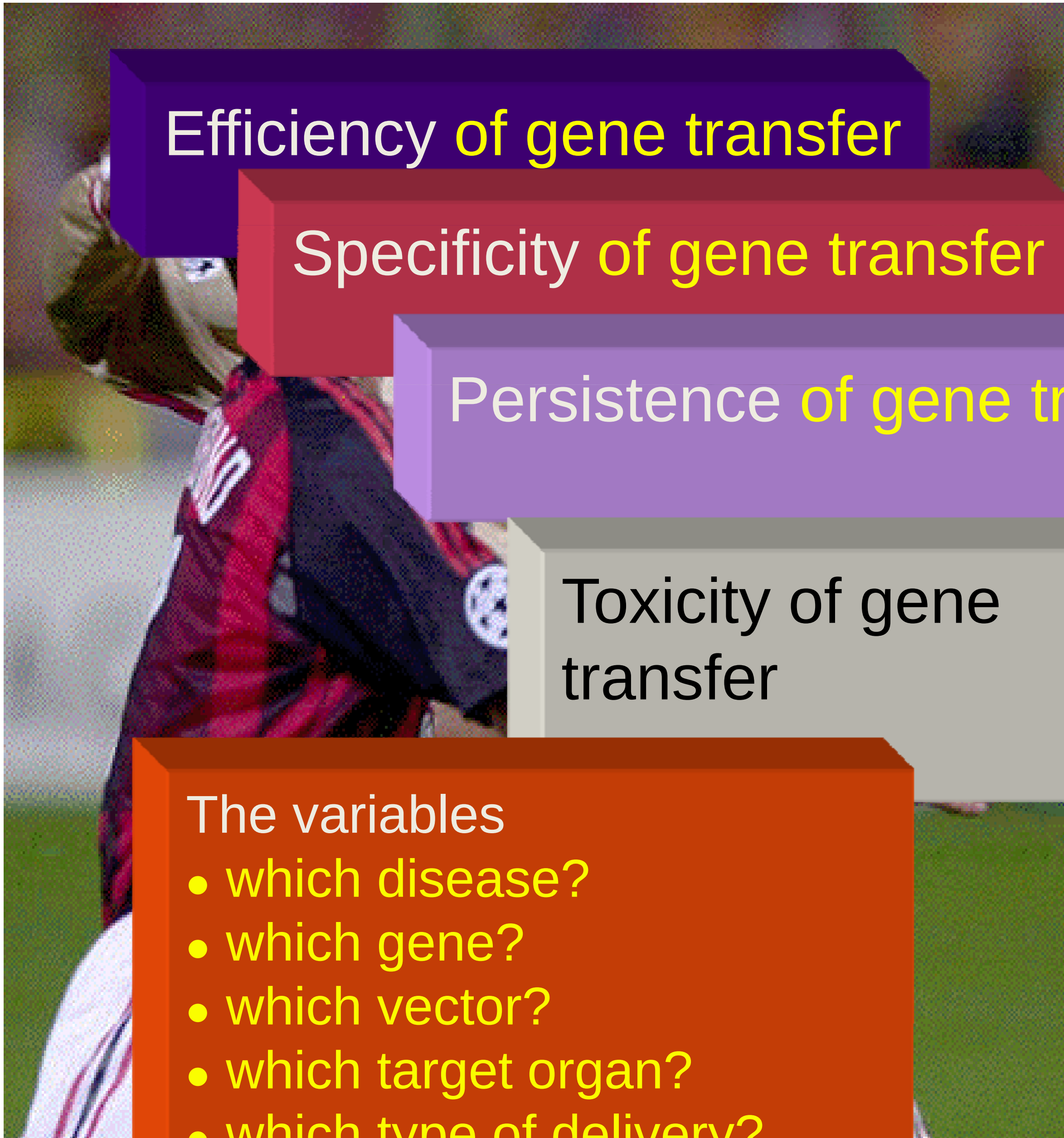
Rischi specifici

- L’ormone della crescita e l’IGF-1 sono potenti mitogeni ed agenti antiapoptotici, che determinano un alto rischio tumorale.
- L’iperespressione di HIF-1 (hypoxia-inducible factor 1) e di fattori angiogenici potrebbero potenzialmente portare ad una miglior vascolarizzazione e promuovere così la crescita tumorale.

- L’iperespressione di Epo ha un elevato numero di rischi:
  - La somministrazione di Epo causa un aumentato ematocrito con conseguente aumento della viscosità del sangue e dello sforzo cardiaco
  - Tra le conseguenze potenziali ci sono il blocco della microcircolazione, l’ictus e l’infarto
  - La produzione di Epo da terapia genica ha determinato anemia in alcuni modelli animali (macachi)

- Il blocco completo dell’attività miostatinica, come visto nei topi knockout per la miostatina, determina una forza muscolare non paragonabile all’aumento volumentrico del muscolo, per cui I topi knockout per la miostatina hanno muscoli più voluminosi, ed una forza solo di poco aumentata rispetto al topo wild-type

The four technical basic questions



Efficiency of gene transfer

Specificity of gene transfer

Persistence of gene trans

Toxicity of gene transfer

The variables
- which disease?
- which gene?
- which vector?
- which target organ?
- which type of delivery?

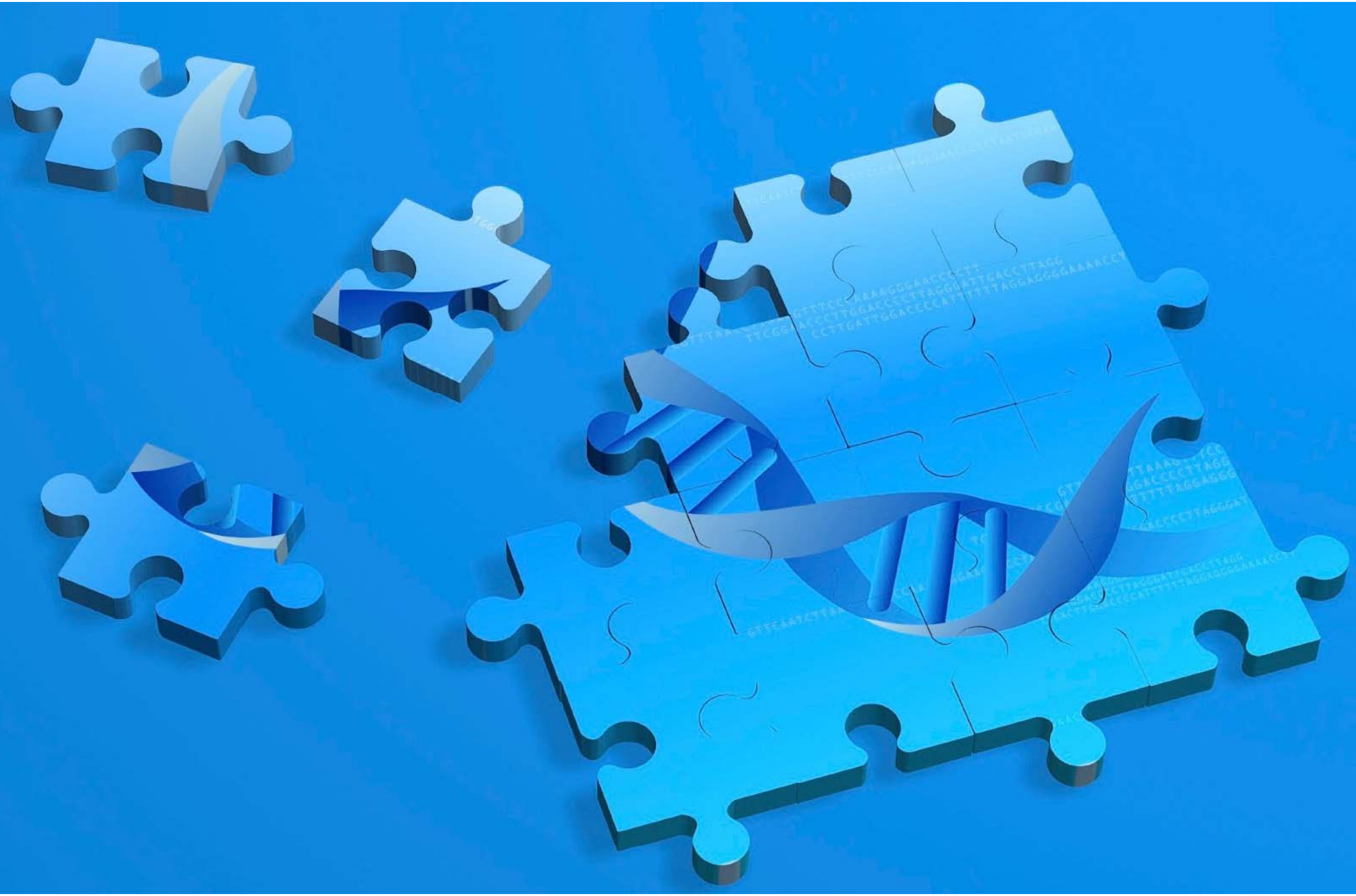
Remember!

Efficiency

Specificity

Persistence

Toxicity



The experiments of Lee Sweeney (2004) have raised further smoke...

Gene transfer of IGF-1 (J. App Physiol 96, 1097 ff (2004))

The features

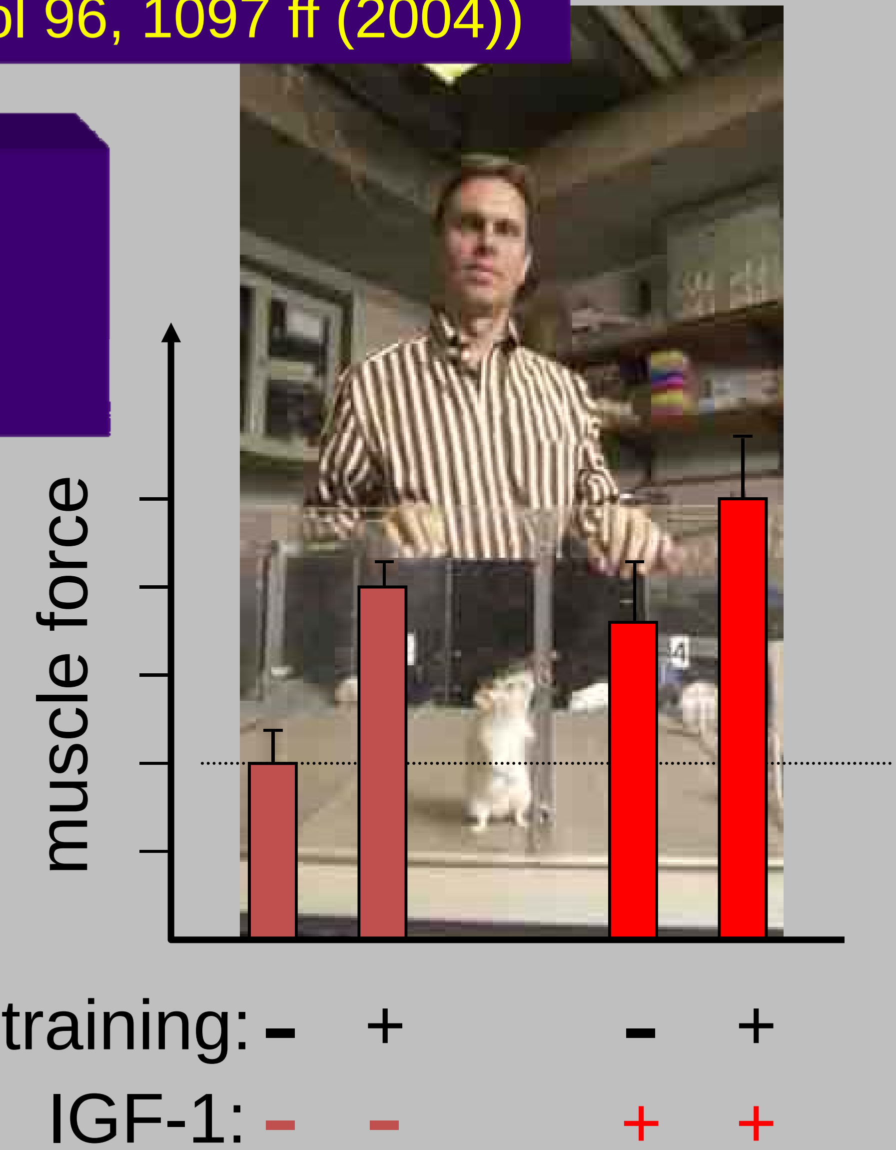
- IGF-1 -> growth factor for muscles
- AAV Vector, intra muscular
- Rat model , + or - training

Results

muscle force and muscle mass increased beyond levels obtained in training

Ergo

ok, Dr. Sweeney, transfer of IGF-1 in rats significantly increases muscle performance,, but...



Which would be the objective limitations of gene doping?

Viral gene transfer

- immune problems
- limited readministration possibilities
- general toxicity, genotoxicity

Nonviral gene transfer

- generally inefficient
- lack of persistence, requires readministration

Strategy-independent problems

- laborious, not readily available
- long term gene expression difficult to control
- irreversible effects or permanent tagging
- asymmetry of effects

Ergo:
risks seem today currently higher than benefits

Detection possibilities of gene doping

- Antibody detection (viral antigens)
- r-nucleic acids detection (PCR)
- recombinant protein / post-translational modification detection (MALDI-TOF)

- Anatomically difficult to detect
(if locally administered)
-> but leaves permanent genetic marking

- Detection of nucleic acids cannot be
fluids (except in early phase after sy
-> might require specific tissue biop

Ergo

- foreign genes detectable only short- term in blood or body fluids, but
- foreign genes detectable long term in tissue biopsies, and
- abnormal gene products detectable (example GT erythropoietin in monkeys)

Comparison of advantages/disadvantages: with respect to conventional Doping

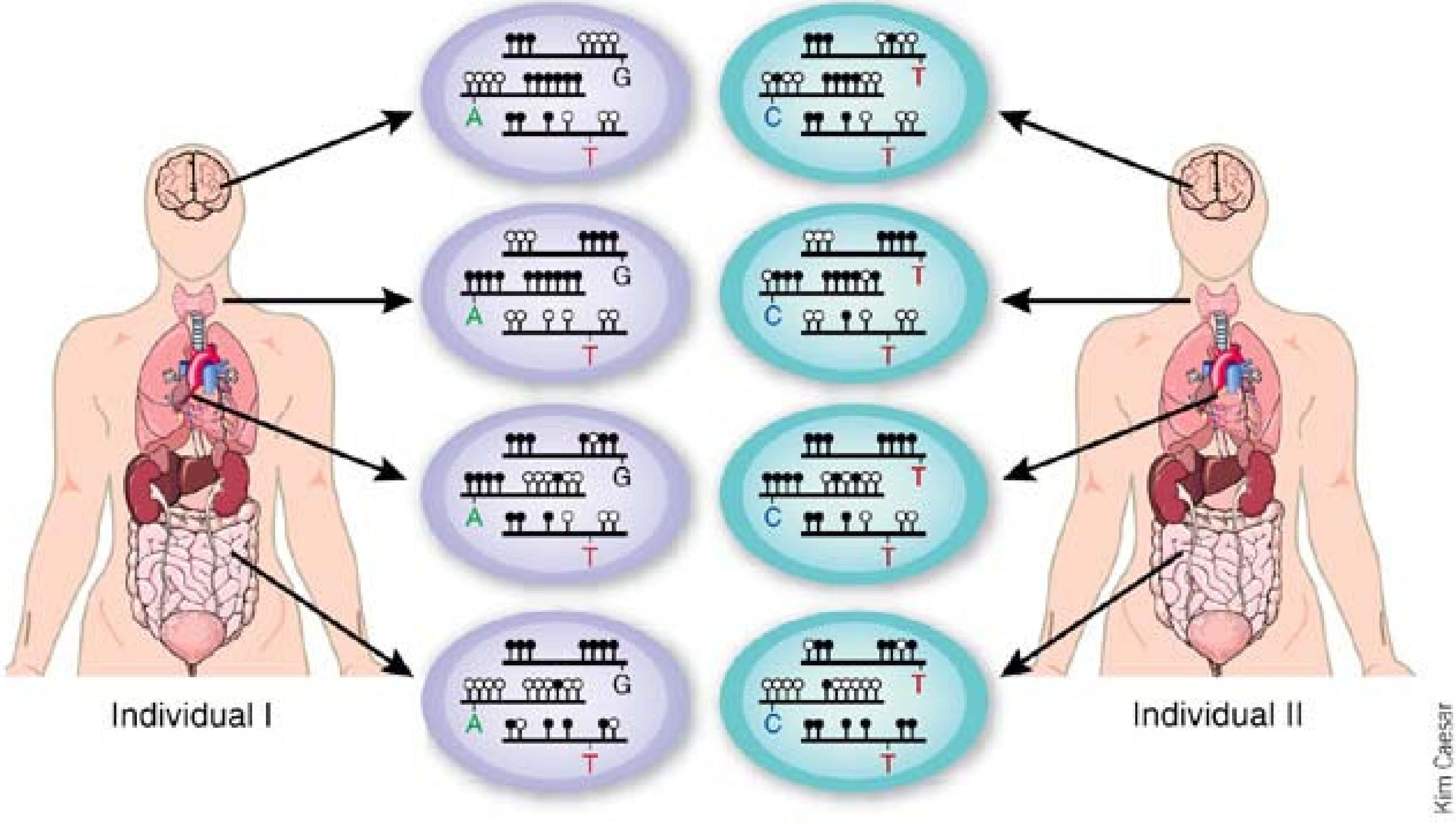
Category	Drug/protein	Gene-
Rapidity of effects	rapid	slow
Reversibility	rapid	slow
Dosage	straightforward	difficult
Complexity of treatm.	simple	complex
Associated risks	depends	high
Concealability	possible	difficult /impossible

From a static to a dynamic view of genetic risk

✓ chromatin differences between individuals can exist independently of DNA sequence polymorphisms

✓ the dynamic quality of epigenetic modification, which stands in contrast to static nucleotide sequence information, provide the basis for an individual’s response to a constantly changing environment

✓ Epigenetic factors affect the expression of drug-metabolizing enzymes, drug transporters, and nuclear receptors that regulate the expression of variuos genes and ultimately affect the response to drug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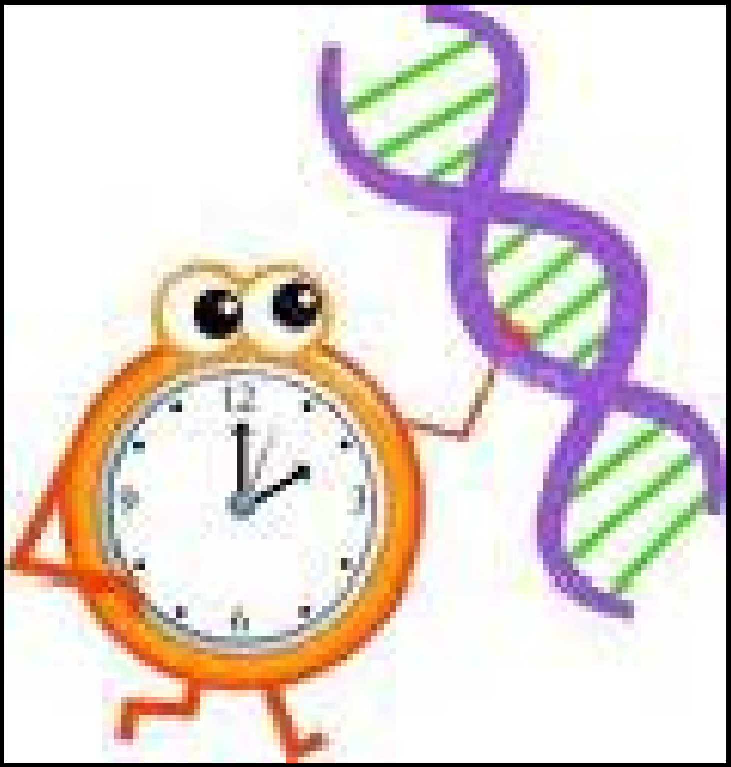
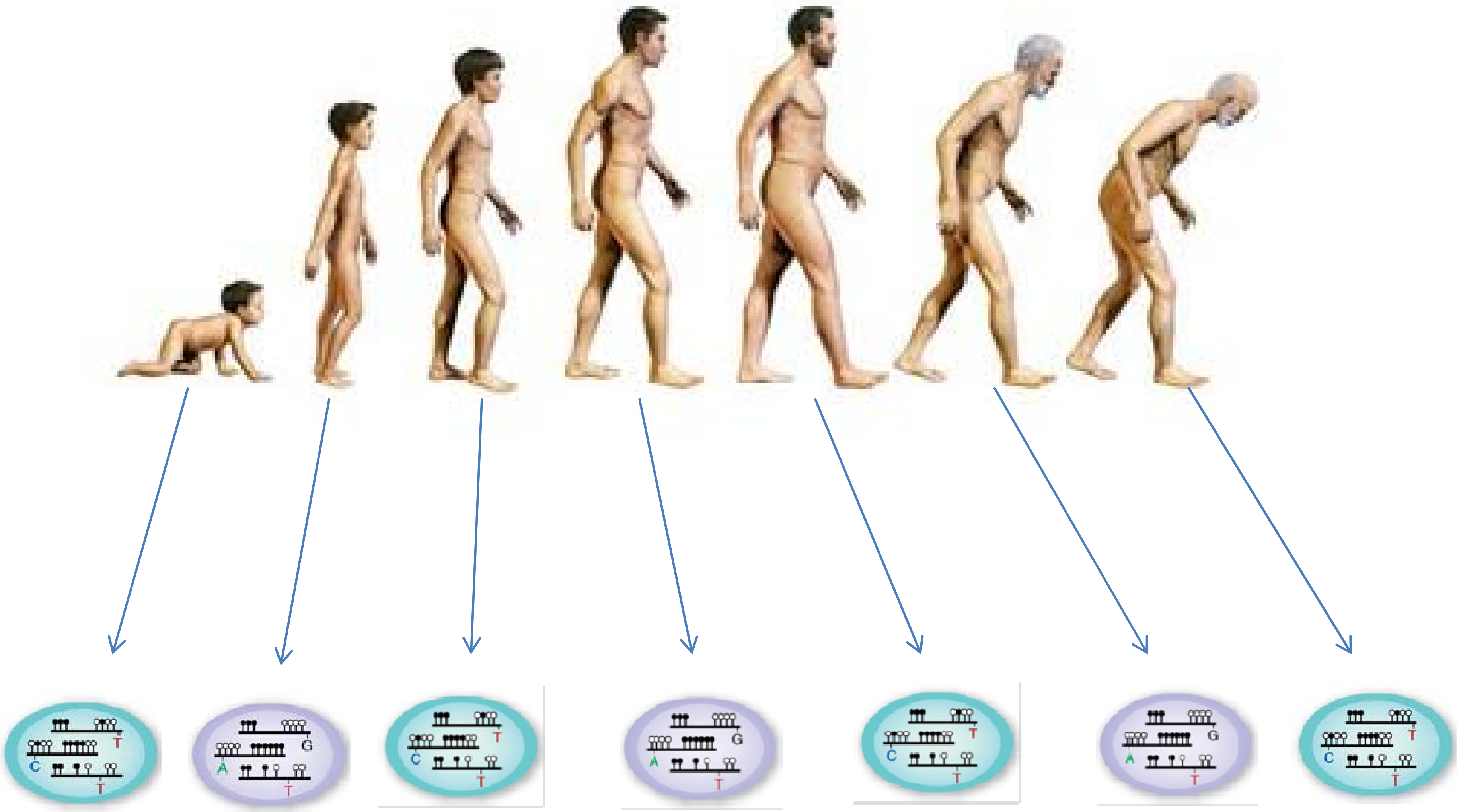
Individual I
Individual II
Kim Caesar

The regulation of genes changes over the time



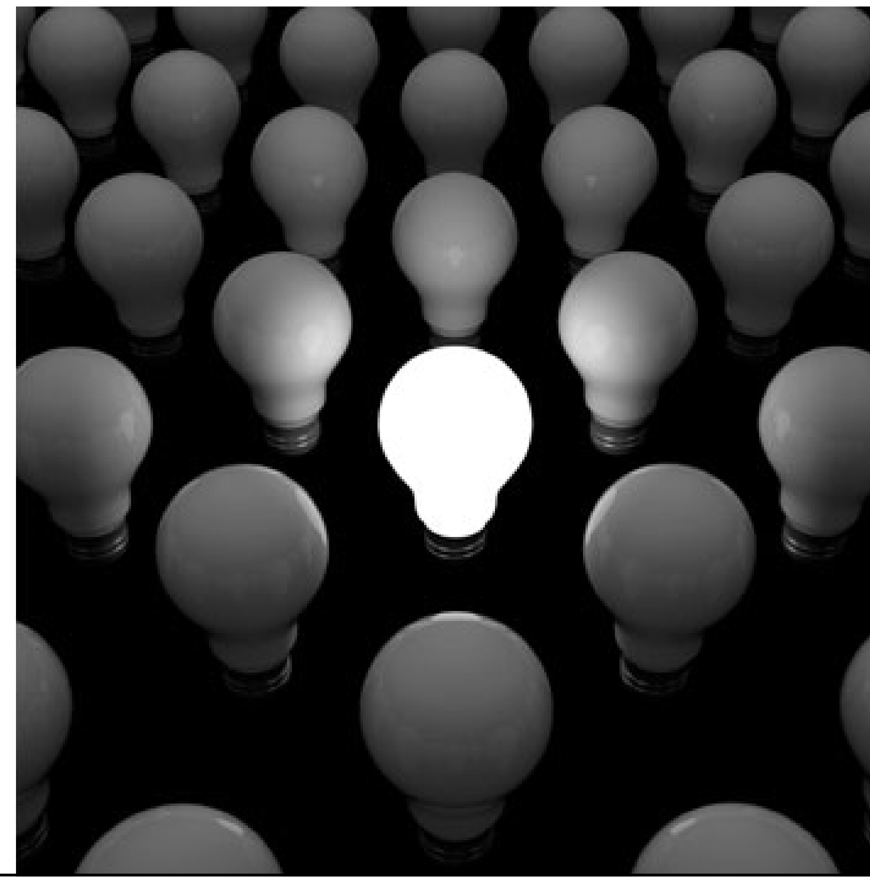


Methylation patterns and their control of gene silencing influence gene expression and cellular function. These variation both environmental and inherited accumulated during the life modify the risk for complex diseases later in life.

Cell Metabolism
Short Article

Acute Exercise Remodels Promoter Methylation in Human Skeletal Muscle

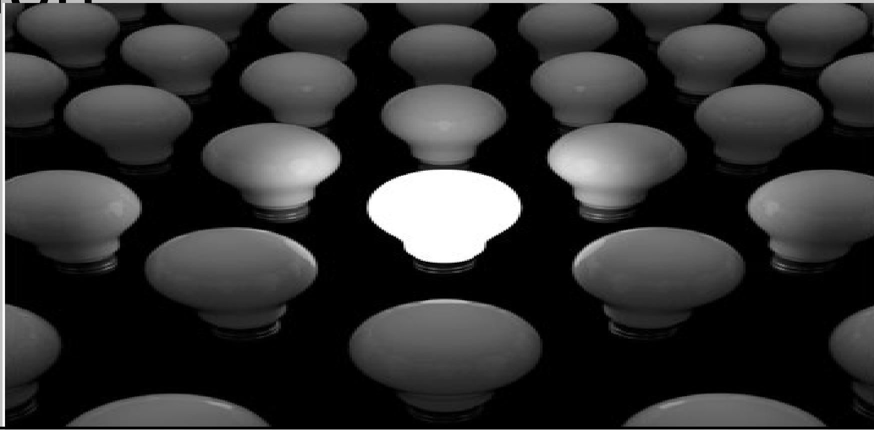
Romain Barrès,[1,4] Jie Yan,[1] Brendan Egan,[1,5] Jonas Thue Treebak,[4] Morten Rasmussen,[4] Tomas Fritz,[3] Kenneth Caidahl,[2] Anna Krook,[1] Donal J. O'Gorman,[5] and Juleen R. Zierath[1,4,*]



- Whole genome methylation was decreased in skeletal muscle biopsies obtained from healthy sedentary men and women after acute exercise.

- Exercise induced a dose-dependent expression of PGC-1a, PDK4, and PPAR-d, together with a marked hypomethylation on each respective promoter.

- Promoter methylation of PGC-1a, PDK4, and PPAR-d was markedly decreased in mouse soleus muscles 45 min after ex vivo contraction.



Sydney Brenner, 1980

- *“Progress in science depends on new techniques, new discoveries, and new ideas, probably in that order”*