Conflict of interests: is a free expertise possible? GMO and NBT as a case study

Yves Bertheau

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GMO and NBT as a case study

Yves Bertheau
Inra – MNHN
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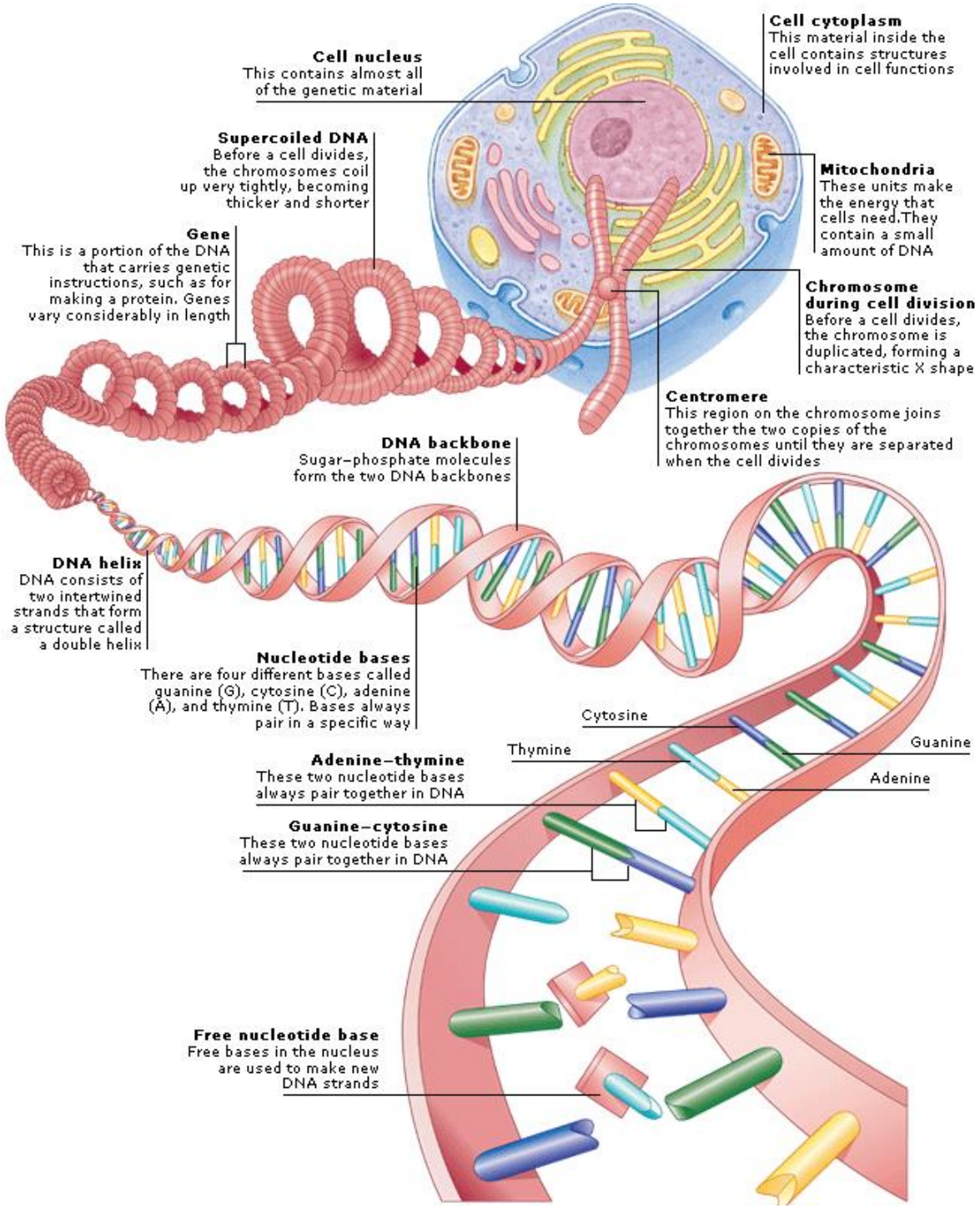
Ethics, Research & Society Workshop
University of Liège

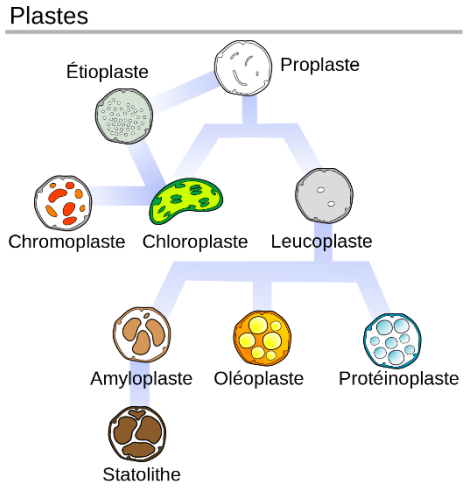
A rapid progression of techniques

- 1970s: appearance of the molecular biology of prokaryotes, with some persistent dogmas over-simplified and overused...
- Horizontal transfers, phylogenetic analyzes, sequencing,
- 1980s: transformation of eukaryotes (Agrobacterium, micro-injection ...)
- 1990s: GMOs, OdMs and meganucleases
- 2000s: Genome editing (ZFN, interfering RNA, OdM) and epigenetics take off ...
- 2010: TALEN, Crispr-endonucleases, epitranscriptomics, the importance of 3D or even 4D organization of nuclei / chromosomes ...

THE LIFE COMPLEXITY

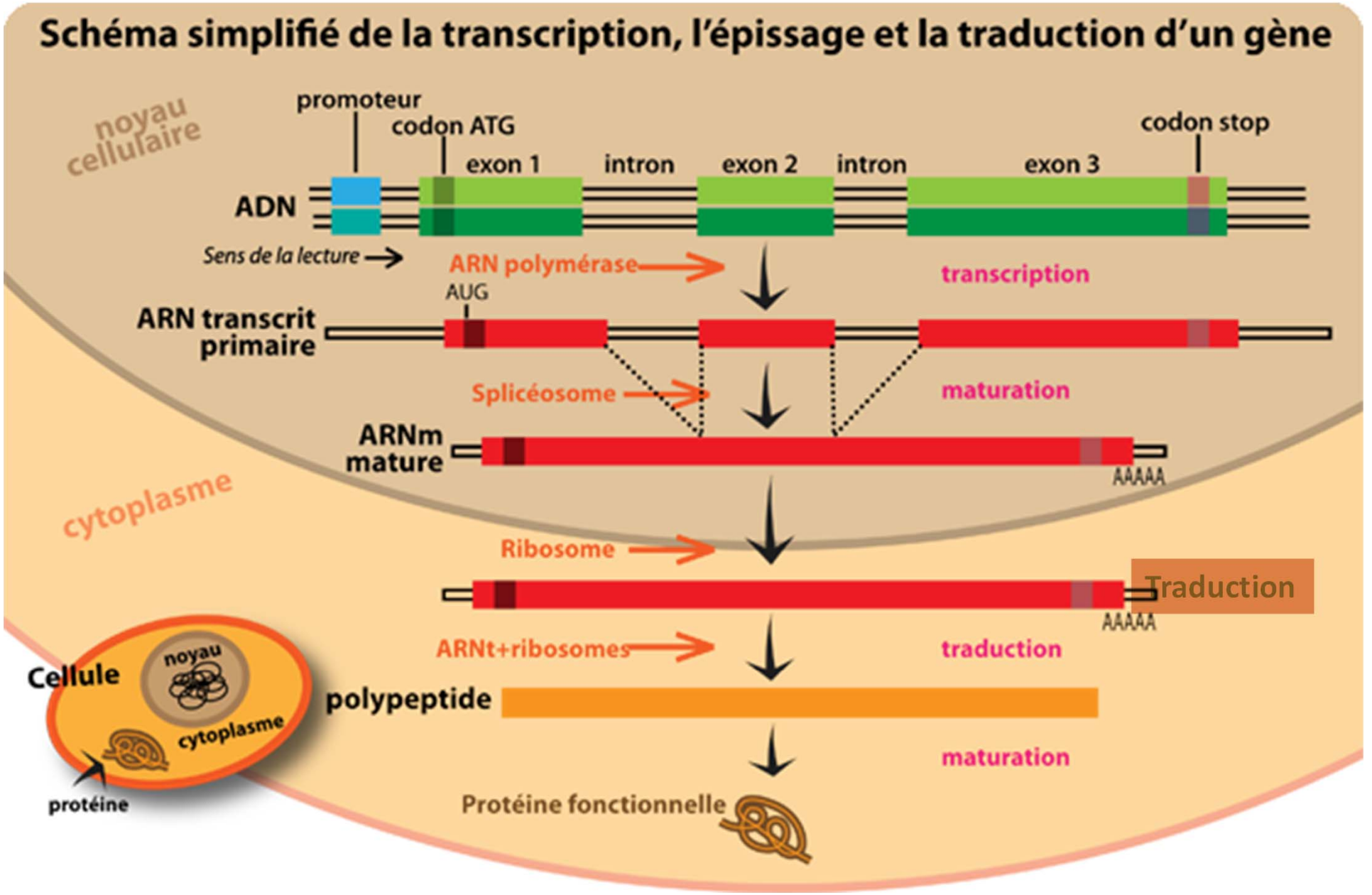


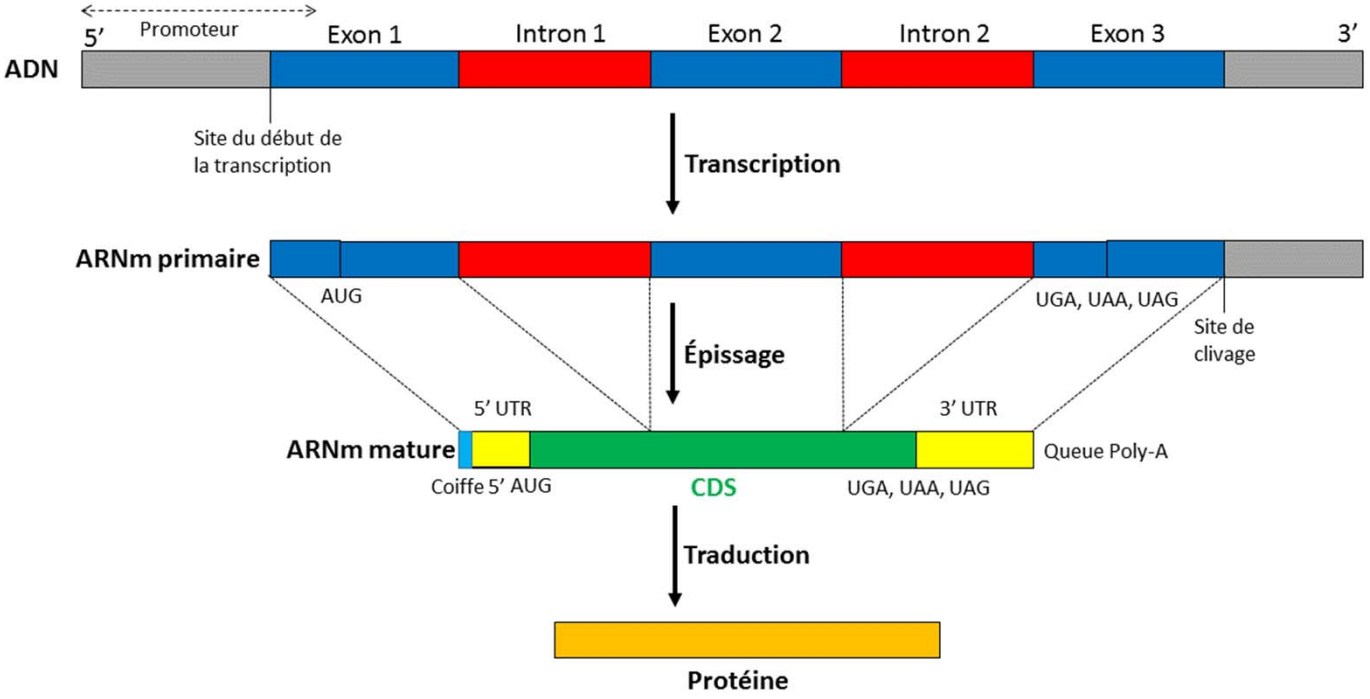
From the nucleotide to the cell then tissues



The majority of considerations will focus on the eukaryotes' nuclei, not on the Organelles' genomes (mitochondria, chloroplasts)

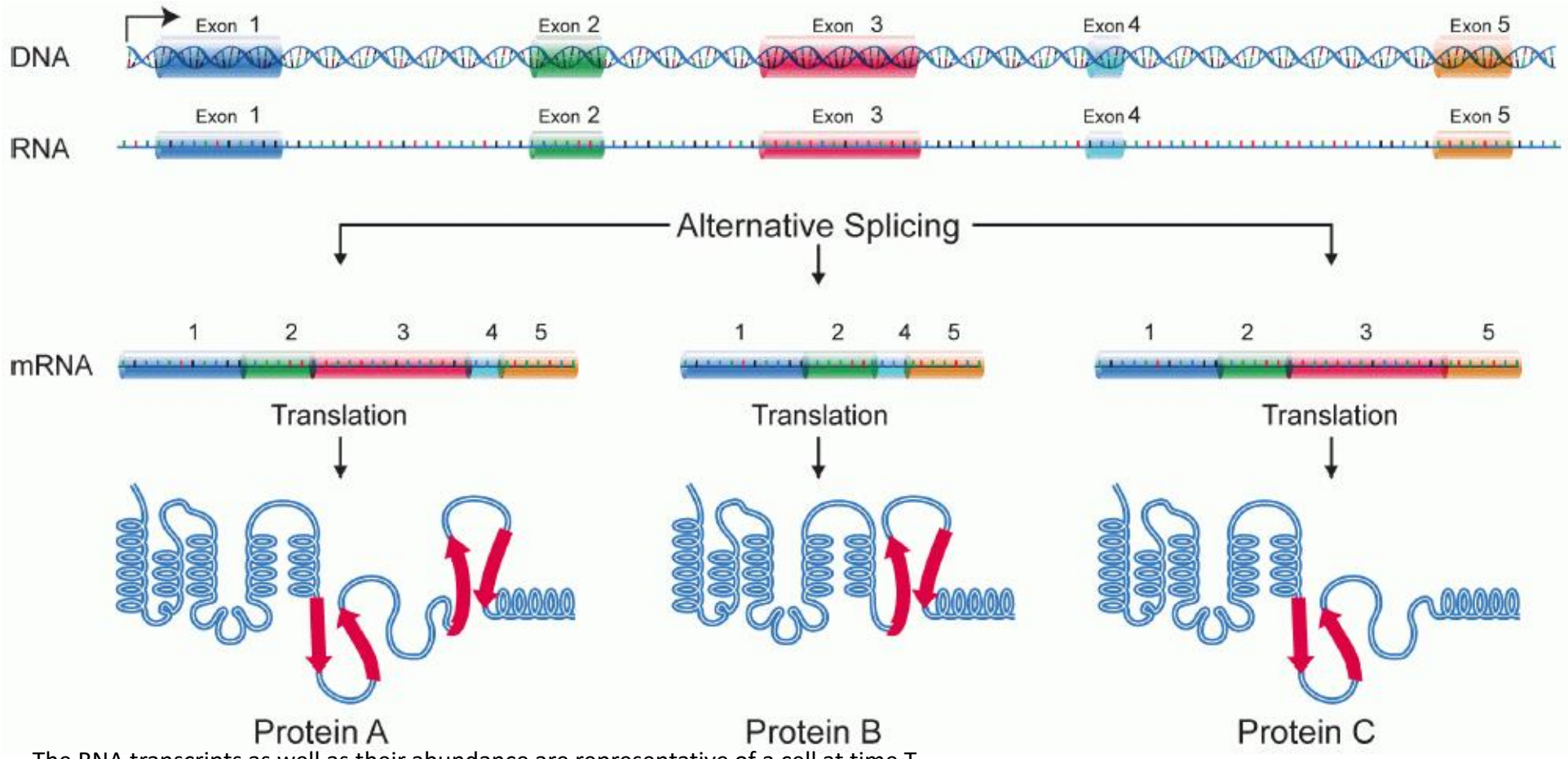
Simplified diagram of a protein's synthesis





Central dogma of genes' splicing in eukaryotes ...

"Central dogma of biology": but in fact ...

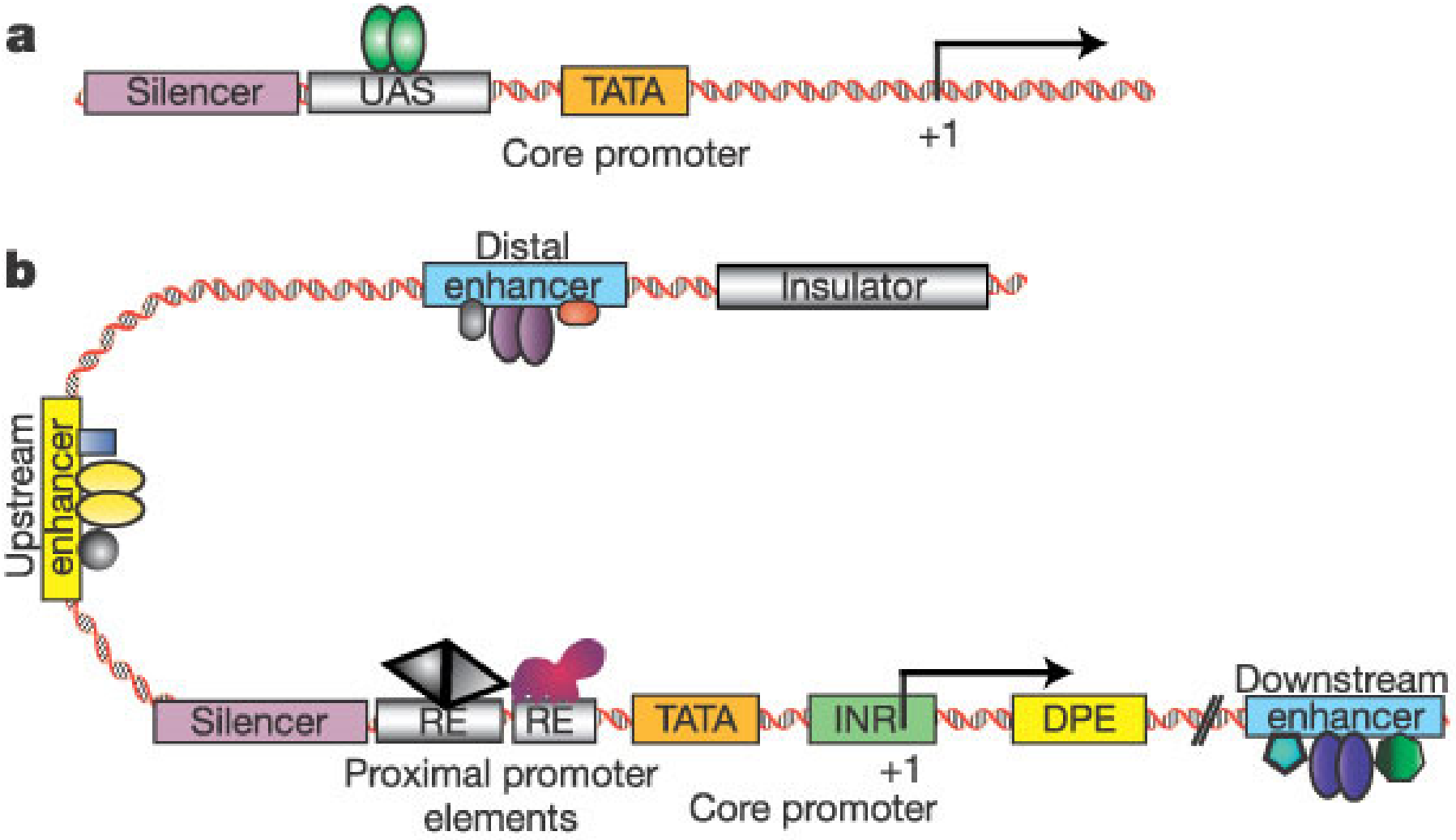


The RNA transcripts as well as their abundance are representative of a cell at time T ...

But in fact :

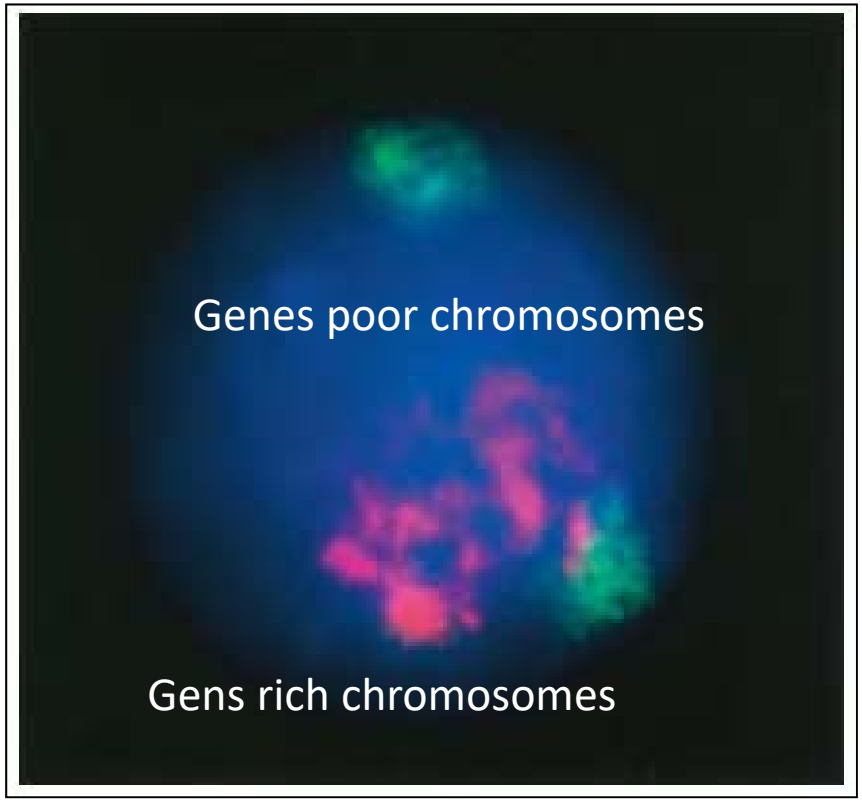
- Alternative splicing produces many isoforms, some of which are pathological (e.g. exon skipping of the Duchenne muscular dystrophy).
- Hence, different abundances for various transcripts, variable according to the tissues
- The transcriptome is a mixture of transcripts of all genes varying according to tissues, age and environmental conditions.
- The human transcriptome consists of tens of thousands of transcripts of 20-25,000 genes (95-98% of non-coding DNA) for more than 100,000 proteins per cell
- Proteins "moonlighting" with a changing function (ex: enzyme) one time then another (ex: structural) ...

Eukaryotic transcriptional unit: the spatio-temporal organization matters



Levine et Tjian Nature, 2003

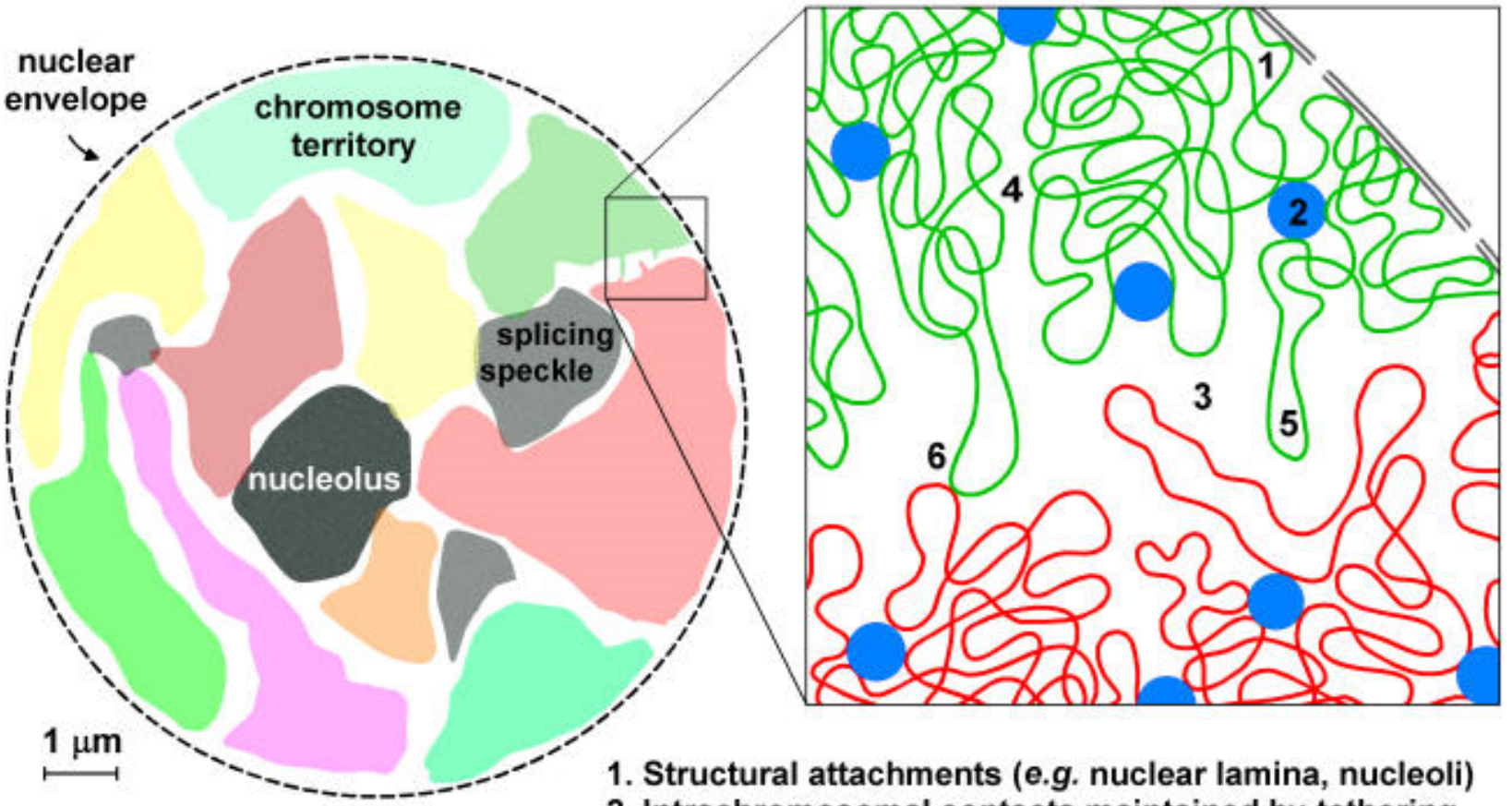
Spatio-temporal organization



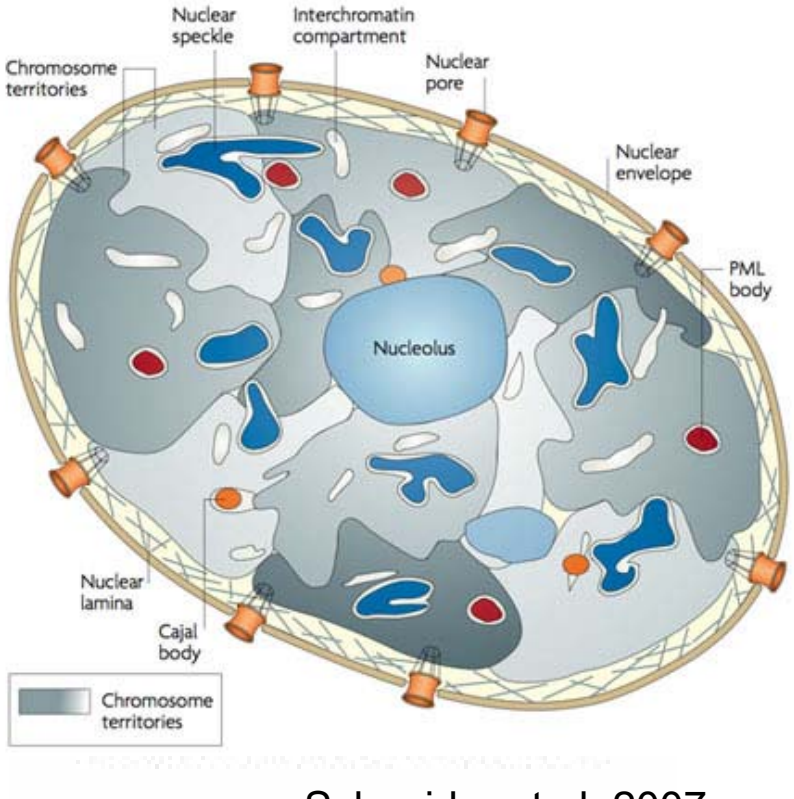
Schneider et al. 2007

Spector et al. 2003

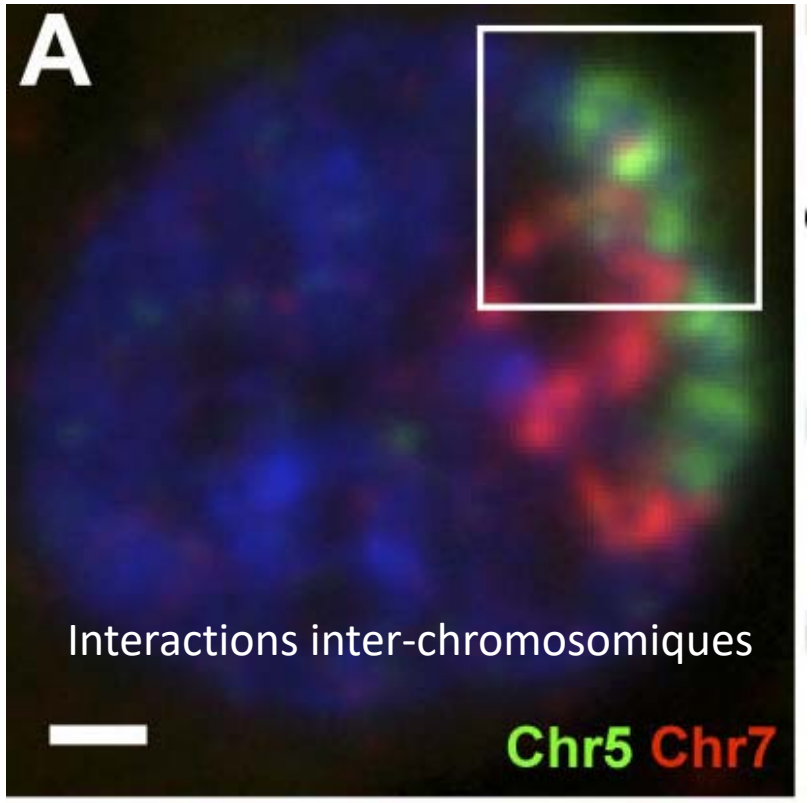
Spatio-temporal organization



Spatio-temporal organization: inter-chromosomal domains (TAD)



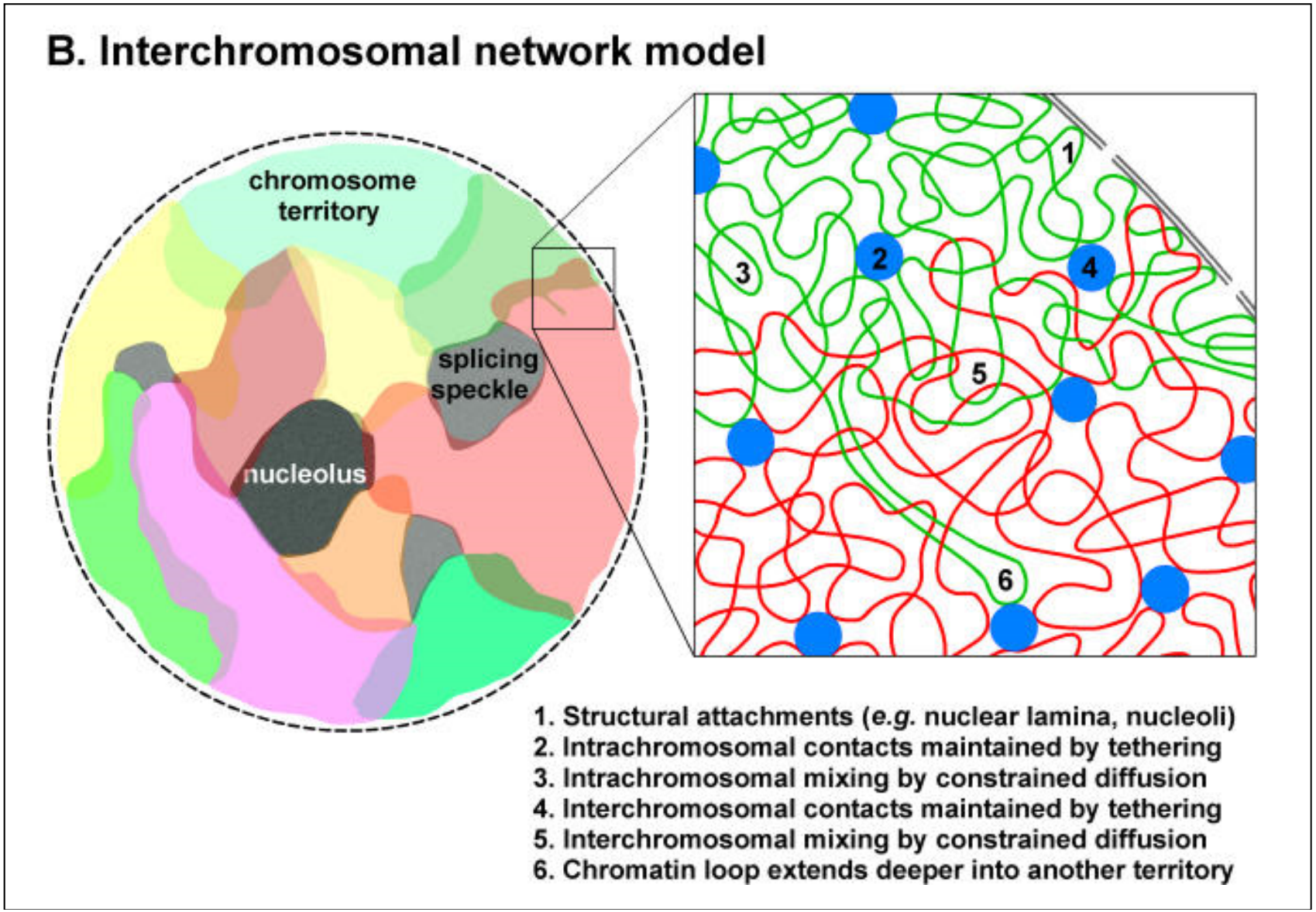
Schneider et al, 2007



Branco et Pombo, 2006

Gene synchronization? Co-regulation?

Spatio-temporal organization: inter-chromosomal domains (TAD)



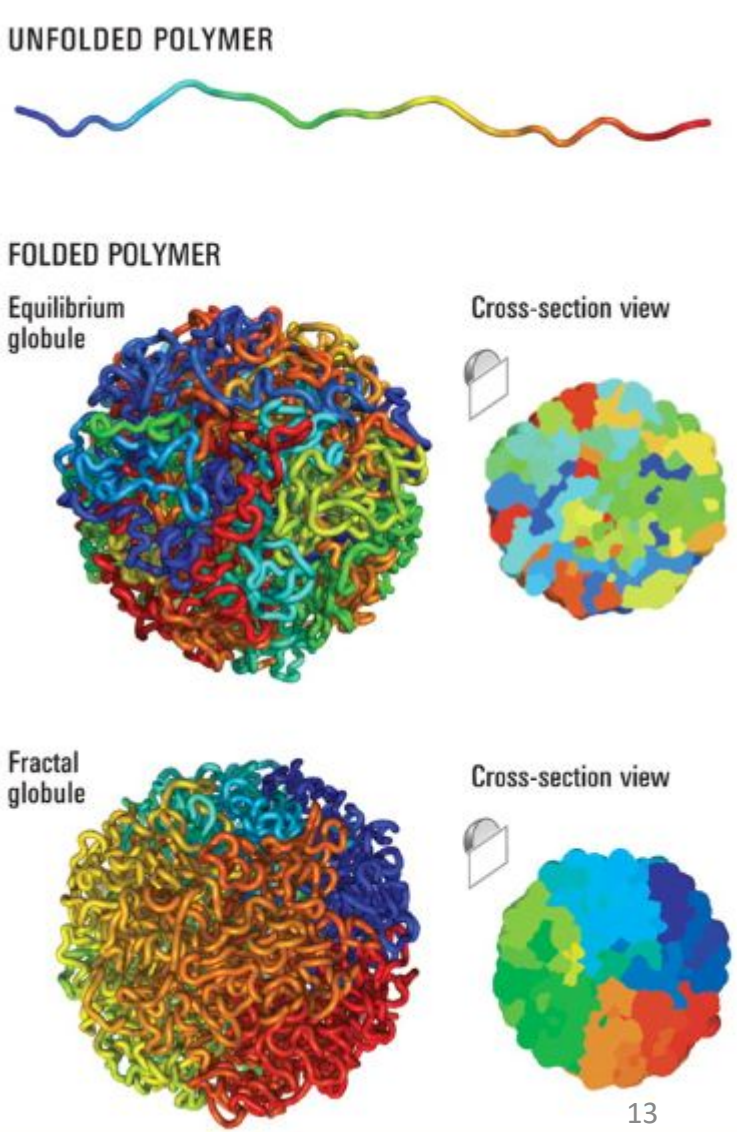
Recall on genomes and epigenomes

- Very large shares of non-coding DNA, ex: man 95-98%, previously thought as "DNA junk" now known as involved in the gene expression regulation
- The "Black matter" (the essential "missing" genes in some organism)
- The poor functional correlation between linear and spatial genomic and epigenomic regions.

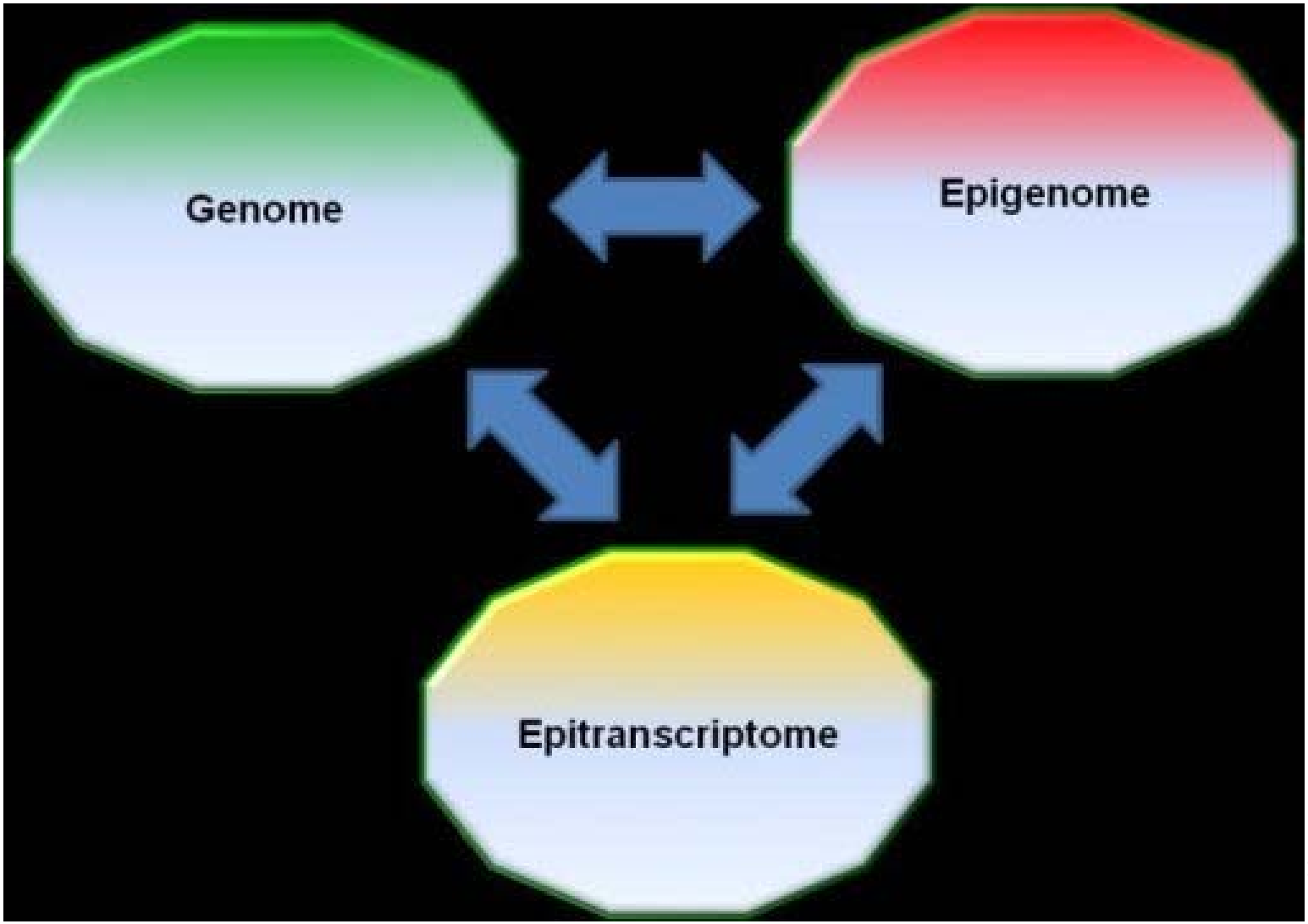
- We are very far from the mechanistic molecular biology of the 70s-80s

- Epigenomes (DNA, Proteins and RNA): we start to know where start (see the conclusions of the EFSA Symposium held in June 2016)

Message to take home: except neutral mutations and those improving the fitness in the current environment, all living organisms do everything to preserve their genomes from mutations (e.g. 'Napoléon's oak')…



Interactions between "domains" still very poorly understood

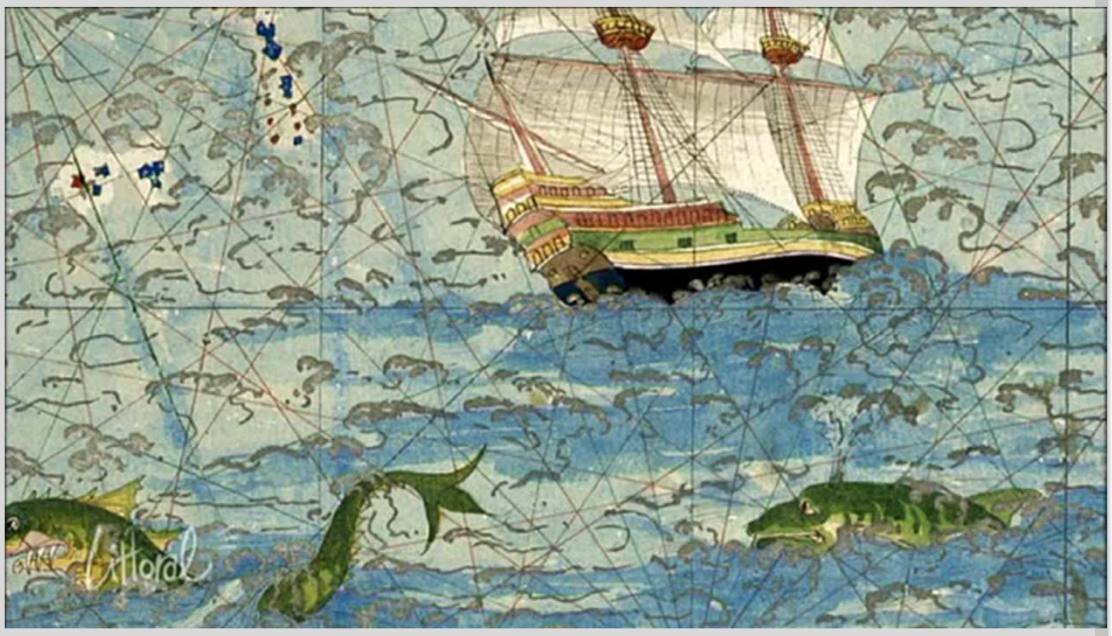


Interactions between "domains" still very poorly known

- DNA and RNA circulate in the blood, placenta and plant vessels: sources of information and expression of genes in distal parts (including graft-scions),
- Nucleic acid exchanges with parasitic plants, sources of adaptation and coevolution,
- Food RNA could regulate the genes of the animal consuming it,
- *C. elegans*: transport of small RNAs inside the cells,
- Epimutations that are transmitted are taken into account in varietal breeding programs, as they may explain heterosis,
- Plant micro-RNAs present in royal jelly induce caste changes in the haemolymph (creation of bee queens),
- SiRNAs of modified plants induce their resistance to insects ...

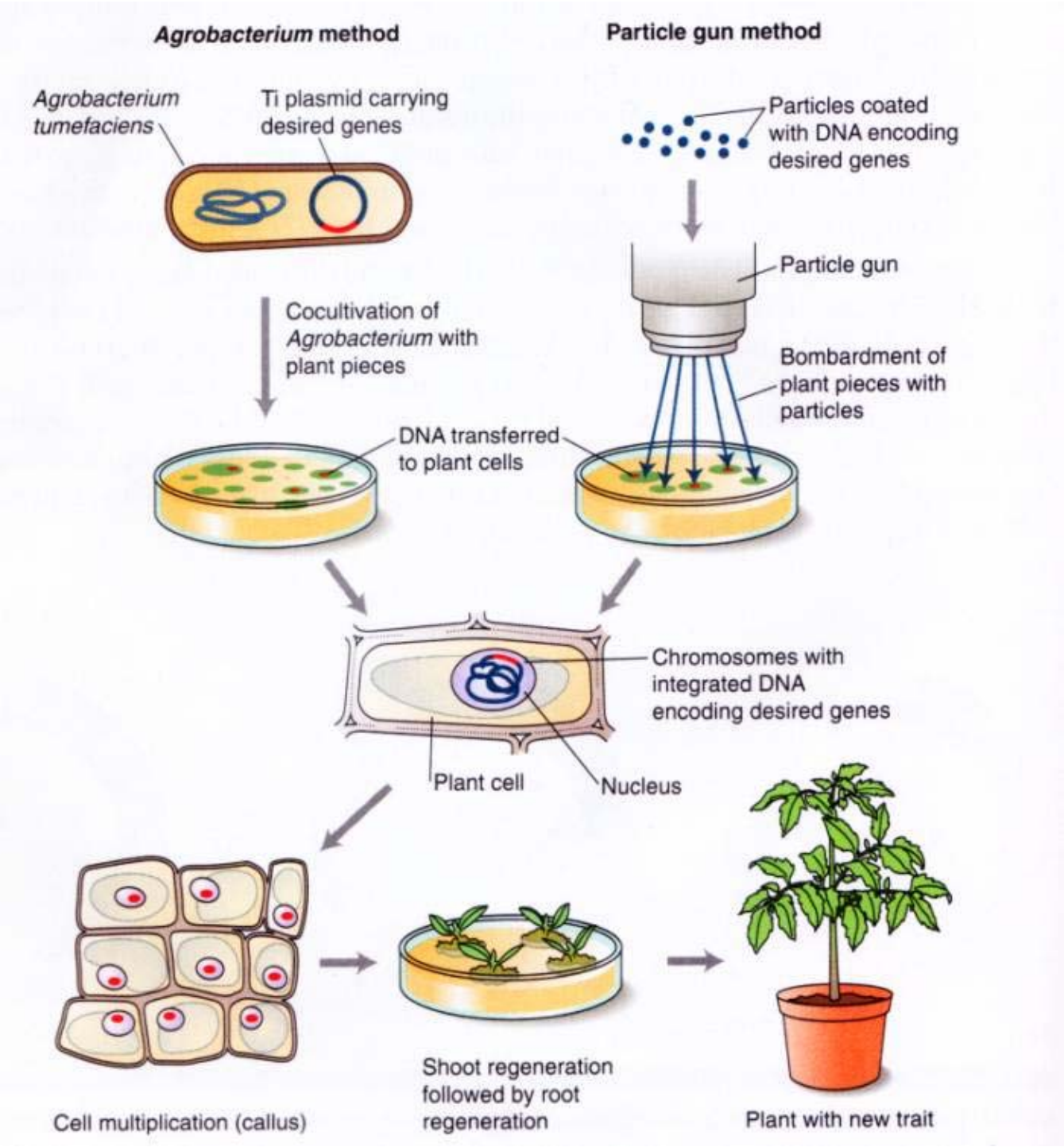
Message to take home: even a single nucleotide change (SNP) may have important effects on either neighboring or distal genetic, epigenetic or epitrancriptomic items, changes which might be visible only in some agro-environmental circumstances…

Conclusion:
Terra incognita



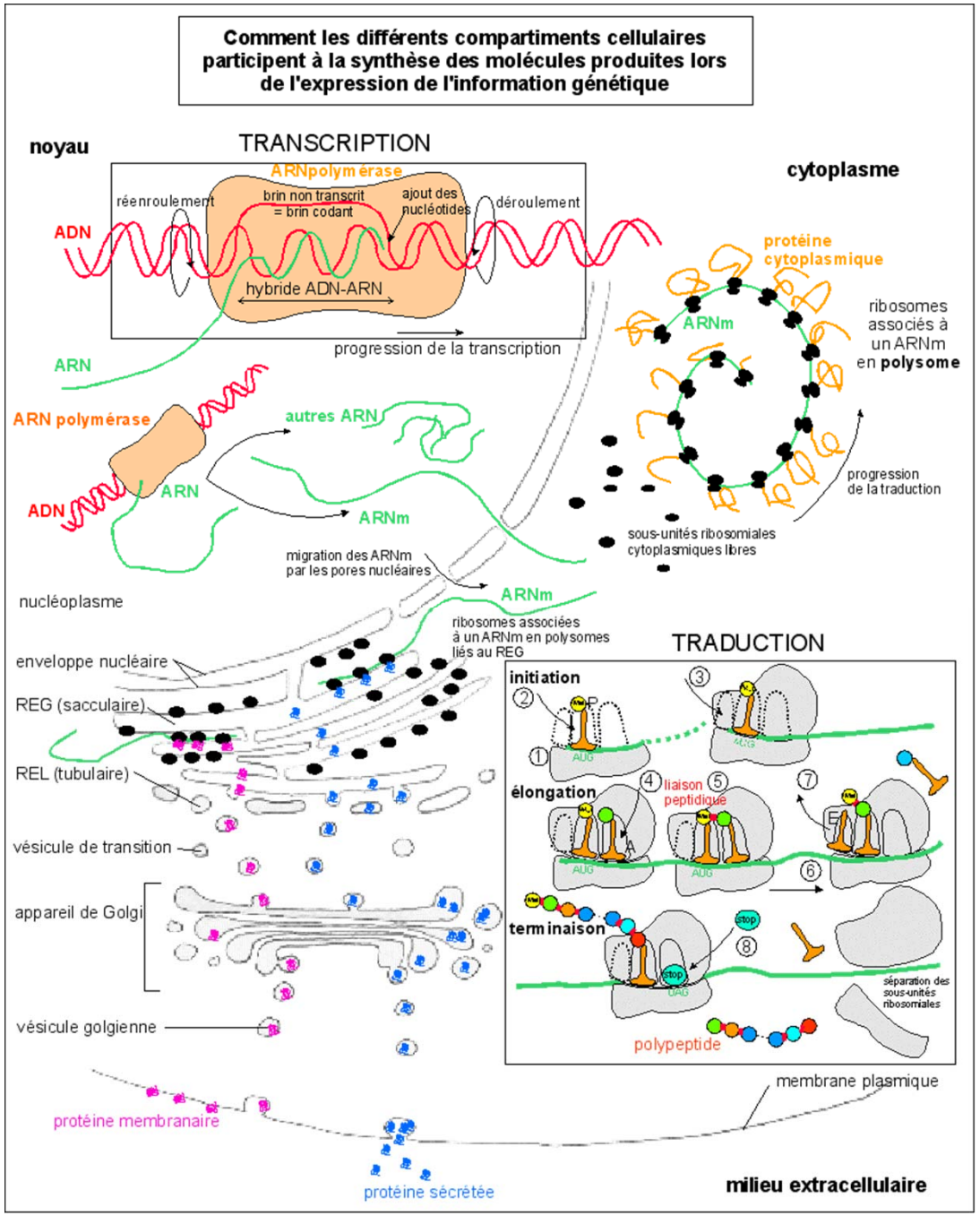
But how do these fabulous changes come about?

RELATED TECHNIQUES

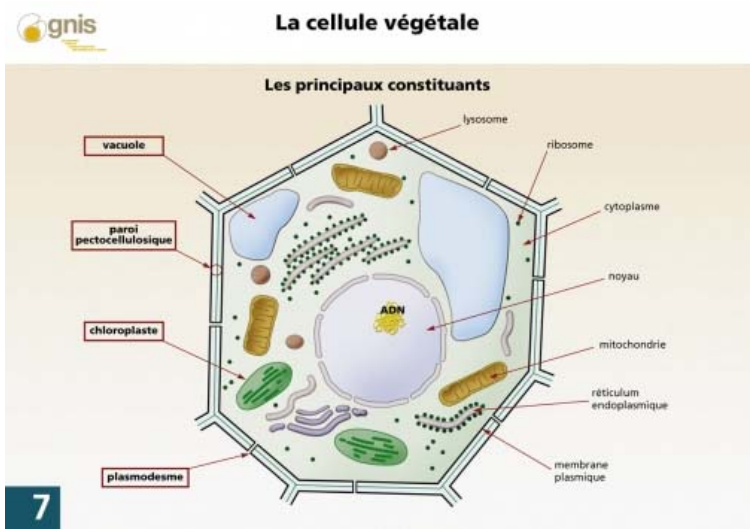


Agrobacterium: phytopathogenic bacteria with many aborted infections as observed in Plants (e.g. sweet potato) ...

A regeneration of plants limited to a few species: little hope to enlarge the circle of elected
given the costs of development, returns on late investments and from the few considerations for publications ...
A specific international meeting held in London (Oct. 2016)



Les différents compartiments cellulaires affectés par les techniques connexes : membranes…



Related techniques

NBTs require the use of "old techniques" used for the transgenesis of already marketed GMOs:

- protoplastisation, vectorization (very large proteins, remains of genome and Agrobacterium plasmid ...), cell cultures, modified cell selection systems, regeneration of non-recalcitrant plants (hence a still limited spectrum of species),
- All stressful techniques inducing mutations and epimutations (up to 35% for cell cultures, *somaclonal mutants*):
  - poorly identifiable (reliable software and reference genomes missing) because often point or indelible mutations, especially in repeated or non-coding regions, problems of translocations and inversions ...
  - Difficult to eliminate (backcrossing by insufficient firms, co-segregations according to characteristics, regions with non-Mendelian inheritance…) leaving millions of pb not "cleared" and poorly controllable (see the software and reference genomes issues)

International meeting held in London in October 2016: laboratories are desperately looking for good, well-trained chefs and regret the lack of schools to train future "chefs" ...

NBT TECHNIQUES

WITH ONLY 2 CASE STUDIES

Initial European work

- Zinc finger nuclease technology (ZFN1-ZFN3) + TALEN+ meganucleases (then Crispr-endonuclease added)
- Oligonucleotide directed mutagenesis (ODM)
- Cisgenesis/ Intragenesis vs. Transgenesis
- RNA-dependent DNA Methylation (RdDM),
- Negative segregants
- Grafting (GM rootstock / non-GM scion)
- Agro-infiltration (Agro-infiltration "*sensu stricto*", Agro-infection, Floral-dip i.e. plant transformation)
- Reverse breeding
- Synthetic biology (later on abandoned as being taken on board by CBD protocol despite SB remains still undefined)

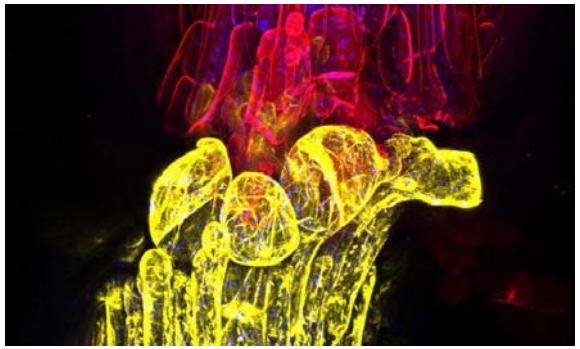
A 2007-2012 Euopean working group
Report not publicly available
But largely commented for its "positive" aspects (ex: JKI)

Grafts: interactions between scions and rootstocks

- pathogens, proteins (eg Cry1Ac), DNA, RNA, hormones... circulate,
- Expression regulation (silencing ...), protein synthesis in scions due to rootstock,
- Genomes communicate with each other with epialleles
  http://phys.org/news/2016-01-grafted-genomes.html#jCp

Products of the non-GM scion (e.g. fruit) cannot be considered as not influenced by the GM rootstock

Credit: Charles Melnyk/University of Cambridge



Sam van Aken, Syracuse University, New York.

Crispr-Cas9 et al…

CRISPR

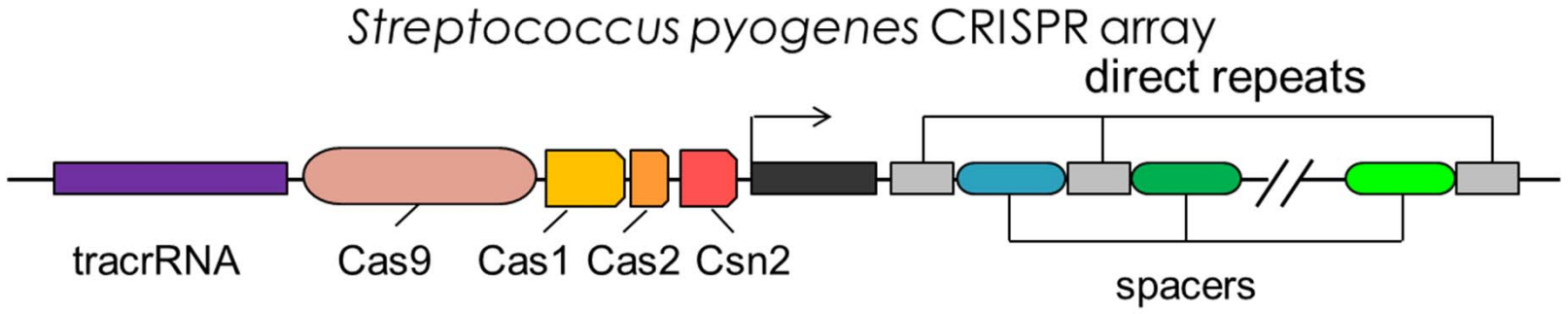
(6 classes, 19 subclasses with little or fully unknown functions)

Clustered Regularly Interspaced Short Palindromic Repeats

"Adaptive acquired immunity" of bacteria against phages
described since 1987, adapted for genome editing in 2012, and eukaryotes
in 2013,

Evolutionary convergences in animals and plants with genome stabilizing systems
ex: mRNAs and transposable elements, small RNAs and DICER ...

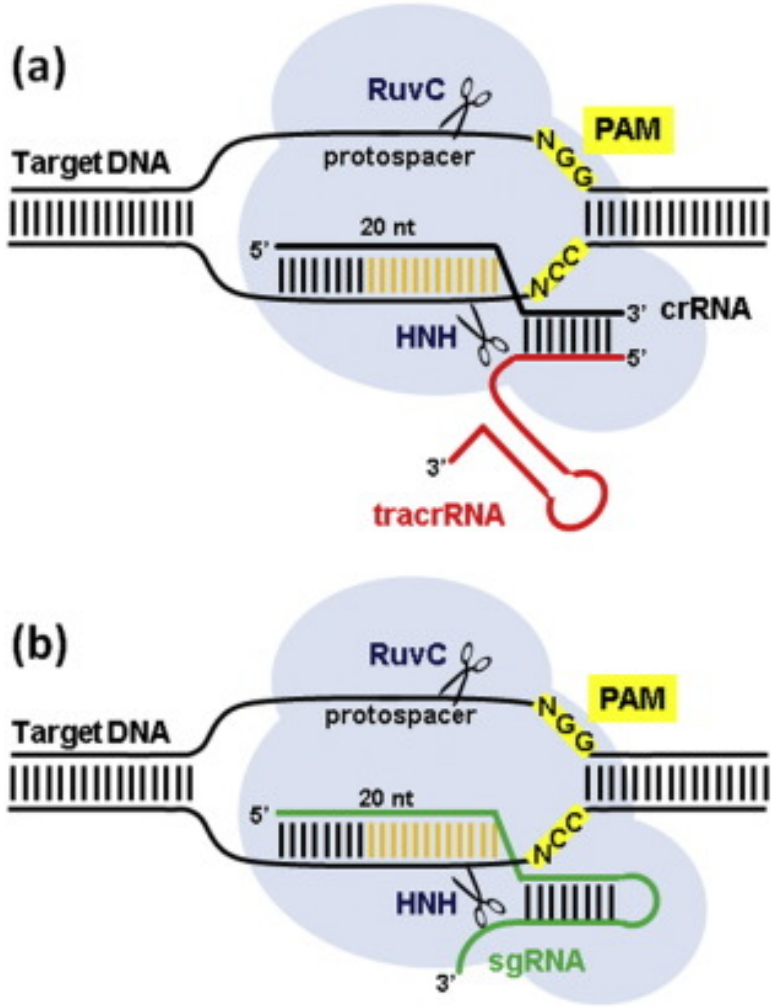
Fundamental objective: to fight invasive DNA



Crispr-Cas9/endonucleases: genomes and épigenomes modifications

natural

artificial



RNA-guided DNA cleavage by Cas9. (a) In the native system, the Cas9 protein (light blue) is guided by a structure formed by a CRISPR RNA (crRNA, in black), which contains a 20-nt segment determining target specificity, and a trans-activating CRISPR RNA... Luisa Bortesi, Rainer Fischer. Biotechnology Advances, Vol. 33, Issue 1, 2015, 41–52

Limitations of action in genomes by PAM sequences inducing a search for other nucleases with other PAM (AT / GC rich) ex: Cas9, C2c1, Cpf1 (1 single RNA); bringing his own PAM...

Limitations of insertion size from where problems for e.g. human modifications,

Unintended effects:

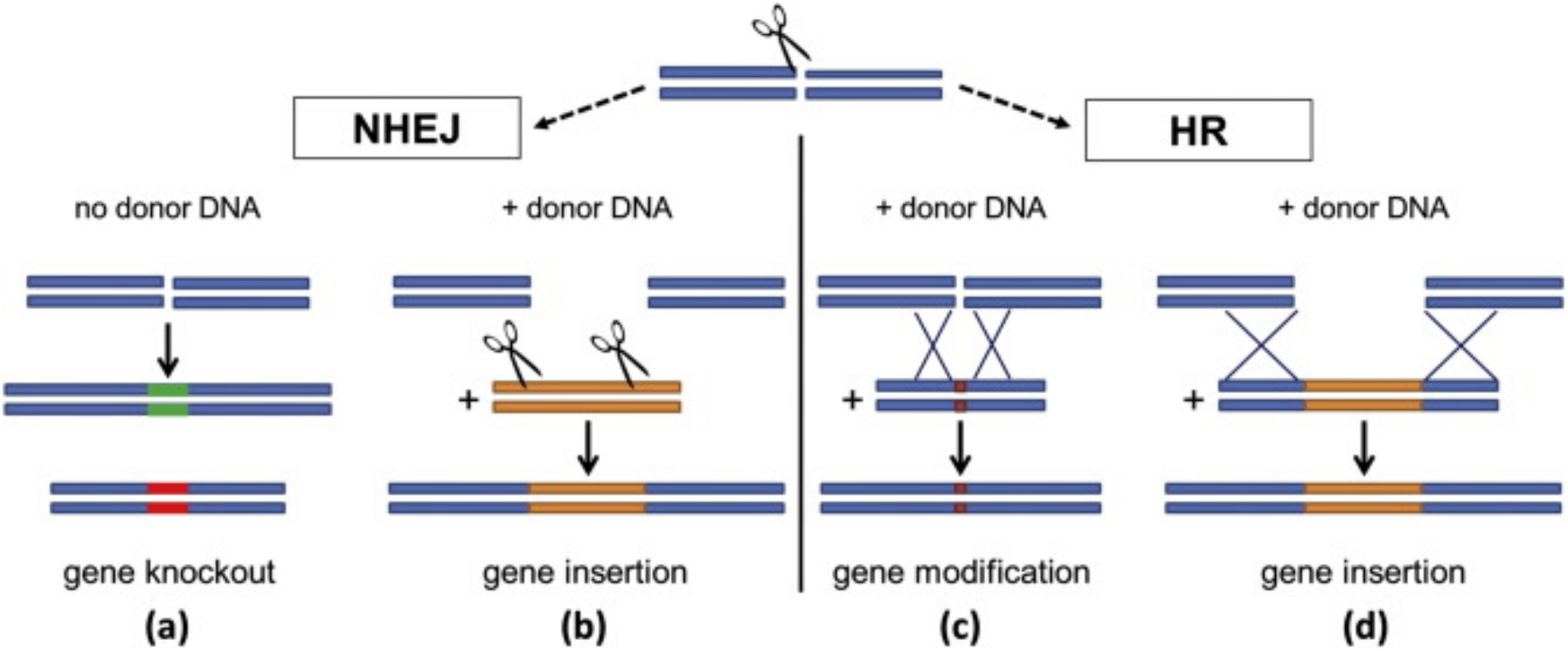
- Off targets because SDN = sequence directed mutagenesis (homologies of sequences) and several thermodynamic considerations...
- Exon skipping (generally not screened)
- Chromosomes' translocations (undetectable by 'low cost' off-targets sequencing)…

Attempts to reduce off-targets by changes -> nickase activity, reduction of reagent quantities, activity duration, RNP (but with contaminating DNA),

C2C2 (Cas13a) and RCas9 for RNA modifications…

Numerous cooking recipes of different labs applied without any quality assurance frame…

NBT such as Crispr-endonuclease are using double strand DNA reparation system



High frequency
Canonical and alternatives (MMEJ)

Low frequency

The challenge: favoring the less efficient HR DNA reparation system

Message to take home: very rapid and numerous dsDNA breaks followed by very rapid fragments sticking without any control of the former vicinity of the sticked fragments nor of the absence of changes at the cutting point…

IDENTIFICATION OF THE INITIALLY USED NBT TECHNIQUES AND DERIVED PRODUCTS

PRINCIPLE OF THE MATRIX APPROCHE

(GATHERING A CONVERGING PROOF NETWORK)

HTTP://WWW.INRA.FR/ENTREPRISES-MONDE-AGRICOLE/RESULTATS-INNOVATION-TRANSFERT/TOUTES-LES-ACTUALITES/DETECTER-LES-OGM-INCONNUS

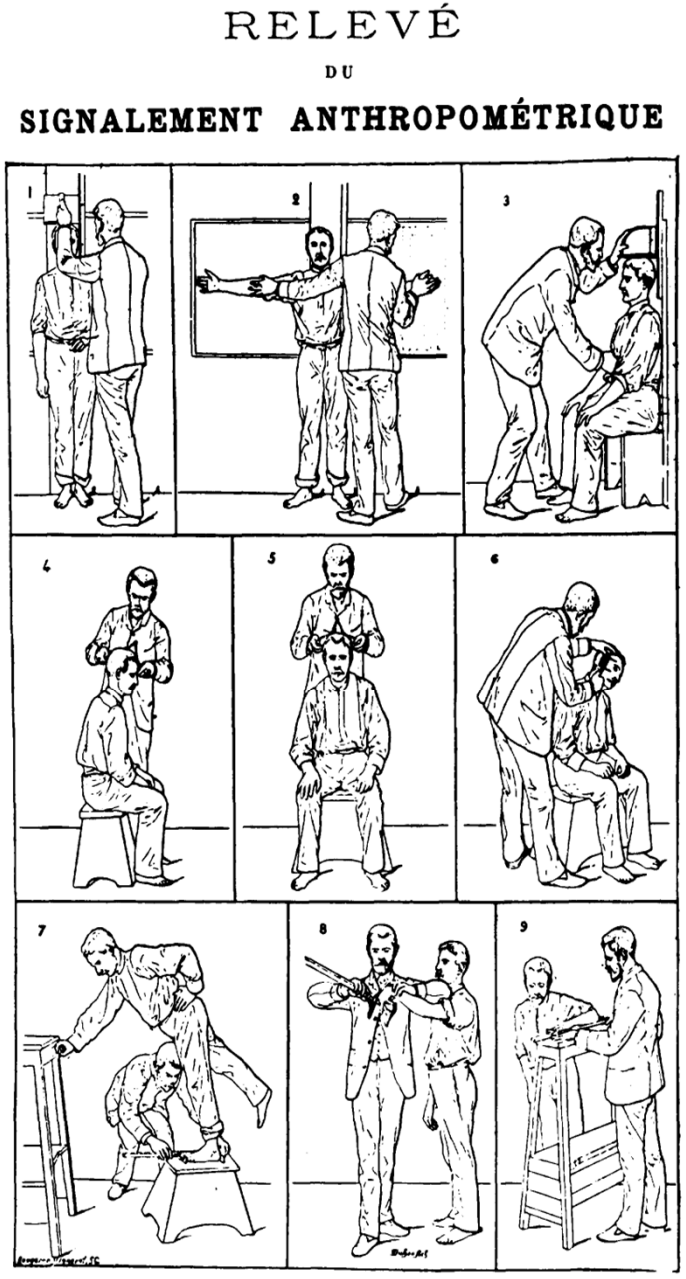
Classic premises of identification

- Principle:
  - Observe / distinguish characters, traits ... eg shape of flowers, animals, hair or feathers ...
  - Inventory the elements: locomotion devices, bone, disposition, ontogenesis ...
  - Classify the elements: phenotypic, genotypic, epigenotypic, epitranscriptomic ...
  - Analyze nucleic acid sequences; proteins, compare DNA / proteins (exon jumps, alternative splicing) ...
  - Combine the elements if necessary according to the desired degree of precision,
  - Correlate, for example in trees (evolutionary tree ...)
- Users: Aristotle, Linnaeus, Jussieu, Darwin ... breeders of seed companies ...

- The identification of NBT techniques and products is only an application of the methods and targets used in taxonomy, phylogeny / cladistics / phenetics / statistics, varietal identification, marker-assisted selection, detection of GMOs ... assisted or not by various statistical tools, databases, decision support systems (DSS) ...

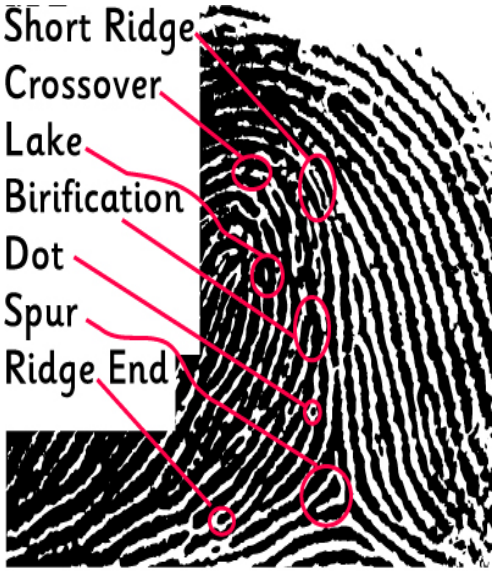
Other examples
Of the matrix approach

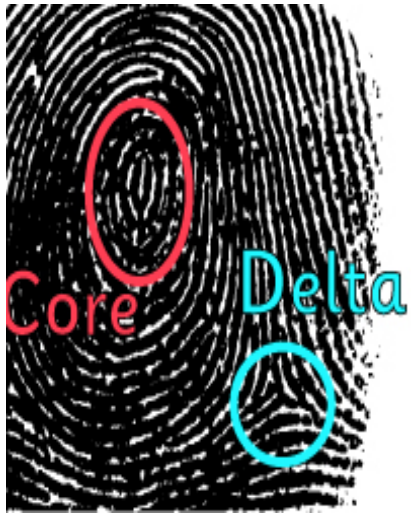
Basic principles of scientific identification as synthesized by Alphonse Bertillon in judicial anthropometry



Another example of the matrix approach

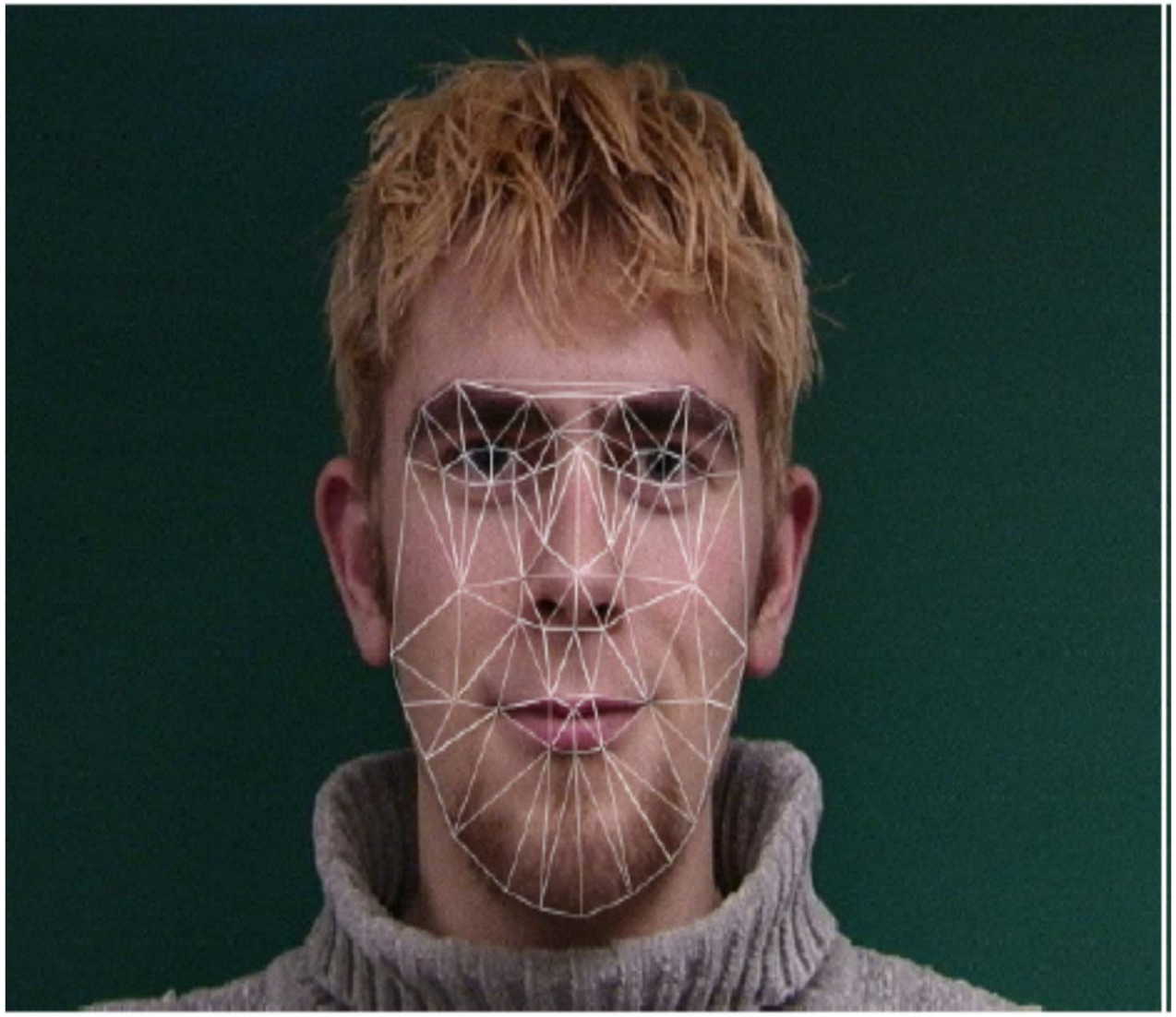
Some characters / traits of differentiation in dactyloscopy





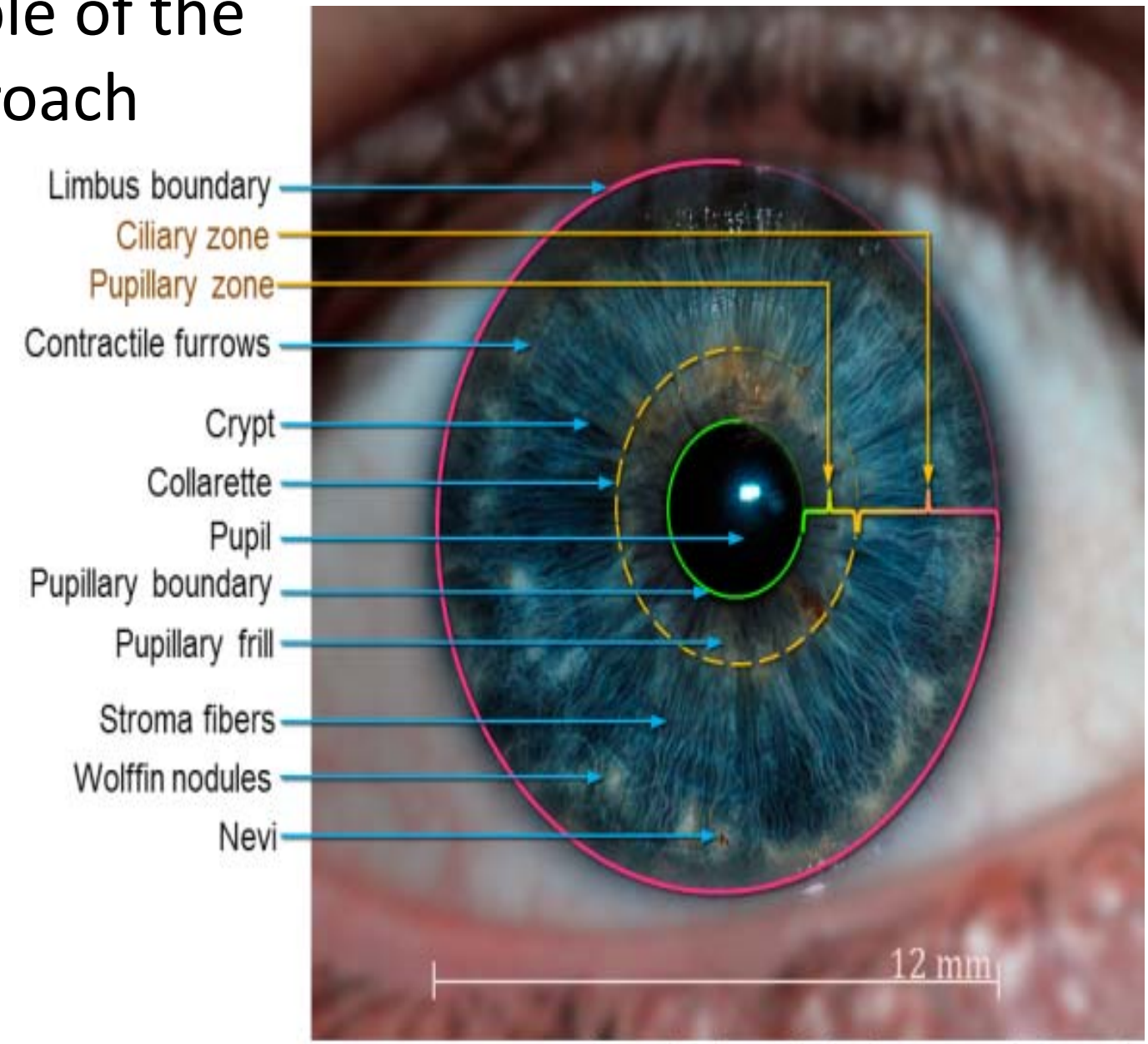
Another example of the matrix approach

Multipoints
facial recognition

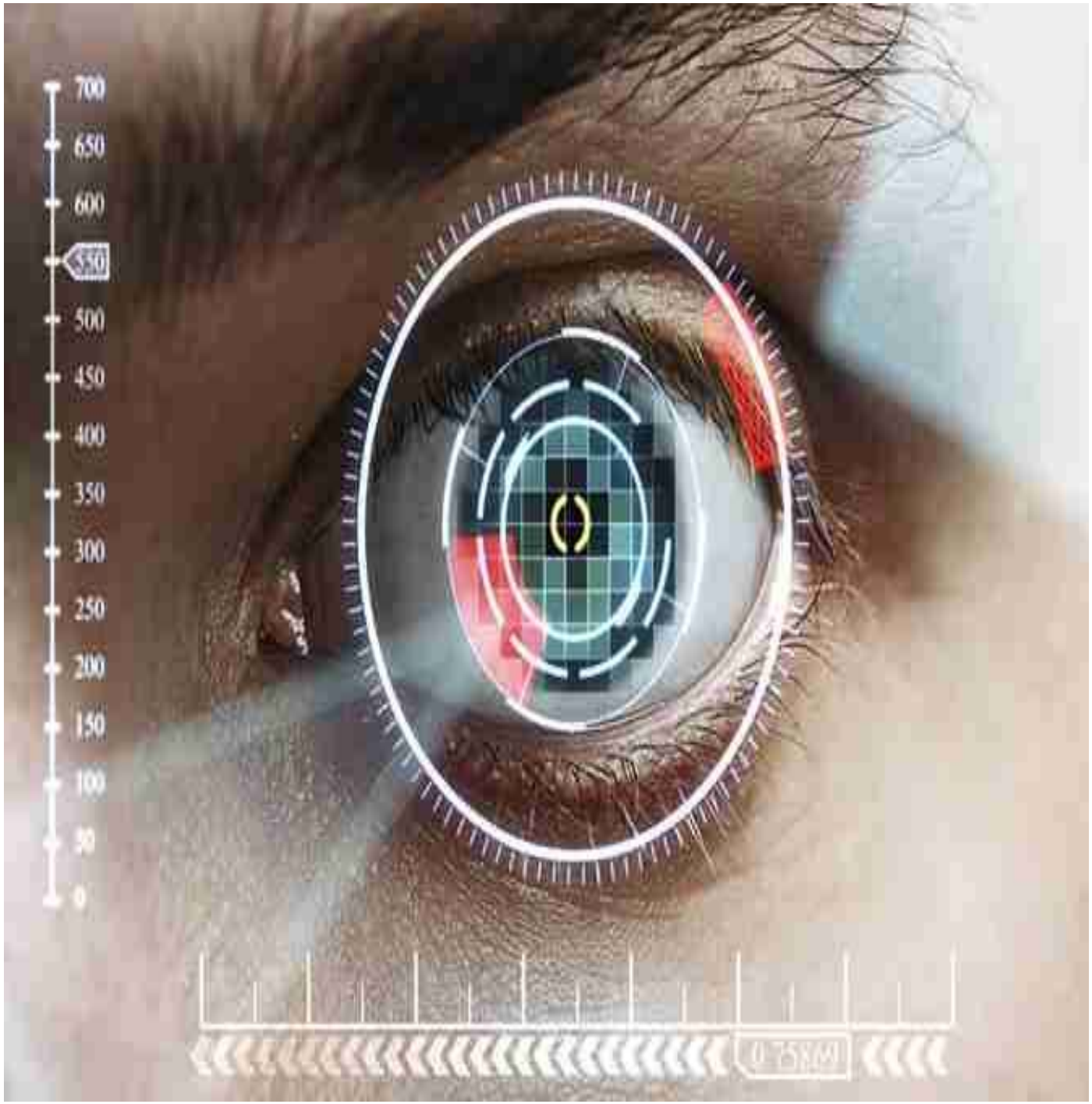


Another example of the matrix approach

Iris recognition



Source (eye image): Dr. Jan Drewes. www.jandrewes.de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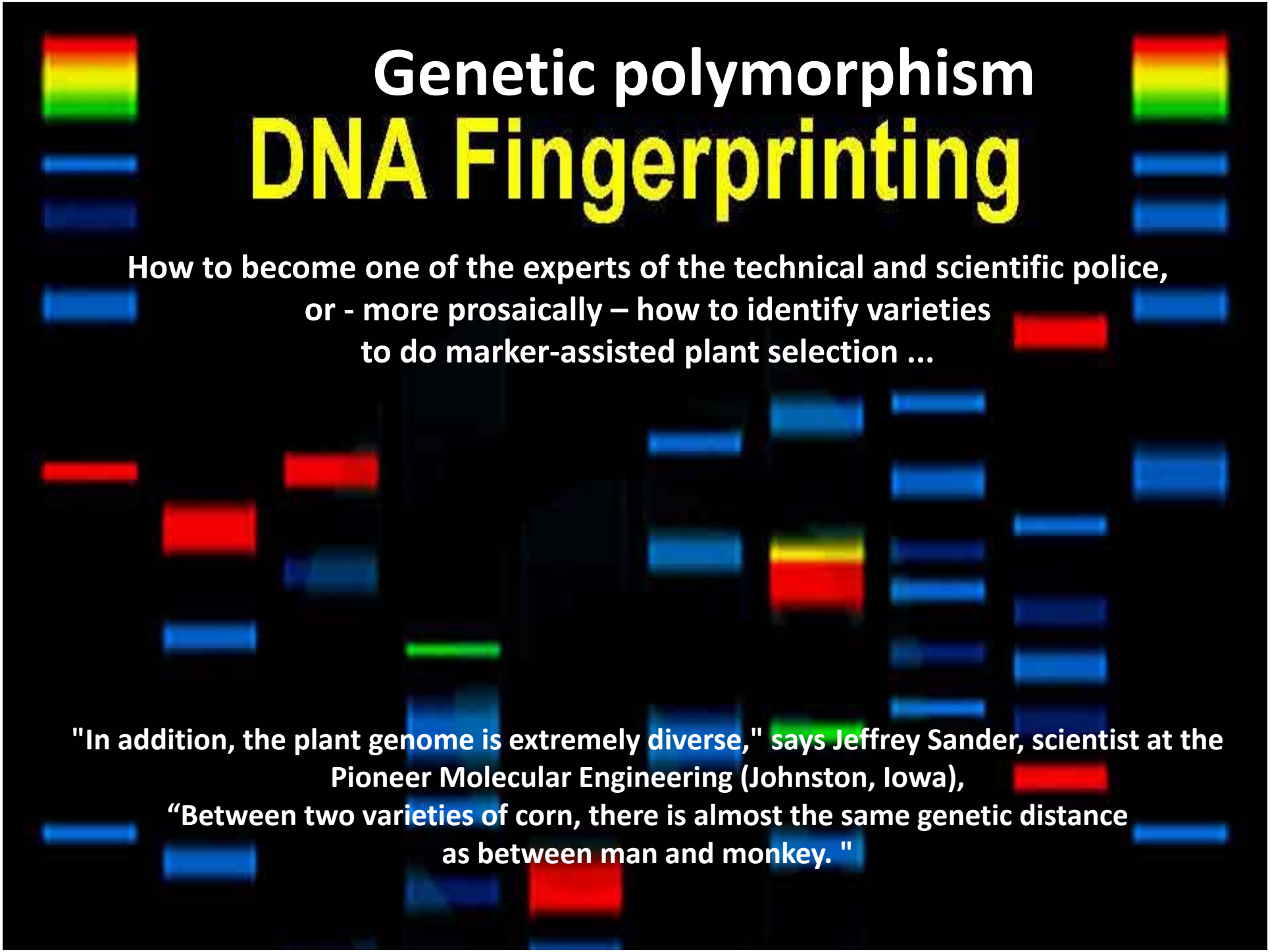
Genetic polymorphism

DNA Fingerprinting

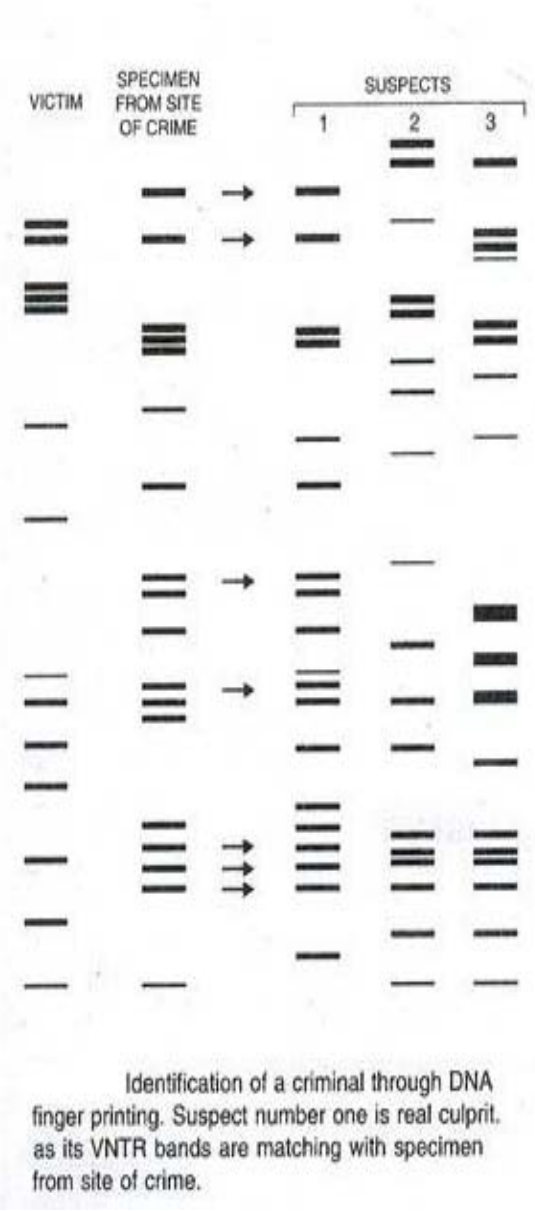
How to become one of the experts of the technical and scientific police,
or - more prosaically – how to identify varieties
to do marker-assisted plant selection ...



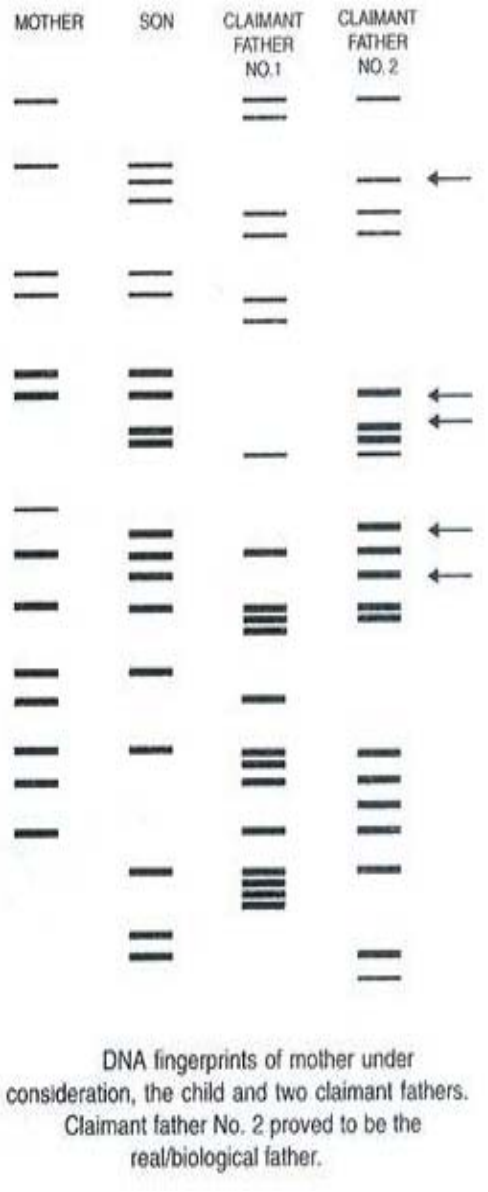
"In addition, the plant genome is extremely diverse," says Jeffrey Sander, scientist at the Pioneer Molecular Engineering (Johnston, Iowa),
“Between two varieties of corn, there is almost the same genetic distance as between man and monkey. "

Another example of the matrix approach

Depending on the context, the "weight of evidence" (here the number of identification bands differentiating individuals in these forensic studies) may differ ...



Identification of a criminal through DNA finger printing. Suspect number one is real culprit. as its VNTR bands are matching with specimen from site of crime.

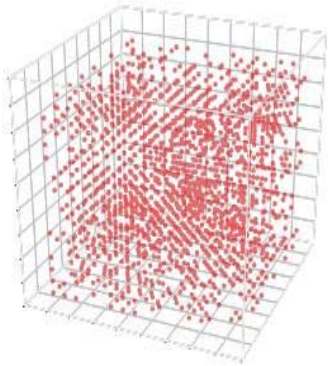


DNA fingerprints of mother under consideration, the child and two claimant fathers. Claimant father No. 2 proved to be the real/biological father.

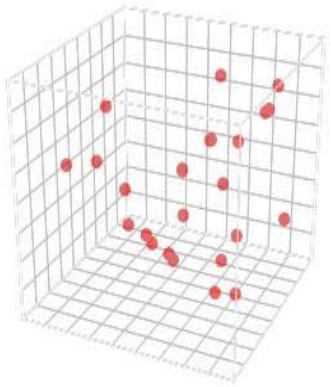
Another example of the matrix approach

Another approach to facial recognition in noisy contexts: similarities of approach between sequencing of genomes and / or epigenomes and scanning recognition methods (eg Viola-Jones method)

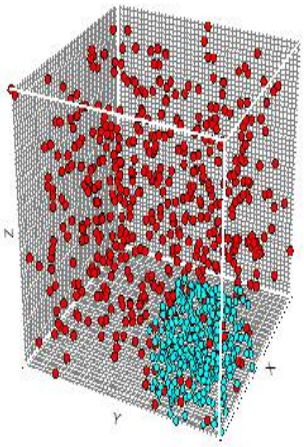
The matrix approach to identify the NBT techniques initially used and the derived products is based on the assemblies of markers of different types, for example in genomes and epigenomes



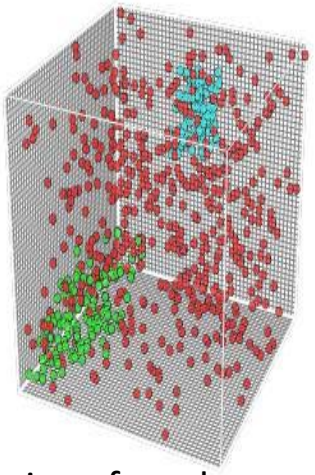
Markers in all genome and epigenomes (ex: used for a MAS, PAM, off-targets, translocation, transversion ... frequency, cartography ...)



Choice of identification markers of the species



Choice of markers differentiating products from *in vitro* techniques



Choice of markers differentiating one (of) technique(s), ex. Crispr-endonuclease (s) natural mutation (s)

- Choice of a combination for unambiguous legal identifications
- Choice of one (or some) relevant marker (eg PCR on targeted mutation-PAM) for routine detections (aspects of cost, speed ...)

Conclusion

- Do not be fooled by the tree that hides the forest:
- An element, alone, isolated, might not be an unambiguous signature (but a border fragment or a rearrangement internal to an insert is sufficient for GMOs of transgenesis ...), this is what you are generally asked to consider for NBT (to make you admit that the modification is natural), not all...
- Various elements collected (see above for NBT and mutagenesis *in vitro* vs. *in vivo*) make it possible to determine the technique employed and then to trace the product by using only a part of the elements (cost aspect, speed... according to the needs of the analysts)
- These matrix approach practices are already in use (detection of known and unknown GMOs, to reduce costs, simplify complex samples detection ...) and made more user-friendly using the labs' databases and DSS ...

DETECTION METHODS

Available methods

- Phenotypic (ex: tolerance to a herbicide, immunology ...)
- Omics (metabolomic modification, proteomics ...)
- Molecular: genomes and epigenomes / epitranscriptomes (DNA, proteins, RNA):
  - DNA, RNA and modified or unmodified proteins
  - Simplex (PCR, LCR, OLA ...) multiplex (SNPLex, DNA chips ...),
  - From the nucleotide (LCR, OLA ...) to the large chromosomal rearrangement (border fragment ...),
  - Isothermal or not (LAMP, NASBA ...)
  - Combined or not (eg SNPLex = LCR + PCR + DNA chip)
  - Sequencing (Sanger, NGS, ChiSeq, RNASeq ...) with or without reference genome,
  - On isolated tissues or cells, nucleus or organelles,
  - In the laboratory or in the field (PCR, LAMP, sequencing ...)
- Using scars and signatures
  - Univocal (s) or multiple (databases and DSS, see ENGL network works and FP6 Co-Extra program)
  - Analyzes with various software (assemblies, comparisons, phylogeny, statisitics, cartographies)
  - Combinable and modular according to the needs: legal identification vs. routine detection

IDENTIFICATION / DETECTION TARGETS

In vitro versus *in vivo*

- It is not forbidden to consider in the modifications of the genomes of the organelles (mitochondria ...) in addition to the nucleus (cf post-traumatic stress war of the Golf ...)
- A fundamental:
  - genomes and epigenomes are stable (in equilibrium as a result of evolution), cf. work on the stability of animal and plant genomes such as "Napoleon Oak", tomato ...
  - only the neutral or pressurized modifications that are transmittable remain.
- Random mutagenesis methods: chemical, physical
  - Types of induced modifications, eg transversion, micro-deletions according to mutagen (EMS, ENU, neutron flux, γ-rays ...)
  - Characterizations (principle and software of tilling / ecotilling, NGS ...): frequencies / statistics, cartographies, hotspots ...
- Related techniques: "scars" (including somaclonal variations following electroporations, cell cultures, regeneration of calls / plants ...), ...)
  - Mutations and random epimutations of DNA, proteins and RNA (the change of a nucleotide can induce mutations and epimutations, positioning in TAD and gene expression ...)
  - Modification of polymorphisms: genetic maps, SNPs, STR repeat sequences, microsat ...
  - Transposable element motions, hotspot studies and recombination coldspots,
  - Traces of elimination of modified cell selection sequences (eg Cre-Lox),

General signatures

- Comparison of « cell lineage », e.g.https://www.pourlascience.fr/sd/genetique/suivre-le-devenir-de-chaque-cellule-du-corps-9810.php) considering nuclear and mitochondrial genomes and epigenomes

NBT signatures

- PAM proximity (sometimes several) and targeted mutation (s) / epimutation (s), eg Crispr-endonuclease,
- Off-targets near PAM, off-targets of RNAi, ZFN and TALEN...
- Insertions of vector residues (eg Agrobacterium genome and plasmid) for SDN, RNAi ...
- Contaminating DNA for RNP systems (see results of DNA insertion in the human genome ...), chromatin modifications,
- Natural or Crispr-based "barcode" for recording changes in genomes and epigenomes and environmental cues ...
- DNA, RNA (including mRNA) and proteins circulating between rootstock and scion,

Conclusion

- The identification of the methods used (in vitro vs in vivo, NBT ...) is possible using the matrix approach already in use for known and unknown GMOs, as in other areas of identification / detection ...
- The techniques and targets used are of the same type as those used by seed companies for varietal identification, SAM ...
- One, or part, of these targets can suffice in routine, so at the least cost as for the current GMOs
- The proof of concept will be accessible
  - As soon as the research programs proposed by ENGL to the European Commission in 2013 are launched,
  - The reference materials will be provided by the companies as for the GMOs of transgenesis (regulations 1829/03 and 1830/03)
- The premises have not been fulfilled in the HCB, Scientific Advice Mechanism European... Reports despite the available time (5 years for the HCB...) and considerable resources (40 experts for the HCB), in absence of elicitation of experts (ex: Q method) and public consultation demonstrate a political choice (see speech JY Le Déaut at OPECST in 2016, comment European Commissioner January 2018 ...) allowed by the use of circular reasoning (a classic of scientific bias)

France as a case study:
NBT at the HCB and the Conseil d'Etat

- Working Group 2008-2015
- December 2015: "discussion on a summary note" of the WG
  - No gvt referral nor self-referral, thus without precise request of work to be performed,
  - "Written in a weekend or so",
  - provided to the Scientific Committee 3 working days before the HCB SC meeting,
  - with less than a third of the final document (remainder to be provided later on),
  - with regulatory and economic considerations of the CEES but not of the CS,
  - provided to CEES the same day (contrary to the procedures' scheme),
  - which will be announced the days after as to be provided as a notice to the government,
  - request for dissent refused
- Communication "double talk" to justify a misuse of procedures,
- Resignation of the CS: an expert *intuitu personae* vs. organizations of the highest quality,
- Status of the document regressed several times on the HCB website but was always presented by the gvt as a "advice" of HCB,
- Request to the gvt to urgently provide the HCB with an official referral on NBT,
- New SC WG on NBT started for writing a new CS document… delivered November 2017 despite a new dissent,
- Ad-hoc working group ('coach', chairs and staff) i.e. a judge and party WG on HCB governance. Conclusions after several months: the rules of procedures have to be applied, the documents have to be provided in due time, procedures have to be transparent, minority opinions have to be taken into account as stated by the procedures...

HCB (High Council for Biotechnologies): "business as usual"

Application to the Conseil d'Etat by organizations against Prime minister decisions of "VrTH" ("hidden GMO") approval,
NBT were included during an exceptional investigation at the helm ('enquête à la barre')
The Conseil d'Etat then sent several preliminary questions to the European Court of Justice (ECJ)
Follow-up of the ECJ general advocate comments delivered on February 2018

Some general questions

- A series of agricultural technical evolutions, often described as revolutions (middle-age, green...),
- A technical evolution of molecular biology: GMOs, then NBT, synthetic biology,
- Related techniques: cooking recipes that have been used for several decades generating mutations and epimutations (see the October 2016 seminar)
- Techniques used without evaluation guidelines on certain impacts (e.g. epigenome, see EFSA symposium, June 2016) nor an appropriate quality assurance scheme,
- Societal issues that led to the refusal by citizens of certain techniques and leading to regulatory questions (what is mutagenesis, GMO or not, exempted or not?) being processed at the level of the ECJ,
- A society where technical progress and innovation are asserted as a source of happiness and wellbeing by both private and public actors (ministries in charge of the environment and agriculture, CTPS vs. Evaluation agency ...)

What can be learned from the current NBT controversy (ZFN, TALEN, Crispr-nuclease) and other techniques (negative segregants, RdDM, RNAi, OdM, grafting ...), copy / paste errors, positions of experts and authorities they control, business lobbies and policies?

NBT : rhetoric and omerta

- Confusing copy-paste, without any updating or critical thinking, ex:
  - the story of the definition of *recombinant DNA* and the nucleotides
  - Traceability / detection / identification, from the industry arguments to the European SAM report,
- From flooding of promises reminiscent of GMOs and cloning promises 30 and 20 years before, with many omissions: breeding acceleration, feeding the world… to a fabulous world 2.0 without genetic diseases nor harmful organisms, around precision medicine
- An abuse of undefined wording such as 'natural' (e.g. use of natural mechanisms such as NHEJ, but current GMOs also use natural DNA reparation mechanisms), a semantics of fight ('new' synonymous with breakage for patents, and 'plant breeding / selection' for falling asleep citizens), 'editing' rather than modification, this despite the many errors, 'precision' despite several unintended effects such as off-target ...
- A focus on 'targeted mutagenesis' not mentioning the unexpected effects of old techniques (protoplastisation, regeneration of plants...) used to generate mutations and new techniques of genome modifications,
- A mechanistic presentation of the 70s-80s of the genome and molecular biology instead of the current one of dynamic networks constantly interacting between genomes of a cell, between cells, between tissues,
- A biased choice of mechanisms and of their relative importance e.g. RdDM (a way of obliterating the problem of gene regulation, pleiotropy):
  - Methylation of DNA as a natural process,
  - Unmodified DNA sequence,
- A mix of confusing situations: old reassuring techniques such as grafting but with GM rootstock without consideration of the remote effects on the scion and its products ...
- Confusion maintained for example by the different definition between countries about e.g. agro-infiltration (agro-infection itself and floral-dip included or not),
- Gibberish confusion with 'concepts' (e.g. cis-, intra- and trans-geneses using the same techniques) presented at the same level as techniques.

A semantic battle and biased rhetoric to mask the regulatory and financial aims

AFTER THE GMO, THE NBT: BIASED SEMANTIC AND RHETORIC BATTLES TO MASK THE REGULATORY AND FINANCIAL ONES

Who's expert in which context?

- As a kid, you can be the expert for the bicycle of your sister,
- Administrations' delegates: OECD, EC's committees… with several instructions,
- As a "connoisseur", such as a scientist or as stakeholders' representative…

But

- Experts are themselves "politicians" (as they are as scientists in their labs) with different opinions and strategies, hidden agendas…
- Experts may be rare, difficult to mobilize, particularly in controversial areas, possible biases in their choice by e.g. the nominated chairs…
- Experts are generally questioned about unstable situations, with large uncertainty margins, affecting different possible scenarios, sometimes with guidelines and norms (e.g. toxicology) not taking into account recent research data…
- Expertise can be also mostly based on stakeholders documents (expertise privatization?) without power of initiating additional research, in very short expertise timing,
- The quality of expert's work is generally not assessed (some are just making summaries instead of applying critical mind or checking the application of legal directives)
- Generally highly depending on the secretariat competence, a staff whose members can publish to express their viewpoints (see e.g. EFSA's staff) and thus influence the experts.

The experts / politicians relatiosnhips

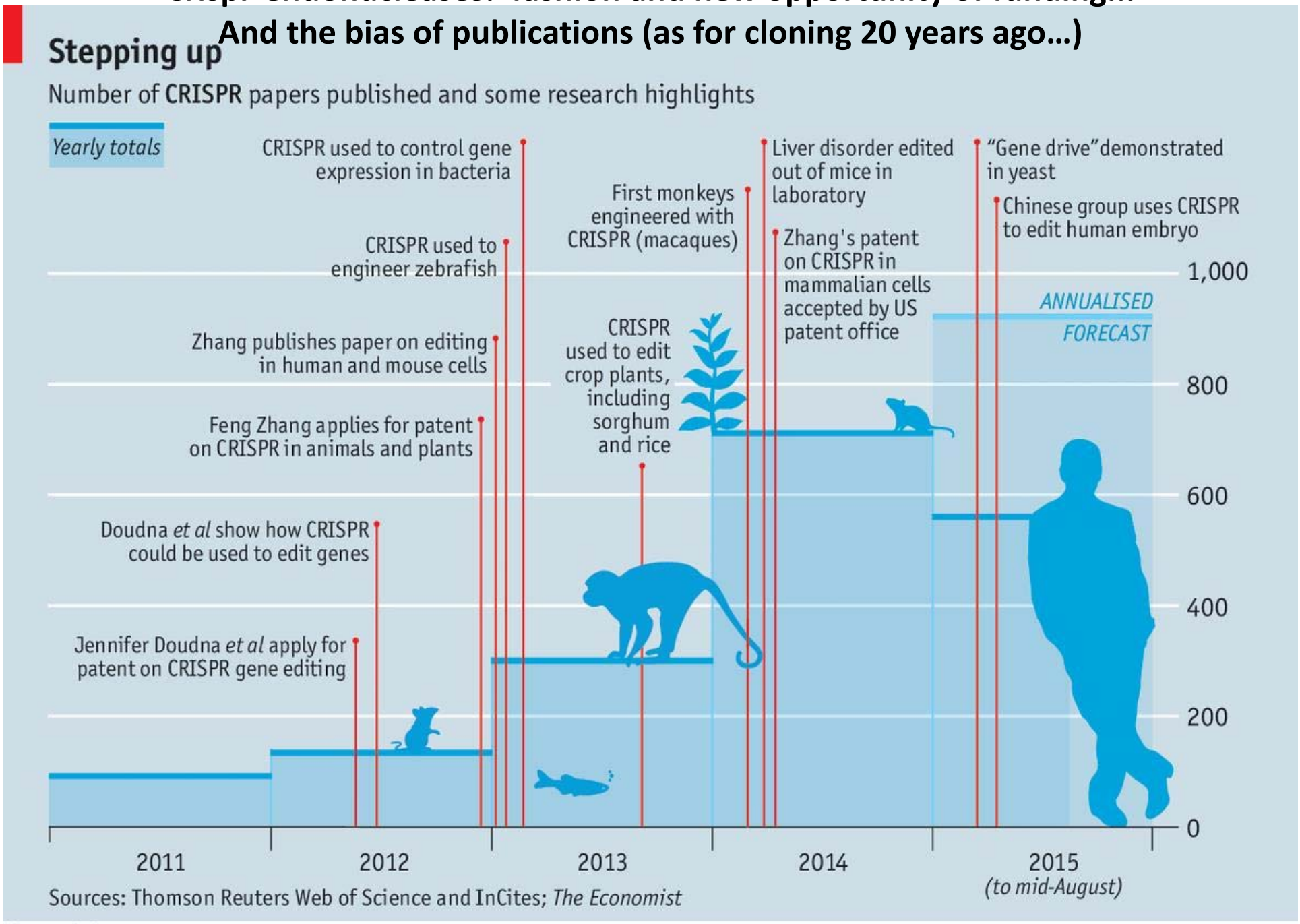
- The enlightened prince, a kind of epistocracy,
- The empire of standards: the legitimacy of “science based” and the full epistocracy. Which expert to choose and who is nominating them?
- The technical democracy: the power of experts decreases, citizens are involved (consensus conference, HCB CEES…). But who is representing? Several limits (see the debate about nanotechnologies in France).

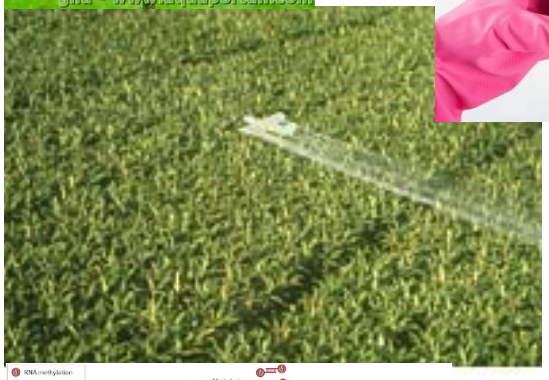
Direct interferences during the expertise

- Social pressures of an economy of promises:
  - Colleagues' interventions about e.g. socio-economic impacts (feeding vs. nurturing the world, children's nutritional deficiencies, supposed huge costs of approval dossiers…),
  - Nationalist arguments: agricultural exportations and trade balance, improving competitiveness, Shangai's ranking of our research,
  - Authority arguments during colleagues' interventions about what "everybody knows", tribunes of renowned scientists of other domaines,
  - The usual and intentional confusion between science and their applications,
  - Number of "successful publications" (forgetting the fashion effect, the funding needs with claims for disruptive technics, the publication biases…)
  - No compliance to scheme rules (e.g. CS HCB meeting on dec. 16, 2015 and following steps) and their control (internal WG's conclusions: to respect the rules of procedure, be transparent, provide documents in time…), independent structure without ethical committee at the difference of Anses (recent dissensus issue)

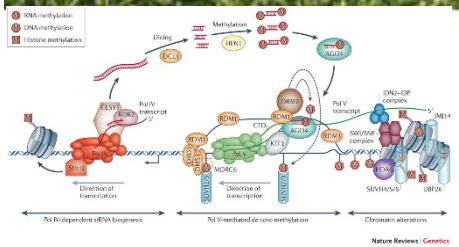
In conclusion: the red Queen race of Alice in Wonderland

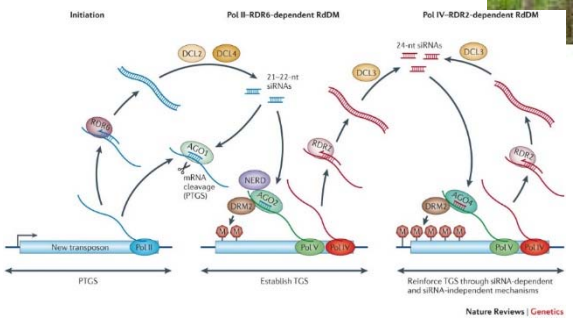
Crispr-endonucleases: fashion and new opportunity of funding...
And the bias of publications (as for cloning 20 years ago…)






A comparison of corn with disease and Bt corn.(Photo by Biotech info center)





Are consumers, but also supply chains, really wishing such changes?

An economy of promises and a race of lies

Genta: 1998-2012

The strange but true tale of a beleaguer with 100 quadrillion shares outstanding.

SPECIAL

Fraud and misconduct in science: the ste cell seduction

Implications for the peer-review process

ETATS-UNIS

Une start-up d'analyses sanguines soupçonnée d'avoir menti sur sa technologie

Par Anaïs Cherif(http://www.liberation.fr/auteur/16518-anais-cherif) — 19 avril 2016 à 17:18

theguardian

What pushes scientists to lie? The disturbing but familiar story of Haruko Obokata

The spectacular fall of the Japanese scientist who claimed to have triggered stem cell abilities in regular body cells is not uncommon in the scientific community. The culprit: carelessness and hubris in the drive to make a historic discovery

N° 2198

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CONSTITUTION DU 4 OCTOBRE 1958

ONZIÈME LÉGISLATURE

Enregistré à la présidence de l'Assemblée nationale le 24 février 2000

Annexe au

OFFICE PARLEMENTAIRE D'ÉV... DES CHOIX SCIENTIFIQUES ET TEC...

RAPPORT

SUR

LE CLONAGE, LA THÉRAPIE CE... ET L'UTILISATION THÉRAPE... DES CELLULES EMBRYONN...

PAR M. Alain CLAEYS, Député.

PAR M. ... Sénateur.

Breakthroughs IN BIOSCIENCE

Cloning: Past, Present, and the Exciting Future

by Marie A. Di Berardino, Ph.D.

too-distant

into the neighborhood ...ill his prescription for a ...s born without. He ...s for blood clotting ...relies on the local ...his medicine. Jimmy ... bag that contains his ... of patches, removes ...from his chest, and ...w one. He adjusts his ...ds out to meet his ...game of touch football. ...e is hemophiliac, Jimmy ...about the bruises and ...ure to get.

...s scheduled to have her ...ically reprogrammed skin ...planted to replace her ...maged heart cells.

...o has Parkinson's ...eives special nerve ...s not concerned about ...ompatibility and rejection, ...ese cells are her own ...reprogrammed skin cells.

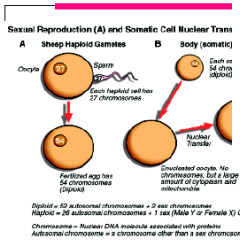
➢ Patients routinely buy anti-cancer or anti-viral drugs in large quantities to treat their conditions.

This is the future. It is what Dolly so wondrously has wrought. Born July 1996, she is the first mammal successfully cloned from an adult cell, one taken from a ewe's mammary gland.

Nuclear transfer

Dolly was not created in the ordinary way. Typically, a lamb is the product of natural reproduction—two germ cells, a sperm from an adult male and an egg (oocyte) from an adult female, fuse at fertilization. Each of these germ cells (the sperm and the oocyte) contributes half the chromosomes needed to create a new individual. Chromosomes are found in the cell's nucleus and they carry DNA, which is the genetic blueprint for an individual.

The process that produced Do... differs from ordinary reproduction... two major ways. First, body (... somatic) cells from ... udder (this is the d... in a culture dish an... The nutrients were ... from the culture, w... cells' growth. One ... growing cells was ... electric jolts) with ... oocyte from which ... been previously rem...



IN FOCUS NEWS

PHARMACEUTICALS

Drug giants turn their backs on RNA interference

A once much-touted technique faces a difficult transition to the clinic.

BY HEIDI LEDFORD

Not long ago, a technique called RNA interference (RNAi) seemed to be on the fast track to commercial success. Its discovery in 1998 revealed a new way to halt the production of specific proteins using specially designed RNA molecules, and it quickly became a favourite tool of basic research. In 2006, the scientists who made the discovery were awarded the Nobel prize for medicine, and the New Jersey-based pharmaceutical giant Merck paid more than US$1 billion to snatch up Sirna Therapeutics in San Francisco, California — one of the first biotechnology companies aiming to

A SAMPLING OF RNAI THERAPIES IN CLINICAL TRIALS

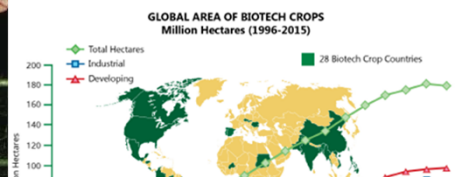
Indication	Company	Clinical phase	Delivery method
Age-related macular degeneration	Quark Pharmaceuticals/ Pfizer/Silence Therapeutics	Phase II	Naked RNA
Diabetic macular oedema	Quark Pharmaceuticals/Pfizer	Phase II	Naked RNA
HIV	Benitec	Phase I	Lentivirus vector
Liver cancer	Alnylam/Takemira	Phase I	Lipid nanoparticle
TTR amyloidosis	Alnylam/Takemira	Phase I	Lipid nanoparticle
Respiratory syncytial virus	Alnylam/Cubist Pharmaceuticals/ Kyowa Hakko Kirin	Phase II	Inhaled naked RNA

than originally thought," says Michael French, ... wary of the RNAi platform, says Josh Schim...

Molecular Therapy

La Recherche
L'ACTUALITÉ DES SCIENCES
MENSUEL N° 394 • FÉVRIER 2006

Dossier spécial
L'avenir du clonage humain
Après le scandale coréen p. 30



Is RNAi Dead?

A recurring theme in the way that many pharmaceutical companies approach new technologies is that they are initially extremely enthusiastic, perhaps excessively so, but then subsequently overreact in the opposite direction, abandoning them when the first bumps in the road come along. Only a few years ago, the affection of big pharma for RNA interference (RNAi) seemed unlimited. Merck had acquired

strategies to improve internal R&D productivi... none of these has shown the hoped-for benefi...

Enter RNAi. RNAi promised rational design with unparalleled specificity and ra... of development, and it obviated the issue ... druggable targets. In theory, a research team ... pick a new drug target and have a lead RNA... specific for its gene ready for human clinical ... within 15 months. A good deal of early pharm...

Authority's arguments

- Usual sentences during sessions: 'everybody knows',
- Appeals to authority such as chair taking the time instead of favoring the expression of diverse opinions,
- Subordinate relationship, funding or positions backlash in future e.g. call for tenders…
- Standardized risk assessment (e.g. toxicology) vs. research issued assessment (e.g. epigenetics without guidelines, effects of fed miRNA on host's genes regulation…),
- Using several cognitive biases.

Experts' integrity

- Direct links of interests and earnings (merchants of doubt…)
- Cherry picking of publications
- Biased information / communication (see the lack of HCB CS to CEES communication on related techniques)
- Circular reasoning (e.g. identification of NBT techniques and derived products, effetcs of genes modification vs. verifications....)

How language matters YB1

- The direct impact of using words and expressions on the reasoning and acceptance of both scientists and laypersons (e.g. new breeding techniques),
- Outrageously simplified language and metaphors (e.g. genome editing),

Diapositive 60

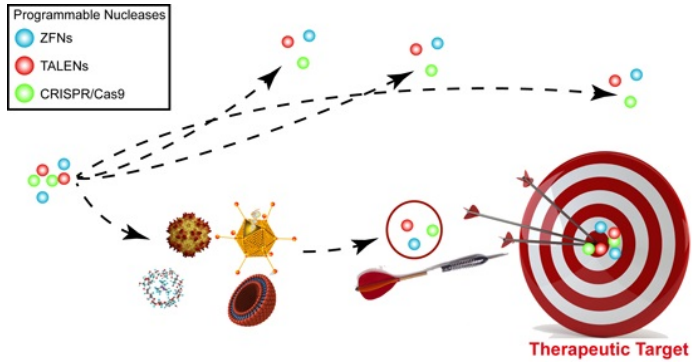
YB1 Yves Bertheau; 28/03/2018

What are precision, unintended modifications and publication's bias?

First training…

Then start ... for missing a lot ...

- Many total or partial homologies in the rest of the genome,
- Thermodynamic considerations,
- Many recipes attempting to reduce the number of off-targets (of a **1,500 factor**...)



- As a result: occasional insertions / deletions or not, chromosomal rearrangements (inversions,
- translocations...), exon skipping effects, epimutations… difficult to predict and detect...
- Additionnally: false positive not checked, circular reasoning accepted…

Finally, only presumed successful data are published: the usual **bias of publication**

Some misleading metaphores

(which influence your perception and then your ways of thinking and finally acting)

SDN: do not foresee an unique and precise cutting

But a series of cuttings (with numerous breaks to rapidly and accurately stick)

Editing the genome ... Waiting for amending electronic and known languages?

What you have effectively to "edit": untranslated handwritten languages…

plus

The promised precise modification?

Several 'off targets' obtained due to rebound effects from homologous Sequences

On a destructed landscape due to related techniques: everything to rebuild

It's rather Staline's organ shots

Targeted mutagenesis: were you thinking about a 'one shot'?

Links of interest

- Direct:
  - e.g. scientist paid by a stakeholder, published results are generally more favorable to the funding companies…
  - Your company or public research institute and its policies, hierarchy and carrier…
- Indirect: lab. funding and temporary positions of team's personnel (e.g. backlash from colleagues in position to influence the results of your research proposals),

Message to take home: you cannot expect a scientist selling all the week a technique, such as NBT, for funding his team to change his/her state of mind and being critical when entering into an expertise's room…

Bioethics

- Enhanced gene drive: organisms' eradication (despite it is already known it is unsustainable) means a privatization of public health policies (see. WHO director's statements at the appearance of Zika, Chikungunya… viruses in Brazil…)
- Plants as a proof of concept, to test the accuracy of the methods,
- Huge demand of perfect children, particularly in some countries…

As for GMOs, large differences observed in polls in western countries about changes of human somatic vs. germinal lines, as well as for pharmaceuticals issued from modified organisms (different 'cost-benefits' analyses).

Recurrent politician interferences

- The abuse of metaphors and other rhetoric effects, oversimplified statements, the confusion between science and applications / innovations,
- Choice of experts and of the committees' chairs, creation of appropriate new structures when political decisions have not been endorsed by the current expertise areas (e.g. European SAM with "high level" experts),
- Requests of changes in e.g. the coexistence issues of GM and non-GM fields production: isolation distances with negotiations between farmers vs. territory management, measurement units to be changed (ENGL advice requested…),
- French OPECST's chair, telling we have to avoid with NBT the current situation of GMOs' refusal,
- European commissioner saying it would prefer NBT product being not traceable (ENGL proposed in 2013 to work on…)…

How to deal with expertise's biases such as links of interest?

- Better recognition of the involvement: emoluments, careers' evaluation…
- Experts' elicitation: Delphi, Q methods (see the current FRB's call for expertise on synthetic biology and environment), involvement of young experts without backlash…
- Improving the weight of evidence? Scientific collective expertise according to recognized quality standards and rules (see e.g. Inra's EsCo), systematic lliterature reviews, metaanalyses… But how could the democratic debate be maintained?

Message to take home: links of interest cannot be fully discarded, we have to find combined ways to 'manage' them and control the success of the procedures.


After decades of expertise, such as for the HCB, i like to remind this sentence of Pierre Gilles de Gennes (Nobel laureate) :
« You know, experts are often like the military.
They are experts of the last war but not of the next… »
THANK YOU FOR YOUR ATTENTION