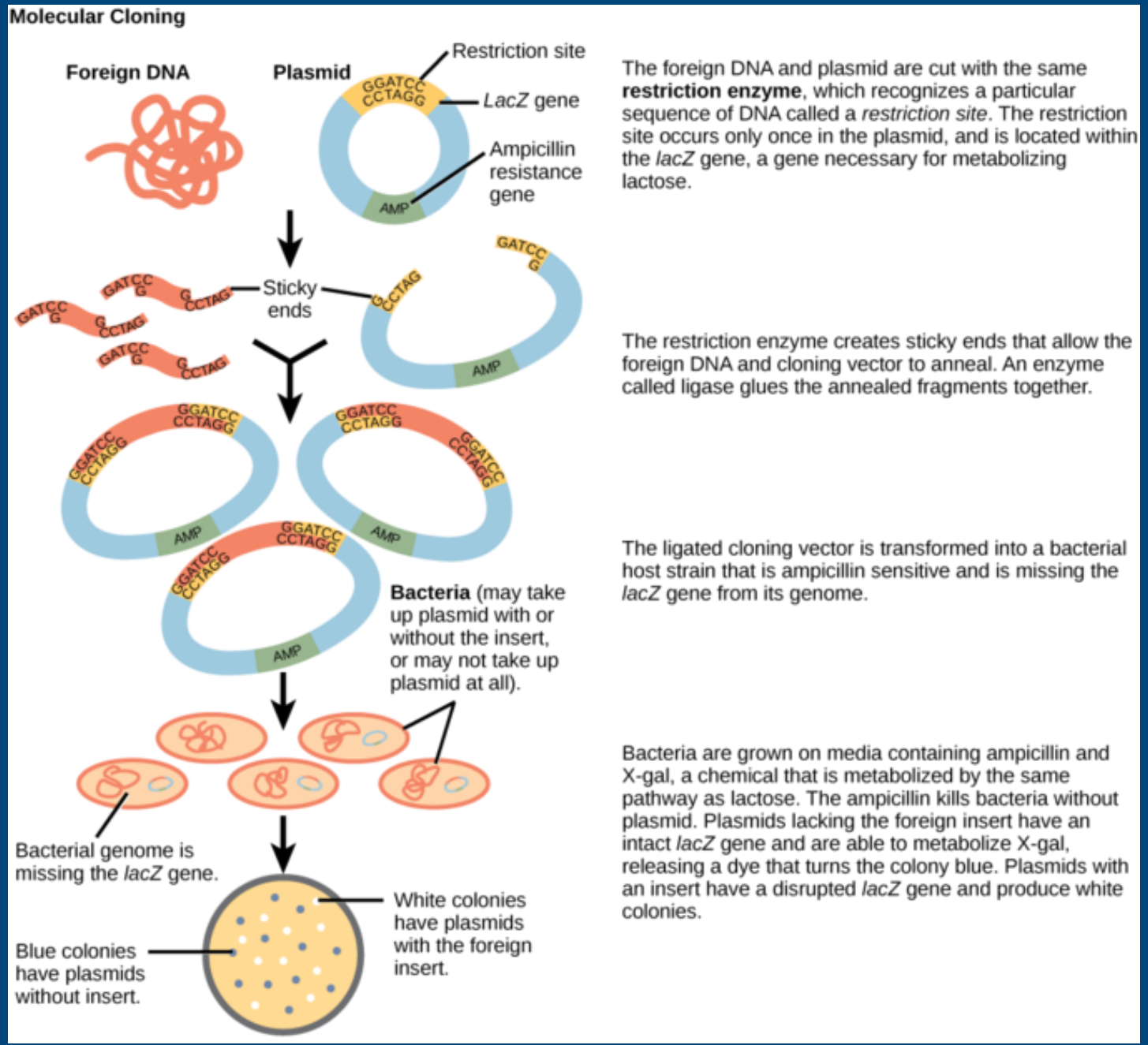
College 2 Biotechnologie.

DNA/RNA-technologie	=> Gentherapie/mRNA therapie
Afweersysteem	=> Immunotherapie
Stamcellen	=> Stamceltherapie
Cybernetica	=> Verbeteren (integratie technologie en biologie)
Synthetic biology	=> Wat is leven?



Klonen van een gen

- Restrictie enzymen knippen op specifieke plekken.

NB Plasmiden en restrictie enzymen komen uit bacterien

⇒boek mol.biol, H.20 (p.332)

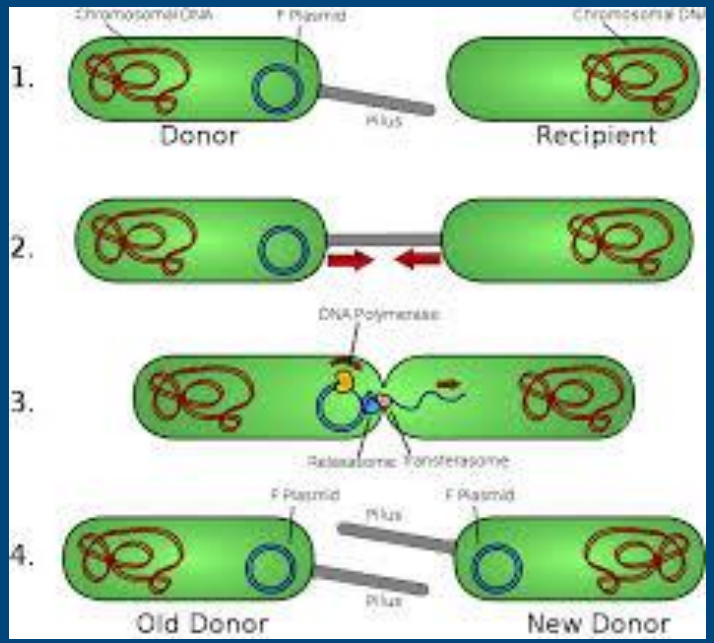
⇒Recombinant DNA tech

- Wordt nu aangevuld met CRISPR
- Resistentie gen vergemakkelijkt selectie

https://bio.libretexts.org/
https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Biology_(Kimball)/11%3A_Genomics/11.01%3A_Recombinant_DNA_and_Gene_Cloning

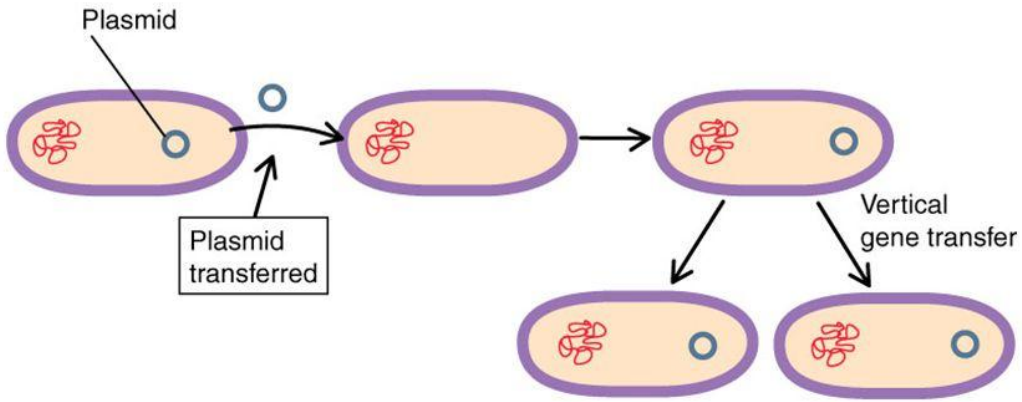
Uitwisselen van DNA is natuurlijk, zelfs bij de mens

(10% DNA in humane genoom is afkomstig van virussen)



Horizontal gene transfer

(b) Horizontal gene transfer



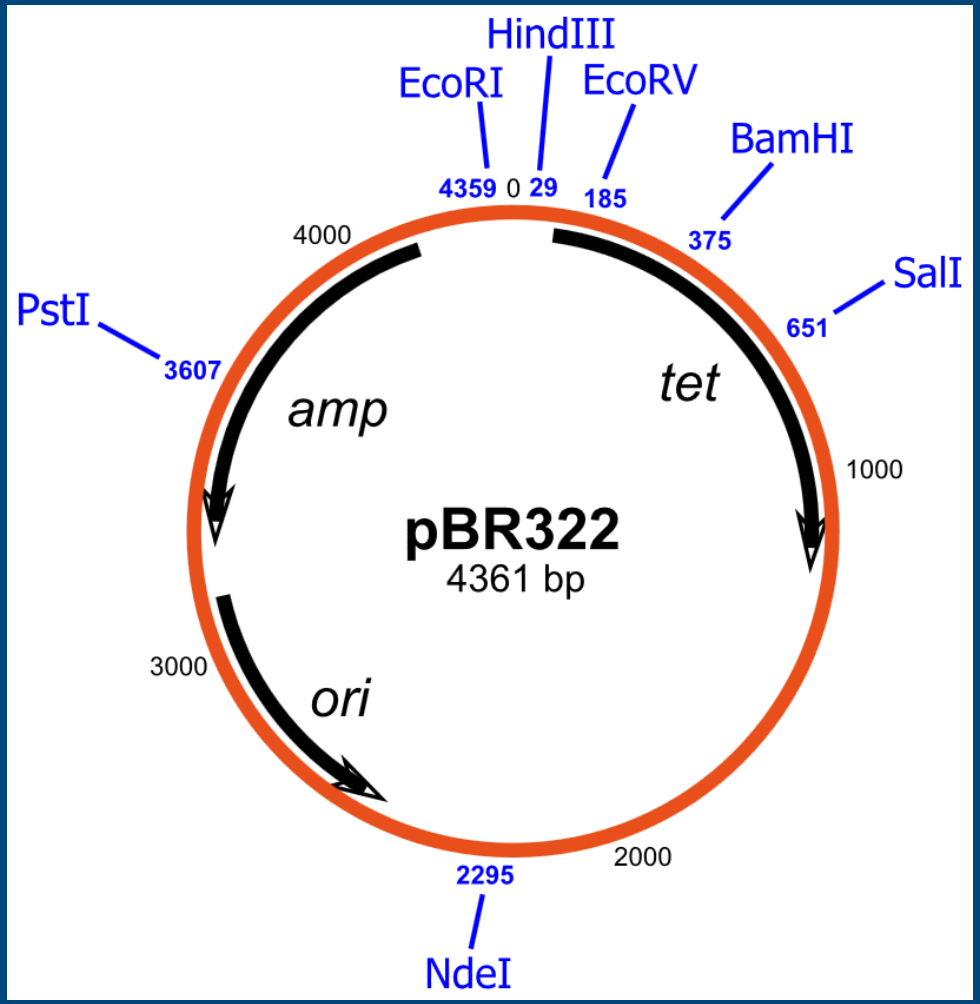
- https://www.bioplek.org/animaties/celtotaal/bacterie.html

Leven is..

- Een informatie systeem
- Manipuleerbaar met informatietechnologie
- Individuele processen kunnen ook buiten een levend systeem plaatsvinden
- Uitwisseling van informatie is natuurlijk

Vector (Plasmide)

- Relatief eenvoudig
- Groot potentieel



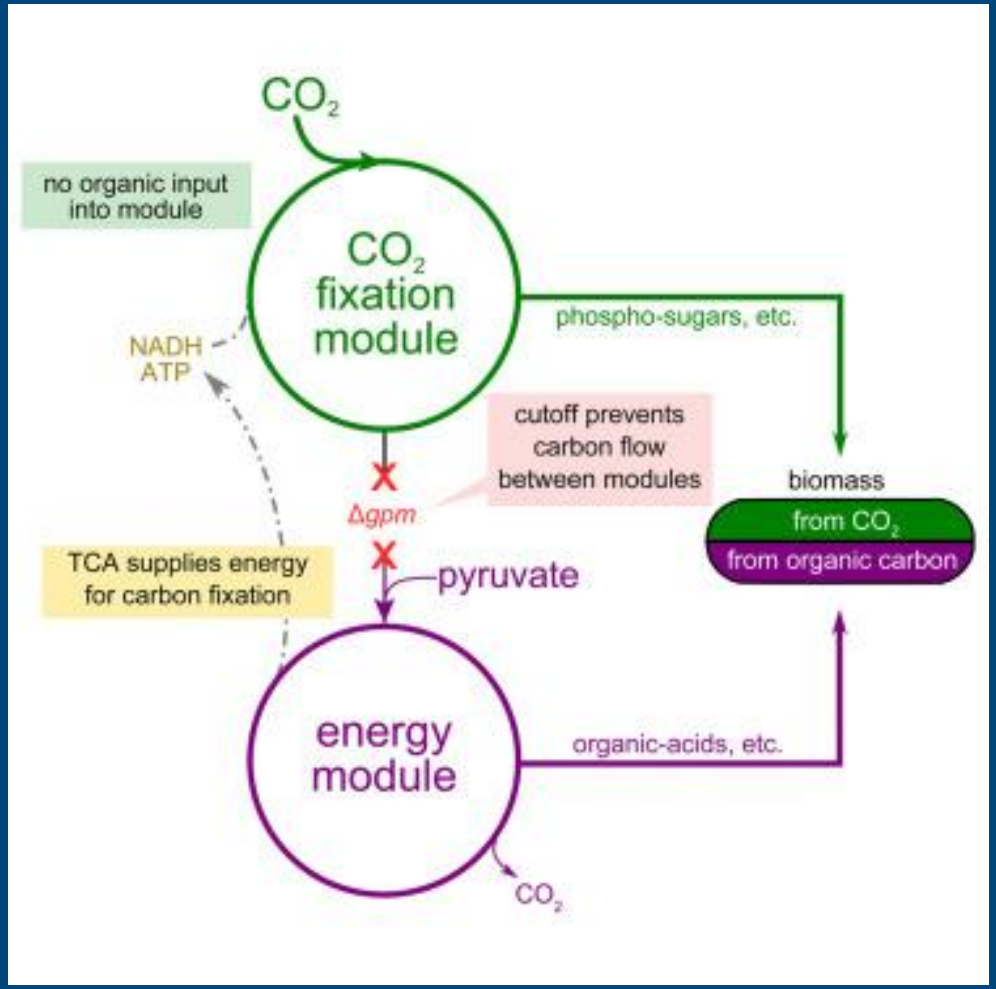
Sugar Synthesis from CO_2 in *Escherichia coli* => Quick fix for the climate !?

Biomass from CO_2 directly by E.coli.

⇒ Toepassing bij CO2 productie
⇒ Of zelfs uit atmosfeer halen

Here, we demonstrate how a combination of rational metabolic rewiring, recombinant expression, and laboratory evolution has led to the biosynthesis of sugars and other major biomass constituents in *E. coli*. But no net. negative CO2

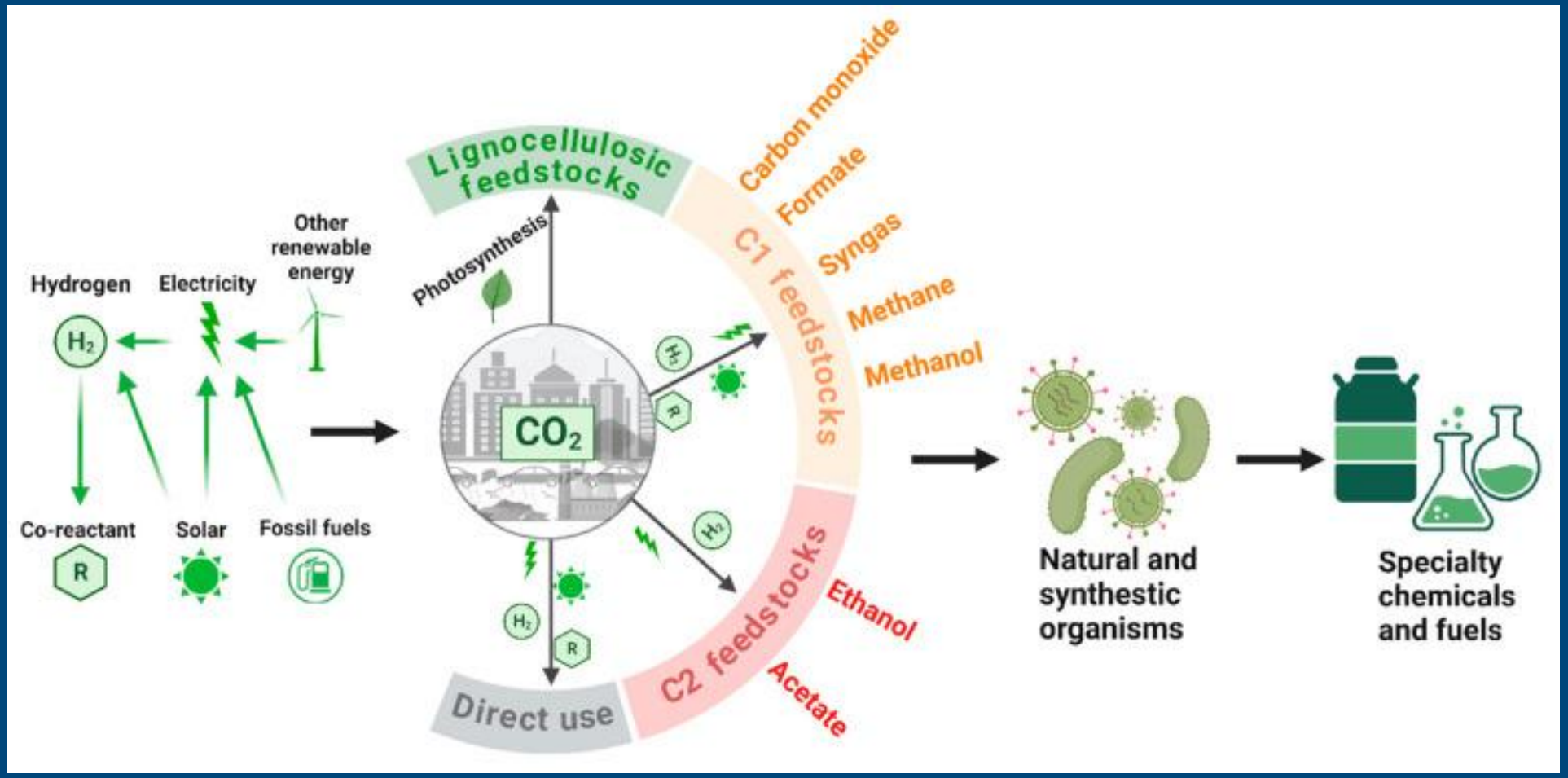
⇒ Biotech kan de wereldproblemen helpen oplossen



https://www.nature.com/articles/d41586-019-03679-x

https://pmc.ncbi.nlm.nih.gov/articles/PMC11446362/

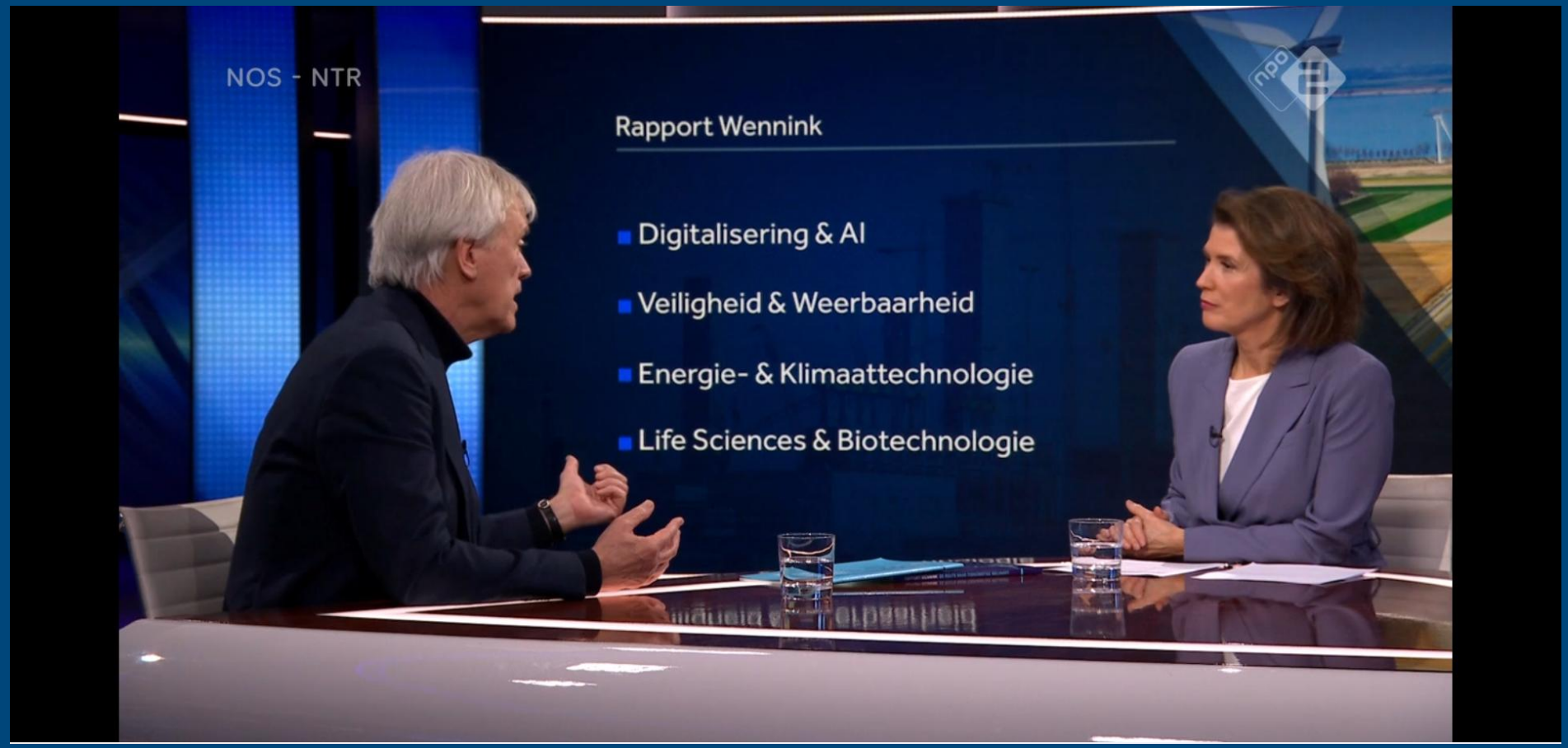
Perspectives for Using CO_2 as a Feedstock for Biomanufacturing of Fuels and Chemicals



https://pmc.ncbi.nlm.nih.gov/articles/PMC10740661/

Biotech als groeimotor

Wennink (ASML)

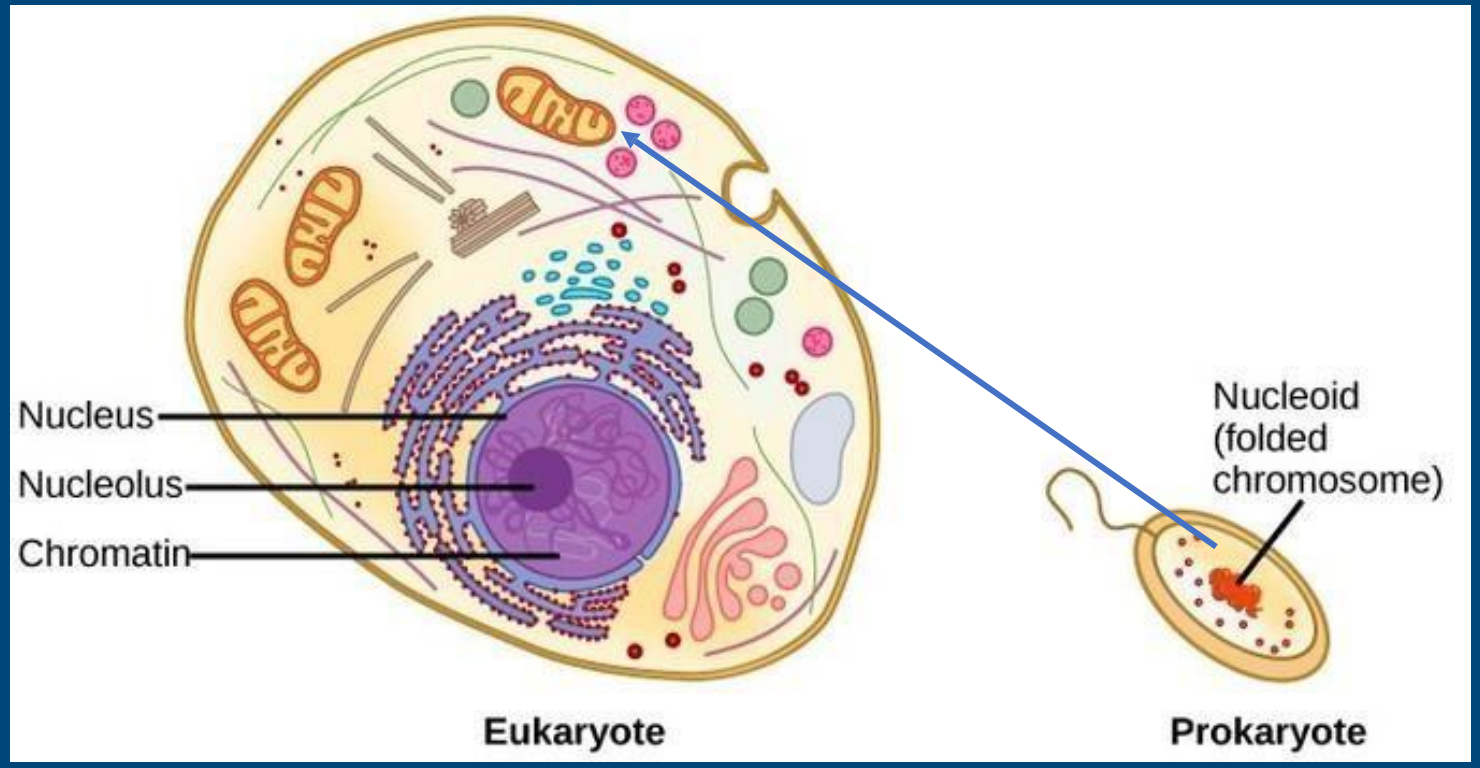


AI can write genomes
how long until it creates synthetic life?

The Evo2 genomic language model can generate short genome sequences, but scientists say further advances are needed to write genomes that will work inside living cells.

https://www.nature.com/articles/d41586-026-00681-y

Eukaryoot v Prokaryoot



Prokaryoot is eigenlijk nog kleiner dan op plaatje

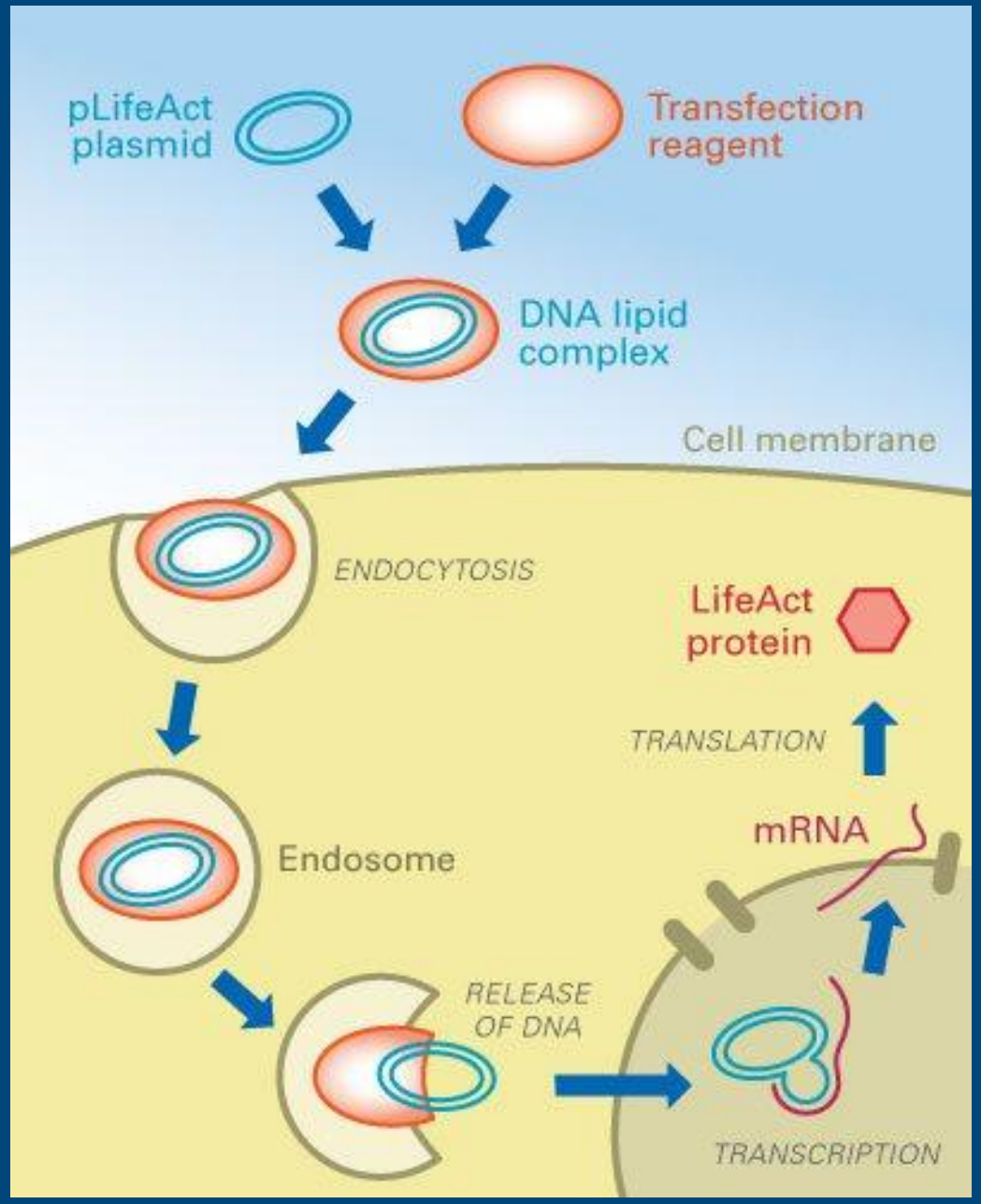
https://www.youtube.com/watch?v=kGd-5HSDo6g
http://www.bioplek.org/animaties/celtotaal/celstart.html

Transfectie

- **Transfection** is the process of deliberately introducing naked or purified nucleic acids into eukaryotic cells
- https://en.wikipedia.org/wiki/Transfection

⇒Bacterie maakt zo bijv. menselijk eiwit

- Transduction is often used to describe virus-mediated gene transfer into prokaryotic cells.



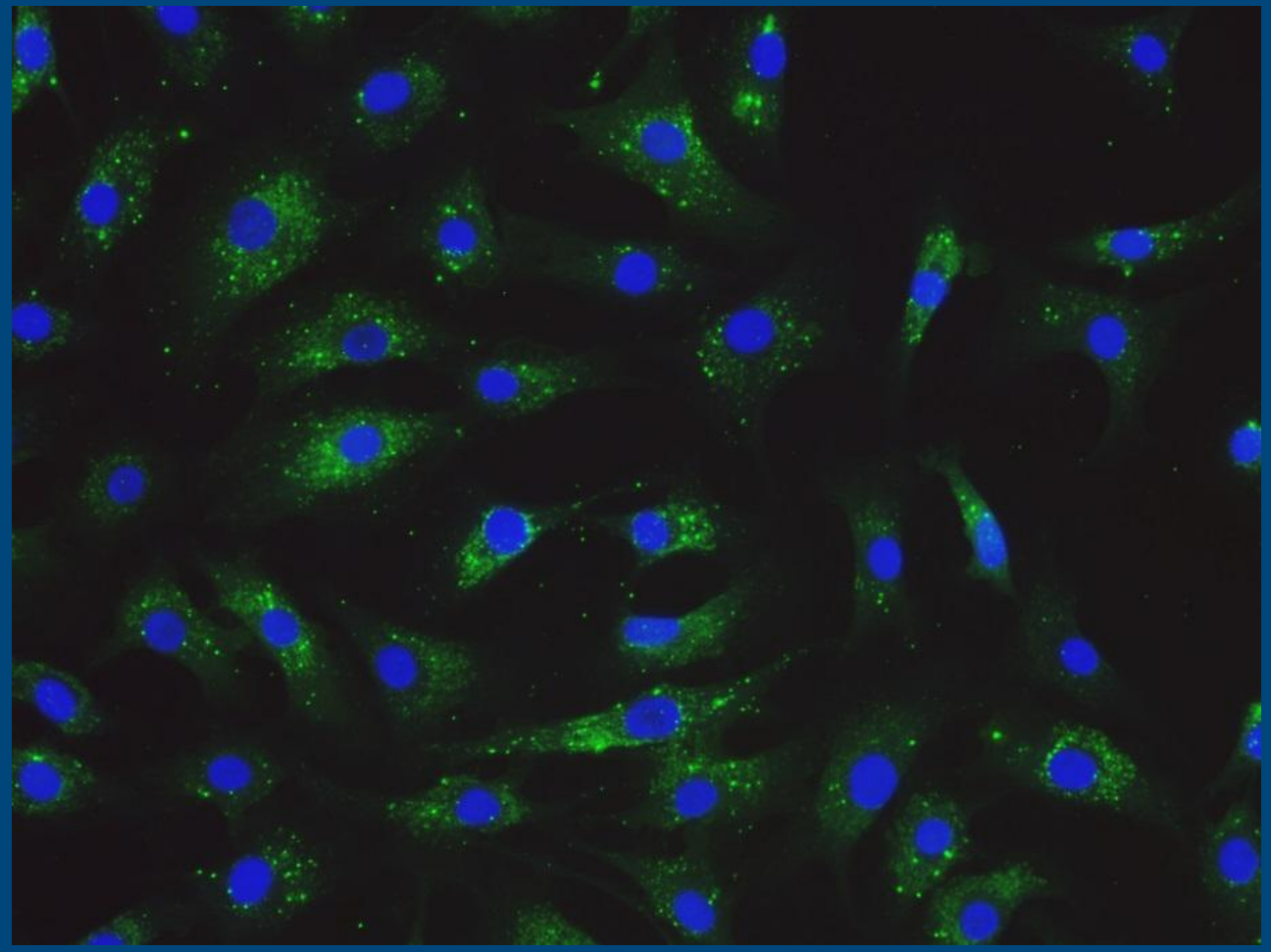
Genetische manipulatie

- Synthese of klonen van gen
- Maken vector
- Transfectie => disruptie van celmembraan

(alleen *nodig bij non-virale vectoren)*

- Selectie en quality control
- Verspreiding
- Eten

- Doel
- Noodzakelijkheid



Vector (Plasmide)

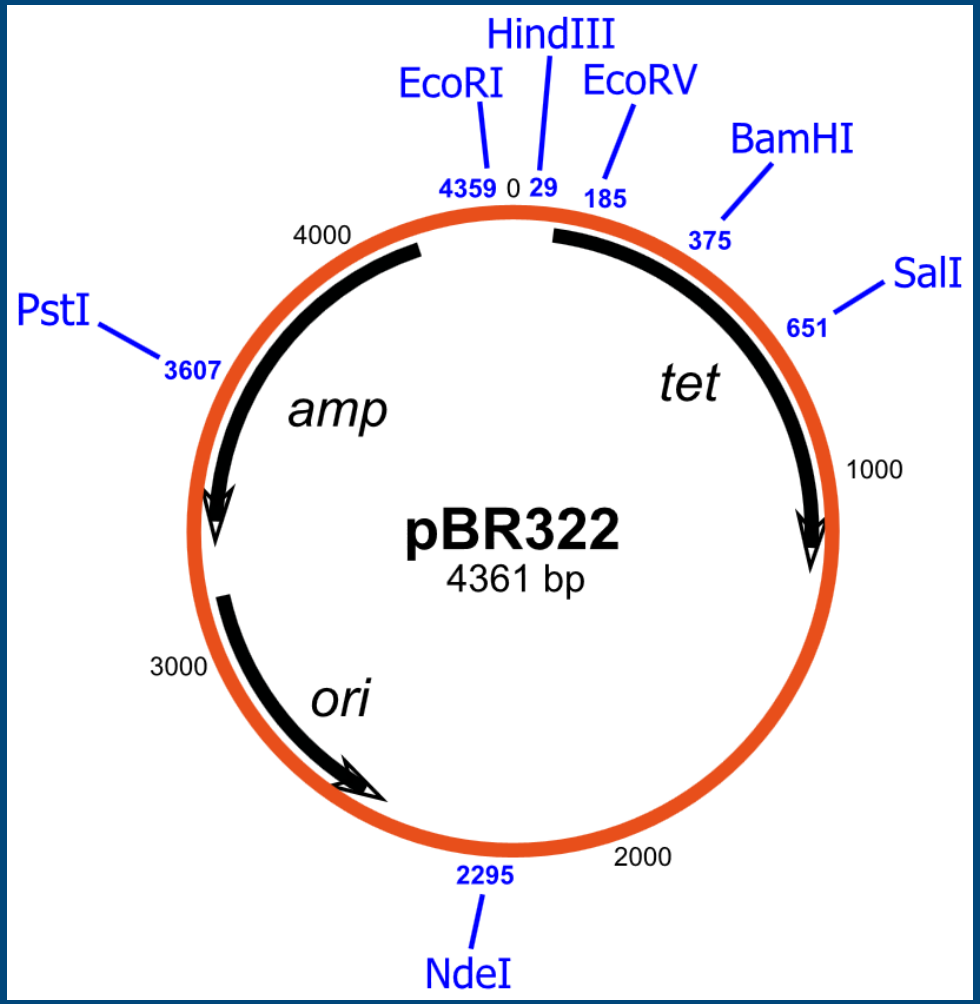
- Meer dan 1 kopie

=> overexpressie

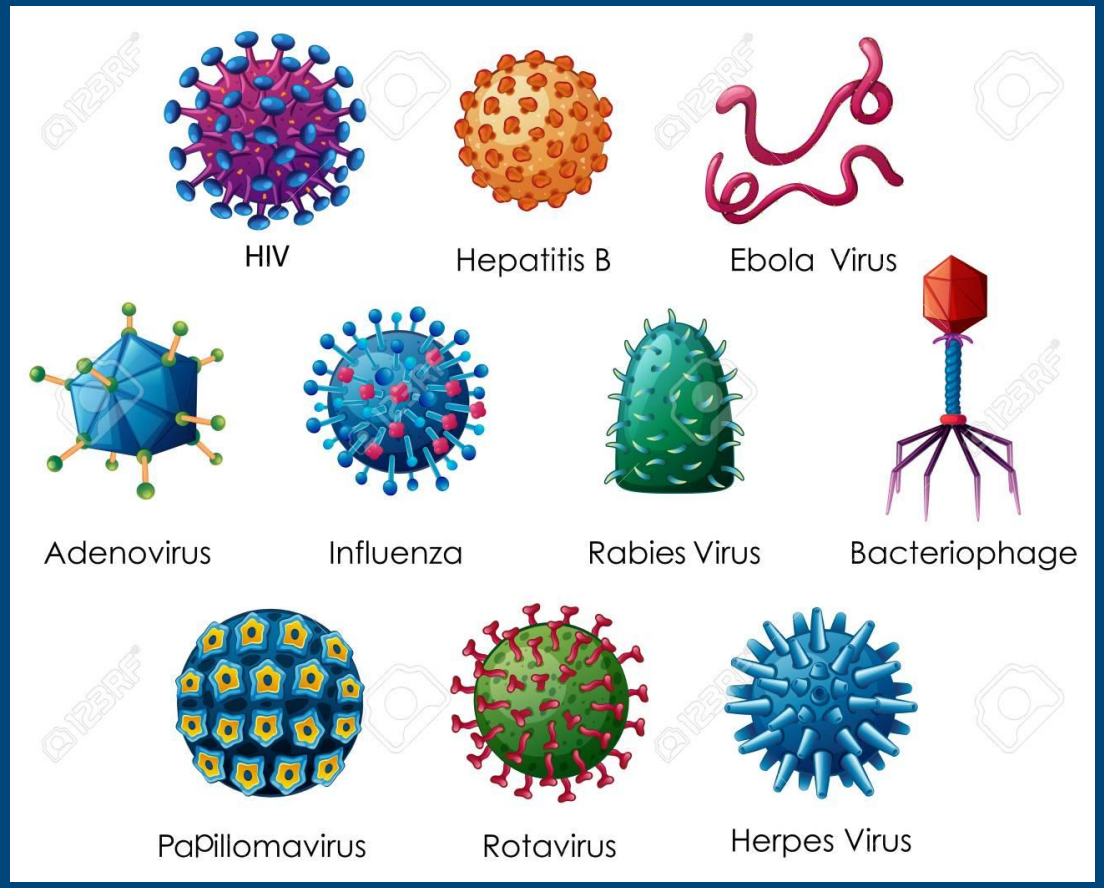
⇒ Genomische instabiliteit

- Kans op problemen in bacterie of plant is klein en niet erg.
- Maar mens en dier wel

- NB Worden wel steeds beter/veiliger

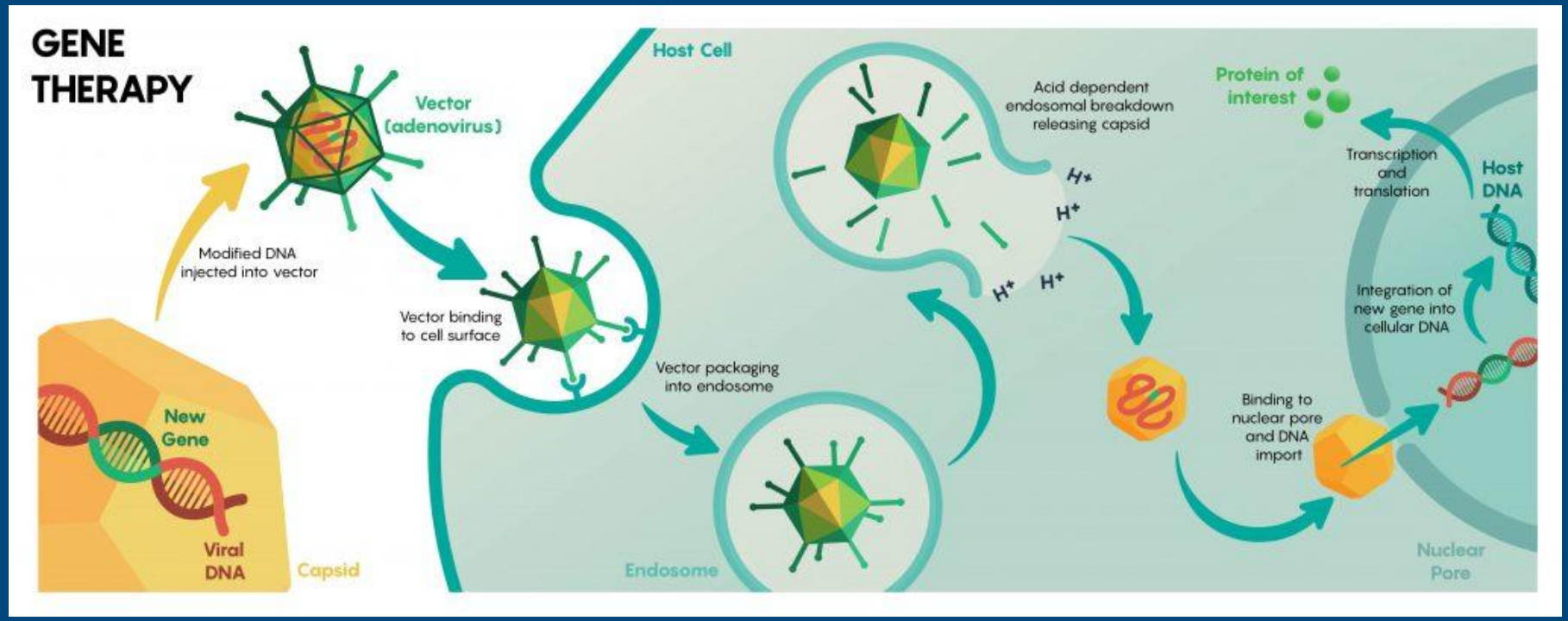


Virus als vector
=> delivery



- Vector = vehikel voor aflevering (delivery)
- Zijn bij uitstek geschikt om genetisch materiaal in menselijke cellen te krijgen (doen ze van nature).
- 10% van ons genoom is oud virus materiaal
- Bij vaccins gebruiken we getemde virussen om virussen te bestrijden
- Maar is ingewikkeld om te maken en te testen (=> recombinatie, waarbij het virus weer helemaal actief wordt uitsluiten)
- Er is vaak al bestaande afweer tegen deze virussen

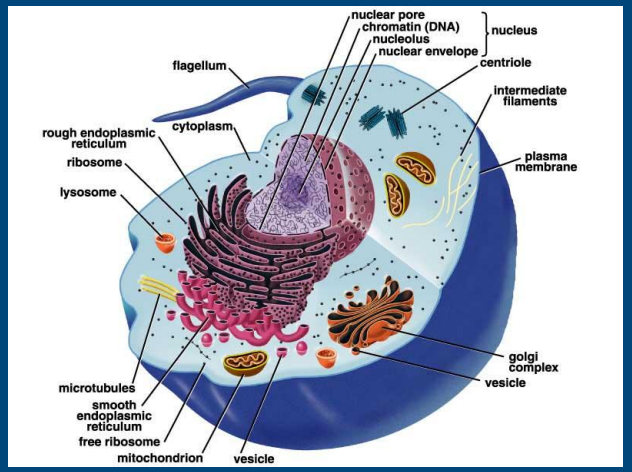
Delivery: 2 traps raket



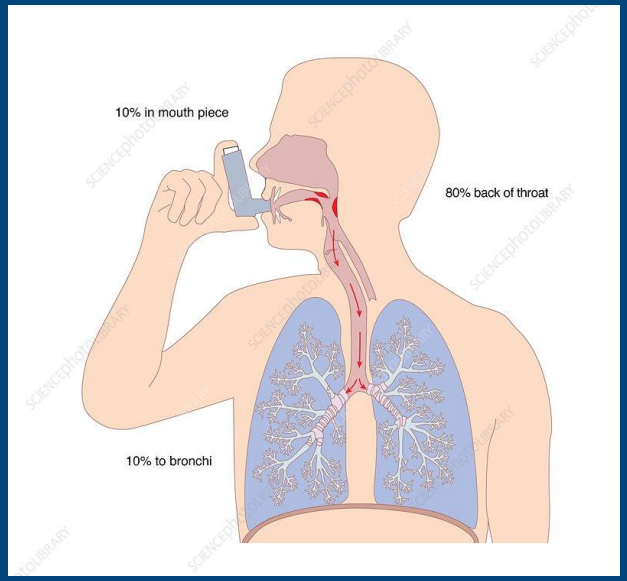
⇒ Homing naar de cel
⇒ Integratie van DNA

The delivery problem

- 1 cel => OK
- Elke cel in kweekbak => al lastig



- Taaislijmziekte => heel lastig
- Heel mens => onmogelijk?



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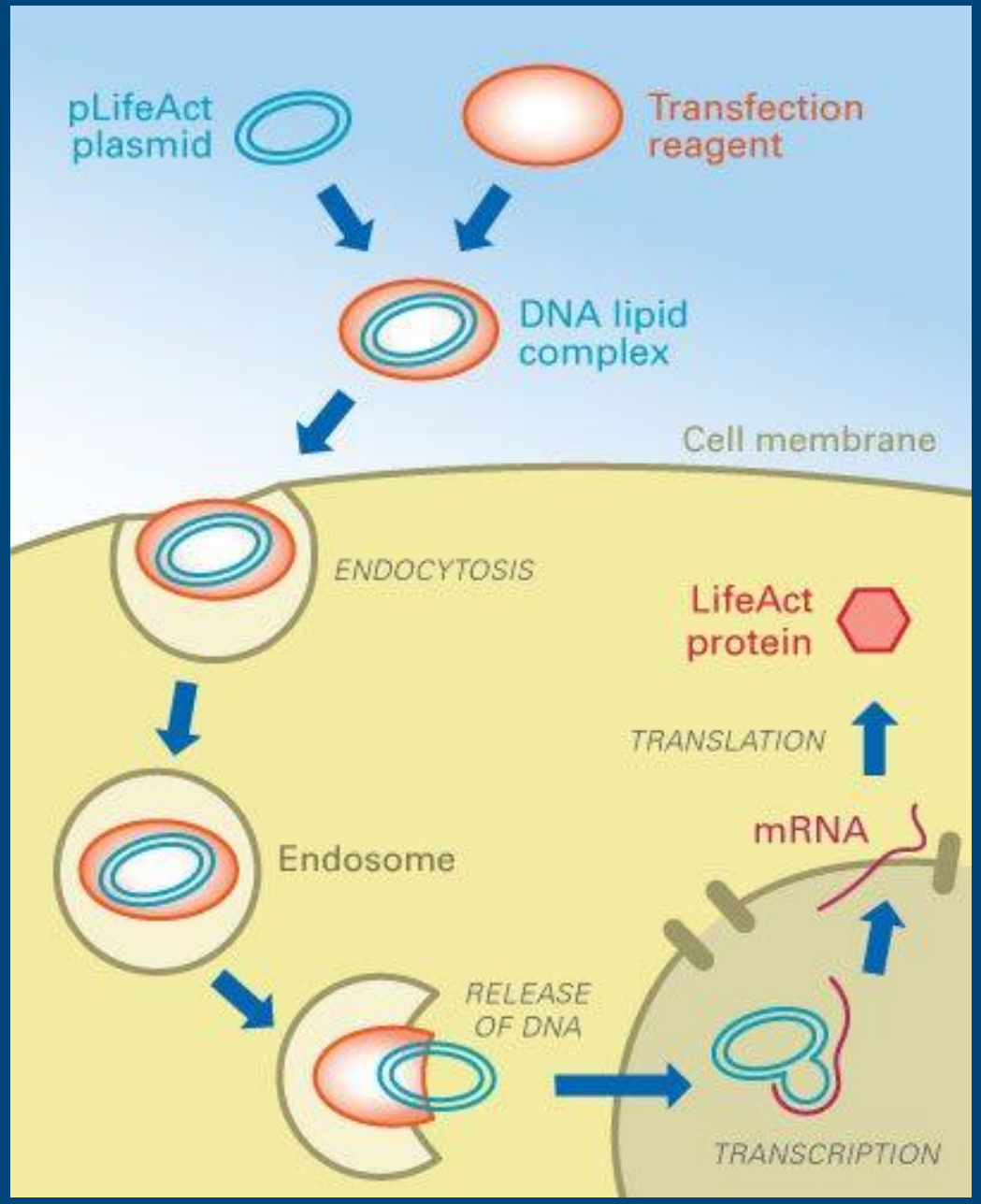
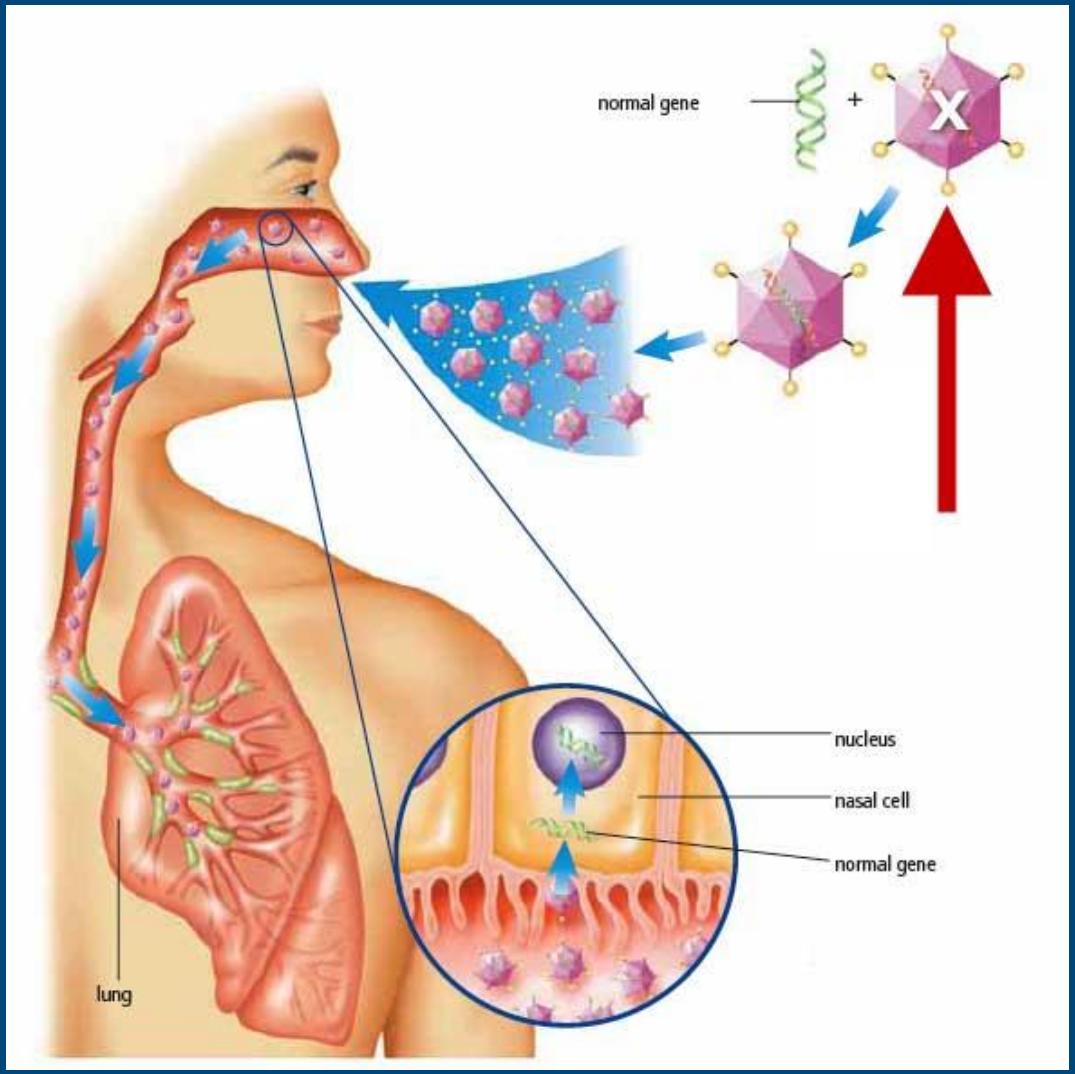


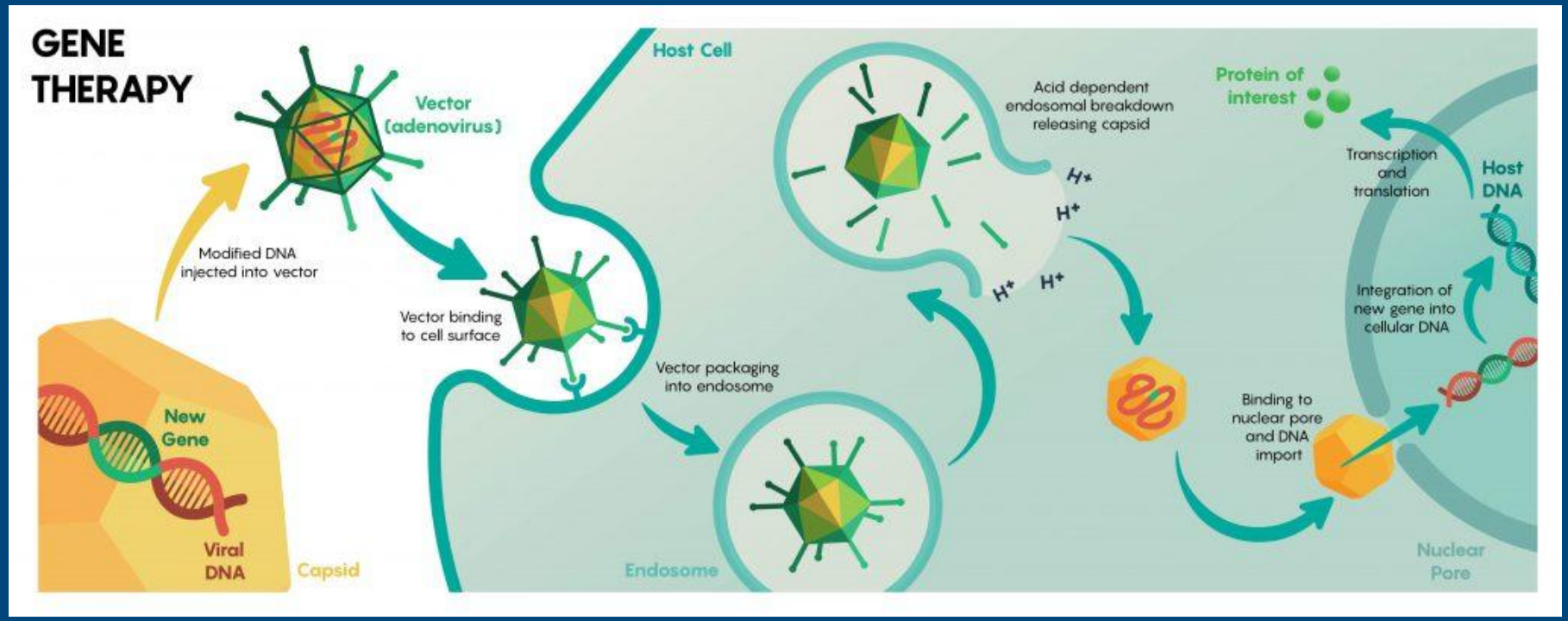
Delivery:
Gene therapy treatment for cystic fibrosis



http://learn.genetics.utah.edu/content/genetherapy/casestudy/

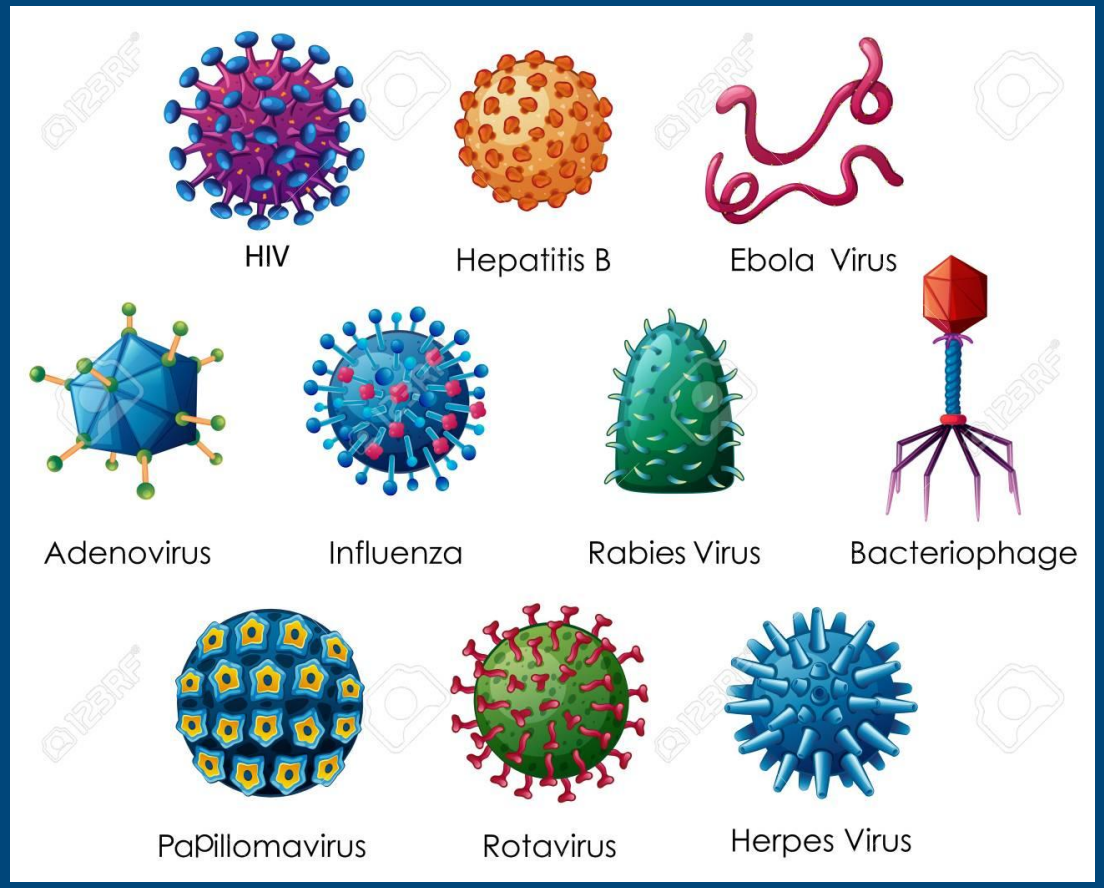
http://perspectivesinmedicine.cshlp.org/content/3/2/a009472.full

Delivery: 2 traps raket



⇒ Homing naar de cel
⇒ Integratie van DNA

Virus als vector
=> delivery



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- Bij vaccins gebruiken we getemde virussen om virussen te bestrijden
- Maar is ingewikkeld om te maken en te testen (=> recombinatie, waarbij het virus weer helemaal actief wordt uitsluiten)
- Insertie op meerdere locaties, en in oncogenen

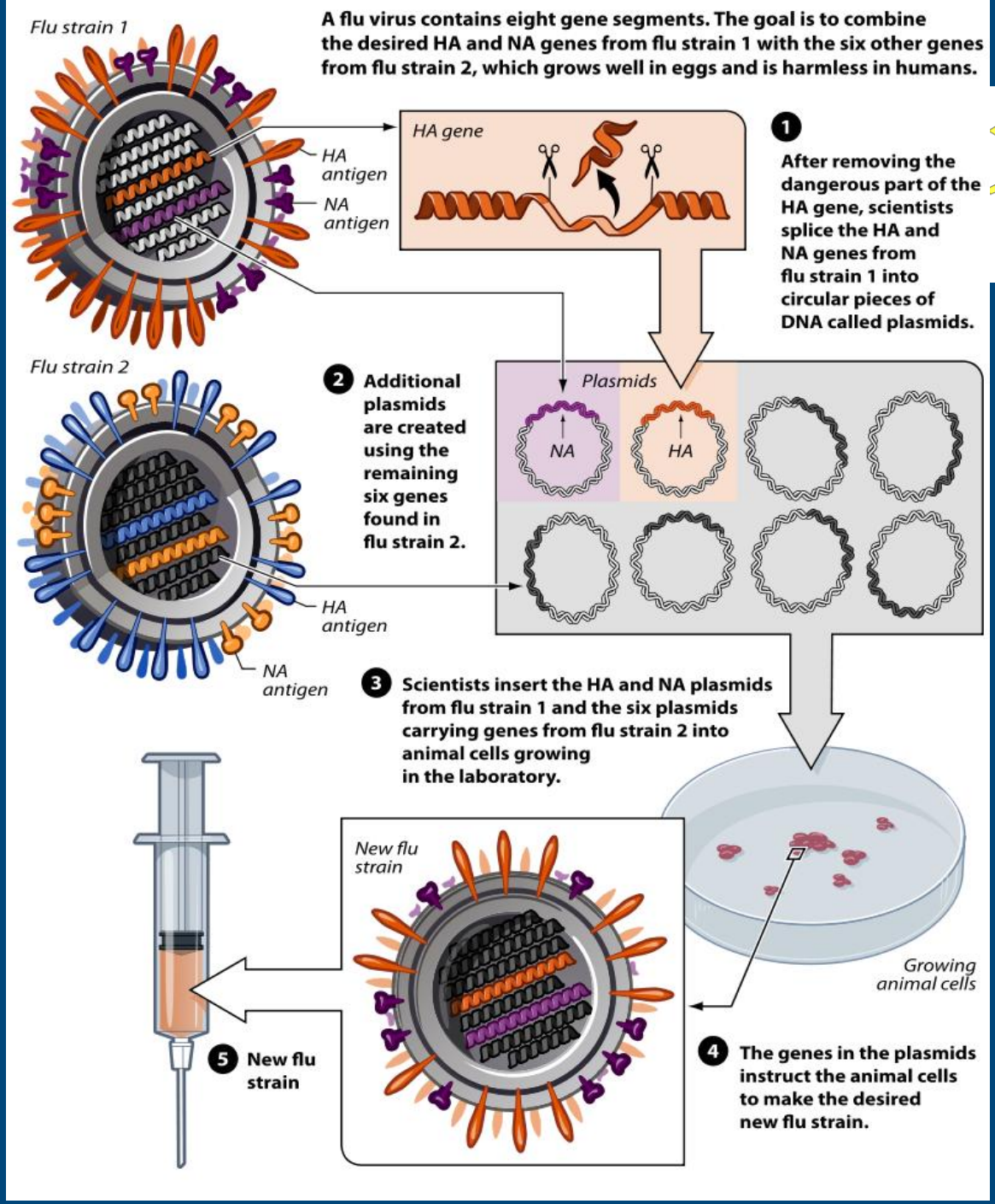
Vector / Vaccin

Moleculaire biologie!

(Knippen/plakken)

Voor het maken van vectoren worden vaak lentivirussen gebruikt, vanwege hun efficientie. Maar dit zijn gevaarlijke klanten. (The best known lentivirus is the Human Immunodeficiency Virus HIV”)

=> Quality Control !



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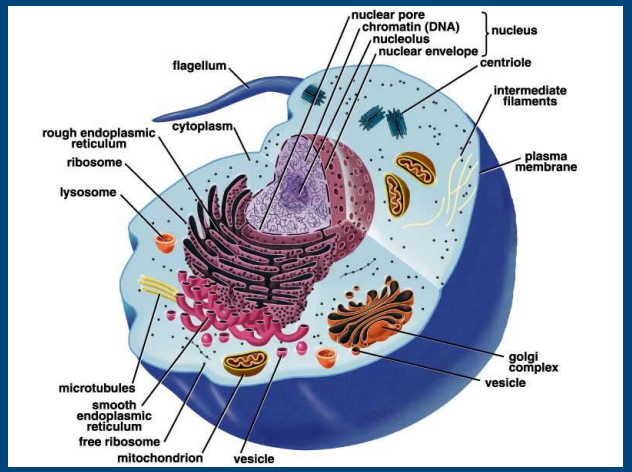

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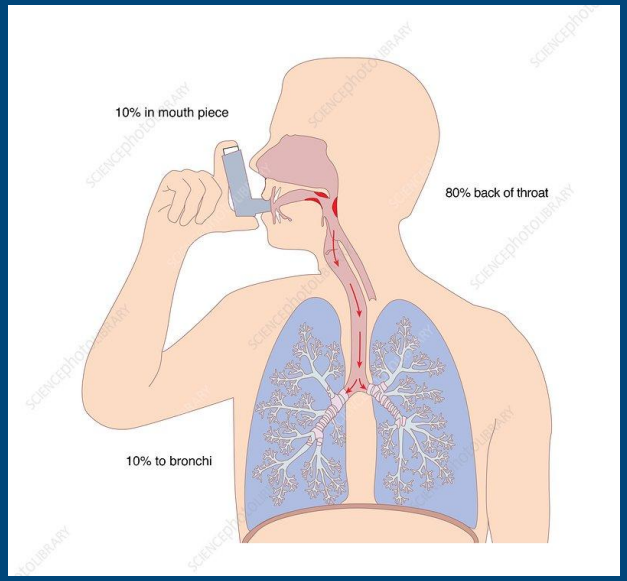
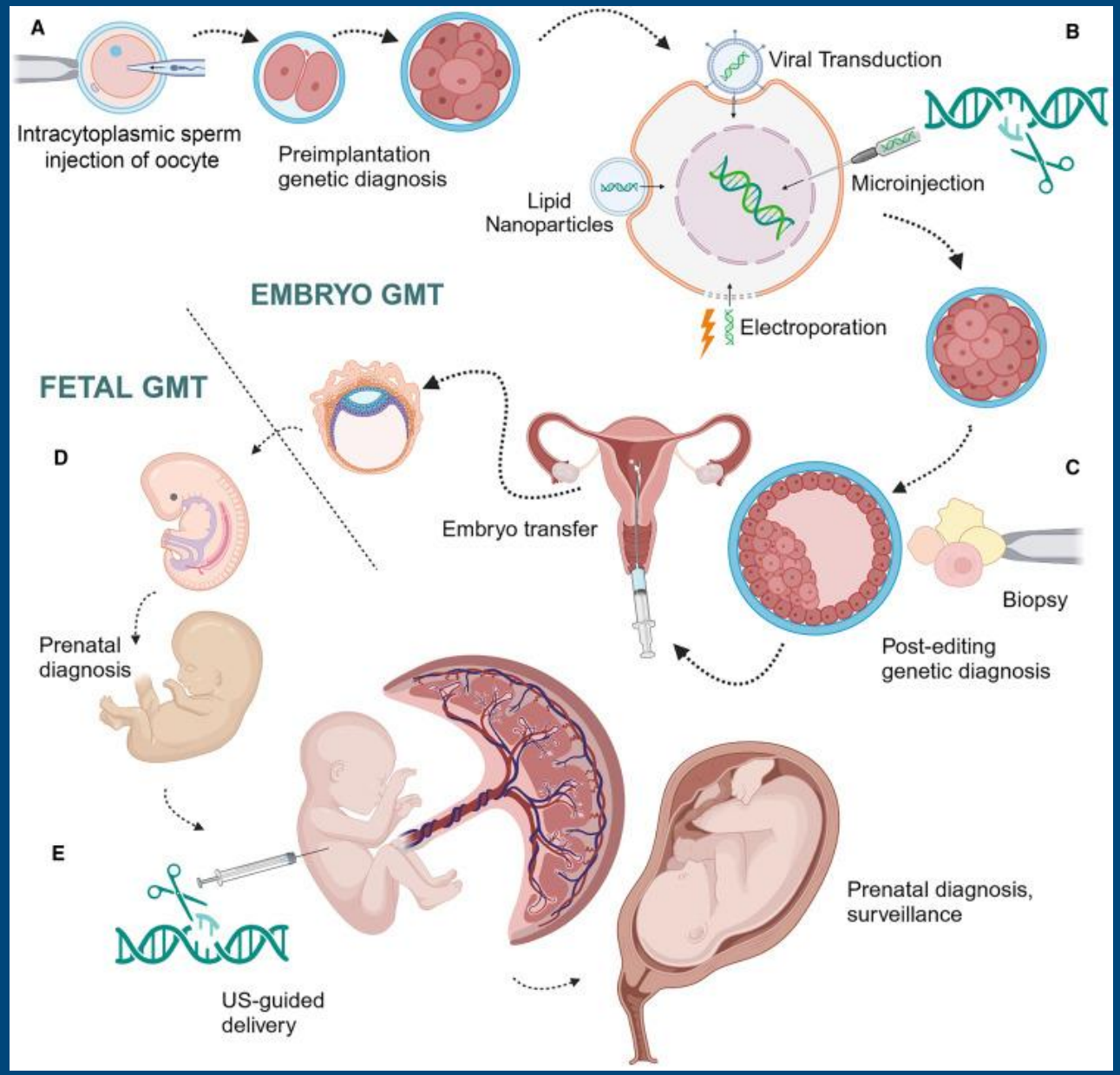
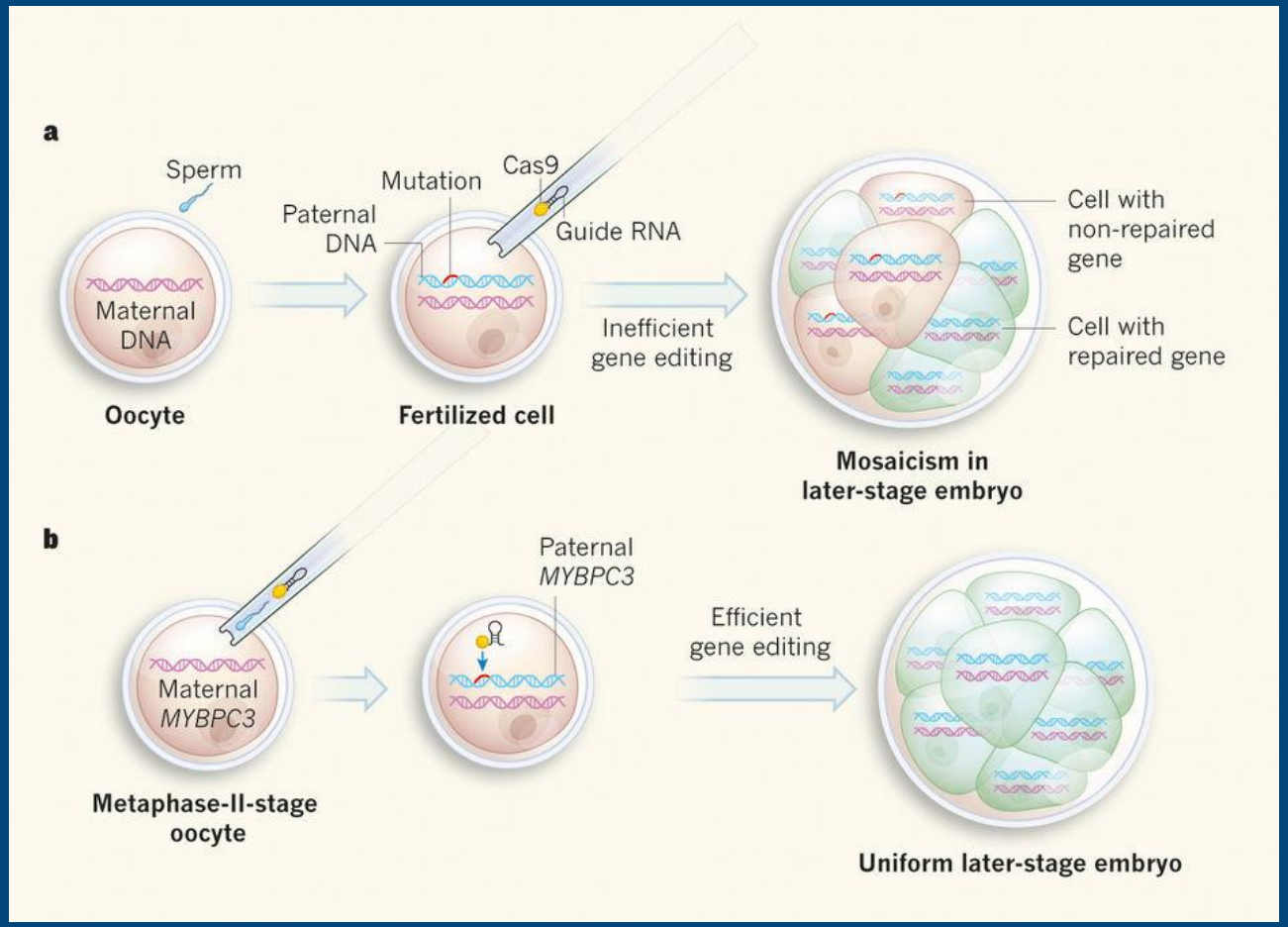


Embryo editing

- https://www.cell.com/molecular-therapy-family/advances/fulltext/S2329-0501%2824%2900045-7



Correction of a pathogenic gene mutation in human embryos using CRISPR

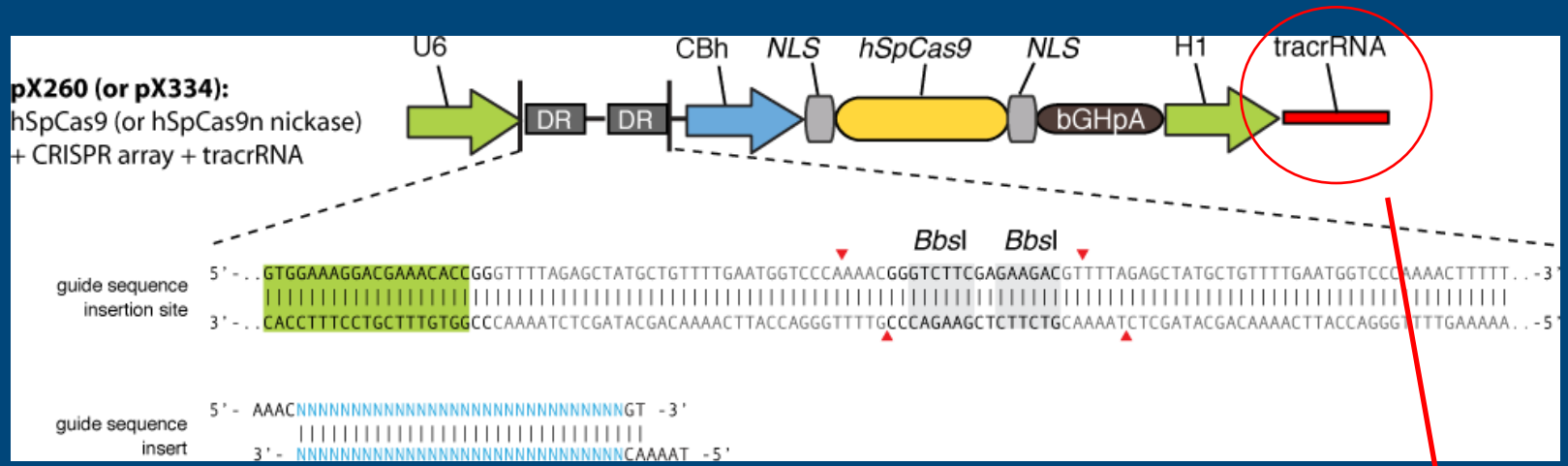


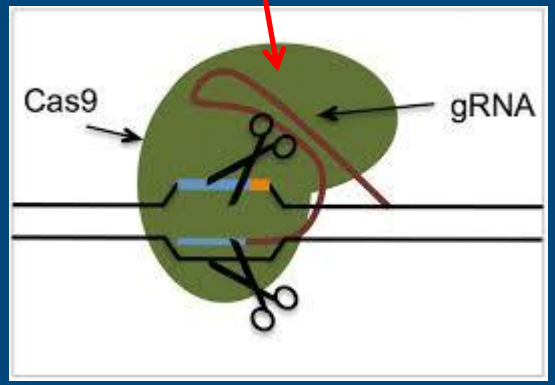
Insertie in elke cel,
en op 1 locatie,
in principe

http://www.nature.com/nature/journal/v548/n7668/fig_tab/nature23533_F1.html

Vector

CRISPR GENOME ENGINEERING TOOLBOX





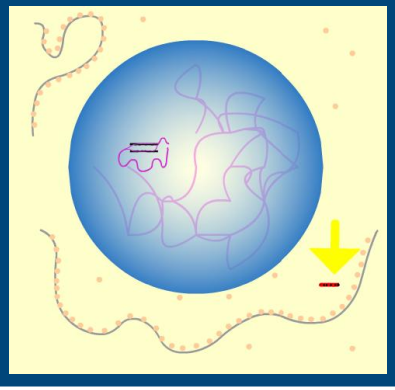
CRISPR (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats) is a microbial nuclease system involved in **defense against invading phages and plasmids**. CRISPR loci in microbial hosts contain a combination of CRISPR-associated (Cas) genes as well as **non-coding RNA elements** capable of programming the specificity of the CRISPR-mediated nucleic acid cleavage. We have recently harnessed the type II CRISPR nuclease system to facilitate genome editing in mammalian cells

The CRISPR/Cas system can be implemented in mammalian cells by co-expressing the bacterial Cas9 nuclease along with **the guide RNA**. Two forms of guide RNAs can be used to facilitate Cas9-mediated genome cleavage, using a CRISPR RNA array and tracrRNA or a **synthetic guide RNA** fusing the CRISPR RNA with the tracrRNA. These two systems are described below.

Bacteriofagen

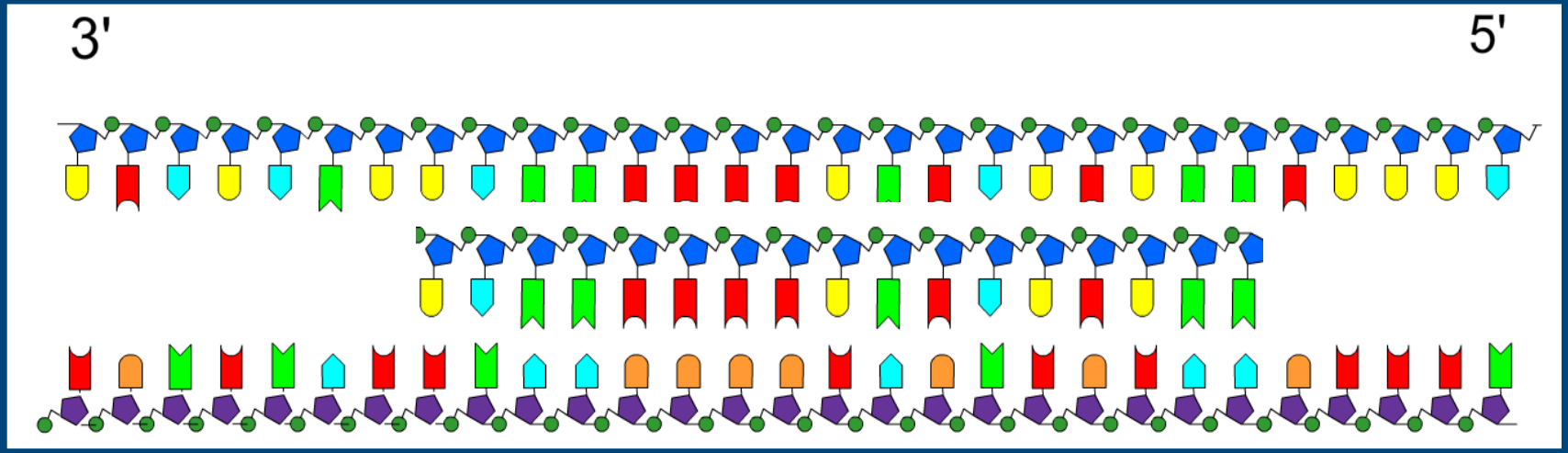
https://youtu.be/MnYppmstxIs
https://youtu.be/4YKFw2KZA5o

Biotech:
guideRNA/primer actie



RNA/
DNA

gRNA

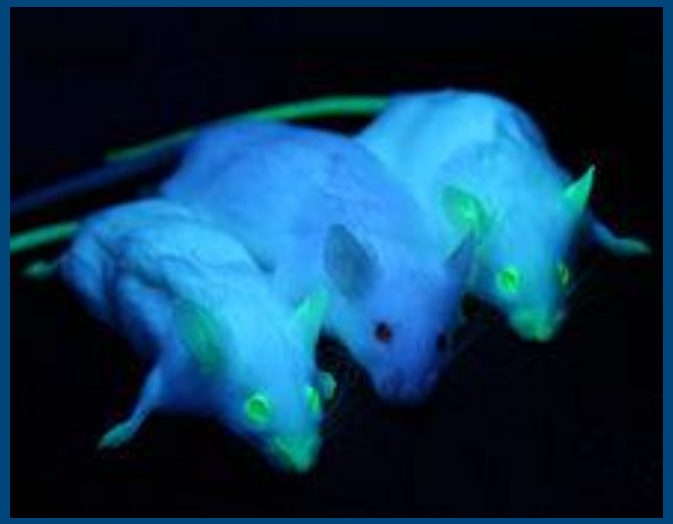


Kans = ¼ voor 1e nucleotide, en ¼ voor 2e nucleotide (er zijn 4 verschillende)
Kans = ¼ x ¼ = $1/4^2$ = 1 op 16 voor 2 nucleotiden ($1/4^n$ voor n nucleotiden)
Kans = $1/4^{20}$ voor 20 nucleotiden = 1/1000.000.000.000
Aantal nucleotiden in het genoom = 3.000.000.000

Klein stukje GuideRNA/primer zorgt al voor specifieke herkenning
=> in principe geen off-target effects

Orgaan v. systemisch (“germ-line”)

GFP-muis



Germ-line:
=> aanpassing van de soort

Handig voor onderzoek, bacterien en planten
Maar niet voor mensen.
Volgende generaties hebben de aanpassing ook
=> verbeteren/eugenetica

Evolutie v Gentechnologie

- Langzaam v Snel
- Slordig v Precies
- Moet functioneel zijn v Kan alles zijn wat je wilt

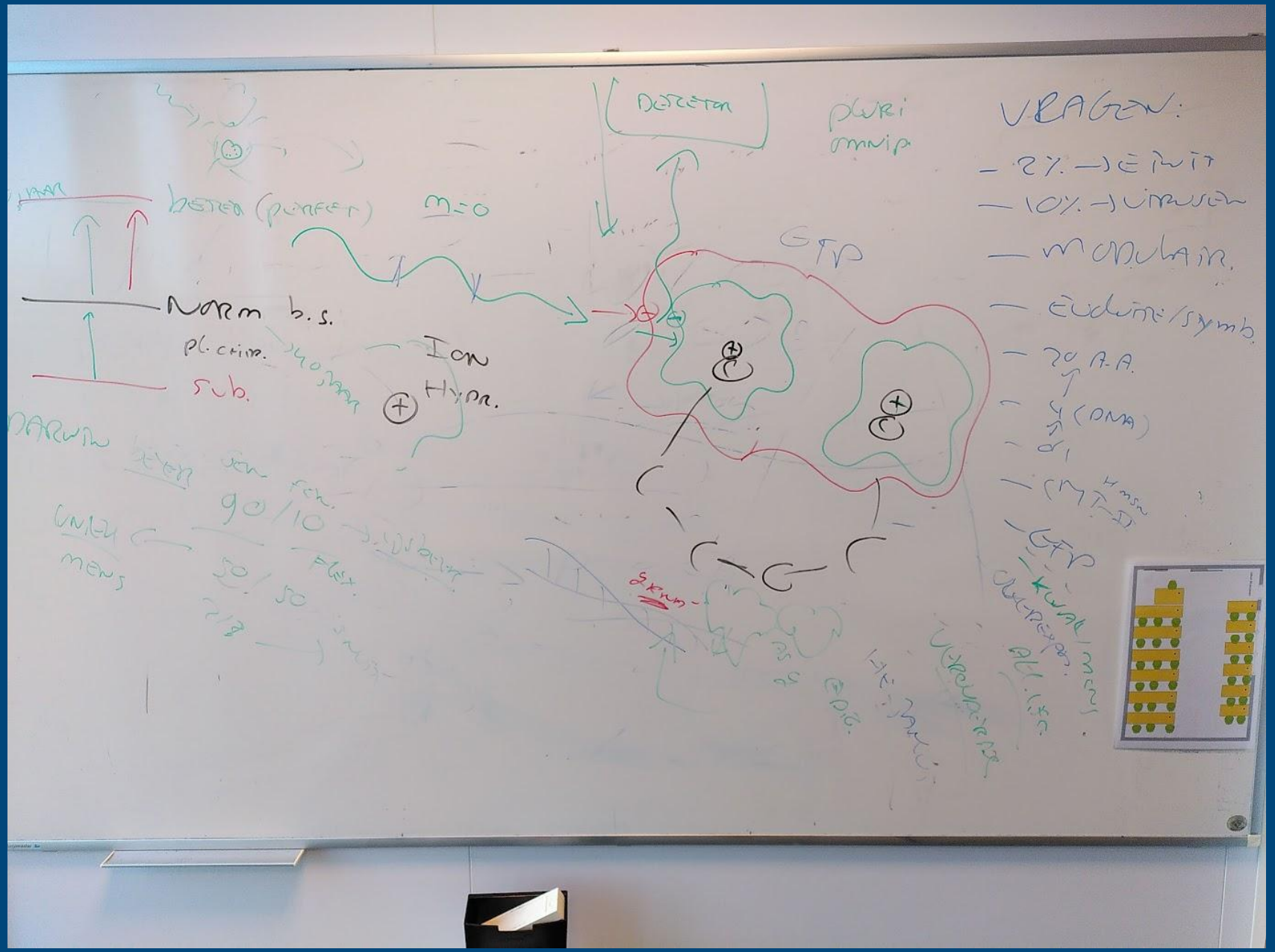
=> Gevaar!

=> ethiek!

Vragen

He Jiankui
Normaal
Moleculen
GFP
Overexpressie
2% genome proteins
10% genome viruses
(Endo)symbiose
Epigenetica
Evolutie
CMT-II
Life is based on feedback
20 Aminozuren
Vertalen codes
Flu pandemic
Wat maakt de mens bijzonder

Pluripotent
=> College 3



The He Jiankui Case (Gene-Edited Babies)

- **The Affair:** In 2018, Chinese researcher **He Jiankui** announced that he had created the world's first gene-edited babies (twin girls) using CRISPR technology.
- **HIV Context:** The stated goal of his research was to modify the *CCR5* gene in embryos to make them resistant to HIV, as the father was HIV-positive.
- **Consequences:** The experiment was widely condemned by the international and Chinese scientific communities as unethical and dangerous. He Jiankui was sentenced to three years in prison and fined for "illegal medical practices".

Normaal is variabel

- Beter

=> nieuwe normaal

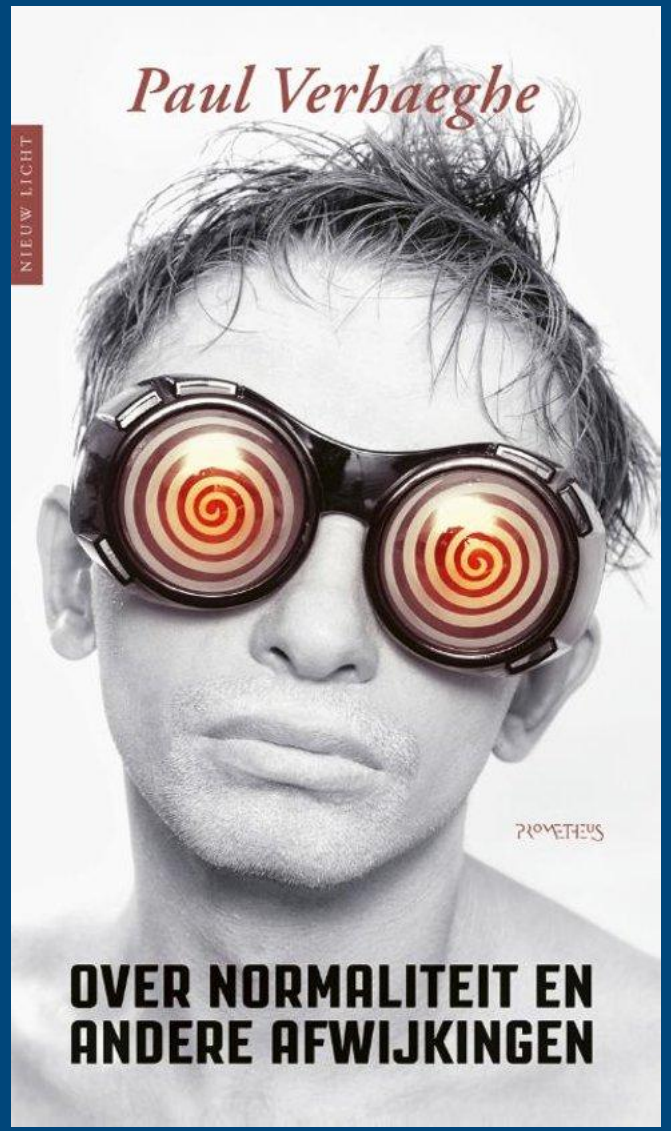
- Normaal
- Slechter

NB Slechter dan normaal repareren is OK. Van normaal naar beter is vaak niet OK

Toch streven we allemaal naar beter (Instagram)?

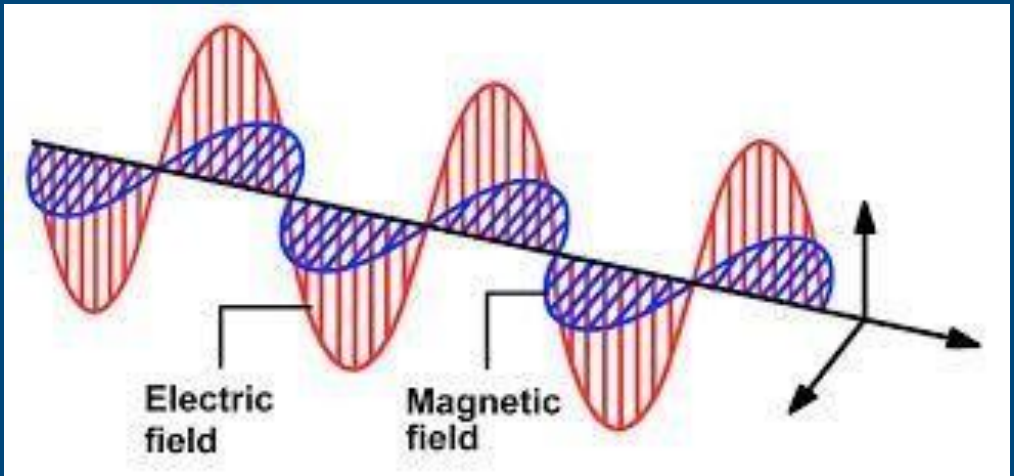
Wat is normaal ?

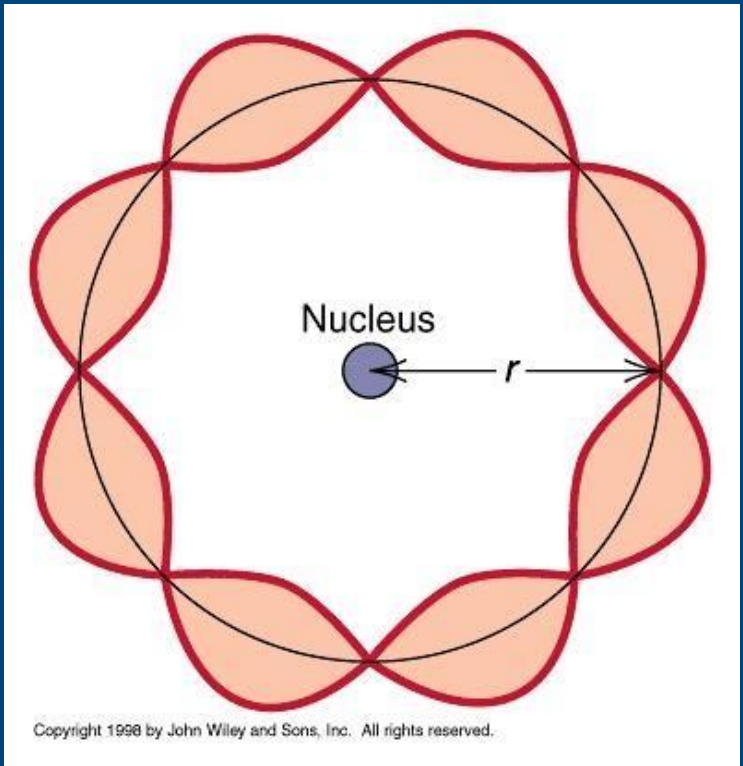
- Auteur: Paul Verhaeghe
- Uitgever: Prometheus
- Nederlands
- 9789044643220
- november 2019



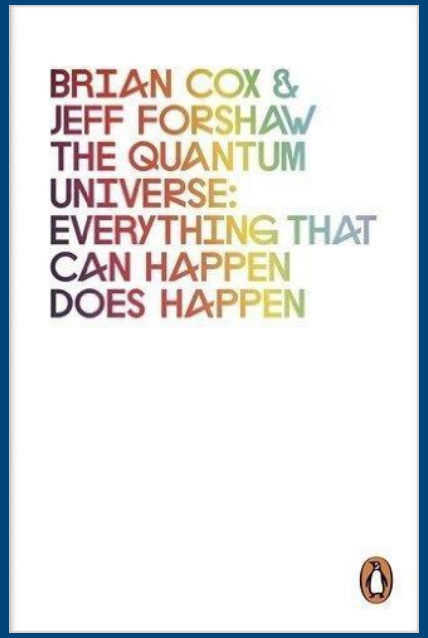
Celbio:

Fotonen en elektronen kunnen energie uitwisselen



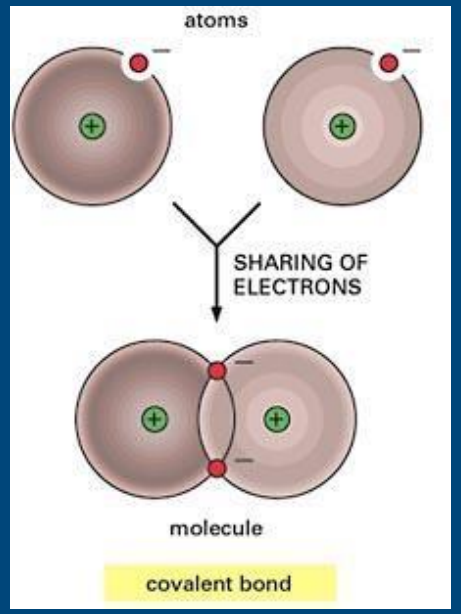


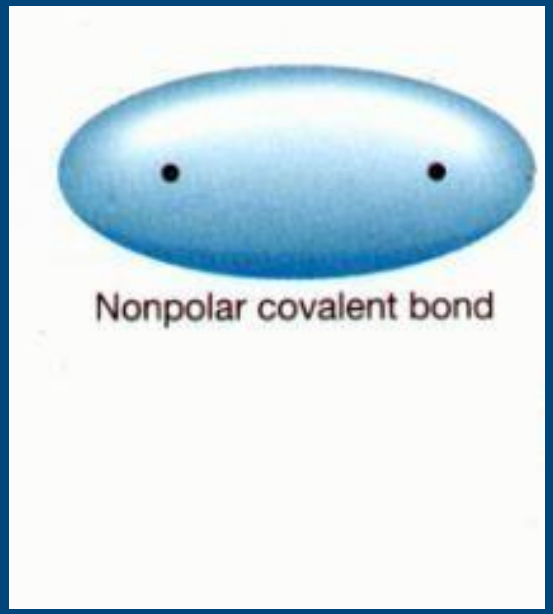
The quantum universe



Icke: Waarom dit kan is de helft van de natuurkunde (fermion/boson)
RL: Electromagnetic force interactions
https://en.wikipedia.org/wiki/Fundamental_interaction

Elektronen (wolken) kunnen zich spreiden
=> kost energie





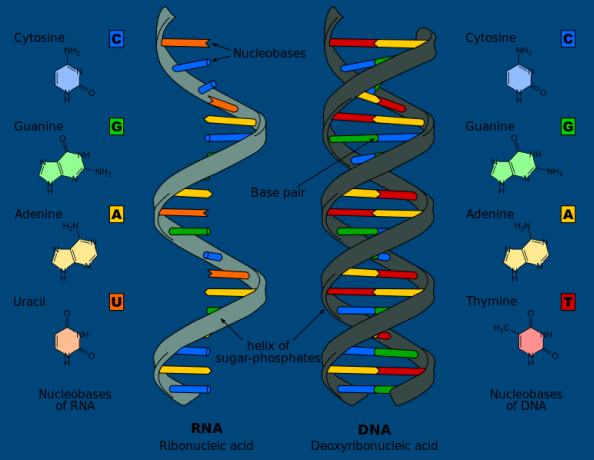
Elastiek vergelijking (geldt alleen voor de energie in het systeem)

=> Covalente binding als opgerekt elastiekje

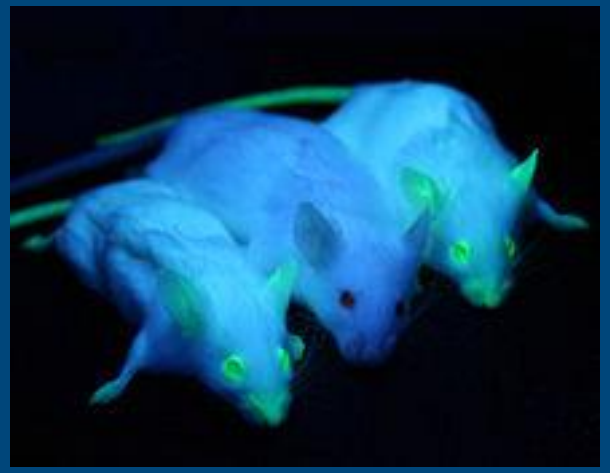
=> In een covalente binding zit energie opgeslagen!

Gen en stamcel techniek

GFP-gen



GGO (Genetisch gemodificeerd organisme)



Beenmerg

Stamcellen

(Green) Fluorescent Proteins

- In cell and molecular biology, the GFP gene is frequently used as a reporter of expression.[7]

- The GFP from *A. victoria* has a major excitation peak at a wavelength of 395 nm and a minor one at 475 nm. Its emission peak is at 509 nm, which is in the lower green portion of the visible spectrum.
- Many mutations have been made, including color mutants; in particular, blue fluorescent protein (EBFP, EBFP2, Azurite, mKalama1), cyan fluorescent protein (ECFP, Cerulean, CyPet, mTurquoise2), and yellow fluorescent protein derivatives (YFP, Citrine, Venus, YPet)
- https://en.wikipedia.org/wiki/Green_fluorescent_protein

Genetische manipulatie

- Overexpressie

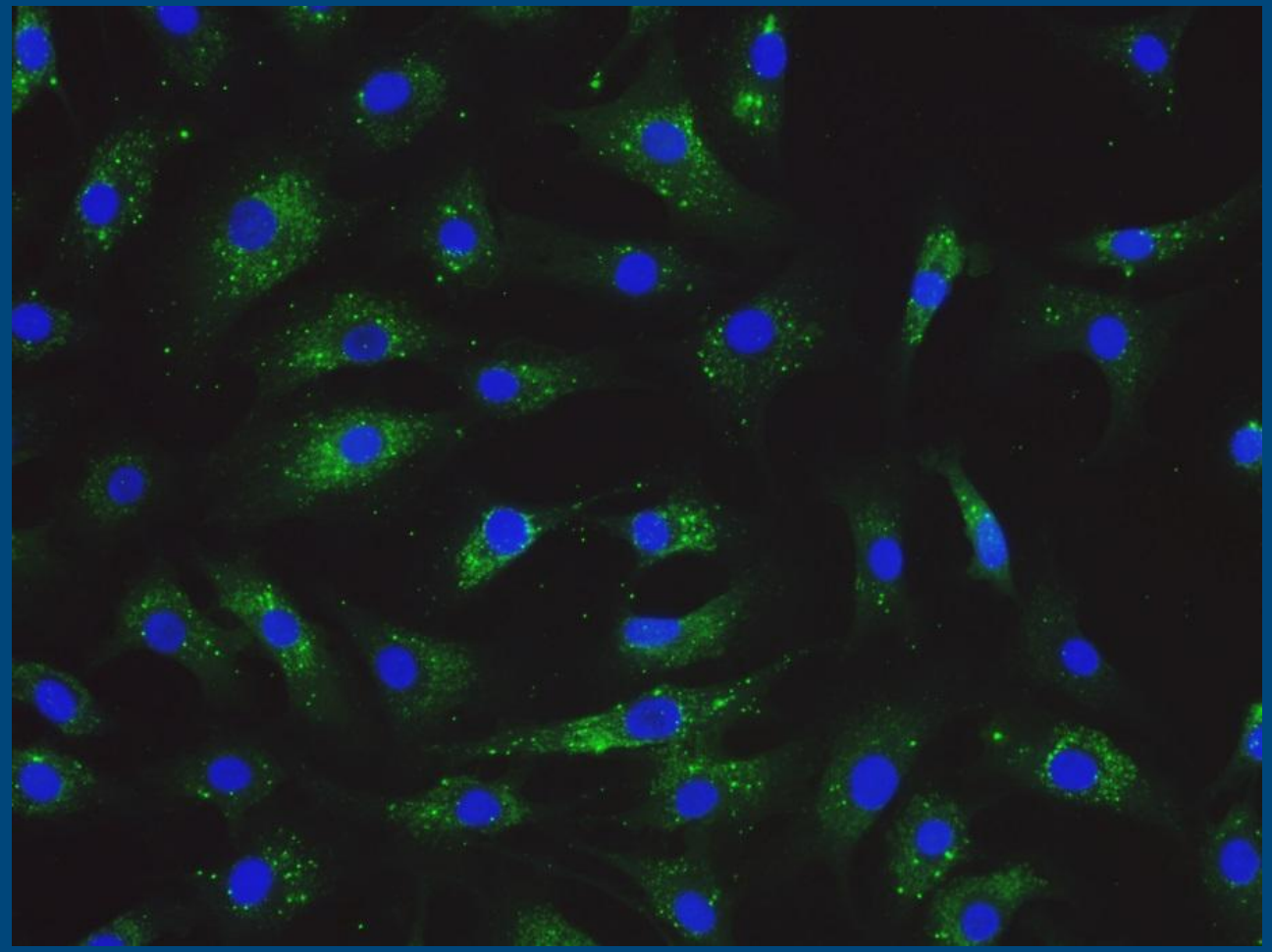
- Toevoegen van meer vector dan nodig

=> grote slagingskans

⇒ lekker makkelijk, maar leidt tot afwijkingen

⇒ niet erg in bacterie of plant

⇒ wel in muis of mens



GM in plants

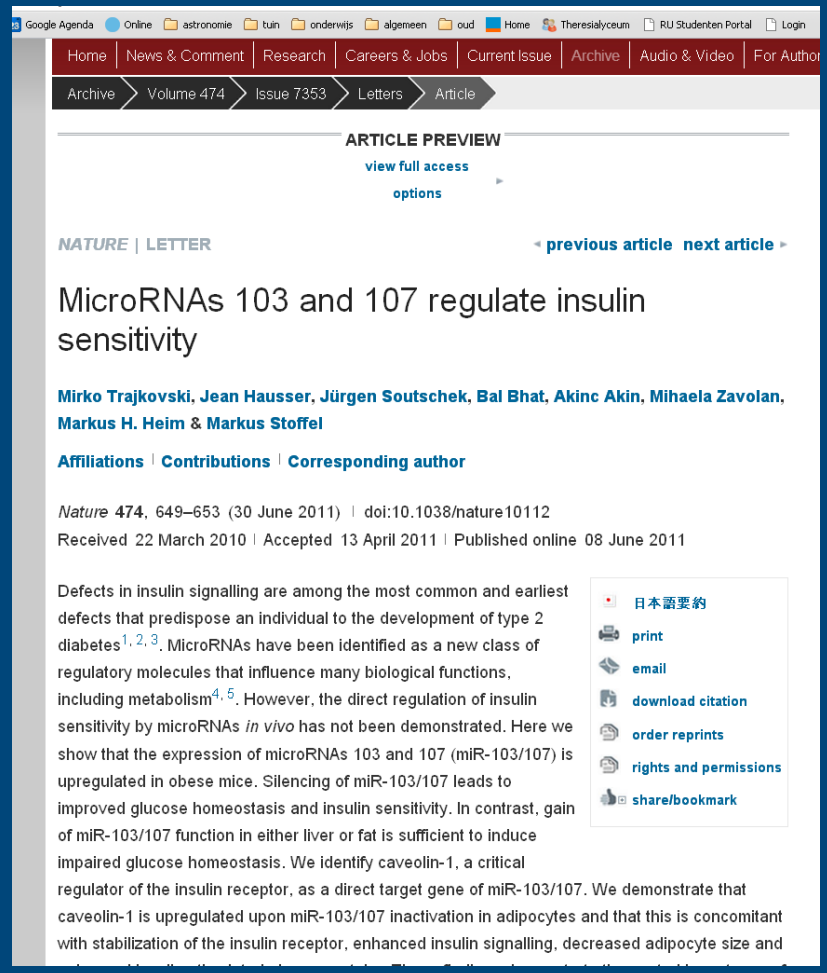
⇒ Veel meer pogingen tegelijk
⇒ QC is veel makkelijker
⇒ Niemand vind het erg als er iets mis gaat met een plantje
⇒ Alleen probleem als modificatie terecht komt in milieu
⇒ Bijv. roundup resistentie

De RNA wereld
=> Epigenetica

Human genome project in 2000:
1-2% DNA codeert voor eiwit (via mRNA).

The Encyclopedia of DNA Elements (ENCODE) project reported in 2012 that over 80% of DNA in the human genome "serves some purpose"
=> no junk

Oa non-coding RNA's (microRNA)
⇒Belangrijke extra regelniveaus in de celkern
=> fine-tuning genexpressie (belangrijk met maar 20.000 genen)
⇒Encode link http://www.nature.com/encode/#/threads



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MicroRNAs 103 and 107 regulate insulin sensitivity

Mirko Trajkovski, Jean Hausser, Jürgen Soutschek, Bal Bhat, Akinc Akin, Mihaela Zavolan, Markus H. Heim & Markus Stoffel

Affiliations | Contributions | Corresponding author

Nature **474**, 649–653 (30 June 2011) | doi:10.1038/nature10112
Received 22 March 2010 | Accepted 13 April 2011 | Published online 08 June 2011

Defects in insulin signalling are among the most common and earliest defects that predispose an individual to the development of type 2 diabetes[1, 2, 3]. MicroRNAs have been identified as a new class of regulatory molecules that influence many biological functions, including metabolism[4, 5]. However, the direct regulation of insulin sensitivity by microRNAs *in vivo* has not been demonstrated. Here we show that the expression of microRNAs 103 and 107 (miR-103/107) is upregulated in obese mice. Silencing of miR-103/107 leads to improved glucose homeostasis and insulin sensitivity. In contrast, gain of miR-103/107 function in either liver or fat is sufficient to induce impaired glucose homeostasis. We identify caveolin-1, a critical regulator of the insulin receptor, as a direct target gene of miR-103/107. We demonstrate that caveolin-1 is upregulated upon miR-103/107 inactivation in adipocytes and that this is concomitant with stabilization of the insulin receptor, enhanced insulin signalling, decreased adipocyte size and

日本語要約
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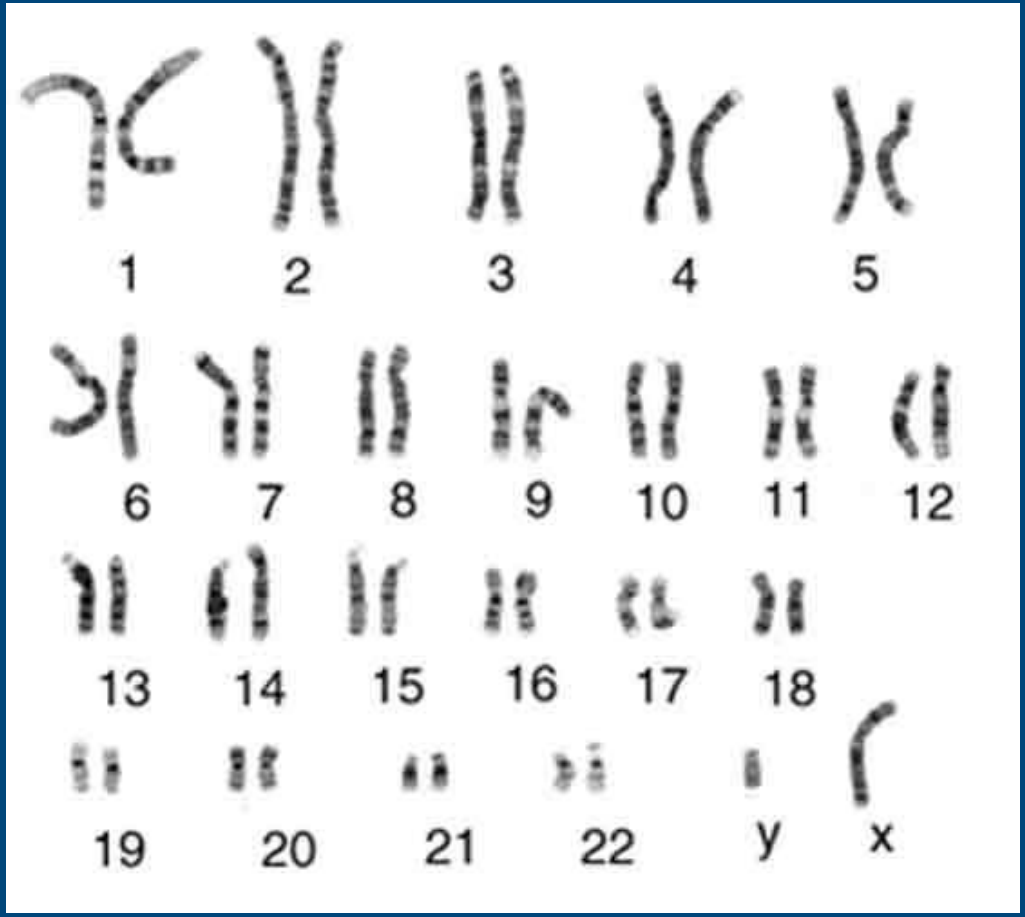
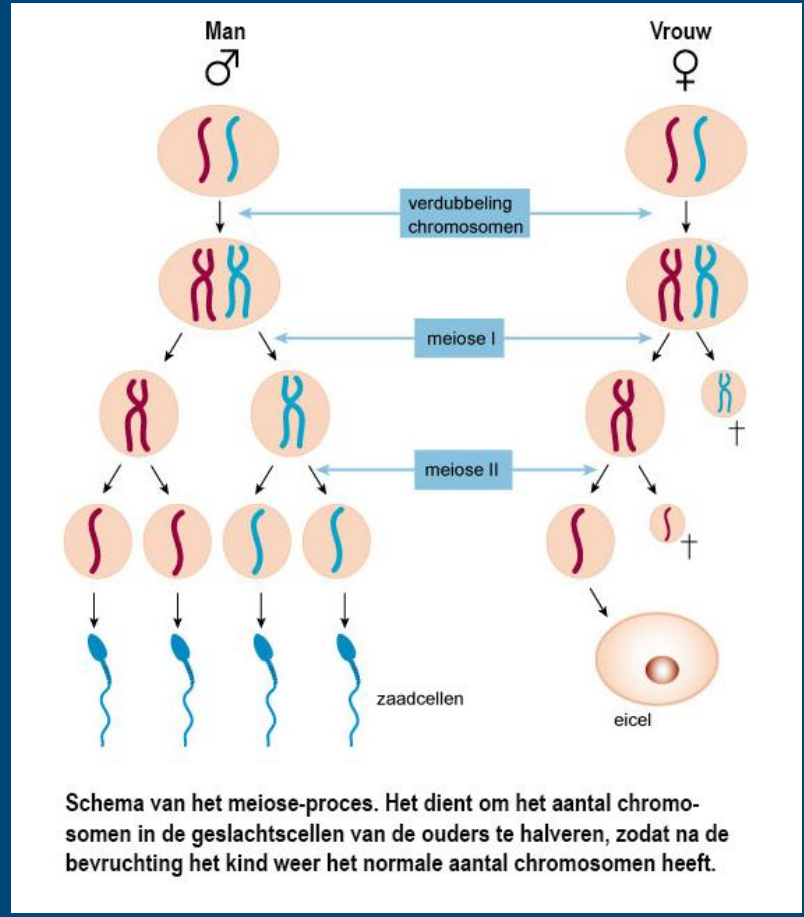
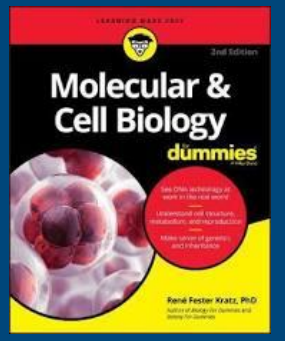
Ouderwetse Genetica: 23 Chromosomen (karyogram)
Alles 2x (behalve XY) => 46 chromatiden





Schema van het meiose-proces. Het dient om het aantal chromosomen in de geslachtscellen van de ouders te halveren, zodat na de bevruchting het kind weer het normale aantal chromosomen heeft.



H.14, p225

https://youtu.be/qCLmR9-YY7o

- Alles dubbel => controle op foutjes
- Sexuele voorplanting => variatie!

Meiose => alles 1x

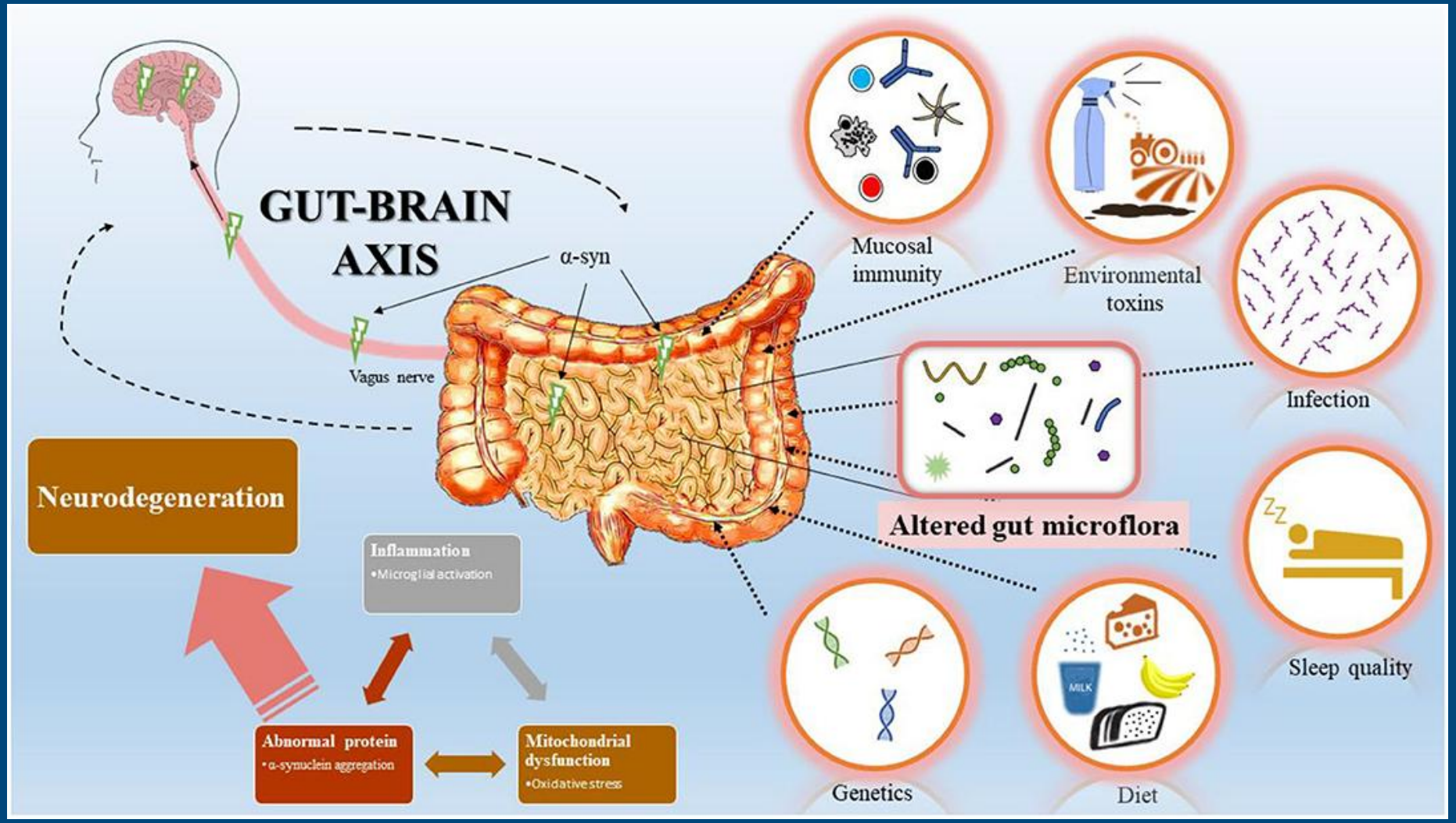
Bevruchting => alles 2x

NB Voor Meiose is er eerst nog een verdubbeling => alles 4x => 4 geslachtscellen met 1x

Gut brain axis

Half of you is not actually you

But does important things for you



The hologenome concept of evolution

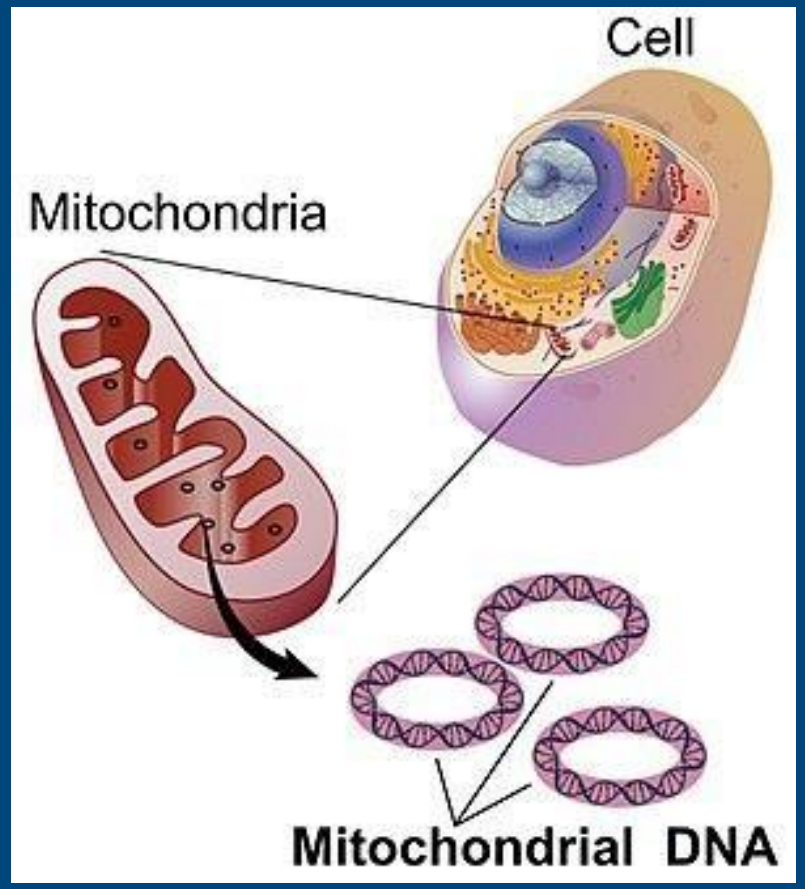
- The host with its microbiome, the holobiont, functions generally as a distinct biological entity anatomically, metabolically, immunologically, during development and in evolution
- Half of you is not actually you (but bacteria, mitochondria, viruses, transposons, fungi)
- Vgl met boom. Daar leeft ook van alles op, en in.

https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-018-0457-9

Leven is..

- Een informatie systeem
- En tegelijkertijd een materieel systeem
- Individuele processen kunnen ook buiten een levend systeem plaatsvinden
- Symbiotisch

Het Mitochondrium:
De energiefabriek van de cel



Mitochondria waren ooit vrij levende organismen, en hebben nog steeds hun eigen DNA.

Nadeel: Het genoom in de celkern en in de mitochondrien moet samenwerken

Gevolg: Al het (eukaryote) leven is symbiotisch
⇒ Endosymbiose (twee levensvormen die samen gaan)
Concept: Leven berust op samenwerking

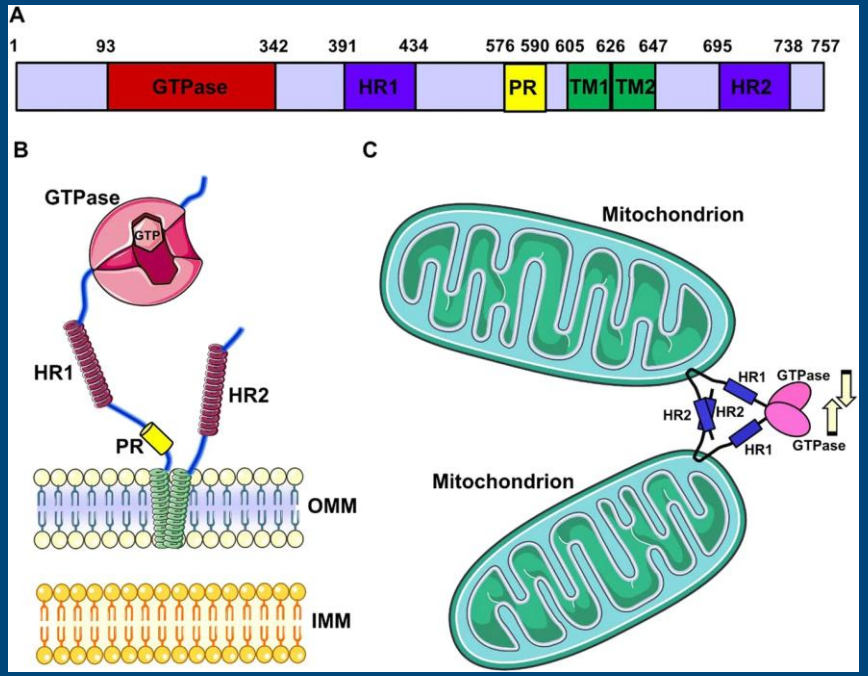
Voordeel: Geen last van sommige beperkingen van de thermodynamica (opp/inhoud)
⇒ Diffusie

https://nl.wikipedia.org/wiki/Endosymbiontentheorie

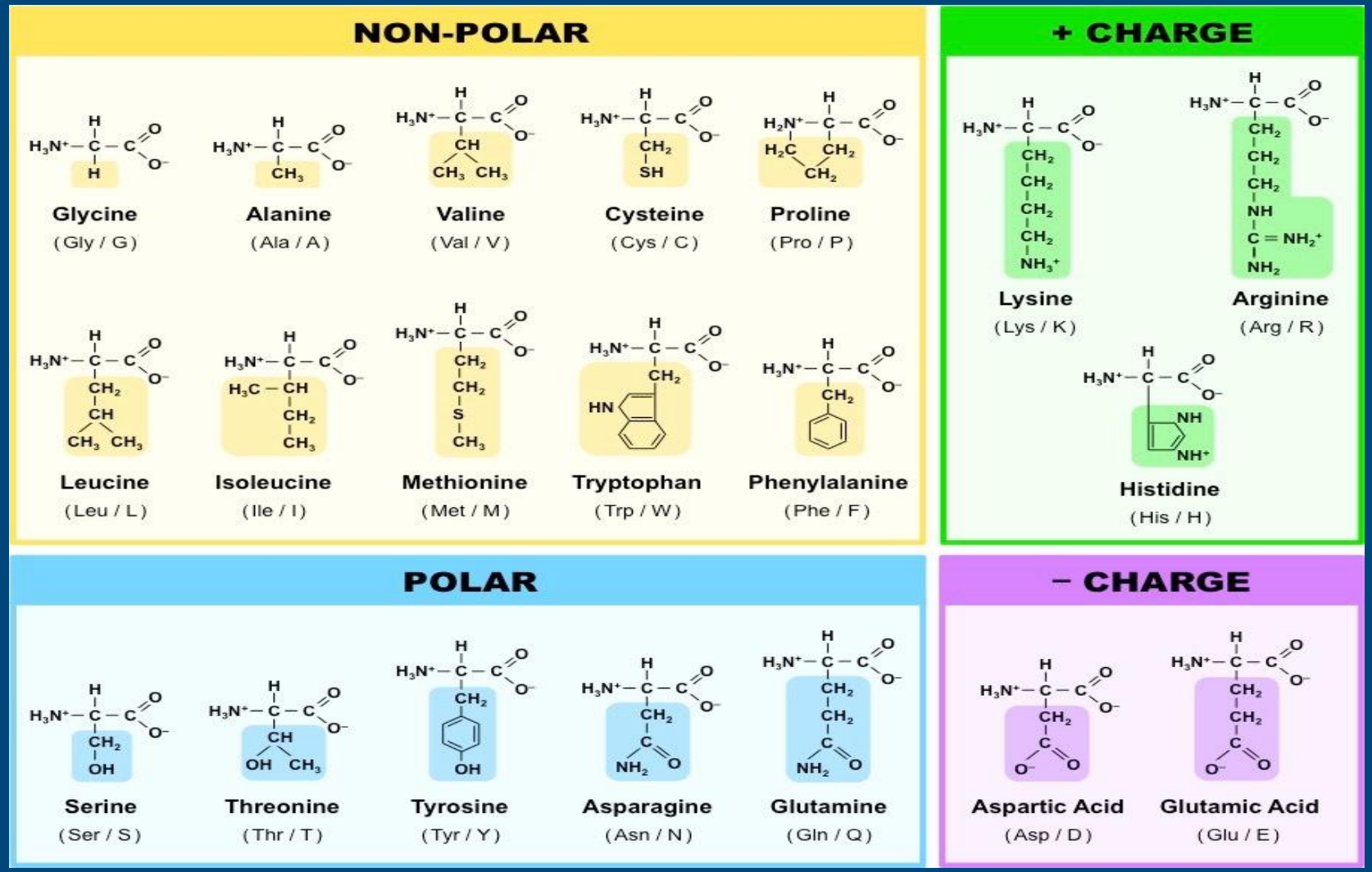
https://nl.wikipedia.org/wiki/Diffusie

CMT 2 / HMSN 2

- CMT/HMSN is de naam voor meerdere erfelijke spierziekten waarbij de zenuwen niet goed werken..
- https://www.spierziekten.nl/diagnose/charcot-marie-tooth-ziekte-van-type-2-cmt-2-hmsn-2/
- CMT2 is caused by a variety of gene mutations. The gene that is mutated determines the subtype of CMT2 that a person has.
- https://cmtausa.org/cmt2/
- https://www.ncbi.nlm.nih.gov/books/NBK1358/
- CMT2 is a hereditary motor and sensory neuropathy caused by mutations in the Mitofusin 2 gene a protein critical for mitochondrial fusion, transport, and energy production.
- https://www.nature.com/articles/s41419-017-0023-6
- **Developing a gene therapy for Charcot-Marie-Tooth disease: progress and challenges**
- https://pubmed.ncbi.nlm.nih.gov/40219666/

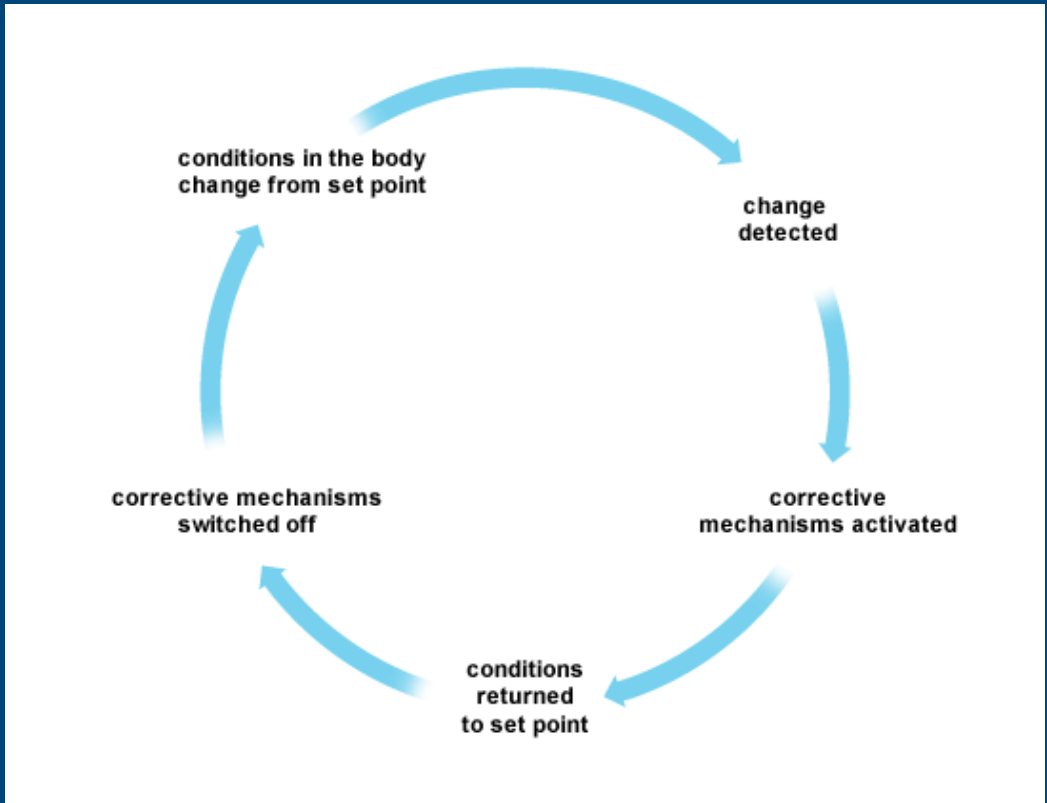


Aminozuren: 20 kralen (of lego-blokjes) waarmee je kunt maken wat je wilt



Polar (polair): elektronen zijn ongelijk verdeeld.

Celbio 4: Feedback is continuous



For example: Biological clock, or Nerve cell firing, where input generates output, which generates input, which generates output…

Analogie is een stad:
Beetje centrale aansturing maar eigenlijk gevolg van activiteit van individuele onderdelen.
Bottom-up ipv top-down (vgl spons)

En, er is altijd iets dat het evenwicht verstoord (al is het maar entropie), dus er is continue correctie

Behoud van ordening mbv feedback (kost energie).
Proces is metastabiel, maar bestaat al 4 miljard jaar

https://www.youtube.com/watch?v=AZUeKoD_3y0

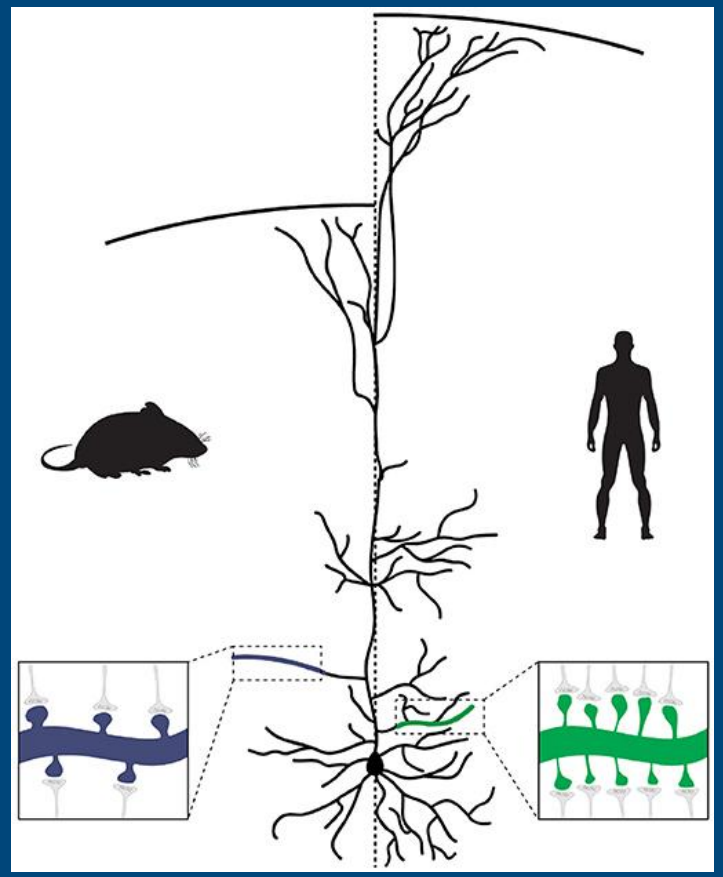
Regelneef

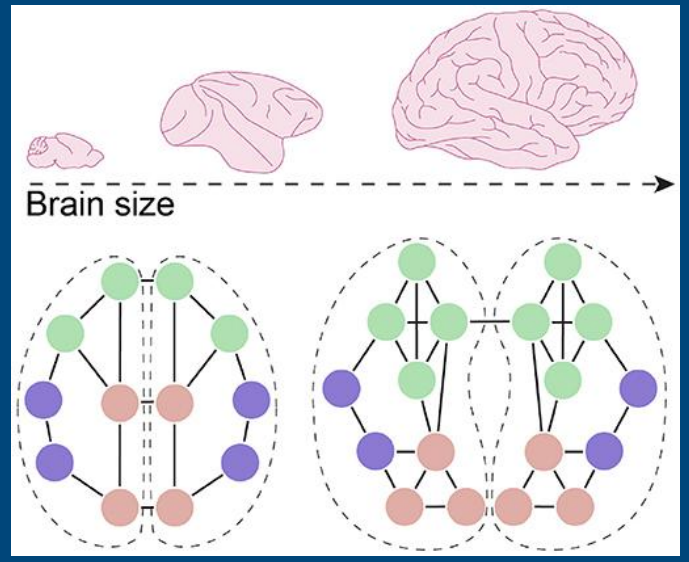
- https://youtu.be/Pw1iMFY56LM?t=365
- Alles top down organiseren is onbegonnen werk
- Leven maakt juist gebruik van natuurwetten, en is zelf organiserend

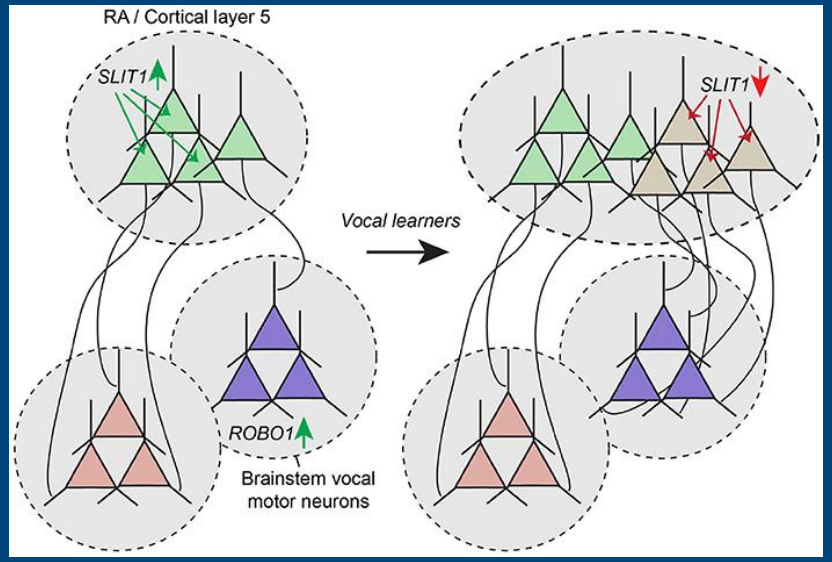
Wat maakt de mens bijzonder:

More connections

More flexible connections







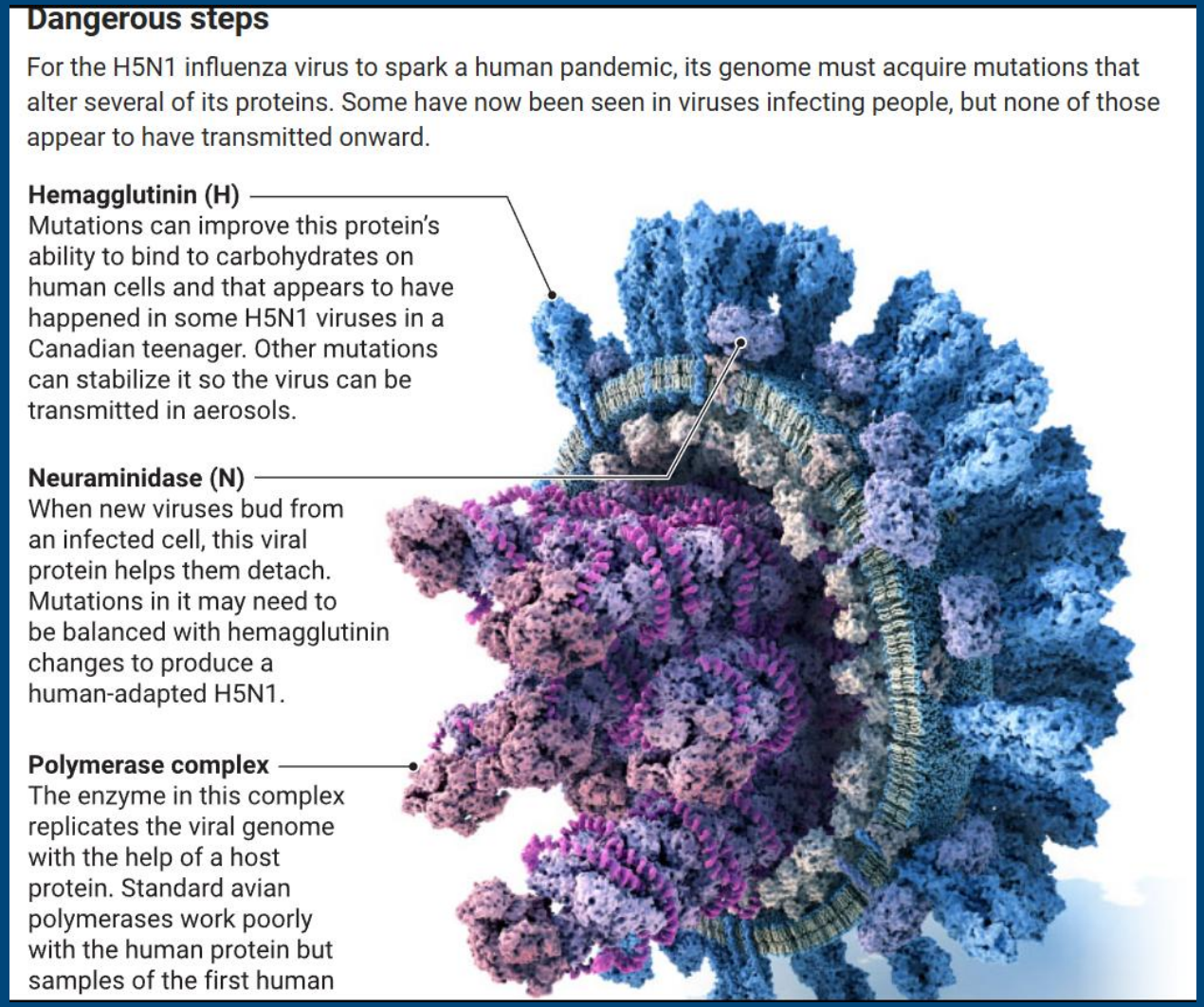
In human pyramidal neurons (PNs), a larger number of branch points leads to a more extensive and complex dendritic tree. Each branch also contains a larger number of synapses, resulting in an overall increase in the number of synapses made onto human PNs. In addition, because of the increased cortical thickness of the human brain, the apical dendrites of human PNs extend over a longer distance to reach layer I.

https://www.frontiersin.org/articles/10.3389/fncir.2021.787164/full

New genes, Synaptic pruning

Why hasn't the bird flu pandemic started?

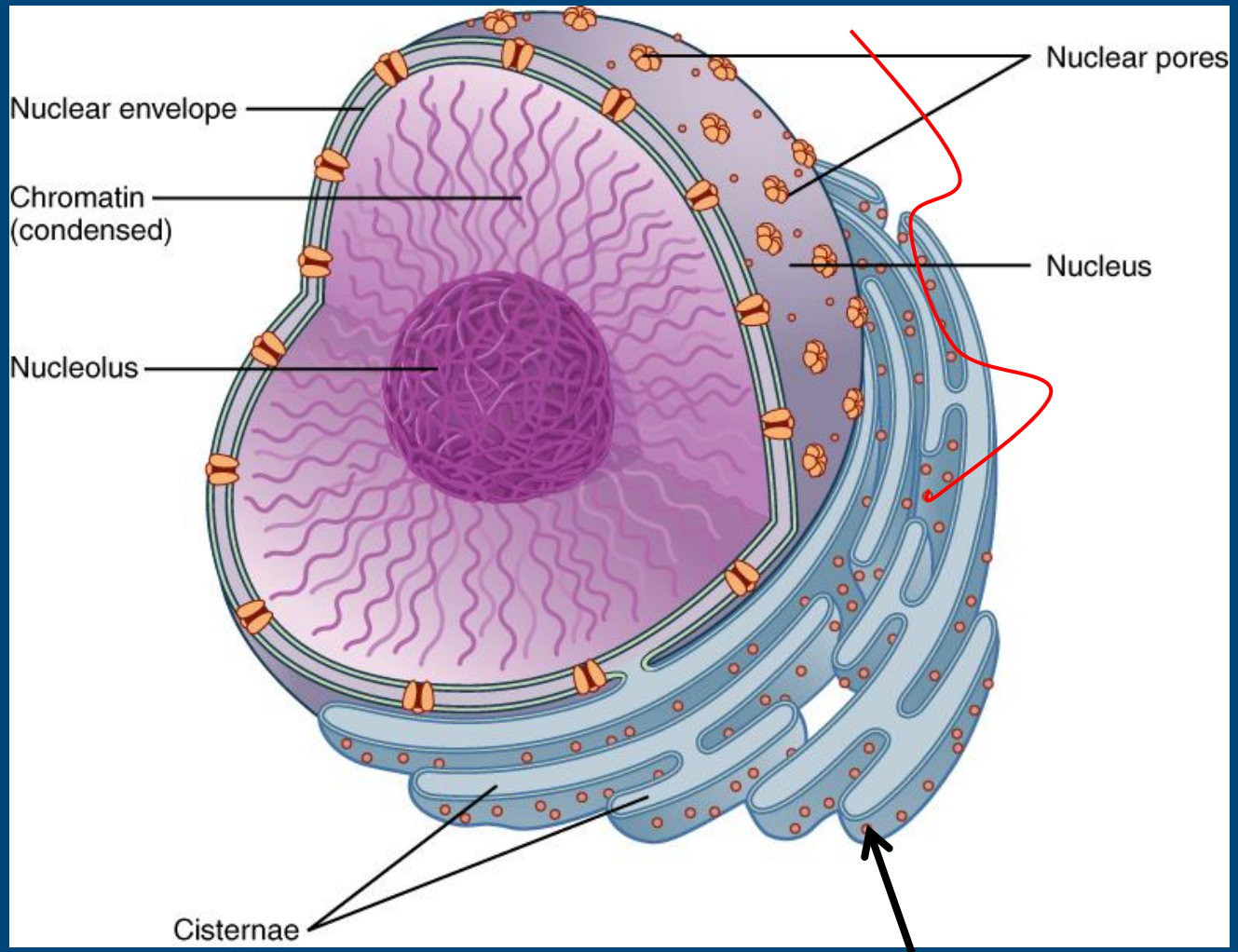
- Some scientists examining mutations found in H5N1 viruses fear major outbreak is imminent but others say pathogen remains unpredictable
- One simple answer is that the virus may just need more time to hit the right combination of mutations. The high mutation rate of influenza viruses should tip the odds in H5N1's favor: "My rule of thumb is that one in 4000 [virus] particles will have a mutation at the amino acid that you are interested in," Paulson says. Indeed, one polymerase mutation the virus likely needs, dubbed 627K because it leads to the amino acid lysine (K) at position 627 of the protein, has been found several times in strains infecting mammals but also in virus isolated from the first human case associated with the U.S. outbreak in dairy cows.



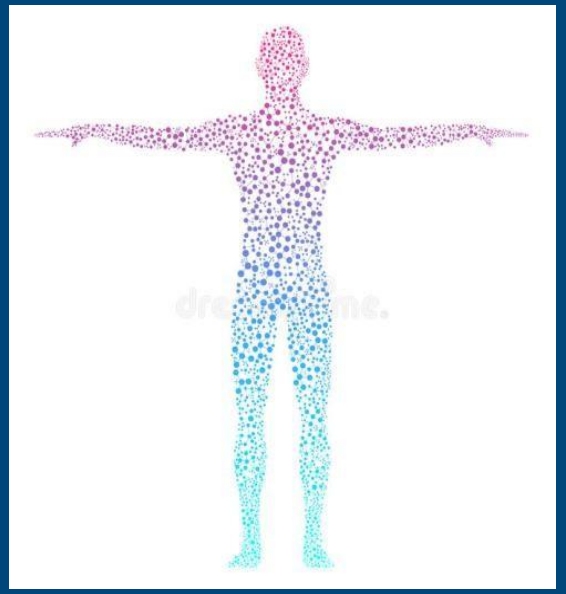
College 3 Biotechnologie.

DNA/RNA-technologie	=> gentherapie/mRNA therapie
Afweersysteem	=> Immunotherapie
Stamcellen	=> stamceltherapie
Cybernetica	=> verbeteren (integratie technologie en biologie)
Synthetic biology	=> wat is leven?

De celkern, met daarin DNA

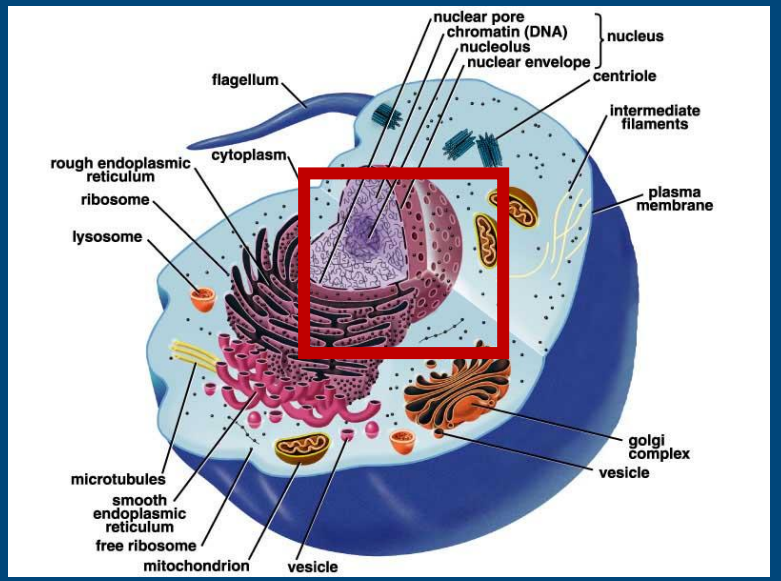


Ruw Endoplasmatisch Reticulum met ribosomen
(mRNA => eiwit)



Leven bestaat uit cellen https://youtu.be/SEejivHRIbE

Een (eukaryote) cel met celkern

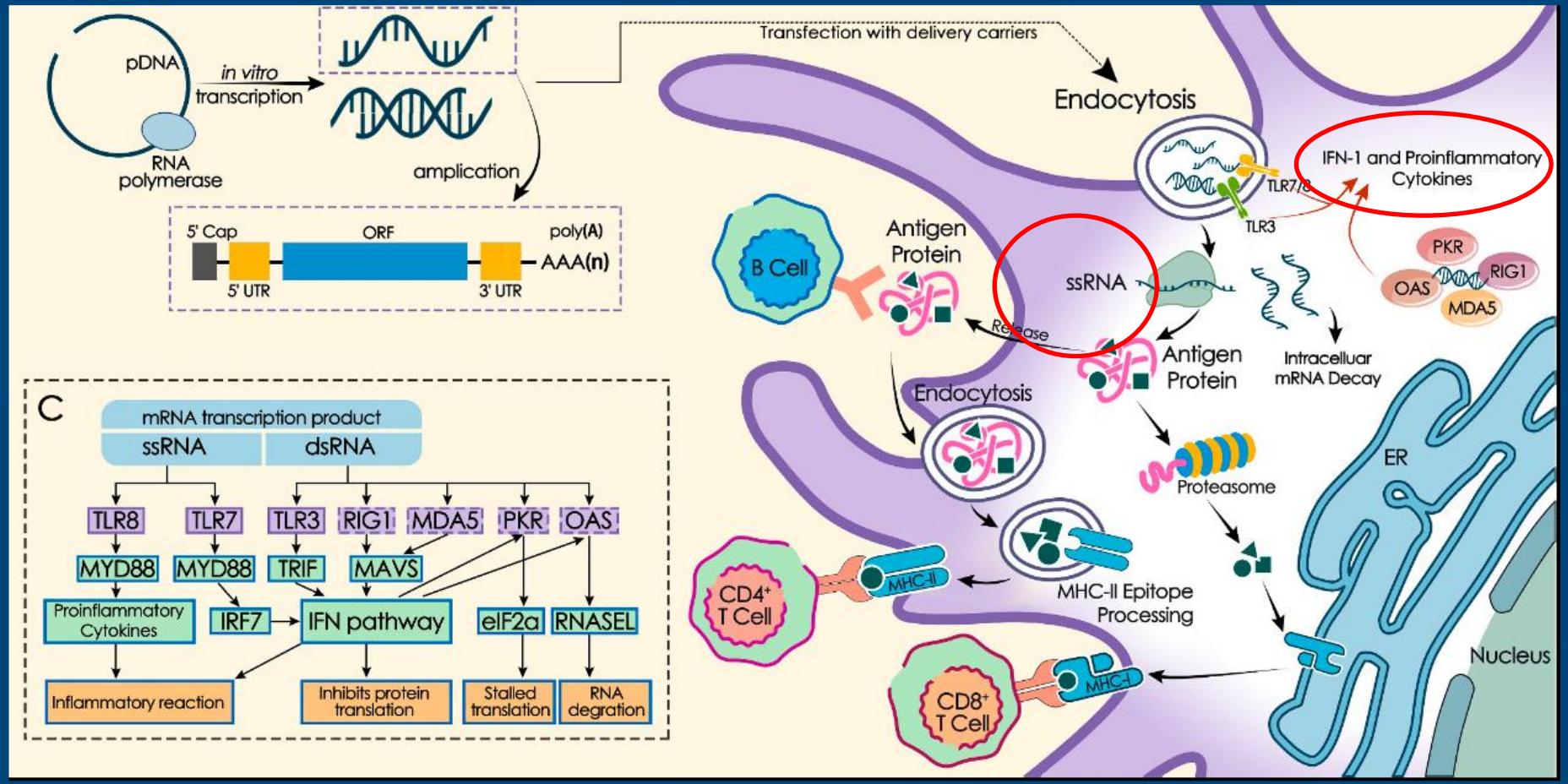


mRNA is transient

- RNA is veel minder stabiel dan DNA
- Wordt snel afgebroken (minuten-dagen)

=> Eigenlijk dus geen gentherapie

mRNA in Vaccin
Direct naar ribosoom, en meer



Werkingsmechanisme is niet helemaal duidelijk!

Intracellulaire afweer (tegen virussen)
De interferon respons

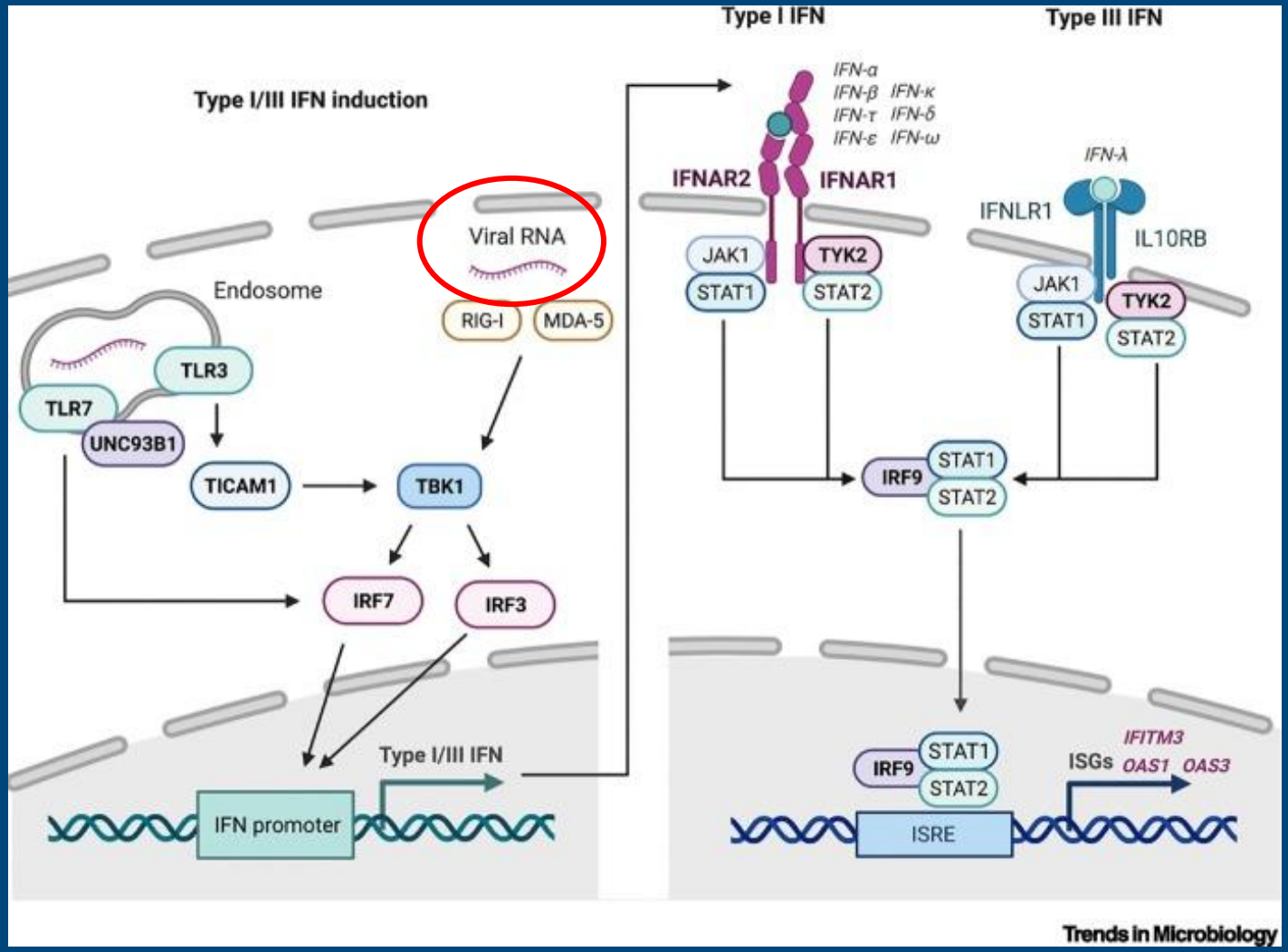
Simplified signaling cascades leading to transcription of type I/III interferon genes following infection with an RNA virus (*left panel*).

Simplified signaling cascades leading to transcription of interferon-stimulated genes (ISGs) following type I/III interferon stimulation (*right panel*).

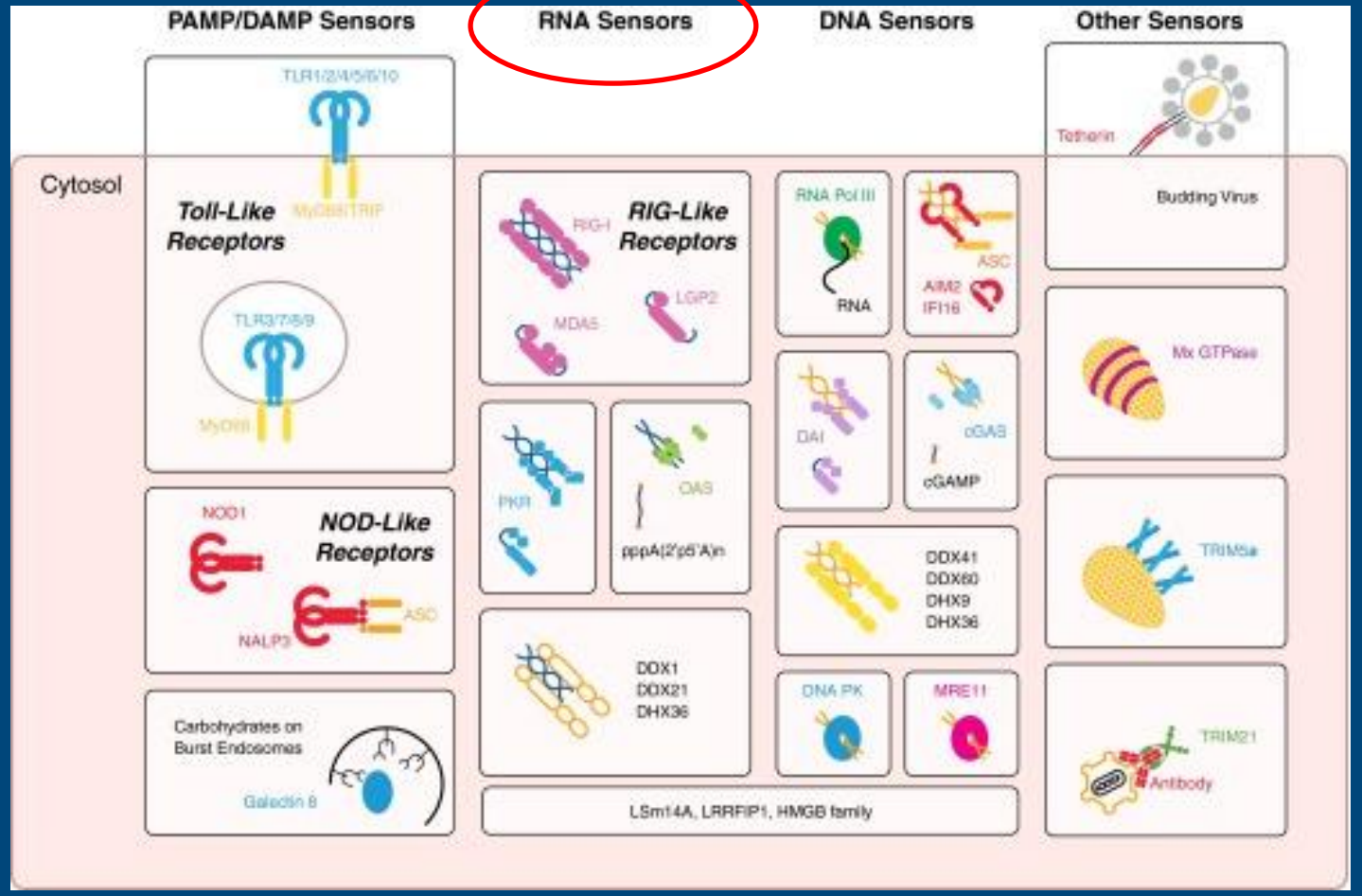
⇒Meer MHC

⇒Pro-inflammatoire cytokines

=> gevaar



Intracellulaire afweer: interferon => aspecifiek!

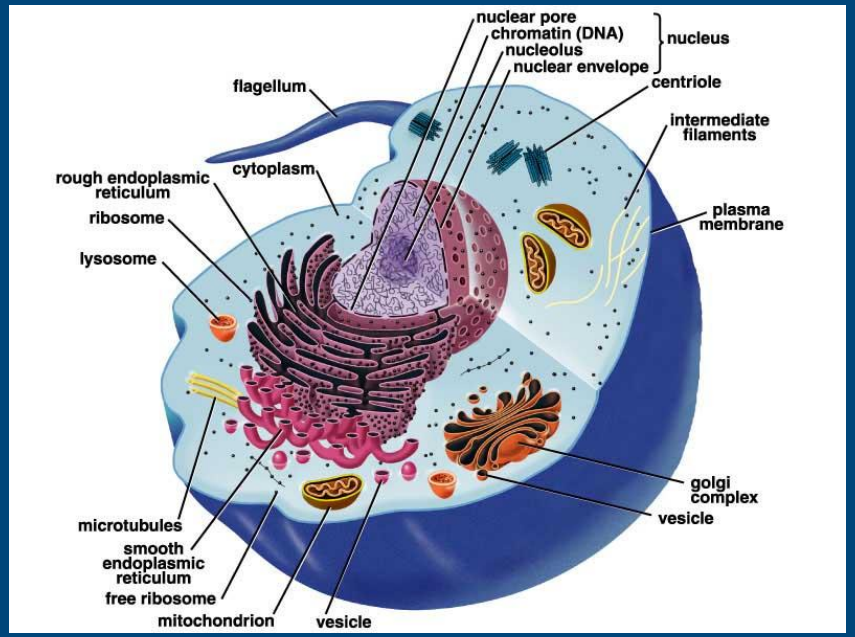


https://www.sciencemag.org/topic/crispr?r3f_986=http://science.sciencemag.org/content/327/5962/167.full

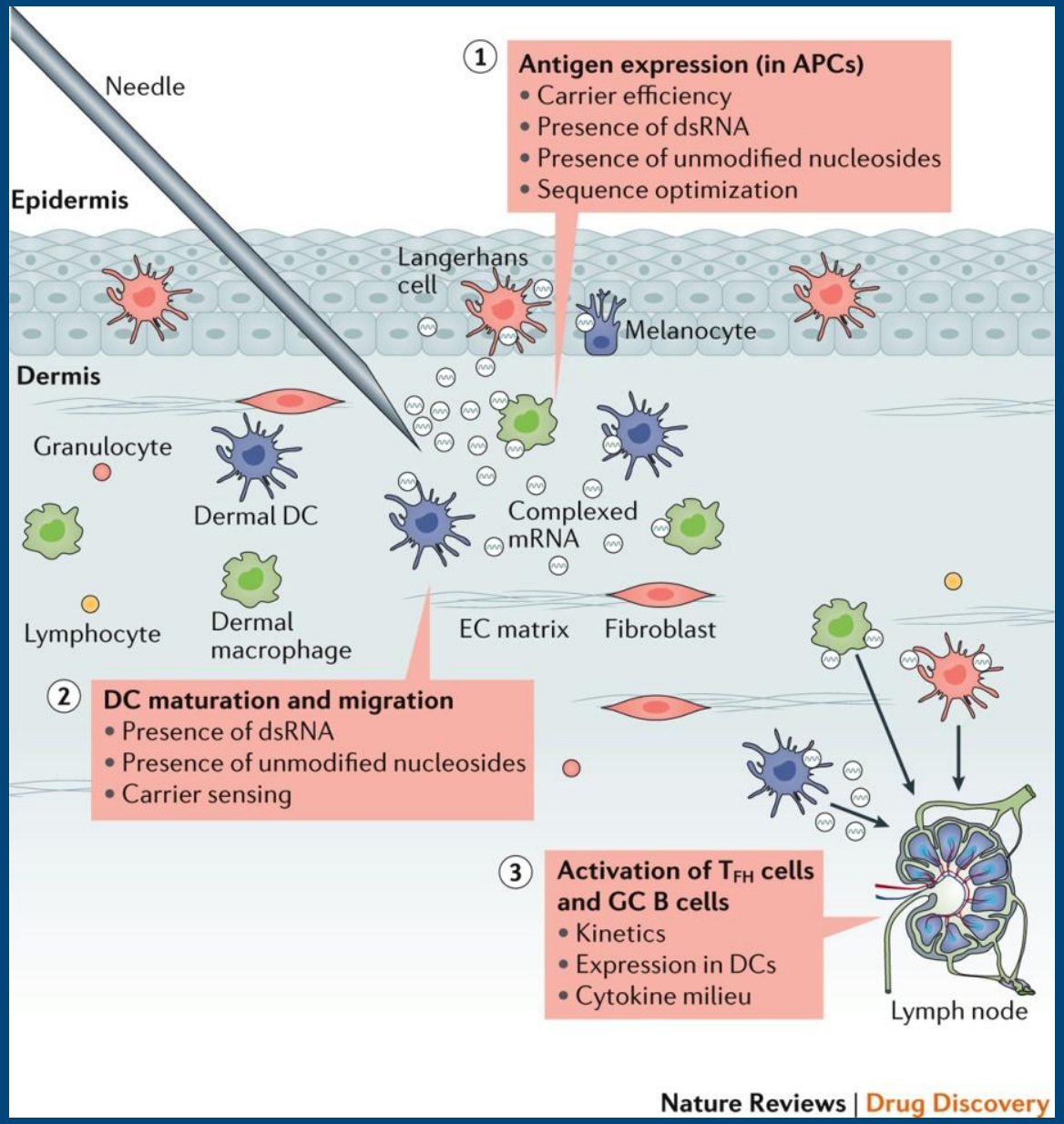
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4192899/

Intracellular pathogen sensors are soluble, cytoplasmic proteins. TLR, NLR, and galectin-8 sensing of burst endosomes detect danger associated with infection.

RNA komt in het cytoplasma normaal niet voor => virus!



The delivery problem, solved



mRNA vaccins
(Moderna, BioNtech)

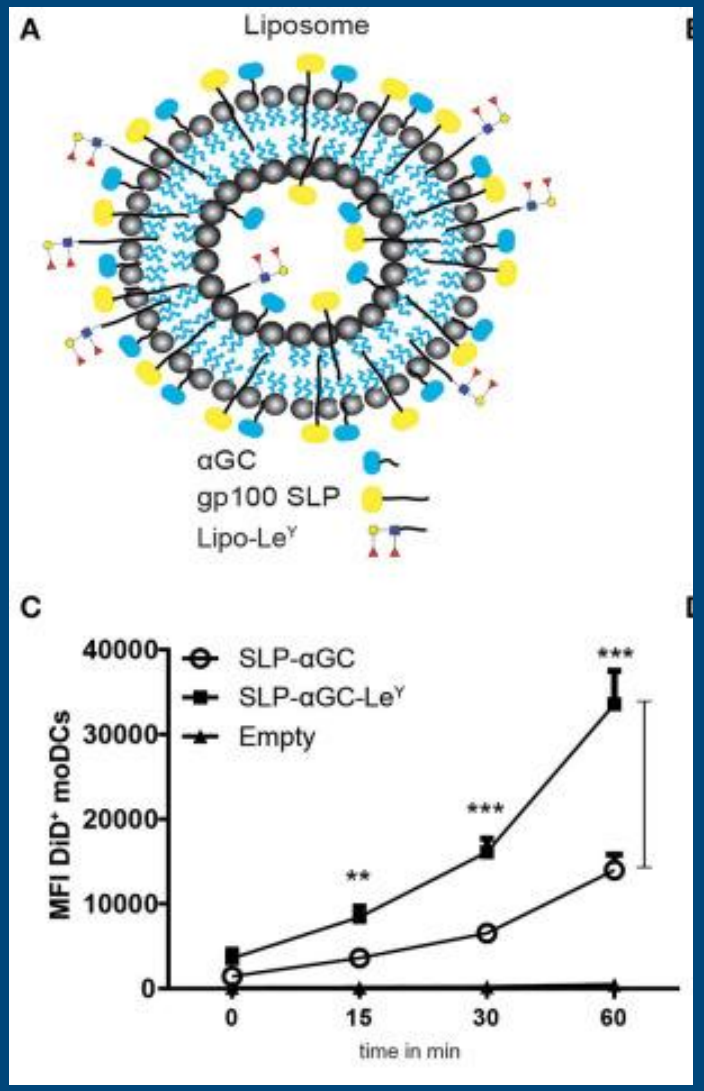
Zijn verpakt in liposomen die specifiek worden opgenomen door DC's in LK, die ze ziet als ziekmaker
=> presentatie in lymfeklier

mRNA zijn zo aangepast (modified nucleosides) dat ze heel efficient worden afgelezen (translatie)
⇒ Expressie van corona eiwit (alleen spike, dus geen volledig virus

Targeting DC's with liposomes => delivery

Dendritic cells (DC) are an attractive target for the initiation of strong immune responses as they possess the capability of capturing antigens and presenting them to specific T-cells for their activation. The skin harbors different types of antigen presenting cells, such as dermal DC (dDC) and Langerhans cells (LC) and is therefore an attractive site for delivery of vaccines. Delivery of vaccines to DC and LC can be addressed by targeting e.g., C-type lectin receptors such as Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN) and Langerin. This can be easily done using their natural glycan ligands, including Lewis Y (Le^Y)

=> MHC-II loading?



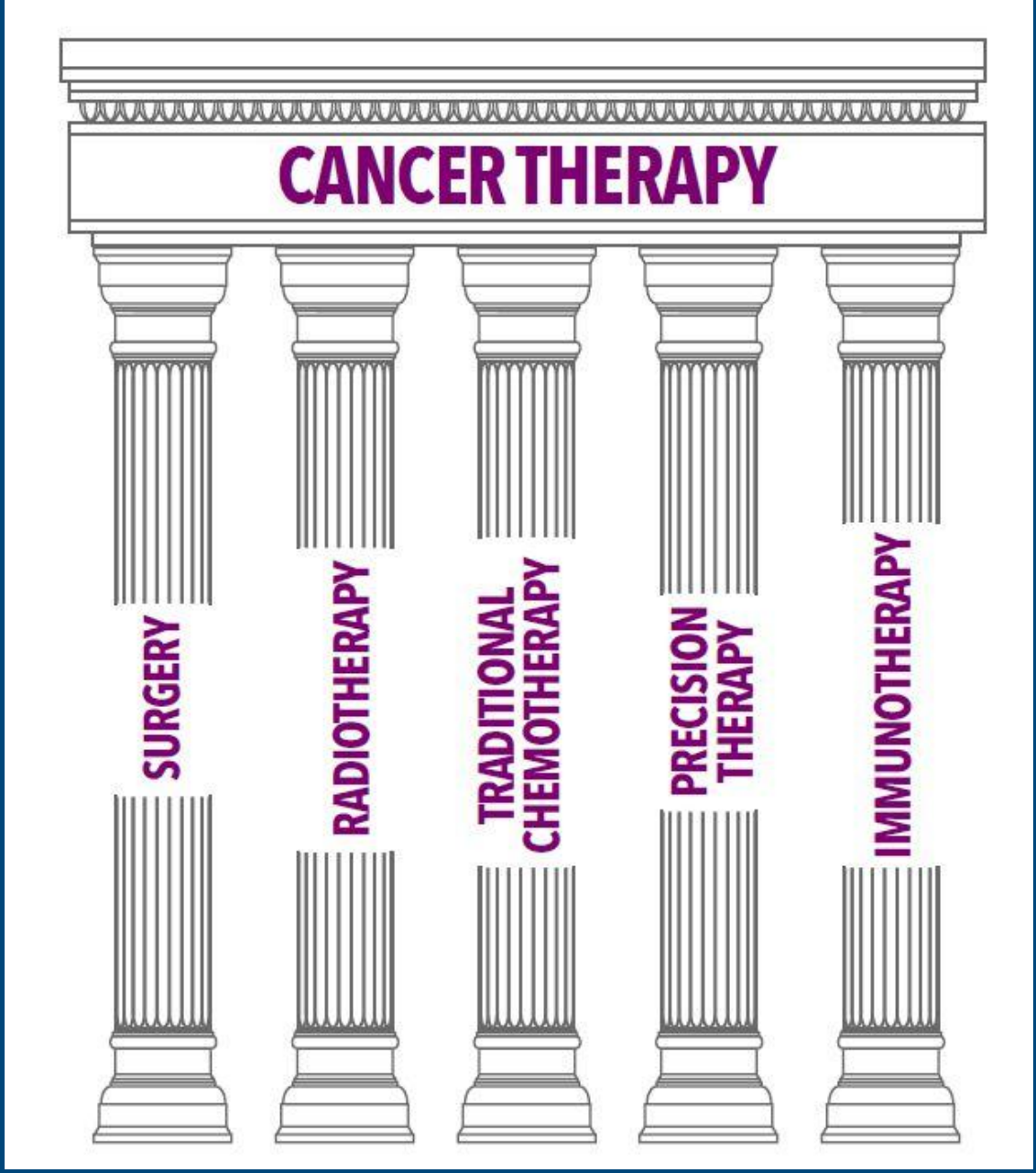
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7267035/

https://www.frontiersin.org/articles/10.3389/fimmu.2018.00590/full

mRNA tegen kanker

- https://youtu.be/R83JCXSjvJo?t=2121

Immunotherapy:



Surgery: wegsnijden

Radio: bestraling

Chemo: doden (alle) snel delende cellen

Precision: doden specifieke cellen (antilichamen)

Immuuntherapie:

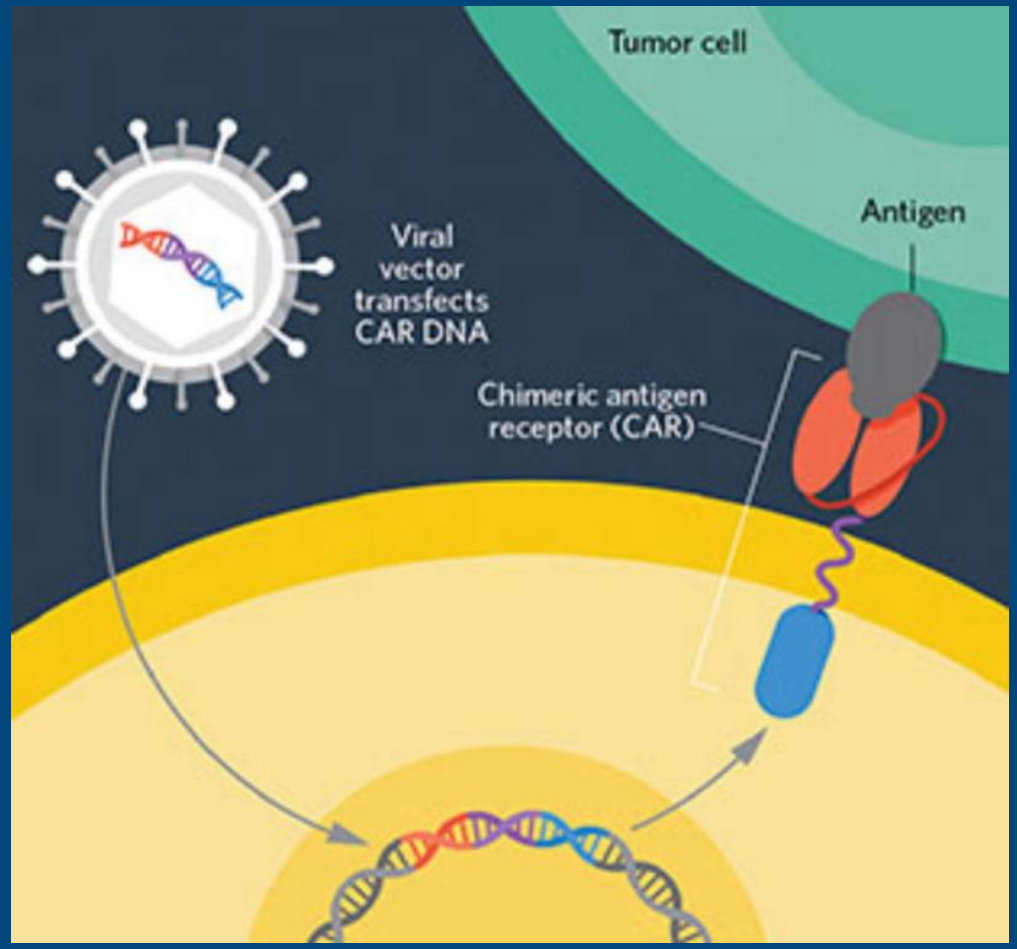
Afweercellen opleiden tot tumor bestrijders

Verminderen supressie (antilichamen)

mRNA

Immunotherapie:
CAR-T

- Opvoeden van de afweer om kanker te herkennen
- Levend medicijn
- Biologische robot
- If there isn't a cure for their cancer today, there's a reasonable chance that around the corner, there's going to be one.



https://www.nature.com/articles/d41586-022-00241-0

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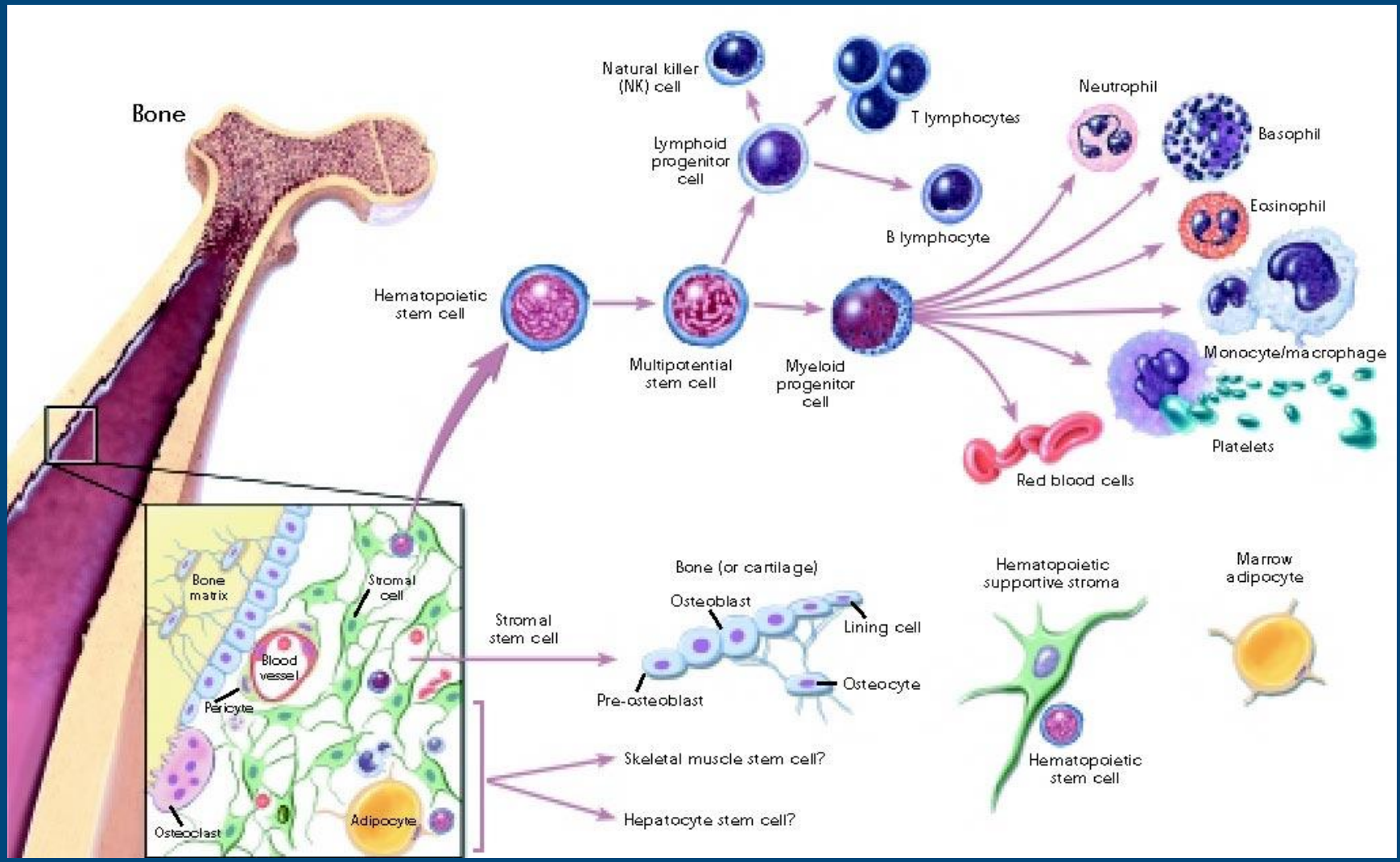
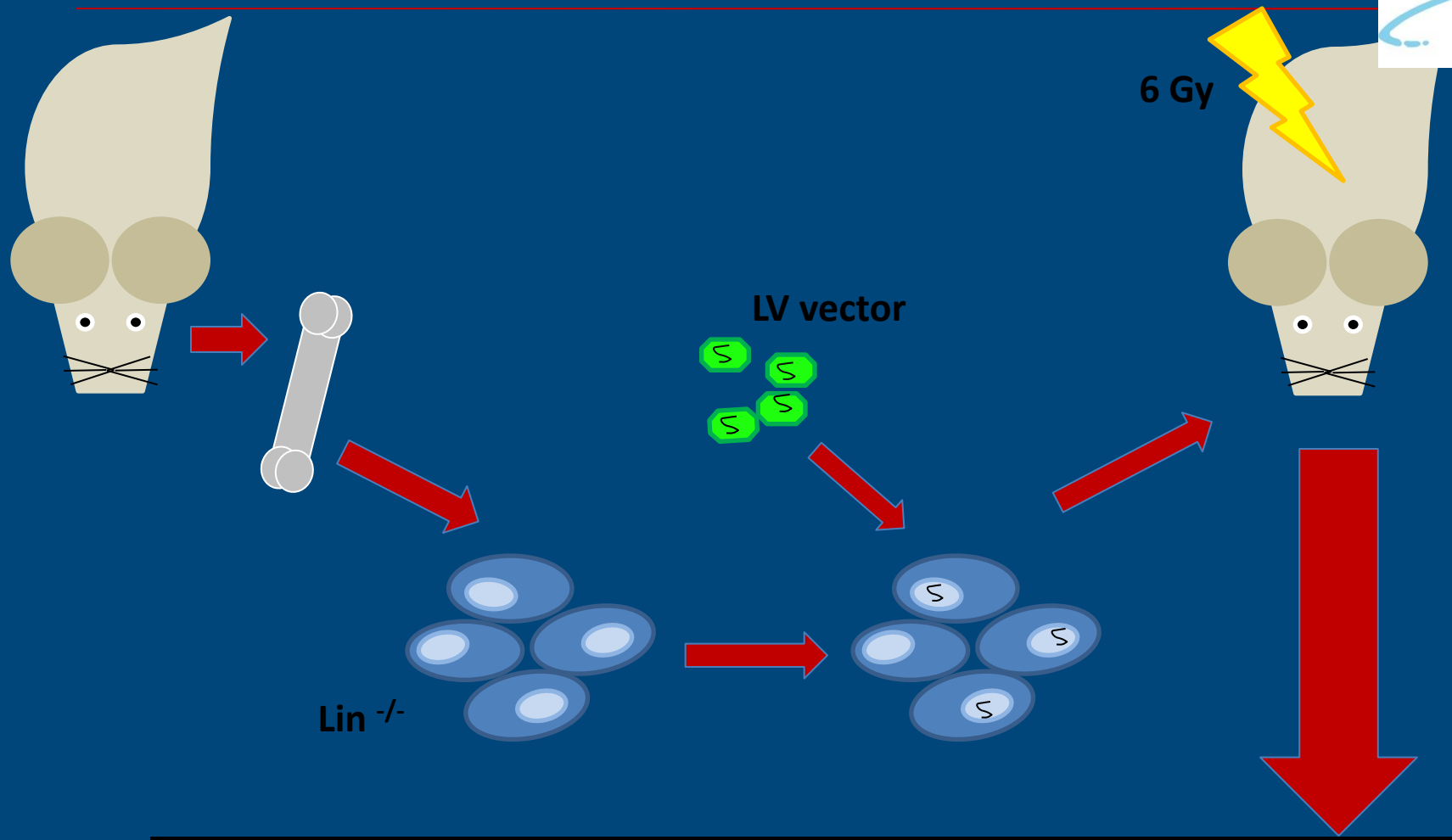
Celbio 2: Stamcellen in het beenmerg



Experimental Approach (ex vivo)



Expression of the transgene in all hematopoietic cells
Ex vivo possible through use of stem cells (quality control)

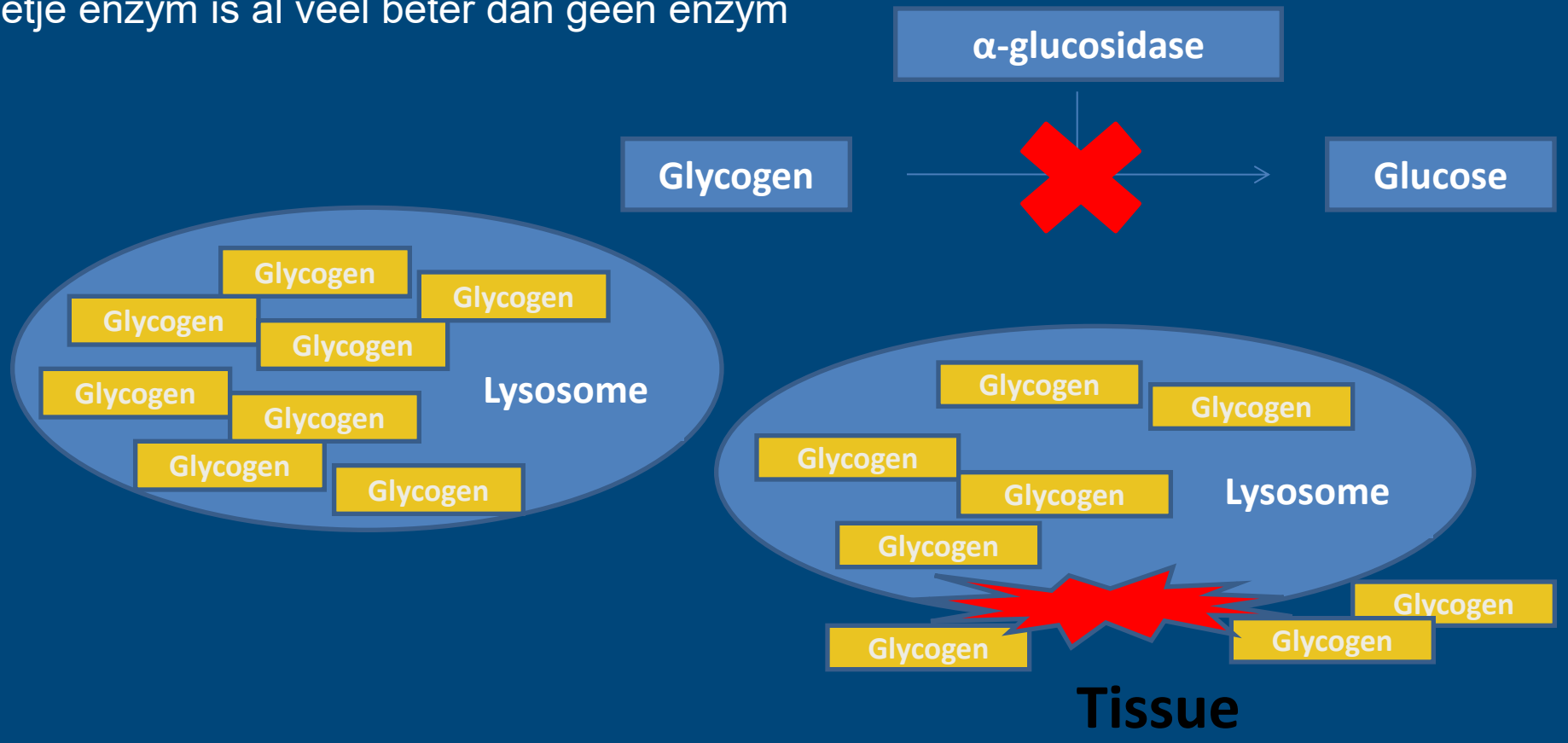
stamcel => college 2

Gen/stamceltherapie: Pompe Disease

Autosomal recessive lysosomal storage disorder

Deficiency in a-glucosidase (GAA)

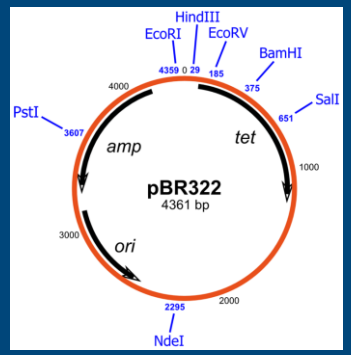
=> Klein beetje enzym is al veel beter dan geen enzym



The delivery problem

- Planten, bacterien

=> plasmide

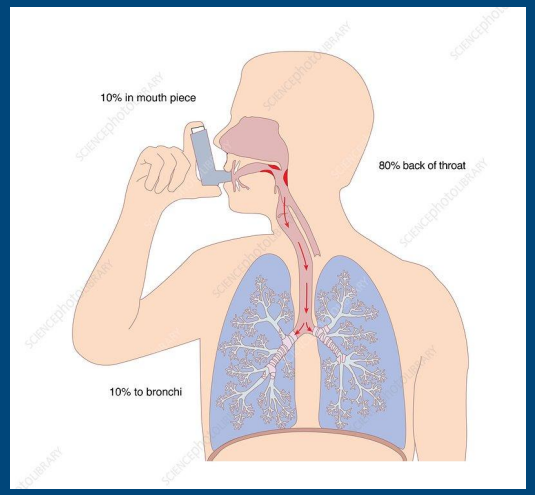


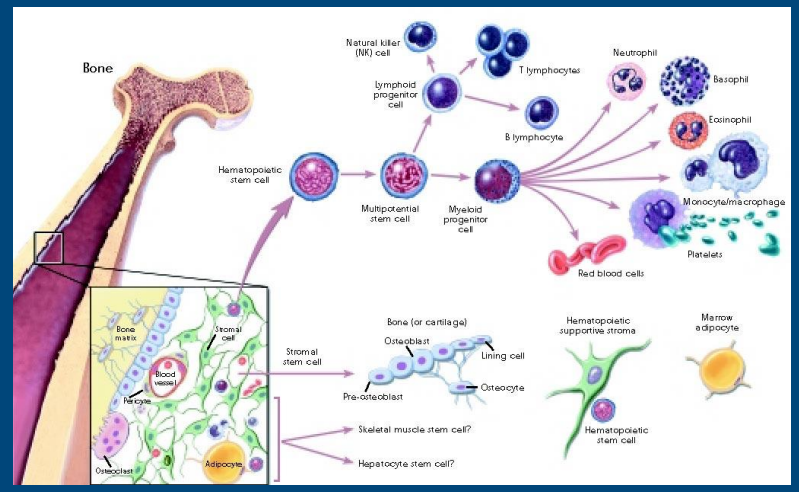
- Mens

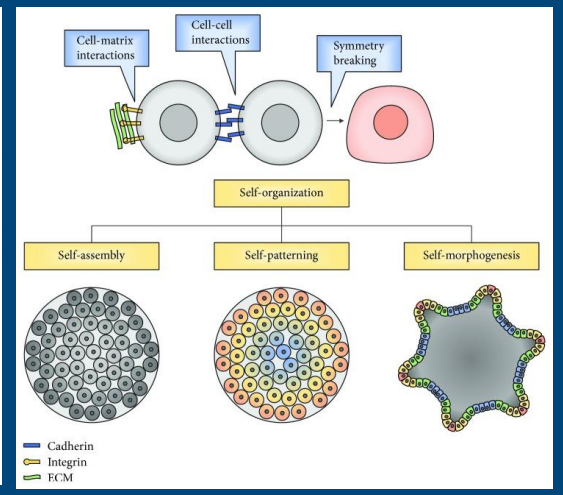
⇒Virus

⇒Stamcel

⇒Organoid (TE)







Stem cell therapies ‘come of age’ with two conditional approvals in Japan

- Induced pluripotent stem cells could help treat diseased hearts and brains

Stem cells provide a potent treatment for frailty

Elderly people with frailty, increased their endurance after a single dose of stem cells.