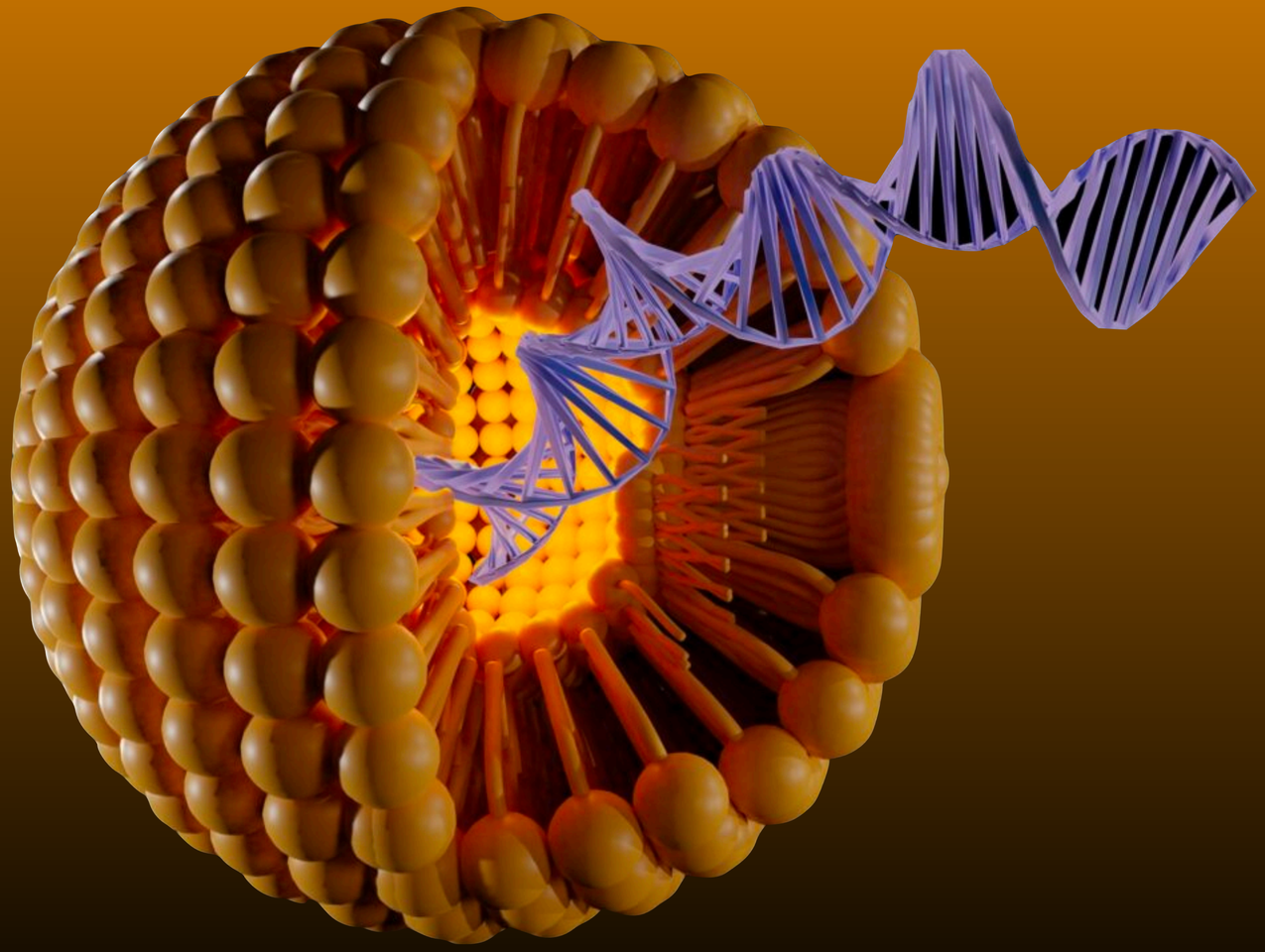
Molekularbiologische Kernschmelze



Frame-Shift Proteins

Zeta-Potential

Plasmide

LNP-Addukte

Promoter

Trojanisches Pferd

Mikrobiom

Nachweis

Frame-Shift Proteins

Zeta-Potential

Plasmide

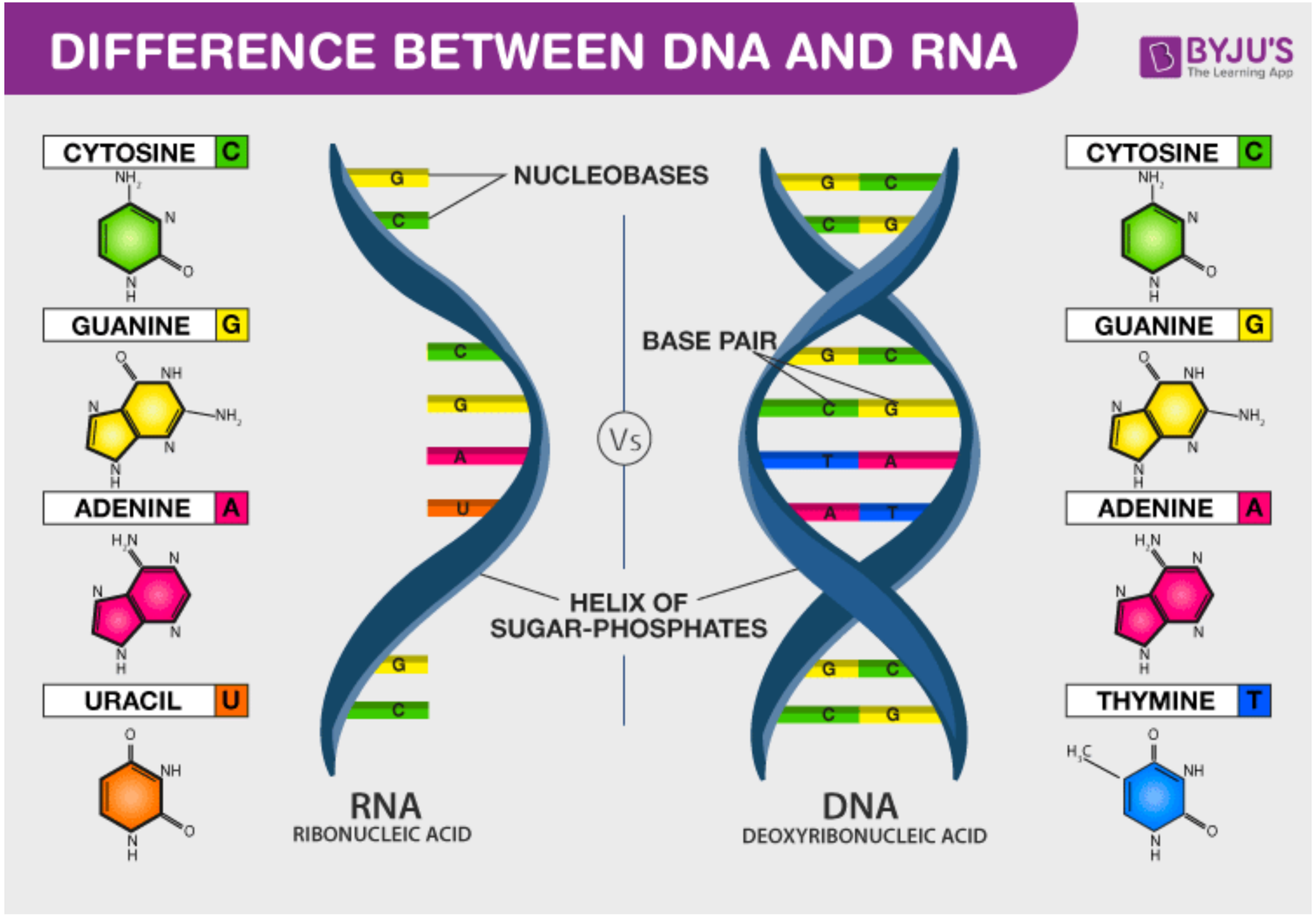
LNP-Addukte

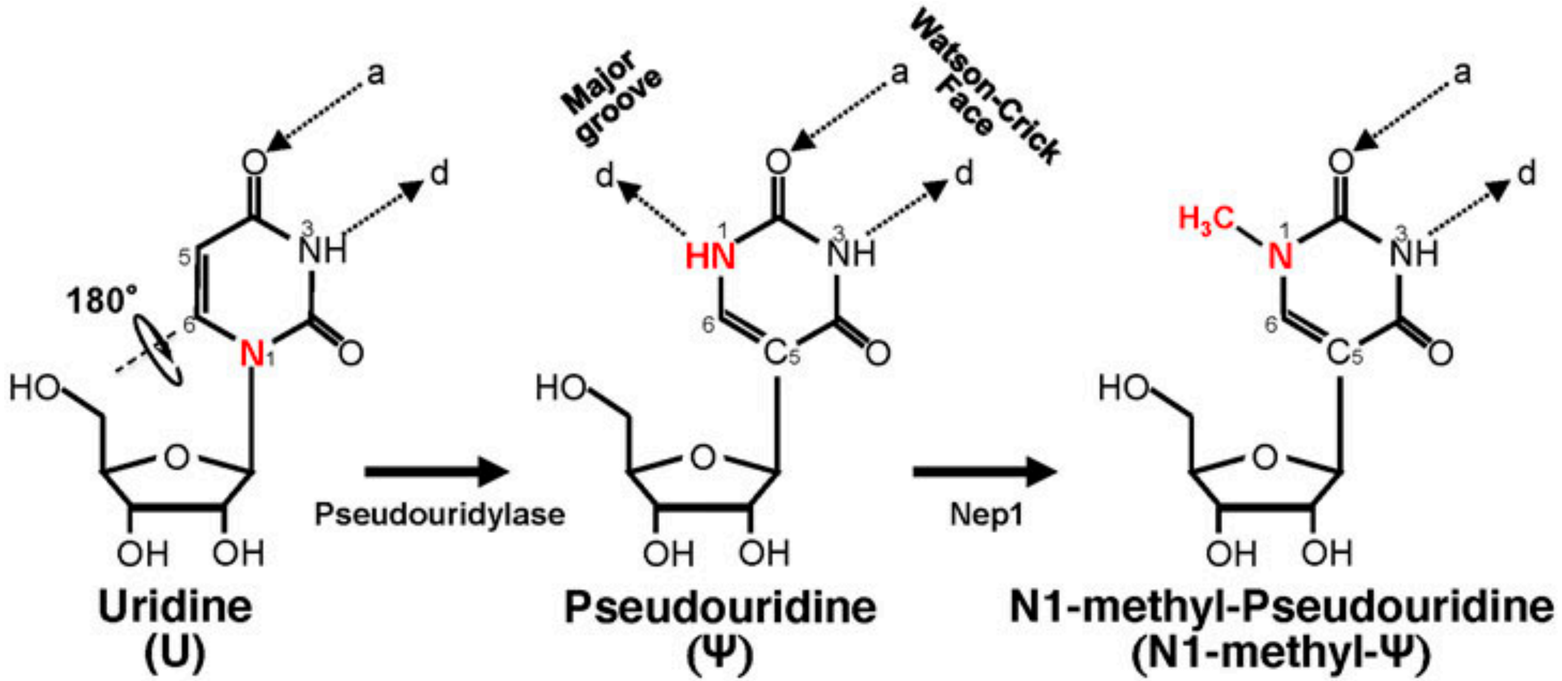
Promoter

Trojanisches Pferd

Mikrobiom

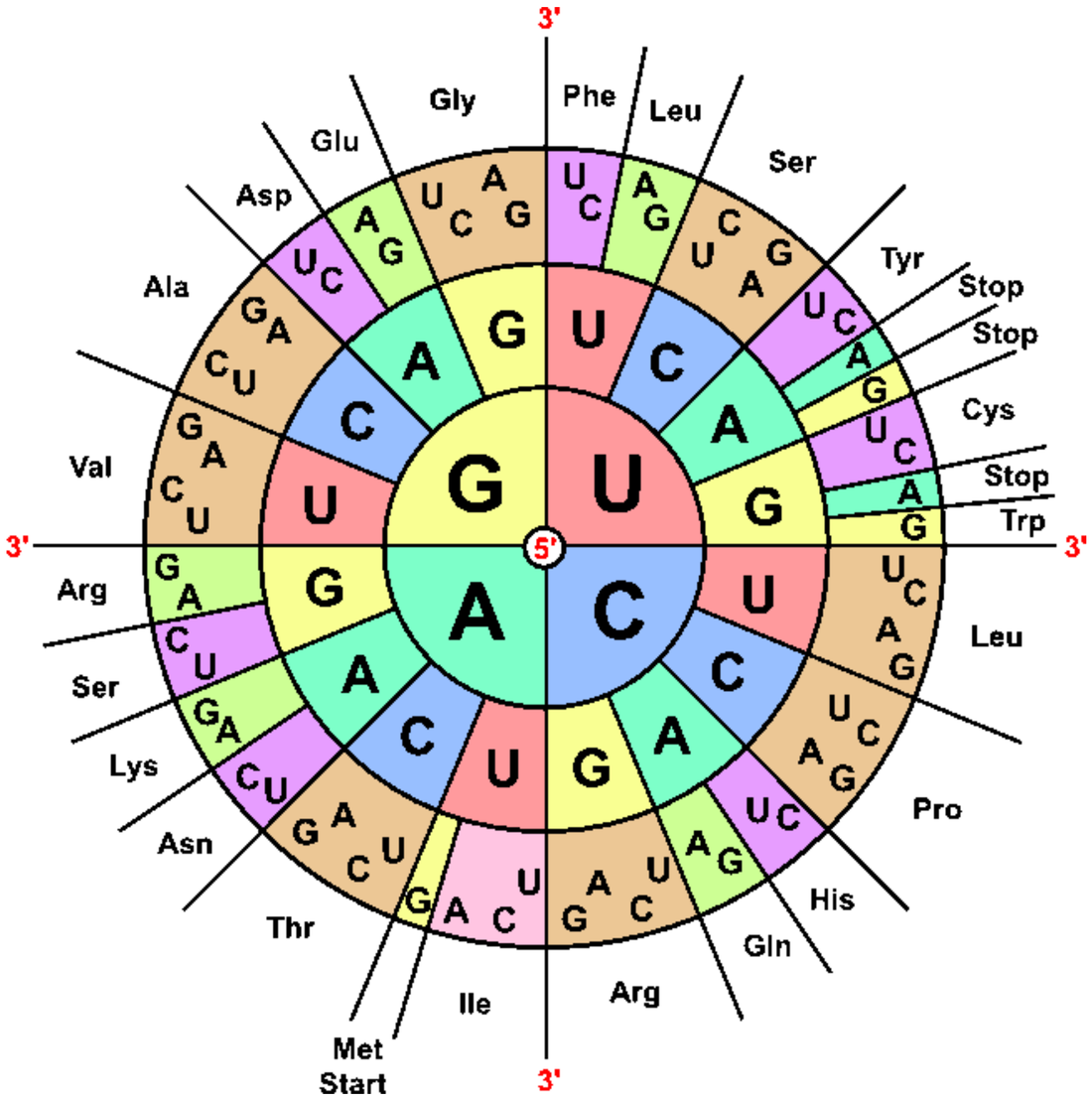
Nachweis


DIFFERENCE BETWEEN DNA AND RNA
BYJU'S
The Learning App
CYTOSINE C
GUANINE G
ADENINE A
URACIL U
NUCLEOBASES
BASE PAIR
Vs
HELIX OF
SUGAR-PHOSPHATES
RNA
RIBONUCLEIC ACID
DNA
DEOXYRIBONUCLEIC ACID
CYTOSINE C
GUANINE G
ADENINE A
THYMINE T



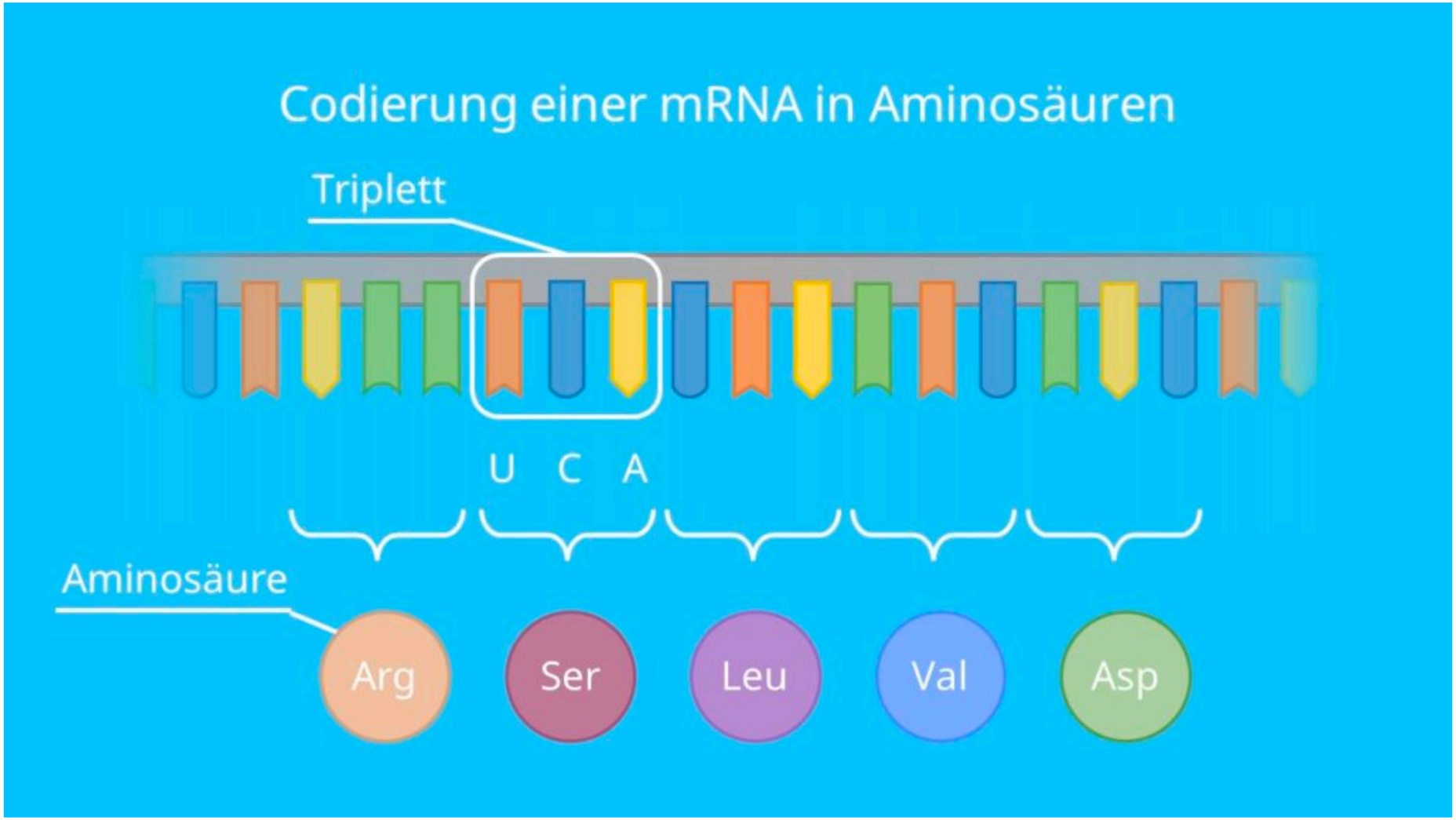
Natural: <5%

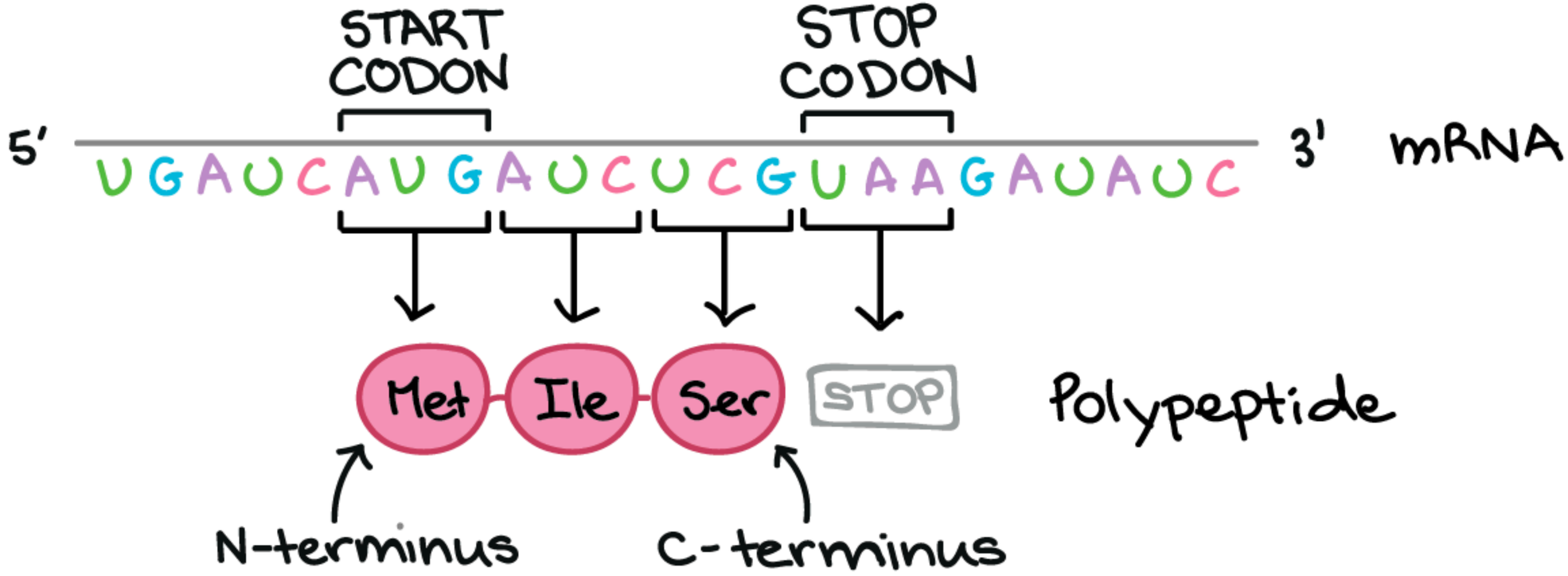
Pfizer: 20%

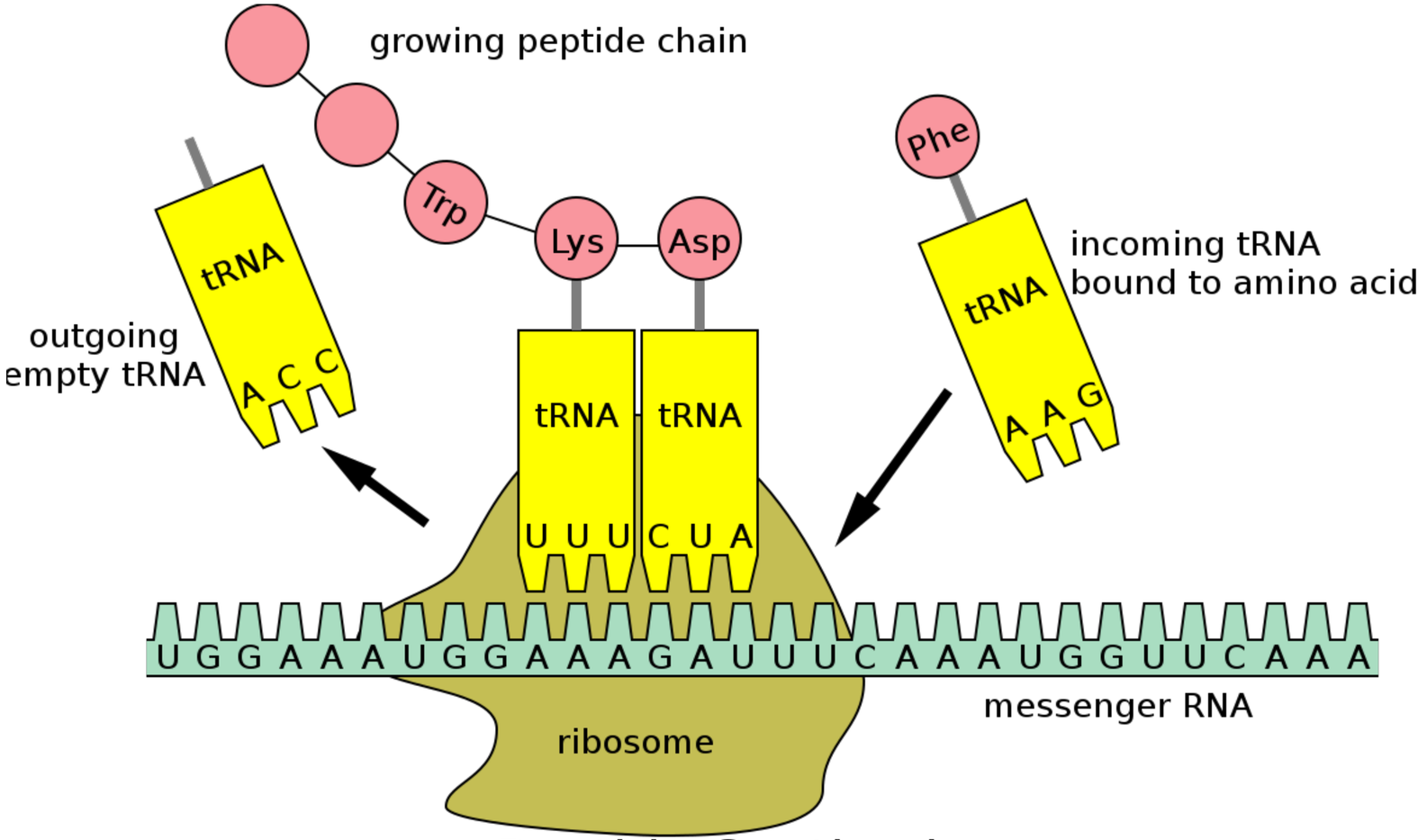


G C A = Ala(nin)

U A G = Stop


Codierung einer mRNA in Aminosäuren
Triplett
U C A
Aminosäure
Arg
Ser
Leu
Val
Asp

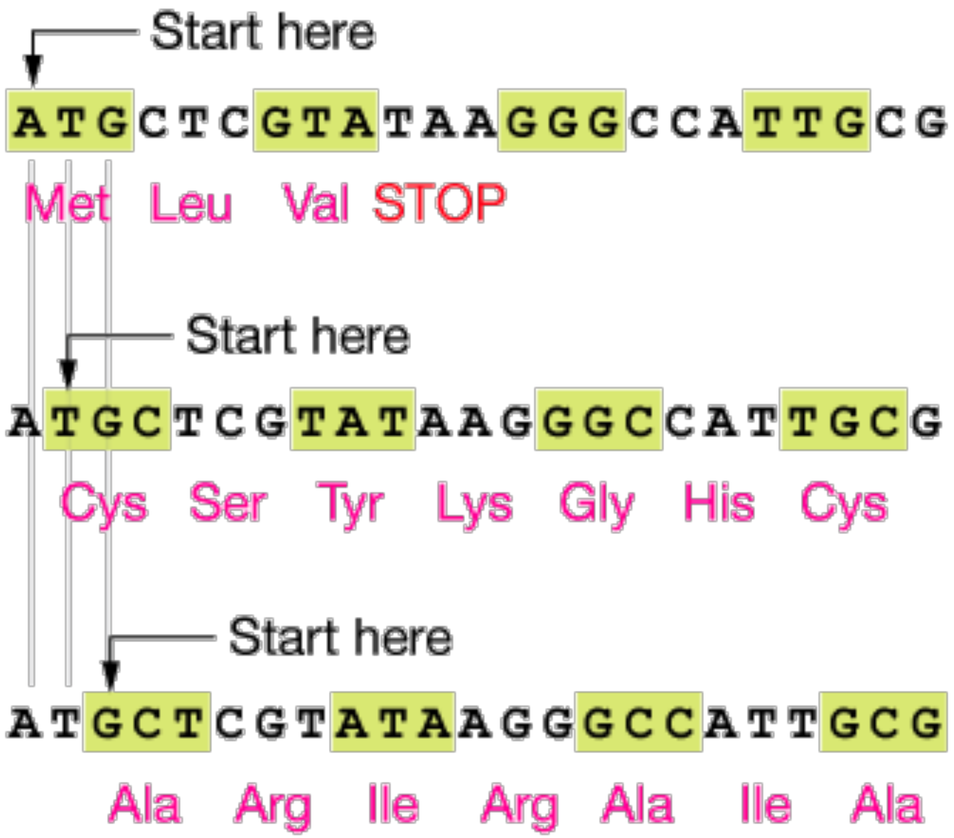

START CODON
STOP CODON
5'
3'
mRNA
U G A U C A U G A U C U C G U A A G A U A U C
Met
Ile
Ser
STOP
Polypeptide
N-terminus
C-terminus



Peptide Synthesis

The sequence

ATGCTCGTATAAGGGCCATTGCG

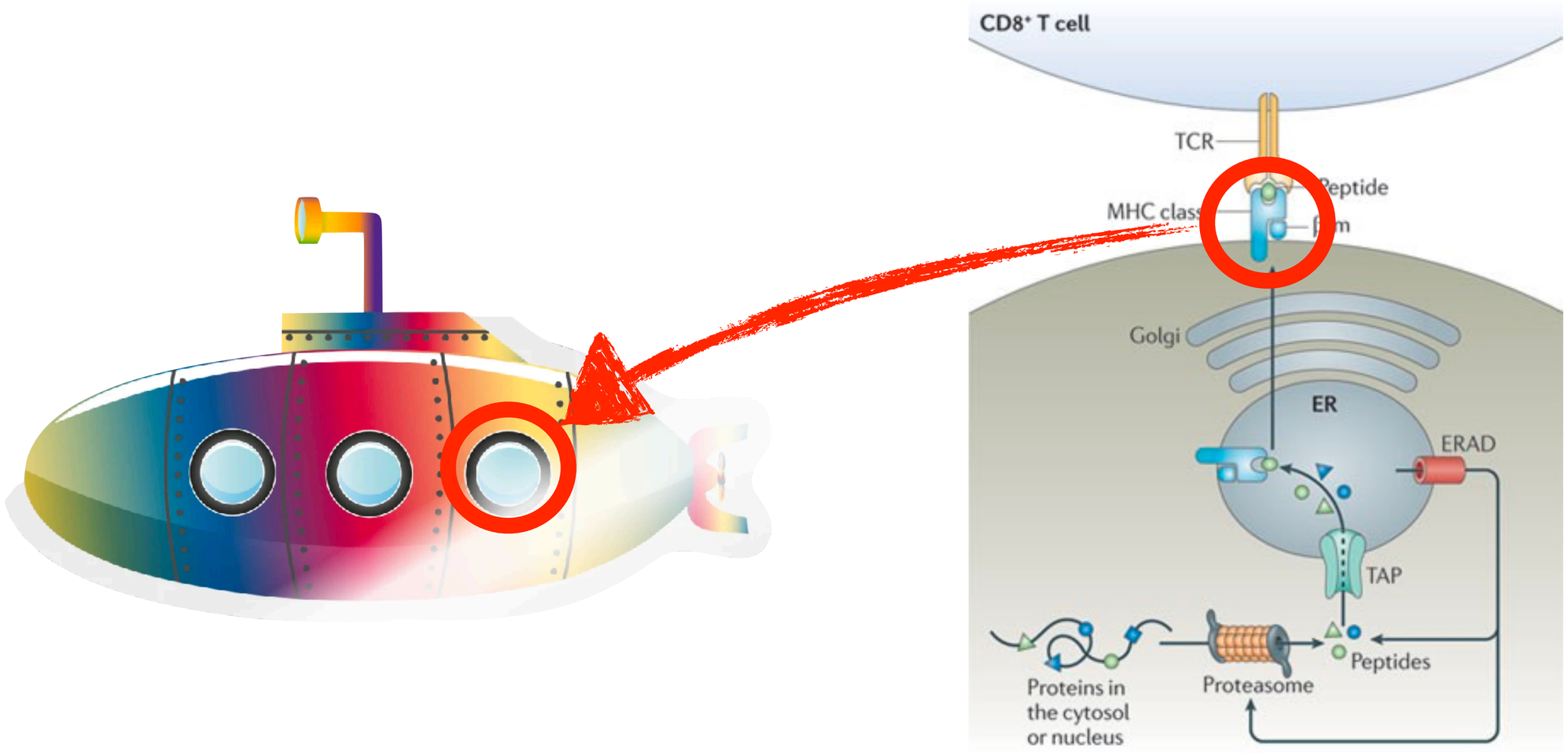


Target Protein

CAT AND DOG SAW BIG HOT SUN

CAT AND DOG SAB IGH OTS UN

Off-Target-Protein



Neefjes (2011); https://doi.org/10.1038/nri3084

nature > articles > article

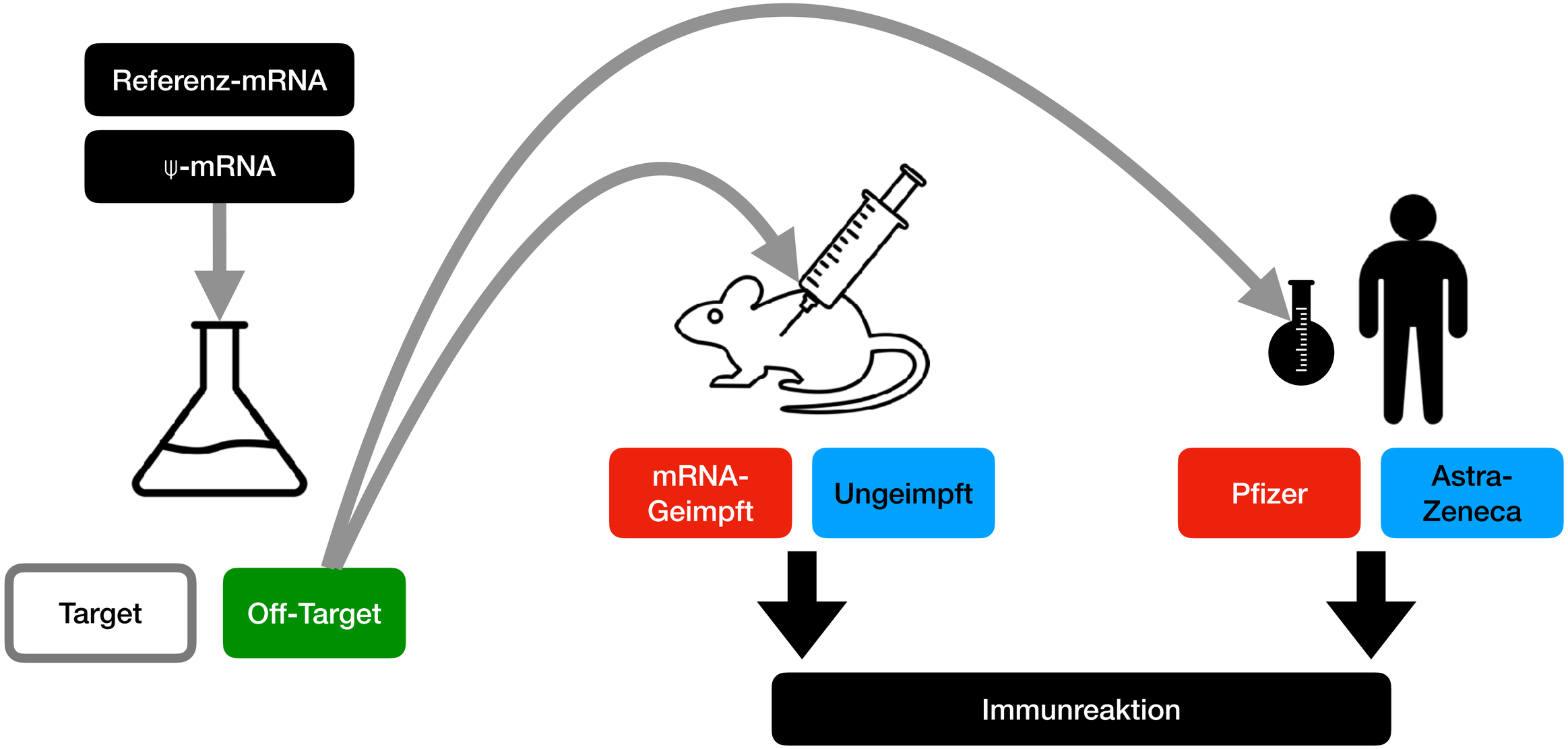
Article | Open access | Published: 06 December 2023

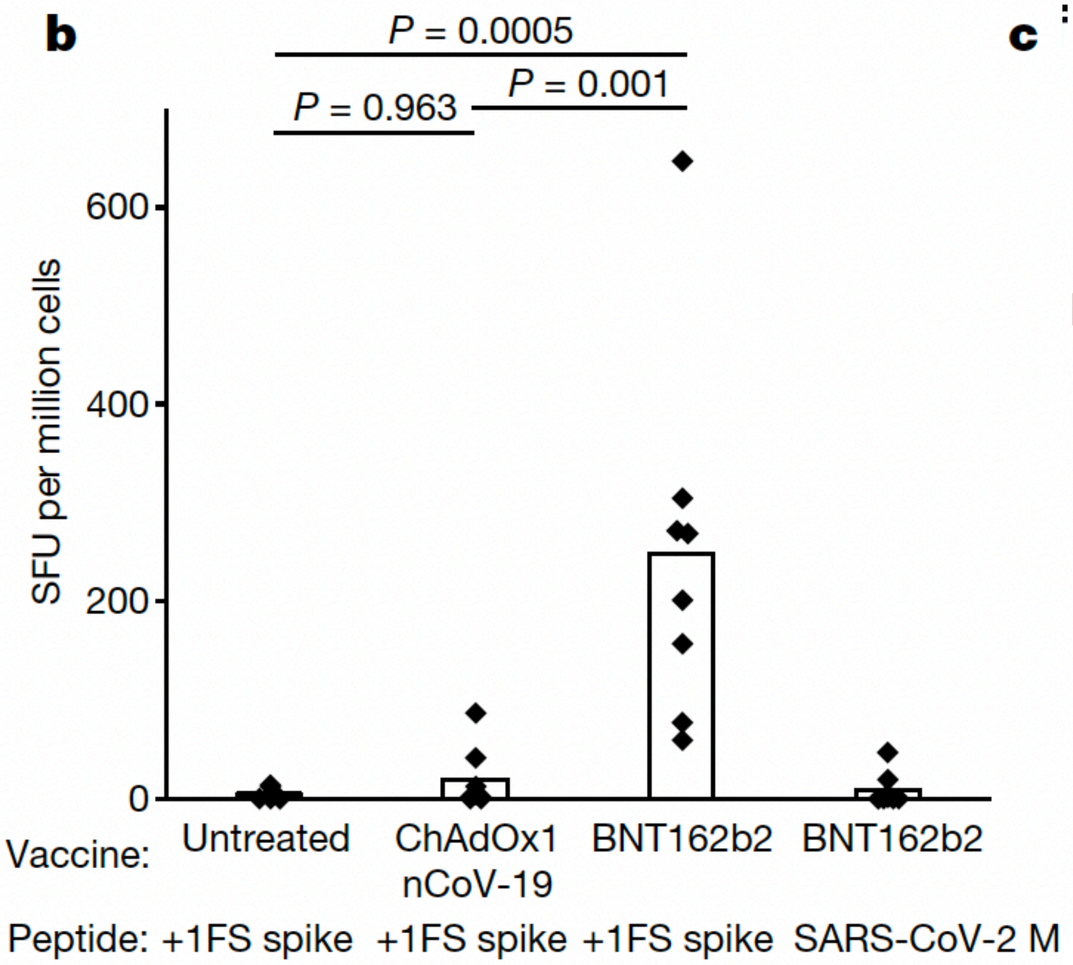
N^1-methylpseudouridylation of mRNA causes +1 ribosomal frameshifting

Thomas E. Mulroney, Tuija Pöyry, Juan Carlos Yam-Puc, Maria Rust, Robert F. Harvey, Lajos Kalmar, Emily Horner, Lucy Booth, Alexander P. Ferreira, Mark Stoneley, Ritwick Sawarkar, Alexander J. Mentzer, Kathryn S. Lilley, C. Mark Smales, Tobias von der Haar, Lance Turtle, Susanna Dunachie, Paul Klenerman, James E. D. Thaventhiran & Anne E. Willis

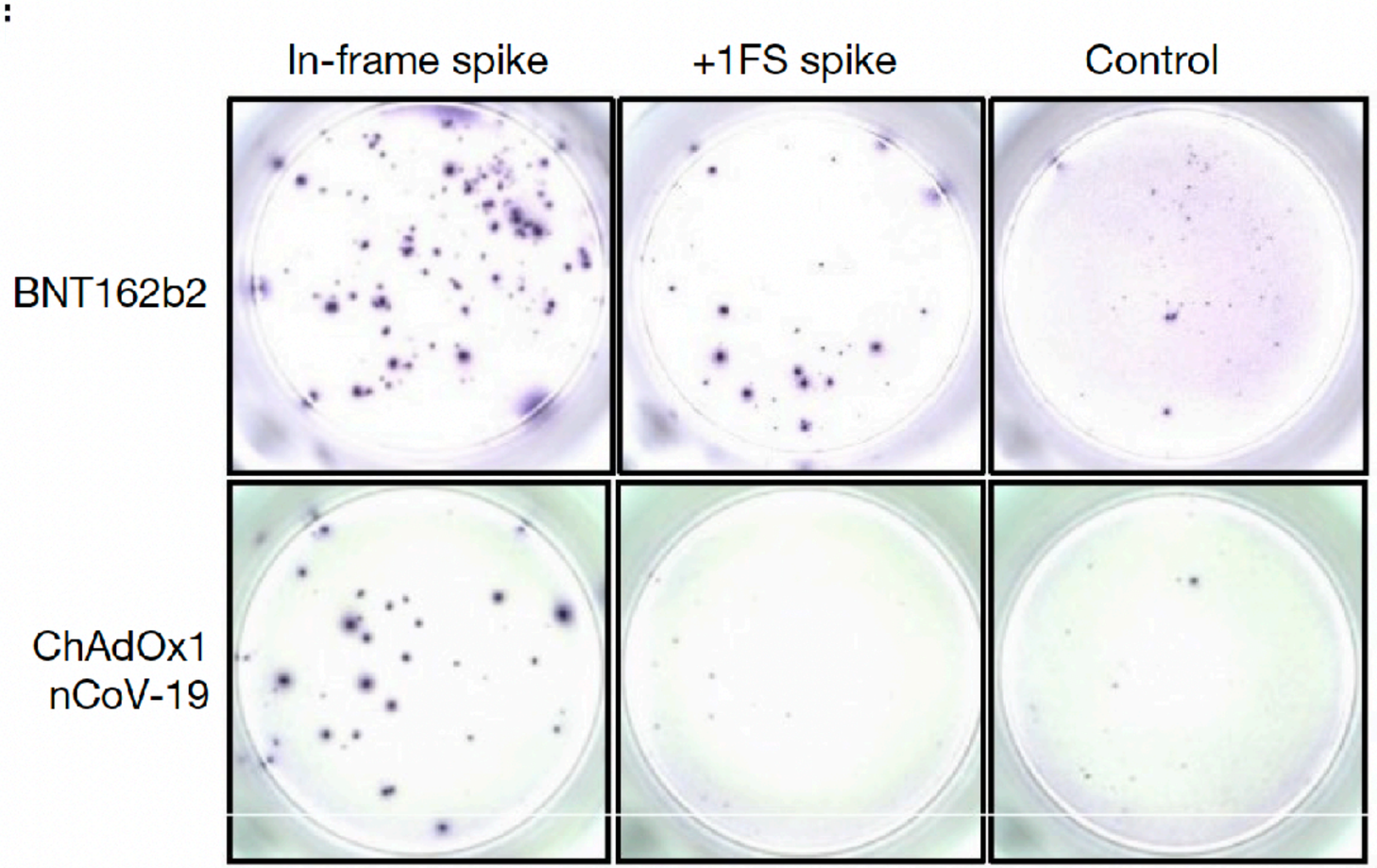
Nature (2023) | Cite this article

https://doi.org/10.1038/s41586-023-06800-3


Referenz-mRNA
ψ-mRNA
Target
Off-Target
mRNA-Geimpft
Ungeimpft
Pfizer
Astra-Zeneca
Immunreaktion



Mäuse



Impflinge

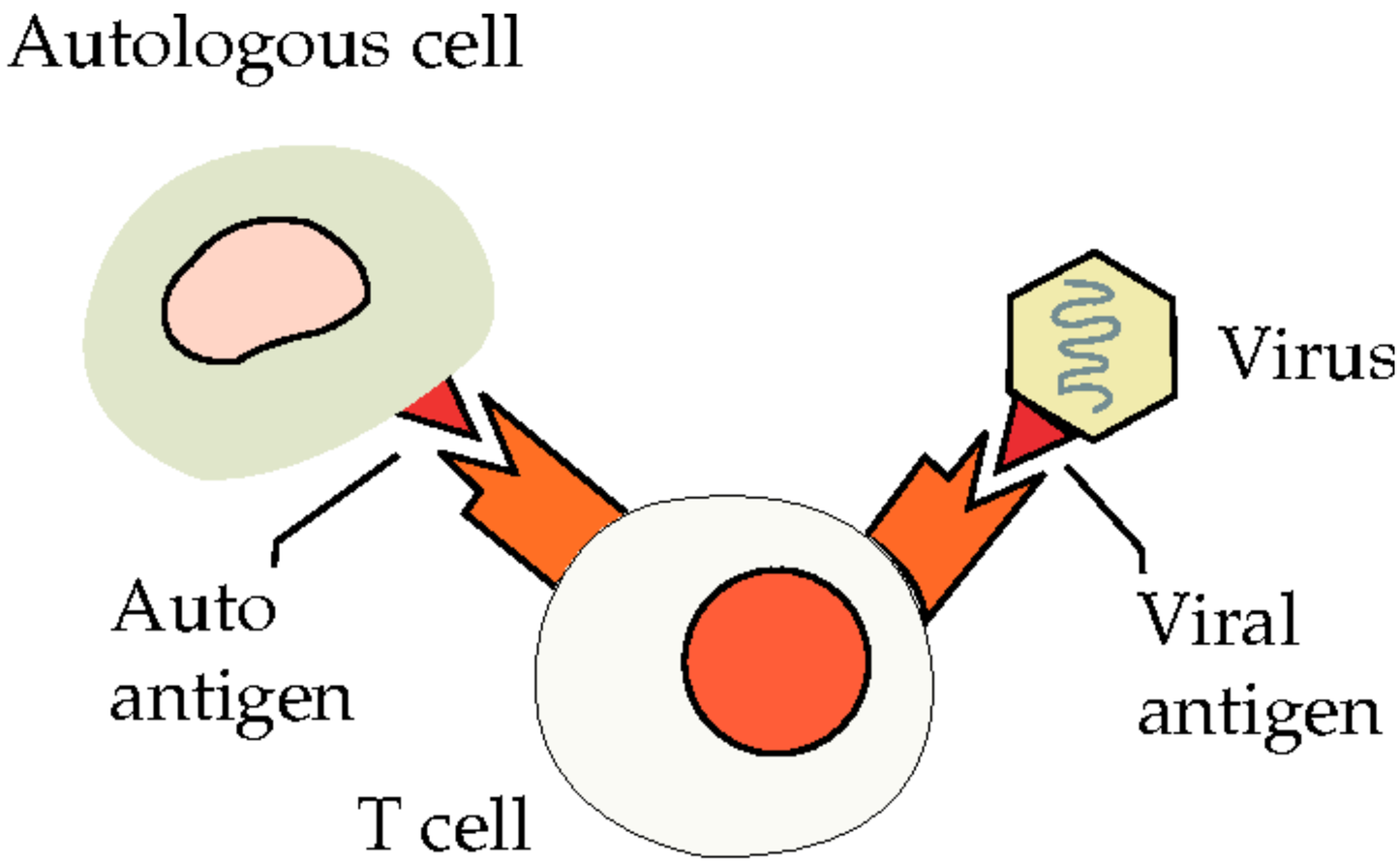
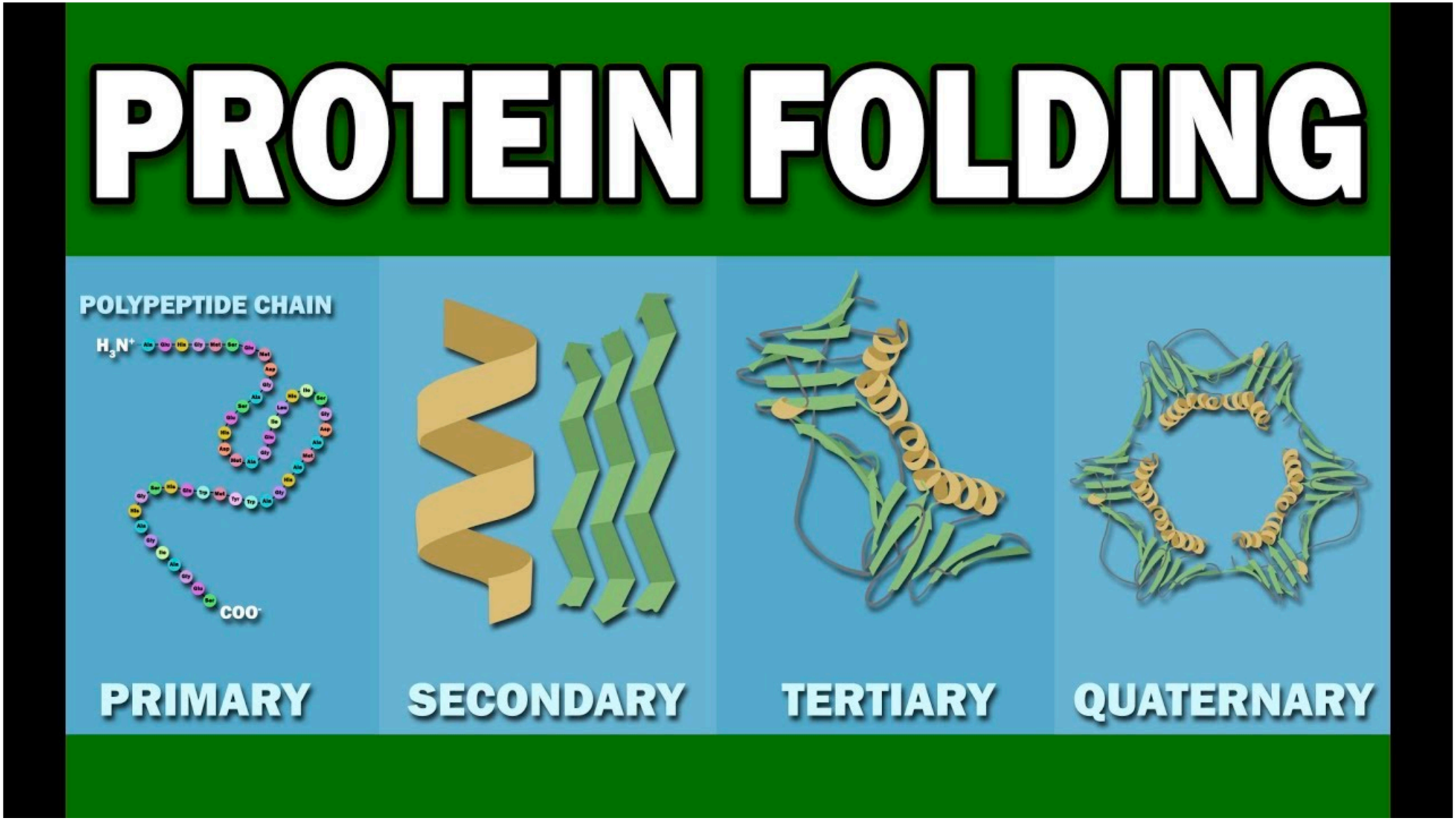
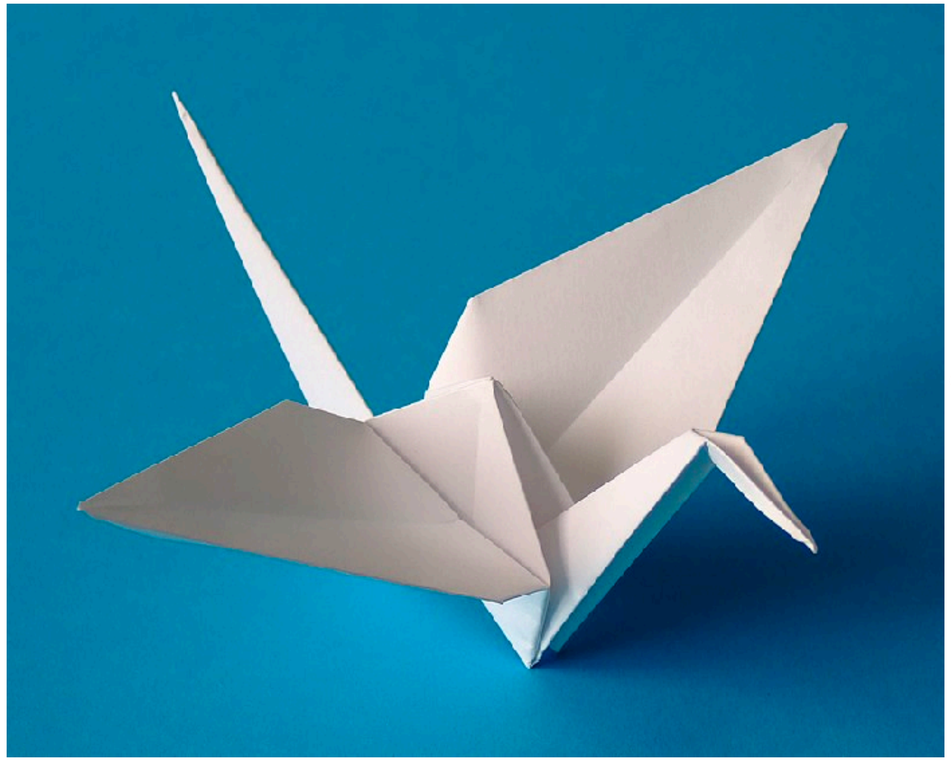
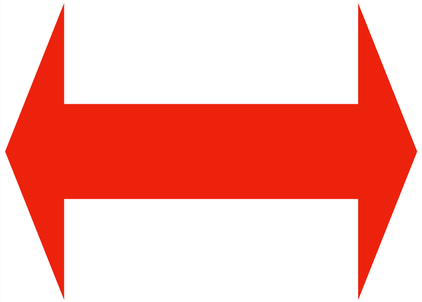


Fig. 1 : Diagram showing Molecular Mimicry Hypothesis. The


PROTEIN FOLDING
POLYPEPTIDE CHAIN
H_3N^+
COO-
PRIMARY
SECONDARY
TERTIARY
QUATERNARY





Frame-Shift Proteins

Zeta-Potential

Plasmide

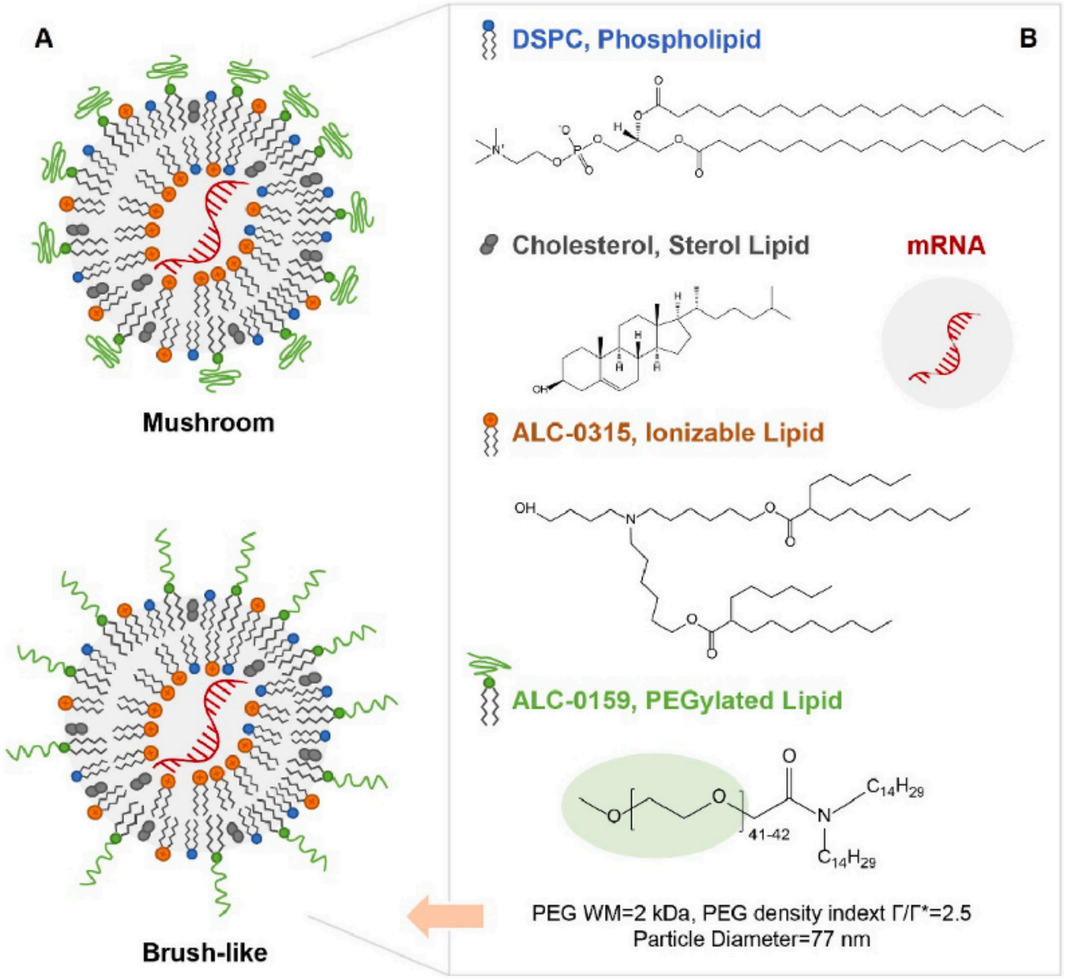
LNP-Addukte

Promoter

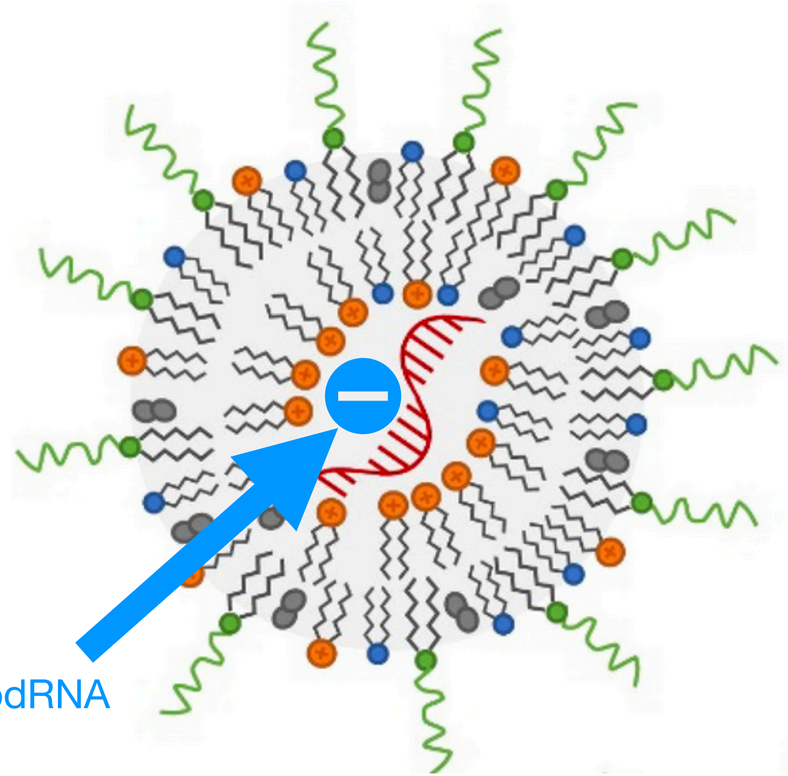
Trojanisches Pferd

Mikrobiom

Nachweis



- Lipide besitzen Ladungen
- Kationisch = positiv
- Anionisch = negativ
- Autonome (da automatische) Zusammenlagerung im Herstellungsprozess
- ALCs originär nicht für Verwendung am Menschen zugelassen > Backdoor-Licensing im Gesamtpaket



Gesamtladung nach außen

= ZETA-Potential

\+ — Lunge

− — Milz, Gefäße, Herz — Endotheliitis, Gerinnsel, Amyloide, …

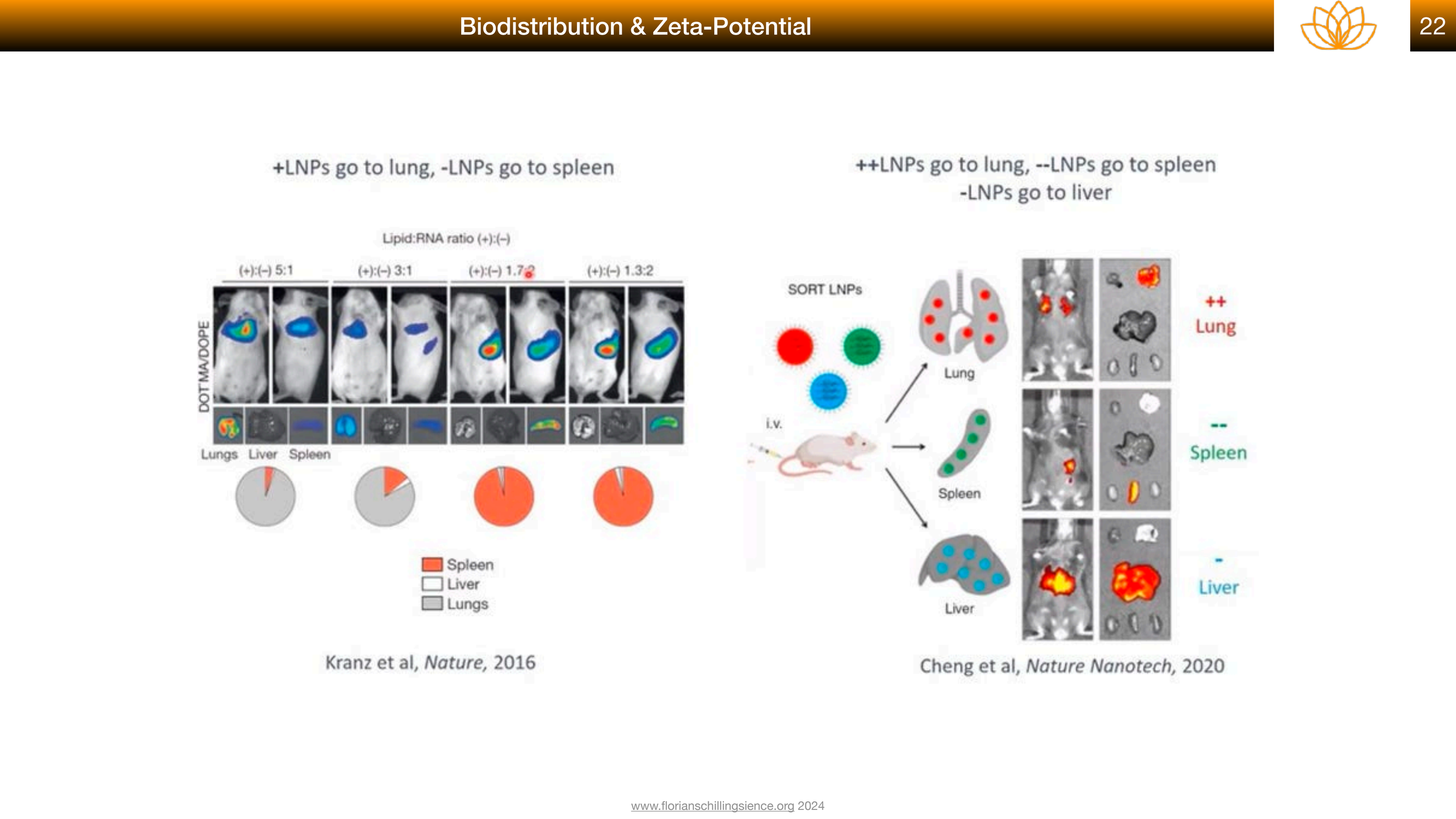
= — Leber

2.6.5.5B. PHARMACOKINETICS: ORGAN DISTRIBUTION CONTINUED

Test Article: [^{3}H]-Labelled LNP-mRNA formulation containing ALC-0315 and ALC-0159
Report Number: 185350

Species (Strain):	Rat (Wistar Han)
Sex/Number of Animals:	Male and female/3 animals/sex/timepoint (21 animals/sex total for the 50 µg dose)
Feeding Condition:	Fed ad libitum
Method of Administration:	Intramuscular injection
Dose:	50 µg [^{3}H]-08-A01-C0 (lot # NC-0552-1)
Number of Doses:	1
Detection:	Radioactivity quantitation using liquid scintillation counting
Sampling Time (hour):	0.25, 1, 2, 4, 8, 24, and 48 hours post-injection

Sample	Mean total lipid concentration (µg lipid equivalent/g (or mL) (males and females combined)							% of administered dose (males and females combined)						
	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h	0.25 h	1 h	2 h	4 h	8 h	24 h	48 h
Adipose tissue	0.057	0.100	0.126	0.128	0.093	0.084	0.181	--	--	--	--	--	--	--
Adrenal glands	0.271	1.48	2.72	2.89	6.80	13.8	18.2	0.001	0.007	0.010	0.015	0.035	0.066	0.106
Bladder	0.041	0.130	0.146	0.167	0.148	0.247	0.365	0.000	0.001	0.001	0.001	0.001	0.002	0.002
Bone (femur)	0.091	0.195	0.266	0.276	0.340	0.342	0.687	--	--	--	--	--	--	--
Bone marrow (femur)	0.479	0.960	1.24	1.24	1.84	2.49	3.77	--	--	--	--	--	--	--
Brain	0.045	0.100	0.138	0.115	0.073	0.069	0.068	0.007	0.013	0.020	0.016	0.011	0.010	0.009
Eyes	0.010	0.035	0.052	0.067	0.059	0.091	0.112	0.000	0.001	0.001	0.002	0.002	0.002	0.003
Heart	0.282	1.03	1.40	0.987	0.790	0.451	0.546							
Injection site	128	394	311	338	213	195	165	19.9	52.6	31.6	28.4	21.9	29.1	24.6
Kidneys	0.391	1.16	2.05	0.924	0.590	0.426	0.425							
Large intestine	0.013	0.048	0.093	0.287	0.649	1.10	1.34	0.008	0.025	0.065	0.192	0.405	0.692	0.762
Liver	0.737	4.63	11.0	16.5	26.5	19.2	24.3	0.602	2.87	7.33	11.9	18.1	15.4	16.2
Lung	0.492	1.21	1.83	1.50	1.15	1.04	1.09	0.052	0.101	0.178	0.169	0.122	0.101	0.101

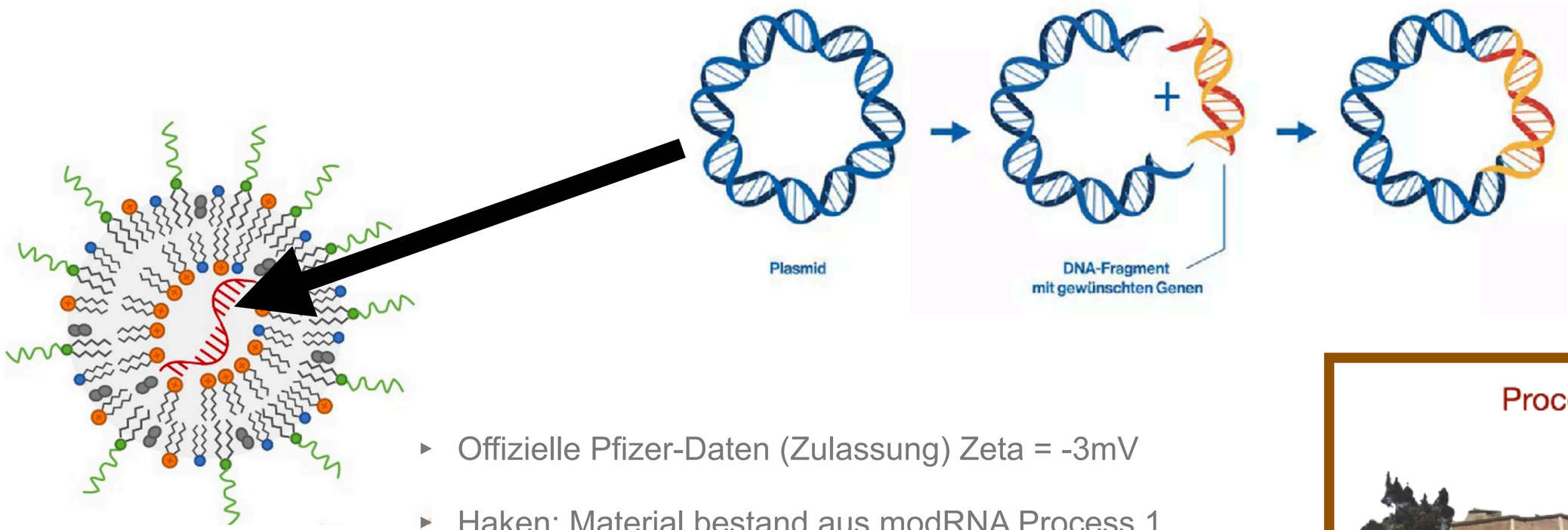


+LNPs go to lung, -LNPs go to spleen

Kranz et al, *Nature*, 2016

++LNPs go to lung, --LNPs go to spleen
-LNPs go to liver

Cheng et al, *Nature Nanotech*, 2020



- Offizielle Pfizer-Daten (Zulassung) Zeta = -3mV
- Haken: Material bestand aus modRNA Process 1
- Roll-Out: Kontamination mit dsDNA aus bakteriellen Plasmiden (Process 2)
- Self-assembly: unbekannt
- Zeta-Potential: unbekannt

https://odysee.com/@florian_schilling_science:d/procedure_2:8

nature > npj vaccines > articles > article

Article | Open access | Published: 27 September 2023

Duration of SARS-CoV-2 mRNA vaccine persistence and factors associated with cardiac involvement in recently vaccinated patients

Aram J. Krauson, Faye Victoria C. Casimero, Zakir Siddiquee & James R. Stone

npj Vaccines **8**, Article number: 141 (2023) | Cite this article

Offiziell (Zulassung, Process 1): -3MV

>> annähernd neutral, d.h. Leber!

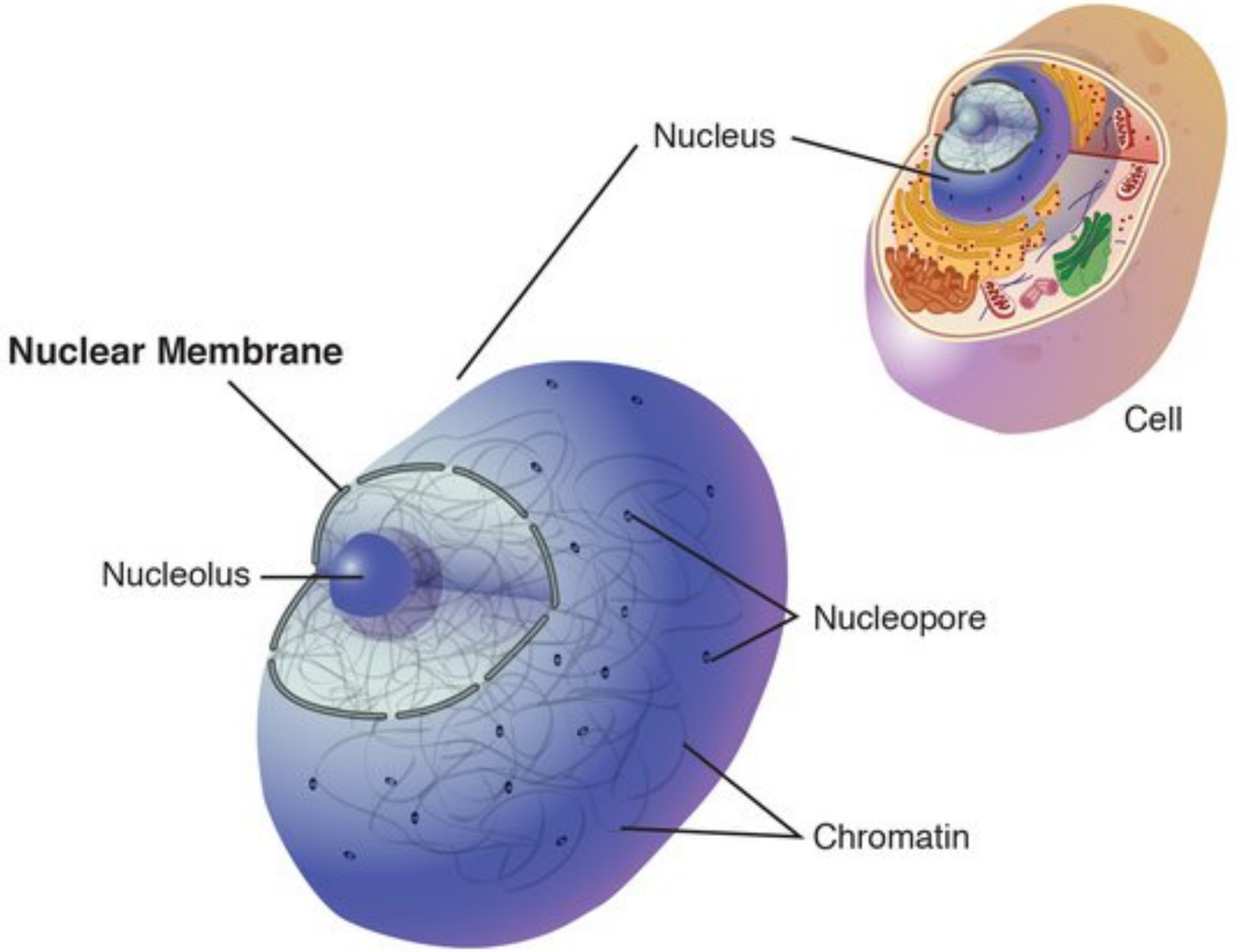
At the start of the COVID-19 pandemic, the BNT162b2 (BioNTech-Pfizer) and mRNA-1273 (Moderna) mRNA vaccines were expediently designed and mass produced. Both vaccines produce the full-length SARS-CoV-2 spike protein for gain of immunity and have greatly reduced mortality and morbidity from SARS-CoV-2 infection. The distribution and duration of SARS-CoV-2 mRNA vaccine persistence in human tissues is unclear. Here, we developed specific RT-qPCR-based assays to detect each mRNA vaccine and screened lymph nodes, liver, spleen, and myocardium from recently vaccinated deceased patients. Vaccine was detected in the axillary lymph nodes in the majority of patients dying within 30 days of vaccination, but not in patients dying more than 30 days from vaccination. Vaccine was not detected in the mediastinal lymph nodes, spleen, or liver. Vaccine was detected in the myocardium in a subset of patients vaccinated within 30 days of death. Cardiac ventricles in which vaccine was detected had healing myocardial injury at the time of vaccination and had more myocardial macrophages than the cardiac ventricles in which vaccine was not detected. These results suggest that SARS-CoV-2 mRNA vaccines routinely persist up to 30 days from vaccination and can be detected in the heart.

PLoS Comput Biol. 2010 Apr; 6(4): e1000747.
Published online 2010 Apr 22. doi: 10.1371/journal.pcbi.1000747
PMCID: PMC2858669
PMID: 20421988

Charge as a Selection Criterion for Translocation through the Nuclear Pore Complex

Lucy J. Colwell,[1] Michael P. Brenner,[1,*] and Katharina Ribbeck[2,*]

- Negative Ladung als Treiber der Translokation in den Nukleus



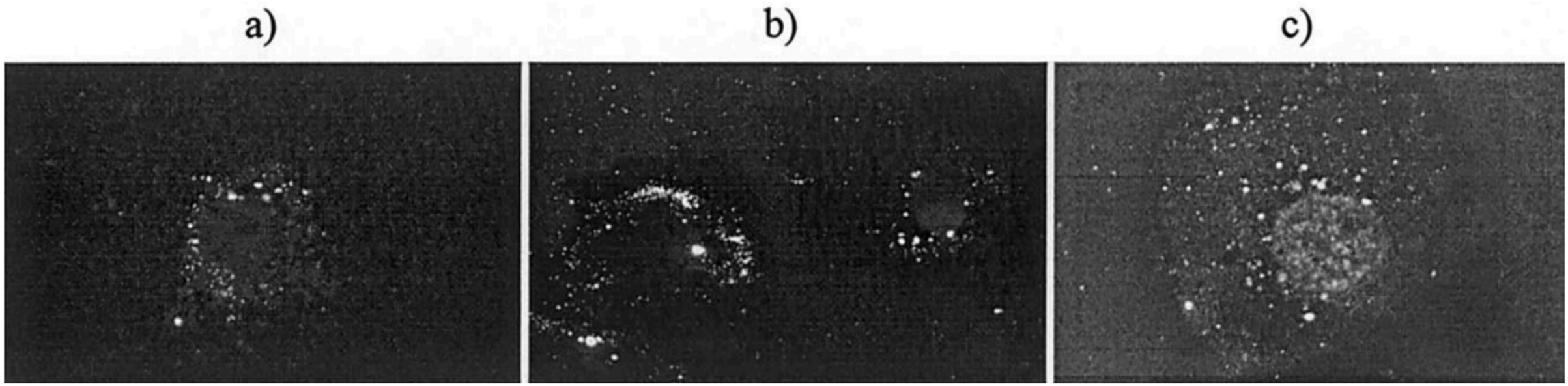
> Int J Pharm. 2000 Mar 10;196(2):251-2. doi: 10.1016/s0378-5173(99)00433-0.

Nuclear gene targeting using negatively charged liposomes

C Welz [1], W Neuhuber, H Schreier, R Repp, W Rascher, A Fahr

Affiliations + expand

PMID: 10699729 DOI: 10.1016/s0378-5173(99)00433-0



Cellular distribution of fluorescence-labelled DNA: DNA-oligonucleotide–protamine-complex with a charge ratio of 3:1 (+/−) mediated by (a) neutral liposomes; (b) negatively charged control liposomes; and (c) AVE™ -3 (negatively charched liposomes)

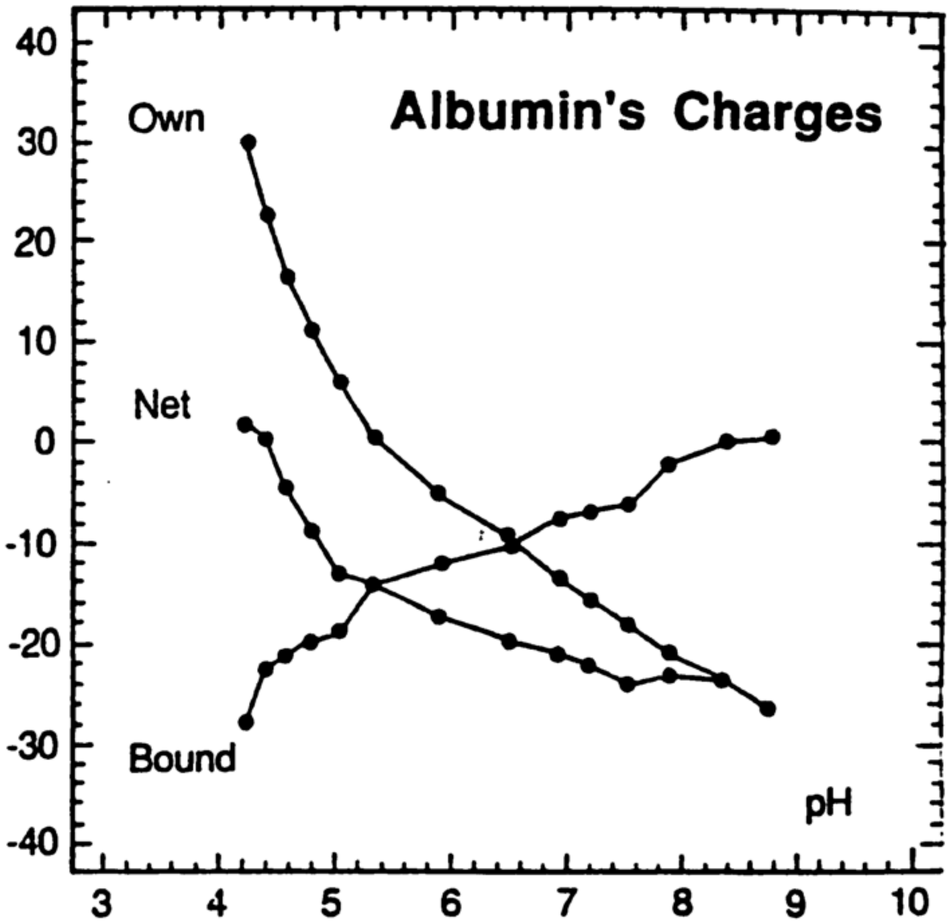


Fig. 4. The number of charges per albumin molecule as a function of pH

Fogh-Andersen et al (1993). Ionic binding, net charge, and Donnan effect of human serum albumin as a function of pH. *Clinical chemistry, 39 1*, 48-52 .

› Med Hypotheses. 2004;62(1):124-9. doi: 10.1016/s0306-9877(03)00314-1.

A hypothesis on the role of the electrical charge of haemoglobin in regulating the erythrocyte shape

P Wong [1]

Affiliations + expand

PMID: 14729017 DOI: 10.1016/s0306-9877(03)00314-1

Abstract

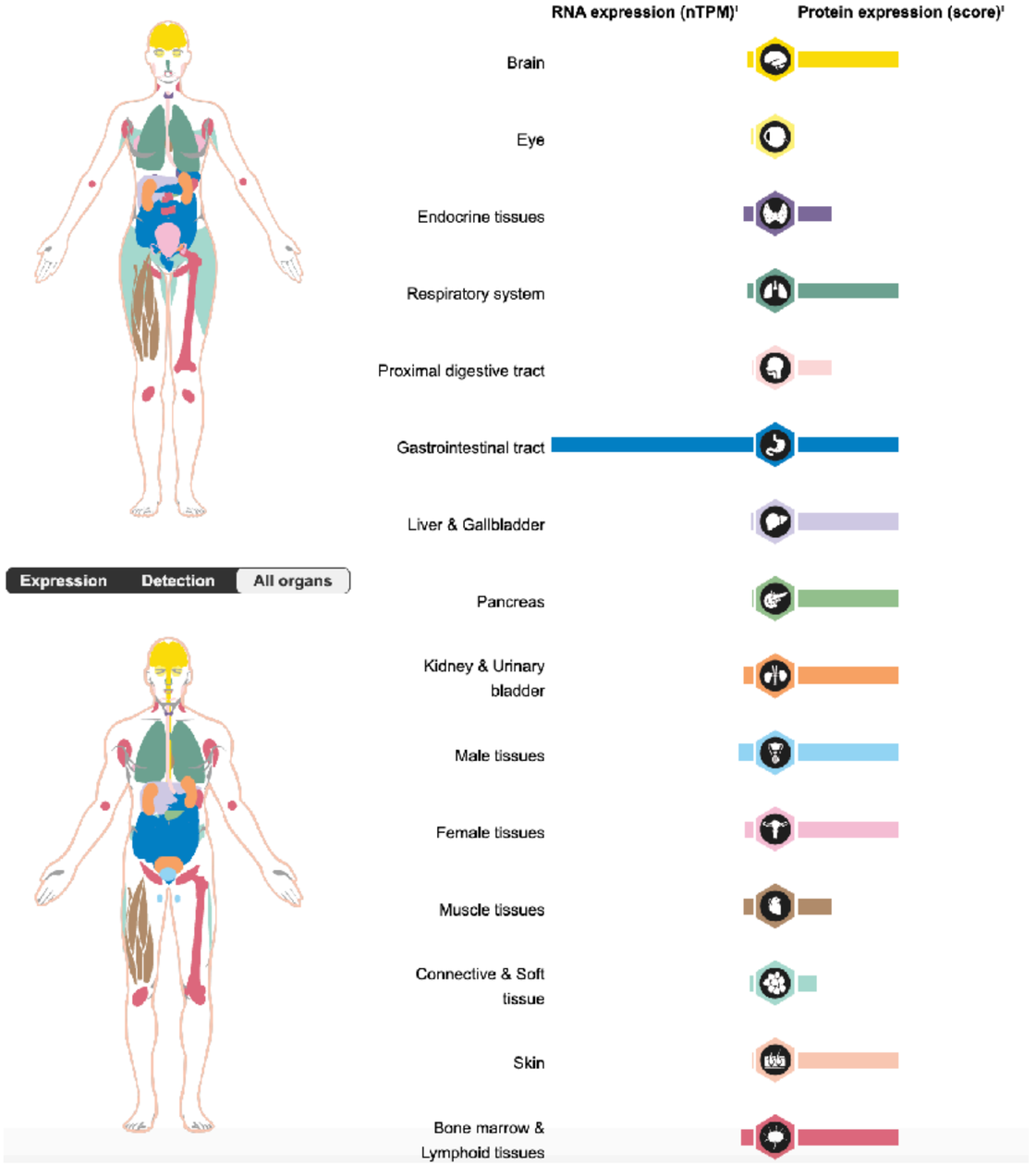
A previously proposed mechanism of erythrocyte shape has been proposed in which the ratio of the two conformations of Band 3, the anion exchange protein, controls the erythrocyte shape by modifying the degree of contraction or relaxation of the membrane skeleton. This mechanism was previously shown to explain several observations related and unrelated to the erythrocyte shape. We show that it can also explain the occurrence of target cells in blood smears of individuals expressing Hb A variants with significantly lower and higher isoelectric points. This would provide further support of its validity and would have the following implications. The electrical charge of of Hb A influences the erythrocyte shape and deformability, thus explaining the low level of expression of Hb A2 with an isoelectric point significantly higher than Hb A. It would suggest that Hb A2 has a physiological function related to the control of the erythrocyte shape. This could be of regulating the activity of the K-Cl cotransport system or of tuning finely the cell pH. A role of haemoglobin in the control of the erythrocyte shape would ensure a more efficient oxygenation of tissues and removal of carbon dioxide generated by tissues. It would also provide a basis for the absence of spreading in populations of some haemoglobin variants differing only by the electrical charge.

Adv Drug Deliv Rev. 2022 Sep; 188: 114416.
Published online 2022 Jul 3. doi: 10.1016/j.addr.2022.114416
PMCID: PMC9250827
PMID: 35787388

The role of lipid components in lipid nanoparticles for vaccines and gene therapy

Camilla Hald Albertsen,[a,1] Jayesh A. Kulkarni,[b,1] Dominik Witzigmann,[b] Marianne Lind,[a] Karsten Petersson,[a] and Jens B. Simonsen[a,*]

- LNP können ApoE rekrutieren
- LNP-ApoE-Komplexe werden via LDL-Rezeptoren (LDLR) aufgenommen
- Nach Endozytose > Freisetzung der modRNA / Plasmide



Frame-Shift Proteins

Zeta-Potential

Plasmide

LNP-Addukte

Promoter

Trojanisches Pferd

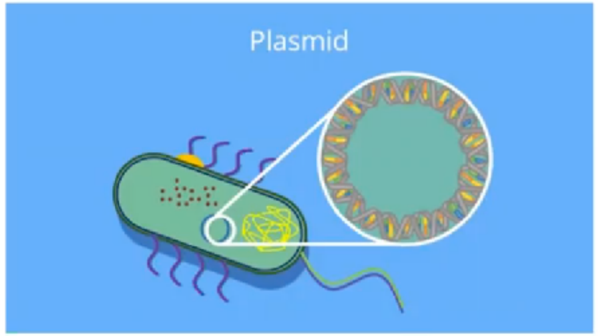
Mikrobiom

Nachweis

Procedure 2

https://odysee.com/@florian_schilling_science:d/procedure_2:8

Plasmide in mRNA-Vakzinen



https://odysee.com/@florian_schilling_science:d/plasmid:9

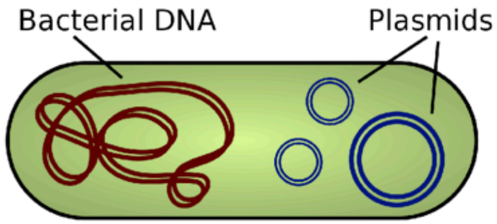
Sequencing of bivalent Moderna and Pfizer mRNA vaccines reveals nanogram to microgram quantities of expression vector dsDNA per dose

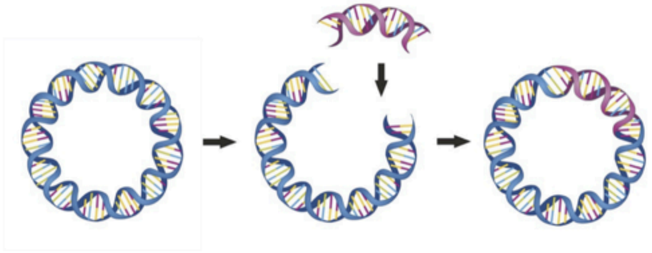
ANANDAMIDE
APR 10, 2023

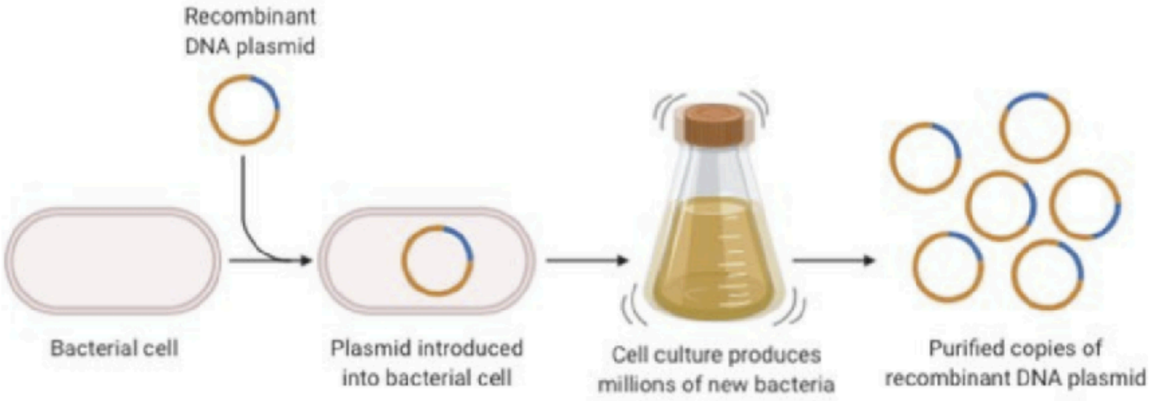
98 24 Share

Sequencing of bivalent Moderna and Pfizer mRNA vaccines reveals nanogram to microgram quantities of expression vector dsDNA per dose

https://anandamide.substack.com/p/sequencing-of-bivalent-moderna-and?utm_source=substack&utm_campaign=post_embed&utm_medium=web


Bacterial DNA
Plasmids




Recombinant
DNA plasmid
Bacterial cell
Plasmid introduced
into bacterial cell
Cell culture produces
millions of new bacteria
Purified copies of
recombinant DNA plasmid

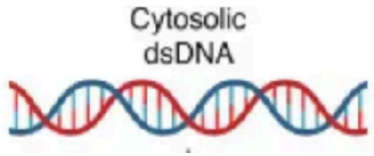
MAPK
>> Zellproliferation

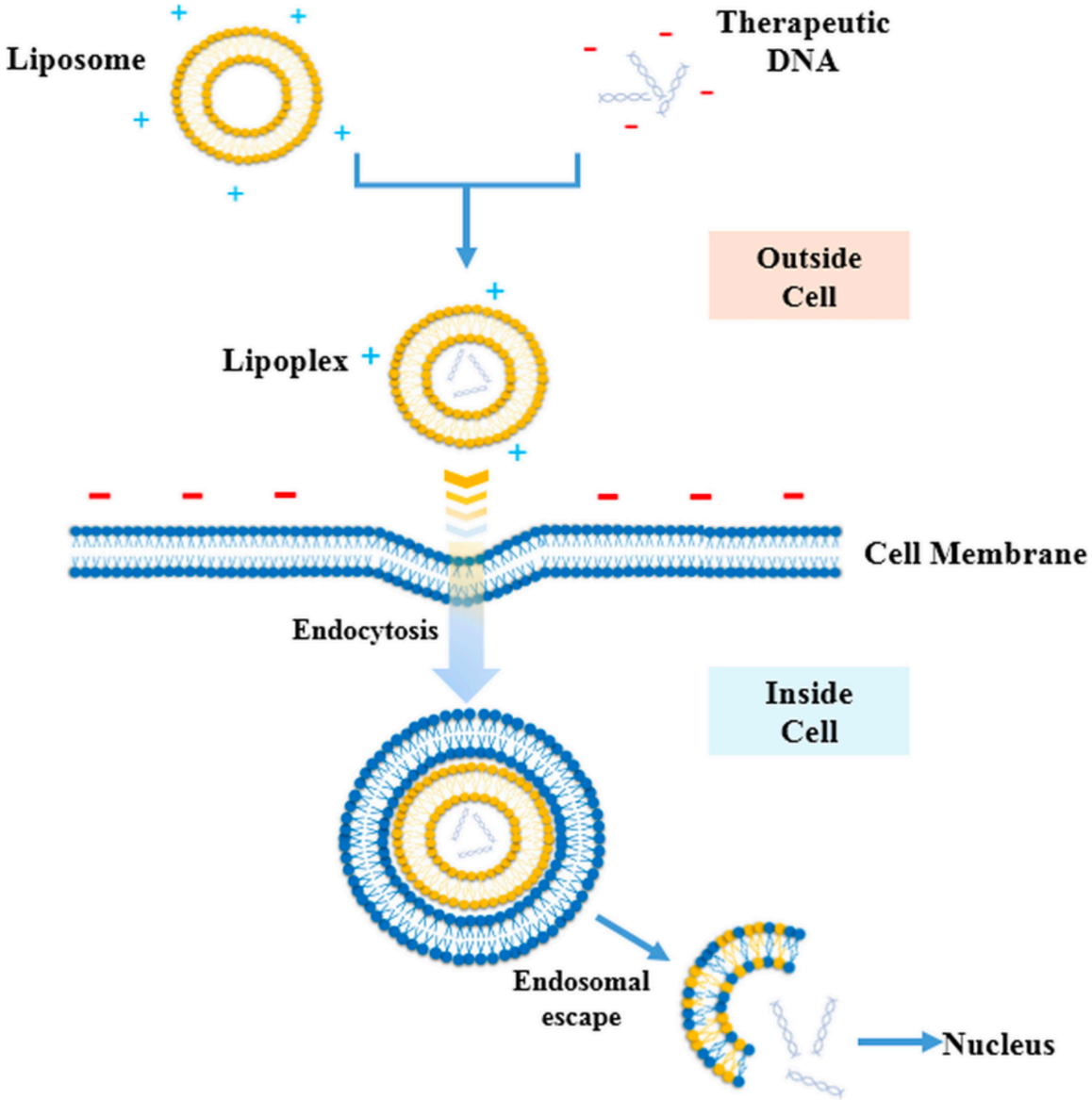
Autoimmunität

Gewebeschäden

Silent inflammation
Neuroinflammation

Zellstoffwechsel
>> Gärungsmodus




Liposome
Therapeutic DNA
Outside Cell
Lipoplex
Cell Membrane
Endocytosis
Inside Cell
Endosomal escape
Nucleus

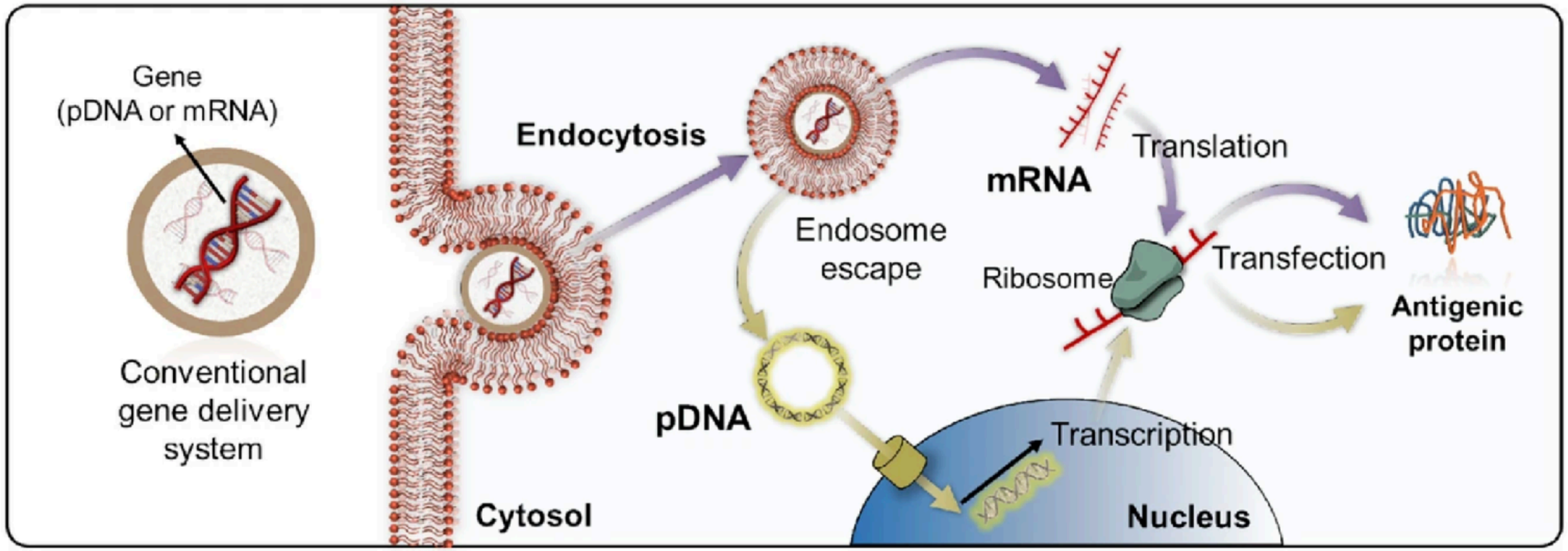
Review | Open access | Published: 07 August 2023

Lipid nanoparticle-based mRNA delivery systems for cancer immunotherapy

Jieun Han, Jaesung Lim, Chi-Pin James Wang, Jun-Hyeok Han, Ha Eun Shin, Se-Na Kim, Dooyong Jeong, Sang Hwi Lee, Bok-Hwan Chun, Chun Gwon Park ✉ & Wooram Park ✉

Nano Convergence **10**, Article number: 36 (2023) | Cite this article

advancement of mRNA therapeutics [15, 16]. mRNA has emerged as an attractive therapeutic agent endowed with unique advantages. It functions within the cytoplasm, thereby eliminating the risk of unintentional gene alterations or mutations as observed with plasmid DNA (pDNA) (Fig. 1). Moreover, mRNA exhibits high efficacy in dividing cells and can be synthesized on a large scale, rendering it a compelling candidate for the development of novel therapeutic



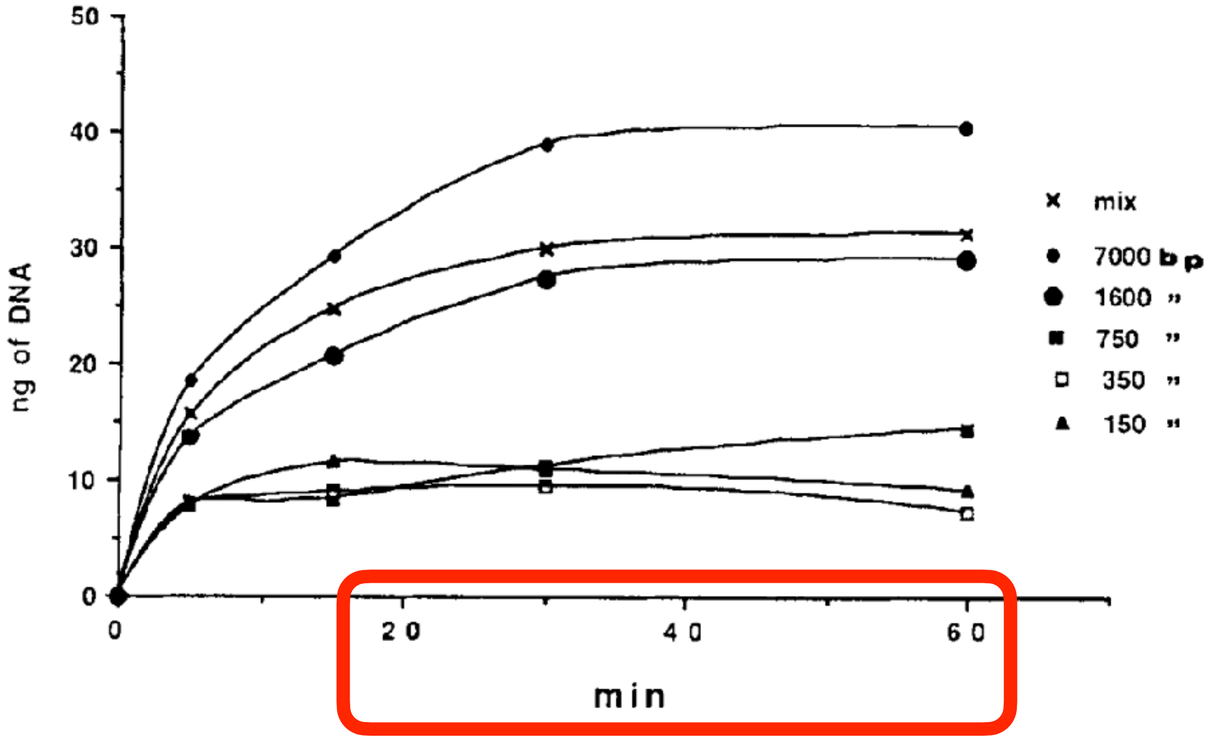
> Mol Reprod Dev. 1992 Mar;31(3):161-9. doi: 10.1002/mrd.1080310302.

The interaction between exogenous DNA and sperm cells

M Lavitrano [1], D French, M Zani, L Frati, C Spadafora

Affiliations + expand

PMID: 1554501 DOI: 10.1002/mrd.1080310302



- Aufnahme externer dsDNA (Plasmide) durch Spermien
- Positive Korrelation der DNA-Aufnahme mit
  - Größe der DNA (bp)
  - Elektro-Negativität des externen Genoms
  - Zeitachse: <1h (!)

Volume 57, Issue 5, 2 June 1989, Pages 717-723

Article

Sperm cells as vectors for introducing foreign DNA into eggs: Genetic transformation of mice

Marialuisa Lavitrano ★ †, Antonella Camaioni †, Vito M. Fazio † §, Susanna Dolci ‡, Maria G. Farace † ¶ ‖, Corrado Spadafora ★ †

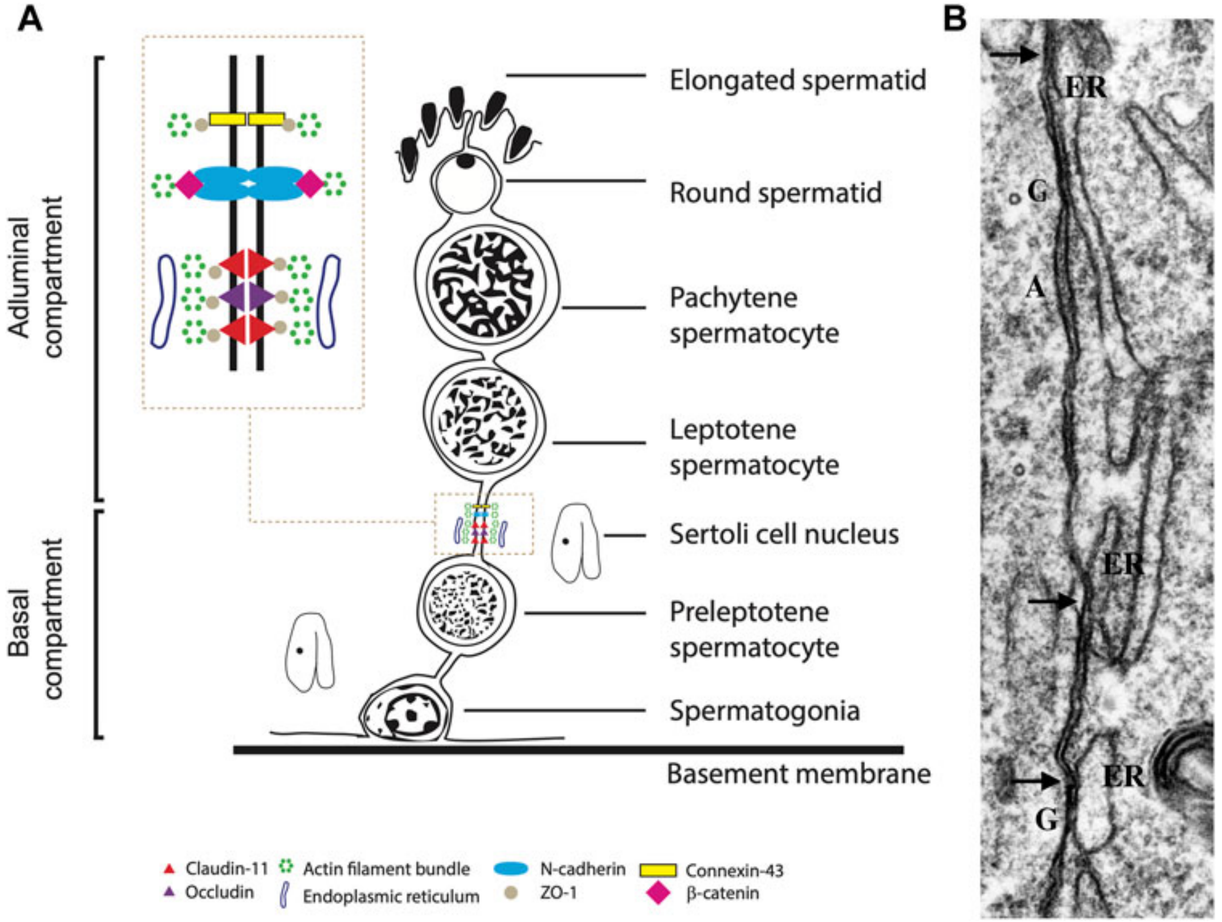
Show more

+ Add to Mendeley Share Cite

https://doi.org/10.1016/0092-8674(89)90787-3

- Inkubation von Spermien mit externer dsDNA (Plasmide) für 15 Minuten
- Anschließend Befruchtung von Eizellen
- Nachweis der Vektor-DNA (d.h. Plasmid-DNA) bei 30% der Nachkommen
- Schlussfolgerung: Geeignete Methode zur „Produktion transgener Mäuse“

- Theoretischer Schutz: Blood-Testis-Barrier (Tight Junctions)
- Porengröße <200nm, LNP-Durchmesser: 50-100nm
- Stärkere Aufnahme bei negativer Ladung
- Expression von ApoE-Rezeptoren in den Hoden
- Nachweis von LNPs in japanischer Biodistrubtions-Studie



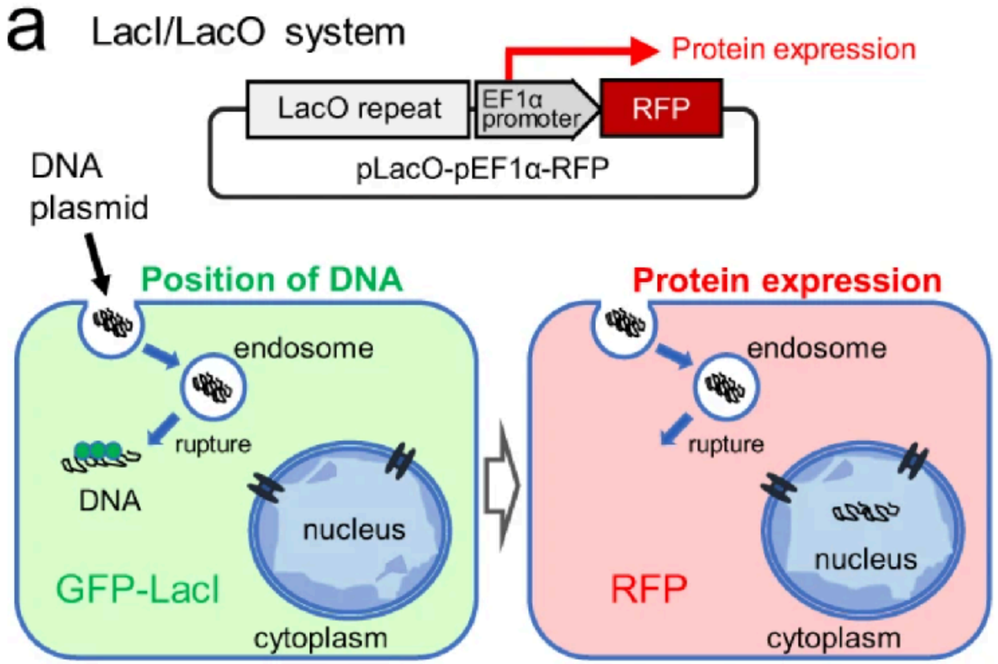
nature › communications biology › articles › article

Article | Open access | Published: 20 January 2022

Transfected plasmid DNA is incorporated into the nucleus via nuclear envelope reformation at telophase

Tokuko Haraguchi ✉, Takako Koujin, Tomoko Shindo, Şükriye Bilir, Hiroko Osakada, Kohei Nishimura, Yasuhiro Hirano, Haruhiko Asakawa, Chie Mori, Shouhei Kobayashi, Yasushi Okada, Yuji Chikashige, Tatsuo Fukagawa, Shinsuke Shibata & Yasushi Hiraoka

Communications Biology **5**, Article number: 78 (2022) | Cite this article



- Lipid Transfection with DNA plasmids
- Fluorescent marking for relase (GFP-LacL) and Expression (RFP)
- Nuclear translocation and expression after mitosis

TECHNICAL ADVANCE | Free Access

Lipofection of plasmid DNA into human mast cell lines using lipid nanoparticles generated by microfluidic mixing

Brett A. Duguay, Kate Wei-Chen Huang, Marianna Kulka

First published: 18 April 2018 | https://doi.org/10.1002/JLB.3TA0517-192R | Citations: 12

Abstract

Mast cells are important immune cells that have significant roles in mediating allergy and asthma. Therefore, studying the molecular mechanisms regulating these and other processes in mast cells is important to elucidate. Methods such as lipofection, transduction, and electroporation are often employed to dissect these mechanisms by disrupting gene expression in mast cell lines. However, as with other leukocytes, human mast cells (HMCs) are often refractory to the delivery of plasmids by lipofection. In this study, we investigated the utility of lipid nanoparticles (LNPs) containing the ionizable cationic lipids 1,2-dioleoyloxy-3-dimethylaminopropane, 1,2-dioleyloxy-3-dimethylaminopropane, or 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane for the delivery of plasmid DNA into HMC lines. Herein, we demonstrate for the first time the use of LNPs to achieve significant and reproducible levels of plasmid DNA transfection in HMC-1.2 and laboratory of allergic diseases 2 (LAD2) cells. These levels reached 53.2% and 16.0% in HMC-1.2 and LAD2 cells, respectively; and outperformed Lipofectamine 3000 in both cases. Moreover, cell viability in the transfected cells remained above 65%

In vivo delivery of plasmid DNA by lipid nanoparticles: the influence of ionizable cationic lipids on organ-selective gene expression†

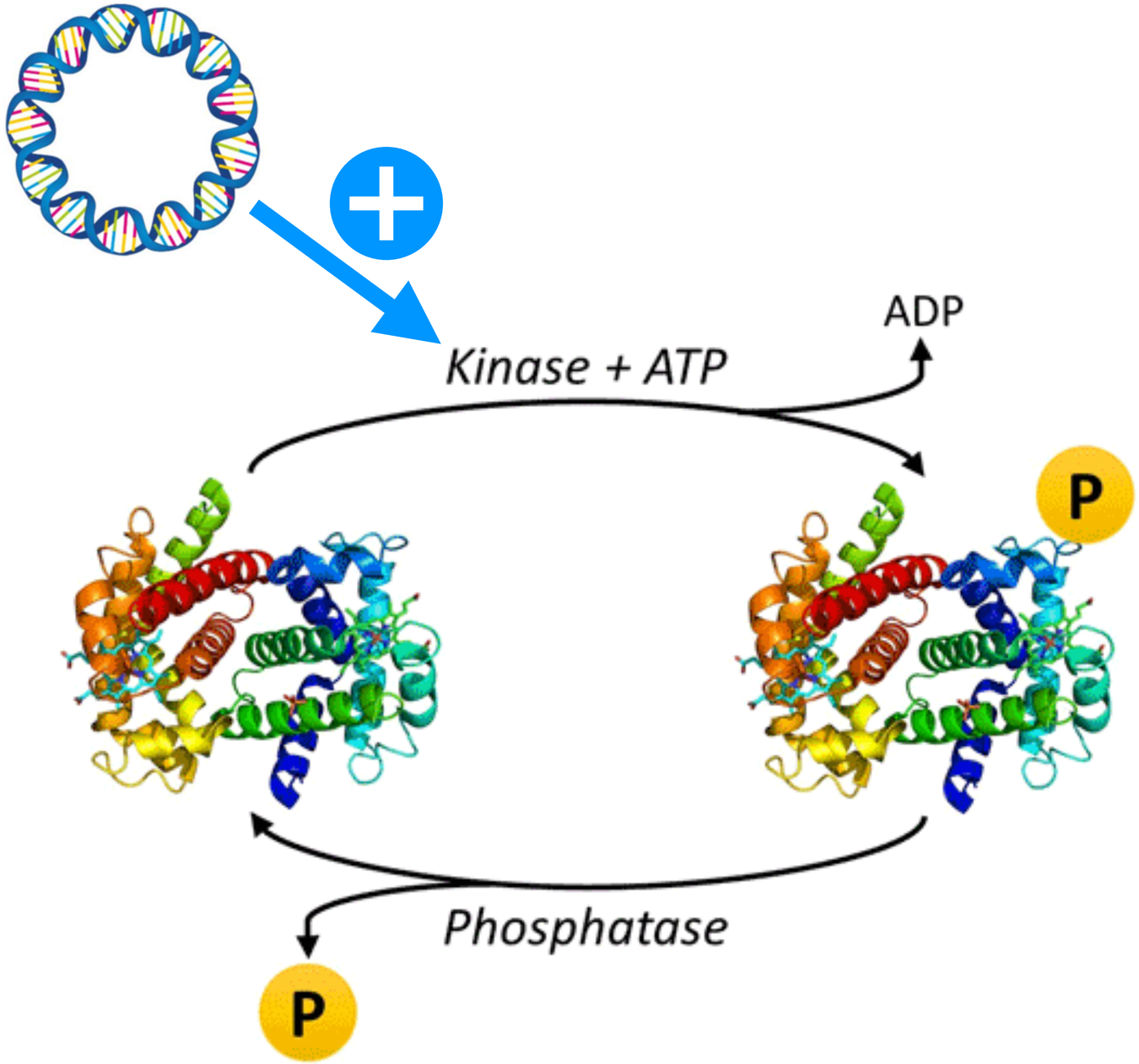
Check for updates

Azizah Algarni, [a] Emily H. Pilkington, [a] Estelle J. A. Suys, [a] Hareth Al-Wassiti, [a] Colin W. Pouton *[a] and Nghia P. Truong *[a]

Author affiliations

Abstract

Ionizable cationic lipids play a critical role in developing new gene therapies for various biomedical applications, including COVID-19 vaccines. However, it remains unclear whether the formulation of lipid nanoparticles (LNPs) using DLin-MC3-DMA, an optimized ionizable lipid clinically used for small interfering RNA (siRNA) therapy, also facilitates high liver-selective transfection of other gene therapies such as plasmid DNA (pDNA). Here we report the first investigation into pDNA transfection efficiency in different mouse organs after intramuscular and intravenous administration of lipid nanoparticles (LNPs) where DLin-MC3-DMA, DLin-KC2-DMA or DODAP are used as the ionizable cationic lipid component of the LNP. We discovered that these three benchmark lipids previously developed for siRNA delivery followed an unexpected characteristic rank order in gene expression efficiency when utilized for pDNA. In



- Phosphorylierung zählt zu den post-translationalen Modifikationen
- Moduliert werden u.a. folgende Protein-Eigenschaften:
  - Lokalisation
  - Stabilität & Form
  - Enzymatische Aktivität

Research Article | 1 January 1985 |

Double-stranded DNA induces the phosphorylation of several proteins including the 90 000 mol. wt. heat-shock protein in animal cell extracts.

A.I. Walker, T. Hunt, R.J. Jackson, and C.W. Anderson | AUTHOR INFORMATION

The EMBO Journal (1985) 4: 139 - 145 | https://doi.org/10.1002/j.1460-2075.1985.tb02328.x

Double-stranded DNA (dsDNA) induces the transfer of phosphate from ATP to several proteins in extracts of widely divergent eukaryotic cells. Extracts of HeLa cells, rabbit reticulocytes, Xenopus eggs and Arbacia eggs all show dsDNA-dependent protein phosphorylation. The mechanism is specific for dsDNA and will not respond to either RNA or single-stranded DNA. One of the proteins which is phosphorylated in response to dsDNA has a subunit mol. wt. of 90 000 and has been identified as a heat-shock protein (hsp90). Although mouse cell extracts were shown to contain hsp90, they failed to show a dsDNA-dependent protein phosphorylation. The observation that dsDNA can modulate the phosphorylation of a set of proteins raises the possibility that dsDNA may play a role as a cellular regulatory signal.

Specifically, protein phosphorylation promotes the occurrence and development of cancer in the following aspects: inducing the cancer cell proliferation, inhibiting the cancer cell apoptosis, inducing cancer cells invasion and metastasis, inducing cancer cells angiogenesis and inducing cancer stem cells proliferation. Therefore, an in-depth understanding of the relevant mechanisms and signal pathways of protein phosphorylation disorders and related regulatory enzymes can help us to further understand the pathogenesis of cancer and screen tumor-related markers and target molecules, which is also of great significance to the research for potential targeted therapeutic antitumor drugs.

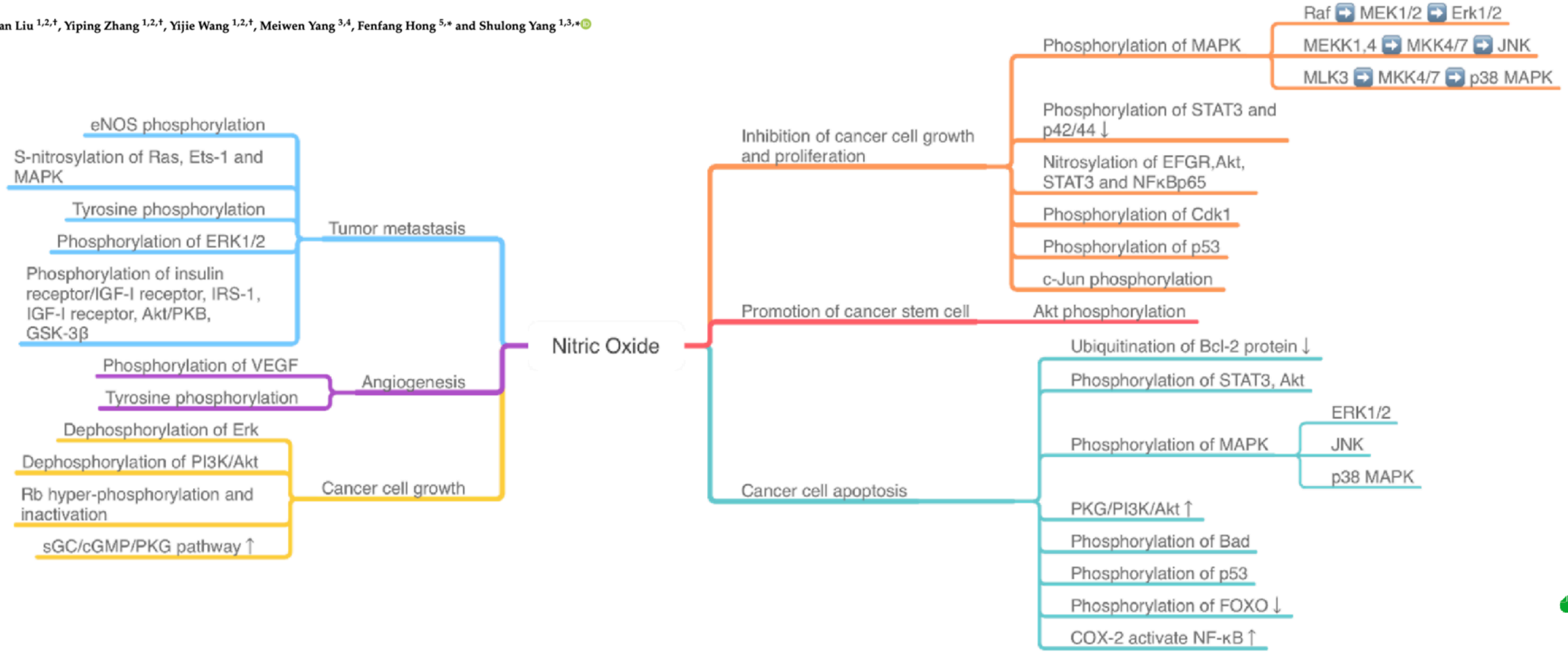
biomolecules

MDPI

Review

Protein Phosphorylation in Cancer: Role of Nitric Oxide Signaling Pathway

Xinran Liu [1,2,†], Yiping Zhang [1,2,†], Yijie Wang [1,2,†], Meiwen Yang [3,4], Fenfang Hong [5,*] and Shulong Yang [1,3,*]



Frame-Shift Proteins

Zeta-Potential

Plasmide

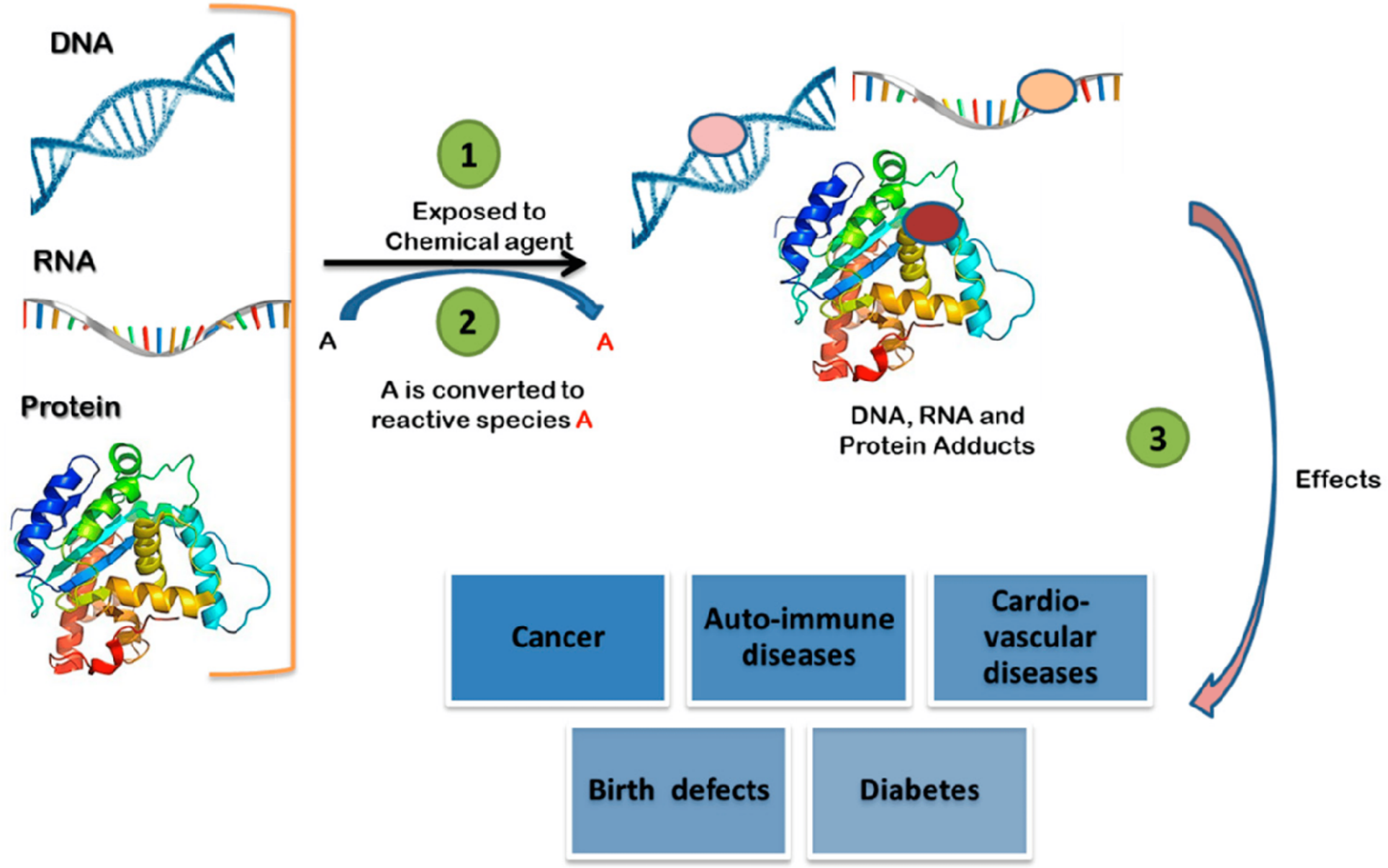
LNP-Addukte

Promoter

Trojanisches Pferd

Mikrobiom

Nachweis


DNA
RNA
Protein
1
Exposed to
Chemical agent
2
A
A
A is converted to
reactive species A
DNA, RNA and
Protein Adducts
3
Effects
Cancer
Auto-immune
diseases
Cardio-
vascular
diseases
Birth defects
Diabetes

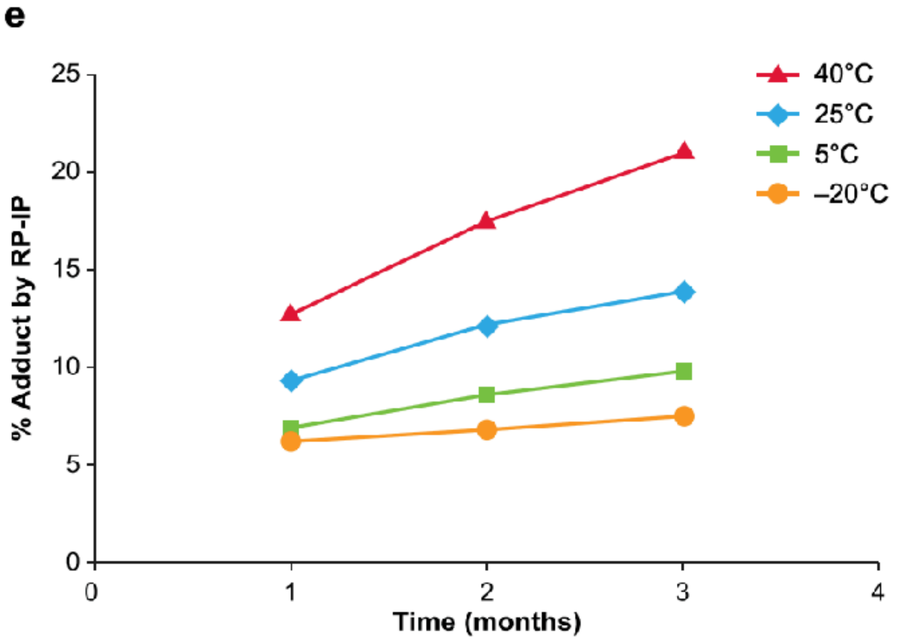
nature > nature communications > articles > article

Article | Open access | Published: 22 November 2021

A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems

Meredith Packer, Dipendra Gyawali, Ravikiran Yerabolu, Joseph Schariter & Phil White

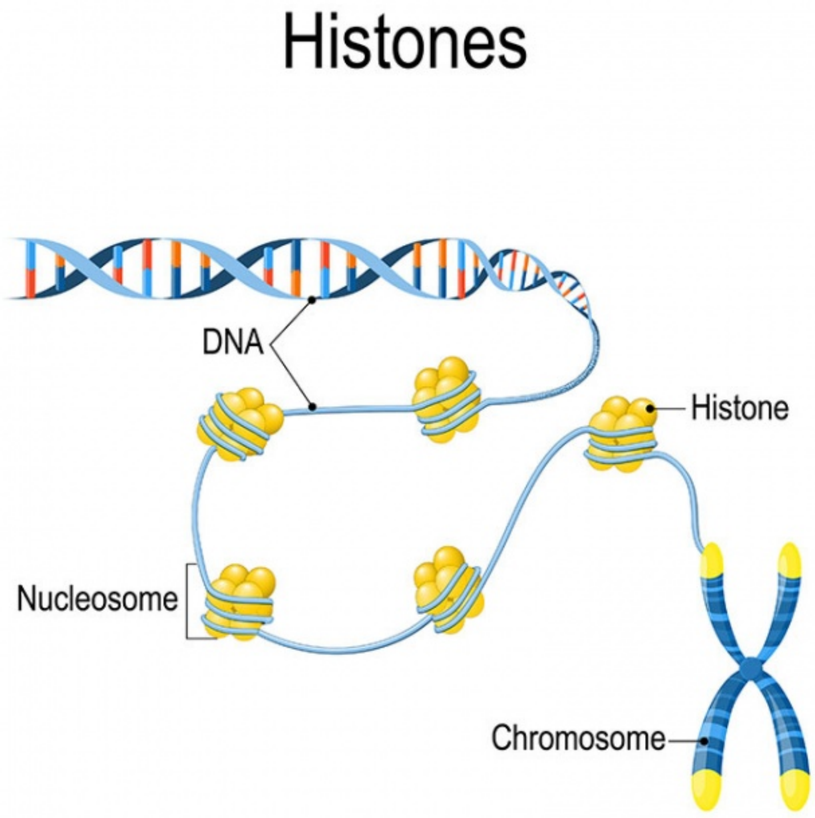
Nature Communications **12**, Article number: 6777 (2021) | Cite this article

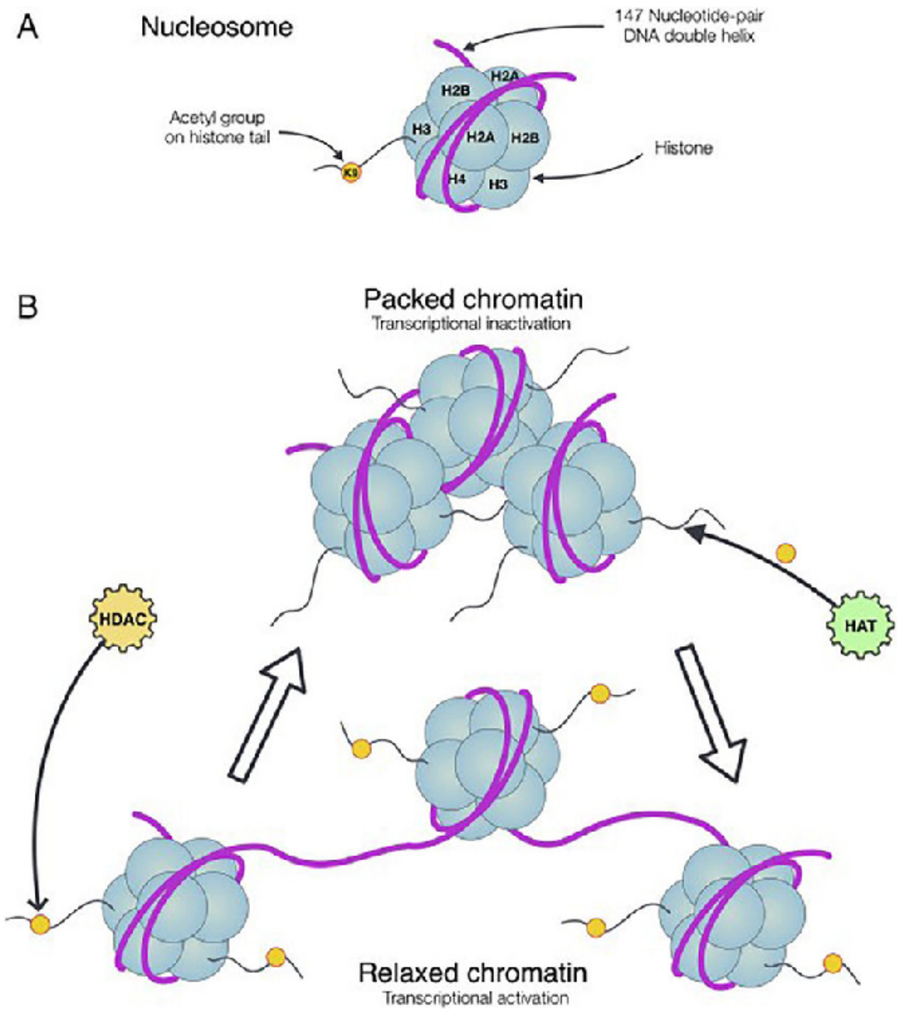


- non coding
- frameshift
- point mutation
- junk protein
- misfolded proteins

Weitere Addukte?

- Die genannten Mechanismen finden instant, bereits während der Produktion, in den LNP statt
- Diese enthalten aber (dank Process 2) nicht nur mRNA - sondern auch dsDNA in Form von Plasmiden
  - Möglichkeit 1: Addukte an der dsDNA
  - Möglichkeit 2: Addukte an der modRNA
  - Möglichkeit 3: Addukte an zellulärer mRNA
  - Möglichkeit 4: Eintritt der LNP in den Nukleus einer geimpften Zelle und LNP-Addukte am nukleären Genom
- Konsequenzen: Potentiell weitreichend, bislang nicht geprüft!
  - Theoretisch: (Kanzerogene) Mutationen?
  - Aberrante Proteine (Funktionsverlust, Amyloide, Autoimmunität, Prionen, etc.)
  - Rescue via DNA-Reparatur unwahrscheinlich, da diese epigenetisch heruntergeregelt wird (vgl. p53)





Verhelstet al (2020). Comprehensive histone epigenetics: A mass spectrometry based screening assay to measure epigenetic toxicity. MethodsX. 7. 10.1016/j.mex.2020.101055.

NANO · MICRO small

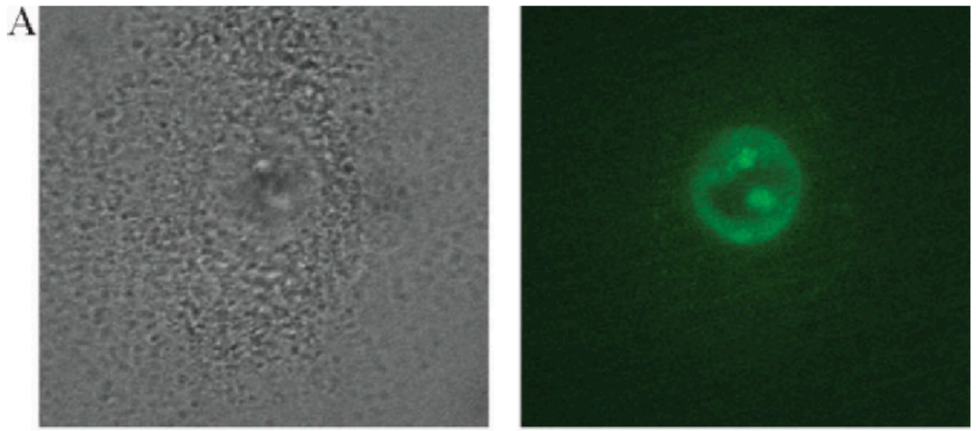
Full Paper

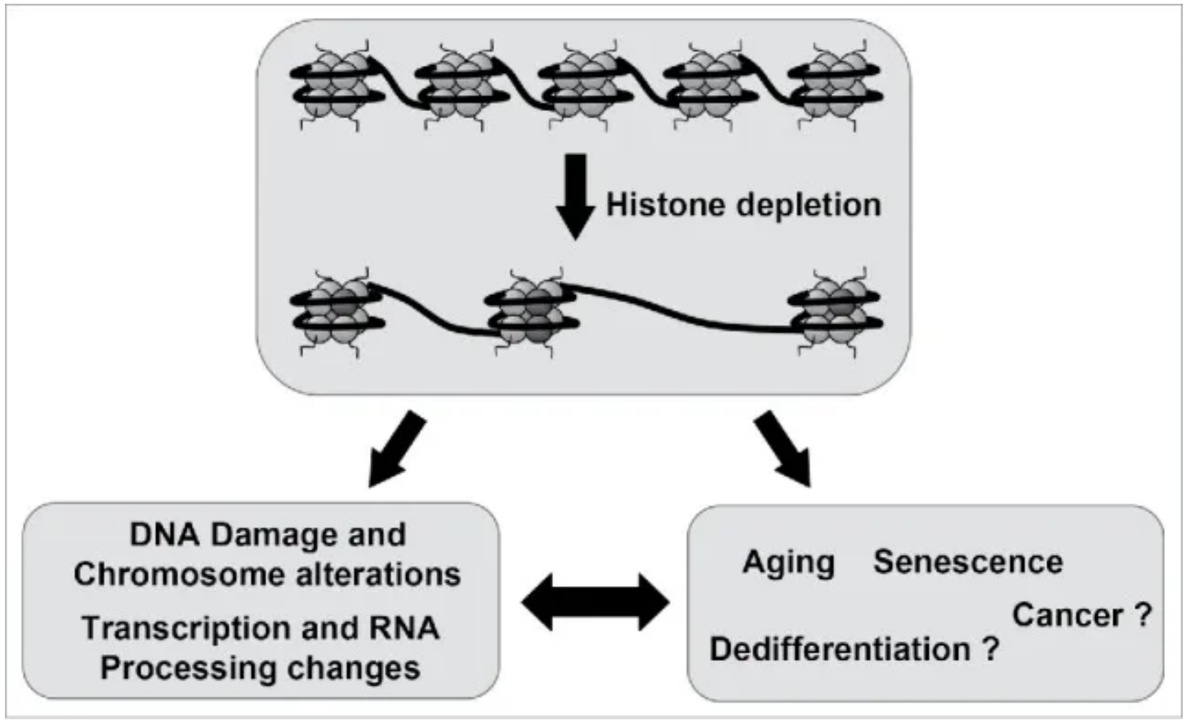
CdTe Nanoparticles Display Tropism to Core Histones and Histone-Rich Cell Organelles

Jennifer Conroy, Stephen J. Byrne, Yurii K. Gun'ko, Yury P. Rakovich, John F. Donegan, Anthony Davies, Dermot Kelleher, Yuri Volkov

First published: 03 November 2008 | https://doi.org/10.1002/smll.200800088 | Citations: 65

- QD (Quantum dots) = Nanoparticles with similar size & charge (highly negative) as LNP





Frame-Shift Proteins

Zeta-Potential

Plasmide

LNP-Addukte

Promoter

Trojanisches Pferd

Mikrobiom

Nachweis

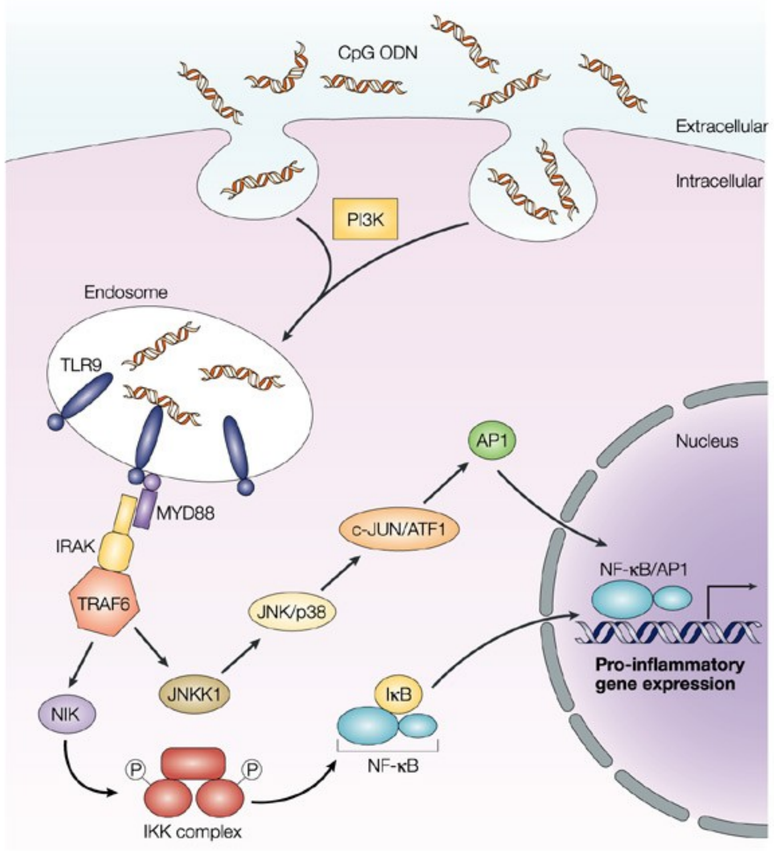
nature › nature reviews immunology › review articles › article

Review Article | Published: 01 April 2004

Immunotherapeutic uses of CpG oligodeoxynucleotides

Dennis M. Klinman

Nature Reviews Immunology **4**, 249–259 (2004) | Cite this article



- Cytidin-Guanosin-Dinukleotide
- Imititation mikrobieller Genomsequenzen
- Starke Reizung des Immunsystems (PRR)

Simon Rothenfußer
Bernd Jahrsdörfer
Anne Krug
Stefan Endres
Gunther Hartmann

CpG-Oligonukleotide: Immuntherapie nach dem Muster bakterieller DNA

Zusammenfassung

DNA wurde bislang vor allem als biologischer Speicher für die Vielzahl der Gene des Erbguts betrachtet. Seit kurzer Zeit wird die funktionelle Bedeutung von DNA weiter gefasst. Unterschiede im Aufbau von bakterieller oder viraler DNA (so genannte CpG-Motive) im Vergleich zur Wirbeltier-DNA ermöglichen dem Immunsystem, diese potenziellen Krankheitserreger aufgrund ihrer DNA-Struktur zu erkennen. CpG-Motive sind nichtmethylierte Cytidin-Guanosin-Dinukleotide mit bestimmten flankierenden Basensequenzen. Synthetische Oligonukleotide, die solche CpG-Motive enthalten, imitieren die Anwesenheit von mikrobieller DNA und induzieren ein charakteristisches Aktivierungsmuster von Immunzellen. Kürzlich wurden ein potentes humanes CpG-Motiv identifiziert und nukleasestabile CpG-Oligonukleotide entwickelt. Die erste klinische Studie mit einem dieser Oligonukleotide hat die günstigen Eigenschaften von CpG-Oligonukleotiden als Vakzine-Adjuvans beim Menschen bestätigt. Derzeit werden CpG-Oligonukleotide zur Therapie von Tumorerkrankungen, Infektionserkrankungen, Allergien und Asthma bronchiale klinisch geprüft.

Schlüsselwörter: Oligonukleotid, bakterielle DNA, bakterielles Lysat, Immuntherapie, Adjuvans

Summary

CpG-Oligonucleotides: Immunotherapy Based on Bacterial DNA

Certain sequences within bacterial or viral DNA serve as a molecular pattern which alerts the immune system against invading pathogens. Detection of microbial DNA is based on cytidine-guanosine (CpG) dinucleotides. These are underrepresented and also selectively methylated in vertebrate DNA but present at expected frequency and unmethylated in bacterial DNA. Oligonucleotides which contain unmethylated CpG dinucleotides within specific flanking bases (CpG motif) mimic microbial DNA and induce a coordinated set of immune responses. Recently, CpG motifs with high activity in the human system have been identified. Based on these motifs nuclease-stable CpG oligonucleotides have been designed which demonstrate potent adjuvant vaccine activity in primates and in humans. Currently, CpG oligonucleotides are under clinical development for infectious disease, cancer, and allergy.

Key words: oligonucleotide, bacterial DNA, bacterial lysate, immunotherapy, adjuvant

Vor über 100 Jahren hat der New Yorker Chirurg William Coley erkannt, dass das Immunsystem in der Lage ist, Tumoren erfolgreich zu bekämpfen. Er beobachtete, dass sich ein Sarkom nach einer bakteriellen Infektion (Erysipel) im Bereich des Tumors zurückbildete. Im Jahr 1891 begann er mit der lokalen Injektion von Bakterien oder bakteriellen Lysaten aus Streptokokkus und Serratia. Diese Behandlung führte bei einem Teil der Patienten zu einer vorübergehenden Rückbildung der Sarkome (3, 24, 35). Dieses Vorgehen stellte die erste Immuntherapie einer Tumorerkrankung dar. Andere konnten die Therapieerfolge in diesem Ausmaß nicht bestätigen. Die Standardisierbarkeit von bakteriellen Lysaten war um die Jahrhundertwende nicht gegeben und die einzelnen Komponenten des Immunsystems waren noch nicht identifiziert. Zudem zeigte die damals neu entwickelte Strahlentherapie von Tumoren gute Resultate und rückte in den Mittelpunkt des Interesses.

Heute werden Bakterienextrakte mit definierter Zusammensetzung in der Therapie von rezidivierenden Infektionen der Luftwege eingesetzt (zum Beispiel Broncho-Vaxom). Im Bereich der Therapie von Tumoren ist die Instillation von teilungsfähigen Tuberkelbakterien (BCG, Bacillus Calmette-Guerin) in die Harnblase Teil der Standardtherapie des oberflächlichen Harnblasenkarzinoms in den Stadien Carcinoma in situ (CIS), T_a und T_1 (zum Beispiel BCG Connaught Immucyst). In einer klinischen Studie bei 254 Patienten mit Kolonkarzinom führte eine adjuvante mehrfache Vakzinierung mit bestrahlten körpereigenen Tumorzellen in Verbindung mit BCG (lebende Tuberkelbakterien) als Adjuvans zu einer Verminderung des Auftretens eines Rezidivs nach kurativer Resektion des Primärtumors (31).

Tabelle
Übersicht zum Aufbau von CpG-DNA

	CG-Dinukleotide	Methylierung am Cytidin
Wirbeltier-DNA	1 von 60 Dinukleotiden	ja
Bakterielle DNA	1 von 16 Dinukleotiden	nein
CpG-Oligonukleotid	vorhanden (CpG-Motive; z. B. 5'...GACGTT...3')	nein

Die Fortschritte auf dem Gebiet der Immunologie haben zu einem besseren Verständnis des Wirkmechanismus von bakteriellen Lysaten geführt. Tokunaga hat 1984 das Lysat von BCG in verschiedene Fraktionen aufgetrennt und deren therapeutische Aktivität in zwei Tumormodellen untersucht. Dabei zeigte überraschenderweise die Fraktion mit der DNA der Bakterien die höchste Aktivität (29). Yamamoto entdeckte 1992, dass die DNA von Bakterien, nicht aber die von Wirbeltieren immunstimulatorische Aktivität (Typ-1-Interferon-Synthese, NK-Zellaktivierung [NK, natürliche Killerzellen])

Abteilung für Klinische Pharmakologie (Leiter: Prof. Dr. med. Stefan Endres) der Medizinischen Klinik Innenstadt (Kommissarischer Direktor: Prof. Dr. med. Detlef Schlöndorff), Klinikum der Ludwig-Maximilians-Universität München

nature > nature reviews molecular cell biology > review articles > article

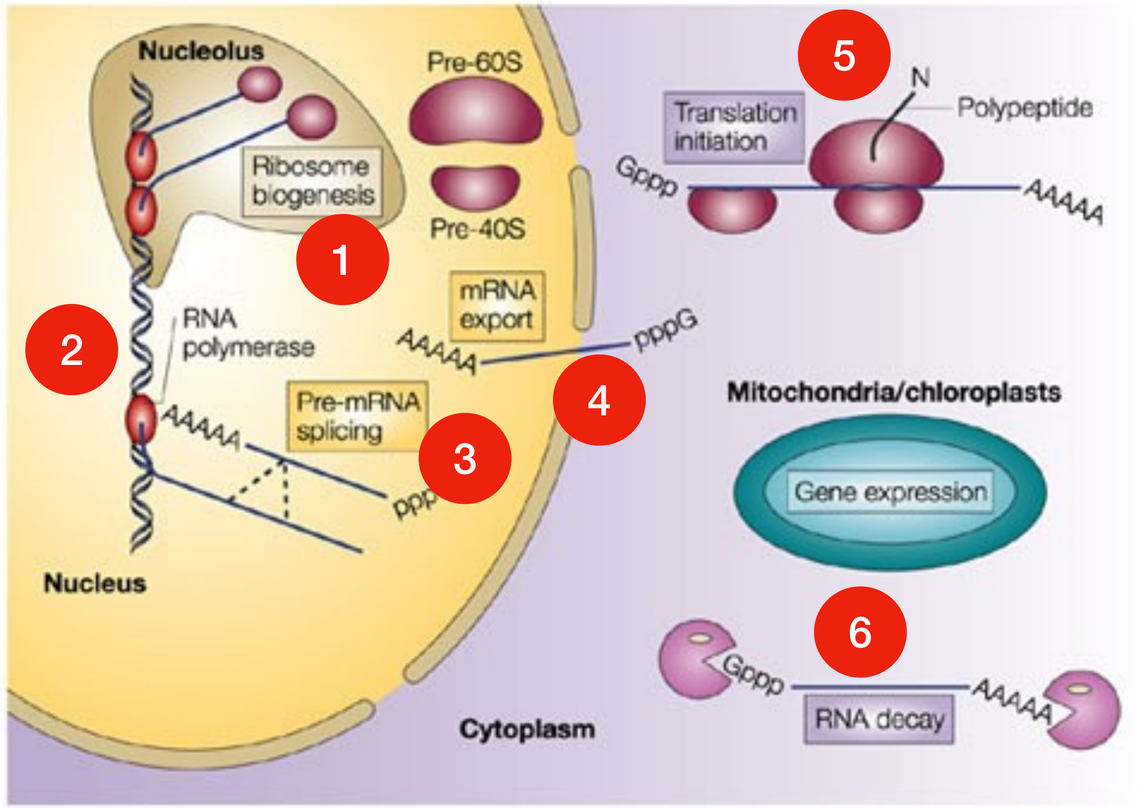
Review Article | Published: 01 March 2004

DEAD-box proteins: the driving forces behind RNA metabolism

Sanda Rocak & Patrick Linder

Nature Reviews Molecular Cell Biology **5**, 232–241 (2004) | Cite this article

- ATP-abhängige RNA-Helicasen
- DEAD nach einer dominanten Aminosäuresequenz (Asp-Glu-Ala-Asp)
- Biogenese Ribosomen 1
- Genexpression 2
- mRNA-Splicing 3
- mRNA-Export ins Zytosol 4
- mRNA-Translation 5
- mRNA-Abbau 6



Nature Reviews | Molecular Cell Biology

RNAbiology

RNA Biol. 2013 Jan 1; 10(1): 121–132.
doi: 10.4161/rna.23312

PMCID: PMC3590229
PMID: 23353573

DEAD box RNA helicase functions in cancer

Frances V. Fuller-Pace

Author information Copyright and License information PMC Disclaimer

Abstract

Go to:

Members of the DEAD box family of RNA helicases are known to be involved in most cellular processes that require manipulation of RNA structure and, in many cases, exhibit other functions in addition to their established ATP-dependent RNA helicase activities. They thus play critical roles in cellular metabolism and in many cases have been implicated in cellular proliferation and/or neoplastic transformation. These proteins generally act as components of multi-protein complexes; therefore their precise role is likely to be influenced by their interacting partners and to be highly context-dependent. This may also provide an explanation for the sometimes conflicting reports suggesting that DEAD box proteins have both pro- and anti-proliferative roles in cancer.

- Sowohl modRNA als auch pDNA können an DEAD-boxes binden > Interaktion!
  - Modulation der DEAD-boxes
  - Modulation der exogenen Nukleotide (z.B. Expression)

Arch Clin Biomed Res 2021; 5 (3): 484-518

DOI: 10.26502/acbr.50170181

Research Article

Huaier Compensates Impaired Signal Transfer Inter/Intra Neurons in Central and Peripheral Nervous Systems

Manami Tanaka[1*], Tomoo Tanaka[1], Fei Teng[2], Hong Lin[2], Ning Li[3], Zhu Luo[3], Sotaro Sadahito[4], Toshiyuki Suzuki[5], Ding Wei[6] and Zhengxin Lu[7]

[1]Bradeion Institute of Medical Sciences, Co. Ltd., Kanagawa 259-1145, Japan

[2]BGI-Shenzhen, Building NO.7, BGI Park, Shenzhen 518083, China

[3]BGI-Japan, Kobe 650-0047, Japan

[4]Department of Surgery, Kameda-Morinosato Hospital, Kanagawa 243-0122, Japan

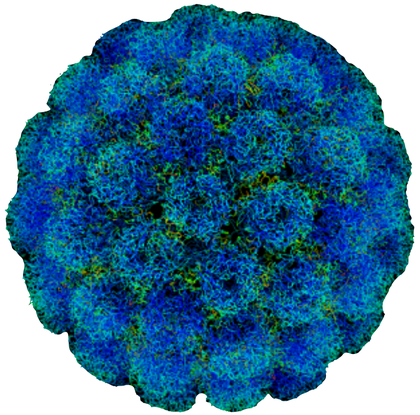
[5]Department of Surgery, Oiso Hospital, Tokai University School of Medicine, Kanagawa 259-0198, Japan

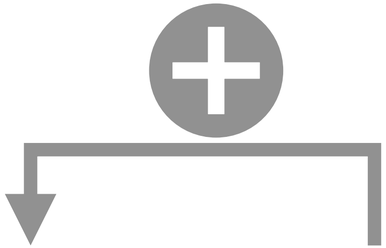
[6]Japan Kampo NewMedicine, Co. Ltd., Tokyo 103-0025, Japan

[7]QiDong Gaitianli Medicines Co. Ltd., Jiangsu Province, China

[*]Corresponding author: Manami Tanaka, Bradeion Institute of Medical Sciences, Co., Ltd., Japan

Received: 30 May 2021; **Accepted:** 17 June 2021; **Published:** 25 June 2021





- Simian Virus 40
- dsDNA-Virus (5.2kb), natürlicher Wirt: Affen
- Entdeckt 1960 - in Nierenzellen, die zur Produktion von Polio-Lebendimpfstoff verwendet wurden
- Genomischer Effekt:
  - Integration ins Wirtsgenom
  - Promoter-Funktion auf Gene proximal der Insertion
  - Immortalisierung der befallenen Zelle
- Verwendung in der Biomedizinischen Forschung & Industrie:
  - Gentransfer
  - Bioreaktoren (gezielte Expressionsförderung z.B. in Plasmiden)

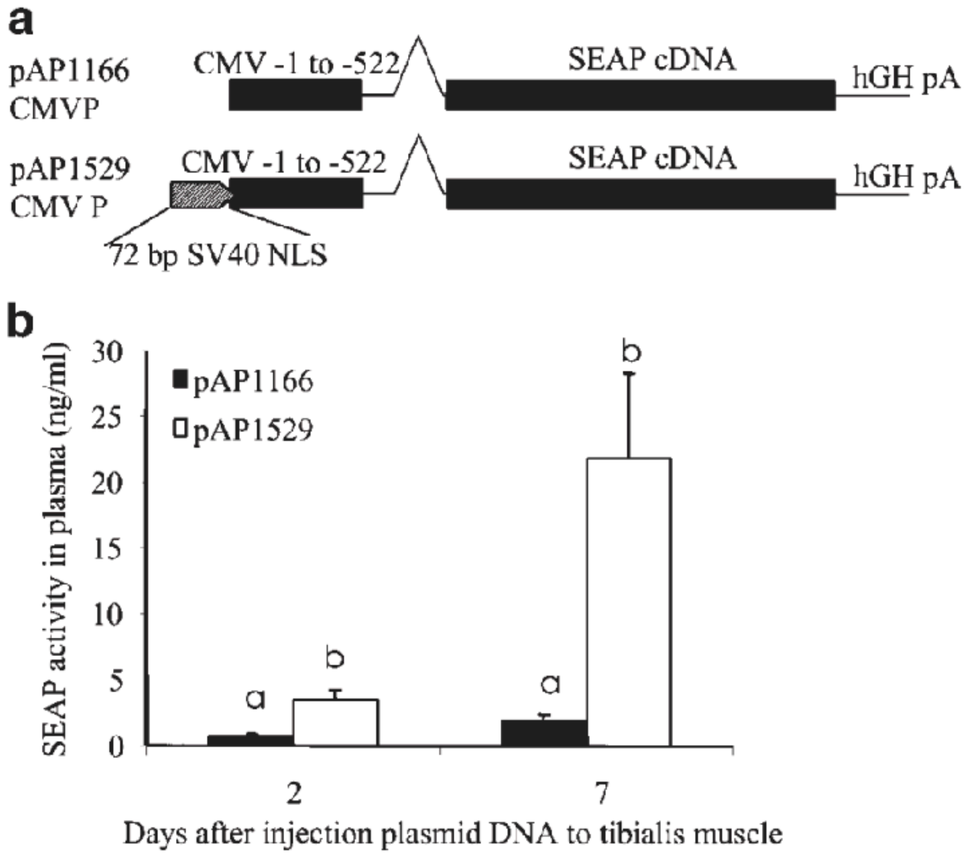
Muscle-specific enhancement of gene expression by incorporation of SV40 enhancer in the expression plasmid

S Li, F C MacLaughlin, J G Fewell, M Gondo, J Wang, F Nicol, D A Dean & L C Smith

Gene Therapy **8**, 494–497 (2001) | Cite this article

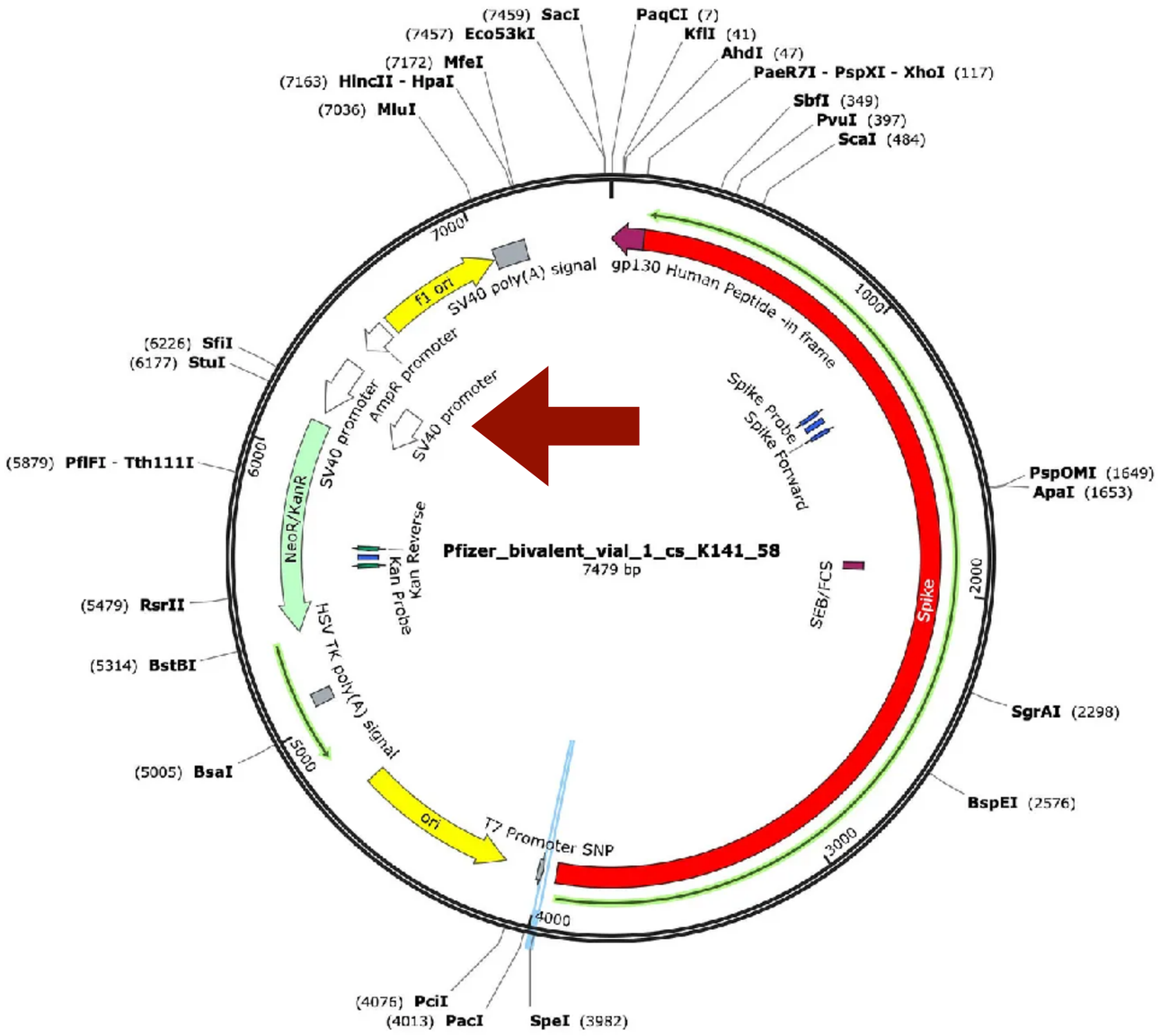
Abstract

Skeletal muscle is established as an ideal tissue for gene delivery to treat systemic diseases. However, the relatively low levels of gene expression obtained from using naturally occurring promoters, including the strong cytomegalovirus (CMV) enhancer/promoter (E/P), have limited the use of muscle as a target tissue. The relatively weak simian virus 40 (SV40) enhancer is known to have dual function promoting localization of DNA to the nucleus and activating transcription. An SV40 enhancer incorporated either at the 5′ end of CMV E/P or



pDNA ohne SV40

pDNA mit SV40



- Process 2 batches (d.h. kommerzieller Roll-Out) enthalten 20-30% Masse-Anteil pDNA
- Kontrolle obliegt den Herstellern, keine autonome Überwachung durch Aufsichtsbehörden
- Gehalt an intakter modRNA <70% war der EMA zum Jahreswechsel 20/21 bekannt
- Folge: Anpassung der Lieferverträge auf niedrigeren Qualitätsstandard
- Effekt von pDNA-geladenen LNP auf menschliche Zellen: Offiziell unbekannt

Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Az.: 6790-10-34

August 1995

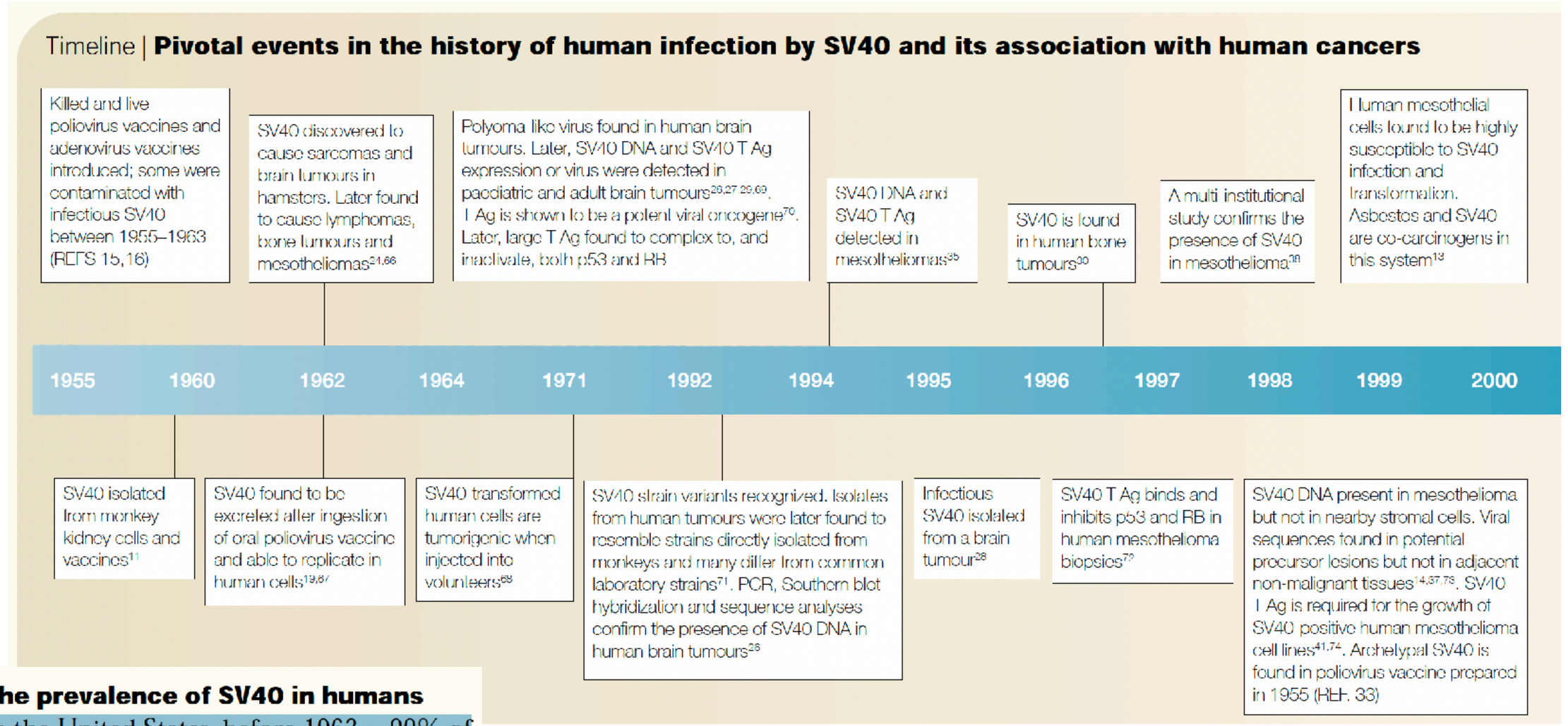
Allgemeine Stellungnahme der ZKBS
zu häufig durchgeführten gentechnischen Arbeiten mit den zugrunde liegenden Kriterien der Vergleichbarkeit:

Gentechnische Arbeiten mit SV40 als Spenderorganismus

3.7. Wird die gesamte genomische Information von SV40 in einen nicht-permissiven Empfängerorganismus der Risikogruppe 1 eingeführt (auch mit weiteren Nukleinsäureabschnitten ohne Gefährdungspotential) und integriert das SV40-Genom in das Wirtschromosom, so ist der gentechnisch veränderte Organismus der **Risikogruppe 1** zuzuordnen, auch wenn das Vektor-Empfänger-System keiner biologischen Sicherheitsmaßnahme entspricht. Gentechnische Arbeiten mit gentechnisch veränderten

Nieren von Rhesusaffen können latent mit SV40 infiziert sein. Als Verunreinigung in Zellkulturen von Affennieren wurde SV40 schließlich um 1960 entdeckt. Diese Entdeckung rief aus zwei Gründen Besorgnis hervor:

1. SV40 war eine nicht erkannte und weit verbreitete Kontamination in viralen Impfstoffen (Poliomyelitis-Impfstoff, Adenovirus-Impfstoff), die auf Affennierenzellen hergestellt wurden und die zwischen 1955 und 1963 weltweit Millionen von Personen verabreicht wurden. Allein in den USA wurde 98 Millionen Personen kontaminierter Poliomyelitis-Impfstoff injiziert, 10 000 Personen erhielten ihn oral, 100 000 Personen wurde kontaminierter Adenovirus-Impfstoff injiziert [6].
2. SV40 hat onkogenes Potential. Nach Injektion in neugeborene Hamster kann es Tumore verursachen. Sein immortalisierendes Potential für menschliche Zellen wurde in-vitro dokumentiert [1, 7, 9, 10].

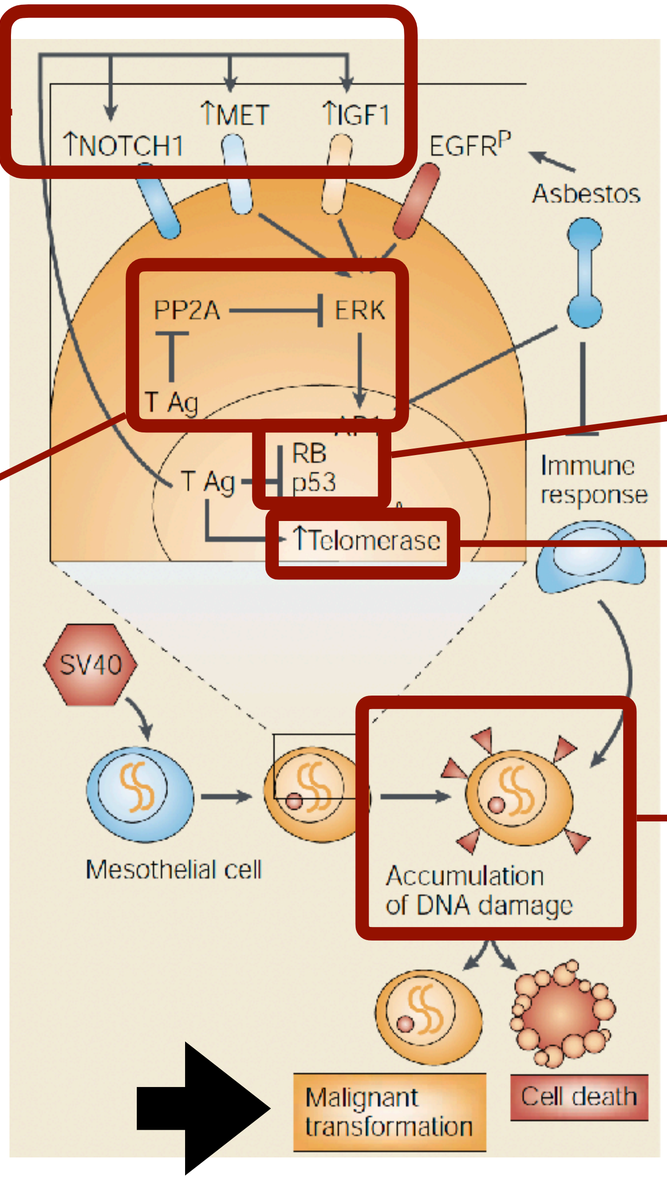


The prevalence of SV40 in humans

In the United States, before 1963, ~90% of children and 60% of adults received one or more contaminated poliovirus vaccinations,

▲ Aktivität von Onkogenenen & pro-proliferativen Signalwegen

Enthemmung ERK (Endstrecke Ras/Raf): Proliferation Angiogenese, Resistenz



▼ DNA-Reparatur

Immortalisierung

Akkumulation von DNA-Schäden

Integration von mit Coronavirus-Impfstoffen kontaminierter DNA in das Genom menschlicher Zelllinien

797

Hiroshi Arakawa
3. März 2024 20:09

https://note.com/hiroshi_arakawa/n/na5d608e4fe9d

Vaccine targeted qPCR of Cancer Cell Lines treated with BNT162b2

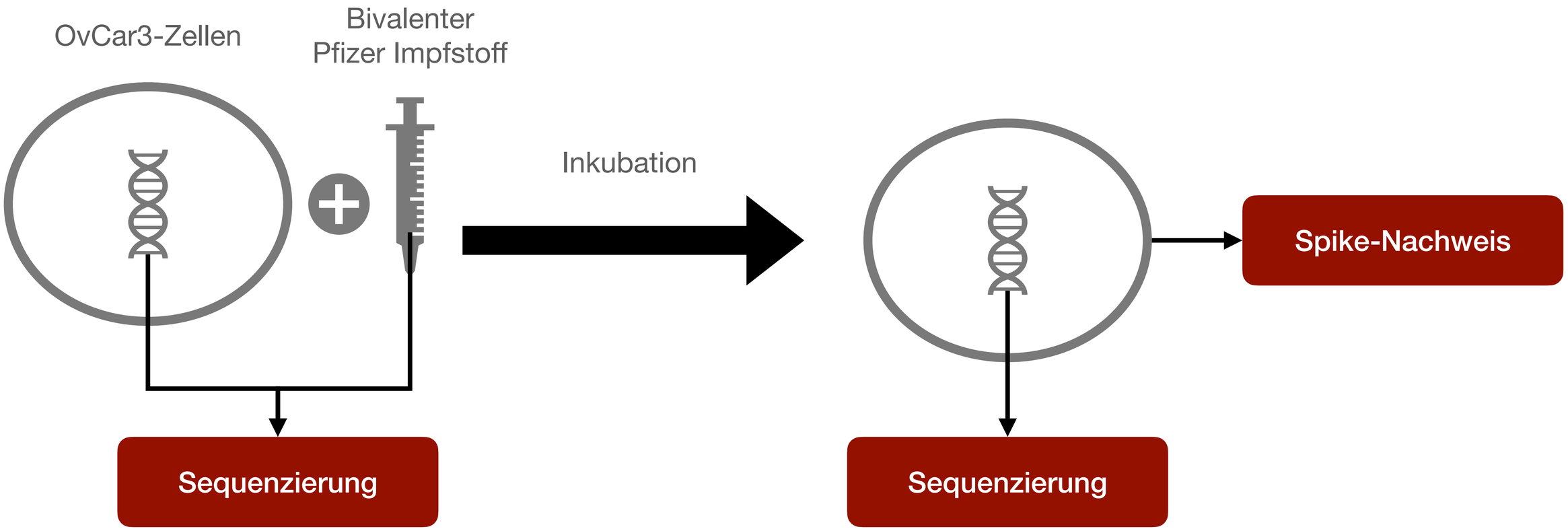
Putative integration events

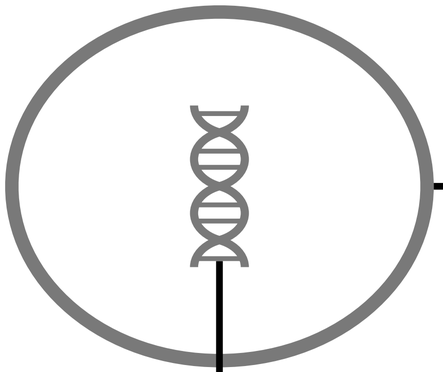
ANANDAMIDE
FEB 25, 2024

https://anandamide.substack.com?utm_source=navbar&utm_medium=web

Apl. Prof. Dr. rer. hum. biol. Ulrike Kämmerer
Wissenschaftliche Mitarbeiterin

In-Vitro-Inkubation von Eierstock-Krebszellen mit Pfizer-Impfstoff
+
Anschließende Sequenzierung & immunhistochemische Färbung


OvCar3-Zellen
Bivalenter
Pfizer Impfstoff
Inkubation
Spike-Nachweis
Sequenzierung
Sequenzierung



Spike-Nachweis

- Anhaltende Produktion

Sequenzierung

FAIM2 is a potential pan-cancer biomarker for prognosis and immune infiltration

Jiayang Cai[1,2†], Zhang Ye[1,2†], Yuanyuan Hu[3], Yixuan Wang[1,2], Liguo Ye[1,2], Lun Gao[1,2], Qian sun[1,2], Shiao Tong[1,2], Zhiqiang Sun[1,2], Ji'an Yang[1,2*] and Qianxue Chen[1,2*]

[1]Department of Neurosurgery, Renmin Hospital of Wuhan University, Wuhan, China, [2]Central Laboratory, Renmin Hospital of Wuhan University, Wuhan, China, [3]Department of Ophthalmology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

- Insertion der pDNA in Chromosom 12 bei Gen FAIM2 (Apoptose-Regulation u.a. via Bax, hohe Korrelation mit Hallmarks maligner Erkrankungen; „Pan-cancer-biomarker)
- 30-fache Anreicherung relativ zum normalen Genom (= forcierte Expression)
- Nachweis von 5 SNPs (= Mutation im Rahmen der Integration)

Frame-Shift Proteins

Zeta-Potential

Plasmide

LNP-Addukte

Promoter

Trojanisches Pferd

Mikrobiom

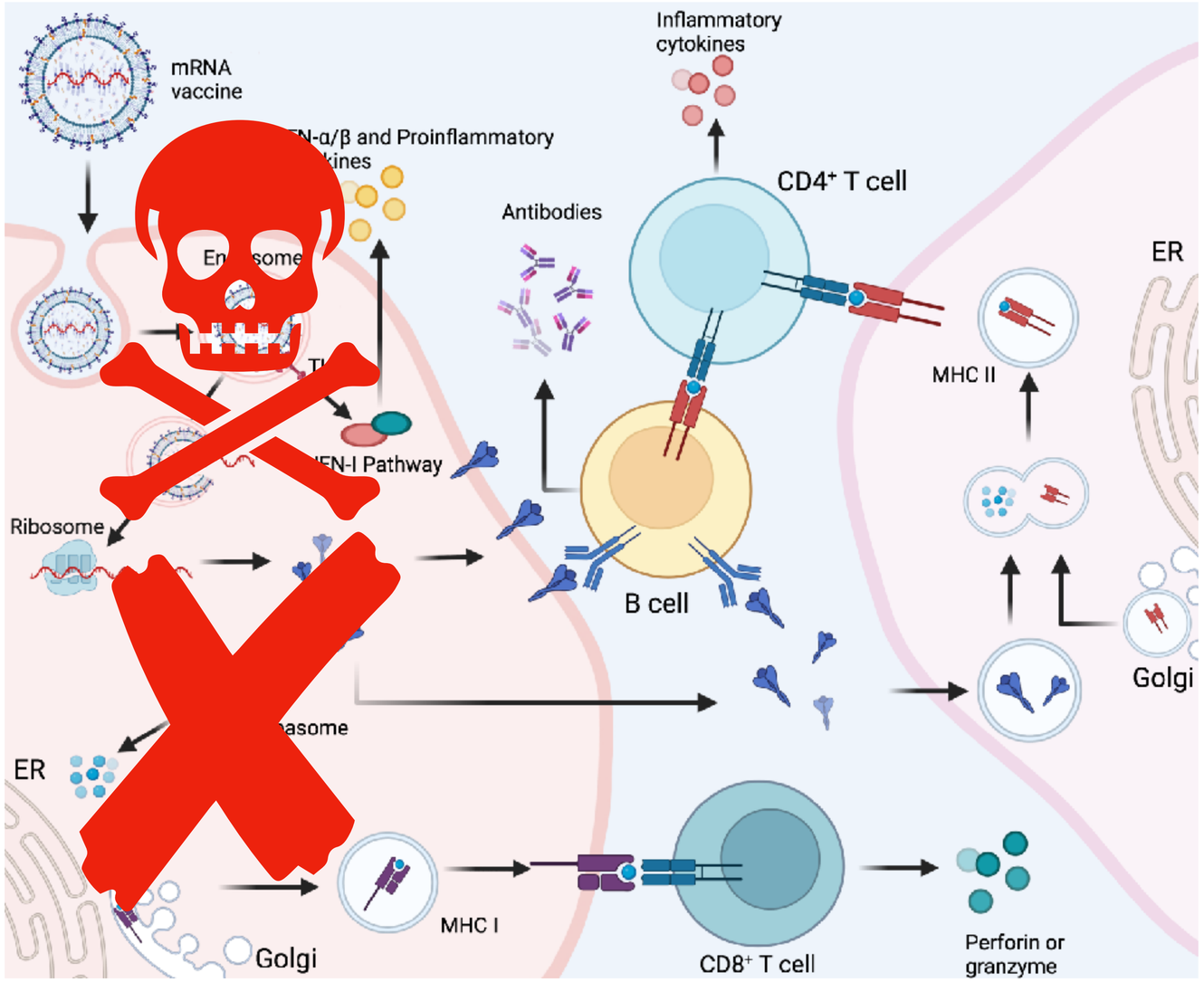
Nachweis

Review Article | Open access | Published: 23 March 2022

Advances in COVID-19 mRNA vaccine development

Enyue Fang, Xiaohui Liu, Miao Li, Zelun Zhang, Lifang Song, Baiyu Zhu, Xiaohong Wu, Jingjing Liu, Danhua Zhao & Yuhua Li

Signal Transduction and Targeted Therapy **7**, Article number: 94 (2022) | Cite this article



Review > Int J Mol Sci. 2020 Jul 16;21(14):5017. doi: 10.3390/ijms21145017.

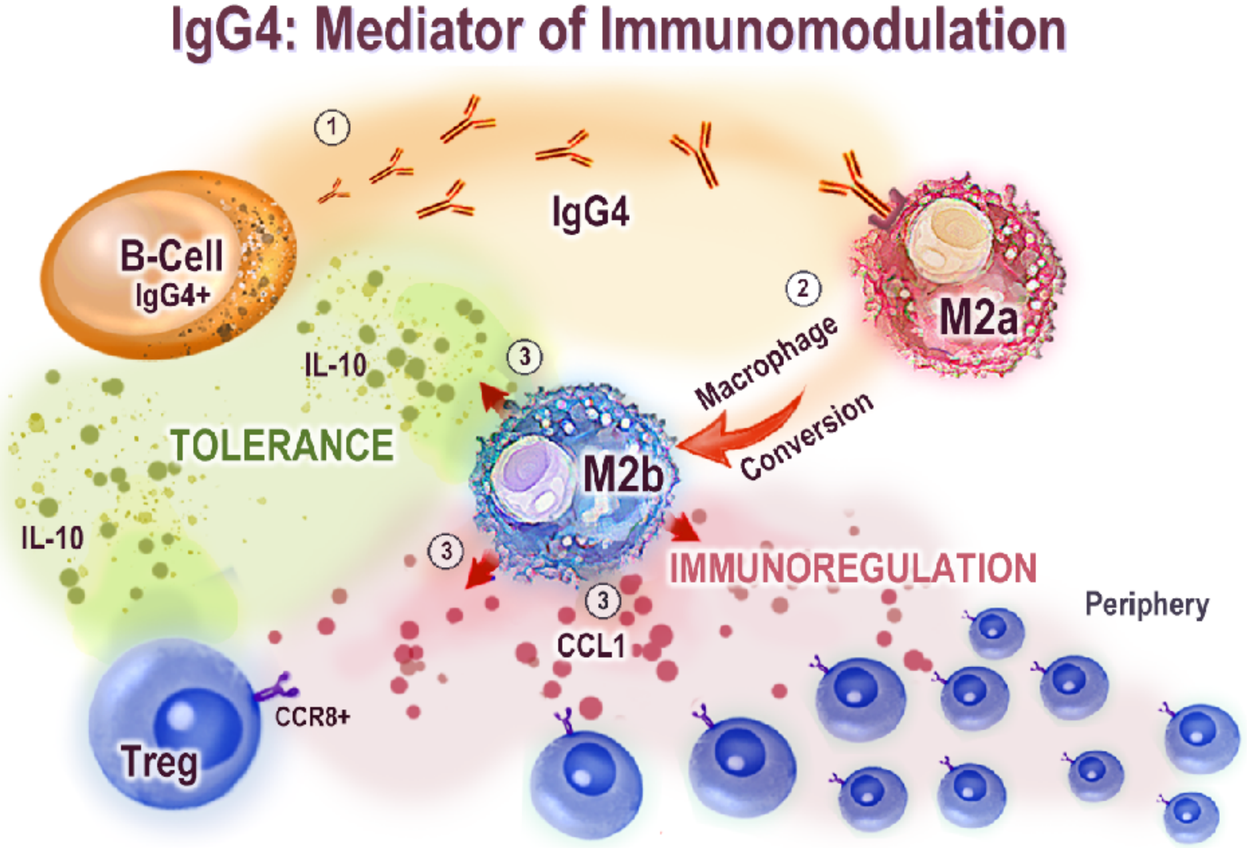
The Role of IgG4 in the Fine Tuning of Tolerance in IgE-Mediated Allergy and Cancer

Rodolfo Bianchini [1 2], Sophia N Karagiannis [3 4], Galateja Jordakieva [5], Erika Jensen-Jarolim [1 2]

Affiliations + expand

PMID: 32708690 PMCID: PMC7404042 DOI: 10.3390/ijms21145017

- Tolerogene Wirkung:
  - T-Reg-Induktion
  - M2-Induktion
  - Verminderte AK-induzierte Phagozytose
  - Verminderte AK-induzierte Komplementaktivierung



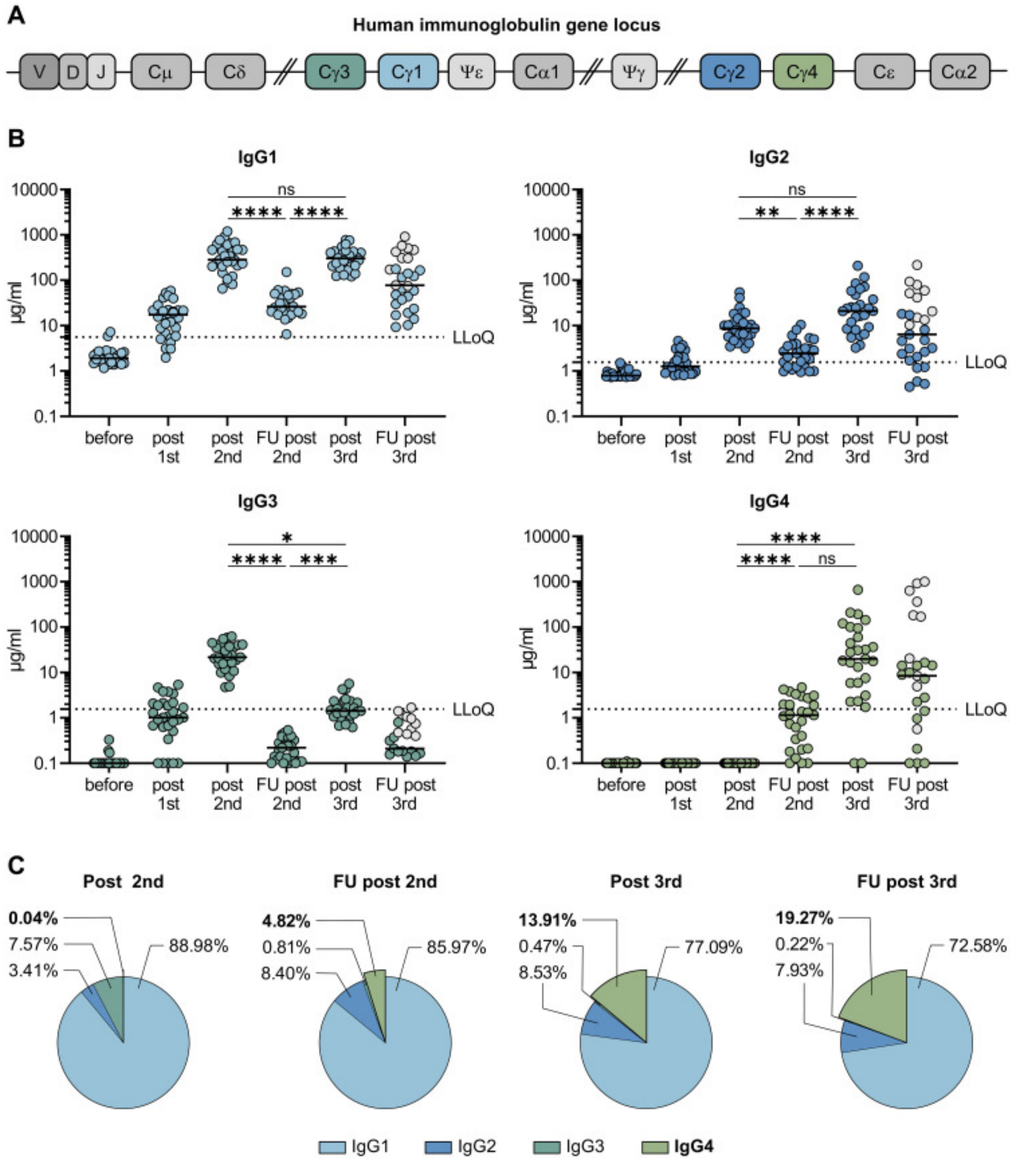
› Sci Immunol. 2023 Jan 27;8(79):eade2798. doi: 10.1126/sciimmunol.ade2798. Epub 2023 Jan 27.

Class switch toward noninflammatory, spike-specific IgG4 antibodies after repeated SARS-CoV-2 mRNA vaccination

Pascal Irrgang [1], Juliane Gerling [2], Katharina Kocher [3], Dennis Lapuente [1], Philipp Steininger [1], Katharina Habenicht [2], Monika Wytopil [1], Stephanie Beileke [1], Simon Schäfer [2], Jahn Zhong [2], George Ssebyatika [4], Thomas Krey [4], Valeria Falcone [5], Christine Schülein [3], Antonia Sophia Peter [1], Krystelle Nganou-Makamdop [1 6], Hartmut Hengel [5], Jürgen Held [3], Christian Bogdan [3 6], Klaus Überla [1 6], Kilian Schober [3 6], Thomas H Winkler [2 6], Matthias Tenbusch [1 6]

Affiliations + expand

PMID: 36548397 PMCID: PMC9847566 DOI: 10.1126/sciimmunol.ade2798



Innate immune suppression by SARS-CoV-2 mRNA vaccinations: The role of G-quadruplexes, exosomes, and MicroRNAs

Stephanie Seneff [a], Greg Nigh [b], Anthony M. Kyriakopoulos [c], Peter A. McCullough [d]

Show more

https://doi.org/10.1016/j.fct.2022.113008

BRIEF RESEARCH REPORT article

Front. Cell. Infect. Microbiol., 10 January 2022
Sec. Virus and Host
Volume 11 - 2021 | https://doi.org/10.3389/fcimb.2021.789462

This article is part of the Research Topic
The Viral Evasion of Antiviral Innate Immunity
View all 71 Articles

SARS-CoV-2 Spike Antagonizes Innate Antiviral Immunity by Targeting Interferon Regulatory Factor 3



Raul S. Freitas† Tyler F. Crum† Kislay Parvatiyar*

Article | Open access | Published: 26 October 2021

Comprehensive investigations revealed consistent pathophysiological alterations after vaccination with COVID-19 vaccines

Jiping Liu, Junbang Wang, Jinfang Xu, Han Xia, Yue Wang, Chunxue Zhang, Wei Chen, Huina Zhang, Qi Liu, Rong Zhu, Yiqi Shi, Zihao Shen, Zhonggang Xing, Wenxia Gao, Liqiang Zhou, Jinliang Shao, Jiayu Shi, Xuejiao Yang, Yaxuan Deng, Li Wu, Quan Lin, Changhong Zheng, Wenmin Zhu, Congrong Wang, ... Zhongmin Liu + Show authors

Cell Discovery **7**, Article number: 99 (2021) | Cite this article

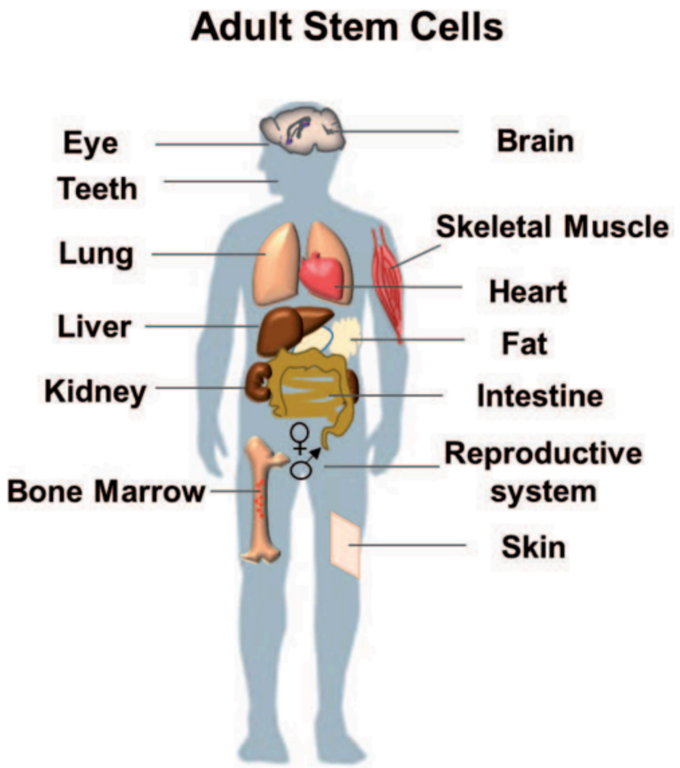
ORIGINAL RESEARCH article

Front. Oncol., 20 December 2022
Sec. Cancer Immunity and Immunotherapy
Volume 12 - 2022 | https://doi.org/10.3389/fonc.2022.975980

This article is part of the Research Topic
The Relationship Between COVID-19 Severity and Cancer Immunity and Immunotherapy
View all 6 articles

Evidence of exhausted lymphocytes after the third anti-SARS-CoV-2 vaccine dose in cancer patients

- Mechanismen sind u.a.:
  - Fehlende Antigen-Präsentation (MHC-I)
  - Expression immunsuppressiver Oberflächenproteine (PD-L, MHC-Ib)
  - Sekretion immunsuppressiver Botenstoffe (TGF-ß)



- Hoden & Ovarien
- Plazenta / Fötus
- Stammzellen
- Cornea
- Gehirnzellen

- Ausbildung von Cancer-Hallmarks in transfektierten Zellen:
  - Hemmung von Tumorsuppressorgenen (z.B. p53/DNA-Reparatur)
  - Hemmung der Apoptose (Bax, Caspasen)
  - Aktivierung onkogener Signalwege (mTOR, Ras/Raf/ERK)
  - Mutationen
  - Immortalisierung
  - Mitochondriopathie: TKTL1-Aktivierung und Laktat-Produktion (>>Warburg-Effekt)

Frame-Shift Proteins

Zeta-Potential

Plasmide

LNP-Addukte

Promoter

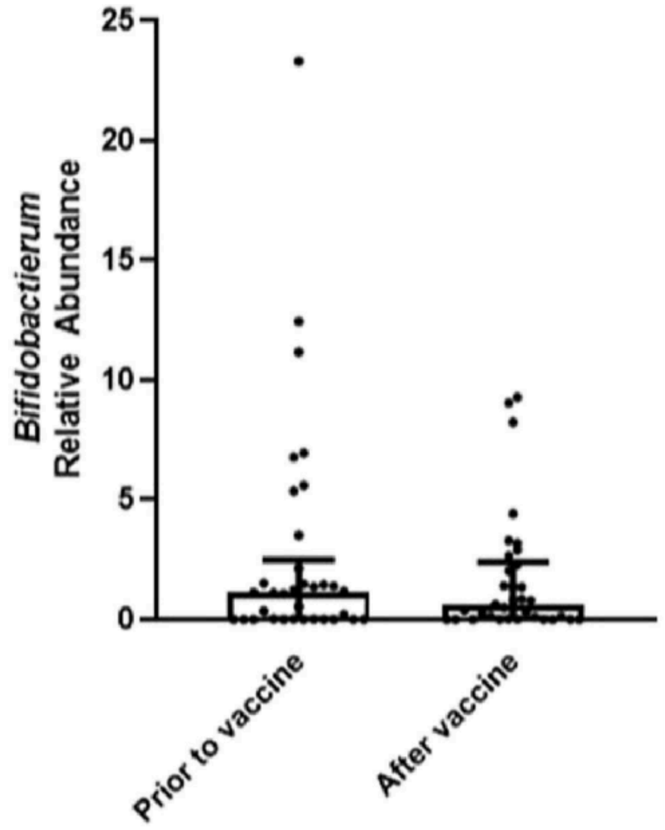
Trojanisches Pferd

Mikrobiom

Nachweis

S227 Messenger RNA SARS-CoV-2 Vaccines Affect the Gut Microbiome

Hazan Sabine; Dave, Sonya; Barrows, Brad; Borody, Thomas J. The American Journal of Gastroenterology; New York Bd. 117, Ausg. 10S, (Oct 2022): e162. DOI:10.14309/01.ajg.0000857548.07509.09



- Abnahme der Bifidobakterien post-vaccination um 50%
- Bedeutung der Bifidos:
  - Wesentlicher Bestandteil der komensalen Flora
  - Wichtig für Immunregulation und Immunkompentenz

Life (Basel). 2023 Sep; 13(9): 1847.
Published online 2023 Aug 31. doi: 10.3390/life13091847

PMCID: PMC10532519
PMID: 37763251

The Role of Bifidobacterium in COVID-19: A Systematic Review

Clarissa Reginato Taufer[1] and Pabulo Henrique Rampelotto[2,3,*]

Einar Ringø, Academic Editor

› Life Sci Alliance. 2024 Feb 5;7(4):e202302529. doi: 10.26508/lsa.202302529. Print 2024 Apr.

Stability of gut microbiome after COVID-19 vaccination in healthy and immuno-compromised individuals

Rebecca H Boston [1], Rui Guan [1], Lajos Kalmar [1], Sina Beier [1], Emily C Horner [1], Nonantzin Beristain-Covarrubias [1], Juan Carlos Yam-Puc [1], Pehuén Pereyra Gerber [2 3], Luisa Faria [1], Anna Kuroshchenkova [1], Anna E Lindell [1], Sonja Blasche [1], Andrea Correa-Noguera [4], Anne Elmer [5], Caroline Saunders [5], Areti Bermperi [5], Sherly Jose [5], Nathalie Kingston [6]; CITIID-NIHR COVID-19 BioResource Collaboration; Sofia Grigoriadou [7], Emily Staples [1], Matthew S Buckland [7 8], Sara Lear [4], Nicholas J Matheson [2 3 9], Vladimir Benes [10], Christine Parkinson [4], James Ed Thaventhiran [11 4], Kiran R Patil [12]

Affiliations + expand

PMID: 38316462 PMCID: PMC10844540 DOI: 10.26508/lsa.202302529

ORIGINAL RESEARCH article

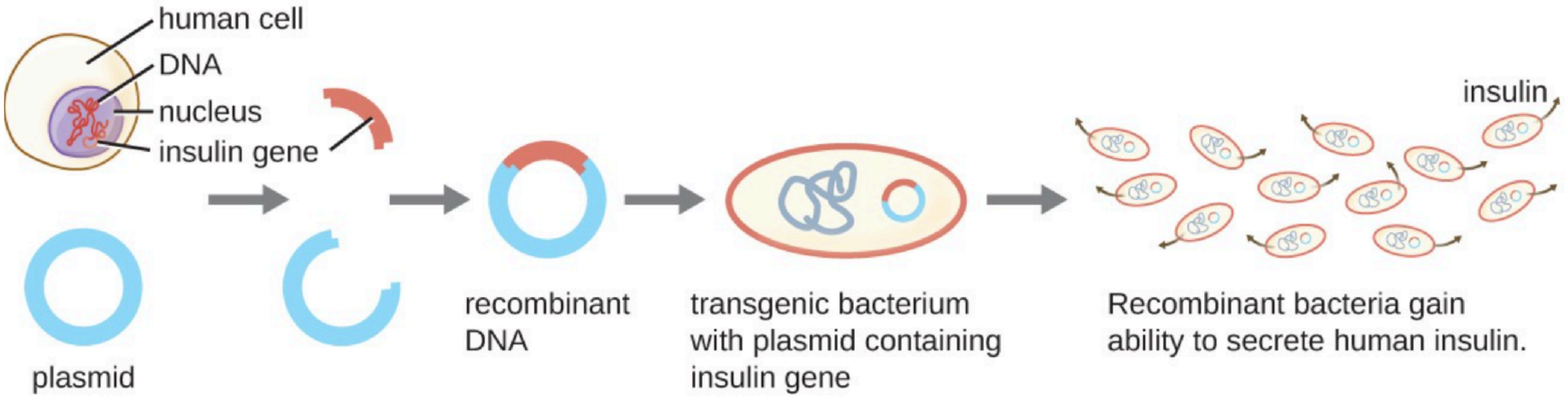
Front. Cell. Infect. Microbiol., 02 August 2023
Sec. Intestinal Microbiome
Volume 13 - 2023 | https://doi.org/10.3389/fcimb.2023.1211348

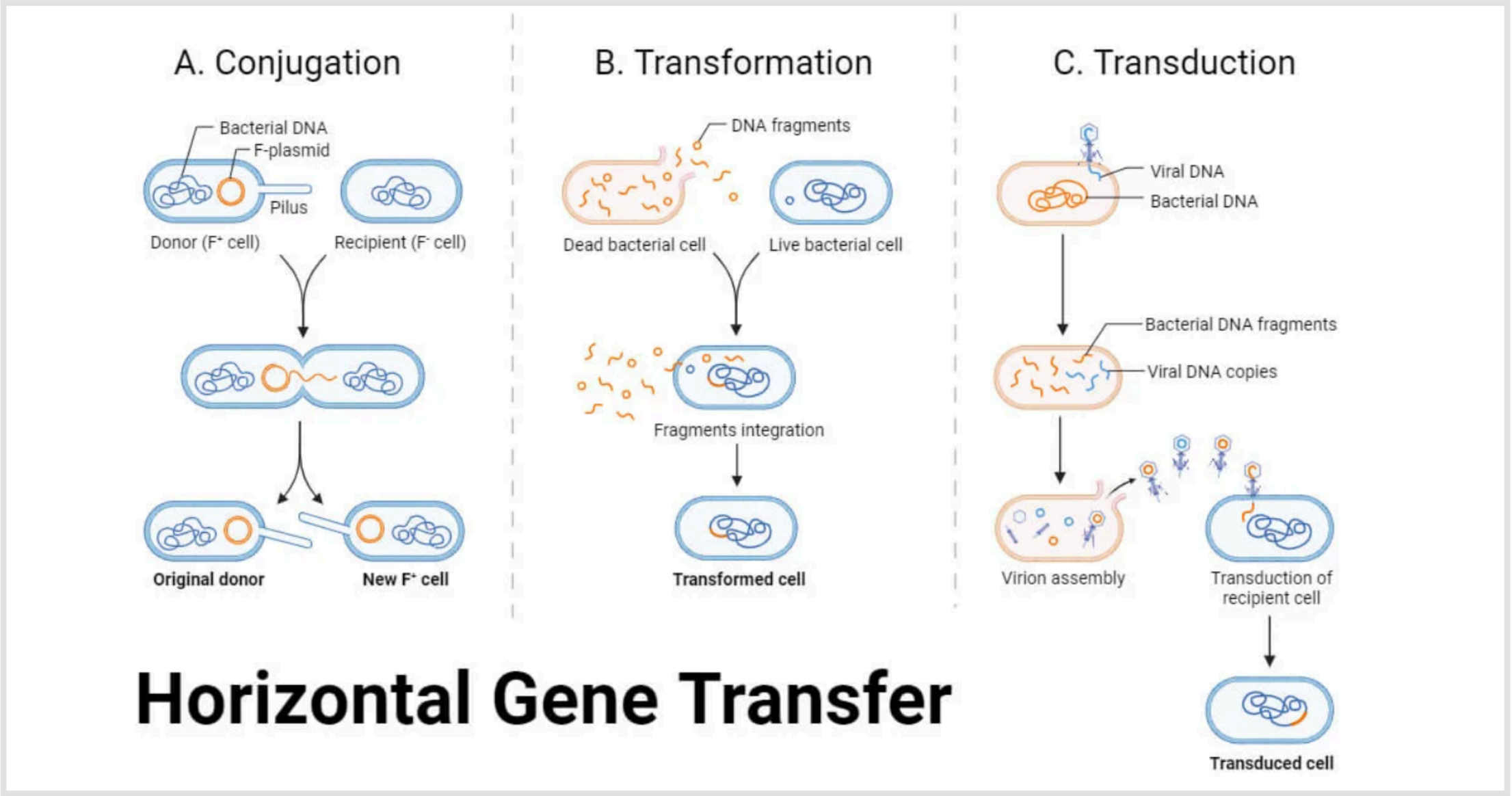
This article is part of the Research Topic
The interplay of gut-microbiome between infection and inflammation
View all 17 Articles ›

COVID-19 alters human microbiomes: a meta-analysis

Rine Christopher Reuben[1,2*] Rémy Beugnon[1,3,4] Stephanie D. Jurburg[1,5*]

[1] German Centre of Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany
[2] Institute of Biology, Leipzig University, Leipzig, Germany
[3] Leipzig Institute for Meteorology, Universität Leipzig, Leipzig, Germany
[4] CEFE, Université de Montpellier, CNRS, EPHE, IRD, Montpellier, France
[5] Department of Environmental Microbiology, Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany


human cell
DNA
nucleus
insulin gene
plasmid
recombinant DNA
transgenic bacterium with plasmid containing insulin gene
insulin
Recombinant bacteria gain ability to secrete human insulin.


A. Conjugation
Bacterial DNA
F-plasmid
Pilus
Donor (F+ cell)
Recipient (F- cell)
Original donor
New F+ cell
B. Transformation
DNA fragments
Dead bacterial cell
Live bacterial cell
Fragments integration
Transformed cell
C. Transduction
Viral DNA
Bacterial DNA
Bacterial DNA fragments
Viral DNA copies
Virion assembly
Transduction of recipient cell
Transduced cell
Horizontal Gene Transfer

- In welchem Ausmaß treten LNP bzw. Lipoplexe im Darm auf?
- Kommt es zur Transfektion im intestinalen Mikrobiom? Falls ja -
  - Nur bestimmte Bakterien?
  - Welchen Effekt hat diese?
  - Replikation der Plasmide?
  - Horizontaler Gentransfer?
  - Produktion von Spike?

Frame-Shift Proteins

Zeta-Potential

Plasmide

LNP-Addukte

Promoter

Trojanisches Pferd

Mikrobiom

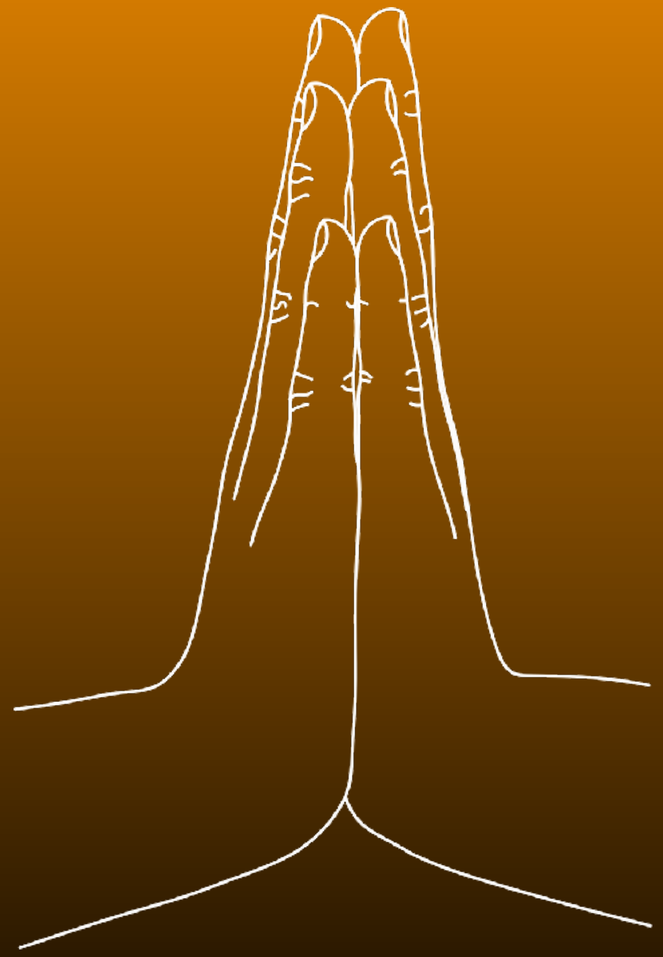
Nachweis

1.1	**Material:** 1x Heparin- oder Citratblut (mind. 4ml) oder Serum (mind. 2ml) bzw. Eluat	**Quantitative Bestimmung des freien SARS-CoV-2 Spikeproteins in Plasma/Serum In Eluat**	Spike
1.2	**Material:** 1x Heparin- oder Citratblut (8ml) oder Serum (mind. 4ml)	**Quantitative Bestimmung des SARS-CoV-2 Spikeproteins in Exosomen**	
1.3	**Material:** 1x Heparin- oder Citratblut (8ml)	**Quantitative Bestimmung des SARS-CoV-2 Spikeproteins in Immunzellen (PBMC)**	
1.4	**Material:** 1xMorgenurin (mind. 5ml)	**Quantitative Bestimmung des SARS-CoV-2 Spikeproteins im Urin**	
1.5	**Material:** 1x Serum (mind. 2ml) bzw. Eluat 1x Serum (mind. 4ml) 1x Heparin- oder Citratblut (8ml)	**Differenzierung des SARS-CoV-2 Spikeproteins (Infektion/Impfung)** **Nur in Verbindung mit 1.1 – 1.3.** • **In Plasma/Serum/Eluat - noch nicht verfügbar** • **In Exosomen - noch nicht verfügbar** • **In Immunzellen 8PBMC) - noch nicht verfügbar**	
2.1	**Material:** 1x Heparin- oder Citratblut (8ml) oder Serum (mind. 4ml)	**Nachweis von Impf-mRNA (Pfizer, Moderna) in Exosomen**	modRNA
2.2	**Material:** 1x Heparin- oder Citratblut (8ml)	**Nachweis von Impf-mRNA (Pfizer, Moderna) in Immunzellen (PBMC)**	
2.3	**Material:** Muttermilch (mind. 4ml)	**Nachweis von Impf-mRNA (Pfizer, Moderna) in Muttermilch**	SC2-RNA
3.1	**Material:** 1x Heparin-oder Citratblut (mind. 8ml) oder Serum (mind. 4ml)	**Nachweis von SARS-CoV-2 RNA im Serum/Plasma (Persistenz), hoch sensitiv**	
3.2	**Material:** 1x Heparin- oder Citratblut (8ml)	**Nachweis von SARS-CoV-2 RNA in Immunzellen (PBMC) (Persistenz)**	
3.3	**Material:** 1x Stuhlprobe (1 g)	**Nachweis von SARS-CoV-2 RNA im Stuhl (Persistenz)**	
3.4	**Material:** 1x Samenflüssigkeit (1ml)	**Nachweis von SARS-CoV-2 RNA in Samenzellen (Persistenz)**	
4.1	**Material:** 1x Heparin- oder Citratblut (8ml) 1x Samenflüssigkeit (4ml) 1x Mundschleimhautabstrich	**Nachweis der SARS-CoV-2 mRNA Expressionsvektoren (Pfizer, Moderna, Janssen)** • **In Immunzellen (PBMC)** • **In Samenzellen** • **In Mundschleimhautzellen**	Plasmide
4.2	**Mögliche Materialien:** 1x Heparin- oder Citratblut (Immunzellen [PBMC]; 8ml), Mundschleimhautabstrich, Samenflüssigkeit (1ml)	**Nachweis von LINE-1 (dieses Enzym ist die Voraussetzung für den Einbau von Impf-mRNA in das menschliche Genom)** **Nachweis der Integration der Impf-mRNA in den Zellkern**	
4.3	**Material:** 1x Stuhlprobe (1 g)	**Nachweis der Expressionsvektoren (Plasmide) von Pfizer/Moderna in Darmbakterien**	

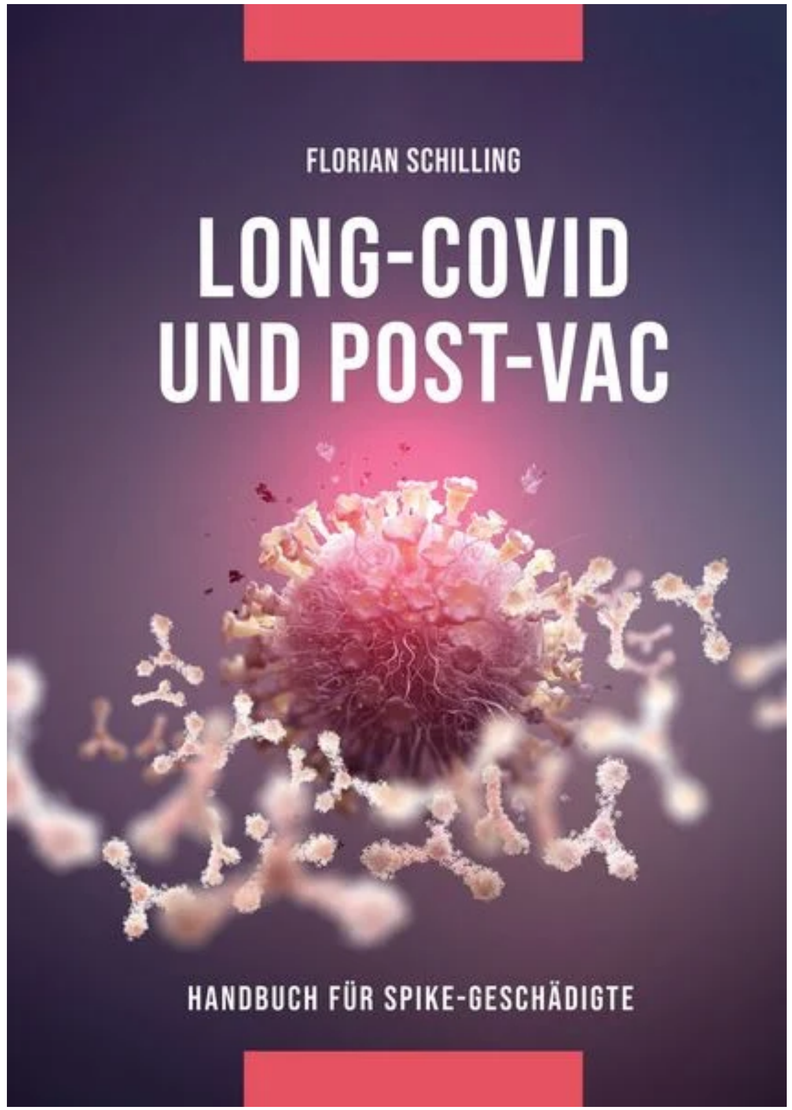
Großes Live-Seminar

Juni 2024

Seid gesegnet



Namasté



https://shop.tredition.com/booktitle/Long-Covid__Post-Vac/W-444-091-402

Workshops

Protokolle

Lives

www.florianschillingscience.org