Developing mRNA for therapy - Not Warp Speed

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Senior Vice President
BioNTech RNA Pharmaceuticals

Reichstein Medal Lecture
September 6, 2021

Timeline - mRNA development for therapy

- 1961 Discovering mRNA
- 1978 mRNA delivery into mammalian cells
- 1984 Synthesizing mRNA in vitro
- 1990 In vivo delivery of mRNA
- 2000 IVT mRNA inflammatory in human cells
- 2005 Nucleoside-modified RNA: non-immunogenic
- 2017 LNP-formulated modRNA for vaccine
- 2021 FDA approval of covid-19 LNP-modRNA vaccine

1961 - Discovery of mRNA

576 NATURE May 13, 1961 VOL. 190

AN UNSTABLE INTERMEDIATE CARRYING INFORMATION FROM GENES TO RIBOSOMES FOR PROTEIN SYNTHESIS

By DR. S. BRENNER
Medical Research Council Unit for Molecular Biology, Cavendish Laboratory, University of Cambridge

DR. F. JACOB
Institut Pasteur, Paris

AND

DR. M. MESELSON
Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, California

UNSTABLE RIBONUCLEIC ACID REVEALED BY PULSE LABELLING OF *ESCHERICHIA COLI*

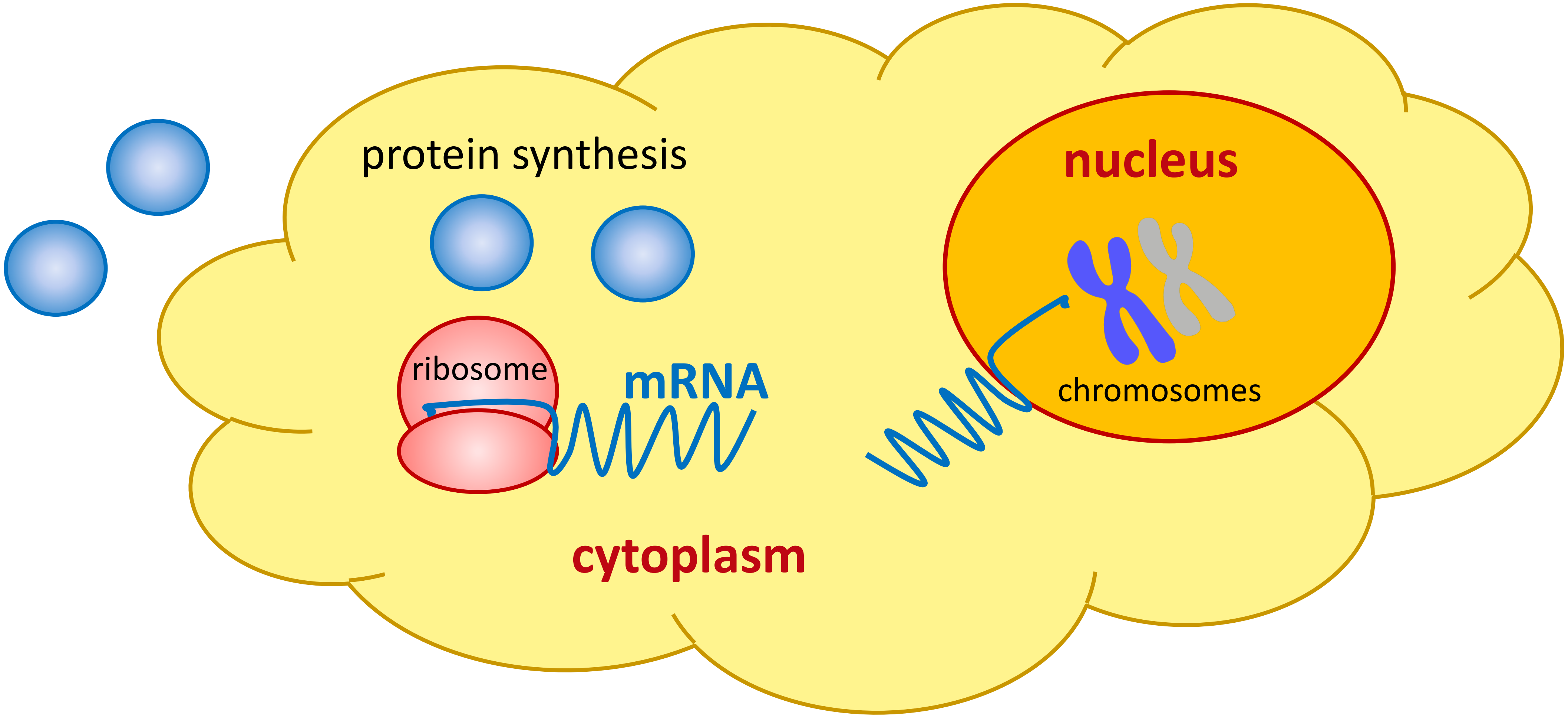
By DRS. FRANCOIS GROS and H. HIATT
The Institut Pasteur, Paris

DR. WALTER GILBERT
Departments of Physics, Harvard University

AND

DR. C. G. KURLAND, R. W. RISEBROUGH and DR. J. D. WATSON
The Biological Laboratories, Harvard University

1961 - Discovery of mRNA



1978 - Liposome-formulated mRNA transfer into mammalian cells

Nature Vol. 274 31 August 1978

Translation of rabbit globin mRNA introduced by liposomes into mouse lymphocytes

GIORGOS J. DIMITRIADIS

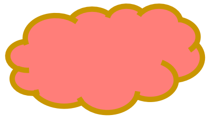
National Institute for Medical Research,
The Ridgeway,
Mill Hill, London NW7, UK

Nature Vol. 274 31 August 1978

Evidence for translation of rabbit globin mRNA after liposome-mediated insertion into a human cell line

MARC J. OSTRO,
DARIO GIACOMONI,
DON LAVELLE,
WILLIAM PAXTON,
SHELDON DRAY

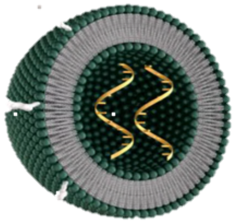
Department of Microbiology and Immunology,
University of Illinois at the Medical Center,
Chicago, Illinois 60612



Rabbit reticulocyte
(precursor of red blood cell)

mRNA isolation

Liposome formulation



Fusion of cells with liposomes

Mouse spleen lymphocytes

immunoprecipitation

globin

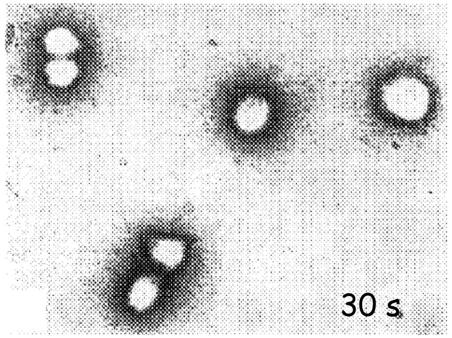
1980 - Liposome-formulated DNA transfer into mammalian cells

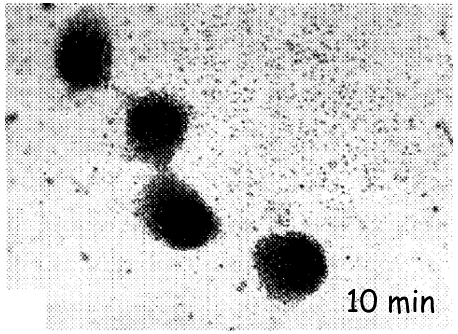
Acta Biochim. et Biophys. Acad. Sci. Hung. Vol. 20 (3–4), pp. 203–211 (1985)

Liposome Mediated DNA-transfer into Mammalian Cells

G. Somlyai, É. Kondorosi, K. Karikó,* E. G. Duda

Institute of Biochemistry and *Institute of Biophysics, Biological Research Center, Szeged, Hungary

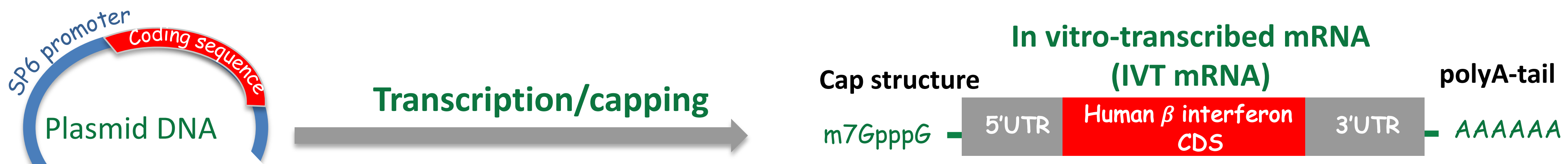




Synthesizing mRNA in vitro
1984

1984 - Synthesizing and evaluating IVT mRNA

The first in vitro synthesis of mRNA and its translation into a functional protein



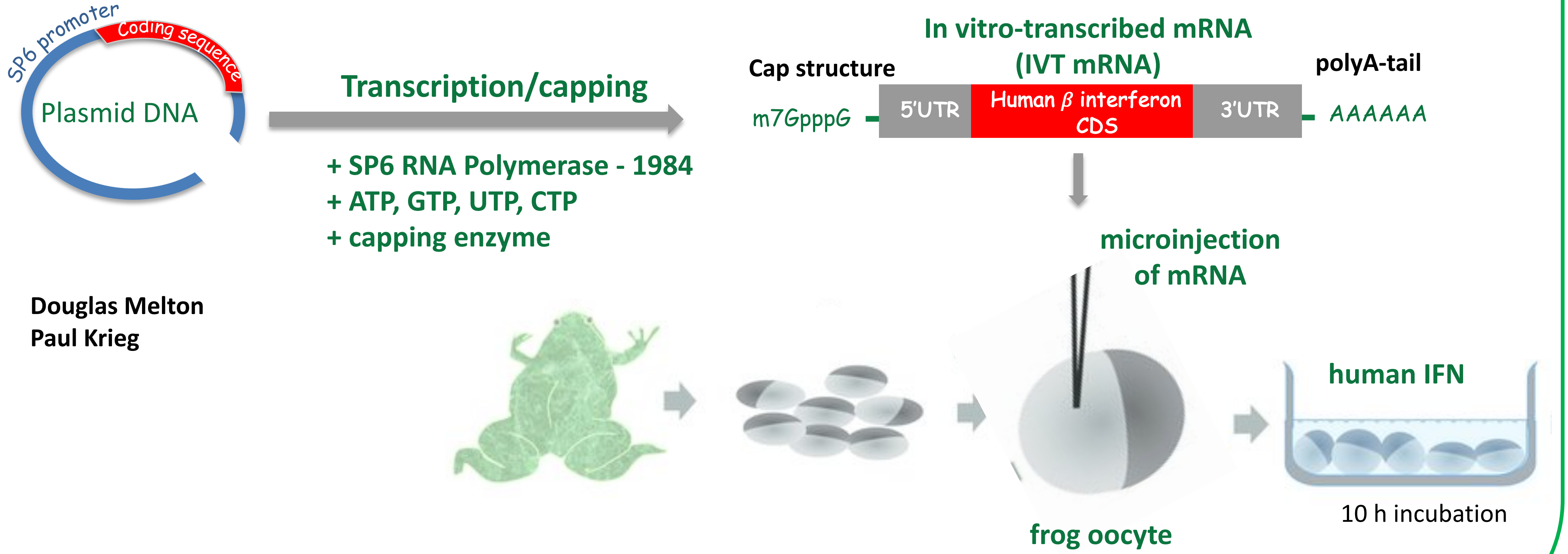
+ SP6 RNA Polymerase - 1984
+ ATP, GTP, UTP, CTP
+ capping enzyme

Douglas Melton
Paul Krieg

Nucleic Acids Res. 1984, 12: 7035, 7057

Synthesizing and evaluating IVT mRNA

The first in vitro synthesis of mRNA and its translation into a functional protein



Douglas Melton
Paul Krieg

Nucleic Acids Res. 1984, 12: 7035, 7057

mRNA delivery in vivo
1990

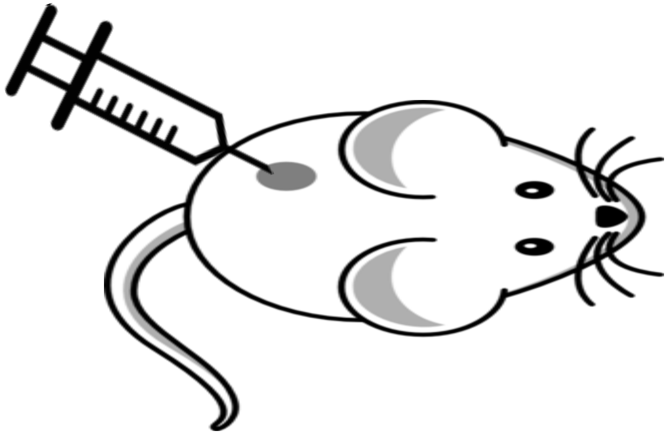
1990 - mRNA delivery in vivo

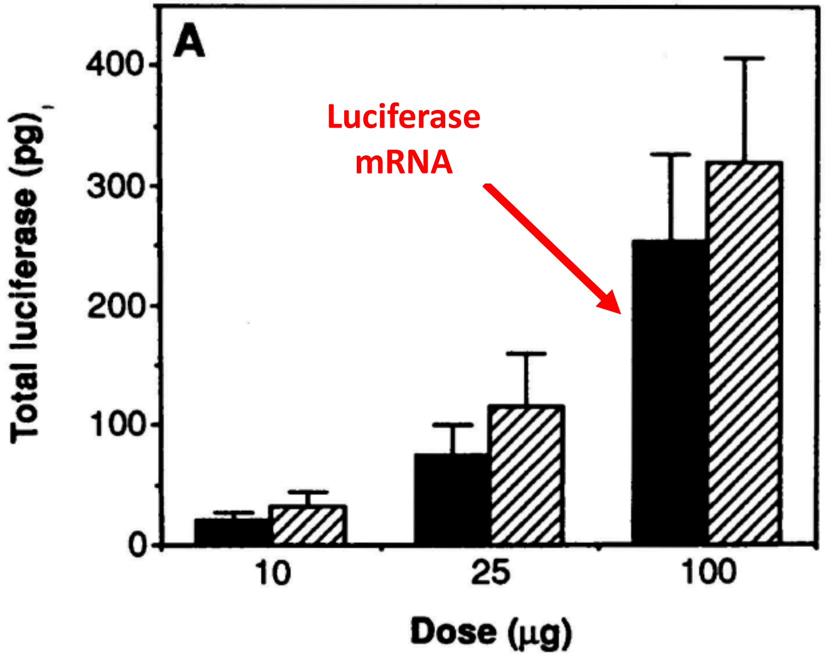
1990 SCIENCE, VOL. 247

Direct Gene Transfer into Mouse Muscle in Vivo

Jon A. Wolff,* Robert W. Malone, Phillip Williams, Wang Chong, Gyula Acsadi, Agnes Jani, Philip L. Felgner

Luciferase mRNA

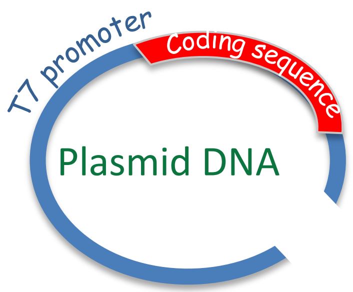




1992-96 mRNA delivery in vivo

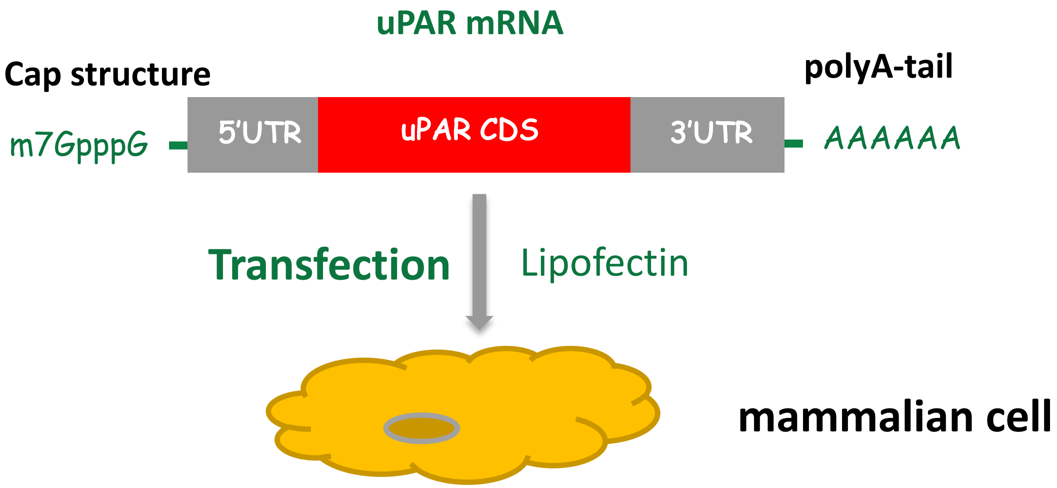
- **1992** Vasopressin mRNA therapy in rats – Bloom and colleagues
  Science 1992, 255: 996
- **1993** NP mRNA for influenza vaccine – Martinon, Meulien and colleagues
  Eur J Immunol 1993, 23: 1719
- **1994** NP saRNA for influenza vaccine – Liljeström and colleagues
  Vaccine 1994, 12: 1510
- **1995** mRNA for cancer vaccine in mice – Conry, Curiel and colleagues
  Cancer Res 1995, 55: 1397
- **1996** mRNA for cancer vaccine human DC – Gilboa and colleagues
  J Exp Med 1996, 184: 465

1990s - Synthesis of mRNA and evaluating in mammalian cells



Transcription

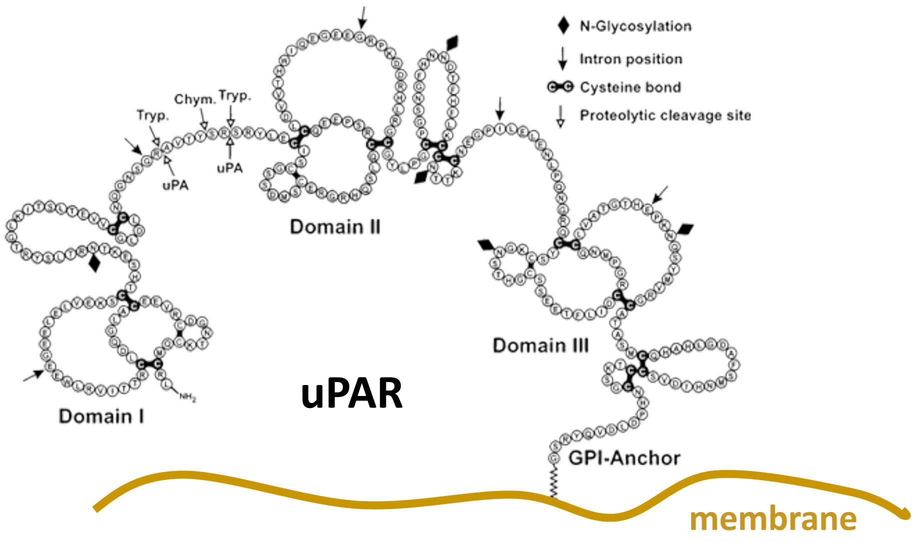
+ T7 RNA Polymerase
+ ATP, GTP, UTP, CTP
+ cap analog



Gene Therapy (1999) 6, 1092–1100

Overexpression of urokinase receptor in mammalian cells following administration of the in vitro transcribed encoding mRNA

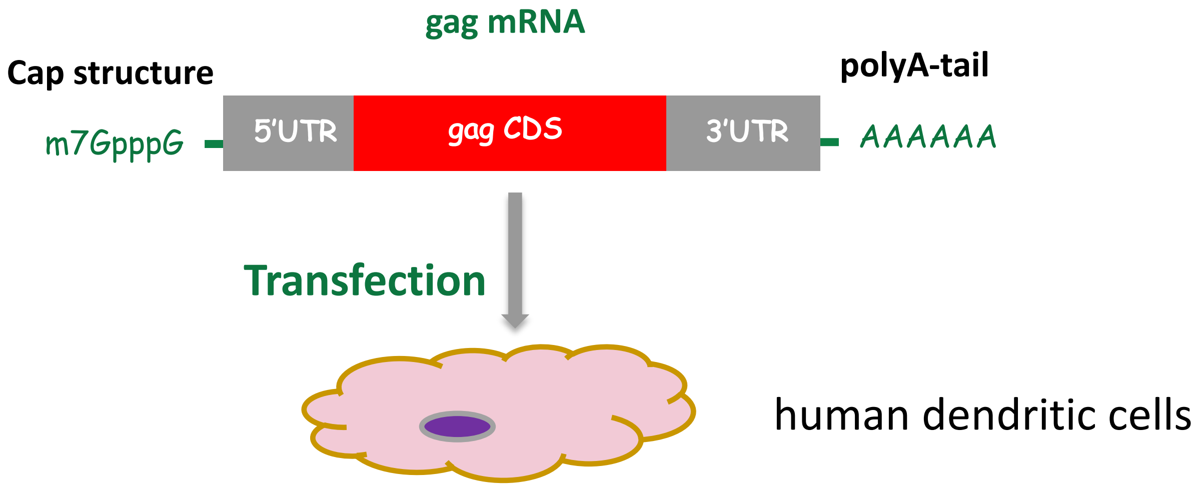
K Karikó[1], A Kuo[2] and ES Barnathan[2]



IVT mRNA induces inflammatory cytokines
2000

2000 - Evaluating gag mRNA in human dendritic cells

Drew Weissman

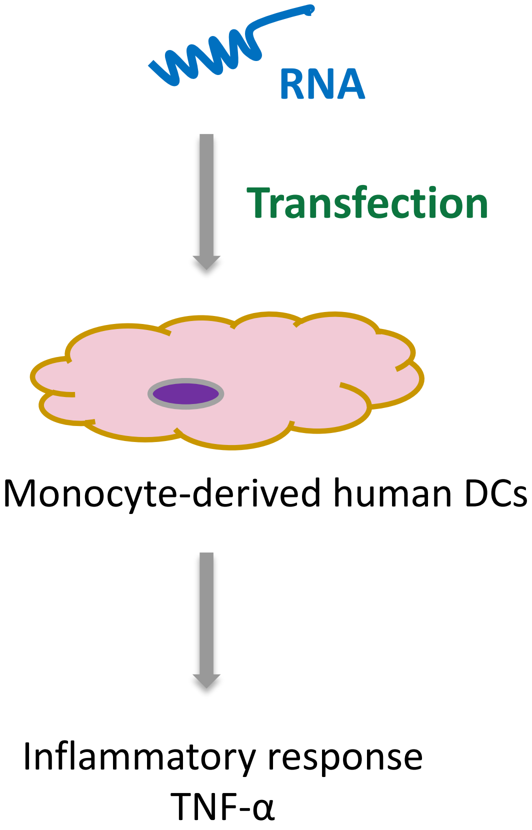


J Immunol 2000; 165:4710-4717

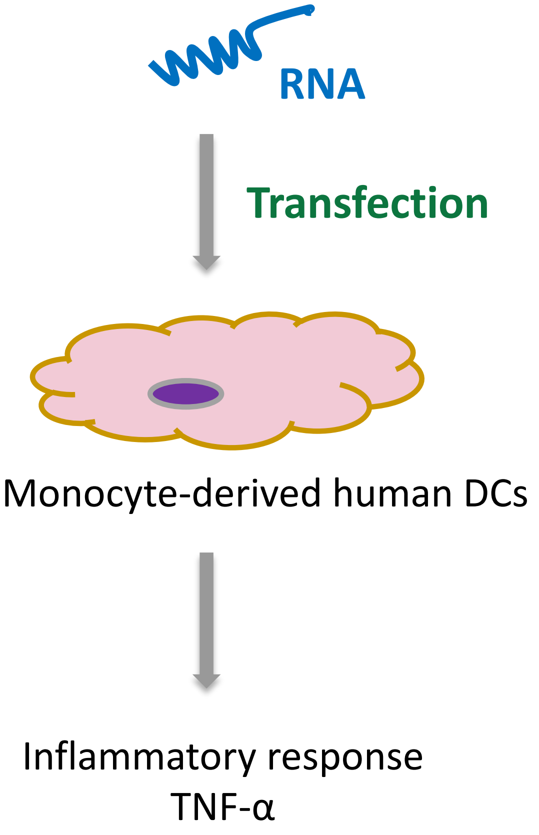
HIV Gag mRNA Transfection of Dendritic Cells (DC) Delivers Encoded Antigen to MHC Class I and II Molecules, Causes DC Maturation, and Induces a Potent Human In Vitro Primary Immune Response[1]

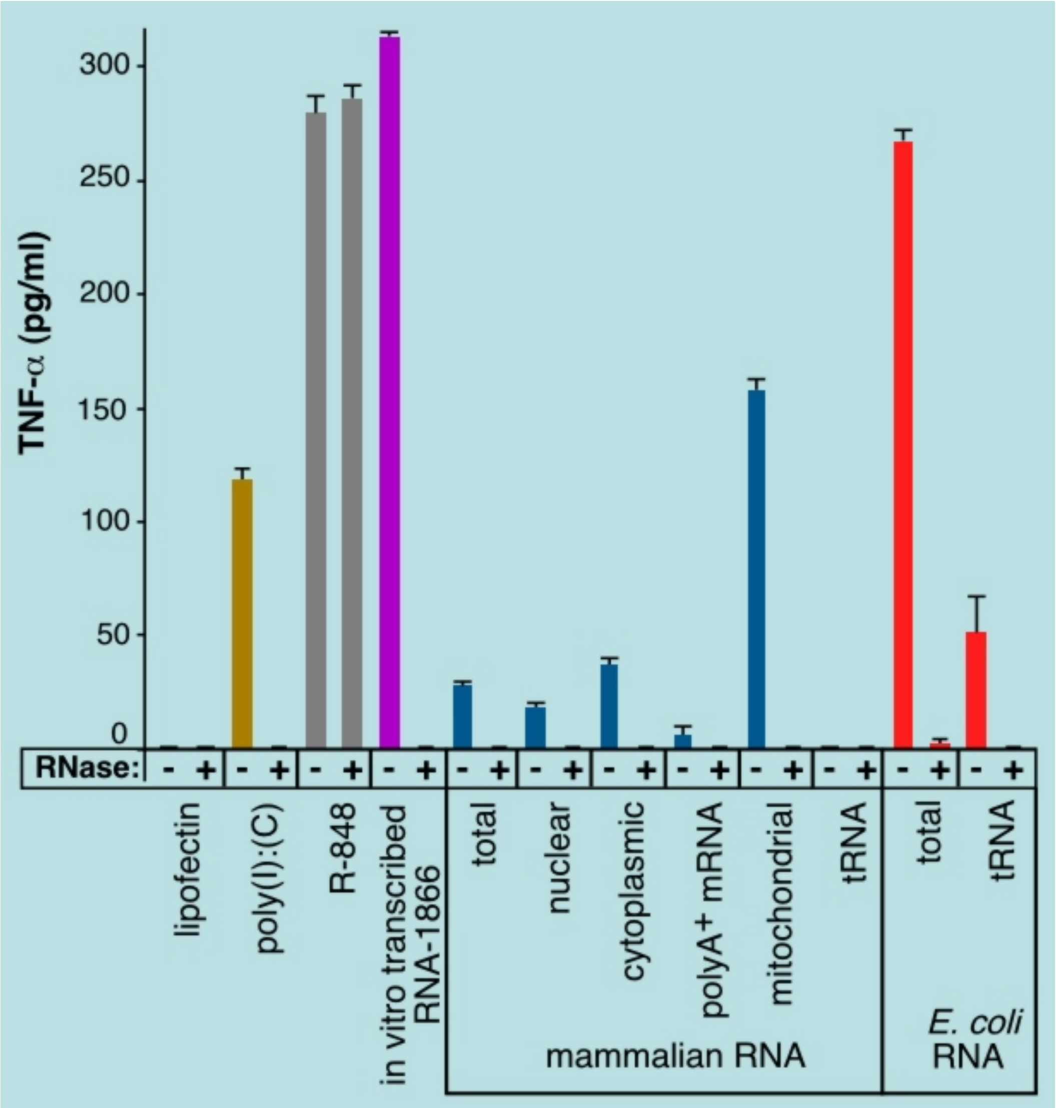
Drew Weissman,[2]* Houping Ni,* David Scales,* Annie Dude,* John Capodici,* Karen McGibney,* Asha Abdool,* Stuart N. Isaacs,* Georgetta Cannon,* and Katalin Karikó[†]

2005 - Natural RNAs are not equally potent activators of DCs



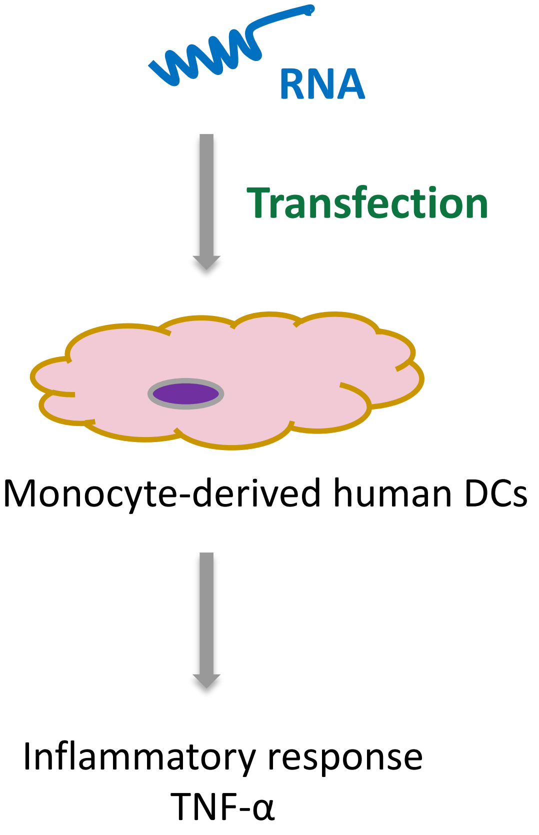
2005 - Natural RNAs are not equally potent activators of DCs

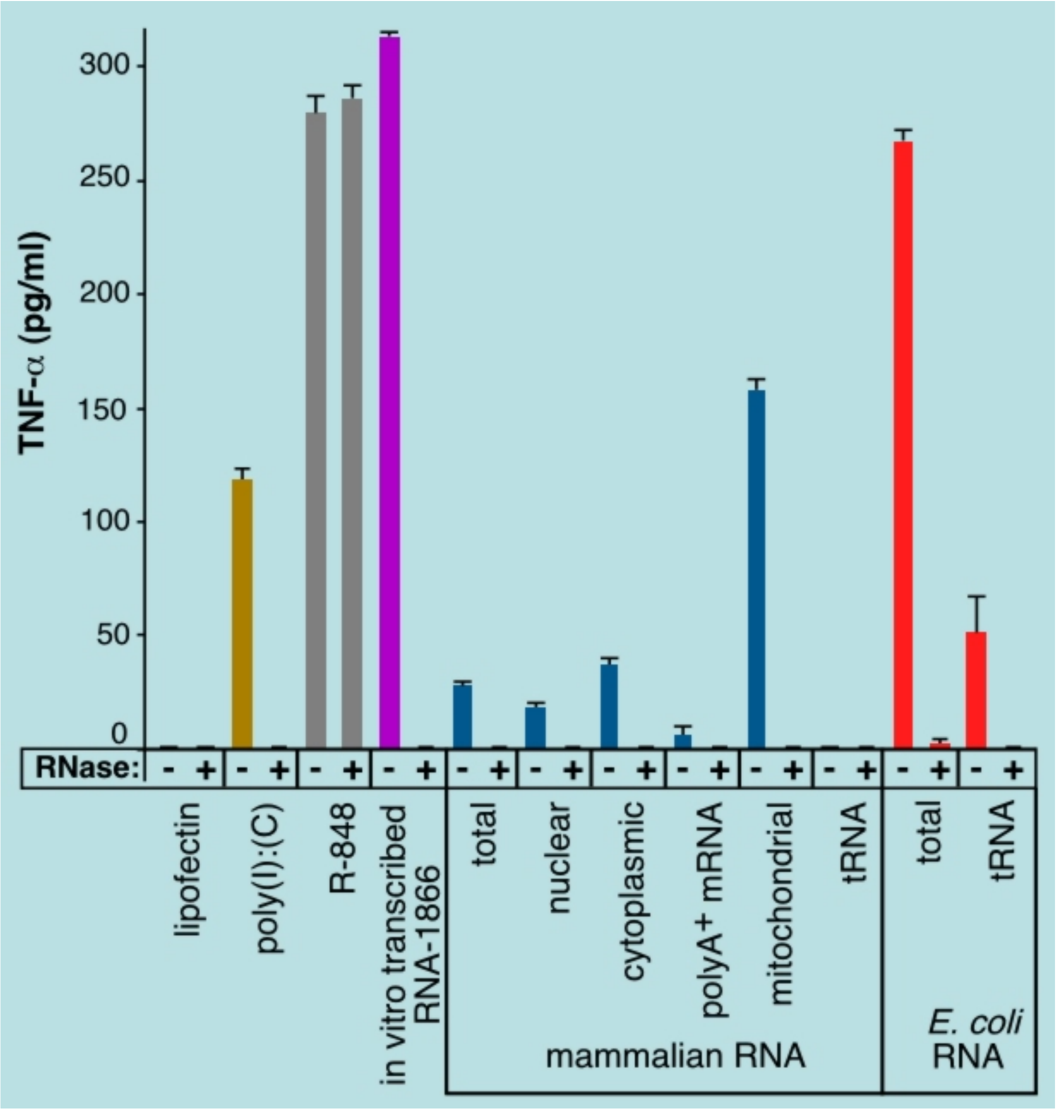




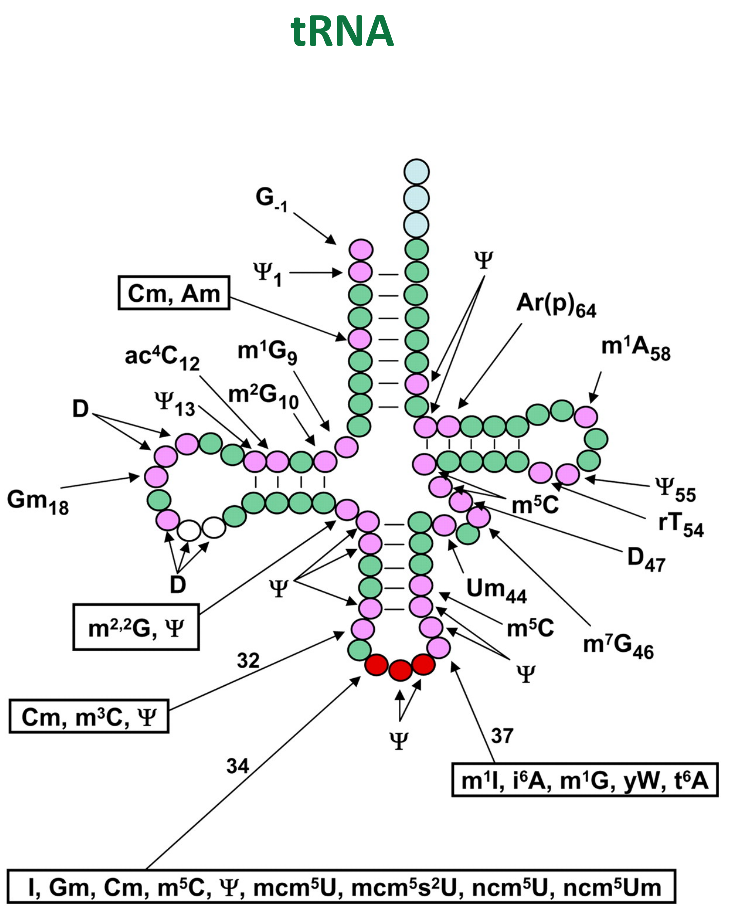
Immunity 2005, 23: 165

2005 - Natural RNAs are not equally potent activators of DCs



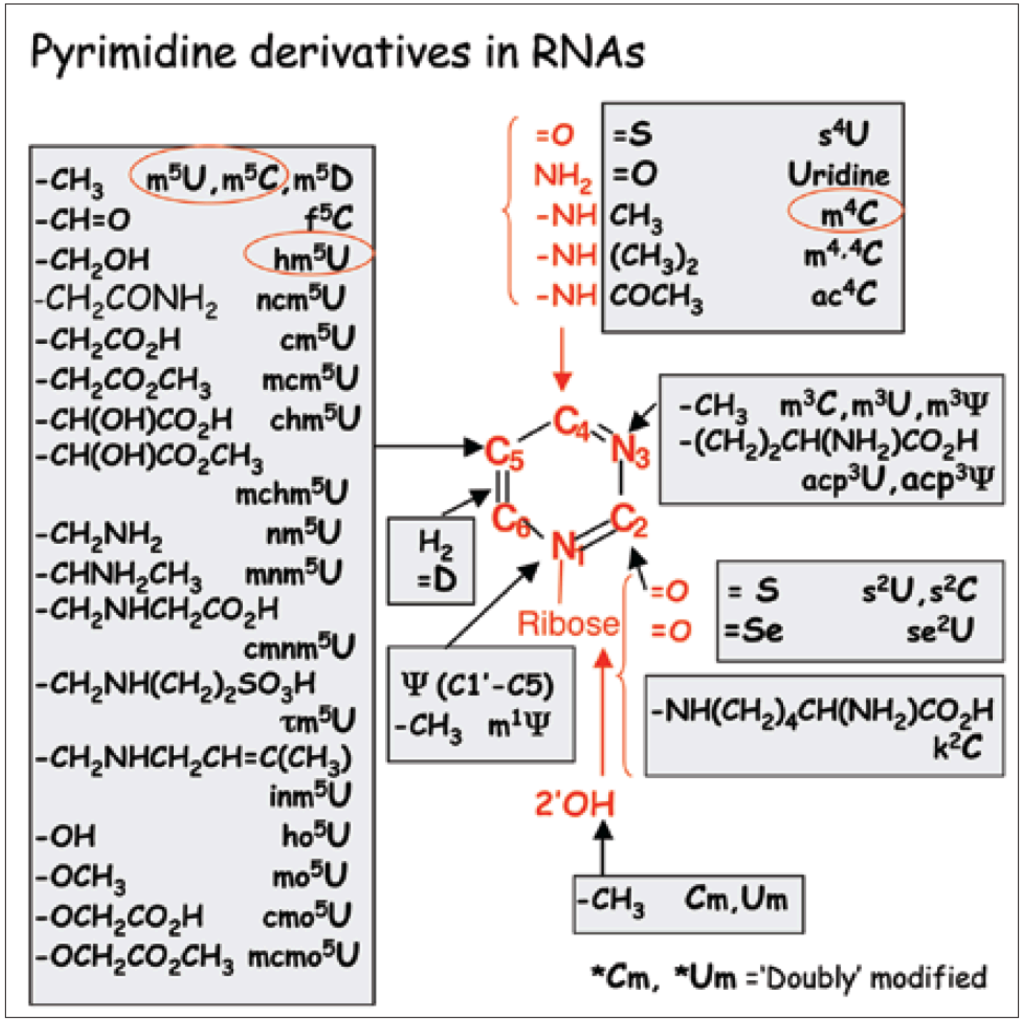


Immunity 2005, 23: 165

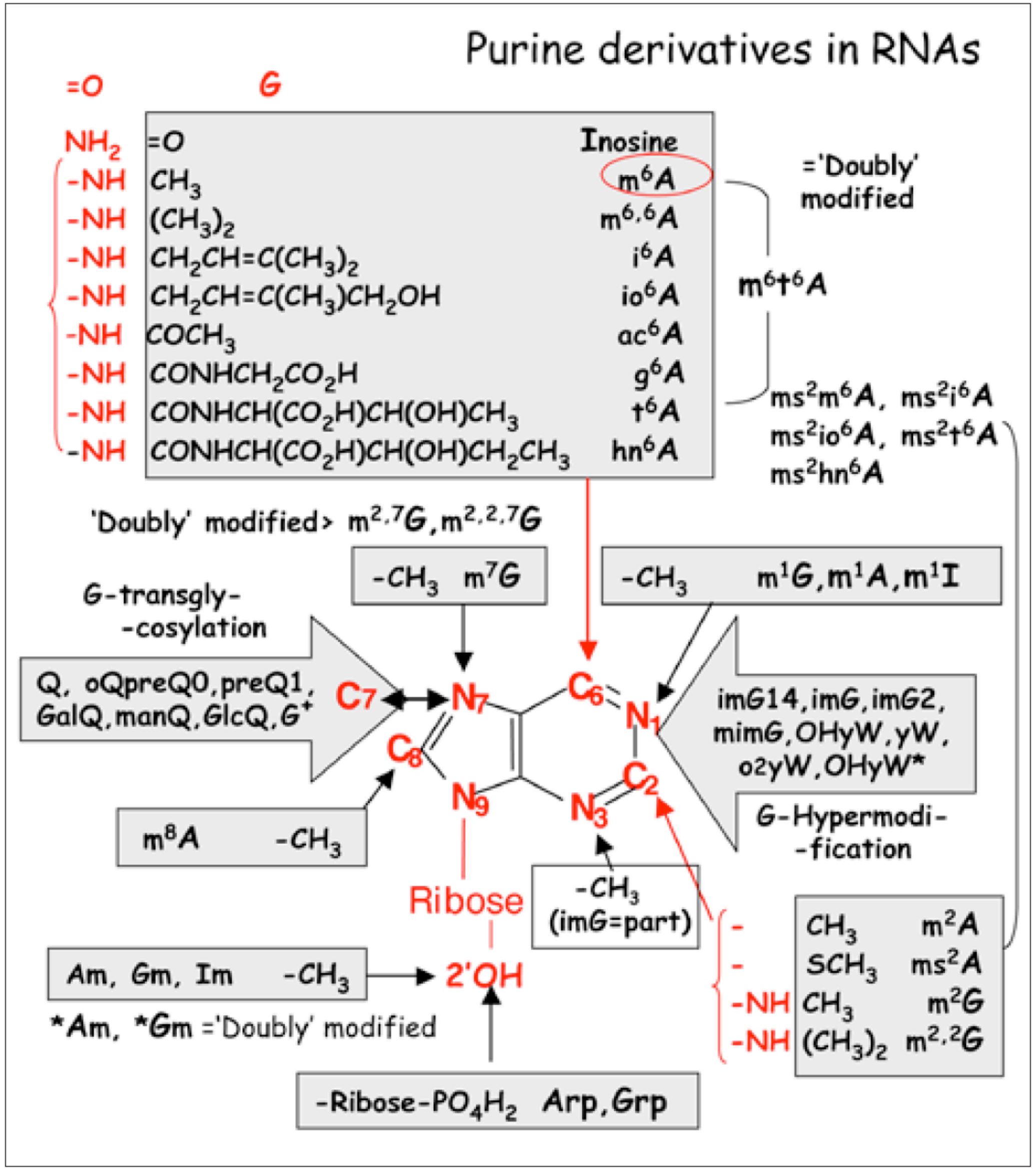


tRNA is enriched in modified nucleosides

108 Naturally-occurring modified nucleosides in RNA



DNA and RNA Modification Enzymes: Structure, Mechanism, Function and Evolution, edited by Henri Grosjean

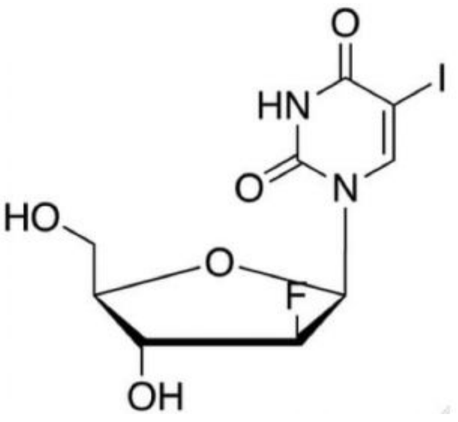


2005 - Incorporation of modified nucleotides into RNA by in vitro transcription

Naturally-occurring modified nucleosides

NTP
m5C m5U Ψ m6A s2U
m1A m1G m7G 2'-*O*-Met-C 2'-*O*-Met-U

Unnatural nucleoside analogs are TOXIC



Fialuridine

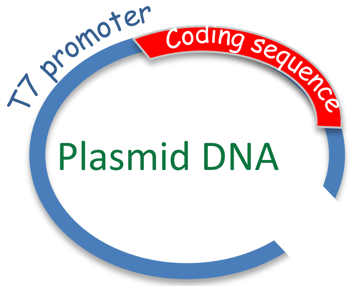
NEJM 1995, 333: 1099

Identification of the Mitochondrial Targeting Signal of the Human Equilibrative Nucleoside Transporter 1 (hENT1)

*IMPLICATIONS FOR INTERSPECIES DIFFERENCES IN MITOCHONDRIAL TOXICITY OF FIALURIDINE**

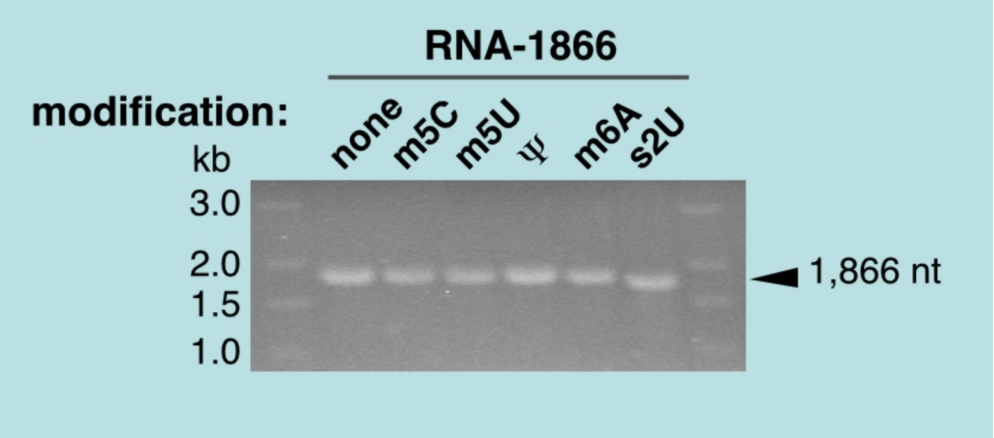
JBC 2006, 28: 16700

2005 - Incorporation of modified nucleotides into RNA by in vitro transcription



Transcription →

+ T7 RNA Polymerase
+ cap analog
+ ATP, GTP, UTP, CTP

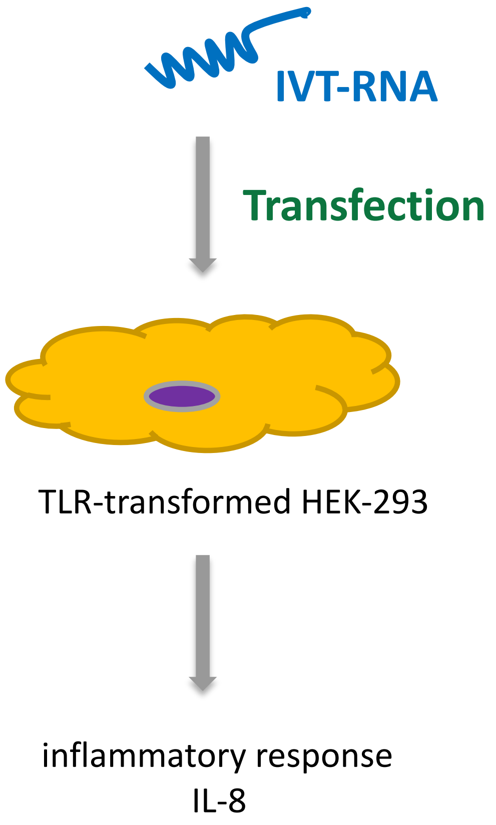


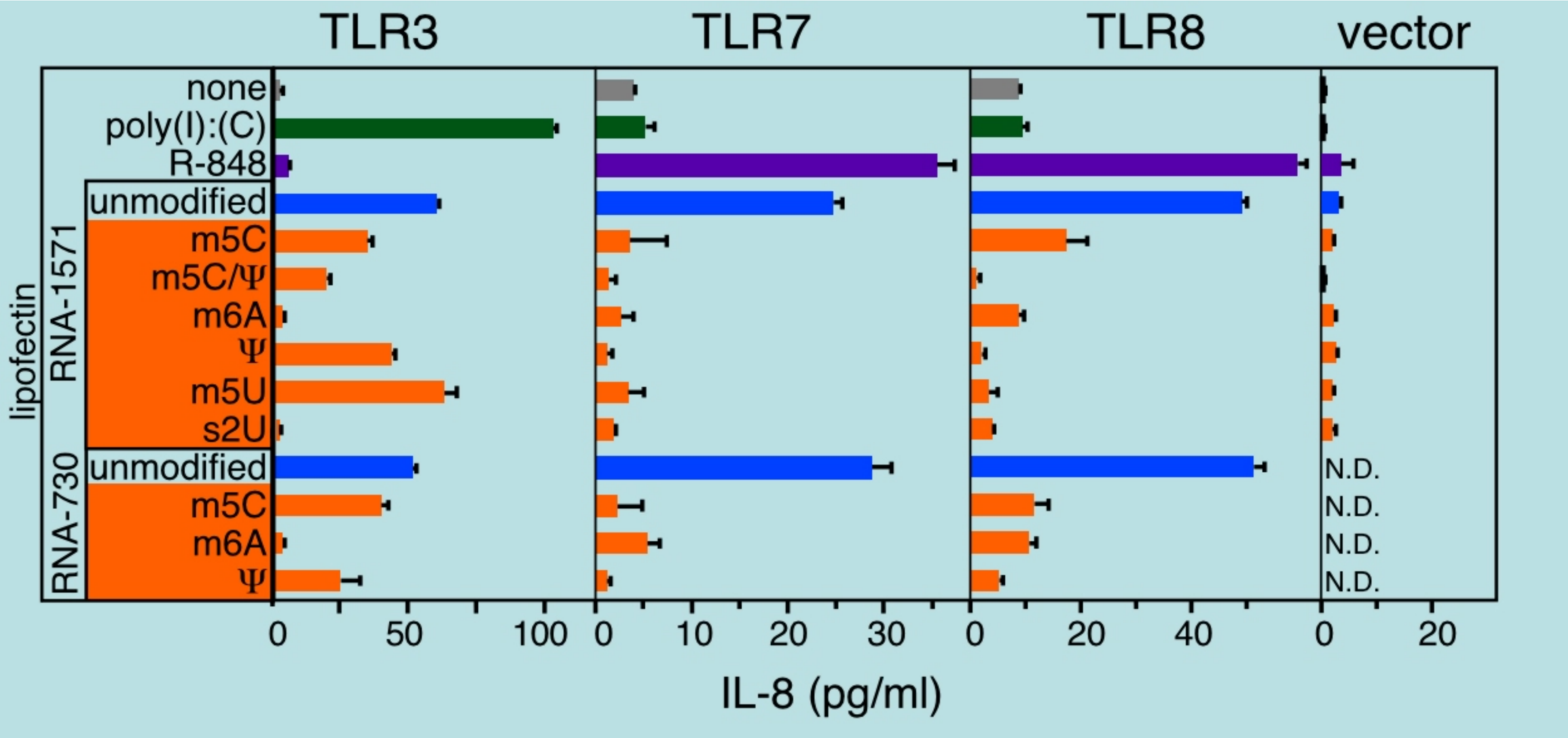
NTP	
m5C m5U Ψ m6A s2U	yes
m1A m1G m7G 2'-*O*-Met-C 2'-*O*-Met-U	no

Immunity 2005, 23: 165

Nucleoside-modified RNA: non immunogenic
2005

2005 - Measuring inflammatory response to modified mRNA





Immunity 2005, 23: 165

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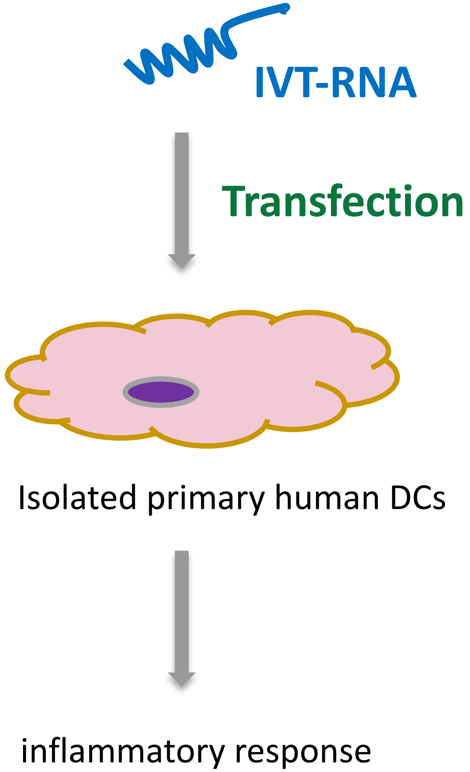
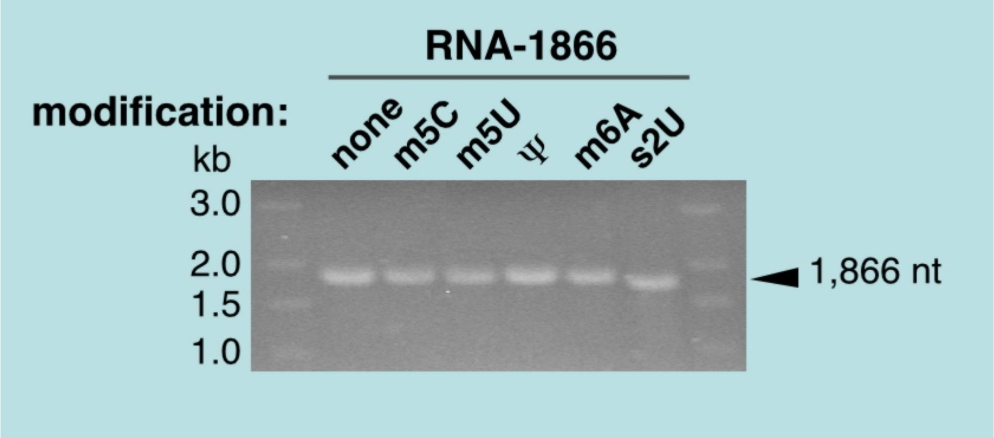
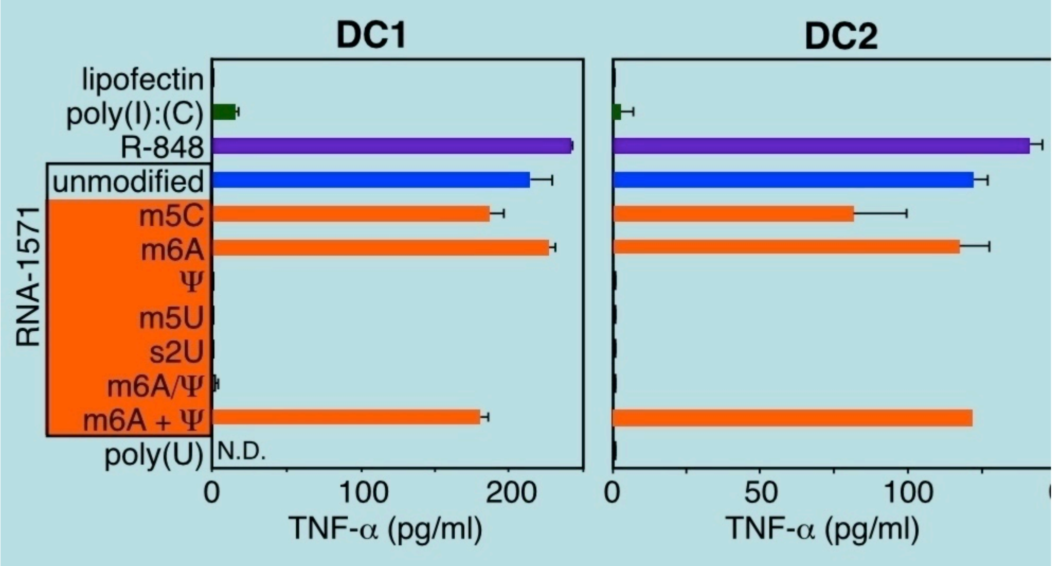
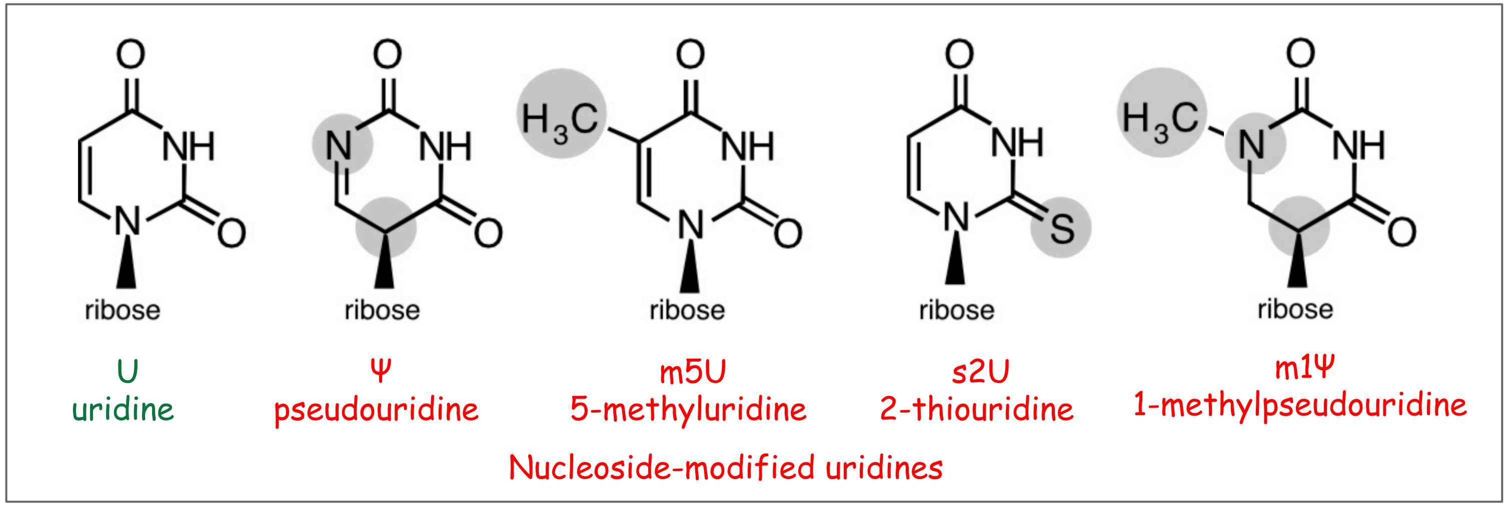


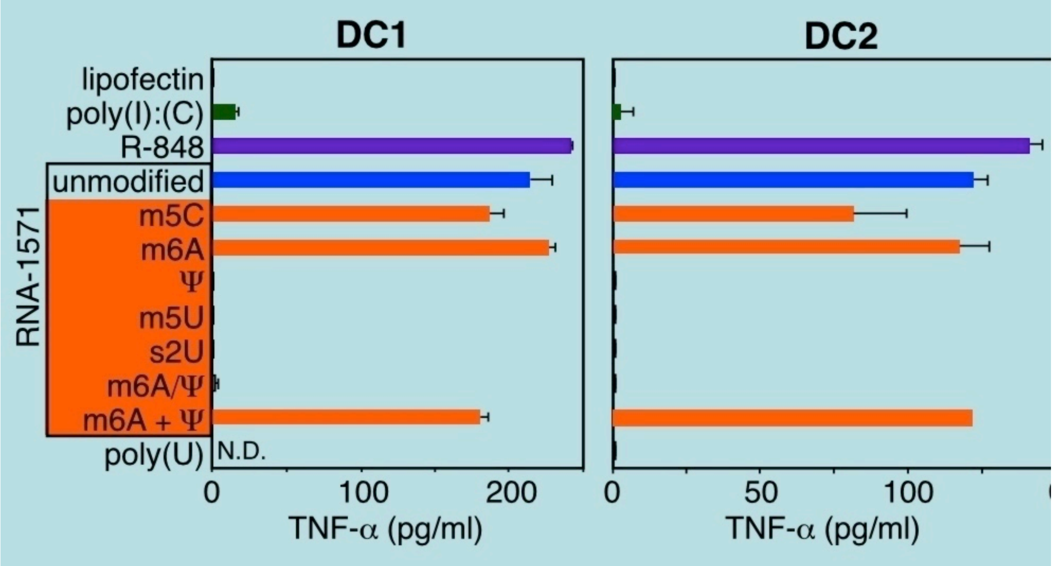




Immunity 2005, 23: 165

2005 - Modified uridine-containing mRNA is non-immunogenic





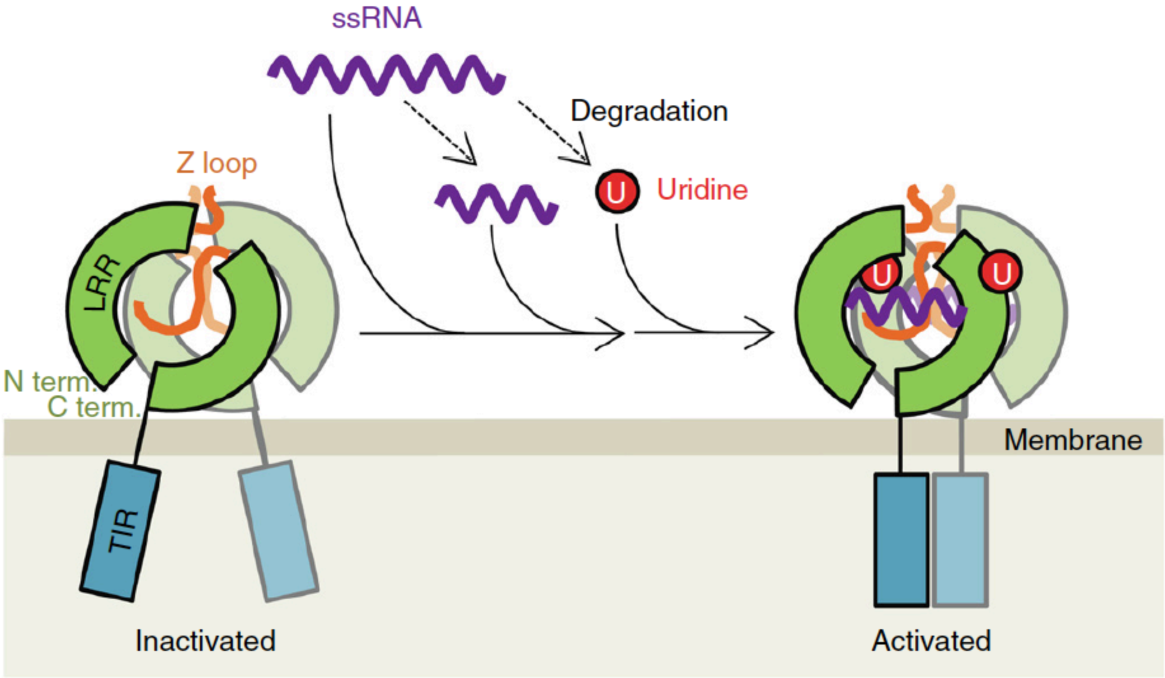
Immunity 2005, 23: 165

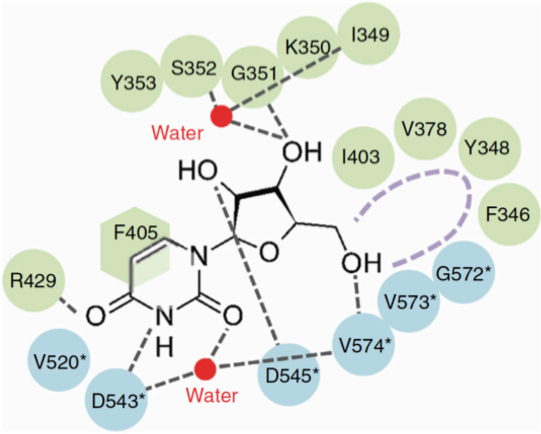
2015-16 Uridine-containing RNA activates TLR7 and TLR8 of immune cells

Crystal structure of TLR7 and TLR8

TLR7 Uridine-containing ssRNA, guanosine *Immunity 2016 – 45:737*

TLR8 Uridine, UpG *Nature Structural & Molecular Biology 2015* ***22****:109*





1963 - Immunogenicity of the U-containing RNA – nitrous acid treatment

JULY 20, 1963 THE LANCET

FOREIGN NUCLEIC ACIDS AS THE STIMULUS TO MAKE INTERFERON

A. ISAACS
M.D. Glasg., Hon. M.D. Louvain

R. A. COX
Ph.D. Birm.

Z. ROTEM*
Ph.D., M.Sc. Jerusalem

OF THE NATIONAL INSTITUTE FOR MEDICAL RESEARCH, MILL HILL, LONDON, N.W.7

antiviral action was produced by cells treated with nitrous-acid-modified R.N.A., but not by cells treated with unmodified R.N.A.

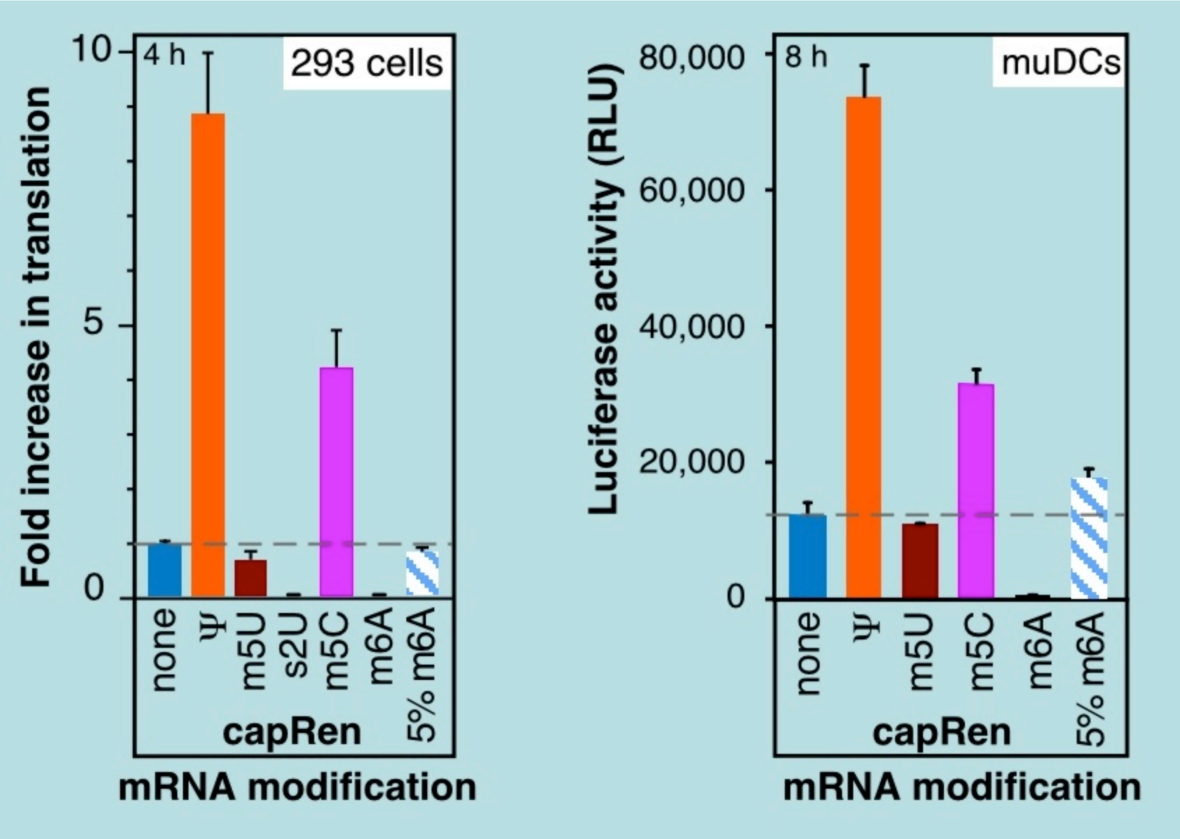
Nitrous acid is known to deaminate adenine to hypo-xanthine, guanine to xanthine, and cytosine to uracil (Schuster and Schramm 1958), and the extent of the

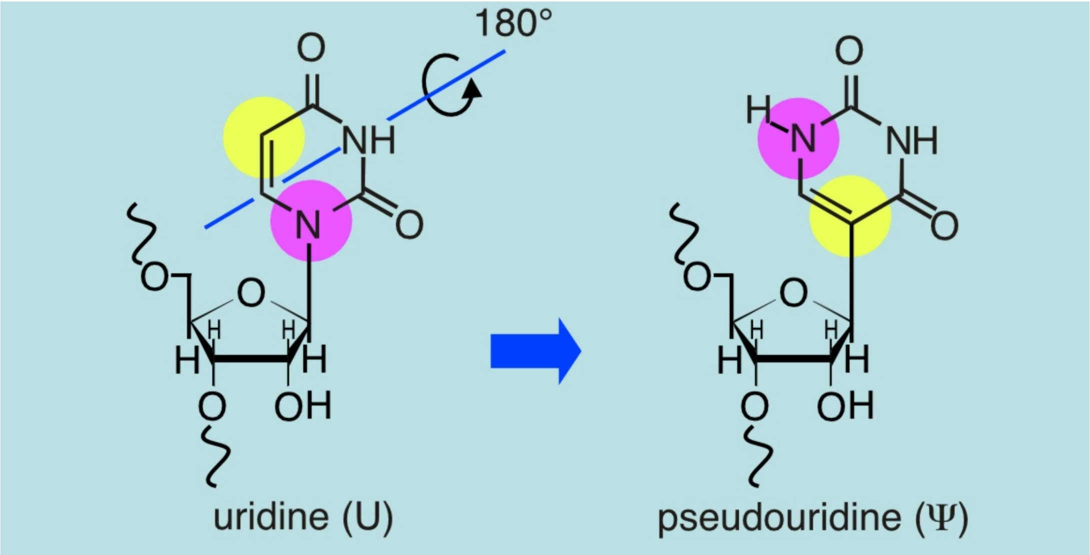
ascertained by the present technique it was necessary to deaminate about 10% of cytosine residues before the R.N.A. was sufficiently foreign to stimulate the production of interferon. These changes are very much greater than

The results support the hypothesis that the production of interferon represents a response of cells to foreign nucleic acids.

A	U	G	C	U	C	G	G	U	G	C	U	C	A	U	C	C	U	G	G	U	C	U	C	C	C	U	G	C	C	C	G	U	Original sequence
Hx	U	G	U	U	U	G	G	U	G	U	U	U	Hx	U	U	U	U	G	G	U	U	U	U	U	U	U	G	U	U	U	G	U	Nitrous acid (conversion C-U) - very immunogenic *The Lancet* **282**, 113 (1963)

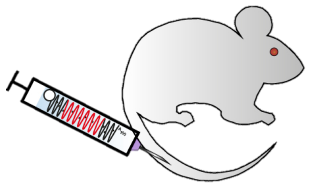
2008 - Superior translation of lipofectin-delivered pseudouridine (Ψ)-modified mRNA

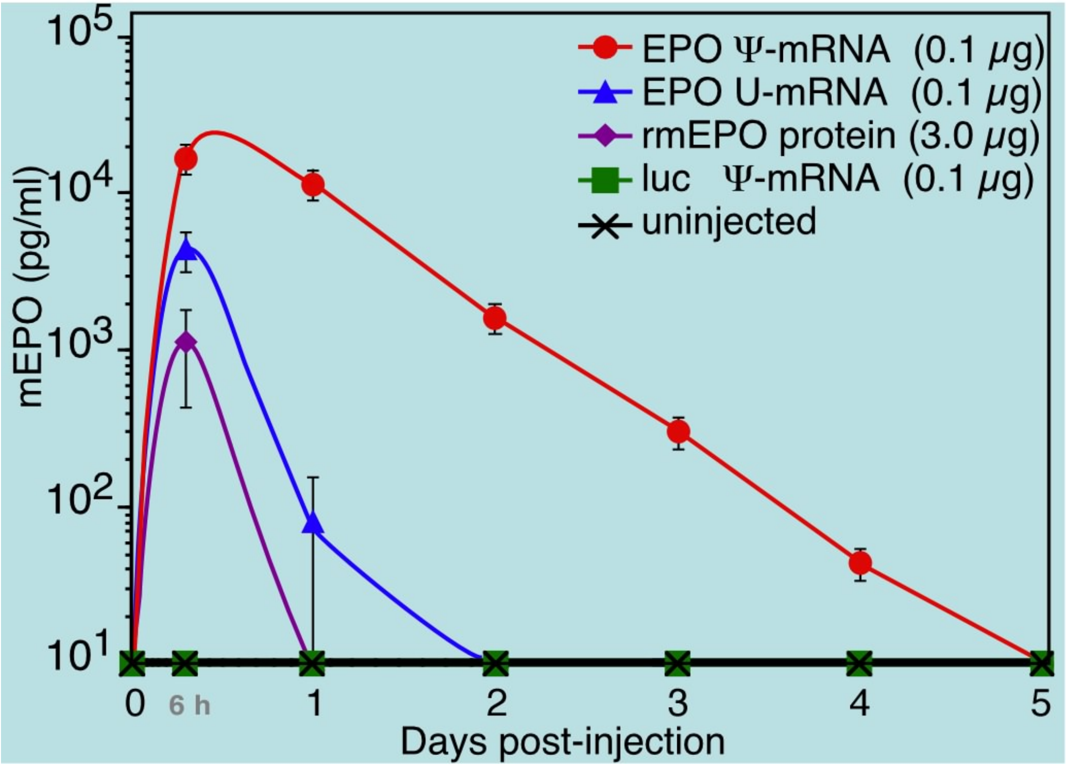


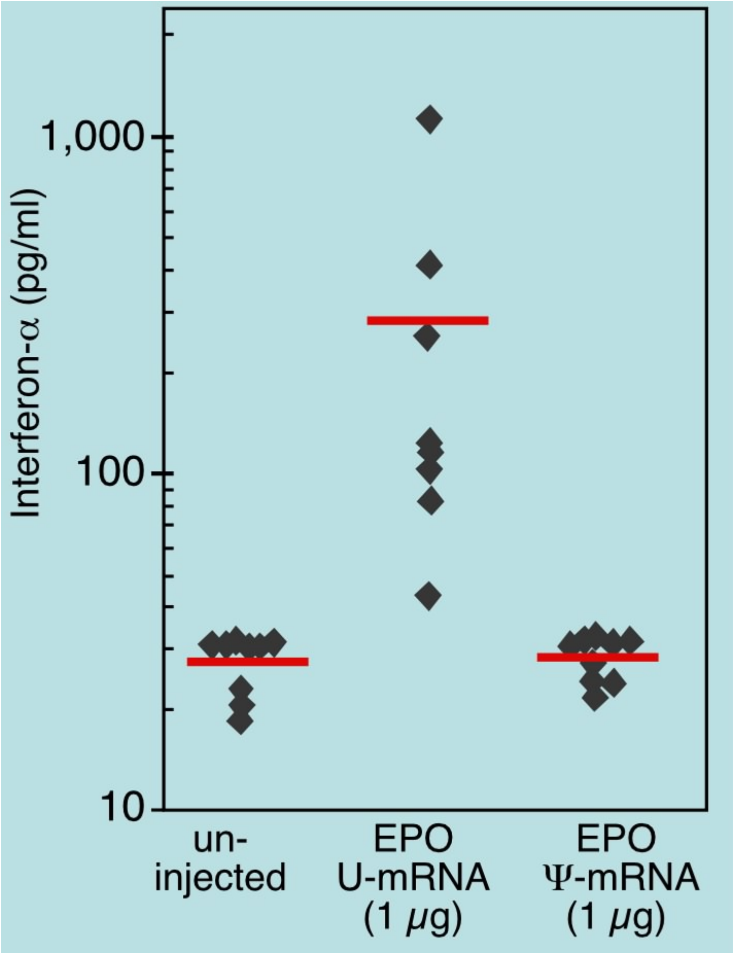


Mol Ther 2008, 16: 1833

2012 - Pseudouridine-containing mEPO mRNA is non-immunogenic in mice

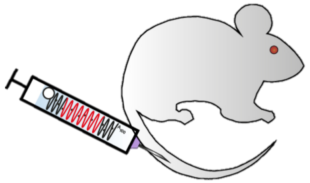


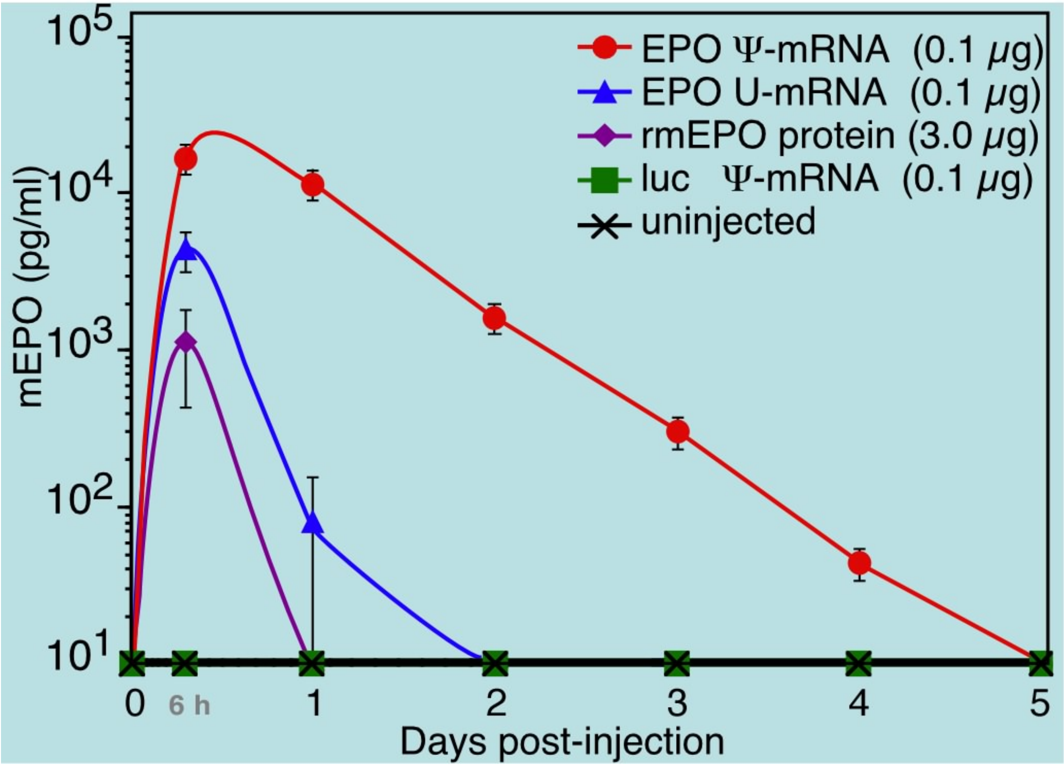


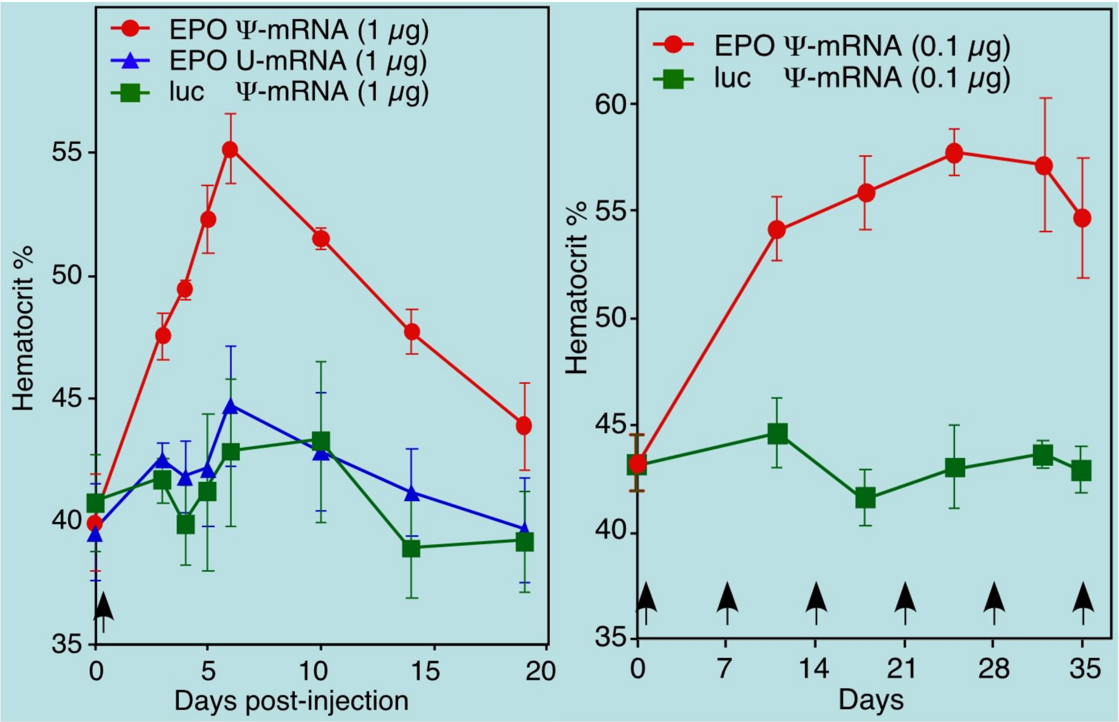


Mol.Therapy 2012, 20: 948

2012 - Increase of hematocrit in mice following mEPO mRNA delivery

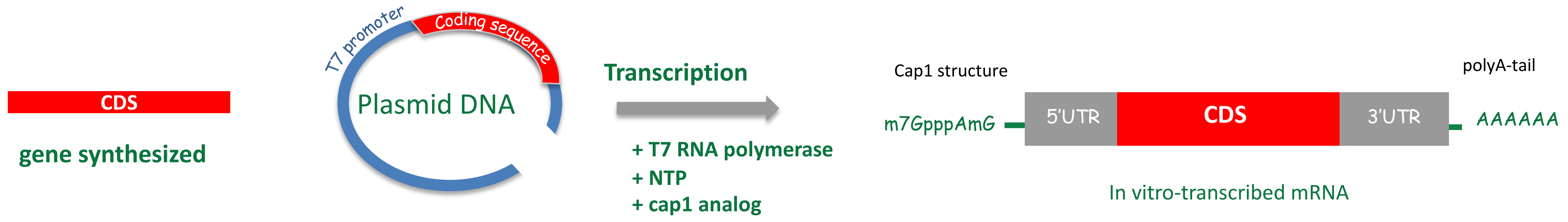


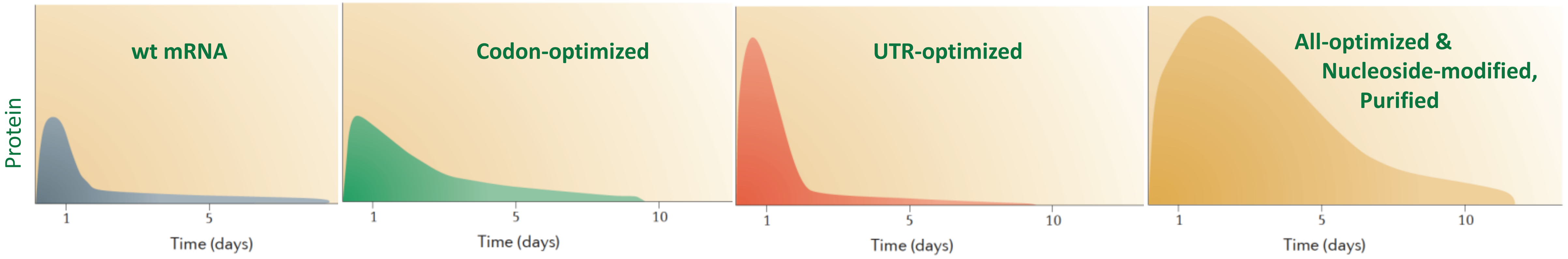




Mol.Therapy 2012, 20: 948

2014 – today Optimizing IVT mRNA





Nature Reviews Drug Discovery 2014, 13: 759 - Updated

Codon-optimization: Approaches to alter the U-content, thus immunogenicity of RNA

Met	Leu	Gly	Ala	His	Pro	Asn	Phe	Ser	Val	Arg	
A U G	C U U	G G U	G C U	C A U	C C U	A A U	U U U	U C U	G U U	C G U	Original sequence immunogenic
A Ψ G	C Ψ Ψ	G G Ψ	G C Ψ	C A Ψ	C C Ψ	A A Ψ	Ψ Ψ Ψ	Ψ C Ψ	G Ψ Ψ	C G Ψ	Ψ-modified none immunogenic *Immunity* **23**, 165 (2005)
A U G	C U A	G G C	G C C	C A C	C C C	A A C	U U C	A G C	G U A	C G C	GC-rich codon-optimized less immunogenic *Mol. Ther.* **23**, 1457 (2015)

AA frequencies in vertebrate

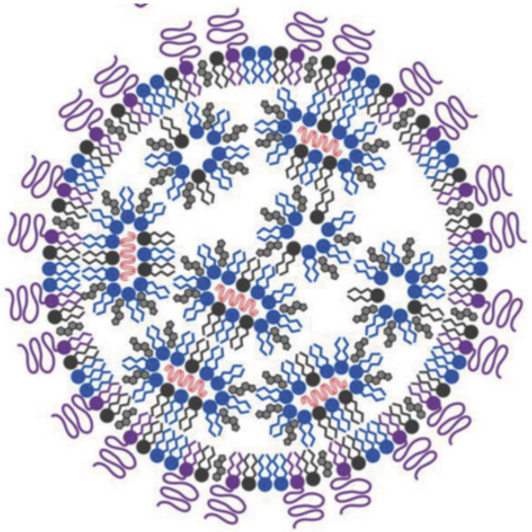
8%	Serine
8%	Leucine
7%	Alanine
7%	Glycine
7%	Lysine
7%	Valine
6%	Threonine
6%	Aspartic Acid
6%	Glutamic Acid
5%	Proline
4%	Asparagine
4%	Arginine
4%	Phenylalanine
4%	Isoleucine
4%	Glutamine
3%	Cysteine
3%	Tyrosine
3%	Histidine
2%	Methionine
1%	Tryptophan
---	Stop Codons
32%	**Total AA**

Minimum 1 U/codon (red)

Minimum 2 U/codon (orange)

LNP-formulated nucleoside-modified mRNA for vaccine
2017

2012 - LNP as a vehicle for delivery of nucleoside-modified mRNA



Ionizable lipids
- Neutral at physiological pH, cationic under acidic conditions
- Form complex with mRNA
- Promote endosomal escape of mRNA

Helper lipids
- Improve the stability of mRNA/LNP complexes
- Promote uptake and endosomal escape

PEGylated lipids
- Prevent rapid clearance

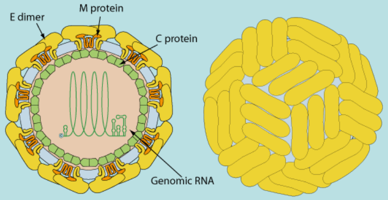
Cholesterol
- Structural integrity of LNPs and endosomal escape

J Controlled Release 2015 217: 345

2017- Generating m1Ψ-mRNA encoding glycoproteins of Zika virus

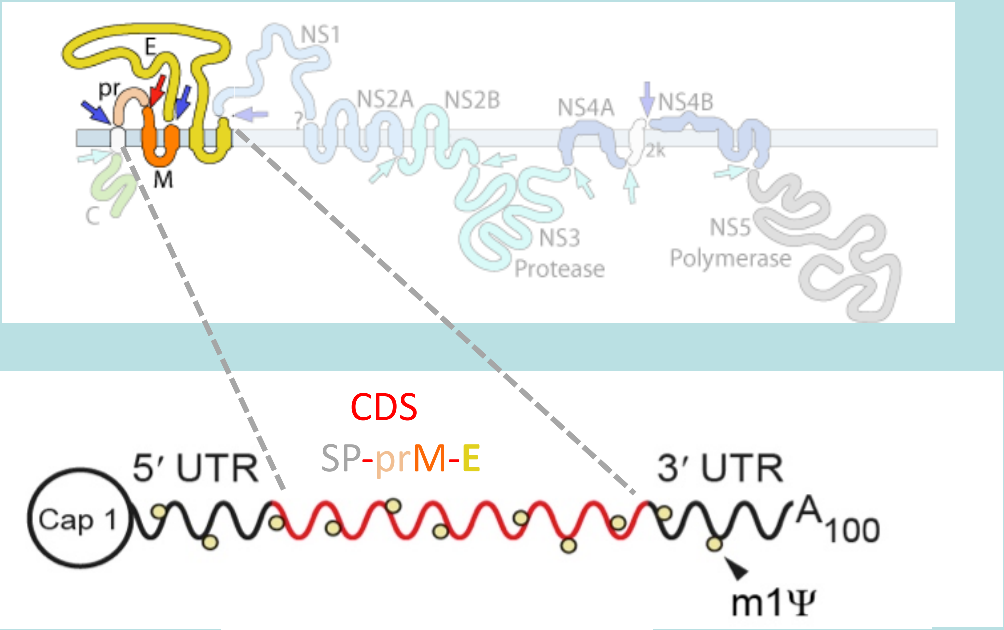
Norbert Pardi

Zika virus

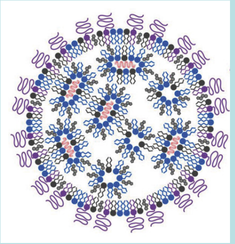


ZIKV protein

IVT m1Ψ-mRNA SP-prM-E



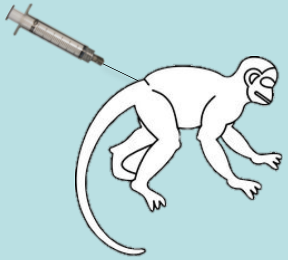
LNP-formulated mRNA



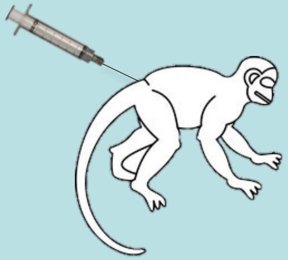
80-100 nm

Nature 2017, 543: 248

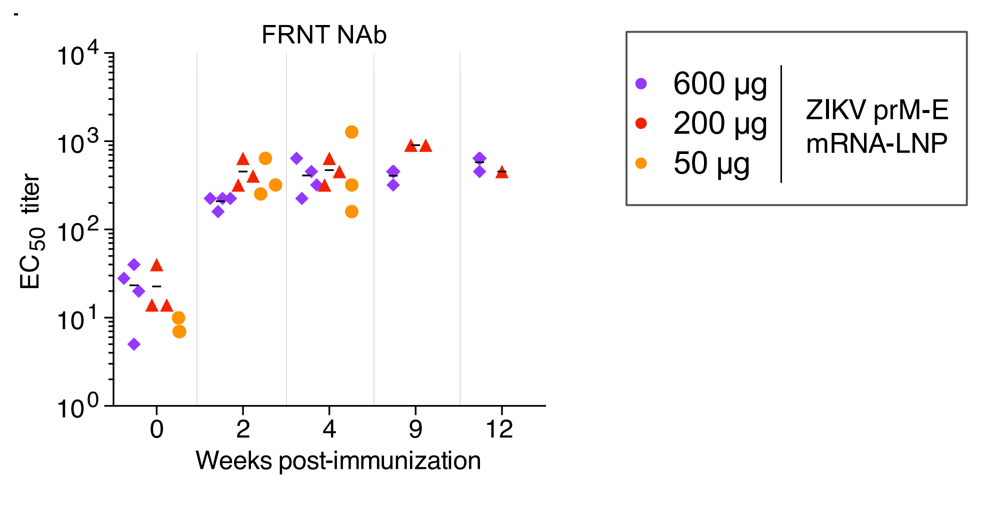
2017 - 50 µg of ZIKV modRNA-LNP protects macaques from ZIKV challenge

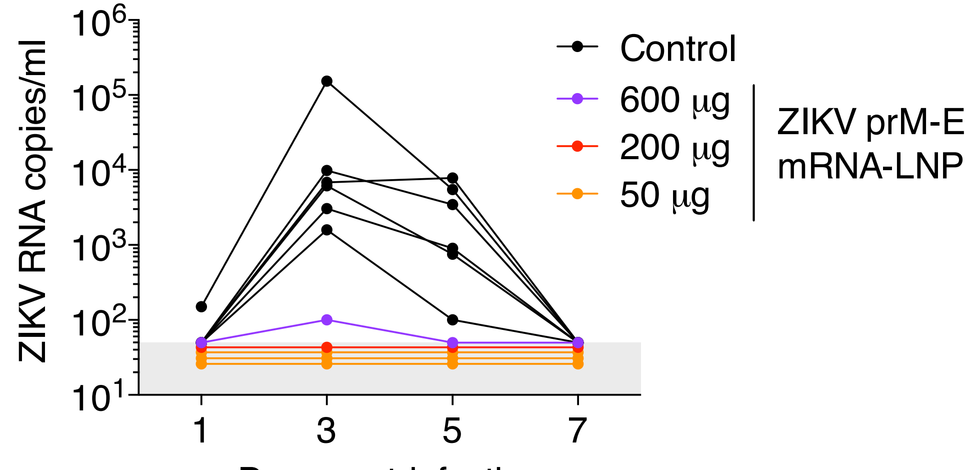


Vaccination i.d.: 50, 200 or 600 µg
ZIKV prM-E m1Ψ-mRNA-LNP
50 µg ~ 0.02 mg/kg



Challenge i.d.: 10,000
$TCID_{50}$
live ZIKV PRVABC59





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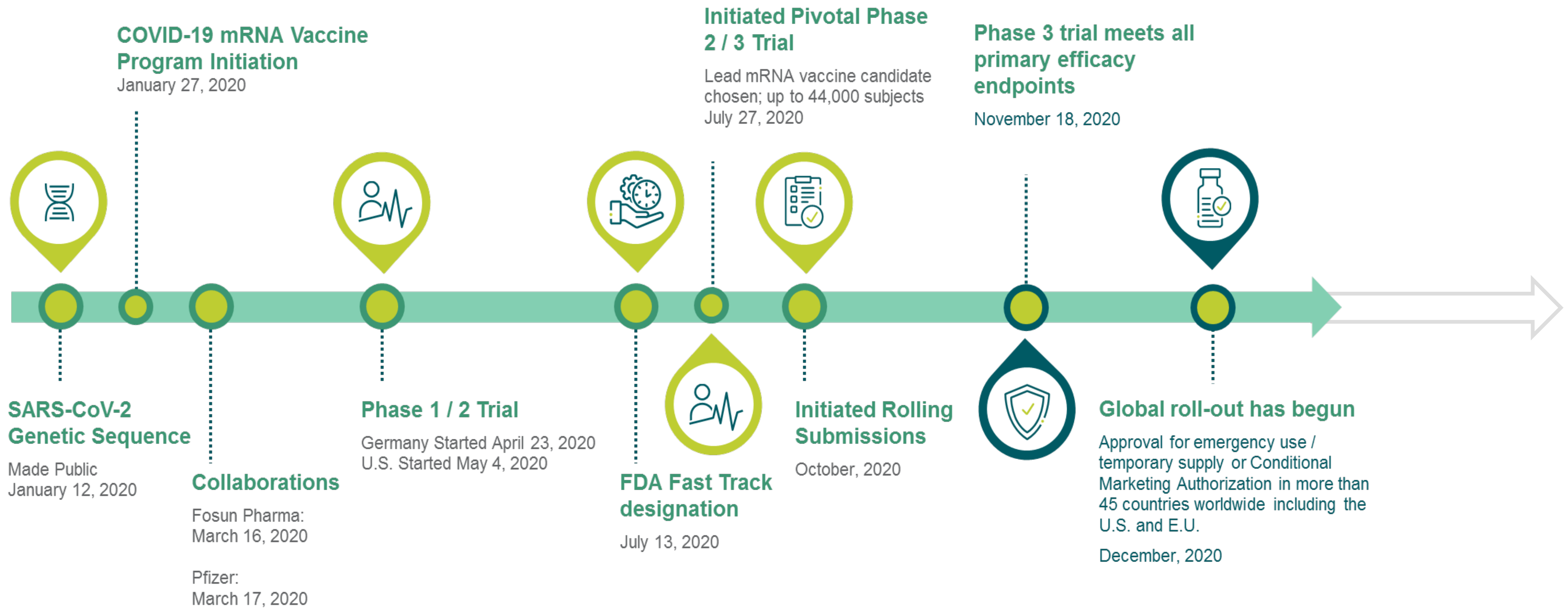
2020 - Clinical development of Comirnaty (BNT162b2)

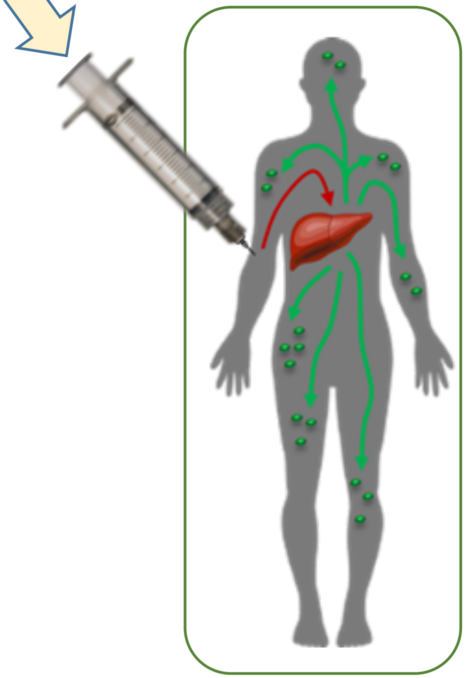


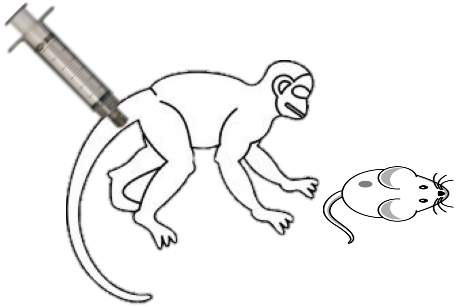
BIONTECH Pfizer

2021 and beyond - mRNA is a new class of medicine

cap1 $^{m7}GpppA_mG$ — 5'UTR | Coding sequence | 3'UTR — **polyA-tail** $A_{70}LinkerA_{30}$

Nucleoside-modified mRNA for therapy





Preclinical studies for

- **vaccination against –** malaria, HSV, HIV, Flu, ZIKV,
- **tolerization for autoimmune diseases–** Multiple scelrosis
- **genome editing of genetic diseases –** sickle cell anemia, HIV
- **Edema –** VEGFC mRNA lymphoid vessel

Clinical trials ongoing for treatment of

- **acute diseases –** VEGFA mRNA heart falilure, wound healing
- **cancer –** vaccines, intratumor injection of cytokine mRNAs
- **infectious diseases –** mAb mRNA
- **genetic diseases –** Cas9 mRNA for genome editing

Thank you for your attention