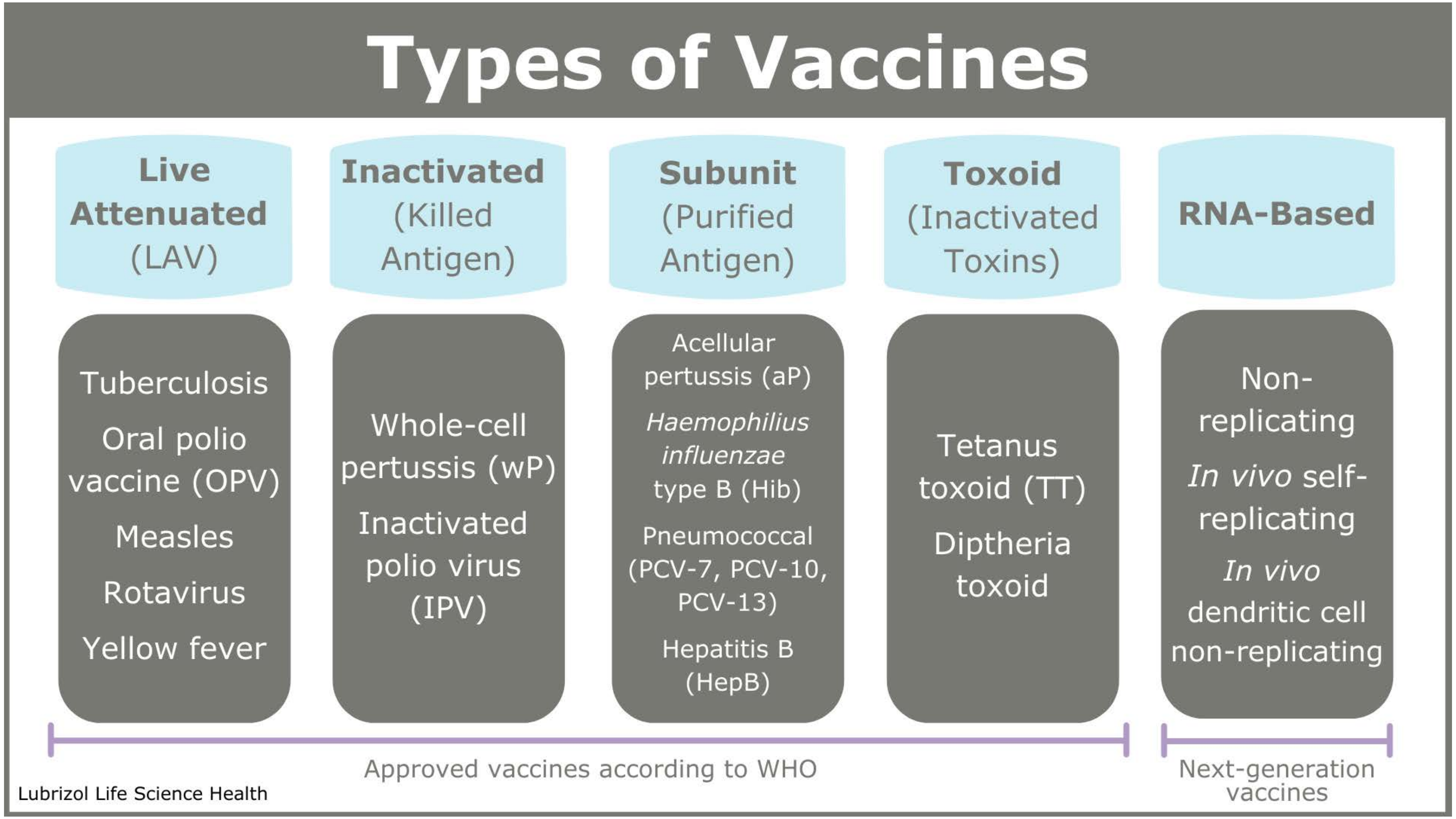
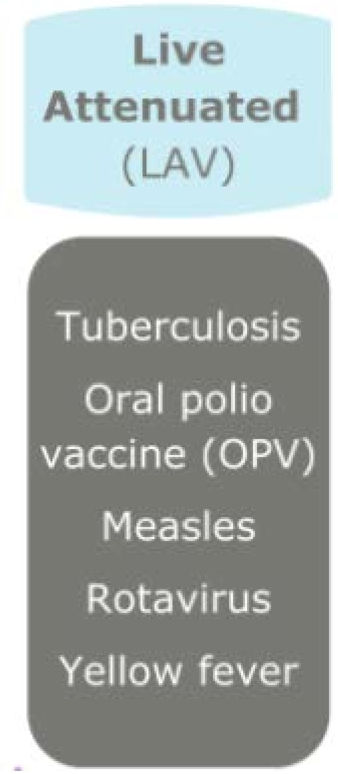
Amplifying RNA Vaccine Development

Journal club

Hui Zhang

29.09.2020


Types of Vaccines
Live Attenuated (LAV)
Inactivated (Killed Antigen)
Subunit (Purified Antigen)
Toxoid (Inactivated Toxins)
RNA-Based
Tuberculosis
Oral polio vaccine (OPV)
Measles
Rotavirus
Yellow fever
Whole-cell pertussis (wP)
Inactivated polio virus (IPV)
Acellular pertussis (aP)
Haemophilius influenzae type B (Hib)
Pneumococcal (PCV-7, PCV-10, PCV-13)
Hepatitis B (HepB)
Tetanus toxoid (TT)
Diptheria toxoid
Non-replicating
In vivo self-replicating
In vivo dendritic cell non-replicating
Approved vaccines according to WHO
Next-generation vaccines
Lubrizol Life Science Health



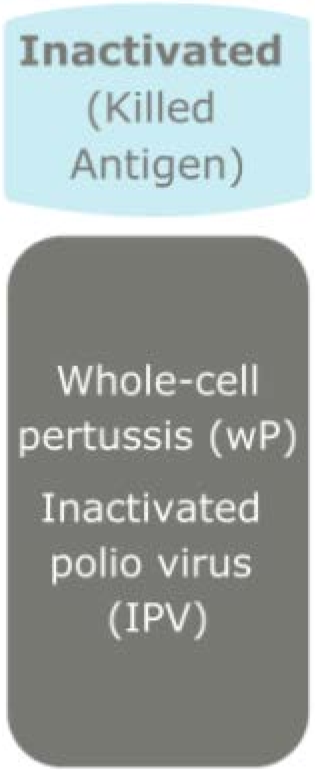
- **A weakened form of the germ.**

Pros

- Strong and long-lasting immune response.
- Just 1 or 2 doses of most live vaccines give a lifetime of protection.

Cons

- Potential harmful to people with weakened immune systems, long-term health problems, or who've had an organ transplant.
- Storage conditions limitations: stay cool.



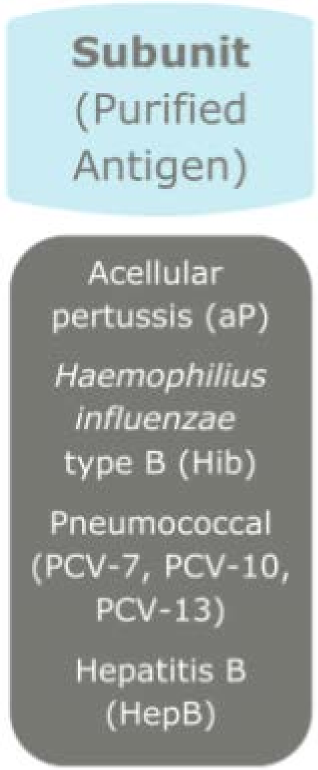
- **The killed version of the germ.**

Cons

- Induced immunity is not as strong as live vaccines.
- Several doses over time (booster shots) in order to get ongoing immunity

Pros

- Safe…



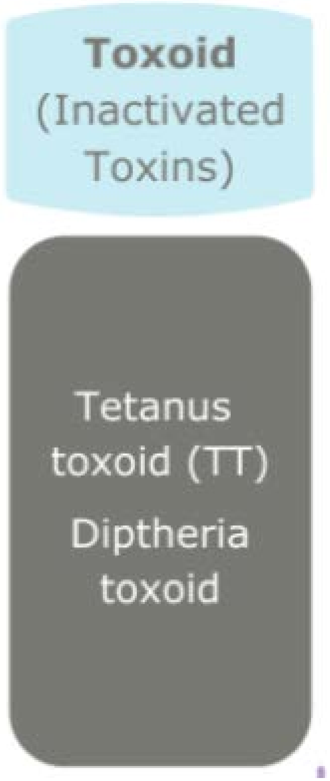
- **Subunit, recombinant, polysaccharide, and conjugate vaccines:** specific pieces of the germ — like its protein, sugar, or capsid

Pros

- Strong immune response targeted to key parts of the germ.
- Broad application: anyone who needs them.

Cons

- Need booster shots to get ongoing protection.



- **A toxin (harmful product) made by the germ.**

Pros

- Immunity to the parts of the germ (toxin) that cause a disease instead of the germ itself.

Cons

- Need booster shots to get ongoing protection.

Newly developed and promising

sequence the genome of a viral pathogen to determine the code for a good antigen.

Purify the mRNA and formulate it as a vaccine.

mRNA translation into antigen *in vivo*.

RNA VS DNA

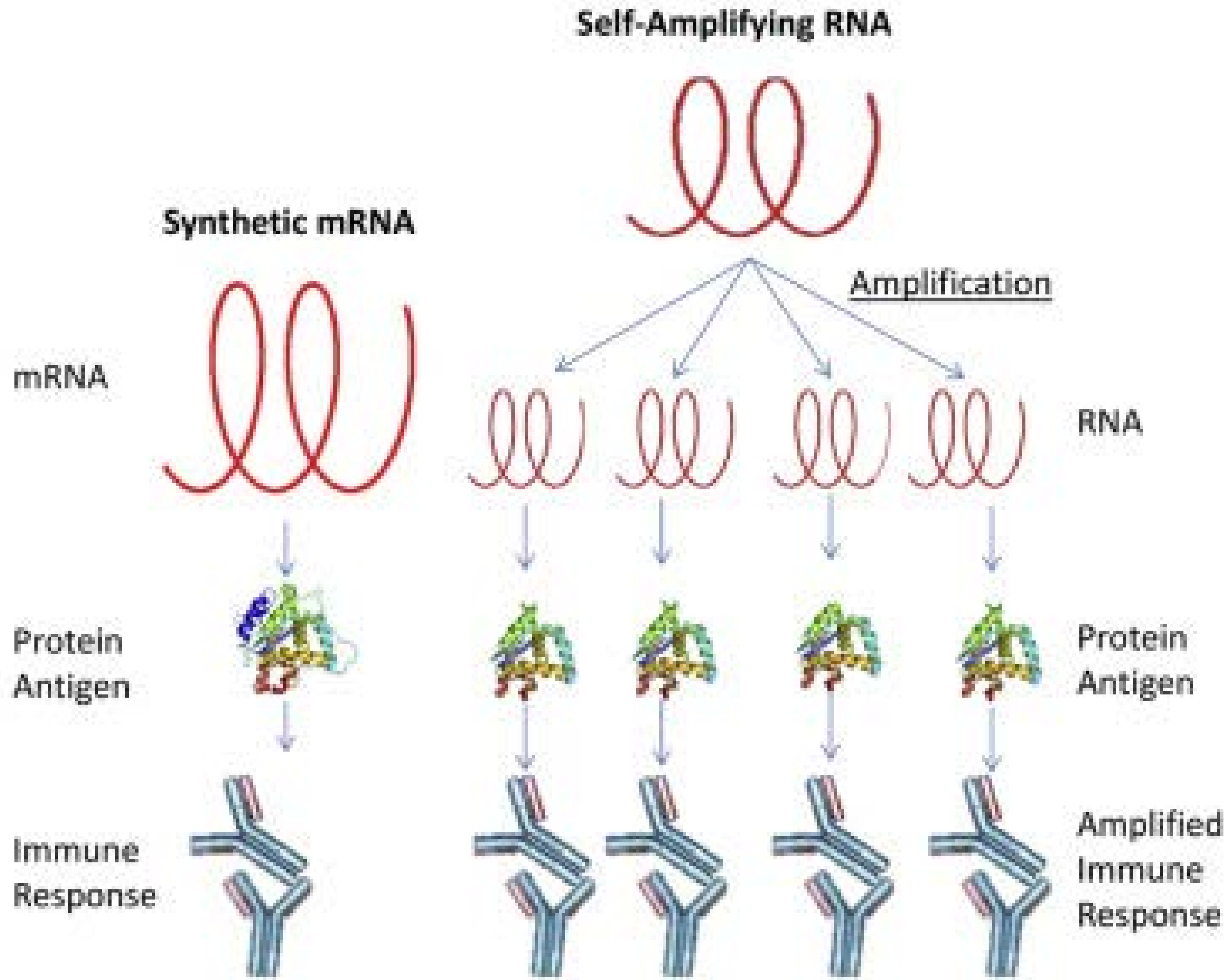
- Do not need to enter the nucleus to express the antigen.
- Avoid the risk of integration of targeted sequence into host cells.

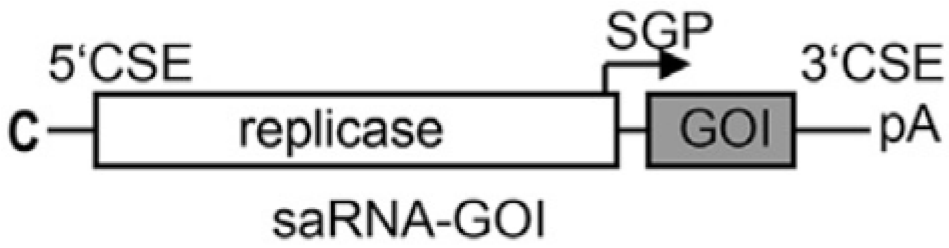
Lubrizol life science health

Molecular Therapy
Original Article

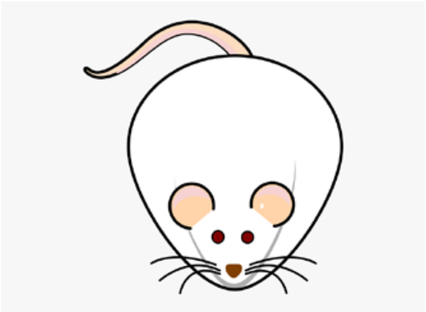
Self-Amplifying RNA Vaccines Give Equivalent Protection against Influenza to mRNA Vaccines but at Much Lower Doses

Annette B. Vogel,[1,5] Laura Lambert,[2] Ekaterina Kinnear,[2] David Busse,[2] Stephanie Erbar,[1] Kerstin C. Reuter,[3] Lena Wicke,[1] Mario Perkovic,[4] Tim Beissert,[4] Heinrich Haas,[1] Stephen T. Reece,[1,6] Ugur Sahin,[3] and John S. Tregoning[2,5]

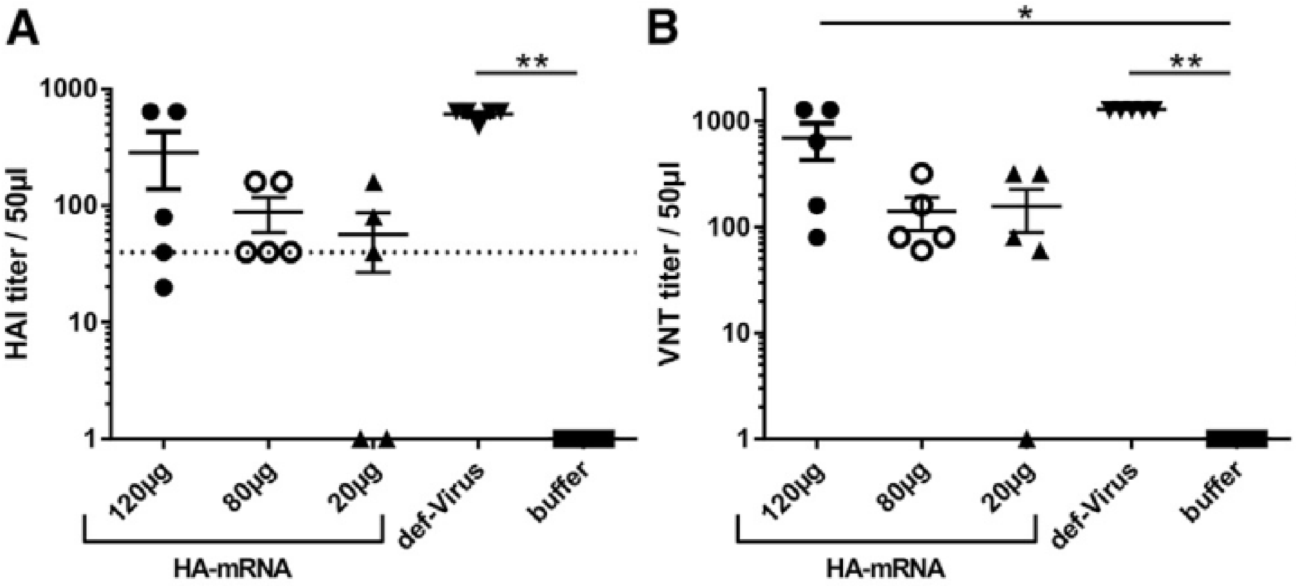

Self-Amplifying RNA
Synthetic mRNA
Amplification
mRNA
RNA
Protein
Antigen
Protein
Antigen
Immune
Response
Amplified
Immune
Response


SGP
5'CSE
3'CSE
C
replicase
GOI
pA
saRNA-GOI

sa-RNA Achieves Equivalent Protection to mRNA but Requires Less RNA



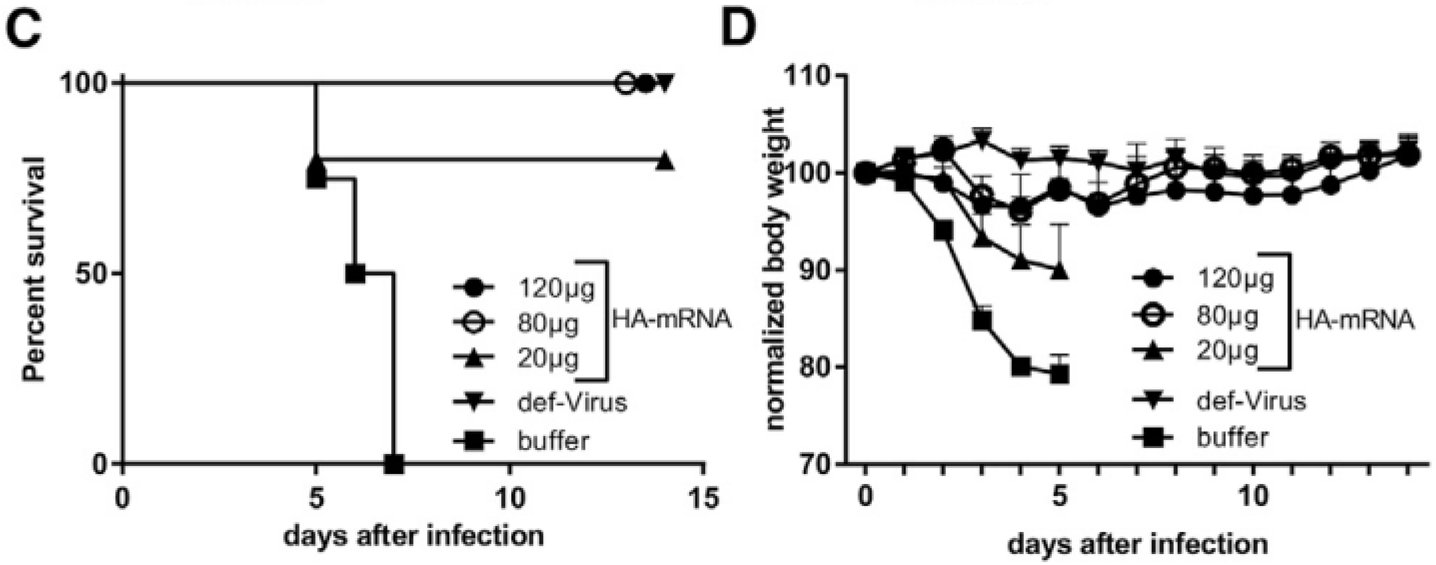
Vaccine procedure:
BALB/c mice
i.m with synthetic mRNA encoding HA from H1N1/PR8
prime-boost regimen: 120, 80, 20ug
Inactivated virus as positive control



Immune response assessment:
hemagglutination inhibition(HA)
viral neutralizing titer(VNT)

Increasing antibody responses with increasing doses

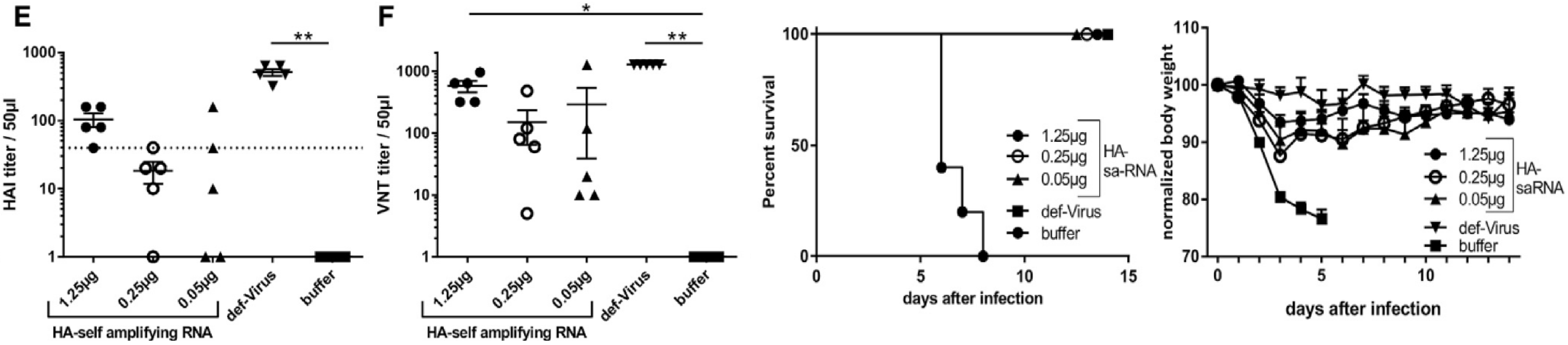
sa-RNA Achieves Equivalent Protection to mRNA but Requires Less RNA



Protection assessment:
Intranasally infection with 10-fold lethal dose of H1N1/PR8
120 and 80 ug groups were fully protected but the 20 ug group was partially protected

sa-RNA Achieves Equivalent Protection to mRNA but Requires Less RNA

Sa-RNA expressing H1N1/PR8 HA antigen



Vaccination induced anti-H1N1/PR8 functional antibody response;
1.25ug dose gave significant response and also full protection;
0.25ug and 0.05ug were partially protected.

sa-RNA Achieves Equivalent Protection to mRNA but Requires Less RNA

Table 1. Comparison of Responses by Different RNA Platforms

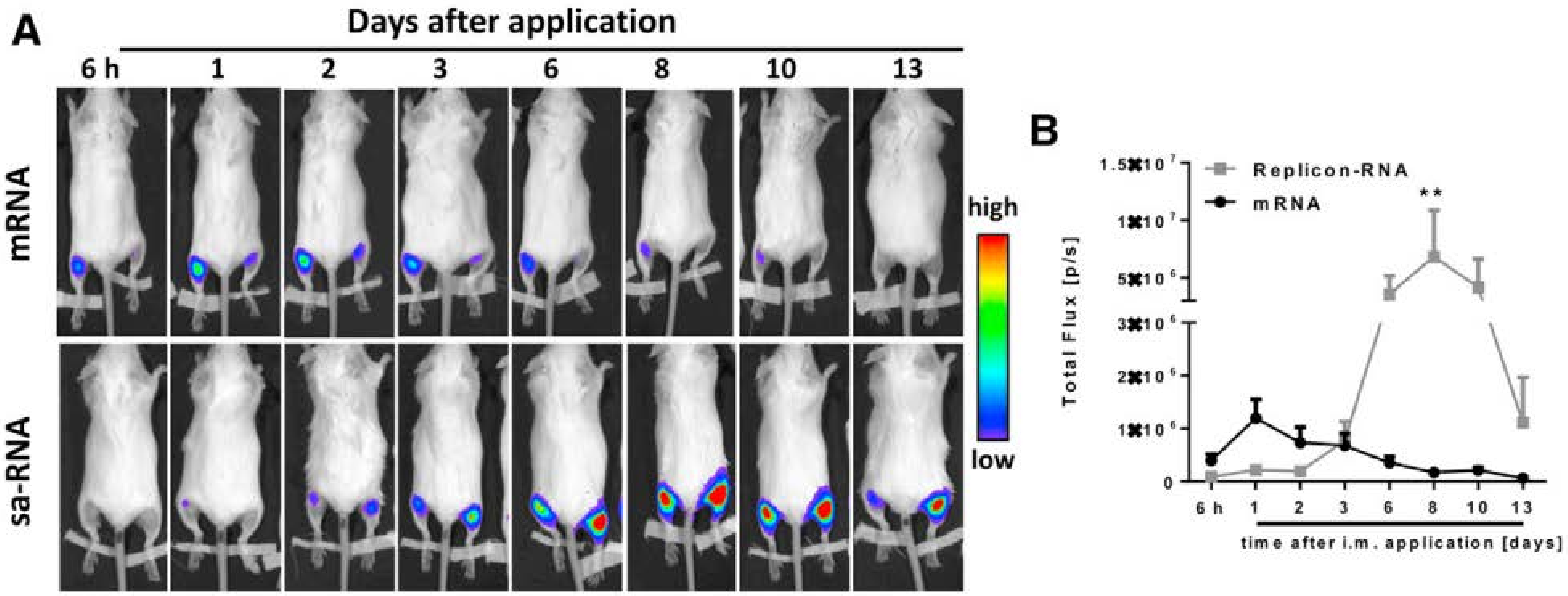
	mRNA			sa-RNA		
Dose	120 µg	80 µg	20 µg	1.25 µg	0.25 µg	0.05 µg
HAI (mean ± SD)	284 ± 325.7	88 ± 65.73	56.4 ± 66.52	104 ± 53.67	18.2 ± 14.53	42.4 ± 67.66
VNT (mean ± SD)	688 ± 581.3	140 ± 107.7	156.2 ± 152.3	576 ± 267.7	149 ± 189.6	288 ± 556.5
Weight d3 p.i.	96.7 ± 6.7	97.6 ± 2.0	93.4 ± 5.3	93.4 ± 2.9	87.6 ± 4.3	90.3 ± 5.6

HAI, hemagglutination inhibition assay titer; p.i., post-infection; VNT, viral neutralizing titer.

64-fold lower dose of sa-RNA than synthetic mRNA was required to give an equivalent protective response

sa-RNA Gives Extended Expression Compared to mRNA

Sa-RNA encoding firefly luciferase genes and visualized with IVIS spectrum in vivo imaging system after intraperitoneally injection of D-luciferin.



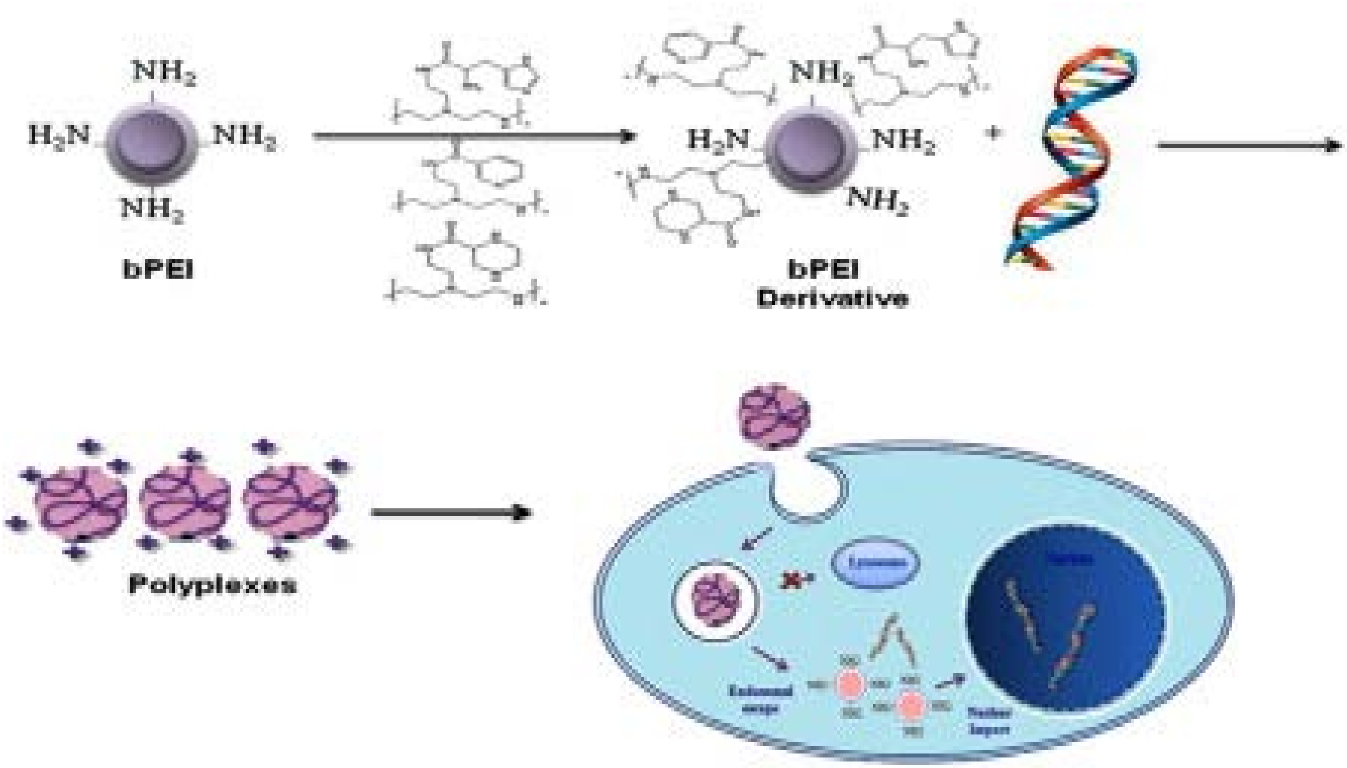
Delayed luciferase expression from sa-RNA, peaking 8 days after mRNA, 5-fold higher peak, 10 days lasting above mRNA.

Current formulation and delivery technologies for mRNA vaccines

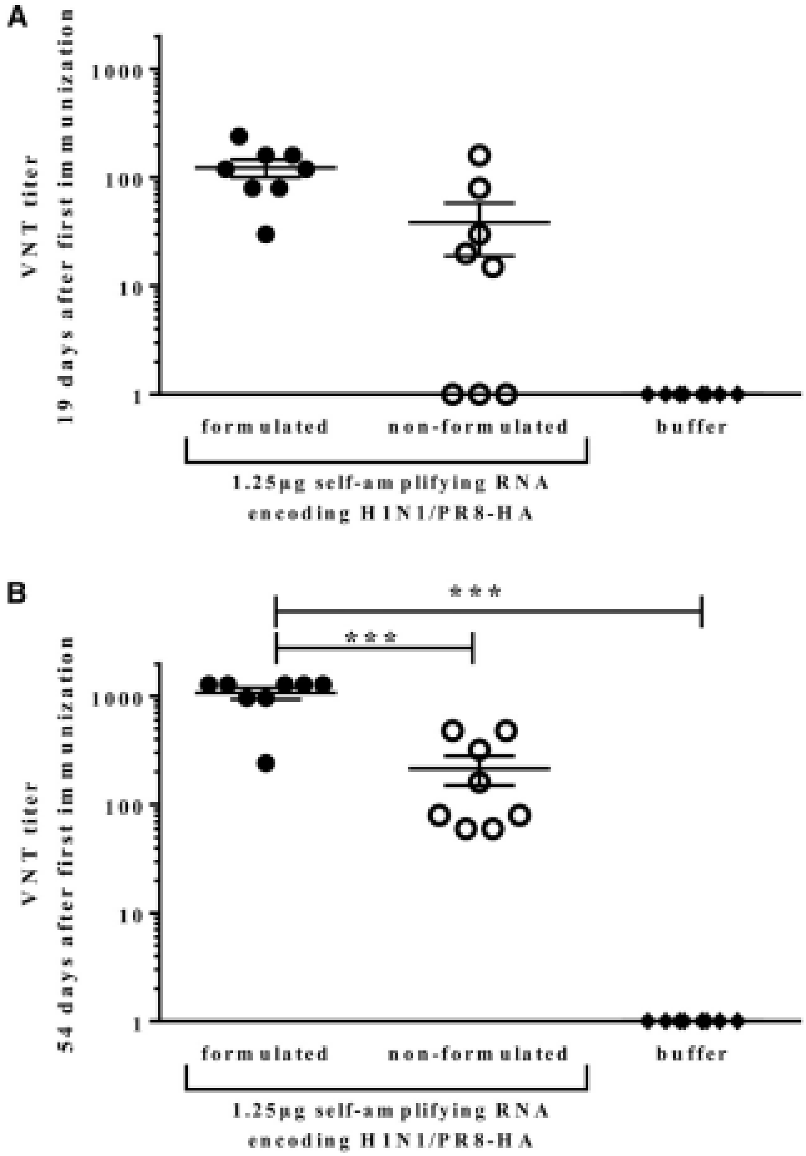
Delivery format	Advantages	Challenges	Readiness for human[a]
Lipid-based nanoparticles	• Protect mRNA from RNase degradation • Efficient intracellular delivery of mRNA • High reproducibility • Easy to scale up	• Potential side effects	Clinical trials
Polymer-based nanoparticles	• Protect mRNA from RNase degradation • Efficient intracellular delivery of mRNA	• Potential side effects • Polydispersity	Preclinical mouse model
Protamine	• Protect mRNA from RNase degradation • Protamine-mRNA complex has adjuvant activity	• Low delivery efficiency • mRNA complexed with protamine is translated poorly	Clinical trials
Other peptides	• Protect mRNA from RNase degradation • Peptides offer many functions to be exploited	• Low delivery efficiency	Preclinical mouse model

C. Zeng et al. 2020

Delivery formulation improves sa-RNA efficacy

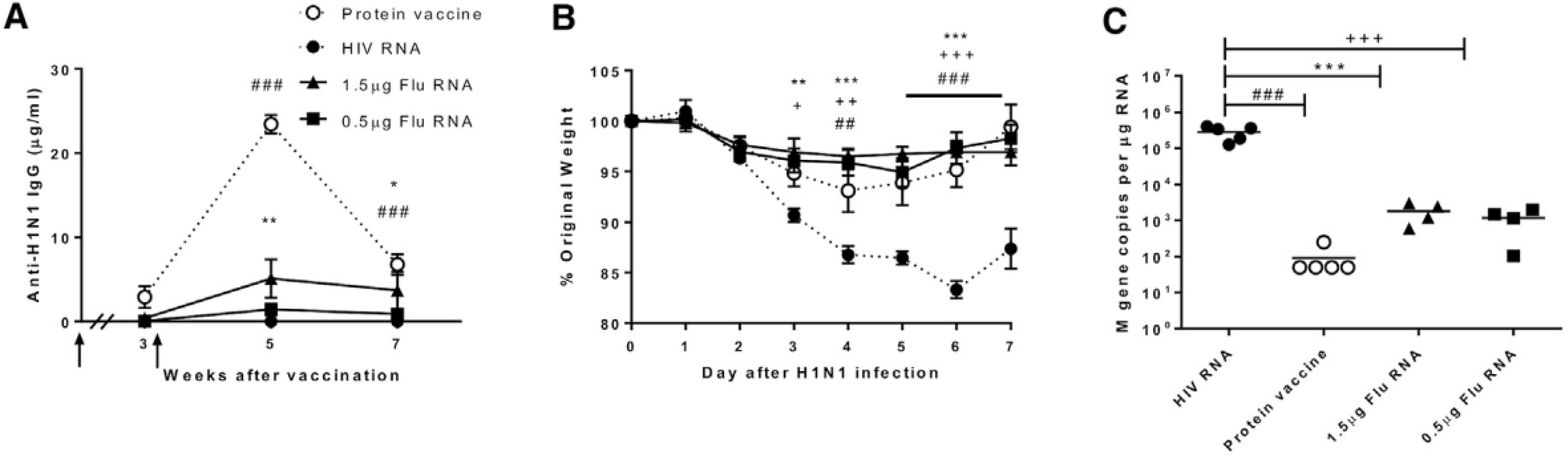


Formulation: PEI, Polyethyleneimine



https://images.app.goo.gl/PbMgtaUd16D6D9fM7

sa-RNA Vaccine Encoding Influenza A Virus HA Protects against Current Seasonal Influenza Strains



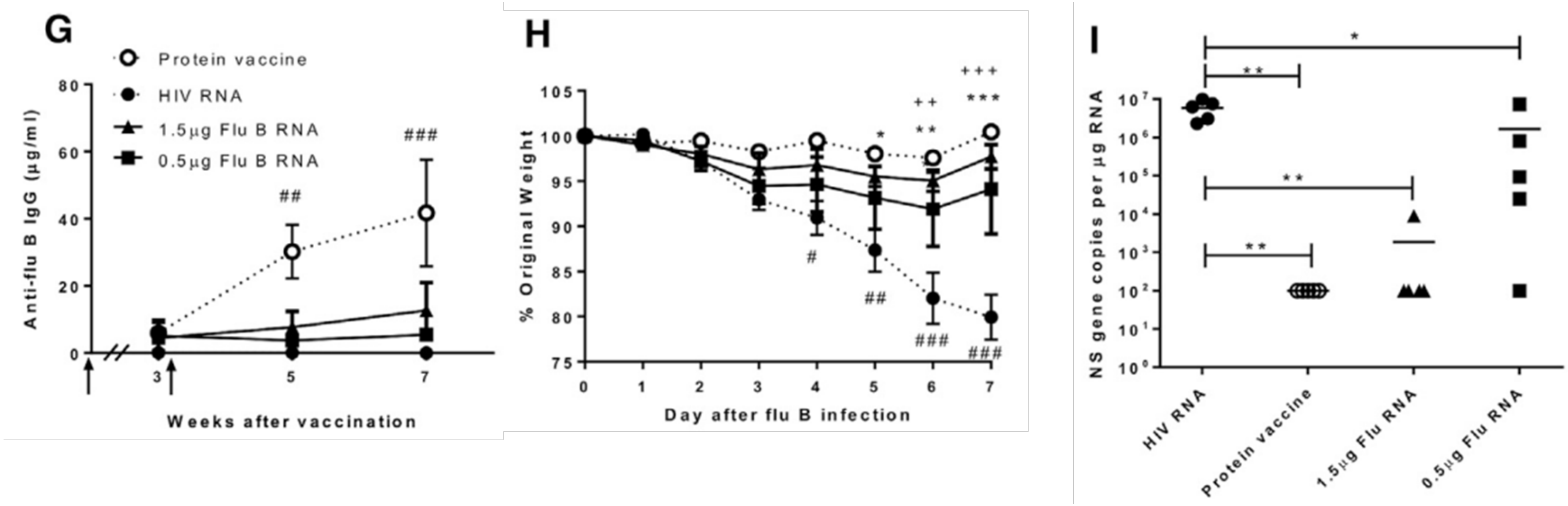
Immune response against influenza

Protective effect against influenza

Reduced M gene in lung

Other influenza strains

B/Massachusetts/2/2012

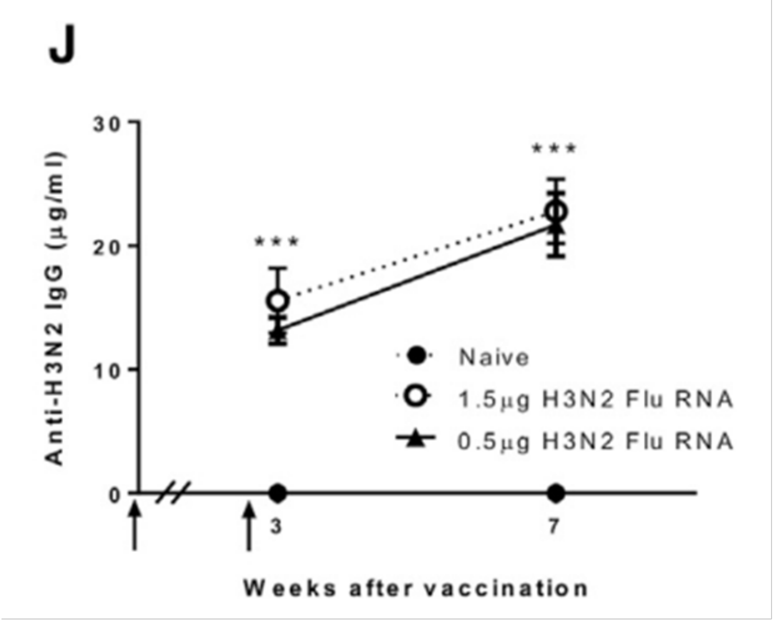


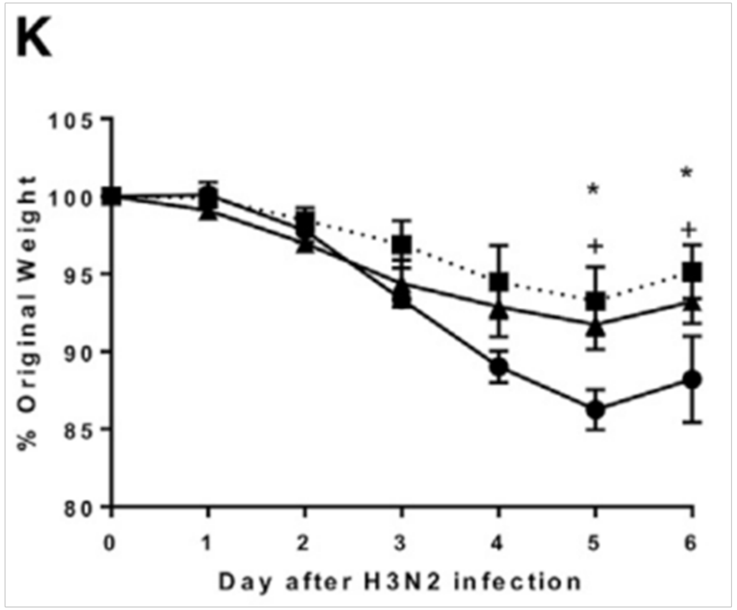
Higher IgG levels from protein vaccine.

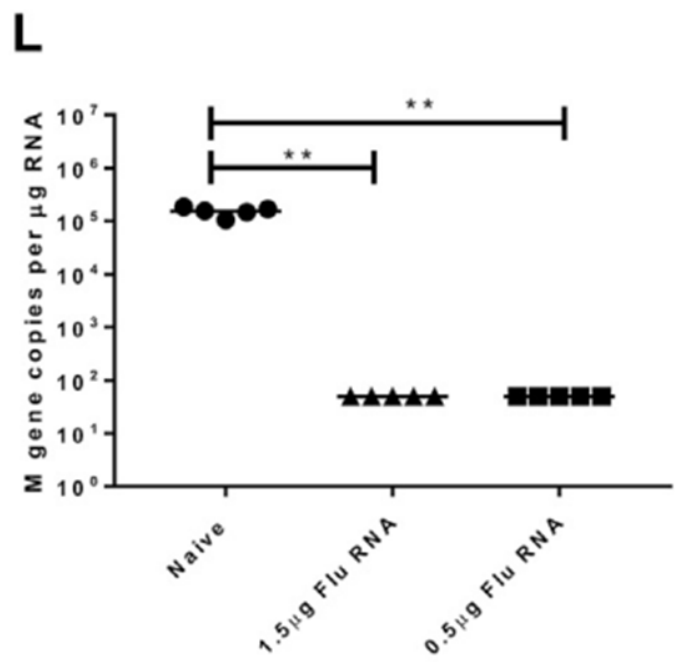
Weight loss protection from high and low dose sa-RNA

Reduced virus gene in lung following both protein and sa-RNA vaccination

Seasonal H3N2







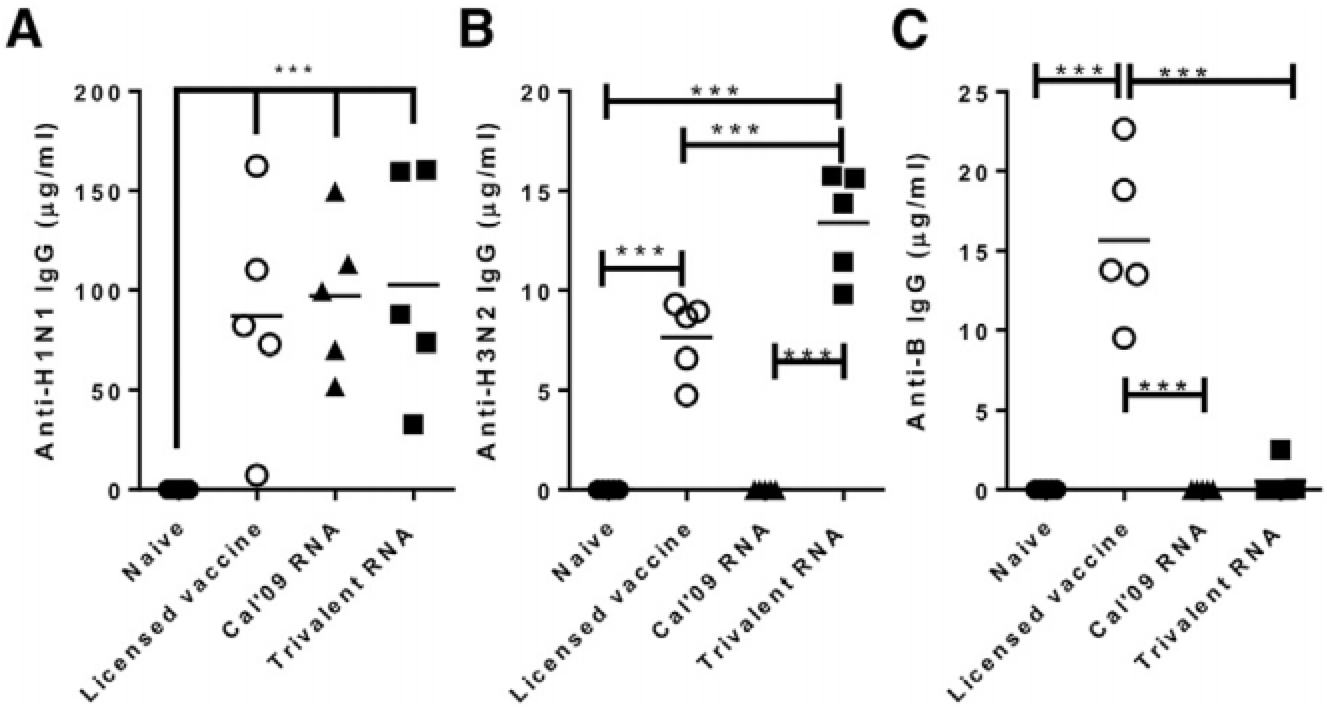
More specific antibody than control

Reduced weight loss

Reduced viral load

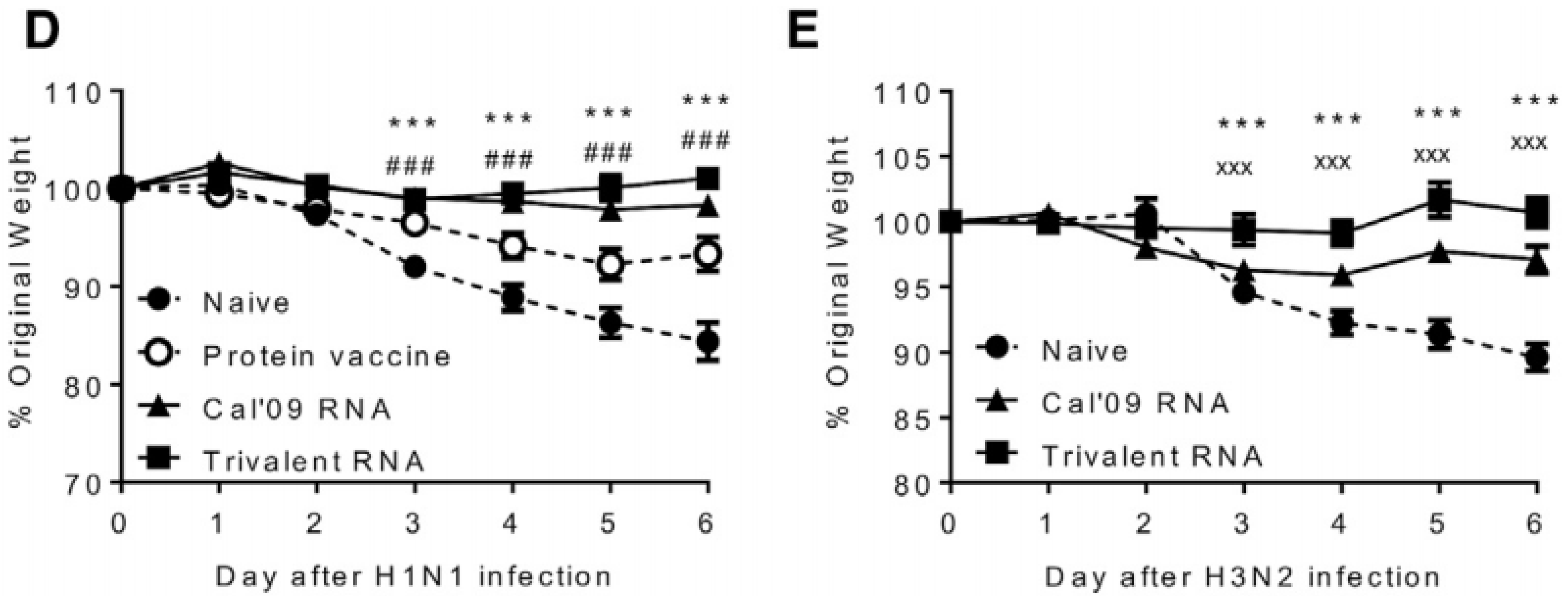
Protection against 3 different strains of influenza from sa-RNA expressing antigen

Trivalent RNA Vaccine

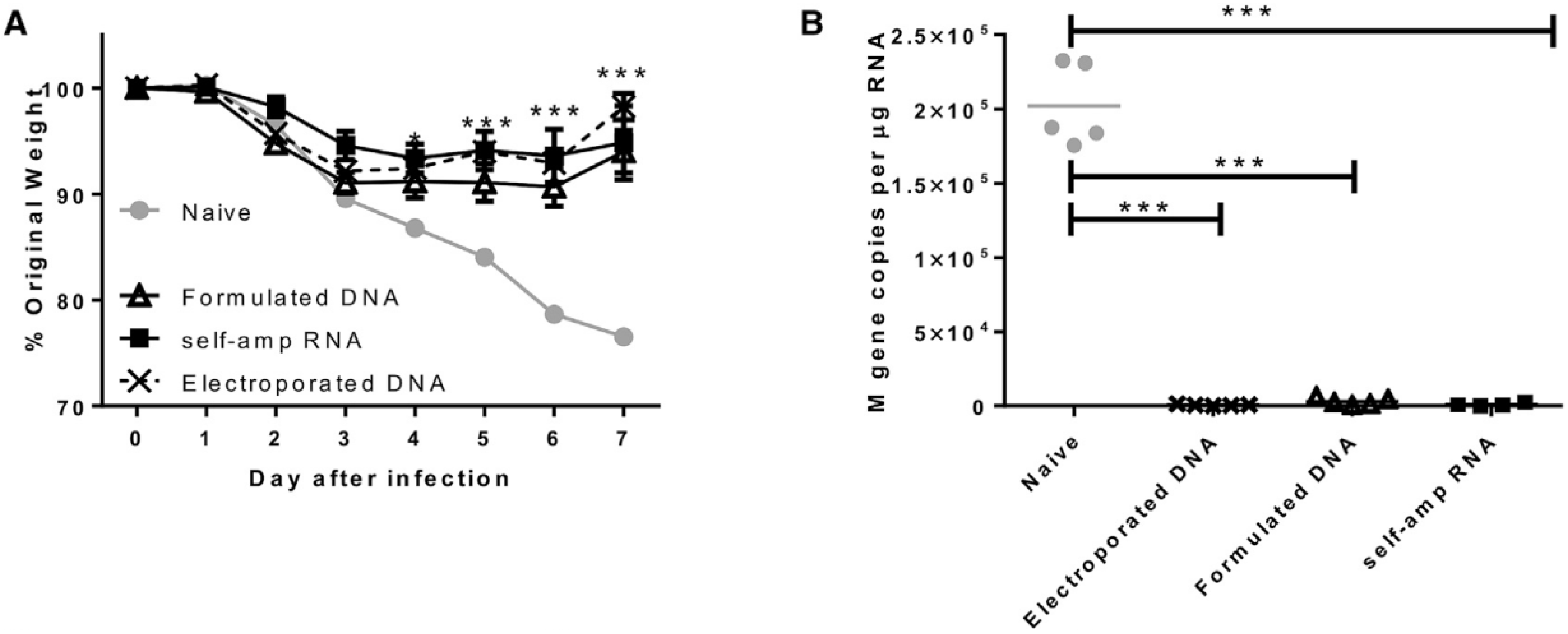


A/Califonia/07/2009 (H1N1) ✓
A/Hong Kong/1/68 (X31, H3N2) ✓
B/Massachusetts/2/2012

Trivalent RNA Vaccine



“single shot” immunity?



A single shot of sa-RNA or DNA encoding HA protects against H1N1 influenza disease, affording protection against weight loss and a significant reduction in viral load.

Current challenges in RNA vaccine design

- Increase RNA stability
  - Nucleoside modifications
- Protein production
  - Self-amplifying RNA
- Improve delivery
  - Lipidic, polymeric nanoparticle delivery

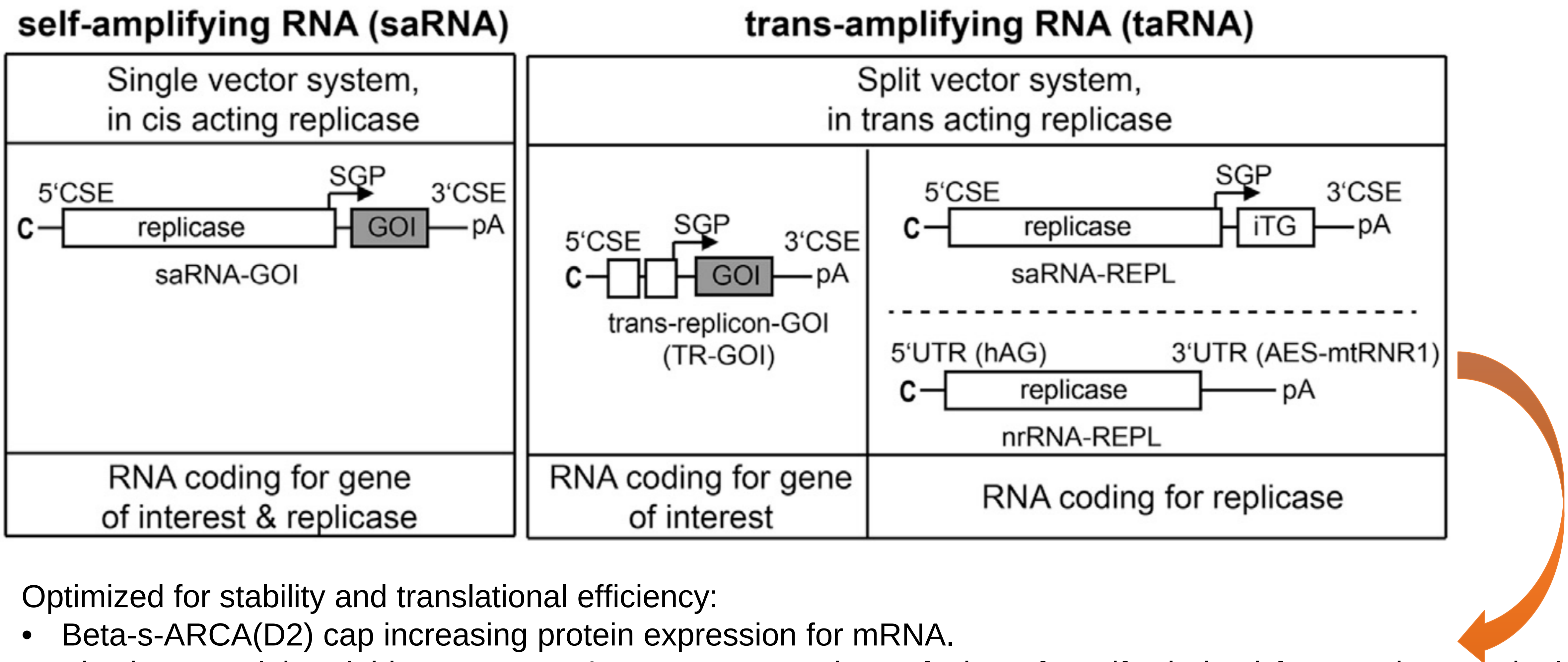
Zhou X, PNAS, 1995;92:3009-13

Molecular Therapy
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

A Trans-amplifying RNA Vaccine Strategy for Induction of Potent Protective Immunity

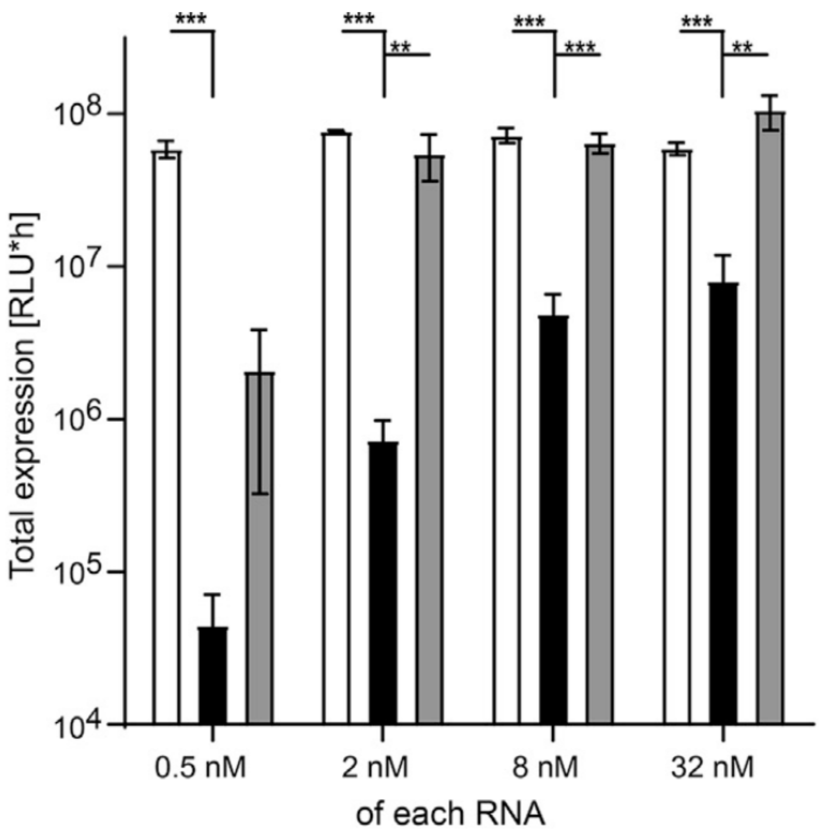
Tim Beissert,[1,4] Mario Perkovic,[1,4] Annette Vogel,[2] Stephanie Erbar,[2] Kerstin C. Walzer,[2] Tina Hempel,[1] Silke Brill,[1] Erik Haefner,[3] René Becker,[1] Özlem Türeci,[2] and Ugur Sahin[1,2,3]



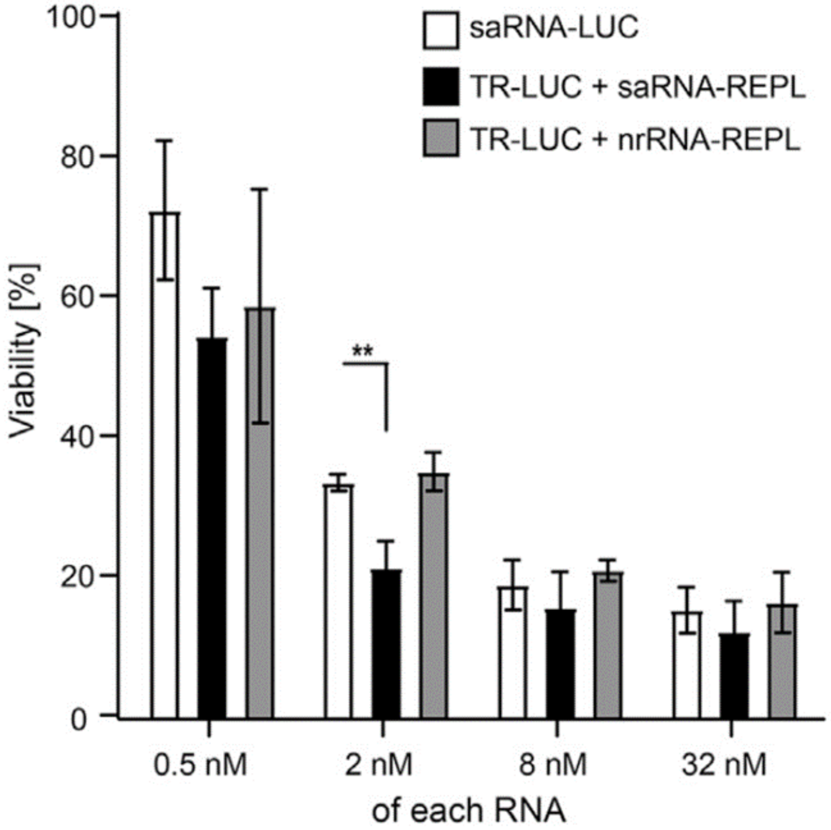
Optimized for stability and translational efficiency:

- Beta-s-ARCA(D2) cap increasing protein expression for mRNA.
- The human alpha-globin 5' UTR, a 3' UTR representing a fusion of motifs derived from amino-terminal enhancer of split (AES) mRNA and mitochondrially encoded 12S rRNA (mtRNR1).
- An unmasked poly(A) tail.

Expression levels of luciferase(reporter) by three different RNA vectors

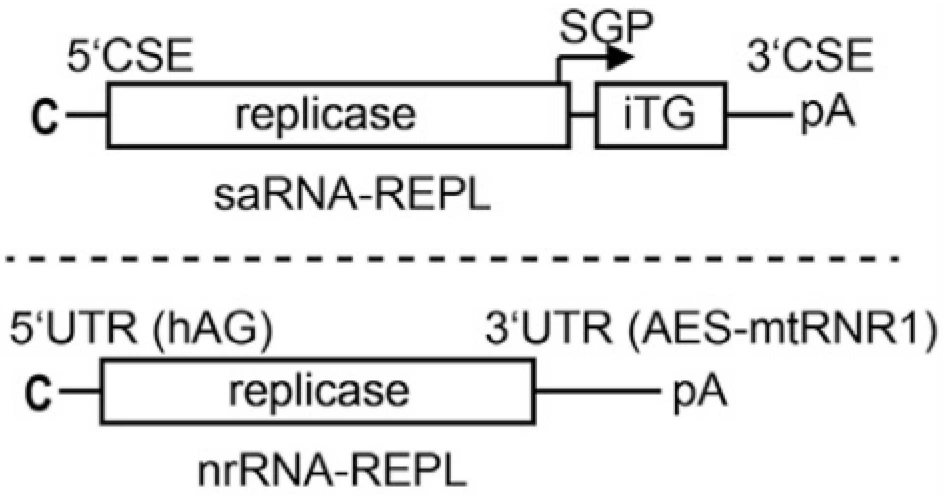


Expression levels achieved by taRNA driven by nrRNA-REPL were comparable to those of the saRNA single vectorsystem.
In contrast, expression levels achieved by taRNA in conjunction with saRNA-REPL did not reach this



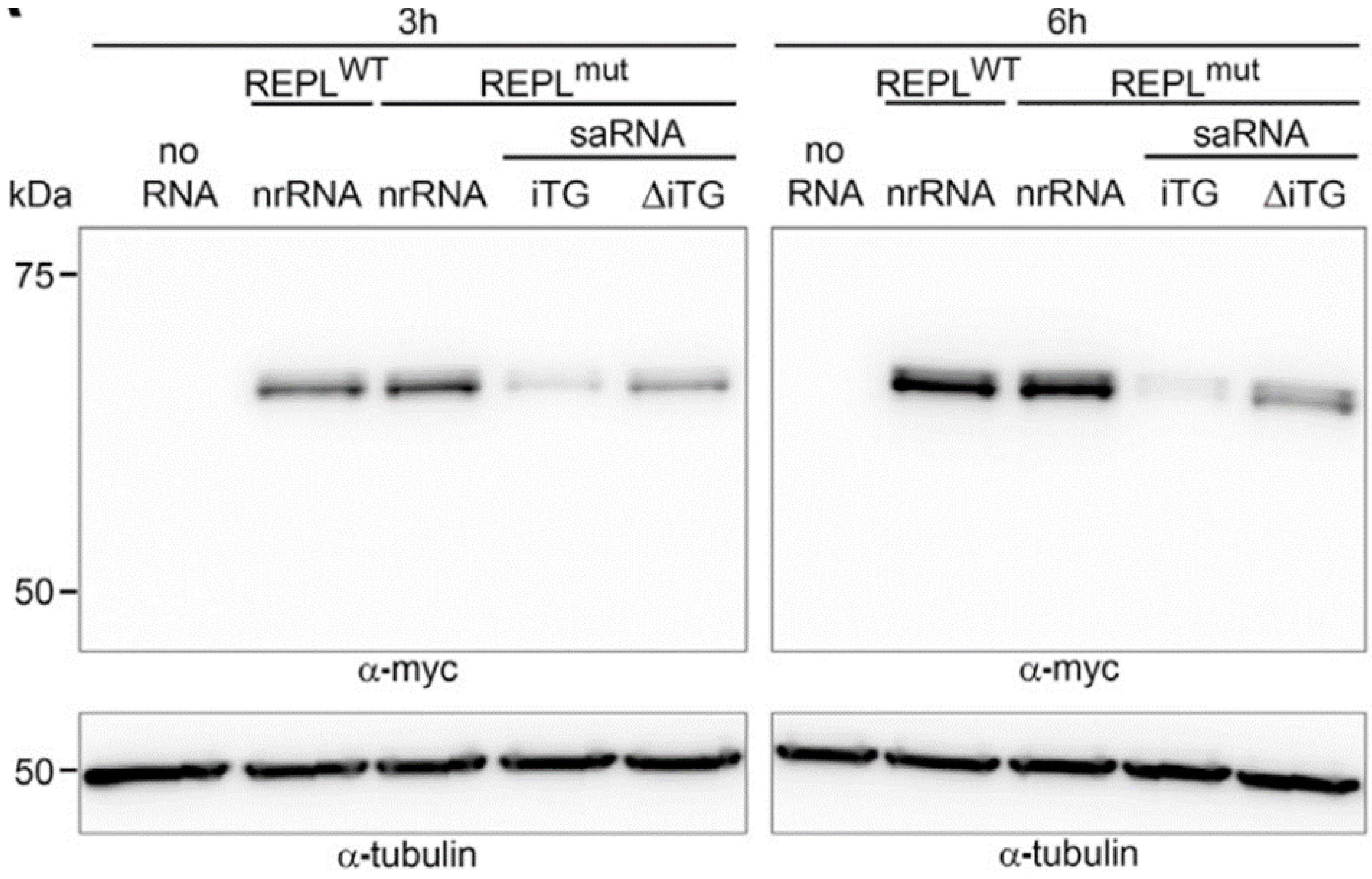
All three systems resulted in reduction of cell viability starting at 24 h after electroporation

Why nrRNA is superior to saRNA in complementing the taRNA split-vector system?

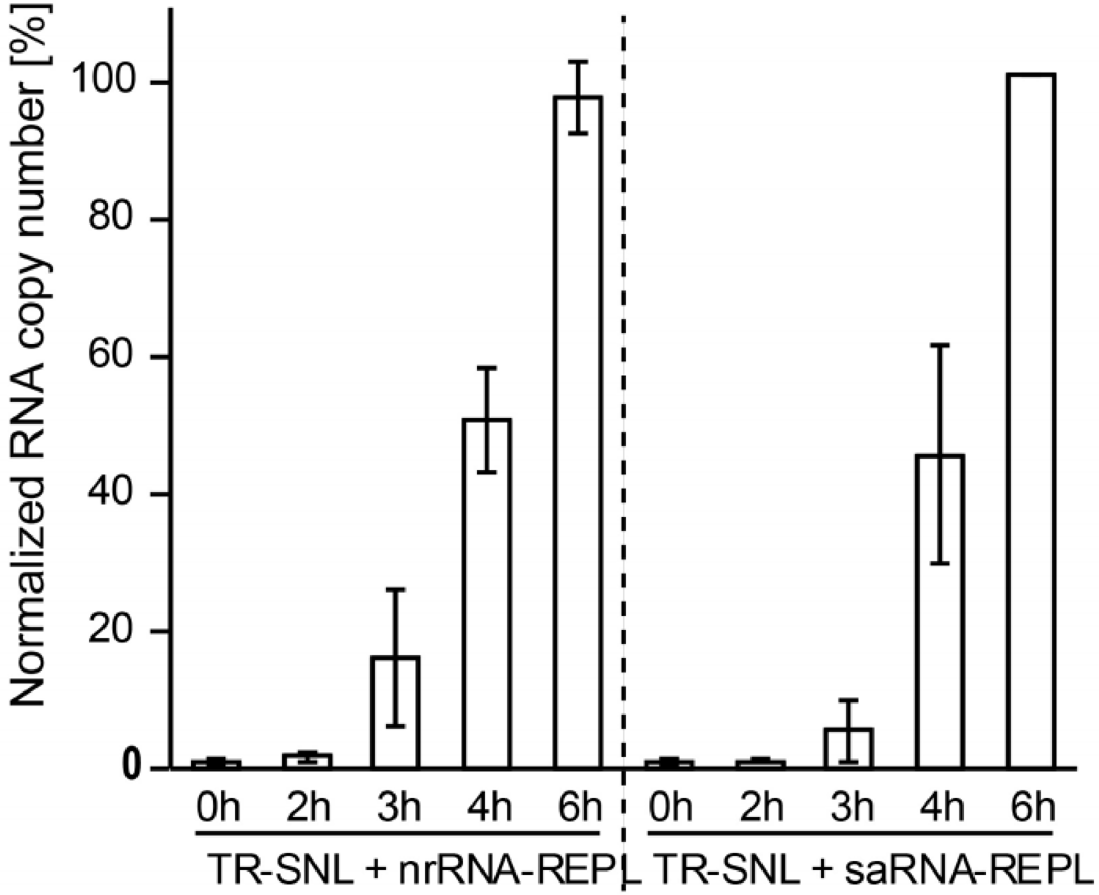


To investigate whether the translation efficiency of the replicase ORF depends on the vector backbone, they introduced 2 essential controls:

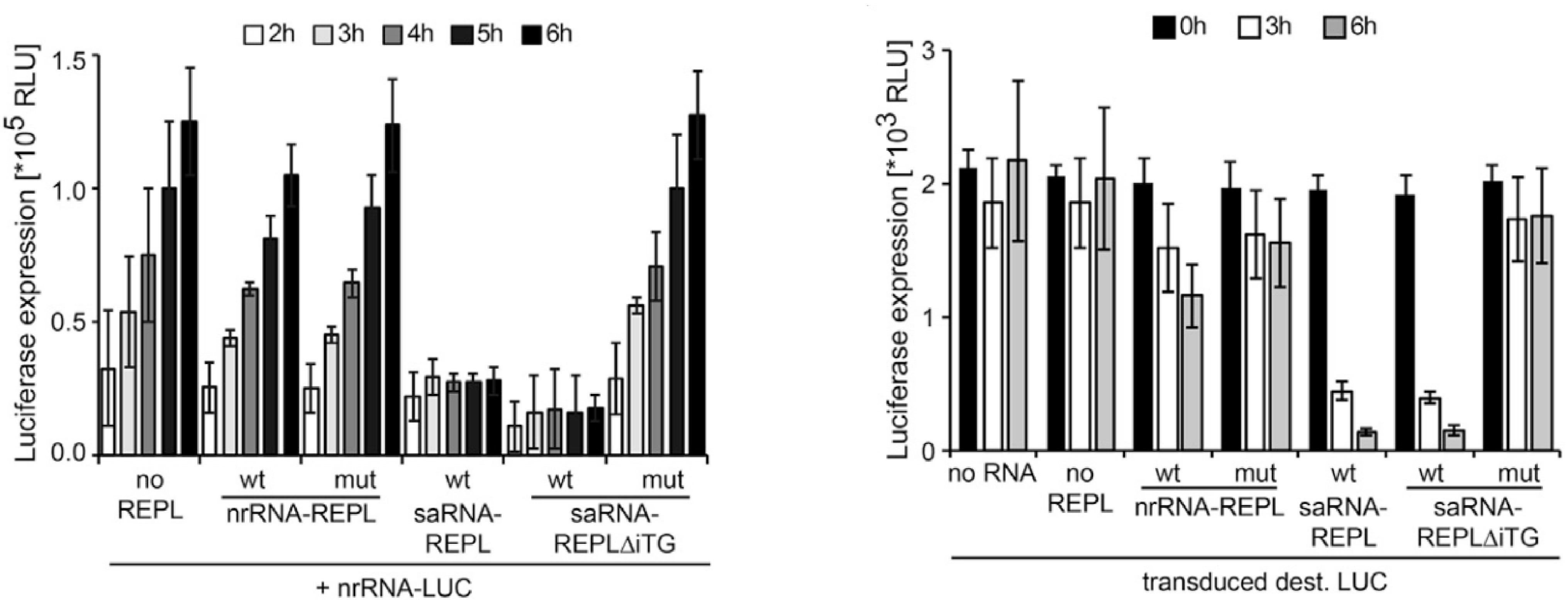
- One control entailed quantifying replicase expression in transfected cells in a model without RNA replication; they used a replicase mutant (mut-REPL), which is deficient in polymerase activity. This enabled the analysis of replicase translation from exclusively in vitro transcribed and transfected RNA molecules and neutralized de novo saRNA synthesis as a confounding factor.
- A saRNA variant with a mutant SGP and full deletion of the transgene ORF (saRNA-REPL ΔiTG) to control for the possibility that the large “unused” second ORF (iTG) downstream of the SGP in saRNA-REPL may impair expression from this construct.



- The amount of replicase protein generated in cells transfected with nrRNA-REPL was the same for wild-type (WT)-and mut-REPL, indicating that the mutation did not affect protein stability.
- Expression of mutant replicase was higher with saRNA lacking the iTG as compared to saRNA encoding an iTG, indicating that nonsense-mediated mRNA decay would affect replicase levels.

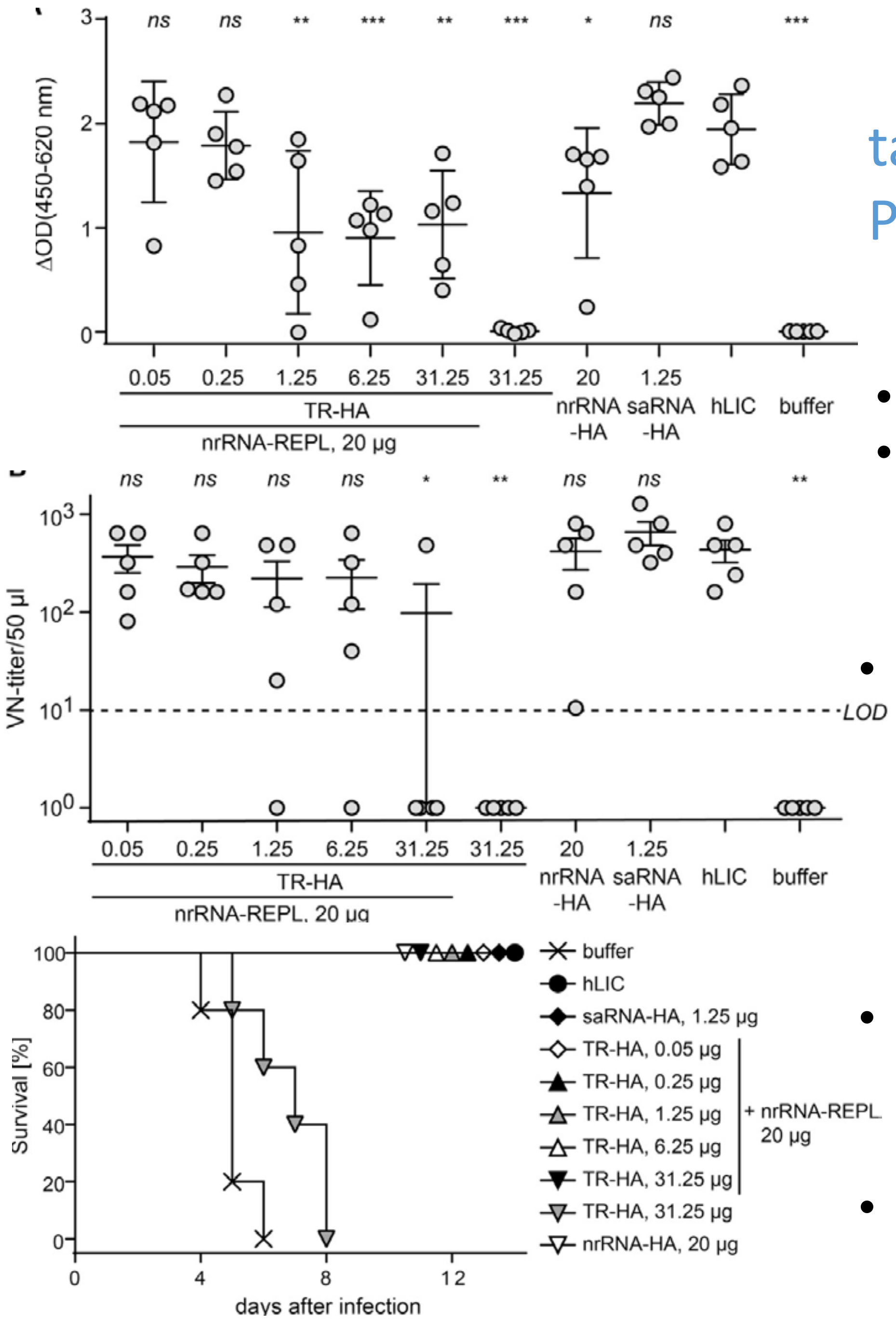


No differences at PCR results → Translational level difference?



- Assess the expression of a co-transfected nrRNA coding for luciferase (nrRNA-LUC) in the presence of either the saRNA or the taRNA split-vector systems.
- Generate a stably transduced BHK-21 cell line expressing destabilized luciferase (Luc2CP) and measured Luc2CP levels in response to saRNA or taRNA transfection.
- The translation of co-transfected nrRNA-LUC was unaffected by taRNA in conjunction with nrRNA-REPL but strongly inhibited when cotransfected with saRNA-REPL or saRNA-REPL-ΔiTG.
- The use of both saRNA versions with WT replicase reduced promoter-driven expression of Luc2CP within 3 h and at a much greater extent than taRNA replication driven by nrRNA-REPL.

These data suggested that saRNA replication rather than TR-replication impaired cellular translation.



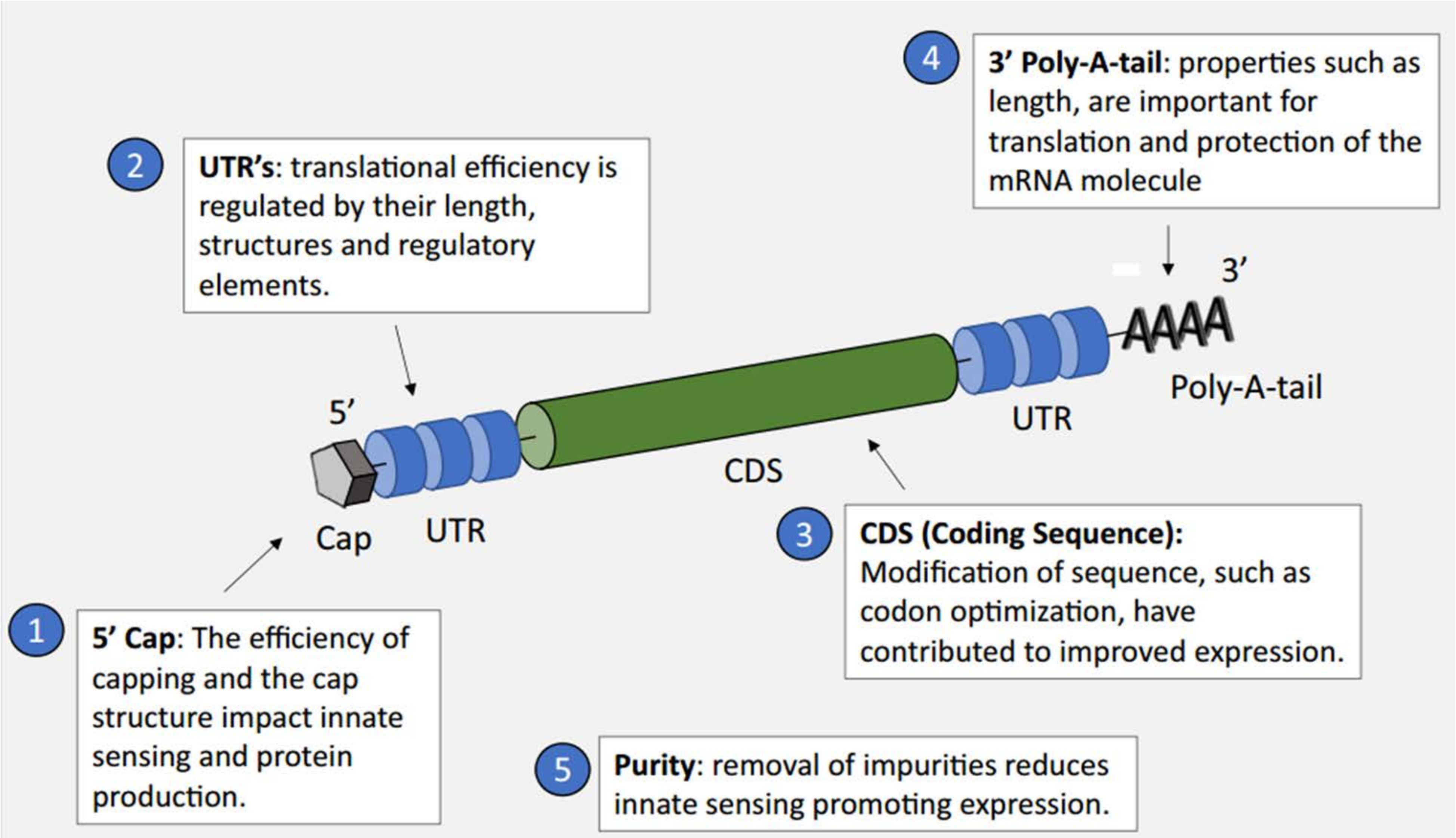
taRNA Profoundly Reduces the Doses Required for Protective Immune Responses

- Immunize mice intradermally with the taRNA split-vector system,
- dose range of 0.05–31.25 mg combined with 20 mg nrRNA-REPL.

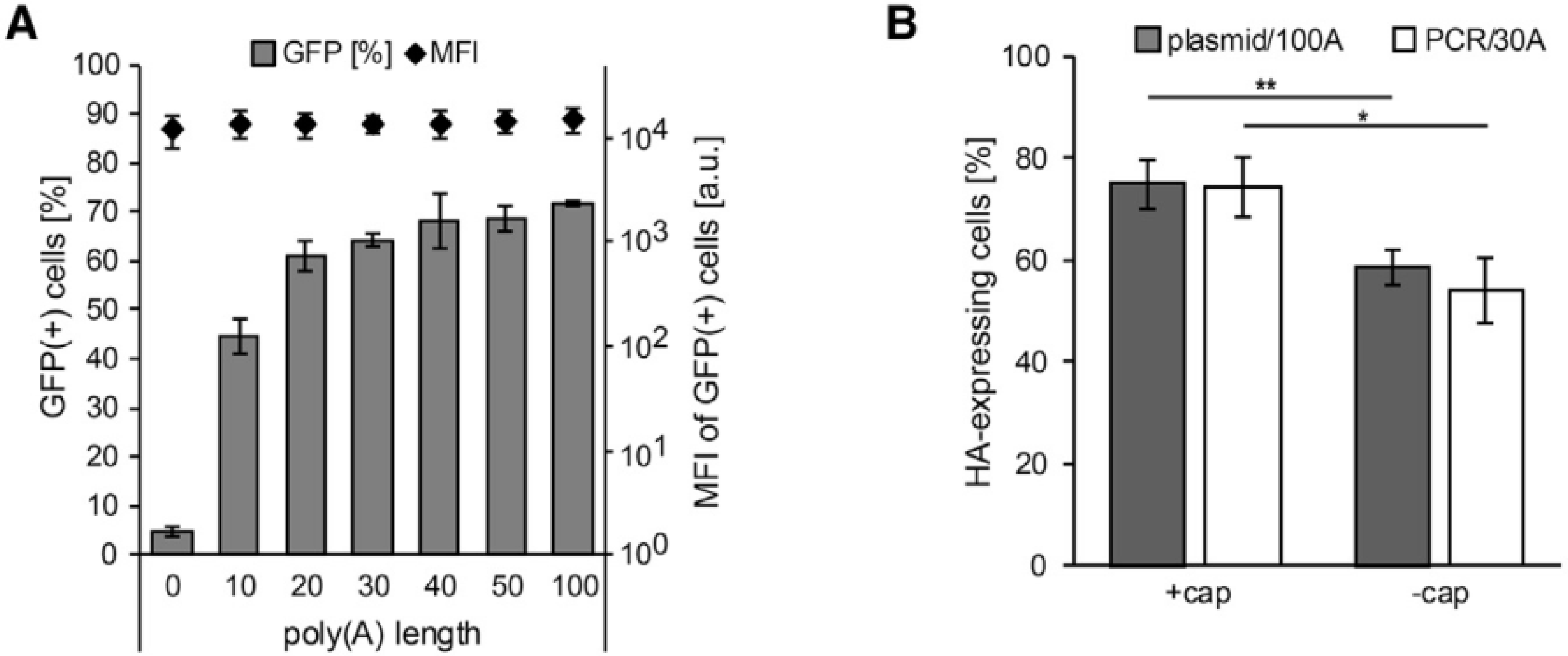
- All groups immunized with taRNA developed HA-specific antibody responses. The two lowest doses of TR-HA (50 and 250 ng) were most effective and did not significantly differ from intramuscularly administered human licensed vaccine.

- TR RNA without nrRNA-REPL did not yield an antibody response.

- In all taRNA-immunized groups, VN antibodies were detected and mice survived influenza virus challenge with minimal loss of body weight and no signs of illness.


4
3' Poly-A-tail: properties such as length, are important for translation and protection of the mRNA molecule
2
UTR's: translational efficiency is regulated by their length, structures and regulatory elements.
3'
AAAA
Poly-A-tail
UTR
5'
CDS
Cap
UTR
3
CDS (Coding Sequence): Modification of sequence, such as codon optimization, have contributed to improved expression.
1
5' Cap: The efficiency of capping and the cap structure impact innate sensing and protein production.
5
Purity: removal of impurities reduces innate sensing promoting expression.

Simplify trans-replicon without Compromising the Immunogenicity of the taRNA Split-Vector Vaccine



Summary

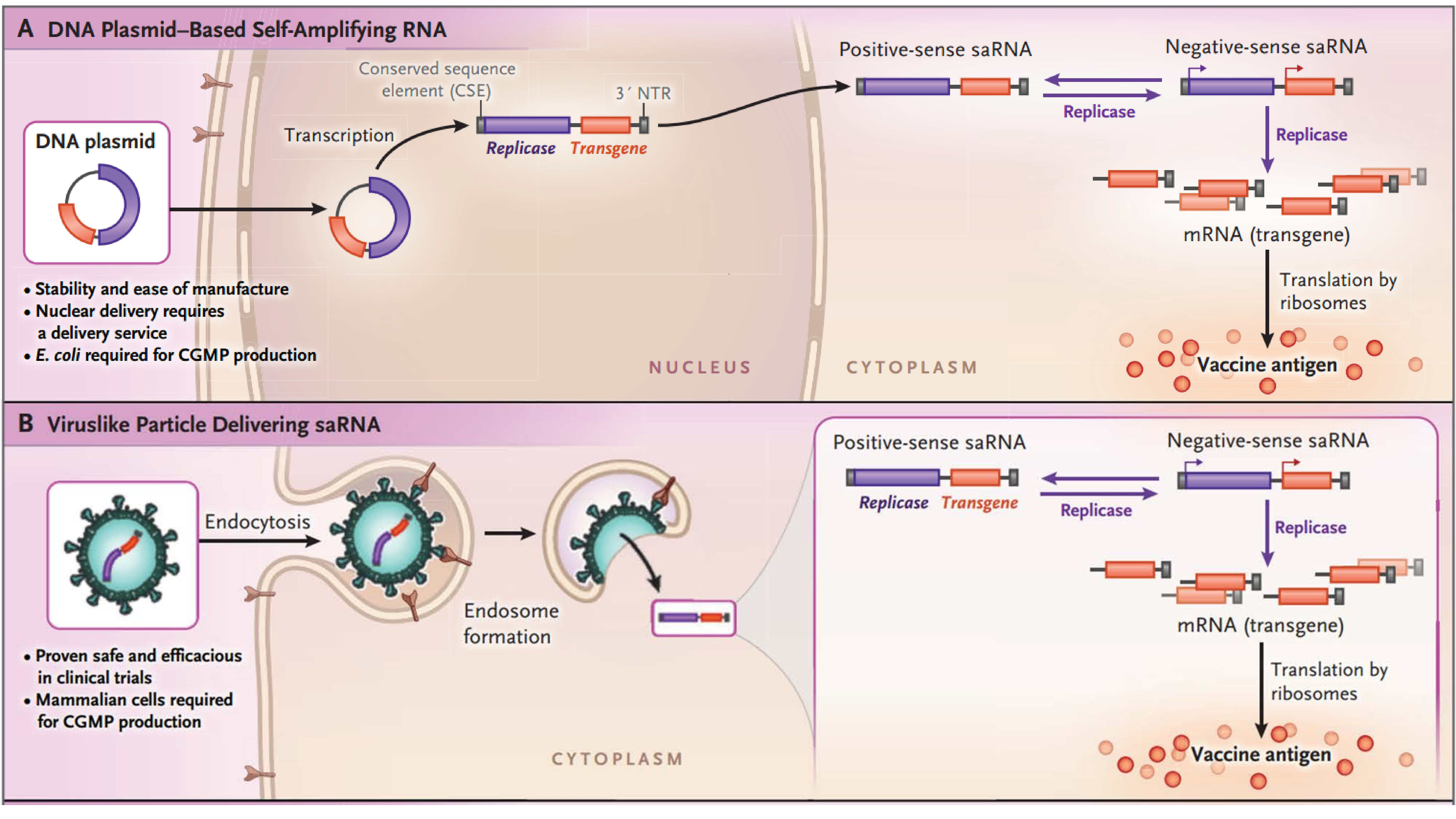
- **Split-vector system**
- safety advantage
  - Avoid the risk incurred with sa-RNA that are engineered to express budding-competent viral glycoproteins which would transfer into new host cells.
- Versatility and efficiency advantage
  - Uncoupled antigen and replicase sequence could be optimized independently.
- Easy, time and cost efficient manufacturability advantage
  - Dose efficiency, shorter RNA sequence to produce.
  - Omitting in vitro capping and shortening poly(A) tails of the TR.
  - Invariable component could be pre-produced at large scale and stored.

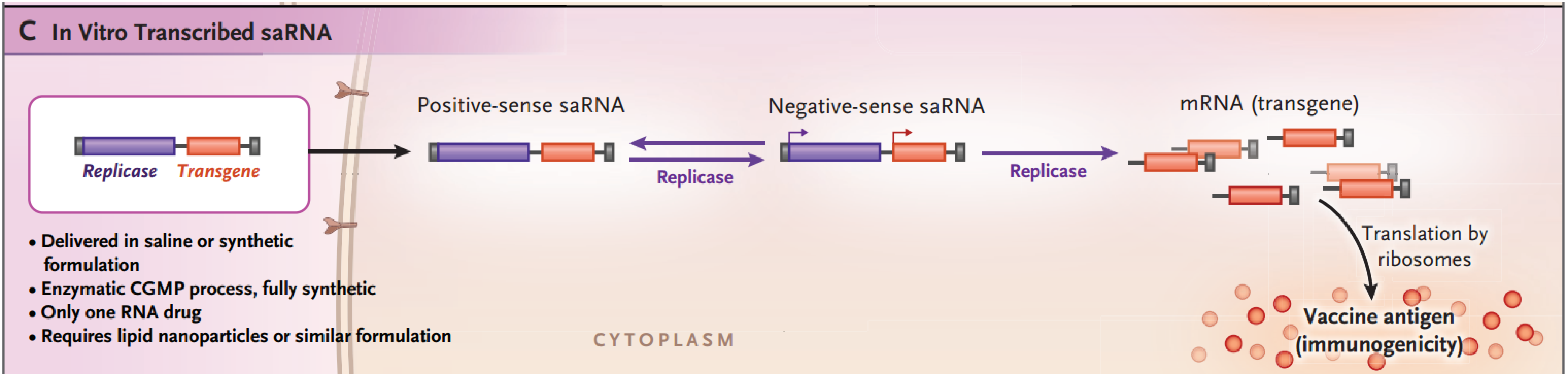
Summary

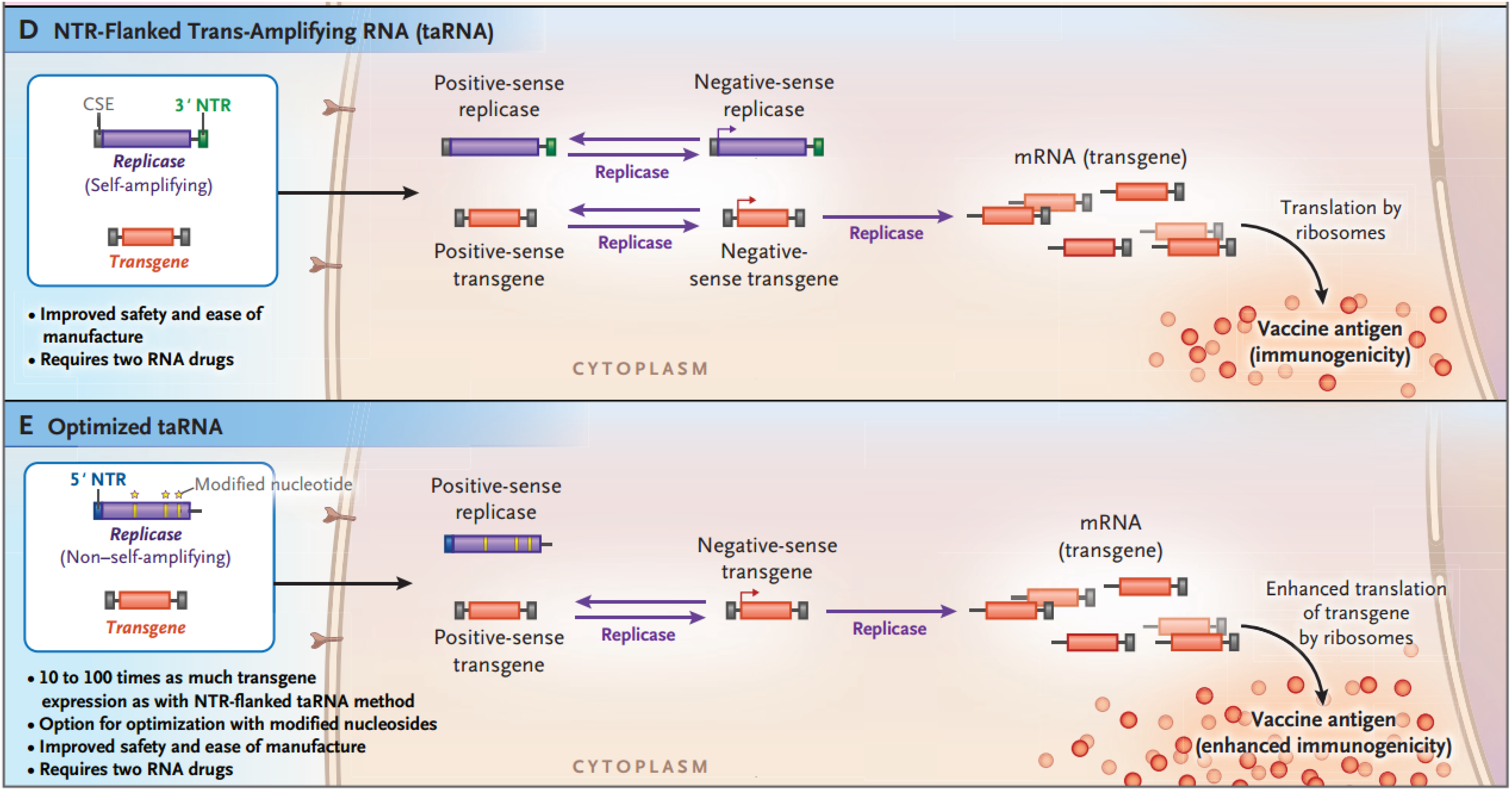
- **Split-vector system**
- Potential drawbacks:
  - The requirement to manufacture two RNA drugs.
  - The complexity for efficiently in vivo delivery of both components into the same cell.

Summary

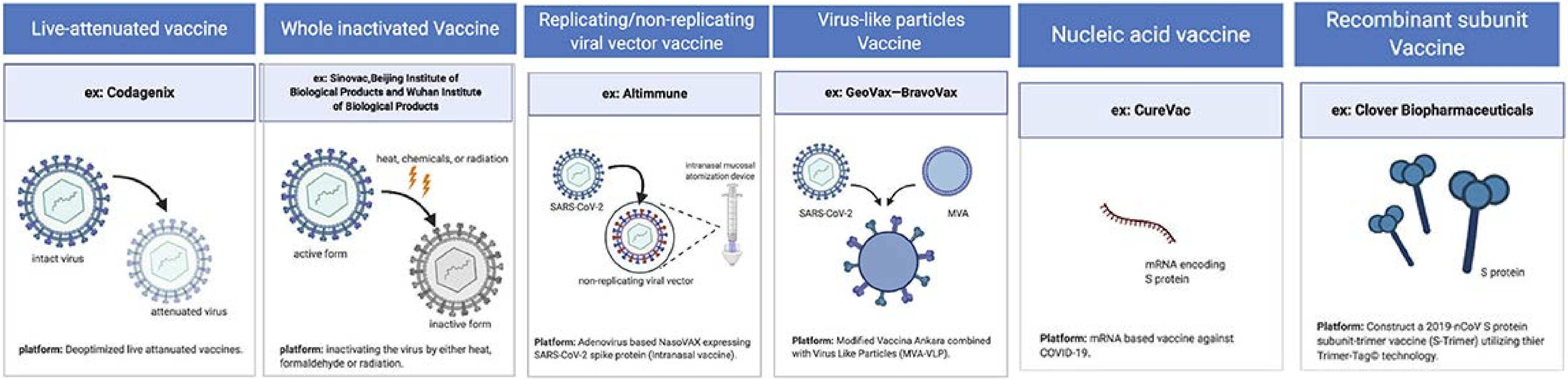
- **Further improvements** based on new mRNA technology for the current approach:
  - Nucleoside modifications
  - Stabilizing sequences
  - Codon optimization of the entire replicon gene


A DNA Plasmid–Based Self-Amplifying RNA
DNA plasmid
Transcription
Conserved sequence element (CSE)
3′ NTR
Replicase
Transgene
Positive-sense saRNA
Negative-sense saRNA
Replicase
Replicase
mRNA (transgene)
Translation by ribosomes
Vaccine antigen
• Stability and ease of manufacture
• Nuclear delivery requires a delivery service
• *E. coli* required for CGMP production
NUCLEUS
CYTOPLASM
B Viruslike Particle Delivering saRNA
Endocytosis
Endosome formation
Positive-sense saRNA
Negative-sense saRNA
Replicase
Transgene
Replicase
Replicase
mRNA (transgene)
Translation by ribosomes
Vaccine antigen
• Proven safe and efficacious in clinical trials
• Mammalian cells required for CGMP production
CYTOPLASM


C In Vitro Transcribed saRNA
Replicase
Transgene
• Delivered in saline or synthetic formulation
• Enzymatic CGMP process, fully synthetic
• Only one RNA drug
• Requires lipid nanoparticles or similar formulation
Positive-sense saRNA
Replicase
Negative-sense saRNA
Replicase
mRNA (transgene)
Translation by ribosomes
Vaccine antigen (immunogenicity)
CYTOPLASM


D NTR-Flanked Trans-Amplifying RNA (taRNA)
CSE
3′ NTR
Replicase
(Self-amplifying)
Transgene
• Improved safety and ease of manufacture
• Requires two RNA drugs
Positive-sense replicase
Negative-sense replicase
Replicase
Positive-sense transgene
Replicase
Negative-sense transgene
Replicase
mRNA (transgene)
Translation by ribosomes
Vaccine antigen (immunogenicity)
CYTOPLASM
E Optimized taRNA
5′ NTR
Modified nucleotide
Replicase
(Non–self-amplifying)
Transgene
• 10 to 100 times as much transgene expression as with NTR-flanked taRNA method
• Option for optimization with modified nucleosides
• Improved safety and ease of manufacture
• Requires two RNA drugs
Positive-sense replicase
Negative-sense transgene
Positive-sense transgene
Replicase
Replicase
mRNA (transgene)
Enhanced translation of transgene by ribosomes
Vaccine antigen (enhanced immunogenicity)
CYTOPLASM

Vaccine platforms for the COVID-19



Alturki Sana O et al Front. Immunol., 19 August 2020

Thank you for your attention!