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SaRNA Vaccines and Their Controlled and Self-Limiting Mechanism

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Abstract

SaRNA (self-amplifying RNA) vaccines are created with the dual purpose of reducing the amount of genetic material inoculated with the vaccination and simultaneously generating large amounts of viral antigens to achieve a strong immune system response. The platform of these vaccines is based on the genome of a specific, suitably modified alphavirus: the Venezuelan equine encephalitis virus VEEV, consisting of a positive single-stranded RNA of about 11 Kbases. It is organized into two open reading frames (ORFs); the first encodes for 4 non-structural proteins that constitute an RNA-dependent RNA polymerase (replicase), the second encodes for the virus' structural proteins, which make-up the capsid and envelope glycoproteins. Once assembled, the viral replicase is able to replicate the entire genome (called replicon, *i.e.* a sequence of genetic material able to replicate itself) in thousands of copies and the researchers thought to exploit this feature by modifying the genome of appropriate strains of VEEV preserving the non-structural replicase genes and by replacing the structural proteins genes with the sequence of a protein of a specific virus, called gene of interest (GOI), capable of behaving like an antigen and triggering a strong immune system response. The self-amplification of this structure within the host cell results in the synthesis of very large amounts of reference antigen, much higher than those synthesized by current mRNA vaccines, thus allowing the inoculation of smaller amounts of encapsulated genetic material carried in lipid nanoparticles (LNPs). In view of the current spread of SARS-CoV-2, the first saRNA vaccine produced and approved worldwide involved the insertion of the RNA sequence encoding for the spike protein as a GOI within the replicon. However, concerns have been raised in the international scientific world regarding the self-amplification mechanism of saRNA vaccines: the aim of this paper is to demonstrate that the self-replication mechanism of the saRNA vaccines is controlled and self-limiting.

Keywords

SaRNA Vaccines, Replication Mechanism, Controlled and Limited, Spike (GOI)

1. Introduction

The world's first approved saRNA vaccine is ARCT-154 (Zapomeran) [1]-[3]. Approved in Japan in November 2023 had later a positive evaluation by the CHMP (Committee for Medicinal Products for Human Use) of the EMA (European Medicines Agency), the vaccine received marketing authorization in December 2024. Subsequently, in February 2025, it was approved for use in adults in the European Union as a vaccine for preventing SARS-CoV-2-caused COVID-19. Compared to previous mRNA vaccines, Kostaive is proposed as a vaccine that requires a smaller amount of genetic material to be inoculated (at least 5 to 50 times less) while producing a much higher antigen yield and eliciting a strong immune response, due to its self-replicating abilities once it enters host cells. Considering that wild-type alphaviruses have cytopathic effects in mammalian cells [4], saRNA vectors are derived from non-cytopathic variants with attenuated properties; the most clinically advanced saRNA vector is based on the attenuated TC83 strain of the VEEV Trinidad Donkey variant [5]. Within the Kostaive vaccine replicon sequence, the VEEV protein-coding sequence has been replaced with the SARS-CoV-2 Spike protein sequence. The mechanism of auto-amplification of saRNA vaccines has raised some concerns in the academic community, particularly regarding the manner in which this auto-replication occurs.

2. Structure of an mRNA: Role of the Cap and Polyadenylated Tail

Each RNA molecule has a specific structure called a Cap at its 5' end. This Cap consists of a guanosine molecule, shown in orange in the figures, which is linked via a triphosphate group to the first nucleotide of the RNA sequence. There are three types of Caps, differentiated by the location of methyl groups. Cap 0 has a methyl group in position 7 of the guanosine, which is called 7-methylguanosine (7mG), and a triphosphate group (7mGppp). Cap 1 has an additional methyl group in position O-2 of the first nucleotide of the sequence. Finally, Cap 2 has a third methyl group in position O-2 of the second nucleotide of the RNA sequence. Cap 0 is not abundant in higher eukaryotes and triggers an innate immune response by binding to cytosolic receptors. Cap 1 is present in all mRNAs of higher eukaryotes (including humans) and promotes translation of RNA into proteins on ribosomes. Cap 2 is found in fewer than 50% of eukaryotic mRNA, and its function is unclear (**Figure 1**).

Understanding the difference in function between caps is essential to explain the controlled and self-limiting cycle of saRNA vaccines. The RNA of a saRNA

vaccines requires a specific 5' cap structure for better stability, better translation, and to evade detection by the host's IFN-induced proteins IFIT1 and IFIT5 (interferon-induced proteins with tetratricopeptide repeats 1 and 5, respectively) that can bind to the 5'-triphosphate end of single-stranded RNA (ssRNA, such as that of alphaviruses) to inhibit RNA translation and prevent viral replication. The Cap at the 5' end of the saRNA vaccine sequence is a special, patented Cap: it is the CleanCap cap 1 AU technology, which cleverly incorporates the 5' AU dinucleotide necessary for efficient alphavirus replication [6].

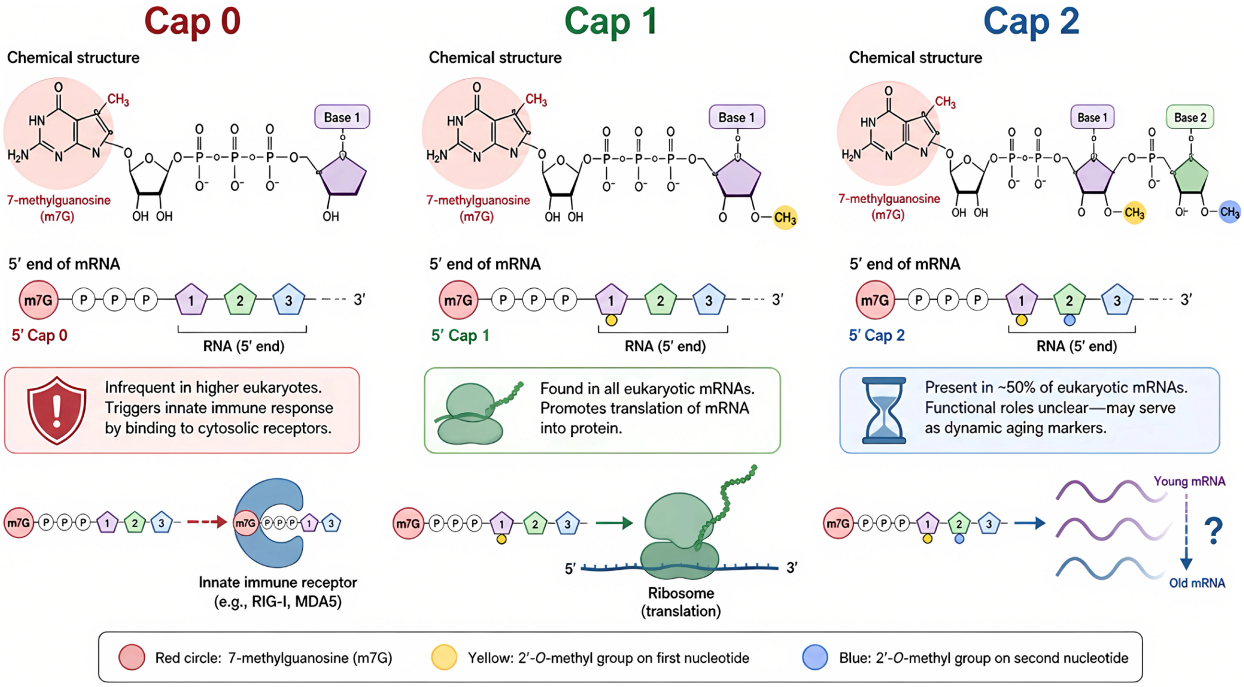
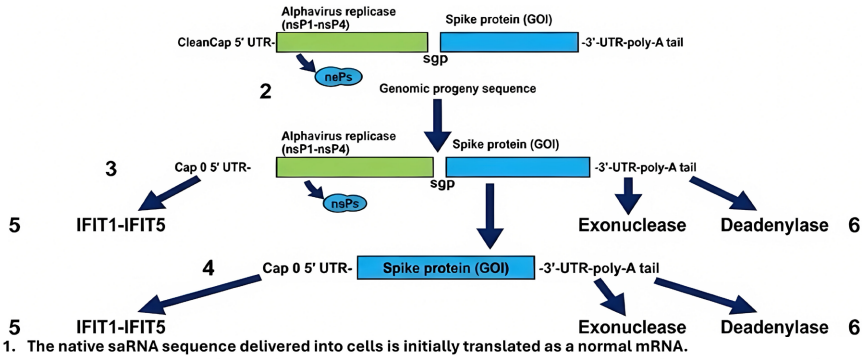


Figure 1. Differences between the various types of Cap.

CleanCap is essentially a modified Cap 1 designed to increase the advantages outlined in priority: significant boost to RNA translation, extremely high stability, and, above all, effective evasion of the innate cellular immune system, because a Cap 1 is interpreted as self [7]. Like all mRNA, that of saRNA vaccines also has regions called untranslated regions (UTRs) at both the 5' and 3' ends, and a polyadenylated tail, *i.e.*, a series of adenines (about 200 in humans), present at the 3' end of the mRNA (Poly-A) which plays a fundamental role in mRNA stability and translation. In the cytoplasm, the poly(A) tail interacts with the poly(A)-binding protein (PABPC), which promotes translation by the 80S ribosome and protects mRNA from degradation. The poly(A) tail also influences mRNA stability: as the tail is gradually shortened by deadenylases such as Carbon Catabolite Repression 4-Negative on TATA-less (Ccr4-Not) and poli-(A) nuclease (Pan2-Pan3), mRNA becomes more susceptible to degradation by exonucleases [8] (**Figure 2**). This shortening of the poly(A) tail is a key step in the regulation of mRNA decay, and the rate of deadenylation is influenced by various factors, including the sequence and structure of the RNA it-

self. When an mRNA is devoid of a poly(A) tail, it is more susceptible to degradation by 3' - 5' exonucleases and exhibits reduced translational efficiency, as ribosomes are less likely to recognize and bind to such molecules.



1. **The native saRNA sequence delivered into cells is initially translated as a normal mRNA.**
2. **Subsequently, once the viral replicase (** nsP1-nsP4) **has assembled, new RNA (**genomic progeny sequence) **is transcribed;** (we have omitted from this diagram the initial replication of a 3'-5' reverse strand of RNA that will be used as a template);
3. **The newly formed RNA is provided with a Cap 0 by the nsP1 subunit of the viral replicase; the newly formed GOI also has a Cap 0;**
4. **The viral replicase will mainly use the sub-genomic promoter** (sgp) **to transcribe many copies of the GOI** (spike) **and simultaneously replicate the entire replicon;**
5. **Cap 0 triggers host cellular responses, and IFIT1 and IFIT5 proteins bind to Cap 0, blocking translation;**
6. **Deadenylases degrade the poly-(A) tail: RNA without a poly-(A) tail is not further translated and is exposed to the action of 3'-5' exonucleases.**

Figure 2. Schematic diagram illustrating the self-limiting replication cycle of saRNA vaccines mediated by cap 0–Induced innate immune recognition and cellular RNA degradation pathways.

3. Role of the Replicon of saRNA Vaccines

The alphavirus replicon (as the saRNA vaccine sequence is identified) consists of 2 open reading frames (ORFs): the first, longer one, starting at the 5' end of the sequence, encodes for the 4 non- structural subunits that make-up the viral replicase, nsP1-nsP4; the second shorter one, preceded by an efficient and specially designed promoter, encodes for the GOI, in the case of the first approved saRNA vaccine, the Spike protein of SARS-CoV-2. Upon delivery into the cytosol, the saRNA uses the replication mechanism of the alphavirus to generate multiple copies of the complete genomic transcript and sub-genomic mRNA. In detail, when the saRNA vaccine sequence enters the cell, the sequence behaves like any mRNA: it arrives at the ribosomes and is translated. The replicase subunits (nsP1-nsP4) are first translated as a non-structural polyprotein and subsequently processed into functional proteins, which then assemble into the actual three-dimensional structure. (Each subunit has its own specific function: viral replicase works a bit like a Swiss army knife). Next, replicase transcribes the positive strand into a negative strand using sequence elements in the 3' untranslated region (UTR); this step is crucial because the 3' - 5' negative strand functions as a template. In fact, using the negative strand and the promoter sequences present in the 5' UTR and the nsP1 region, replicase synthesizes a new genomic positive strand. Furthermore, the replicase uses the subgenomic promoter to generate more subgenomic plus-strand GOI transcripts (the subgenomic promoter allows the viral replicase to initiate transcription from an internal point in the strand), and thus more subge-

nomic RNA (Spike mRNA) is generated than genomic RNA. Higher levels of sub-genomic mRNA encoding GOI result in increased protein translation and thus of spike protein. Upon delivery into the cytosol, the saRNA then utilizes the replication mechanism of the alphavirus to generate more copies of the complete genomic transcript and sub-genomic mRNA (**Figure 3**). As a consequence of the self-replicating capacity of the saRNA vaccine, the saRNA sequence carried in cells with the engineered and patented Cap 1 Clean Cap structure in nascent transcripts is replicated with a Cap 0 structure, since the capping of the newly synthesized genomic and sub-genomic RNA is assembled by the nsP1 subunit of the alphavirus replicase, which provides a Cap 0 to the newly formed RNAs [9]; but Cap 0 is recognized as non-self by RIG-I [10]. RIG-1 recognizes both Cap 0 structures and short dsRNA filaments with 5'-triphosphate ends and initiates an immune signalling cascade by binding to the MAVS protein on the mitochondria, thereby inducing the expression of type I interferons; type I interferons, such as IFN-alpha and IFN-beta, induce, via the JAK-STAT signalling pathway, the transcription of genes encoding human proteins with 1 - 5 tetratricopeptide repeats (IFIT1 and IFIT5, respectively) [11]. The IFIT1 protein possesses a specific, extended hydrophobic pocket that can accommodate Cap 0; by binding to it, it blocks its translation [12] [13]. The IFIT5 protein cannot bind to Cap 0 but is exclusively responsible for sequestering RNA with a 5'-triphosphate end in order to activate the innate immune response. Both can inhibit the translation of viral proteins by rendering the template RNA unavailable and by competing directly with the eukaryotic initiation factor 4F (eIF4F) complex of the Cap-binding initiation factor.

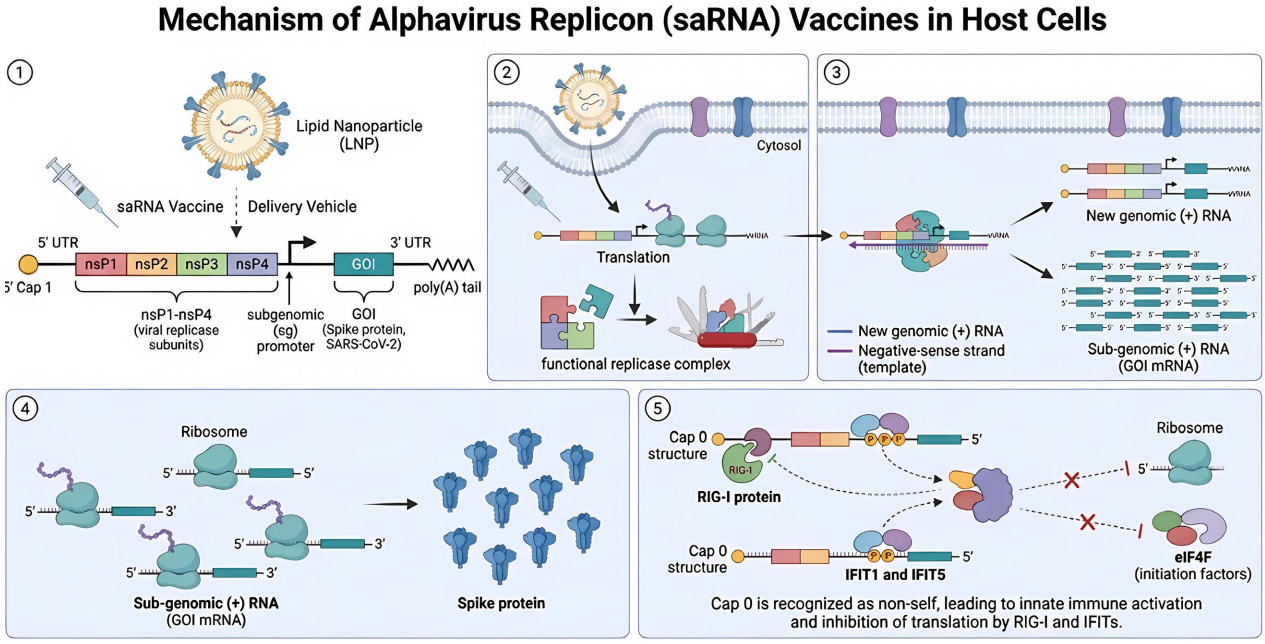


Figure 3. Self-amplifying RNA (saRNA) vaccines leverage the alphavirus replicon to drive robust expression of the protein antigen (Spike) while also activating innate immunity via Cap 0 RNA sensing, contributing to both strong translation and immune surveillance.

4. Genomic and Sub-Genomic Copies of the saRNA Vaccines Are Recognized as Non-Self

An RNA with Cap 0 in a eukaryotic cell acts as an immunostimulant [14] [15]; both single-stranded (ssRNA, such as that of alphaviruses) and double-stranded (dsRNA) RNA with Cap 0 can act as a pathogen-associated molecular pattern (PAMP), in short, a danger signal. This means that the immune system recognizes specific RNA structures as indicators of foreign or damaged material; recognition of these RNAs triggers innates immune responses, including the production of type I interferons and pro-inflammatory cytokines [16]. There are specific receptors for the recognition of foreign RNA, such as Toll-like receptors (TLRs), TLR3, TLR7 and TLR8 [17] which are endosomal sensor; RIG-I-like receptors (RLR), Retinoic acid-inducible gene I (RIG-1), Melanoma differentiation-associated gene 5 (MDA5) and Laboratory of Genetics and Physiology 2 (LGP2) are cytosolic receptors. During saRNA replication, TLR3 binds to the dsRNA (double-stranded RNA) that forms as a replicative intermediate of the saRNA replicon transported or re-internalized into endosomes [18]; TLR7 and TLR8 bind to the ssRNA (single-stranded RNA) sequences present in the initial saRNA strand and in the sub-genomic transcripts passing through the endosomes [19]; RIG-I preferentially recognizes short cytosolic transcription/replication products of dsRNA with 5'-triphosphate ends or regions of ssRNA with partial double-stranded structures generated by the replicase in the cytoplasm; MDA5 binds to long, high-molecular-weight dsRNA intermediates (>1 kb) that accumulate extensively in the cytosol during the intense amplification of the saRNA template; LGP2 also interacts with cytosolic dsRNA, regulating the intensity of the response triggered by RIG-I and MDA5 in response to saRNA replication intermediates [20]. Nucleotide-binding domain-like receptors (NLRs, NOD-like receptors) also play a role in RNA detection, particularly in relation to RNA helicases. Furthermore, an ssRNA such as that of alphaviruses can be a substrate for ribonuclease L (RNase L), an enzyme that, activated by oligoadenylate synthase 1 (OAS 1) degrades RNA. After RNA recognition, these receptors activate downstream signaling pathways, such as the RIG-I-like receptor (RLR) pathway. This pathway involves proteins such as Interferon-beta promoter stimulator-1 (IPS-1) also known as Mitochondrial Antiviral Signaling protein (MAVS), Virus Induced Signaling Adaptor (VISA) or Caspase Activation Recruitment Domain Adaptor inducing IFN-β (CARDIF), all of which are essential proteins for innate antiviral immunity. Activation of these pathways leads to the production of type I interferons (IFN) and other pro-inflammatory cytokines, and interferons play a crucial role in the activation of the adaptive immune response. In essence then, the genomic and sub-genomic saRNA vaccine transcripts generated by alphavirus replicase are recognized by the host cell as foreign RNA of viral origin, triggering an immune response that leads to its degradation.

5. Conclusions

The saRNA vaccine sequence was designed to maximize the self-replicating char-

acteristics of the RNA-dependent RNA polymerase (replicase) of the VEEV alphavirus, thereby ensuring significant increases in protein translation and self-amplification. At the 5' end, the saRNA vaccine sequence has a specifically engineered and patented Cap 1 (CleanCap), which is recognizable as self and able to evade the host's immune defenses. Together with a poly-A tail, this confers stability to the RNA and allows efficient self-replication cycles to begin. Due to the presence of a promoter between the genomic and sub-genomic sequences of the replicon, the viral replicase is facilitated to replicate the sub-genomic sequence (GOI) of the spike (the subgenomic promoter allows the viral replicase to initiate transcription from an internal point in the strand). Consequently, many more copies of the latter are produced than of the entire genomic sequence. Due to self-replication, newly synthesized genomic and sub-genomic sequences must be provided with a new cap, for which the nsP1 subunit of the alphavirus replicase is responsible, which then adds a Cap 0 to the 5' end of the new RNA strands. Cap 0 stimulates innate immune responses by binding to cytosolic receptors and is destined to be quickly deactivated by specific IFN-induced proteins. Based on all these observations, the mechanism of the saRNA vaccines is therefore considered to be controlled and self-limiting. The statement that self-amplifying RNA (saRNA) replication is a controlled and self-limiting mechanism, rather than an indefinite or autonomous cycle, is heavily supported by empirical evidence regarding its kinetics, magnitude, and cellular tropism. Instead of a single, universal molecular "stop switch", saRNA expression naturally tapers off through a multi-layered combination of host innate immune sensing, intracellular RNA decay pathways, and the exhaustion of finite cellular resources.

Self-amplifyng RNA (saRNA) replication mechanism step by step:

a) Direct Evidence of Kinetic Control.

Duration: Transient Window of Expression.

Unlike DNA-based vectors or integrating viruses, saRNA expression is strictly temporary. Longitudinal studies tracking saRNA encoding reporter genes (such as luciferase or green fluorescent protein) demonstrate a distinct kinetic curve [21] [22]. Typicalli for peak expression occurs between days 3 and 7 post-transfection and the expression progressively drops after the peak. Viral replicon RNA is virtually undetectable or returns to baseline levels within 2 to 4 weeks in most immunocompetent models [23]-[25].

Magnitude: Homeostatic Ceiling.

The intracellular copy number of saRNA does not expand exponentially without bound. The magnitude of replication hits a strict physiological ceiling. The viral RNA-dependent RNA polymerase (RdRp) produces sub-genomic transcripts at high volumes early on, but the absolute abundance of positive-strand and negative-strand replication intermediates plateaus. This stabilization proves that homeostatic regulatory mechanisms actively outpace or suppress the replication machinery before it can cause unregulated cellular lysis.

Cell-Type Dependence: Tropism and Microenvironment.

The longevity and peak output of saRNA vary dramatically depending on the host cell type, strictly tying its replication kinetics to cellular context [26]: in primary cells or specialized immune cells (like dendritic cells), saRNA expression is intense but brief due to hyper-vigilant defense systems. In contrast, signal-deficient or transformed cell lines (such as BHK-21 or Vero cells, which lack robust interferon pathways) support significantly prolonged and higher magnitude saRNA persistence [27]-[29]. Highly proliferative cells dilute saRNA copies through cell division, whereas quiescent or post-mitotic cells (e.g., myocytes in muscle tissue delivery) exhibit a longer duration of translation, proving that replication is bound by host cell turnover rates [30].

b) Contributing Mechanisms Behind Self-Limitation

Rather than relying on a predetermined genetic termination signal encoded in the RNA, saRNA replication is choked out by three distinct, overlapping physiological pressures: the very act of replication generates molecular signatures that host cells evolved to destroy. The replication cycle produces double-stranded RNA (dsRNA) intermediates and 5'-triphosphate single-stranded RNA; these are rapidly detected by endosomal TLR3 and cytosolic sensors like RIG-I and MDA5 [31]. This sensing triggers a massive wave of Type I Interferons (IFN-α/β). IFNs act in an autocrine and paracrine manner to upregulate hundreds of Interferon-Stimulated Genes (ISGs). Proteins like IFIT1 and IFIT5 bind and sequester foreign RNA structures, while the OAS/RNase L pathway systematically cleaves viral RNA strands, directly shutting down the replication cycle [32] [33].

Intracellular RNA Decay2

saRNA must constantly compete with endogenous transcripts for stabilit, leaving it vulnerable to standard host clearance mechanisms. Over time, cellular deadenylation complexes strip the poly(A) tail of the saRNA, and decapping enzymes remove the protective 5' cap. This exposes the saRNA backbone to rapid 5'\(\rightarrow\) 3' degradation by XRN1 and 3'\(\rightarrow \) 5' degradation by the exosome complex. Intense saRNA replication activates PKR (Protein Kinase R), which phosphorylates eIF2α. This halts global translation and forces both the saRNA and its translating ribosomes into stress granules, physically isolating the transcripts from the replication machinery [34]-[38].

Exhaustion of Cellular Resources

Replication is an energetically expensive process that ultimately starves itself out by consuming finite cellular machinery [39]. The viral RdRp rapidly consumes intracellular pools of ribonucleotide triphosphates (NTPs). As ATP, GTP, CTP, and UTP are depleted by high-magnitude replication and transcription, the enzymatic velocity of the RdRp drops. The host cell features a fixed number of functional ribosomes, tRNAs, and initiation factors (like eIF4F). Because saRNA subgenomic promoters drive massive translation of the encoded transgene, they induce severe ribosomal competition. Eventually, the metabolic stress compromises host cell viability or translation efficiency, imposing a structural limit on how long

the cell can sustain saRNA production [40]-[42]. In simple terms, the saRNA replication process is like a controlled assembly line; this prevents the entire process from going haywire and producing excessive amounts of anything.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Paul-Chima, U.O., Ben, O.M., Fabian, C.O., Nnenna, U.J. and Chinyere, N.U. (2026) Self-Amplifying RNA (saRNA) and Circular RNA (circRNA) Vaccines: Progress, Evidence Gaps, and Translational Pathways for Durable and Scalable Immunization. *Human Vaccines & Immunotherapeutics*, **22**, Article ID: 2661120. https://doi.org/10.1080/21645515.2026.2661120

[2] Pietilä, M.K., Hellström, K. and Ahola, T. (2017) Alphavirus Polymerase and RNA Replication. *Virus Research*, **234**, 44-57. https://doi.org/10.1016/j.virusres.2017.01.007

[3] Hồ, N.T., Hughes, S.G., Ta, V.T., Phan, L.T., Đỗ, Q., Nguyễn, T.V., *et al.* (2024) Safety, Immunogenicity and Efficacy of the Self-Amplifying mRNA ARCT-154 COVID-19 Vaccine: Pooled Phase 1, 2, 3a and 3b Randomized, Controlled Trials. *Nature Communications*, **15**, Article No. 4081. https://doi.org/10.1038/s41467-024-47905-1

[4] Shin, G., Yost, S.A., Miller, M.T., Elrod, E.J., Grakoui, A. and Marcotrigiano, J. (2012) Structural and Functional Insights into Alphavirus Polyprotein Processing and Pathogenesis. *Proceedings of the National Academy of Sciences*, **109**, 16534-16539. https://doi.org/10.1073/pnas.1210418109

[5] Kinney, R.M., Johnson, B.J.B., Welch, J.B., Tsuchiya, K.R. and Trent, D.W. (1989) The Full-Length Nucleotide Sequences of the Virulent Trinidad Donkey Strain of Venezuelan Equine Encephalitis Virus and Its Attenuated Vaccine Derivative, Strain TC-83. *Virology*, **170**, 19-30. https://doi.org/10.1016/0042-6822(89)90347-4

[6] Casmil, I.C., Jin, J., Won, E., Huang, C., Liao, S., Cha-Molstad, H., *et al.* (2025) The Advent of Clinical Self-Amplifying RNA Vaccines. *Molecular Therapy*, **33**, 2565-2582. https://doi.org/10.1016/j.ymthe.2025.03.060

[7] Tassinari, V., Cerboni, C. and Soriani, A. (2022) Self or Non-Self? It Is Also a Matter of RNA Recognition and Editing by ADAR1. *Biology*, **11**, Article No. 568. https://doi.org/10.3390/biology11040568

[8] Wolf, J. and Passmore, L.A. (2014) mRNA Deadenylation by Pan2-Pan3. *Biochemical Society Transactions*, **42**, 184-187. https://doi.org/10.1042/bst20130211

[9] Ferreira-Ramos, A.S., Li, C., Eydoux, C., Contreras, J.M., Morice, C., Quérat, G., *et al.* (2019) Approved Drugs Screening against the nsP1 Capping Enzyme of Venezuelan Equine Encephalitis Virus Using an Immuno-Based Assay. *Antiviral Research*, **163**, 59-69. https://doi.org/10.1016/j.antiviral.2019.01.003

[10] Bowie, A.G. and Fitzgerald, K.A. (2007) RIG-I: Tri-Ing to Discriminate between Self and Non-Self RNA. *Trends in Immunology*, **28**, 147-150. https://doi.org/10.1016/j.it.2007.02.002

[11] Miedziak, B., Dobieżyńska, A., Darżynkiewicz, Z.M., Bartkowska, J., Miszkiewicz, J., Kowalska, J., *et al.* (2019) Kinetic Analysis of IFIT1 and IFIT5 Interactions with Different Native and Engineered RNAs and Its Consequences for Designing mRNA-Based Therapeutics. *RNA*, **26**, 58-68. https://doi.org/10.1261/rna.073304.119

[12] Kumar, P., Sweeney, T.R., Skabkin, M.A., Skabkina, O.V., Hellen, C.U.T. and Pestova, T.V. (2013) Inhibition of Translation by IFIT Family Members Is Determined by Their Ability to Interact Selectively with the 5'-Terminal Regions of cap0-, cap1- and 5'ppp- mRNAs. *Nucleic Acids Research*, **42**, 3228-3245. https://doi.org/10.1093/nar/gkt1321

[13] Abbas, Y.M., Laudenbach, B.T., Martínez-Montero, S., Cencic, R., Habjan, M., Pichlmair, A., *et al.* (2017) Structure of Human IFIT1 with Capped RNA Reveals Adaptable mRNA Binding and Mechanisms for Sensing N1 and N2 Ribose 2'-O Methylations. *Proceedings of the National Academy of Sciences*, **114**, E2106-E2115. https://doi.org/10.1073/pnas.1612444114

[14] de Alwis, R., Gan, E.S., Chen, S., Leong, Y.S., Tan, H.C., Zhang, S.L., *et al.* (2021) A Single Dose of Self-Transcribing and Replicating RNA-Based SARS-CoV-2 Vaccine Produces Protective Adaptive Immunity in Mice. *Molecular Therapy*, **29**, 1970-1983. https://doi.org/10.1016/j.ymthe.2021.04.001

[15] Uehata, T. and Takeuchi, O. (2020) RNA Recognition and Immunity-Innate Immune Sensing and Its Posttranscriptional Regulation Mechanisms. *Cells*, **9**, Article No. 1701. https://doi.org/10.3390/cells9071701

[16] Santhakumar, D., Rohaim, M.A.M.S., Hussein, H.A., Hawes, P., Ferreira, H.L., Behboudi, S., *et al.* (2018) Chicken Interferon-Induced Protein with Tetratricopeptide Repeats 5 Antagonizes Replication of RNA Viruses. *Scientific Reports*, **8**, Article No. 6794. https://doi.org/10.1038/s41598-018-24905-y

[17] Chattopadhyay, S. and Sen, G.C. (2014) dsRNA-Activation of TLR3 and RLR Signaling: Gene Induction-Dependent and Independent Effects. *Journal of Interferon & Cytokine Research*, **34**, 427-436. https://doi.org/10.1089/jir.2014.0034

[18] Kunyk, D., Plotnikova, M., Bespalov, M., Shevyrev, D., Klotchenko, S., Ivanov, R., *et al.* (2025) The Interplay between Therapeutic Self-Amplifying RNA and the Innate Immune System: Balancing Efficiency and Reactogenicity. *International Journal of Molecular Sciences*, **26**, Article No. 8986. https://doi.org/10.3390/ijms26188986

[19] Blakney, A.K., Ip, S. and Geall, A.J. (2021) An Update on Self-Amplifying mRNA Vaccine Development. *Vaccines*, **9**, Article No. 97. https://doi.org/10.3390/vaccines9020097

[20] Swiecki, M., McCartney, S.A., Wang, Y. and Colonna, M. (2011) TLR7/9 versus TLR3/MDA5 Signaling during Virus Infections and Diabetes. *Journal of Leukocyte Biology*, **90**, 691-701. https://doi.org/10.1189/jlb.0311166

[21] Wang, F., Wang, L., Zou, X., Duan, S., Li, Z., Deng, Z., *et al.* (2019) Advances in CRISPR-Cas Systems for RNA Targeting, Tracking and Editing. *Biotechnology Advances*, **37**, 708-729. https://doi.org/10.1016/j.biotechadv.2019.03.016

[22] Lundstrom, K. (2016) Self-Replicating RNA Viral Vectors in Vaccine Development and Gene Therapy. *Future Virology*, **11**, 345-356. https://doi.org/10.2217/fvl-2016-0028

[23] Currier, R.B., Calvete, J.J., Sanz, L., Harrison, R.A., Rowley, P.D. and Wagstaff, S.C. (2012) Unusual Stability of Messenger RNA in Snake Venom Reveals Gene Expression Dynamics of Venom Replenishment. *PLOS ONE*, **7**, e41888. https://doi.org/10.1371/journal.pone.0041888

[24] Gu, Y., Choi, J., Mutha, D., Wu, C., Ganem, N.J., Grinstaff, M.W. and Wong, W.W. (2026) Self-Amplifying RNA-Based CAR T Cell Therapy with Enhanced Duration and Multi-Genic Logic Functions. https://pmc.ncbi.nlm.nih.gov/articles/PMC12934621/

[25] Kamat, A., Joseph, A.M., Rathour, D. and Badrinarayanan, A. (2025) Variability in Intrinsic Promoter Strength Underlies the Temporal Hierarchy of the Caulobacter SOS Response Induction. *PLOS Biology*, **23**, e3003557. https://doi.org/10.1371/journal.pbio.3003557

[26] Waterhouse, P.M., Wang, M. and Finnegan, E.J. (2001) Role of Short RNAs in Gene Silencing. *Trends in Plant Science*, **6**, 297-301. https://doi.org/10.1016/s1360-1385(01)01989-6

[27] Federico, M. (2025) The Potential of Extracellular Vesicle-Mediated Spread of Self-Amplifying RNA and a Way to Mitigate It. *International Journal of Molecular Sciences*, **26**, Article No. 5118. https://doi.org/10.3390/ijms26115118

[28] Beissert, T., Koste, L., Perkovic, M., Walzer, K.C., Erbar, S., Selmi, A., *et al.* (2017) Improvement of *in Vivo* Expression of Genes Delivered by Self-Amplifying RNA Using Vaccinia Virus Immune Evasion Proteins. *Human Gene Therapy*, **28**, 1138-1146. https://doi.org/10.1089/hum.2017.121

[29] Curcio, J.S.d., Silva, L.d.C., Novaes, E. and Silveira-Lacerda, E.d.P. (2026) Differential Expression of miRNAs in Vero Cells after Mayaro Virus Infection. *Memórias do Instituto Oswaldo Cruz*, **121**, e250177. https://doi.org/10.1590/0074-02760250177

[30] Aufiero, S., Reckman, Y.J., Pinto, Y.M. and Creemers, E.E. (2019) Circular RNAs Open a New Chapter in Cardiovascular Biology. *Nature Reviews Cardiology*, **16**, 503-514. https://doi.org/10.1038/s41569-019-0185-2

[31] Ying, H., Zaks, T.Z., Wang, R., Irvine, K.R., Kammula, U.S., Marincola, F.M., *et al.* (1999) Cancer Therapy Using a Self-Replicating RNA Vaccine. *Nature Medicine*, **5**, 823-827. https://doi.org/10.1038/10548

[32] Han, D., Zhang, B., Wang, Z. and Mi, Y. (2025) Cell-Autonomous Immunity: From Cytosolic Sensing to Self-Defense. *International Journal of Molecular Sciences*, **26**, Article No. 4025. https://doi.org/10.3390/ijms26094025

[33] Chan, Y.K. and Gack, M.U. (2016) Viral Evasion of Intracellular DNA and RNA Sensing. *Nature Reviews Microbiology*, **14**, 360-373. https://doi.org/10.1038/nrmicro.2016.45

[34] Opyrchal, M., Anderson, J.R., Sokoloski, K.J., Wilusz, C.J. and Wilusz, J. (2005) A Cell-Free mRNA Stability Assay Reveals Conservation of the Enzymes and Mechanisms of mRNA Decay between Mosquito and Mammalian Cell Lines. *Insect Biochemistry and Molecular Biology*, **35**, 1321-1334. https://doi.org/10.1016/j.ibmb.2005.08.004

[35] Mata, J., Marguerat, S. and Bähler, J. (2005) Post-Transcriptional Control of Gene Expression: A Genome-Wide Perspective. *Trends in Biochemical Sciences*, **30**, 506-514. https://doi.org/10.1016/j.tibs.2005.07.005

[36] Maździarz, M.A., Krawczyk, K., Lepiarczyk, E., Paukszto, Ł., Makowczenko, K.G., Moczulska, B., *et al.* (2025) Poly(A) Tail Dynamics, Non-Adenine Incorporation and Alternative Polyadenylation Shape the Host Transcriptome in COVID-19 Pathogenesis. *Scientific Reports*, **15**, Article No. 37986. https://doi.org/10.1038/s41598-025-21969-5

[37] Gaglia, M.M. and Glaunsinger, B.A. (2010) Viruses and the Cellular RNA Decay Machinery. *WIREs RNA*, **1**, 47-59. https://doi.org/10.1002/wrna.3

[38] White, E.J.F., Brewer, G. and Wilson, G.M. (2013) Post-Transcriptional Control of Gene Expression by AUF1: Mechanisms, Physiological Targets, and Regulation. *Biochimica et Biophysica Acta* (*BBA*)—*Gene Regulatory Mechanisms*, **1829**, 680-688. https://doi.org/10.1016/j.bbagrm.2012.12.002

[39] Serdyuk, A. and Allers, T. (2025) DNA Replication in Time and Space: The Archaeal Dimension. *DNA*, **5**, Article No. 24. https://doi.org/10.3390/dna5020024

[40] Loan Young, T., Chang Wang, K., James Varley, A. and Li, B. (2023) Clinical Delivery of Circular RNA: Lessons Learned from RNA Drug Development. *Advanced Drug Delivery Reviews*, **197**, Article ID: 114826. https://doi.org/10.1016/j.addr.2023.114826

[41] Della Santina, C.M., Ploessl, D.S., Lindsay-Mosher, N., *et al.* (2025) Self-Amplifying RNA Enables Rapid, Durable, Integration-Free Programming of hiPSCs. https://doi.org/10.1101/2025.10.24.684179

[42] Roux, C., Etienne, T.A., Hajnsdorf, E., Ropers, D., Carpousis, A.J., Cocaign-Bousquet, M., *et al.* (2022) The Essential Role of mRNA Degradation in Understanding and Engineering *E. coli* Metabolism. *Biotechnology Advances*, **54**, Article ID: 107805. https://doi.org/10.1016/j.biotechadv.2021.107805