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Synonymous Sequence Design in Therapeutic mRNA: A Mechanistic Framework for Balancing Transcript Stability, Translation Kinetics, Cotranslational Folding, and Functional Protein Production

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ABSTRACT

Therapeutic messenger RNA (mRNA) is frequently redesigned through synonymous coding substitutions intended to improve stability and expression without changing the encoded amino-acid sequence. Yet RNA persistence, ribosomal translation, nascent-chain folding, and biologically active protein accumulation are distinct outcomes; optimization of one cannot guarantee improvement of the others. This mechanism-based theoretical article integrates experimental and computational evidence on codon choice, RNA structure, decoding state, translational quality control, and cotranslational folding. We argue that sequence designs may change rank when evaluated by functional protein output instead of RNA half-life or bulk expression, provided that folding, processing, and activity are sensitive to the translation history of otherwise identical polypeptides. The account separates established sequence-dependent mechanisms from the proposed coupling among intermediate processes. It identifies contextual dependence on cell state, nucleoside chemistry, delivery, and measurement timescale, while treating direct therapeutic validation as outstanding. A falsifiable, matched-construct approach is proposed for distinguishing RNA abundance, productive translation, maturation efficiency, and active-product formation. Synonymous sequence selection should consequently be judged against the function required of the encoded therapeutic product, with gains in stability or quantity interpreted as conditional evidence.

Key words: *Therapeutic mrna, Synonymous codon design, RNA decay, Translation elongation, Cotranslational folding, Functional protein activity*

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INTRODUCTION

Therapeutic mRNA is an engineered molecular medicine whose coding sequence operates together with untranslated regions, nucleotide chemistry, formulation, and target-cell physiology. Design choices can alter both expression and the persistence of the administered RNA [1]. Computational optimization of coding sequence and RNA secondary structure has yielded improved molecular stability and vaccine-related readouts in studied systems [2]. Such achievements make synonymous rewriting attractive, although immunogenicity or total antigen expression cannot be treated as a general assay of correctly folded, pharmacologically functional protein.

A common design assumption is that greater transcript lifetime or translation gives a proportionate gain in useful output. Codon optimality, however, depends on cellular decoding machinery and can influence mRNA decay [3].

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Synthetic mRNA structure has been linked experimentally to functional half-life and protein expression [4]. Human-cell experiments likewise connect codon identity, ongoing translation, and RNA turnover [5]. These findings establish several causal routes into RNA and protein abundance, not their equivalence. A long-lived transcript may initiate inefficiently; a ribosome-rich transcript may complete fewer chains per unit time; chains released at similar rates may differ in maturation or activity.

The central theoretical problem is therefore a ranking problem. For two RNAs encoding the same polypeptide, when will the construct ranked higher for RNA persistence or measured protein amount also rank higher for desired biological activity? This analysis treats synonymous sequence change as an intervention and the intermediary molecular states as separable observable variables. It does not claim to have performed an experiment or to identify an optimal sequence for every therapeutic class. Its value is to specify a mechanistic condition under which a familiar optimization criterion fails and the measurements needed to detect that failure.

Synonymous sequence variation as a therapeutic mrna design variable

A synonymous intervention substitutes codons while keeping amino-acid identity, reading-frame length, and intended protein sequence fixed. It changes nucleotide composition, local and long-range pairing, codon-pair neighborhoods, and potentially interactions with RNA-binding proteins. Computational sequence design therefore spans several objectives. Recent reviews describe folding-aware codon algorithms [6], and an integrated design server offers predicted structures and translation-related scores [7]. Scores are design hypotheses, since an ensemble predicted in solution is neither the complete intracellular RNA state nor a direct measurement of productive protein synthesis.

Useful interventions must distinguish coding-sequence variables from adjacent engineering choices. Codon-derived sequence representations can carry information relevant to protein engineering [8], but a learned association does not prove a particular molecular pathway. Deep-learning optimization of five-prime untranslated regions can increase gene-editing expression in tested systems [9]; this alters initiation context without necessarily changing synonymous codons. Combinatorial mRNA design experiments have jointly varied structural and translation-related determinants [10]. A study that changes UTR, cap, nucleoside chemistry, or carrier simultaneously with codons cannot attribute its eventual outcome to synonymous substitution alone.

For the present theory, a valid comparison is a matched pair or library of coding-region variants encoding an identical protein, with the noncoding architecture, poly(A) region, chemical modifications, manufacturing quality, administered molar RNA quantity, and delivery exposure controlled as far as experimentally feasible. When those controls cannot be held fixed, factorial comparison and direct measurements of delivered intact transcript are required. The intervention is thus defined by the physical RNA actually entering a translating cell, not by an abstract codon-adaptation index. Cell-type-specific tRNA pools, translational stress, intracellular RNA compartment, and sampling time belong to the explanatory context.

Current evidence on sequence-dependent rna and protein production

Sequence-sensitive RNA persistence can be measured without assuming that protein measurements report the same phenomenon. Massively parallel synthetic-RNA kinetic experiments have combined degradation measurements, biophysical descriptors, and machine learning to characterize sequence-associated stability [11]. Findings about codon usage in human genes also implicate nuclear processes [12]. That result matters as an alternative explanation for endogenous transcripts, but nuclear transcription, processing, and export do not directly reproduce the entry route of cytoplasmically delivered in-vitro-transcribed therapeutic RNA.

Decoding context supplies an independent axis. During oligodendrocyte differentiation, altered tRNA modifications were linked to changes in codon-optimality-mediated decay [13]. Massively parallel analyses identify RNA sequence motifs that activate ribosome-associated quality-control responses [14]. These findings caution against treating codon preference as a fixed property of a codon table. A sequence may behave differently when tRNA modification, surveillance capacity, or cell state changes, even before protein folding is considered.

Engineered tRNA modification has been reported to enhance translation of messages enriched in corresponding codons [15]. Separately, studies of pseudouridine and N1-methylpseudouridine indicate that chemically modified bases can change decoding behavior during translation [16]. The coupling of translation and RNA turnover is itself supported by mechanistic synthesis across cellular systems [17]. These observations motivate distinct measurements of intact RNA per cell, initiation, ribosomal residence, full-length product release, fidelity, and protein lifetime. Protein abundance alone collapses those stages into a single end-point signal.

There is an additional numerator problem: ribosome profiling generally measures protected footprints, whereas productive flux concerns completed chains released over time. Increased occupancy can reflect more initiation, slower elongation, pausing, or quality-control accumulation. For therapeutic design, an assay that measures fluorescent reporter intensity at one time point is therefore an incomplete proxy for the amount of chemically correct, properly matured active protein. The evidence from sequence perturbations is organized by the particular experimental distinction it can support in **Table 1.**

Table 1. Synonymous-sequence evidence separated by measured molecular state and permissible pharmaceutical inference

Perturbation or evidence unit	Directly observed or modeled quantity	Transfer to therapeutic IVT mRNA	Unresolved mechanism or excluded claim
Synthetic RNA decay panels	Sequence-associated degradation kinetics and model predictors	Relevant to stability screening under the tested chemistry and assay	Does not identify per-molecule activity or in-cell translation capacity
Endogenous human codon usage	Gene-expression associations involving nuclear and cytoplasmic processes	Useful for specifying alternative pathways in producer cells	Nuclear processing cannot be assigned to cytoplasm-delivered IVT RNA
Cell-state/tRNA modification changes	Context-sensitive codon-mediated RNA decay	Motivates decoding-state measurements in target cells	No universal codon ranking across differentiation states
Ribosomal surveillance motifs	Quality-control sensitivity to local translated sequence features	Flags sequence regions requiring orthogonal translation assays	Motif association alone does not prove reduced mature protein
Engineered tRNA and modified bases	Codon-correlated translation changes and decoding effects	Highlights chemistry × cell-context interactions	Translation amount, reading-frame accuracy and function are separate

Unresolved relationships among transcript stability, translation, and protein function

Even a precise measure of RNA decay and productive translation need not predict protein-specific activity. Reviews of codon-dependent protein folding describe pathways by which the tempo of synthesis and the local nascent-chain environment can influence structural outcomes [18]. Experiments with synonymous mutations have shown long-timescale changes in enzyme structure and function in a nontherapeutic model [19]. The transfer claim is limited: such observations demonstrate biological plausibility, not that each recoded in-vitro-transcribed therapeutic message produces a different conformational ensemble.

The ribosome also senses the peptide being made. Human-cell experiments implicate nascent-peptide features in translational control of RNA stability [20]. Physical studies reveal that the ribosomal environment can reshape folding thermodynamics of an emerging polypeptide [21]. Chaperone-resolved experiments further show how folding events can be assisted at the ribosome [22]. As the primary amino-acid sequence is identical in a clean synonymous comparison, peptide-dependent mechanisms may be common to both variants; their interaction with different dwell-time profiles is the unresolved question.

Three measurement gaps are especially consequential. First, functional RNA lifetime is not simply the decay constant of extracted bulk RNA; it also depends on whether intact molecules remain accessible for translation. Second, polysome density does not distinguish rapid productive ribosome traffic from prolonged occupancy. Third, immunoblot intensity, reporter fluorescence, antigen immunoreactivity, and purified-protein mass may agree while specific enzymatic activity or correct molecular processing differs. Protein maturation can also be rescued by chaperones or obscured by degradation, making a transient cotranslational change invisible at a late sampling point.

A mechanistic inference about codon-dependent folding requires paired evidence: matched amino-acid sequence, altered translation kinetics, a demonstrable structural or processing change, and a corresponding activity measure. Without that chain, RNA structure and delivery effects remain viable explanations of apparent protein gains. This diagnostic gap motivates investigation of competing routes, not a presumption that recoding changes native folding.

Competing mechanisms and design trade-offs in therapeutic mRNA

Codon choice can alter the relative balance between translational elongation and RNA degradation. Reviews of codon-optimality-mediated decay describe pathways linking ribosomal progression and transcript stability [23].

Simulations addressing near-cognate tRNA competition also suggest that decoding times are sensitive to kinetic competition beyond simplistic abundant-versus-rare codon rankings [24]. A slower region might trigger surveillance, influence folding exposure time, or merely reflect a changed cellular tRNA state. Those alternatives predict different patterns of full-length product release and RNA loss.

Composition and position further complicate attribution. Experiments in bacteria identify start-of-coding-sequence base composition as a determinant of the initiation–degradation balance [25], whereas human-cell studies associate GC content with mRNA storage and decay pathways [26]. Work on leading codons similarly illustrates strong positional effects on translation in bacterial systems [27]. These findings identify candidate mechanisms and instruct the design of controls; neither bacterial initiation architecture nor population-level GC correlations should be imported unchanged into a chemically modified mammalian therapeutic construct.

Chemical state creates a particularly important competing pathway. N1-methylpseudouridine-containing transcripts have been shown to permit +1 ribosomal frameshifting at susceptible contexts, with immune responses to frameshifted products reported after vaccination [28]. This observation raises a testable fidelity concern; it does not demonstrate a clinical efficacy decrement for any proposed synonymous redesign. Frame maintenance and correct protein sequence must therefore be distinguished from total translation and from protein folding: reduced yield of intended protein may arise from decoding errors even when correctly translated chains mature normally.

The resulting trade-offs cannot be ranked along a universal "more optimal codons" axis. A construct can increase measured RNA lifetime because of changed folding or binding yet show little difference in active-protein production; apparent ribosome accumulation can reflect stalling; a change in secreted protein amount can reflect export or degradation. **Table 2** treats these as competing explanatory signatures, showing which inference each pattern leaves open. A valid pharmaceutical choice depends on the target product, the expected cell context, and whether the expression metric measures the desired protein species.

Table 2. Alternative mechanistic readings of discordant synonymous-mRNA performance signatures

Observed readout contrast	Mechanism consistent with signature	Competitive explanation requiring control	Functional-product implication
RNA residence increases; active protein remains level	Reduced decay without greater productive translation	Translation-inaccessible RNA fraction or saturation	Longer apparent lifetime need not increase useful output
Ribosome occupancy increases; full-length release does not	Elongation delay or ribosomal pausing	Higher initiation with downstream bottleneck	Footprint density is not chain-completion flux
Protein mass increases; specific activity decreases	Altered folding/processing or changed mature conformer fraction	Assay interference, aggregation or altered localization	Rank of expression can reverse relative to functional output
Full-length signal decreases with modified nucleosides	Context-sensitive decoding or reading-frame error	RNA degradation, initiation suppression or delivery inequality	Correct amino-acid sequence must be assessed separately
Cell type changes sequence ranking	Different tRNA, chaperone or decay environments	Unequal delivered dose or stress response	Sequence merit may be tissue- and state-conditional

Development of the proposed mechanistic sequence-design framework

The hidden assumption in ordinary expression-centered sequence optimization is rank preservation: among synonymous RNAs encoding the same protein, the design generating more intact RNA or more immunoreactive protein is expected to generate more useful active product. The broader literature recognizes functional consequences of synonymous variation across multiple systems [29], while eukaryotic RNA decay and quality-control pathways can remove or retain messages on different mechanistic grounds [30]. Together, these findings justify questioning rank preservation. They do not establish that rank inversions are frequent in therapeutic delivery.

The proposed causal account places four experimentally distinct variables between administered RNA and useful output: the time-dependent number of translation-competent intact messages; completed, in-frame polypeptide release per message; the fraction of released chains that mature into the intended molecular species; and functional activity per mature species under the relevant assay. The instantaneous rate of generating activity may be regarded as a conditional product of these terms, with each factor dependent on sequence, cell state, and exposure history.

This is a decomposition for measurement, not an empirically fitted equation or an assumption of biological independence. Accumulated activity adds protein trafficking, clearance, and inactivation over time.

Position-specific chemical modifications can independently affect RNA stability and translation [31]. New generative codon optimization methods have also reported benefits in tested translation and therapeutic models [32]. Their existence makes explicit comparison of sequence-only and chemistry-dependent mechanisms necessary. The decisive prediction here is conditional: a variant may outrank another for transcript persistence or bulk protein signal while ranking below it for correctly matured active protein if the intervention changes completion, processing, folding yield, or fidelity sufficiently. Conversely, if maturation and activity remain constant across synonymous variants, abundance-based ranking can remain adequate within that experimental context.

For purposes of falsification, this account would be weakened if carefully matched variants spanning divergent RNA structures and ribosome transit profiles consistently retained the same functional ranking after normalization for intact cellular RNA and productive chain release. Observation of different activity alone would not settle causality: unequal delivery, altered RNA exposure, or off-target cell stress must first be ruled out. **Figure 1** represents the proposed dependencies, the better-supported molecular routes, and the points at which measured expression ceases to warrant inference about functional product.

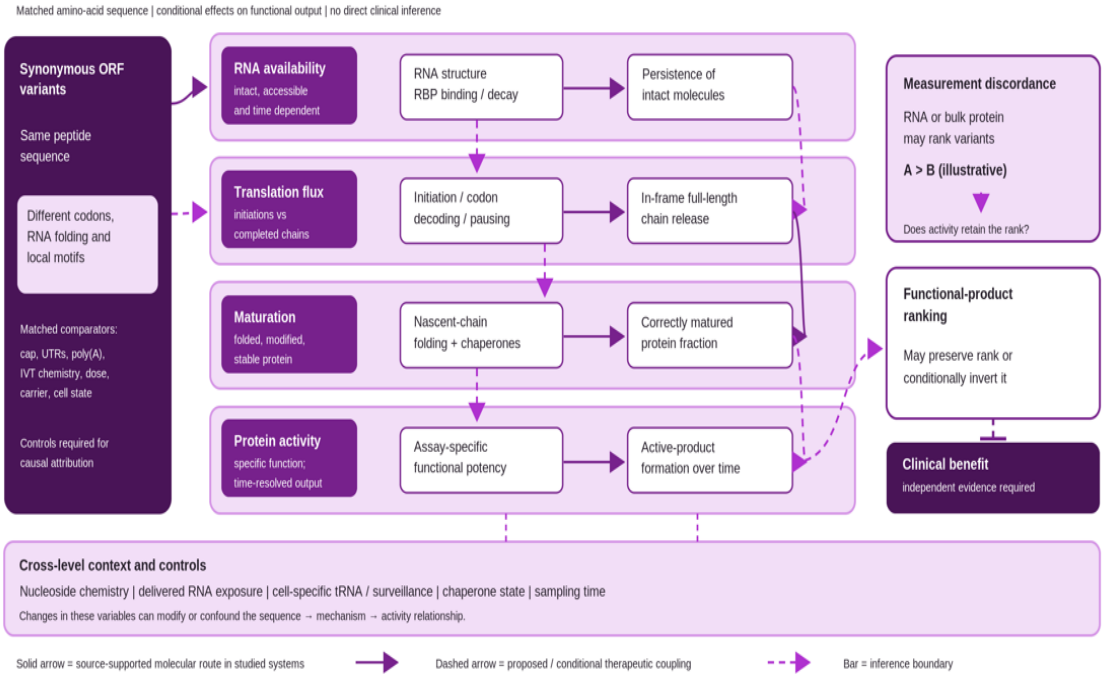


Figure 1. Synonymous coding choices can separate RNA persistence, ribosome throughput, and active-protein yield across biological levels.

A multilevel mechanistic relationship map running from matched synonymous coding-region variants (left) through RNA-state and translation-state processes (center) to mature and functionally active protein (right). Solid arrows indicate routes supported by mechanism-focused evidence in at least one studied experimental system; dashed arrows mark proposed cross-level couplings or rank-inversion pathways that require matched therapeutic-mRNA validation. Open-ended bars indicate constraints rather than additional productive flows. The upper enclosure isolates RNA sequence and chemical state, the middle enclosure distinguishes initiation, elongation, reading-frame fidelity and ribosome release, and the lower enclosure distinguishes cotranslational maturation from measured functional activity. Contextual bands identify cell-state decoding machinery, chaperones, delivery and sampling time. No arrow transfers an experimental observation directly to clinical efficacy. IVT, in-vitro-transcribed; ORF, open reading frame; RBP, RNA-binding protein.

Implications for pharmaceutical biotechnology and functional product evaluation

The relevant pharmaceutical output depends on the encoded product. Vaccination can depend on antigen availability, presentation and immune activation, whereas a replacement enzyme or locally acting protein may require sustained exposure to a correctly processed molecular species. Clinical experience with mRNA vaccines

and immunotherapies illustrates that formulation, dose, intended tissue and biological endpoint change what successful expression means [33]. No generic conversion factor maps fluorescence or protein concentration onto clinical benefit. The construct-level estimand proposed here is time-integrated formation of active, correctly specified protein at the relevant site, qualified by delivered RNA exposure and downstream disposition.

Human cancer data confirm that synonymous changes can have biological consequences, including altered RNA structure and expression in selected settings [34]. Such observations do not establish what happens when an engineered, modified mRNA bypasses nuclear transcription and splicing. For product development, the essential distinction is between assay-defined protein amount and molecularly verified active product. Identity and sequence fidelity, correct disulfide or other maturation state where applicable, aggregation, localization, and target-specific activity should be evaluated with methods appropriate to the therapeutic molecule; a single universal panel is unjustified.

Sequence-and-structure design reviews identify opportunities to optimize stability and translation and acknowledge the difficulty of balancing competing objectives [35]. Accordingly, selection among synonymous candidates should use a predeclared function-relevant primary endpoint and secondary mechanistic readouts. A design showing superior transcript persistence can still be valuable when expression duration is limiting, even if a folding hypothesis proves false. Conversely, activity normalized by completed full-length chains may identify a quality liability invisible to bulk expression. These criteria are proposed decision rules, not clinically established release specifications or regulatory thresholds.

Manufacturing comparability also requires specifying the level at which two products are called equivalent. Identical amino-acid sequence at the design stage does not ensure identical released product when decoding fidelity, secretion, or proteolysis differs. A stability gain observed before formulation may vanish after encapsulation or cellular delivery, so sequence-level analytics should be linked to controlled material comparators. At the selection point, a candidate can be preferred only for the endpoint it has demonstrated, with unanswered potency and disposition questions stated separately.

Testable propositions and mechanism-discriminating evidence

The principal proposition is conditional rank nonpreservation: among synonymous variants encoding one amino-acid sequence, their ordering by intracellular intact-RNA exposure or immunoreactive protein amount need not match their ordering by accumulated active protein. A stringent experiment would randomize a synonymous coding-region library while holding cap, untranslated regions, poly(A), modified-nucleoside fraction, formulation, RNA integrity, endotoxin/impurity burden and administered molar dose constant. Equal delivered cellular RNA must be measured, not presumed. Codon-dependent decay observed in human cells [5] and combined sequence perturbation strategies [10] motivate the intervention, whereas modification-associated frameshifting [28] requires explicit fidelity controls.

A second proposition concerns mechanism identification. If two candidates have different ribosomal occupancy but indistinguishable full-length release per intact message, the occupancy difference should not be described as increased translation productivity. Time-resolved decay, calibrated ribosome profiling, independent nascent-chain release measurements, proteomic identity and target-specific activity are complementary. Comparative folding or aggregation measurements would test whether a residual activity difference follows maturation, while the mechanistic literature on folding at ribosomes [18] and on decoding kinetics [24] only supports plausibility of that pathway. Where matched variants exhibit equal mature-protein fraction and equal activity per completed chain, the folding-dependent component of the account is weakened.

Experimental normalization must avoid ratios with unstable denominators. Specific activity per immunoreactive mass is informative only when the antibody detects the same molecular species across variants; flux per total extracted RNA is misleading if some molecules are fragmented or compartmentalized. Orthogonal assays should estimate in-frame chain release and active-product production over aligned temporal windows, with prespecified calibration and detection limits. Independent manufacturing batches, blinded sample coding, and interval estimates would help prevent assay noise from being mistaken for a sequence-sensitive mechanism.

A third proposition tests context interaction and a nearby rival. Repeat the construct comparison across target-relevant cell states and at more than one sampling time, then independently change nucleoside chemistry in a factorial design. A codon effect that disappears after matching RNA exposure favors an RNA-availability explanation; one that remains after normalizing completed chains but tracks folding or maturation supports an additional protein-quality mechanism. Sequence-structure optimization [2], modified-base decoding effects [16], and generative codon design [32] motivate such comparisons, but do not supply their outcomes. **Table 3** specifies

competing observations that would count against each proposed route. Pre-register null, reversed and cell-specific results, and assess assay artifacts before assigning biological causality.

Table 3. Matched synonymous-mRNA experiments that could separate expression gains from functional-product mechanisms

Proposed construct contrast	Paired measurements required	Mechanistic result being tested	Finding favoring a rival or null explanation
Synonymous ORF variants; same chemical state and delivery	Intracellular intact-RNA time course; protein release per intact RNA	RNA-residence ranking differs from chain-production ranking	All output differences vanish after equalizing delivered intact RNA
Variant pairs with different ribosome footprint density	Ribosome occupancy; transit or runoff; full-length chains over time	Increased occupancy reflects pausing without greater completion	Occupancy difference matches increased initiation and chain release
Equal completed-chain yield from synonymous variants	Proteomic identity; folding/aggregation; mature-fraction assay; specific activity	Activity rank inversion arises downstream of chain release	Correctly matured fractions and activity per chain are equivalent
Synonymous variants crossed with nucleoside chemistry	Frame-resolved proteomics; in-frame completion; transcript exposure	Sequence and chemical state jointly influence translation fidelity	Fidelity change follows chemistry without sequence interaction
Matched sequences in contrasting target-cell states	Cellular tRNA and stress state; decay; completed chains; activity	Cell environment changes preferred construct ranking	Ranking persists and mechanistic parameters do not interact

Discussion, biological context, and future research

The mechanistic problem is that synonymous design changes several molecular properties at once, while therapeutic decision-making often observes only one downstream signal. The preceding account resolves that ambiguity by treating intracellular transcript availability, ribosome transit, intended full-length chain release, correct maturation and activity as different empirical objects. Their relations can be interrogated serially without assuming that a fitted prediction of one variable identifies the others. A folding-mediated inversion of candidate ranking remains a prospective outcome; the framework also permits straightforward preservation of ranking, which would be informative rather than a failure to observe an effect.

Context may determine whether a mechanistic difference is large enough to matter. Differentiation-dependent tRNA changes can alter codon-associated decay [13]; ribosome-associated quality-control responses depend on sequence context in studied systems [14]; and folding is shaped by chaperone engagement on the ribosome [22]. However, neither a particular yeast reporter phenotype nor a protein-folding mechanism demonstrated outside therapeutic cells should be elevated to a universal predictor of a human drug. Comparisons should therefore prioritize the cell populations intended to express the medicine, including their stress state and duration of exposure.

Translational interpretation adds a second separation. Reading-frame errors under modified-nucleoside conditions deserve direct analytical assessment [28], yet that concern should not be conflated with efficacy loss. The clinical evidence base for mRNA platforms spans vaccine and immunotherapeutic indications with different dose and delivery requirements [33]. Research that couples rigorously matched sequence libraries to chemical identity, cell-specific functional assays and appropriate in-vivo distribution measurements could establish which intermediary observations are decision-relevant. No single favorable in-vitro potency ratio would, by itself, identify a clinical dose or predict patient benefit.

A discriminating next experiment could compare a protein with measurable folding-sensitive activity and a separately quantified structural state against a reference protein whose maturation is insensitive to altered ribosomal transit under the same conditions. A context-dependent change in activity restricted to the former would strengthen a maturation interpretation. Even then, altered proteolytic clearance or intracellular trafficking would remain possible until separately measured. The aim is to compare mechanisms with contrasting predictions, not to choose a reporter that predictably responds to synonymous recoding.

Limitations and framework boundaries

This is a nonempirical mechanism-based theory, supported by studies with heterogeneous interventions and organisms. Endogenous human codon effects can include nuclear regulation [12], which cytoplasmically delivered IVT mRNA ordinarily bypasses. Position-specific ribose modifications [31] alter chemistry as well as sequence. Cancer-associated synonymous mutations [34] provide important examples of functional genomic variation but do not establish an mRNA medicine's folding or pharmacology. Accordingly, the account applies most directly to carefully controlled synonymous coding-region comparisons that preserve amino-acid sequence and distinguish the delivered molecular state.

Several observability limits remain. RNA stability measured by degradation or reporter persistence may differ from translation-competent RNA lifetime [4]. A mechanistic review of translation-coupled decay does not furnish a universal rate law for every cell type [17]. Folding-associated changes in one enzyme cannot be extrapolated to all secreted proteins, antigens, or intracellular replacements [19]. Mass-spectrometric coverage, differential extraction, fluorophore maturation, polysome traffic and time-point selection can all bias the inferred chain. The account predicts conditions warranting tests; it supplies neither numerical boundaries for sequence optimization nor validated clinical decision thresholds.

Finally, a ranked sequence design may be distinguishable in a cell assay while clinically unimportant once tissue distribution, exposure duration, and response saturation are considered. The proposed outcome hierarchy must be bounded by pharmacodynamic mode of action: catalytic proteins, cell-surface ligands, and vaccine antigens require different functional assays. Innate immune sensing, glycosylation, and adaptive immune responses may depend on host biology and formulation beyond the coding region. Those dependencies prohibit extrapolation from the present theoretical comparisons to patient-level effect size or therapeutic benefit.

CONCLUSION

Synonymous sequence design can alter the availability and decoding of therapeutic mRNA, yet these changes occupy different stages of active-product formation. Evidence supports context-sensitive links among RNA structure, turnover, translation and nascent-chain behavior; the extent to which their combined effects reorder therapeutic constructs by protein function remains unresolved. The central discriminating prediction is that a rank difference can persist after transcript exposure and full-length chain production are controlled, provided maturation or specific activity is altered. Conversely, convergence of function after those controls would narrow the mechanism to RNA availability or productive translation. Designing and evaluating therapeutic mRNA against the action required of its protein product is therefore a defensible development principle, while claims of improved clinical efficacy require separate evidence.

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