A Report on "A Small Polymerase Ribozyme That Can Synthesize Itself and Its Complementary Strand" by Gianni et al. (2026)

Reviewer 2

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> I am wiser than this person; for it is likely that neither of us knows anything fine and good, but he thinks he knows something when he does not know it, whereas I, just as I do not know, do not think I know, either. I seem, then, to be wiser than him in this small way, at least: that what I do not know, I do not think I know, either.
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> Plato, *The Apology of Socrates*, 21d

> To err is human. All human knowledge is fallible and therefore uncertain. It follows that we must distinguish sharply between truth and certainty. That to err is human means not only that we must constantly struggle against error, but also that, even when we have taken the greatest care, we cannot be completely certain that we have not made a mistake.
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> Karl Popper, 'Knowledge and the Shaping of Reality'

Overview

Citation: Gianni, E., Kwok, S. L. Y., Wan, C. J. K., Goeij, K., Clifton, B. E., Colizzi, E. S., Attwater, J., and Holliger, P. (2026). A Small Polymerase Ribozyme That Can Synthesize Itself and Its Complementary Strand. Unpublished paper.

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Abstract Summary: The paper describes the discovery of QT45, a small 45-nucleotide RNA polymerase ribozyme, which was found from random sequence pools and can catalyze general RNA-templated RNA synthesis using trinucleotide triphosphate substrates. This small ribozyme is capable of synthesizing both its complementary strand and a copy of itself, suggesting that polymerase ribozymes are more abundant in RNA sequence space than previously thought.

Key Methodology: De novo selection from random RNA sequence pools, in vitro evolution, RNA-templated RNA synthesis using triplet substrates in eutectic ice, deep sequencing, and computational modeling of the error threshold.

Research Question: Can RNA polymerase activity be encoded by shorter RNA motifs to reconcile the competing requirements of short length for emergence and complex activity for self-replication in the origin of life?

Summary

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Gianni et al. report the discovery of QT45, a 45-nucleotide RNA polymerase ribozyme selected from random sequence pools. The authors claim this small RNA motif can catalyze the synthesis of both its complementary strand and a copy of itself using trinucleotide triphosphate substrates in eutectic ice conditions. This finding challenges previous assumptions that RNA polymerase activity requires large, structurally complex ribozymes (typically 150–300 nucleotides) and suggests that such functional motifs may be more abundant in RNA sequence space than previously thought. The study also proposes a mechanism for self-replication that relies on modulating the equilibrium between folded (catalytic) and unfolded (template) RNA conformations through substrate concentration.

The most significant challenge to the claim of a "chemical system capable of self-replication" is the kinetic viability of the system described. The authors report synthesis yields of approximately 0.17% to 0.24% over a 72-day incubation period for the self-synthesis reactions (pp. 4–5). In contrast, they report a half-life for the ribozyme of approximately 117 days under standard reaction conditions (p. 2). A comparison of these rates indicates that degradation significantly outpaces synthesis; during the 72 days required to synthesize a minute fraction of new material, roughly 35% of the initial ribozyme population would degrade based on the reported half-life. Furthermore, the stability measurements were conducted in high-salt conditions (50 mM MgCl2), whereas the replication reactions required low-salt conditions (0.4 mM MgCl2) to function (p. 12, Supp. p. 8). Since RNA hydrolysis is often dependent on metal ion concentration, the actual stability during replication is uncertain. While the authors acknowledge that "further improvements in synthetic efficiency and fidelity [are] likely required to overcome chemical degradation" (p. 5), the data present a

system that is slowly decaying rather than proliferating.

There is also a statistical tension in the reported fidelity metrics. For the synthesis of a 33-nucleotide hammerhead ribozyme, the authors report that deep sequencing yielded "42.2% reads of perfect full-length seq0-HH products" (p. 3). However, they also estimate the "average per nucleotide fidelity... to be 92.6%" (p. 4). Assuming a uniform error distribution over the extension length (33 nucleotides), a 92.6% fidelity would theoretically result in only about 7.9% perfect full-length products. This discrepancy suggests that errors are likely not distributed uniformly or that there is a significant "survivorship bias," where sequences containing errors stall and fail to reach full length. The authors note this stalling effect can have a "purifying effect" (p. 4). While this mechanism improves the quality of the final product, it implies that the intrinsic fidelity of the polymerase may be lower than the final product analysis suggests and that the effective yield is severely constrained by the stalling of error-prone sequences.

The evolutionary potential of the system is explored through a fitness landscape and quasispecies model. It is important to note that the fitness values used in this model are derived from a proxy activity—primer extension on a short, simple template—rather than a direct measure of self-replication fitness (p. 3, Supp. p. 17). While the authors are transparent about this limitation, stating they use these values "despite the mismatch" (Supp. p. 17), it limits the strength of the conclusions regarding the error threshold and the maintenance of the ribozyme in a competitive environment.

Despite these kinetic and statistical issues, the core discovery of QT45 remains a significant contribution to the field. The demonstration that a 45-nucleotide RNA can perform the complex functions of primer-template binding, regiospecific catalysis, and general RNA synthesis refines the theoretical minimum size for an RNA polymerase. The authors' strategy of using substrate concentration to shift the thermodynamic equilibrium between the ribozyme's folded state and its template state offers an experimentally validated solution to the "paradoxical requirements" of RNA self-

replication (pp. 1, 4). The work successfully establishes that the catalytic capacity for replication can be encoded in a small motif, even if the specific system described has not yet closed the kinetic loop required for self-sustained evolution.

The Bottom Line

Gianni et al. convincingly demonstrate that a small, 45-nucleotide RNA motif can catalyze the synthesis of its own complementary strand and a copy of itself, significantly reducing the size complexity previously thought necessary for RNA polymerase activity. However, the system is not yet a self-sustaining replicator; the rate of RNA synthesis is orders of magnitude slower than the rate of degradation, resulting in a net loss of genetic material over time. Additionally, statistical discrepancies in fidelity metrics and the reliance on proxy fitness measures suggest that the evolutionary dynamics of this system may be more precarious than the models imply.

Potential Issues

Kinetic inviability due to low synthesis yield and high degradation rate: The paper's central claim of demonstrating the components of a self-replication cycle is undermined by a significant kinetic problem: the rate of RNA synthesis appears to be orders of magnitude lower than the rate of RNA degradation. The study reports synthesis yields of approximately 0.17–0.24% over 72 days for the two strands of the ribozyme (pp. 4–5). In contrast, it reports a half-life for the ribozyme of approximately 117 days under "standard reaction conditions" (p. 2, Supp. p. 26). A simple calculation shows that in 72 days, approximately 34.6% of the initial ribozyme population would degrade. This means that for every 10,000 ribozymes, roughly 3,460 would degrade while only about 17–24 new copies are synthesized, resulting in a catastrophic net loss of information. This discrepancy is complicated by the fact that the half-life was measured in a high-salt buffer (50 mM MgCl2), whereas the self-replication reactions were performed in a low-salt buffer (0.4 mM MgCl2) required for catalytic activity (p. 12, Supp. p. 8). While lower magnesium concentrations generally increase the chemical stability of RNA by slowing hydrolysis, the use of different conditions for the stability and activity assays means the reported half-life may not accurately reflect the rate of degradation during the replication experiment. The authors acknowledge this limitation, stating that "further improvements in synthetic efficiency and fidelity [are] likely required to overcome chemical degradation and achieve self-sustained replication" (p. 5). However, the sheer magnitude of the kinetic gap suggests that the current system is not merely inefficient but fundamentally non-viable as a self-sustaining replicator.

Statistical discrepancy in fidelity metrics: There is an apparent statistical tension between two key metrics reported for the synthesis of a 33-nucleotide hammerhead ribozyme. The paper reports that deep sequencing yielded "42.2% reads of perfect full-length seq0-HH products" (p. 3). From this same data, it estimates the "average

per nucleotide fidelity for full-length product synthesis by QT45 to be 92.6%" (p. 4). These two figures are difficult to reconcile under a simple model of uniform error distribution. The synthesis involves extending a primer using 11 trinucleotide substrates, meaning 33 nucleotides are synthesized (Fig. 3B, C, p. 11). Assuming errors occur independently, the expected fraction of perfect products would be 0.926^{33}, which is approximately 7.9%. This is substantially lower than the reported 42.2%. Conversely, to achieve 42.2% perfect products over 33 nucleotides, the implied per-nucleotide fidelity would need to be approximately 97.4% ($0.422^{(1/33)}$). While a non-uniform error distribution could potentially explain this discrepancy, the text does not provide such an explanation. This ambiguity makes the "average" fidelity metric difficult to interpret and potentially weakens the quantitative basis of the subsequent error threshold analysis.

Use of a proxy for self-replication fitness in the evolutionary model: The paper presents a detailed fitness landscape and a quasispecies model to explore the evolutionary dynamics of the QT45 ribozyme (pp. 3, 5). However, the fitness values used in this model are not derived from a direct measure of self-replication fitness. Instead, they are based on the ribozyme's ability to perform primer extension on a short, simple, and unrelated template ("a template encoding 3 UGC triplets," p. 3). Fitness in a true self-replication context depends on a more complex set of properties—including the ability to fold correctly, bind its own complement, process a structured template, and release the product—that are not captured by this simple proxy. The authors are transparent about this limitation in the supplementary text, stating that they use these fitness values "despite the mismatch, as they are the only quantitative data we have for this large number of genotypes" (Supp. p. 17). While this transparency is commendable, the mismatch between the measured activity and the modeled process remains a central caveat for the conclusions drawn from the model regarding the system's evolutionary potential and error threshold.

Potential overestimation of fidelity due to survivorship bias: The reported fidelity

of the ribozyme may be an overestimation due to a "survivorship bias" in the population of molecules that reach full length. The authors note that stalling of synthesis after a misincorporation can have a "purifying effect," leading to "an improved overall fidelity of full-length products at the expense of synthetic yield" (p. 4). Given the extremely low final yields (~0.2%), this trade-off could be a dominant feature of the system. It is possible that the ribozyme's intrinsic error rate is much higher, but that the vast majority of error-containing strands stall and are never detected as full-length products. The small fraction of strands that do finish would therefore represent a heavily biased population of "survivors." While the authors acknowledge the stalling effect, the full implication—that the true catalytic efficiency and intrinsic fidelity of the system might be significantly lower than the reported values—is a key consideration for the system's viability.

Lack of robustness testing: The self-replication reactions were demonstrated under a single, highly optimized set of conditions, including specific concentrations of RNA, substrates, salts, and pH, all conducted in eutectic ice for a fixed duration (p. 12). For the findings to be broadly relevant to the variable and heterogeneous environments of the early Earth, the system would need to exhibit some degree of robustness to changes in these parameters. The paper does not explore how the system performs outside this narrow experimental window. This makes it difficult to assess whether the observed activity is a fragile laboratory phenomenon or a more generalizable process that could plausibly have occurred under prebiotic conditions. The authors do acknowledge that "further improvements" are needed to achieve a self-sustaining system, which would implicitly include the need for greater robustness (p. 5).

Minor presentation and comparison issues: There are minor issues in the presentation of data that make direct comparisons and a full assessment of the system's performance more difficult. First, the paper's comparisons to the benchmark 5TU ribozyme may be confounded by the use of conditions that are suboptimal for the

benchmark. For instance, the fidelity of 5TU was measured at pH 9, which the authors note is different from the pH 8.3 conditions in which it was evolved and likely contributes to its lower-than-expected performance (p. 4). Second, the paper provides an inconsistent level of detail for the fidelity of the two self-synthesis reactions. For the synthesis of the complementary (-) strand, it reports both the percentage of perfect products and a per-nucleotide fidelity (p. 4), but for the more complex (+) strand synthesis, it reports only the percentage of perfect products (p. 5). While the authors are transparent about the conditions and provide a valid raw metric, these presentational choices complicate a direct comparison of the system's accuracy across its full cycle.

Future Research

Kinetic optimization: Future work should focus on bridging the gap between synthesis and degradation rates. This could involve screening for variants with faster catalytic rates or identifying reaction conditions that further stabilize the RNA against hydrolysis without inhibiting catalytic activity, perhaps by exploring alternative eutectic phases or co-solutes that might exist in prebiotic environments.

Direct fitness assays: To validate the evolutionary models, research should move beyond proxy measures and attempt to measure fitness in a true replication context. This could involve competitive replication assays where variants must complete a full replication cycle to be detected, thereby capturing the complex trade-offs between folding, template accessibility, and product release that are not captured by simple primer extension assays.

Robustness testing: The current system relies on highly specific, optimized conditions. Future studies should test the QT45 ribozyme's activity across a broader range of pH, temperature, and ionic concentrations to determine if this catalytic motif is robust enough to function in the heterogeneous and variable environments plausible on the early Earth.

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