A Report on "Cleavage of mRNAs by a Minority of Pachytene piRNAs Improves Sperm Fitness" by Cecchini 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: Cecchini, K., Zamani, M., Ajaykumar, N., Vega-Badillo, J., Bagci, A., Bailey, S., Zamore, P. D., and Gainetdinov, I. (2026). Cleavage of mRNAs by a Minority of Pachytene piRNAs Improves Sperm Fitness. *Nature*, pp. 1-9.

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Abstract Summary: Most mouse pachytene piRNAs are 'selfish' and promote their own production, but a minority reduce target mRNA abundance via endonucleolytic cleavage, which is essential for male fertility. This explains the lack of conservation of most piRNA sequences, as the non-functional majority persists because the functional minority supports sperm fitness.

Key Methodology: Mouse genetics, sperm functional assays, various RNA sequencing methods (small RNA-seq, RNA-seq, ribosome footprinting, GRO-seq), in vitro cleavage assays.

Research Question: What is the biological function and mechanism of action of pachytene piRNAs, especially given their rapid sequence divergence?

Summary

Is It Credible?

Cecchini et al. present a provocative challenge to the assumption that the massive repertoire of pachytene piRNAs in mammals serves a collective biological purpose. Instead, they propose a "selfish" model, arguing that the vast majority (approximately 99%) of these small RNAs are non-functional byproducts of a system maintained only because a "tiny minority" (around 1%) are essential for sperm fitness (p. 1). The authors support this through an extensive combination of genetic knockouts, fertility assays, and molecular profiling, concluding that these functional piRNAs regulate targets "exclusively through endonucleolytic cleavage" (p. 3). While the study provides robust evidence for the redundancy of piRNA loci and the mechanism of target slicing, the broader evolutionary conclusions rely heavily on negative evidence and specific detection thresholds.

The characterization of 99% of piRNAs as "selfish" or non-functional is an interpretive narrative that may outpace the data. This quantitative conclusion rests on the identification of cleavage products; however, the authors acknowledge that for low-abundance transcripts, cleavage products are "probably too low to permit detection" (p. 5). Consequently, the "1%" figure represents a technical lower bound rather than a definitive biological census. It is plausible that a larger fraction of piRNAs regulate targets that simply fall below the sensitivity of the current assays. Therefore, while the data supports the idea that *many* piRNAs lack obvious targets, the leap to a "selfish" evolutionary model is a theoretical extrapolation. The rapid divergence of piRNA sequences supports the idea of weak selective pressure, but it does not strictly prove the "selfish" hypothesis over alternative explanations, such as a system that is inherently noisy by design (p. 6).

The study's mechanistic claims are generally strong but perhaps overstated in

their exclusivity. The demonstration that piRNAs direct cleavage (slicing) is well-supported by the detection of 5′-monophosphorylated fragments and *in vitro* assays (p. 5). However, the assertion that they regulate targets "exclusively" via this mechanism and "neither activate nor repress mRNA translation" is a strong negative claim based on the analysis of a subset of predicted targets (pp. 1, 3). While the authors failed to find evidence for miRNA-like destabilization or translational regulation in their specific candidates, this does not definitively rule out these mechanisms for the entire repertoire, particularly given the complexity of RNA regulation (p. 7).

Finally, the genetic evidence for redundancy among piRNA loci is compelling, particularly the finding that single mutants have mild phenotypes while double and triple mutants exhibit severe fertility defects (p. 2). A minor methodological caveat exists regarding the *pi2* locus. While the authors rigorously used two independent alleles to control for off-target effects in *pi9* and *pi17* mutants, they generated only one allele for the *pi2* mutant (p. 14). As the *pi2*-deficient triple mutant showed the most severe phenotype (near sterility), the lack of a second allele control for this specific locus introduces a degree of uncertainty regarding whether the intensified defect is solely due to the loss of *pi2* piRNAs or potential off-target CRISPR effects. Despite these limitations, the core finding that a minority of piRNAs are critical for spermatogenesis appears credible.

The Bottom Line

Cecchini et al. convincingly demonstrate that mammalian pachytene piRNA loci are functionally redundant and that endonucleolytic cleavage is a primary mechanism of action for the essential subset of these RNAs. However, the headline conclusion that 99% of the repertoire is "selfish" junk is likely an overstatement derived from the sensitivity limits of current detection methods. Readers should view the "selfish"

model as a compelling theoretical framework rather than a proven biological fact.

Potential Issues

The "selfish" piRNA model is a speculative evolutionary narrative: The article's central conclusion is that a vast, non-functional majority of pachytene piRNAs are evolutionarily retained because their biogenesis is mechanistically linked to a tiny, essential minority that improves sperm fitness. This "selfish" or "piRNA addiction" model is a compelling interpretive framework, but it is presented as a conclusion rather than a hypothesis tested against a specific null model (p. 8). For instance, the data are also consistent with a simpler model where the piRNA biogenesis system is inherently noisy, producing vast numbers of non-functional transcripts as byproducts, a small fraction of which happen to acquire a function by chance. The article's evolutionary argument—that piRNAs capable of altering target abundance are rare because they are "deleterious and are removed through purifying selection"—is also a theoretical assertion to explain the observed rarity, rather than a conclusion based on direct evidence of purifying selection (p. 1). While proposing such models is appropriate for a discussion section, the strength of the evolutionary claims may exceed the direct evidence provided, which is based on a relatively small number of identified functional targets from which a model for the entire piRNA repertoire over millions of years is extrapolated (p. 4).

The number of functional piRNAs is likely underestimated due to technical detection limits: The article's quantitative argument rests on the finding that only a "tiny minority" (around 1%) of piRNAs have a detectable function (pp. 1, 5). This estimate is based on identifying the 5′-monophosphorylated cleavage products of target mRNAs. However, the study acknowledges that this method is insensitive to the cleavage of low-abundance transcripts, whose products would be too rare to be reliably detected. The authors note this limitation when discussing the `pi6` locus, stating that for some unexplained targets, their low abundance is "probably too low to permit detection of the short-lived cleavage products" (p. 5, Supplementary

Discussion p. 2). This means the reported number of functional piRNAs is a technical lower bound, not a definitive count. The true fraction of piRNAs with cleavage targets is likely higher than 1%, which would not invalidate the qualitative conclusion that most are non-functional, but it does introduce uncertainty into the precise proportions that underpin the "selfish" model.

Conclusions involving the `pi2` locus are potentially confounded by a lack of off-target controls: The study commendably controlled for off-target effects of Cas9 for the `pi9` and `pi17` loci by generating two independent alleles with different sgRNAs and considering only molecular changes observed in both. However, this critical control was not implemented for the `pi2` locus, which is a key component of the triple mutant (`pi2-/-pi9-/-pi17-/-`) that exhibits the most severe infertility phenotype. The article acknowledges this limitation in the Methods section, stating that "only one allele of pi2-/- was generated" (p. 14). Without this control, it is difficult to definitively attribute the molecular changes identified as specific to `pi2`, or the additive effect on fertility in the triple mutant, to the on-target deletion of `pi2` piRNAs rather than a potential off-target effect of the sgRNAs used (p. 4). While the findings are consistent with the more rigorously controlled data, this methodological gap weakens the certainty of conclusions that specifically rely on the `pi2` mutant.

The claim of exclusive regulation via cleavage may be an overstatement: The article concludes that pachytene piRNAs regulate their targets "mainly—perhaps exclusively—... through siRNA-like endonucleolytic cleavage" and finds no evidence for other proposed mechanisms like miRNA-like destabilization or translational activation (p. 8). While the evidence for cleavage is strong, the dismissal of all other possibilities may be too definitive. For example, the test for a miRNA-like mechanism was based on analyzing a set of 24 putative targets for the most abundant piRNAs and finding no effect (p. 7). While these were argued to be the most biologically plausible candidates, this analysis does not exhaustively rule out the possibility that other piRNAs could act via this or other mechanisms under different

pairing rules or in different contexts. The use of strong words like "exclusively" may therefore overstate the certainty of the findings (p. 8).

Analytical choices and presentation issues: Several minor issues related to analytical transparency and presentation appear in the article. First, the analysis relies on several numerical thresholds—such as the 1.25-fold change to classify targets as regulated and the 8-fold decrease to identify cleavage products—that are presented without explicit justification or sensitivity analysis (pp. 4–5). While using such cutoffs is standard practice, the results are contingent on these specific choices. Second, the article presents different sample sizes for its core sequencing experiments, which, while not contradictory, could be clarified. For instance, the standard RNA-seq analysis in Figure 2 used varying sample sizes for mutant groups (ranging from n=7 to n=10) compared to n=7 for controls, whereas the 5'-monophosphate RNA-seq analysis (Figure 3) relied on permutations of 4 control and 4 mutant replicates. These represent distinct experiments, but the rationale for the different sample sizes is not provided. Third, the method for calculating the total number of "piRNAs removed" in combined mutants (Fig. 1a) is not documented in the main text or figure caption, leaving the origin of these numbers unclear. These issues do not invalidate the study's main conclusions but represent minor gaps in methodological transparency and presentation.

Future Research

Validation of the "selfish" hypothesis: Future work could empirically test the "selfish" model by engineering a mouse strain where the sequences of the putative non-functional majority (the 99%) are scrambled or deleted, while preserving the sequences of the identified functional minority (the 1%) and the overall architecture of the piRNA clusters. If the "selfish" model holds, such a mouse should remain fertile; if fertility is compromised, it would suggest that the "junk" piRNAs play a collective structural or regulatory role that is currently unappreciated.

High-sensitivity target detection: To address the potential underestimation of functional piRNAs due to detection limits, researchers could employ targeted degradome sequencing or ligase-mediated approaches specifically designed to capture low-abundance 5′-monophosphorylated cleavage products. This would help determine whether the "1%" figure is a biological reality or a technical artifact, providing a more accurate census of the functional piRNA repertoire.

Rigorous control for `pi2`: To definitively confirm the contribution of the `pi2` locus to the severe infertility phenotype observed in the triple mutant, a second, independent `pi2` knockout allele should be generated using distinct guide RNAs. This would rule out the possibility of off-target effects confounding the interpretation of the additive genetic interactions reported in this study.

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