



How Do New Immune-Response Genes Arise? Inflammation-Induced Trans-Splicing as a Source of Functional Innovation

Abdolkarim Sheikhi ^{1,2,*}, Myron R. Szewczuk ²

¹Department of Immunology, Dezful University of Medical Sciences, Dezful, Iran

²Department of Biomedical & Molecular Sciences, Queen's University, Kingston, 64616 - 43993, Canada

*Corresponding Author: Department of Immunology, School of Medicine, Dezful University of Medical Sciences, Dezful, Iran. Email: sheikhi@queensu.ca

Received: 11 September, 2026; Accepted: 13 September, 2026

Keywords: Immune Response Genes, Trans-Splicing, Chimeric RNA, Inflammation-Induced Interchromosomal Contacts

Dear Editor,

Does the emergence of a new gene product necessarily require mutation, gene duplication, or structural rearrangement of DNA? A recent study by Venezia and colleagues, published in *Nature*, offers an unexpected answer: mammalian cells can generate a functional protein with a novel architecture by joining transcripts derived from two separate genes without creating a new genomic locus (1). This finding expands the protein-coding capacity of the genome from a fixed catalog of genes into a dynamic system influenced by cellular state and the three-dimensional organization of chromatin.

Under the classical model of gene expression, each messenger RNA originates from a single genomic locus, whereas protein diversity is generated primarily through alternative cis-splicing of exons belonging to that gene. Trans-splicing—the joining of sequences from separate precursor RNAs—has long been recognized in several unicellular organisms and invertebrates, and isolated examples have also been reported in healthy mammalian cells (2, 3). Nevertheless, the physiological relevance of mammalian chimeric RNAs has remained controversial. Reverse transcriptases can switch templates during complementary DNA synthesis and thereby generate artificial fusion transcripts. Moreover, it has been unclear whether naturally occurring chimeric RNAs are translated into stable proteins with biological functions or merely represent transcriptional noise.

To address these limitations, Venezia et al. used nanopore-based direct RNA sequencing, which reads native RNA molecules without prior conversion to complementary DNA. Their findings were subsequently

assessed using short-read RNA sequencing, NanoString analysis, junction-specific polymerase chain reaction, and Sanger sequencing. This integrated approach identified 30,390 candidate chimeric RNAs across different activation states of mouse macrophages, approximately 88% of which involved genes located on different chromosomes (1). Importantly, the limited sequencing depth and modest agreement among computational detection tools mean that this number should not be interpreted as representing 30,390 confirmed functional transcripts. Only a subset was supported by orthogonal validation.

The most compelling functional example was *Gsdmd-Tmem106a*, a chimeric transcript generated through the precise joining of *Gsdmd* exon 2 to *Tmem106a* exon 6. Its expression increased following macrophage stimulation with lipopolysaccharide and peaked after approximately six hours. The same junction was detected in several genetically distinct mouse strains. The transcript was also induced in vivo in lung macrophages following influenza A infection, in brain macrophages and microglia during *Escherichia coli* meningitis, and in peritoneal macrophages following lipopolysaccharide administration (1).

Chromatin-conformation analyses indicated that inflammation increased physical contacts between chimeric-RNA parent genes located on different chromosomes. These interactions depended on the genome-organizing protein CTCF. Depletion of CTCF reduced both inflammation-induced interchromosomal contacts and *Gsdmd-Tmem106a* formation without comparably suppressing the expression of either parent gene. Inhibition of the canonical spliceosome also abolished formation of the chimeric transcript. Together,

these observations support a model in which inflammatory signaling reorganizes chromatin architecture, brings selected genes into spatial proximity, and enables their nascent transcripts to undergo regulated trans-splicing.

The biological importance of this process became clearer when the investigators demonstrated that *Gsdmd-Tmem106a* encodes a stable protein. The *Tmem106a*-derived sequence is translated in an alternative reading frame, producing a novel C terminus absent from the annotated mouse proteome. The resulting GSDMD-TMEM106A protein localizes to the plasma membrane and, following inflammasome activation, interacts with the canonical N-terminal fragments of gasdermin D.

Gasdermin D is a central executor of pyroptosis. Its cleavage by inflammatory caspases releases an N-terminal pore-forming fragment that oligomerizes in cellular membranes, permitting the release of interleukin-1 β and interleukin-18 and ultimately promoting inflammatory cell death (4,5). GSDMD-TMEM106A does not replace canonical gasdermin D or independently initiate pore formation. Instead, it acts as a membrane-localized cofactor that accelerates the recruitment and assembly of GSDMD N-terminal fragments. Selective loss of the chimeric protein reduced early cytokine release and slowed membrane permeabilization and pyroptosis without abolishing these processes. Thus, the protein appears to amplify, rather than enable, the canonical gasdermin pathway.

The in vivo findings reveal the double-edged nature of this mechanism. Mice unable to produce functional GSDMD-TMEM106A developed greater bacterial burdens in the liver and spleen after infection with *Salmonella* Typhimurium. Conversely, these animals were protected against hypothermia and mortality following high-dose lipopolysaccharide administration. Chimeric mRNA overexpression produced reciprocal effects: it improved antibacterial defense but intensified systemic inflammation and lethality after lipopolysaccharide exposure. GSDMD-TMEM106A therefore appears to regulate a biological trade-off between rapid pathogen control and potentially fatal immunopathology.

Two issues must be considered before translating these findings into clinical applications. First, the specific GSDMD-TMEM106A transcript was not detected in the human monocyte-derived macrophages examined in this study. Although more than 900 human chimeric RNAs were identified and 33 parent-gene pairs were shared between mice and humans, the translation and biological activity of most human candidates remain unknown. It would therefore be premature to regard GSDMD-TMEM106A itself as a therapeutic target in human

inflammatory disease. Future investigations should prioritize the identification and functional validation of inflammation-regulated chimeric proteins in human cells and patient samples.

Second, the lethal inflammatory model was induced by high-dose lipopolysaccharide and more closely represents experimental endotoxemia than the heterogeneous syndrome of human sepsis. It does not reproduce all clinically relevant features of infection-driven sepsis, including live pathogen replication, interpatient variability, organ-specific responses, and the transition from hyperinflammation to immunosuppression. Validation in infection-based and polymicrobial models, followed by studies in human sepsis cohorts, will be necessary to establish clinical relevance.

Despite these limitations, chimeric RNAs offer an intriguing therapeutic opportunity. Their unique fusion junctions could potentially be targeted using junction-specific small interfering RNAs or antisense oligonucleotides, enabling selective inhibition of the inflammatory chimera while preserving the canonical parent proteins. However, the timing of such an intervention would be critical. Early inhibition might impair pathogen clearance, whereas later inhibition during uncontrolled inflammation might reduce tissue injury and mortality.

The answer to the question posed in the title therefore requires an important distinction: the study does not demonstrate the birth of a new gene at the DNA level. Rather, it reveals how inflammation can generate a new, context-dependent gene product through transient genome reorganization and regulated trans-splicing. This observation invites us to move beyond the conventional question of how many genes the genome contains and instead ask how many functional proteins a cell can assemble from existing genes under different physiological conditions. Answering this broader question may uncover an unexplored layer of immune regulation and identify new mechanisms underlying infection, autoinflammatory disease, and sepsis.

Footnotes

AI Use Disclosure: The authors declare that no generative AI tools were used in the creation of this article.

Authors' Contribution: Conceptualization: A. S. and M. R. S.; Investigation: A. S.; Writing – Original Draft Preparation: A. S.; Writing – Review & Editing: A. S. and M. R. S.; All authors have read and agreed to the published version of the manuscript.

Conflict of Interests Statement: The authors declare no conflict of interest.

Funding/Support: No specific funding was received for this work.

References

1. Venezia O, Kane H, Du G, Coughlan HD, Johanson TM, Wang H, et al. Functional chimeric mRNAs encode proteins in mammalian immunity. *Nature*. 2026. [PubMed ID: 42686912]. <https://doi.org/10.1038/s41586-026-10982-x>.
2. Li H, Wang J, Mor G, Sklar J. A neoplastic gene fusion mimics trans-splicing of RNAs in normal human cells. *Science*. 2008;**321**(5894):1357-1362. [PubMed ID: 18772439]. <https://doi.org/10.1126/science.1156725>.
3. Singh S, Qin F, Kumar S, Elfman J, Lin E, Pham LP, et al. The landscape of chimeric RNAs in non-diseased tissues and cells. *Nucleic Acids Res*. 2020;**48**(4):1764-1778. [PubMed ID: 31965184]. [PubMed Central ID: PMC7038929]. <https://doi.org/10.1093/nar/gkz1223>.
4. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature*. 2015;**526**(7575):660-665. [PubMed ID: 26375003]. <https://doi.org/10.1038/nature15514>.
5. Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H, et al. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. *Nature*. 2016;**535**(7610):153-158. [PubMed ID: 27383986]. [PubMed Central ID: PMC5539988]. <https://doi.org/10.1038/nature18629>.