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RNA "COURIERs": Enabling synthetic cell-to-cell communication in human cells

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The development of molecular couriers to selectively package, export, and recover RNA molecules within human cells is a significant challenge. In this issue of *Cell*, Horns et al.[1] introduce cellular RNA exporters, termed COURIERs, that package, secrete, and protect RNA cargo and establish the foundation for sophisticated cell-to-cell RNA communication.

The capability to sample and measure RNA within live cells in real time is a valuable asset that can allow immediate insights into the gene expression patterns within cells. Real-time RNA analysis opens new possibilities for studying cellular responses, identifying key regulatory mechanisms, and developing targeted interventions. In this issue of *Cell*, Horns et al. describe the development of a novel programmable RNA export system that can be used to selectively package, protect, and secrete RNA molecules in human cells[1] (Figure 1).

To engineer RNA COURIERs (controlled output and uptake of RNA for interrogation, expression, and regulation), the authors drew inspiration from viruses, which have evolved naturally to efficiently transport RNA. Viruses are composed of a viral genome enclosed by a protective capsid protein coat. To ensure survival and proliferation, the capsid must fulfill three essential roles: it needs to capture and package the viral genome efficiently, safeguard the nucleic acid content from environmental factors, and facilitate the entry and release of the genome into the cytoplasm of a host cell.

Using these key principles, Horns et al. optimized an RNA exporter derived from Moloney-murine leukemia virus (MMLV) capsid protein. Their initial step was to define the specificity of the RNA capture using the well-studied MS2 bacteriophage coat protein (MCP) system. To

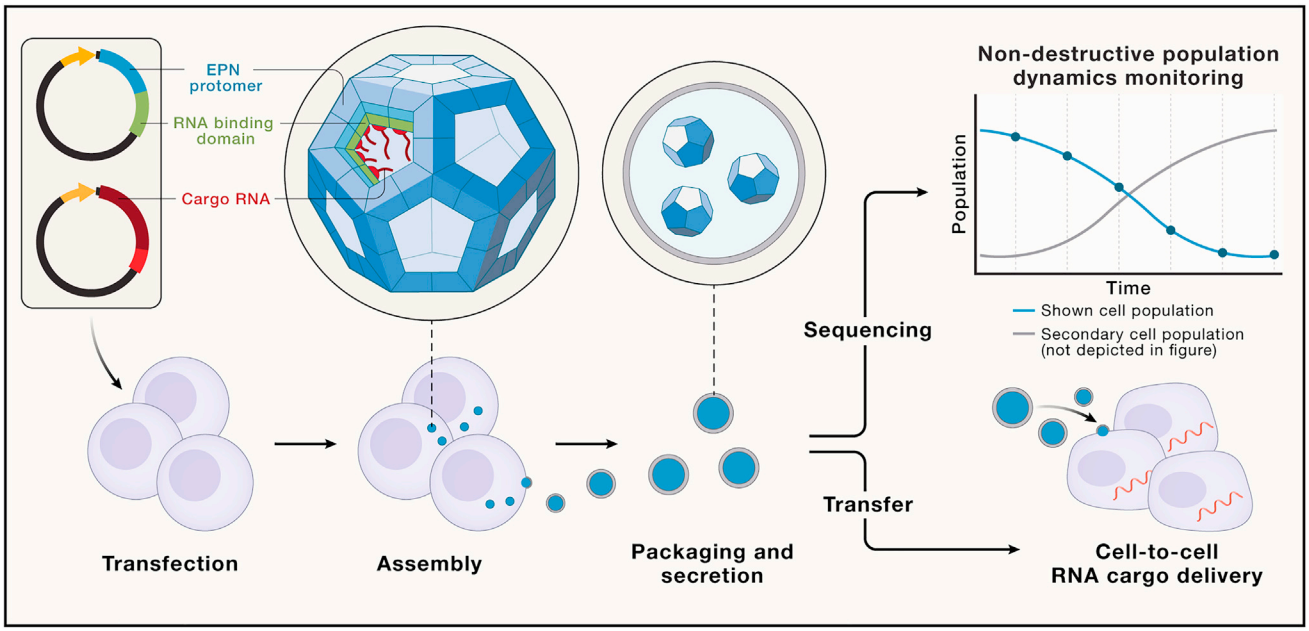

Figure 1. COURIER: Controlled output and uptake of RNA for interrogation, expression, and regulation

The vectors encoding engineered nanocage protomer and cargo RNA are transfected into cells. The protomers self-assemble to form a dodecahedral structure with cavities that accommodate RNA-binding protein and the RNA cargo. The nanocages are then packaged and secreted in extracellular vesicles. Horns et al. isolated the vesicles to track clonal abundance over time by sequencing the barcode RNA cargo and to achieve cell-to-cell delivery of the mRNA transcript by transferring conditioned media directly to recipient cells.

facilitate engineering, the authors then switched to the human immunodeficiency virus (HIV) capsid, which enabled them to replace the nucleocapsid domain with a leucine zipper homo-oligomerization domain to prevent nonspecific RNA packaging. The optimized viral RNA COURIER was capable of transporting desired RNA with undetectable levels of nonspecific export activity while protecting the payload from RNases.

As a first demonstration of the technology, the authors studied cell population dynamics via longitudinal sampling of the exported RNA. Two cell populations, resistant to two different antibiotics, were each labeled with MS2-tagged barcoded libraries and co-cultured under antibiotic selection. Horns et al. found that the exported RNA can accurately and reproducibly capture the changes in the clonal abundances.

While the exporters derived from retroviral capsids indeed facilitated enrichment of the target RNA, the authors noted that their attempts to increase the specificity simultaneously reduced the on-target binding efficiency. To resolve this issue, Horns et al. turned to enveloped protein nanocages (EPNs).[2,3] EPNs are designed and built using custom synthetic protein building blocks and exhibit a cage-like structure with a hollow interior, providing a confined space for various functional payloads, such as nucleic acids, drugs, imaging agents, and other active molecules. In EPNs, the protein nanocage is comprised of protomer subunits that self-assemble into a highly ordered dodecahedral structure with large internal cavities. Both the N and Cтermini of the nanocage protomer are amenable to fusions with protein domains. Additionally, they are engineered to be highly stable and resistant to various environmental conditions and can be secreted from mammalian cells as extracellular vesicles.[4] This unique combination of properties allows EPNs to provide a robust scaffold for RNA export.

Horns et al. designed the next iteration of RNA COURIERs by fusing functional domains to the N and C termini of the core I3-01 self-assembling nanocage scaffold. The authors fully capitalized on the modular nature of the EPN protomer, experimenting with an array of constructs with a membrane-binding domain ($Gag_{2–6}$, $Lyn_{2–13}$, $PLC\delta_{11–140}$), an endosomal sorting recruiting element (HIV-1 $p6_{Gag}$ peptide), and an RNA-binding domain (MCP) in different arrangements. The final design, EPN24-MCP, achieved RNA export efficiency comparable to that of a retroviral capsid with the highest on-target enrichment rate (MMLV-Gag) while also matching the negligible off-target export rate of the optimized viral RNA COURIER (GagZip-MCP).

The EPN nanocages, similar to the viral RNA COURIERs, are expelled via endosomal sorting complexes required for transport (ESCRT) machinery. The authors further manipulated this pathway to influence the RNA export rate. Intriguingly, the modulators involved in the budding of extracellular vesicle (CIT and NEDD4L) were found to enhance the export rate.

To achieve cell-to-cell delivery of RNA cargo, Horns et al. co-transfected viral fusogens with EPN24-MCP to facilitate fusion to the recipient cells. To test the efficiency of delivery, the mRNA transcript coding for Cre recombinase was tagged with MS2 for packaging in sender cells. Upon transferring the conditioned media of the sender cells to the recipient cells harboring a Cre-activable fluorescent reporter, the authors observed reporter activation comparable to direct mRNA

transfection. They also demonstrated that multiple cargos can be delivered simultaneously by packaging two distinct fluorescent reporter mRNA molecules and showing strong co-expression of the reporters upon delivery.

The study by Horns et al. stands out as a pioneering endeavor in the creation of a synthetic RNA export/delivery system, representing a significant leap forward in the field of bioengineering, where RNA is gaining momentum as a potent therapeutic agent. To further advance this technology, future research should focus on evaluating the compatibility of the RNA COURIER system with a wide range of RNA-based molecules, with particular attention to cargo stability, loading capacity, and delivery efficacy. Furthermore, it is crucial to assess the performance in various *in vitro* and *in vivo* models while also exploring options for engineering EPNs to enhance delivery and specificity properties.

Moreover, the analysis of exported RNA opens exciting new avenues for investigating intricate cell-to-cell interactions and population dynamics. By harnessing the power of the RNA COURIER system, researchers can gain valuable insights into the impact of clonal differences on global gene expression and cellular phenotypes, with direct impact on basic biology and therapeutics. This technology can redefine cell-based therapies by delivering cells preloaded with therapeutic RNAs that continuously regulate the transcriptome of target cells and their local environment. Adjusting the RNA landscape in tissues will also unravel a range of basic biology questions related to development and adaptation to environmental cues.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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