can be screened. Substantially increasing genome coverage will require the use of target sequences that can be edited relatively efficently (for example, 30%)—so that relatively low-depth sequencing per sgRNA can determine the editing efficiencies—in addition to using a large number of cells and high-depth sequencing.

Finally, more cell types and target sequences could be examined. The new studies[1–3] conducted Repair-seq in a few immortalized cell lines at one or four target sequences per genome editor. Exploring more cell types, including primary cultured cells and cells in animals, at more target sequences would identify editing determinants in diverse genetic backgrounds and in the context of different transcriptional profiles. ❐

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References

1. Koblan, L. W. et al. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-021-00938-z (2021).
2. Hussmann, J. A. et al. *Cell* **184**, 5653–5669 (2021).
3. Chen, P. J. et al. *Cell* **184**, 5635–5652 (2021).
4. Komor, A. C. et al. *Nature* **533**, 420–424 (2016).
5. Anzalone, A. V. et al. *Nature* **576**, 149–157 (2019).
6. Kim, H. K. et al. *Nat. Methods* **14**, 153–159 (2017).
7. Arbab, M. et al. *Cell* **182**, 463–480 (2020).
8. Song, M. et al. *Nat. Biotechnol.* **38**, 1037–1043 (2020).
9. Gilbert, L. A. et al. *Cell* **154**, 442–451 (2013).
10. Gilbert, L. A. et al. *Cell* **159**, 647–661 (2014).
11. Nelson, J. W. et al. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-021-01039-7 (2021).

Check for updates

RNA THERAPEUTICS

RNA delivery with a human virus-like particle

RNA cargo is transferred into cultured cells using a fully human delivery system.

Anna Gutkin, Daniel Rosenblum and Dan Peer

RNA is emerging as a powerful therapeutic modality in applications ranging from vaccines to protein replacement therapies. Yet in many applications beyond vaccines, a central obstacle to clinical development is the lack of efficient methods to deliver RNA to specific tissues and cells. In a recent paper in *Science*, Segel et al.[1] report a novel RNA delivery strategy that is borrowed from the human genome. The approach uses a protein derived from a human retrovirus with the rare capacity to package its RNA and transport it outside the cell in virus-like particles (VLPs). The authors show that their approach, called 'selective endogenous encapsidation for cellular delivery' (SEND), enables delivery of exogenous mRNA cargos, such as Cre and Cas9, into cells in vitro without the use of non-human components. Although this delivery strategy is still in its infancy, as a fully human system it may prove to be a safer alternative to current methods.

Currently, the most widely used RNA delivery method is lipid nanoparticles made from natural and synthetic amino ionizible lipids. Lipid nanoparticles fueled the remarkable success of the SARS-CoV-2 mRNA vaccines, but for other applications they have several shortcomings. These include uncertainty about their safety and efficacy for repeated dosing and for crossing biological barriers to target specific cell types.

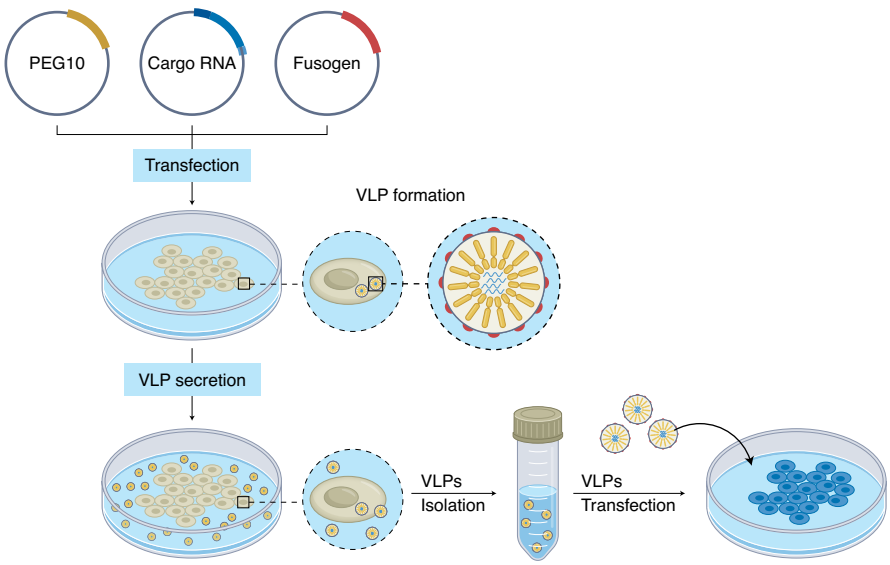

Fig. 1 | Delivery of mRNA cargo by virus-like particles derived from endogenous retroelements. Schematic of the 'selective endogenous encapsidation for cellular delivery' (SEND) system. PEG10, cargo RNA and fusogen vectors are transfected into cells. Inside cells, the PEG10 proteins pack the cargo mRNA and assemble into virus-like particles (VLPs) that are secreted to the growth medium in extracellular vesicles. The medium is then collected and the VLPs are isolated by ultracentrifugation. Finally, the target cells are transfected with the VLPs. Portions of this figure were created with BioRender.com.

Virus sequences incorporated throughout the human genome raise the tantalizing possibility that their natural functions could be harnessed to deliver therapeutic RNA. Retroelements account for about 8% of the human genome[2]. Although most

endogenous retroviral genes have lost their functions, some continue to have roles in human physiology. Several retroelements have been reported to retain some of their ancient functionality, such as binding and transferring mRNA and forming capsids within the cell[2].

To find candidate retroelement genes suitable for RNA delivery, Segel et al.[1] surveyed conserved endogenous retroelements, focusing on homologs of structural retroviral Gag proteins that contain the core capsid domain. This domain protects the genome of both retrotransposons and retroviruses by forming VLPs, suggesting that proteins that contain it might be able to transfer other RNAs. The authors narrowed down their search to proteins that are conserved between human and mouse and have detectable RNA levels, because such proteins are more likely to have retained some functionality in mammalian cells. They screened their leading hits in bacteria and mammalian cells to determine whether they are secreted in extracellular vesicles as VLPs. The protein most highly enriched in the VLP fraction was mouse (*Mus musculus*) PEG10, which is also detected at appreciable levels in mouse serum. Moreover, the VLPs formed by the PEG10 protein contained the full-length *Peg10* mRNA transcript.

To investigate whether these mouse PEG10 VLPs could incorporate unrelated RNAs, Segel et al.[1] flanked a Cre recombinase coding sequence with *Peg10* 5′ and 3′ untranslated regions (UTRs), and co-transfected the construct together with PEG10 into Neuro2a mouse neuroblastoma cells. They also engineered the VLPs by adding the fusogen vesicular stomatitis virus envelope protein (VSVg) to facilitate cellular delivery. Strikingly, PEG10 VLPs with VSVg were secreted in extracellular vesicles and transferred the Cre mRNA into loxP–GFP cells (Fig. 1). This observation suggested that adding *Peg10* UTRs to the mRNA cargo enables the PEG10 VLPs to transfer an mRNA of choice, and that the viral fusogenic protein is required for cell entry. Human PEG10, similarly to the mouse ortholog, could form VLPs and transfer mRNA.

This combination of PEG10, modified mRNA and fusogen forms the SEND system. To make the system fully endogenous, Segel et al.[1] evaluated murine and human fusogens that might replace VSVg. They focused on syncytin, an endogenous fusogenic transmembrane protein that evolved from retroviral elements, which has been used to pseudotype lentiviruses for nucleic acid delivery. The authors found that the fusogenic syncytin proteins in mouse, SYNA and SYNB, had a similar expression pattern to mouse PEG10, and that mouse SYNA could successfully replace VSVg in the transfer of Cre mRNA to tail-tip fibroblasts. The human syncytins (ERVW-1 and ERVFRD-1) operate in a similar fashion, which establishes SEND as a fully human system for functional gene transfer, at least in vitro.

To test the modularity of SEND, the authors also used it to deliver the large SpCas9 mRNA and tested its functionality by evaluating gene disruption in Neuro2a mouse neuroblastoma cells constitutively expressing a single-guide RNA (sgRNA) against *Kras*. The SEND system delivered the Cas9 mRNA cargo and caused a remarkable 60% gene editing in the *Kras* locus in the recipient cells. However, SEND failed to deliver sgRNA cargo to Cas9-expressing cells. Therefore, the authors combined the sgRNA and Cas9 mRNA to create an all-in-one vector. This vector facilitated 30% *Kras* gene editing in Neuro2a cells using the mouse SEND system and 40% *VEGFA* gene editing in HEK293 cells using the human SEND system.

The study by Segel et al.[1] is notable as the first example of an endogenous system able to package, secrete and deliver specific mRNAs. Before practical uses can be envisaged, extensive further testing is needed. The SEND system was studied only in vitro, and it must be evaluated in vivo. As previously reported[3], mouse PEG10 has multiple roles in the placenta and neuronal development, and it is unknown whether adding external PEG10 protein might affect its native functions. Additional questions concern possible autoimmune responses when an endogenous protein is expressed in a different biological context, as well as biodistribution, toxicity, efficacy and scalability.

Future work should also determine how the SEND system compares to existing mRNA delivery systems, including the lipid nanoparticles used in SARS-CoV-2 vaccines[4,5] and many other approaches now in clinical testing[6]. It will be important to understand whether the system possesses intrinsic cell-type specificity and whether such specificity could be engineered. The next generation of lipid nanoparticles includes targeting strategies that have recently shown cell-type specificity, potent efficacy and safety in various animal models of inflammation, cancer and genetic disorders using mRNA alone or in combination with sgRNA to knockout cancer genes[7–10]. Nonetheless, the SEND system could become a safer and even more efficient alternative. After further development, it may have advantages in addressing biological questions, delivering vaccines and treating diseases, with particular relevance to chronic diseases that require lifelong therapies. ❐

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References

1. Segel, M. et al. *Science* **373**, 882–889 (2021).
2. Feschotte, C. & Gilbert, C. *Nat. Rev. Genet.* **13**, 283–296 (2012).
3. Ono, R. et al. *Nat. Genet.* **38**, 101–106 (2006).
4. Baden, L. R. et al. *N. Engl. J. Med.* **384**, 403–416 (2021).
5. Polack, F. P. et al. *N. Engl. J. Med.* **383**, 2603–2615 (2020).
6. Rosenblum, D., Gutkin, A., Dammes, N. & Peer, D. *Adv. Drug Deliv. Rev.* **154-155**, 176–186 (2020).
7. Kedmi, R. et al. *Nat. Nanotechnol.* **13**, 214–219 (2018).
8. Veiga, N. et al. *J. Control. Release* **313**, 33–41 (2019).
9. Rosenblum, D. et al. *Sci. Adv.* **6**, eabc9450 (2020).
10. Dammes, N. et al. *Nat. Nanotechnol.* **16**, 1030–1038 (2021).

Competing interests

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