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An AAV library for retinal cell expression in mouse, macaque, and man

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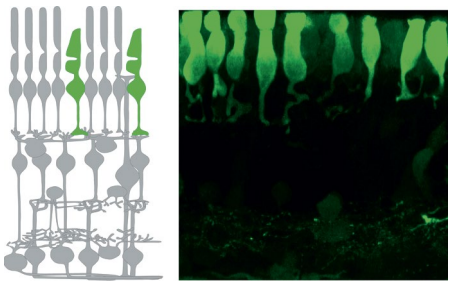
"Gene therapy is a hot topic," says Josephine Jüttner, a research associate in Botond Roska's lab at the Institute of Molecular and Clinical Ophthalmology in Basel, Switzerland. Ideas abound for gene therapies to treat retinal diseases—the lab's research focus—but Jüttner notes that they kept finding themselves running in to a fairly fundamental obstacle: getting those therapies where they need to be.

"We were limited in the available tools," Jüttner says. She and her colleagues rely on adeno-associated viruses (AAVs) to ferry genes into organisms. Such viral vectors have featured in a number of clinical trials, including a retinal therapy approved by the US FDA in 2017. But efficiently targeting particular cell types remains tricky.

A major goal for any gene therapy is cell-type specificity; should a therapeutic gene be expressed where it's not actually wanted—say, healthy cells, rather than diseased ones—the patient receiving the therapy could end up with potentially deleterious off-target effects. Knowing that a gene is being expressed where desired is also an important consideration for researchers studying more basic scientific applications, such as the development of vision. Transgenic animals that express genes in particular places are possible, but developing them can be a costly and time-consuming undertaking—particularly for those who want to study larger animals, such as nonhuman primates. If they can accurately and efficiently target particular cells, viral vectors could be a less resource-intensive option.

To accomplish cell-type specificity, some researchers have been manipulating the capsid of the virus—the protein shell that covers it and mediates its entry into cells—such that the AAVs can only enter certain cells in the first place to deliver their cargo. Jüttner and her colleagues have been taking a somewhat different tack, instead manipulating the promoter sequence of the viral construct itself, with the idea that only AAVs whose promoter is active in a targeted cell would be able to express the genes they carry there.

The problem? Knowing what that sequence should actually be. "Cell-type specific expression is not yet super well understood," says Jüttner. Targeting specific cell types would take a little luck, and a big library. Writing in *Nature Neuroscience*,



Confocal image showing a synthetic AAV promoter at work in human retinal cells. Credit: Jüttner, J. et al. (2019). Springer Nature

Jüttner and her colleagues report the results of their eight year effort to build and test 230 AAVs with unique synthetic promoter sequences for use in the retina.

They used four design strategies to construct the AAVs in the library. The first strategy involved using sequences in close proximity to a known retinal cell-type specific gene, identified by prior work from the lab that looked at gene expression patterns in the mouse retina. In the second strategy, they focused on sequences that appear to be conserved across different species. Strategy three was based on known transcription factor binding sites and the fourth, on epigenetic research that hinted at genomic regions at which transcription factors might bind.

For this proof-of-concept work, all the viral vectors carried a green fluorescent protein as a visual readout of where the tool was ending up. Jüttner admits to being a little skeptical as they started working through all that they had synthesized, but she ended up seeing a surprising amount of glowing green. "That kept me going," she says. "I was always excited to go to the microscope with the next set of promoters."

Ultimately, about half of the AAVs the lab built—including some with entirely artificial sequences—were active four weeks after injection in various areas and different subsets of cells in the mouse retina. The lab then took the active AAVs into cynomolgus macaques and human retinal cells. Not everything made the jump—some of the best murine performers for example proved inefficient in the other species, Jüttner says—though there was correlation in the success rate between the two primates.

The tools will help the lab with basic research in mouse, Jüttner says, but the lack of translation from mouse to primates has them re-thinking their conditions for testing therapeutic vectors intended for human use. As they design new AAVs, they are doing so with human gene expression profiles in mind and will be screening therapies first in human cells or retinal organoids, before taking the most promising candidates into preclinical defined studies in macaque.

It was not a light undertaking, but others in the community welcome the effort. "I think having this resource, and particularly the validation across these different animal models, is unbelievably valuable," commented Luk Vandenberghe from Harvard. Based on Jüttner et al.'s efforts to design and characterize so many variants, others can now select the construct that is most appropriate for their research applications. "It's not that there's one promoter that rises up here—it is really the entire catalog," he says.

"From a basic science perspective, there are some exciting results," noted Greg Field from Duke University. For example, some of the viruses appear to target amacrine cells, one of the least well-understood retinal cell classes. "The tools developed in this study will certainly lead to some new discoveries about interesting cell types in the retina and how they contribute to vision," he says, noting it's likely his lab will leverage the library in the future.

The Roska lab is not stopping here. They plan to design even more AAVs as time goes on, in the hopes of targeting cells and regions that the AAVs in the current library missed, as well as to improve some of the current variants further. For example, they'd like to make some of the promoter sequences smaller. "The smaller the promoter, the more space you have in the AAV for the therapeutic gene," says Jüttner. The library will thus continue to grow, though the lab is already actively using the tools in follow up studies.

In the meantime, information about the AAVs and the corresponding confocal images from each species tested is available online at https://data.fmi.ch/promoterDB/.

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