GENETIC TECHNIQUES

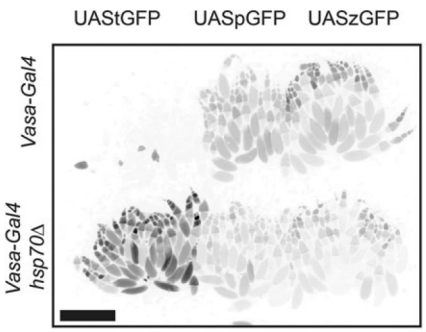
Gal4-UAS gets a germline update

DeLuca, S.Z. and Spradling, A.C. *Genetics* **209**, 381–387 (2018).

The Gal4-UAS system has been a go-to tool for researchers working with *Drosophila melanogaster* for nearly thirty years. GAL4, a transcription factor originating in yeast, will pair with an upstream activation sequence, or UAS, to manipulate the expression of a gene of interest anywhere in the fly, with a notable exception: the female germline. The original vector, developed in 1993 and known as UASt, works well in somatic cells but is only minimally effective in eggs. To get around the issue with manipulating genes in eggs, other fly researchers subsequently developed a vector called UASp; this version suffices in the female germline, but not in somatic cells. The discrepancy means different tools are often needed, depending on where a researcher may want to work.

"The tool should have worked fine in eggs," says developmental biologist Steven DeLuca, yet "nobody knew why it didn't." DeLuca, a postdoctoral fellow in Allan Spradling's lab at the Carnegie Institute for Science in Baltimore, studies how the positions of genes in the genome can influence their expression and inheritance. The existing Gal4-UAS tools worked well enough to study how a gene's location affects its expression, and he didn't originally set out to improve upon them. But a side observation caught his attention. While working through a random genetic screen in which he mutated fly genes one-by-one, he noticed one that, no matter where he placed a UASt reporter, some mutants always seemed to improve its expression. "We were looking for something that affects specific places in the genome, but we found something that affects every place," he recalls. "It was a little serendipity."

A tool that could work across all cells was possible, so they dug a little deeper into the existing constructs, confirming previous results that UASt works best in somatic cells and UASp in the female germline. Building upon prior knowledge about the fly's immune system, they hypothesized that the issue was with part of the UASt sequence that encodes the promoter of Hsp70. It just so happens that this is picked up by the female fly's defense system against viruses and transposons. "Animals have ways to detect foreign genes," DeLuca says, noting that germlines are particularly sensitive—viruses often replicate in germlines, and the animal fights that replication by silencing the expression of foreign DNA with the help of regulatory proteins and piRNAs. Somatic cells don't carry such immune defenses, and detection in male germlines is just different enough from that of females to not pick up the offending sequence in UASt.



GFP expression in fly ovary across the three vectors in wild-type (top) and Hsp70-free (bottom) fly lines. Reproduced from DeLuca and Spradling (2018), GSA.

To confirm, DeLuca and Spradling created fly lines that could not produce any piRNAs against Hsp70. Gal4-UASt in these animals worked just fine, promoting the expression of a green fluorescent protein (GFP) equally well across both somatic and egg cells.

To manipulate expression simultaneously in both types of cells without needing to knock out an entire component of the immune system, they also developed a new UAS vector that could instead simply evade detection. The shortest Hsp70 piRNAs are 23 nucleotides long, so DeLuca and Spradling took the 5'-UTR sequence of UASt and clipped it down to just 19 nucleotides. The result is a trimmed sequence that is too short for the piRNA to find and silence. They dubbed their new vector "UASz."

They then compared all three constructs—UASt, UASp, and UASz. GFP expression was about four times higher in all tissue with UASz than UASp, and equivalent in most—though not quite all—somatic tissues to UASt. "We couldn't know exactly what other things we deleted when we deleted the target of the defense system," DeLuca says; further tweaking could improve the UASz construct further.

Even if it's not perfect, getting the tool out there quickly was a priority for DeLuca. Given how many researchers who work with *Drosophila* rely on genetic manipulations, he wanted his single-vector tool to be available for others to adopt if they found it useful too. He and Spradling posted a preprint that made it to publication in *Genetics* in just two months, and in that short time he has received many requests from the community.

Though yet to have results in transgenic animals, Lynn Cooley's lab at Yale is already trying out the new vector. Andrea Brand, who was involved in the original development of UASt, commended DeLuca and Spradling's elegant efforts to uncover why UASt is repressed in the germline and the two solutions (UASt used in a Hsp70 deletion background and the new UASz vector) they offer in the manuscript. "Their analysis and new vector are both very helpful contributions to the field," she commented via email.

"This whole project was one of those things that sort of happened by luck, but it was worth going through it because of the value to the fly community," DeLuca says. Though happy to contribute to the greater fly good, he's looking forward to using the new construct for the areas of research he finds most interesting. For example, he wants to study the importance of timing of protein destruction: some proteins are present very early in development, but are then absent at later stages. UASz gives him a new way to keep those proteins around from the earliest stages of development, a feat that wasn't easily possible before.

Ellen P. Neff

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