EXPERIMENTAL ORGANISMS

CRISPR in cephalopods yields the first knockout squid

Crawford, K. et al. *Curr Biol* (2020). https://doi.org/10.1016/j.cub.2020.06.099

Karen Crawford and the longfin inshore squid, *Doryteuthis pealeii*, go way back. Though her career has also seen her studying limb regeneration in axolotls and embryogenesis in chicks, she's spent 26 summers now studying the squid, venturing north from St. Mary's College of Maryland to spend several weeks during each of those years at the Marine Biological Laboratory (MBL) in Woods Hole, Massachusetts.

From a first introduction to the squid—the prominent axons of which contributed to Nobel Prize-winning neuroscience work in 1963—during an MBL embryology course in 1983, Crawford has worked on an *in vitro* fertilization method for culturing the cephalopod and has been piecing together a fate map of its cells. Her latest work with collaborators in Joshua Rosenthal's lab at MBL applies CRISPR-Cas9 gene editing tools to knock-out effect—the first demonstration of such editing in a cephalopod.

As with other organisms throughout the animal kingdom to which it has been applied, the CRISPR-Cas9 system works well. "If you can get the protein and the right guide into your cells, you're going to learn something," says Crawford. In the case of the squid, the team developed an appropriate guide to knock out the gene for Tryptophan 2,3 Dioxygenase (TDO), an enzyme involved in the production of the pigmented ommochromes found in the animals' eyes and chromatophores, the structures that enable adult squid to camouflage themselves. No TDO, no (or, depending on the developmental stage of the embryo at injection, very little) pigmentation; genotyping of edited embryos revealed knockout efficiencies greater than 90%.

The hard part, rather, is getting the constructs into the squid in the first place. The developing embryos are surrounded by a rubbery protective layer called the chorion, through which needles alone cannot penetrate. In order to inject fluorescent dyes for fate mapping, Crawford had spent years perfecting her own unique method for tearing tiny holes into the chorion, when another MBL fellow, Antonio Giraldez from Yale, introduced her to the micro-scissors he had designed for working with *Xenopus* embryos. "They let me get through the chorion," Crawford says—and importantly, others could use them to get through too.

Pigmented no more | A control (left) and CRISPR-edited (right) squid embryo. Credit: K. Crawford (2020). Elsevier, Inc

Working with the micro-scissors still takes some finesse—cut too much and the embryo can extrude out through the resulting hole; too little, and the entry point won't be big enough to accommodate the needle. But get it right, and the hole will close up cleanly after injection. Crawford notes a sense of serendipity of meeting the right people at the right time to develop the CRISPR protocol, but timing is also an important consideration for each editing experiment.

Within the first few hours after fertilization, a thin layer called a blastodisc forms and pulls away slightly from the chorion; it is in to this layer that the editing machinery must be injected. If you miss the blastodisc with the needle, nothing will happen, but pass through it into the yolk and you can kill the developing embryo. Wait too long—beyond about three hours of development—and the resulting squid will be patchwork mosaics of edited cells. To knock out genes in the entire animal, it must receive its CRISPR injection before its first embryonic cleavage—when that blastodisc is just beginning to form.

Crawford and the team spent two summers perfecting the process, culminating in the pigment-less squid. It's a proof-of-concept that opens a lot of genetic doors. "We've got the ball now," says Crawford. "Pick your gene." Researchers at MBL have been designing and starting to test guides for targeting additional genes in the squid; Crawford has over a dozen experiments ongoing into the fall that research technician Carrie Albertin will follow up on as the edited embryos develop.

Using the existing fate maps as a guide, researchers should also be able to target those edits to particular places, such as the animals' brains or tentacles. "Folks that have questions specific to those areas, I can direct them," says Crawford. And while making the injections at the single-cell stage yields knockout animals in which the whole organism is affected by the edits, the mosaicism produced by later injections can offer a unique look at what a particular gene is doing.

"The first cleavage separates right and left," says Crawford. "If you inject one cell of the two-cell embryo, you can basically knock that gene out on one side, and the other side of the embryo develops…as your control." From four cells, the squid can be divided into quarters; from 8 cells, parts of the eye, brain, and digestive tract start to emerge; from 16 cells, you can follow the majority of cell fates, she says.

Though the life cycle of this squid remains to be closed—the animals need the deep, dark waters of the North Atlantic to reproduce, so subsequent transgenic generations aren't yet possible—a similar process should be feasible for relatives that can be bred in the lab, such as the bobtail squid, *Euprymna scolopes*. "It's not straightforward for everybody, but if you're tenacious, it's probably doable for most organisms," Crawford says.

With CRISPR technology and ever more genomes being sequenced, Crawford looks forward to work to come. "My sense is that we're going to see this level of investigations in lots of different organisms, which is really going to fill in this beautiful jigsaw puzzle of science," she says. "A lot of us are filling in the sky…some are lucky enough that they're working on the house and the trees and the faces of the people."

But every so often, one finds an edge. "For me, this study is kind of an edge piece," she says. "It allows others to realize that they can do this as well."

Ellen P. Neff

Published online: 10 September 2020
https://doi.org/10.1038/s41684-020-0655-5