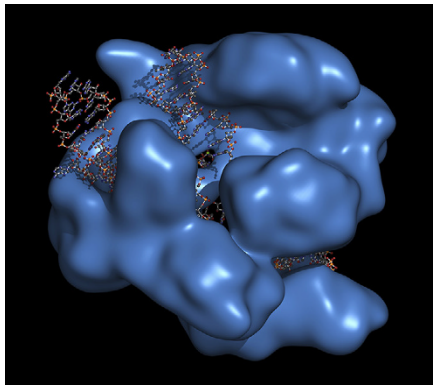
Putting the brakes on CRISPR-Cas9 gene drive systems

Genetic engineering of animals, plants and microorganisms has been a mainstay technique in biomedical science and comparative medicine for decades and has greatly increased our knowledge of disease-states and basic biological function. Recent discoveries and breakthroughs in genetic engineering, including zinc finger nucleases, transcription activator-like effector nucleases and clustered regularly interspaced palindromic repeats (CRISPR), have made it easier and faster than ever to produce powerful genetic models. The potential for this technology extends beyond biomedical research and into the biotechnology sector where researchers are already seeking applications for engineering pest-resistant crops and drug-producing yeast.

However, as gene editing technologies have grown faster and easier to use, so too have concerns about potential unintended consequences. 'Gene drive systems' that use CRISPR and the associated Cas9 endonuclease enable edited genes to be inherited and passed along through a population at exponential rates that are much higher than those of typical Mendelian inheritance.



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While this can be beneficial, for instance when changing a malfunctioning gene that is associated with a specific disease, if an off-target gene is accidentally altered and then spread through a population, it could have harmful and permanent consequences.

To address these issues, James DiCarlo and colleagues at the Wyss Institute for Biologically Inspired Engineering at Harvard University (Cambridge, MA), developed an experimental paradigm with the CRISPR-Cas9 gene drive system that minimizes the risk of synthetic genes escaping into wild populations (*Nat. Biotechnol.* **33**, 1250–1255; 2015). Typically, gene drive systems based on CRISPR-Cas9 are self-sufficient, with all the components built-in that are necessary for targeting and driving a specific gene. DiCarlo *et al.* split the drive system into two separate components: the Cas9 endonuclease, which physically cuts targeted genes, and the guide RNA that is necessary to drive the inheritance of the edited gene. This split-drive system ensures that even if genetically altered yeast were to escape from the lab, mating with wild-type yeast would quickly separate Cas9 from the RNA drive, greatly slowing the spread of altered genes through the wild population and minimizing their impact. "The gene drive research community has been actively discussing what should be done to safeguard shared ecosystems, and now we have demonstrated that the proposed safeguards work extremely well and should therefore be used by every gene drive researcher in every relevant lab organism", commented Kevin Esvelt, a senior author of the study, in a press release.

Dustin M. Graham

EXPLORING THE ROLE OF SYNAPTIC LAYERING

Throughout the brain in a broad range of animal species, synaptic connections between neurons are arranged in layers, or laminae. Laminae are formed by the axons of input neurons connecting with the dendrites of target neurons, and laminae are distinctly organized such that a single lamina contains synapses that share similar functional properties. While research over the last few decades has elucidated many of the molecular mechanisms that guide the formation of this synaptic layering, the purpose of synaptic lamination remains largely unclear.

In a recent study, researchers Nikolas Nikolaou and Martin P. Meyer of King's College London (UK) asked whether synaptic lamination is crucial to the development and function of neural circuits (*Neuron* **88**, 999–1013; 2015). The authors addressed this question using a mutant zebrafish line that lacks the typically organized synaptic lamination of input axons from retinal ganglion cells (RGC) of the eye into the optic tectum, a brain structure that controls higher level visual functions such as prey capture.

Neurons in the optic tectum are direction-selective, meaning that they respond specifically to visual stimuli moving in one direction. This directional specificity in tectal neurons is driven by precise connections with RGCs that are themselves direction-selective. By comparing direction-selectivity in tectal neurons at early and late stages of synapse formation, Nikolaou and Meyer found that although the absence of synaptic layering slowed circuit development in the mutant zebrafish, ultimately the tectal circuits in mutant and wild-type zebrafish became functionally indistinguishable. Nikolaou and Meyer also found that although the mutant zebrafish never regained synaptic lamination, the tectal circuits exhibited full functional recovery of direction-selectivity. When they examined how such a recovery could occur, the authors discovered that the direction-selective tectal neurons adjusted their dendrites to find and connect with incoming RGC axons that they needed to pair with in order to receive directional information. Nikolaou and Meyer attribute this connection in the absence of laminar organization to structural plasticity, or the ability of neurons to alter their morphology and synapses.

These findings demonstrate that the developing brain can overcome the loss of even fundamental and conserved processes such as synaptic lamination. This work suggests that one important purpose of synaptic lamination might be to speed the development of neuronal circuits, and it shows that if given enough time, a disordered brain can set itself straight.

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