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FIRST CUT

Spotlight on Genetic Design in a Spotted Wing Crop Killer

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Writing in this issue, Omar Akbari, Max Scott, and colleagues pave the way for facile genetic manipulation of a non-model organism, spotted wing drosophila.

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Humans and insects have a love–hate relationship. Insects provide important ecosystem services in aquatic and terrestrial environments. However, they negatively impact human society through their role as disease vectors, agricultural pests, and invasive species. Finding the right balance between protecting the beneficial impacts and mitigating the negative impacts is challenging.

Our current relationship with insects is tenuous. Insects remain the deadliest animals on the planet, with vector-borne diseases such as malaria, dengue, and zika responsible for more than one million deaths per year. Agricultural pests are responsible for destroying 5–20% of global grain crops annually, and this is expected to increase by a further 10–25% with each degree Celsius of global warming.[1] Moreover, beneficial insects are dying off at unprecedented rates due to things such as global warming, habitat destruction, and widespread use of chemical pesticides. We are in the midst of an alarming decline in insect abundance worldwide, losing an estimated 1–2% of insect biomass annually.[2]

Genetically engineered (GE) insects are likely to have an important role alongside other control strategies in future integrated pest management plans. There are a growing number of strategies for genetic biocontrol, wherein a pest organism is essentially converted into a pesticide. GE biocontrol agents can be released to mate with their wild counterparts to decrease the population directly through hybrid lethality or to introduce genes that will ultimately decrease local pest populations. Such technologies have already been field tested in several countries to combat mosquitoes that vector disease. Laboratory proof-of-concepts are being developed for an array of agricultural pests as well as invasive species. Future opportunities to use genetic engineering to protect beneficial insect populations from the deleterious effects of climate change or habitat loss are also possible.

Realizing the full potential of insect biotechnology for environmental applications requires that scientists and engineers break away from model laboratory organisms and into diverse organisms around the tree of life. This is happening for many areas of biotechnology. In industrial biomanufacturing, companies and research groups are moving away from the comfortable hosts such as *Escherichia coli* and baker's yeast in favor of microbes with novel metabolic capabilities or environmental tolerances. In plant biotechnology, tools validated in model monocot and dicots need to be translated to crop species to realize their larger impact.

Moving from model to non-model species is not a trivial undertaking. The species needs to be domesticated to the point that it can be reared in the lab through its entire life cycle. The mortality rate at each life stage needs to be sufficiently low so that early-stage transgenic embryos (which are usually generated with an efficiency of <10% for non-model organisms) are not lost through stochastic mortality before they can be outcrossed to generate a stable line. The myriad tools available for model species—reporter gene constructs, balancer chromosomes, genetic markers with easily scored phenotypes, and transgenic lines bearing chromosomal integration sites—do not exist. Without these tools, more time and resources need to be spent validating and tracking edited chromosomes than are spent on the actual editing process.

In theory, groups seeking to branch out into non-model species can accelerate the domestication process by which these tools are created using the recent advances in genetic engineering. This is akin to building the airplane while you are flying it, and a great example is highlighted in this issue of *The CRISPR Journal*. A collaborative team comprising researchers from UC San Diego and North Carolina State University build and

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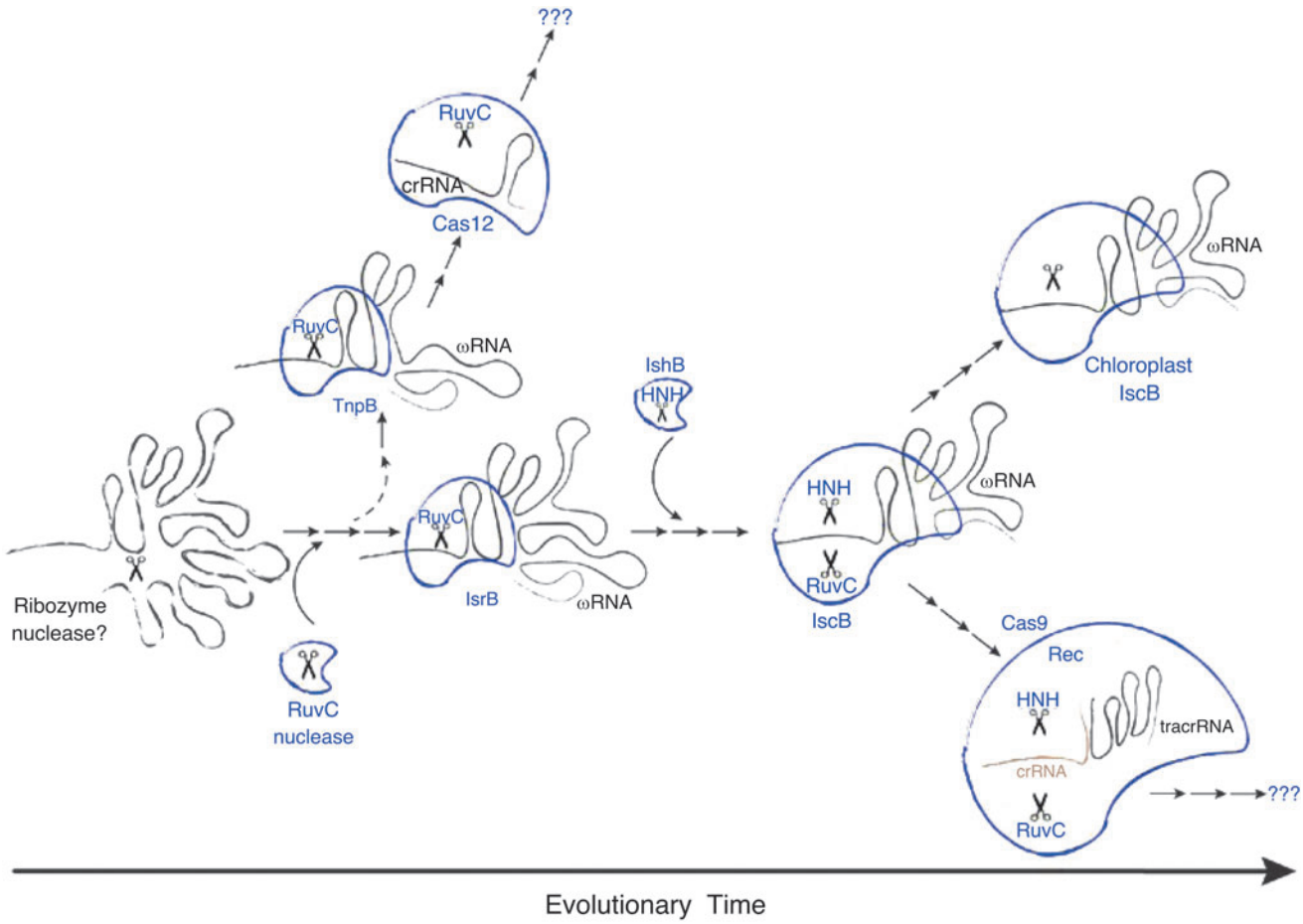

FIG. 1. Artistic representation of Cas9, Cas12, and chloroplast IscB evolution from the IS200/IS605 transposon. An ancient non-coding RNA co-evolved with a mobile RuvC nuclease to give rise to TnpB and IsrB, the ancestors of Cas12 and Cas9, respectively. The insertion or recombination of IsrB with IshB gave rise to IscB. With time and selection, the IscB lineage gave rise to the Cas9 genome editors of today.

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Putting the Puzzle Together

With the ancestral RNA in hand, it was back to the origin of Cas9 (Fig. 1). The authors found two IscB homologs that fit into the evolutionary trajectory of Cas9: IsrB (~350 aa proteins containing the PLMP and the split RuvC domains but lacking the HNH domain) and the even smaller IshB (~180 aa proteins containing only the HNH domain).

Supported by a thorough evolutionary analysis, the authors posit that IsrBs associated with an ωRNA likely represent the ancestral state. Over time, the IsrBs got more complex, gaining an HNH domain from another mobile genetic element or via recombination with an IshB, establishing the IscB family. These events were likely followed by the debut of CRISPR arrays. The Cas9 lineage originated from one particular CRISPR-ωRNA cluster as a result of IscB losing its characteristic PLMP domain and the ωRNA evolving into the shorter tracrRNA. Remarkably, throughout evolution, the ωRNA lost structural complexity and decreased in size, while the accompanying nucleases expanded in size and complexity, consistent with the RNA world hypothesis. Finally, an explosion in diversity giving rise to the broadly distributed type II CRISPR-Cas9 and the panel of gene editing tools we use today was fueled by association of these RNA-guided nucleases with the adaptation machinery.

More to Life than Cas9

Besides our workhorse Cas9, type V Cas12 systems are also deserving of an origin story, given how powerful a tool they are for diagnostics and genome editing. Altae-Tran *et al.* saw that besides IscB and IsrB families, the majority of IS200/IS605 systems encoded RuvC-like TnpB nucleases, previously proposed to be the ancestors of type V effectors[2] due to their association with ncRNAs[9,10] of unknown

function. The authors confirmed this hypothesis by demonstrating that these ncRNAs form a unique group of ωRNAs and guide the TnpB nuclease to nick DNA that is complementary to the variable region of ωRNA.

Beyond the evolutionary origins of prokaryotic CRISPR systems, the authors made one more important discovery that should not be overlooked. For the first time, they identified an active CRISPR-like system inside the cell of a eukaryotic organism. The authors found an *iscB* locus encoding an intact IscB protein and ωRNA in the chloroplast genome of green alga, and they showed that the IscB is guided by ωRNA to cut complementary DNA. While technically of prokaryotic origin, it nonetheless highlights the possibility that CRISPR systems have been co-opted for functions inside a eukaryotic organism.

Sometimes biology can appear remarkably ''unintelligent.'' RUBISCO is a great example where evolution's solution to compensate for a superbly inefficient enzyme was increasing its abundance. In contrast, the serendipitous but repeated association of transposon-encoded nucleases with proximal ncRNA could only be described as an evolutionary masterstroke that ushered in a new era of advanced microbial warfare. As the pieces of the Cas9 and Cas12 origin puzzle begin to fall into place, we are still left to ponder how little we know about the origins of other CRISPR systems. Where are the ancient transposons encoding immature HEPN nucleases that explain the beginning of type VI CRISPR-Cas13? What of the type I and type III systems that arose from ancient stress-response systems? More intriguingly, where do the non-coding RNAs originate and how much farther back do we have to look to find an immune system that's entirely protein free? Moreover, what was the role of the ncRNA before its association with nucleases?

One thing is for sure: as we continue to mine deeper, we will continue to unearth novel tools and remarkable evolutionary insights.

References

1. Kapitonov VV, Makarova KS, Koonin EV. ISC, a novel group of bacterial and archaeal DNA transposons that encode Cas9 homologs. *J Bacteriol* 2016;198:797–807. DOI: 10.1128/JB.00783-15.
2. Shmakov S, Smargon A, Scott D, et al. Diversity and evolution of class 2 CRISPR-Cas systems. *Nat Rev Microbiol* 2017;15:169–182. DOI: 10.1038/nrmicro.2016.184.
3. Koonin EV, Makarova KS. Origins and evolution of CRISPR-Cas systems. *Philos Trans R Soc Lond B Biol Sci* 2019;374:20180087. DOI: 10.1098/rstb.2018.0087.
4. Altae-Tran H, Kannan S, Esra Demircioglu F, et al. The widespread IS200/605 transposon family encodes diverse programmable RNA-guided endonucleases. *Science* 2021 Sep 9 [Epub ahead of print]; DOI: 10.1126/science.abj6856.
5. Deltcheva E, Chylinski K, Sharma CM, et al. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature* 2011;471:602–607. DOI: 10.1038/nature09886.
6. Briner AE, Donohoue PD, Gomaa AA, et al. Guide RNA functional modules direct Cas9 activity and orthogonality. *Mol Cell* 2014;56:333–339. DOI: 10.1016/j.molcel.2014.09.019.
7. Workman RE, Pammi T, Nguyen BTK, et al. A natural single-guide RNA repurposes Cas9 to autoregulate CRISPR-Cas expression. *Cell* 2021;184:675–688.e19. DOI: 10.1016/j.cell.2020.12.017.
8. Jinek M, Chylinski K, Fonfara I, et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012;337:816–821. DOI: 10.1126/science.1225829.
9. Gomes-Filho JV, Zaramela LS, Italiani VC da S, et al. Sense overlapping transcripts in IS1341-type transposase genes are functional non-coding RNAs in archaea. *RNA Biol* 2015;12:490–500. DOI: 10.1080/15476286.2015.1019998.
10. Weinberg Z, Lünse CE, Corbino KA, et al. Detection of 224 candidate structured RNAs by comparative analysis of specific subsets of intergenic regions. *Nucleic Acids Res* 2017;45:10811–10823. DOI: 10.1093/nar/gkx699.

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