

## CRISPR-Cas Changing Biology?

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There's been a lot of excitement about the new gene-editing tool CRISPR-Cas9. Discussion of the technology has largely focused on its precision, accuracy, customizability, and affordability. But the CRISPR-Cas system from which the technology was derived has a fascinating life of its own. The work of Eugene V. Koonin's lab is mapping the rich histories of CRISPR-Cas systems in microbial populations. In "CRISPR: A New Principle of Genome Engineering Linked to Conceptual Shifts in Evolutionary Biology," Koonin argues that fundamental research studying adaptive immune mechanisms has (among other things) illuminated "fundamental principles of genome manipulation." I think Koonin's discussion provides important philosophical insights for how we should understand the significance of CRISPR-Cas systems, and the technologies derived from them. Yet the analysis he provides is only part of a larger story that fully captures the biological significance that CRISPR-Cas systems represent. There is also a human element to the CRISPR-Cas story that concerns its development as a technology. Accounting for the human history of CRISPR-Cas reveals that the story Koonin provides requires greater nuance. I'll show how CRISPR-Cas technologies are not "natural" genome editing systems but are partly artifacts of human ingenuity. Furthermore, I'll argue that when it comes to the story of CRISPR-Cas, fundamental and applied research are importantly intertwined.

CRISPR-Cas systems are encoded in the DNA of microbial populations and can function as adaptive immune mechanisms. These systems consist of a suite of Cas proteins and a CRISPR array. Ideally, the CRISPR array contains genetic information from past encounters with invasive parasites. Koonin helpfully describes the CRISPR-Cas immune defense as having three stages: "1) adaptation, 2) expression/processing, and 3) interference." In the adaptation stage, a distinct set of Cas proteins cleave out a portion of DNA from foreign genetic invaders and insert it into the CRISPR array as a spacer. Typically, the CRISPR array consists of numerous spacers from diverse invaders. During the expression/processing stage, the full CRISPR array is transcribed and distinct Cas proteins process the transcript into many smaller CRISPR RNAs (crRNAs). And finally, the interference stage is when the crRNAs identify genetic material with a matching spacer sequence, marking it for degradation by yet another set of Cas proteins. It is possible for genetic spacers to come from the system's own genome. When this happens, the CRISPR-Cas system can target the host organism which it originally evolved to protect; thereby compromising the organism's fitness.

One of Koonin's central points is that open-ended exploration (what he calls fundamental research) of CRISPR-Cas systems has shed light on a basic principle of genome manipulation. Comparative genomic research has uncovered longstanding coevolutionary relationships between mobile genetic elements (viruses, plasmids, and transposons) and microbes containing CRISPR-Cas systems. Many of the Cas proteins that serve as defenses against genetic parasites in microbial immune systems are homologous to proteins that serve as offensive "weapons" in the very parasites that threaten to infect microbes (Koonin et al. 2017). It appears that microbes have developed what Koonin calls a "guns for hire" strategy where offensive

functional modules employed by infectious pathogens are recruited and exapted to serve as part of a host's immune defense. Many of the functional modules that have been incorporated into CRISPR-Cas systems help make it an effective immune defense by enabling the precise rearrangement of genomic information. According to Koonin, this is what makes CRISPR-Cas a “naturally evolved genome editing toolkit.” The “guns for hire” strategy is not unique to the immune mechanisms of microbes, however. The author argues that many molecular mechanisms distinctive for their ability to precisely target and rearrange genomic information – such as, toxin-antitoxin systems, restriction-modification modules, piRNA machinery, etc. – are evolutionarily linked to mobile genetic elements in prokaryotes and eukaryotes.

Appreciation of CRISPR-Cas' evolutionary significance is certainly indispensable for understanding how it has become an effective genome editing mechanism; however, our understanding of the CRISPR-Cas *technology* requires a bit more nuance. Most molecular technologies were, at one point in scientific history, components of naturally evolved systems. Prior to becoming a technology, biologists modify naturally evolved components to engineer a product that helps meet human needs. This very much describes the applied research programs that made CRISPR-Cas9 into the gene editing tool. When it comes to gene-editing, biologists value improved observational abilities, greater control, and more precise ways to intervene on a wide range of living systems. Many of the modifications done to CRISPR-Cas systems are attempts to make progress towards these goals. When developers of the CRISPR-Cas9 technology adapted the Cas9 protein to carry a signal that ensures its transport into the nucleus of eukaryotic cells, biologists expanded what was biologically possible for CRISPR-Cas9. Now CRISPR-Cas9 can operate in organisms beyond the microbial populations in which it evolved (Cong et al. 2013). A host of further modifications have simplified the mechanism and improved its accuracy (Jinek et al. 2012). Some of the capacities that CRISPR-Cas technologies now possess are abilities that the naturally evolved systems were unlikely to develop independently of human intervention. Thus, the CRISPR-Cas9 system used by researchers in the lab is not a “naturally evolved genome editing toolkit.” Instead it is, in part, an artifact of human ingenuity.

The applied research programs that develop and use CRISPR-Cas technologies have the potential for advancing our knowledge of living organisms in at least two ways. First, it is common for researchers to discover new things about the naturally evolved mechanism from which a technology is derived. Indeed, this has already happened for the CRISPR-Cas9 technology. Efforts to expand and enhance the capabilities of the Cas9 nuclease resulted in discovery of critical amino acid substitutions that enhance its activities (Kleinstiver et al. 2015). This knowledge has been used in conjunction with protein imaging techniques to enrich models of Cas9's structure and function. Second, advancements in technology can make the study of complex biological systems more tractable. CRISPR-Cas9 has made the study of gene function(s) in living organisms by means of knockout studies a more affordable and facile experimental approach (Doench 2018). Technologies often expand what is biologically possible in laboratory settings. In doing so, they unlock new research avenues – even avenues in fundamental research – by making previously unobservable phenomena available for study.

As Koonin acknowledges, there is disproportionate institutional support for applied research programs. Increasingly, funding for fundamental research that engages in open-ended exploration is lost to applied research that holds promise for future commercial products. The multimillion dollar industry that sells and develops CRISPR-Cas technologies is an illustrative example of applied research receiving considerable amounts of funding. To add insult to injury, it is largely the applied side of CRISPR-Cas research that has captured the attention and

imagination of many scientists, journalists, and science fiction writers. (There's even a popular action film entitled "Rampage" featuring CRISPR-Cas9 technology as a major plot device!) Yet as Koonin points out, the CRISPR-Cas9 technology was only possible with the aid of fundamental research.

One can acknowledge the problems of lopsided support for applied research and still appreciate that the story of CRISPR is not "primarily about research into fundamental biological mechanisms." When it comes to CRISPR-Cas, fundamental and applied research are intertwined. The products of applied research often make fundamental research possible and fundamental research is often crucial for applied research to generate new products. The restriction enzymes and genome sequencing techniques that aided in open-ended exploration of the CRISPR-Cas system are, in part, technological products of applied research programs (Lander 2016). And, as Koonin has illustrated, fundamental research in the biological sciences has been crucial for identifying a host of naturally evolved components – like CRISPR-Cas, green fluorescent protein, RNAi, restriction enzymes, and more – whose capabilities are amenable to use and development as experimental tools. There may be good reasons for distinguishing between fundamental and applied research; however, their inherent entanglement means that both are crucial for our understanding of scientific and technological progress.

Biological technologies are philosophically interesting because of how they're made and what they make possible. The molecular tools of biology often require feats of human ingenuity to transform naturally evolved components into partially artificial products. The products of this type of research program are often necessary for the success of fundamental research programs. Furthermore, the making of molecular tools also generates new insights into the naturally evolved component(s) from which the technology is derived. This means that an adequate understanding of a biological technology requires an appreciation of both the component's natural and engineered history. The CRISPR-Cas technologies are no exception.

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