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Applications of CRISPR Technologies Across the Food Supply Chain

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Abstract

The food industry faces a 2050 deadline for the advancement and expansion of the food supply chain to support the world's growing population. Improvements are needed across crops, livestock, and microbes to achieve this goal. Since 2005, researchers have been attempting to make the necessary strides to reach this milestone, but attempts have fallen short. With the introduction of clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) proteins, the food production field is now able to achieve some of its most exciting advancements since the Green Revolution. This review introduces the concept of applying CRISPR-Cas technology as a genome-editing tool for use in the food supply chain, focusing on its implementation to date in crop, livestock, and microbe production, advancement of products to market, and regulatory and societal hurdles that need to be overcome.

INTRODUCTION

With a continuously expanding world population, the food supply chain faces some of its greatest challenges since the Green Revolution. It has been predicted that total crop production must double from the 2005 levels by 2050 to meet the needs of the world population (Tilman et al. 2011). It has been ten years since this prediction was made and crop production is still struggling to meet this goal. Models have estimated that a 2.4% annual increase in crop yield is necessary to reach the 2050 milestone. Unfortunately, a 2013 report revealed that none of the top produced crops in the world are anywhere close to this number (Ray et al. 2013). There are many contributing factors to this lack of increase in crop production. One factor is the amount of arable land available for crops. It has been determined that clearing more land is not the most desirable option. Although increasing arable land may not be viable, recent projections from the United States Department of Agriculture (USDA) show that the number of acres planted will remain stable (USDA 2018b). Moving past arable land, many crops across the globe struggle to thrive because of disease and stress conditions (Ma et al. 2018). Additional strains to the food supply chain are being anticipated. As nations become more affluent, increased demand for livestock is likely. This not only means that livestock production must increase but consequently that more crops will be needed to support the corresponding increase in feed consumption. Increases in demands for cleaner energy also mean that more crops will be outsourced for biofuels (USDA 2018b). Overall, changes must be made to meet global needs. Because of the hurdles listed above, most research is being focused on improving crops to increase yield. Many techniques enable such improvements, but most researchers are turning to the promise of genome editing.

Genome editing can be achieved through various methodologies, but the three most common are zinc finger nucleases (ZFNs), transcription activator–like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPRs). CRISPR has emerged as the clear leader among the three technologies. It was first fully characterized as an adaptive immune response in bacteria in 2007, when it was established as a phage-resistance mechanism in yogurt cultures (Barrangou et al. 2007). Shortly thereafter, researchers elucidated the CRISPR-Cas (CRISPR-associated) mechanism and repackaged this molecular machinery as a genome-editing tool, and the CRISPR craze was born (Pennisi 2013). Many factors have contributed to CRISPR's unprecedented level of popularity across scientific disciplines, not the least of which has been the ease of access—or democratization—of CRISPR. Addgene, a nonprofit plasmid repository, has made CRISPR readily accessible for $65 per plasmid. This universal access has opened up research avenues to companies, academics, and nonprofits alike, and already 3,400 laboratories have received CRISPR shipments (LaManna & Barrangou 2018). The popularity of CRISPR lies not only in its accessibility but most importantly in its success. Shown to be easy to use, highly specific, and programmable, CRISPR has become the tool of choice for many researchers. CRISPR efficacy has been demonstrated in many model organisms and industrial workhorses used in biotechnology, medicine, and agriculture. Although still relatively new to the agriculture field, CRISPR has shown great promise in its ability to overcome many of the hurdles facing the industry, specifically, how to improve crop yield to avoid the upcoming food gap crisis. Perhaps it is fitting that the most promising tool in agriculture was first discovered in the food industry.

In this review, we examine CRISPR as it applies to the food supply chain. We begin with CRISPR biology to examine what has enabled its successful transition from an adaptive immune response to a genome-editing tool. We also discuss unique applications and approaches, beyond the basic uses of genome editing, that can be further taken advantage of. We then detail the successful application of CRISPR in crops, livestock, and microbes. CRISPR has presented unique opportunities to researchers in these areas and has easily outpaced incumbent technologies. However,

the fast pace of adoption has prevented regulatory agencies from keeping up with the CRISPR craze. We discuss impacts CRISPR will likely have on the regulatory process and how societal concerns may affect future paths.

CRISPR BIOLOGY

The knowledge of CRISPR biology has rapidly expanded since 1987, when it was first noted as unusual repetitive sequences in bacterial genomes (Ishino et al. 1987). In the early stages of CRISPR discovery, three main types were identified: Type I, II, and III. Today the different CRISPR systems are divided into 2 classes, 6 types, and 23 subtypes, with additional putative types to be validated (Koonin et al. 2017). Class 1 is characterized by a multiunit effector complex. This class consists of Types I, III, and IV. Class 2 is characterized by single protein effectors. This class consists of Type II, V, and VI (Makarova et al. 2011a,b). The single protein effector of Class 2 has garnered a great deal of attention, specifically in terms of genome engineering. The Type II signature protein Cas9 is the most widely popular and most used effector protein of the CRISPR-Cas family and as such is the main focus of this review. Regardless of the class, all CRISPR systems are DNA encoded, RNA mediated, and nucleic acid targeting (Barrangou et al. 2007, Brouns et al. 2008, Hale et al. 2009, Marraffini & Sontheimer 2008). Each CRISPR-Cas locus consists of the *cas* genes and the CRISPR array. The *cas* genes are specific to each CRISPR type, whereas the CRISPR array consists of the leader, which drives transcription of the array, and the repeats and spacers (Horvath & Barrangou 2010). **Figure 1*a*** depicts a canonical Type II CRISPR locus. Spacers are derived from invading mobile genetic elements (MGEs) and act as the memory for the adaptive immune response. CRISPR biology occurs in three stages: adaptation, expression, and interference.

Adaptation

Adaptation has two main steps: the acquisition of new spacers and the integration of spacers into the genome. **Figure 1*b*** depicts the canonical Type II adaptation stage. This stage is directed by the nearly universal Cas1–Cas2 proteins (Type VI is the exception) (Makarova et al. 2015). Acquisition occurs when foreign DNA enters the cell and is interrogated by Cas. The system copies a small piece of the foreign DNA and incorporates it into the host's genome. DNA from an invading source is termed a protospacer, whereas incorporated DNA is termed a spacer (Deveau et al. 2008). Spacers are added iteratively, with new spacers consistently being added at the leader end of the repeat–spacer array (Barrangou et al. 2013). For the Type II system, a protospacer is identified via the presence of a signature known as the protospacer adjacent motif (PAM; 2–7-nt PAM sequence) (Horvath et al. 2008). This acts as a recognition signal for the target sequence. It is also useful in distinguishing self from nonself, as the PAM is not incorporated into the CRISPR locus (Marraffini & Sontheimer 2010). This is important for subsequent infections. Additionally, as the spacers are added consecutively and consistently, the array becomes a recorded history of infection events for the organism (Andersson & Banfield 2008, Tyson & Banfield 2008). This can then be used to identify related strains and their history.

Expression

The next stage is expression or biogenesis of CRISPR RNA (crRNA). Transcription begins at the leader end of the repeat–spacer array. The leader sequence, which is immediately adjacent to the array, contains a promoter sequence for the expression of crRNA (Carte et al. 2014).

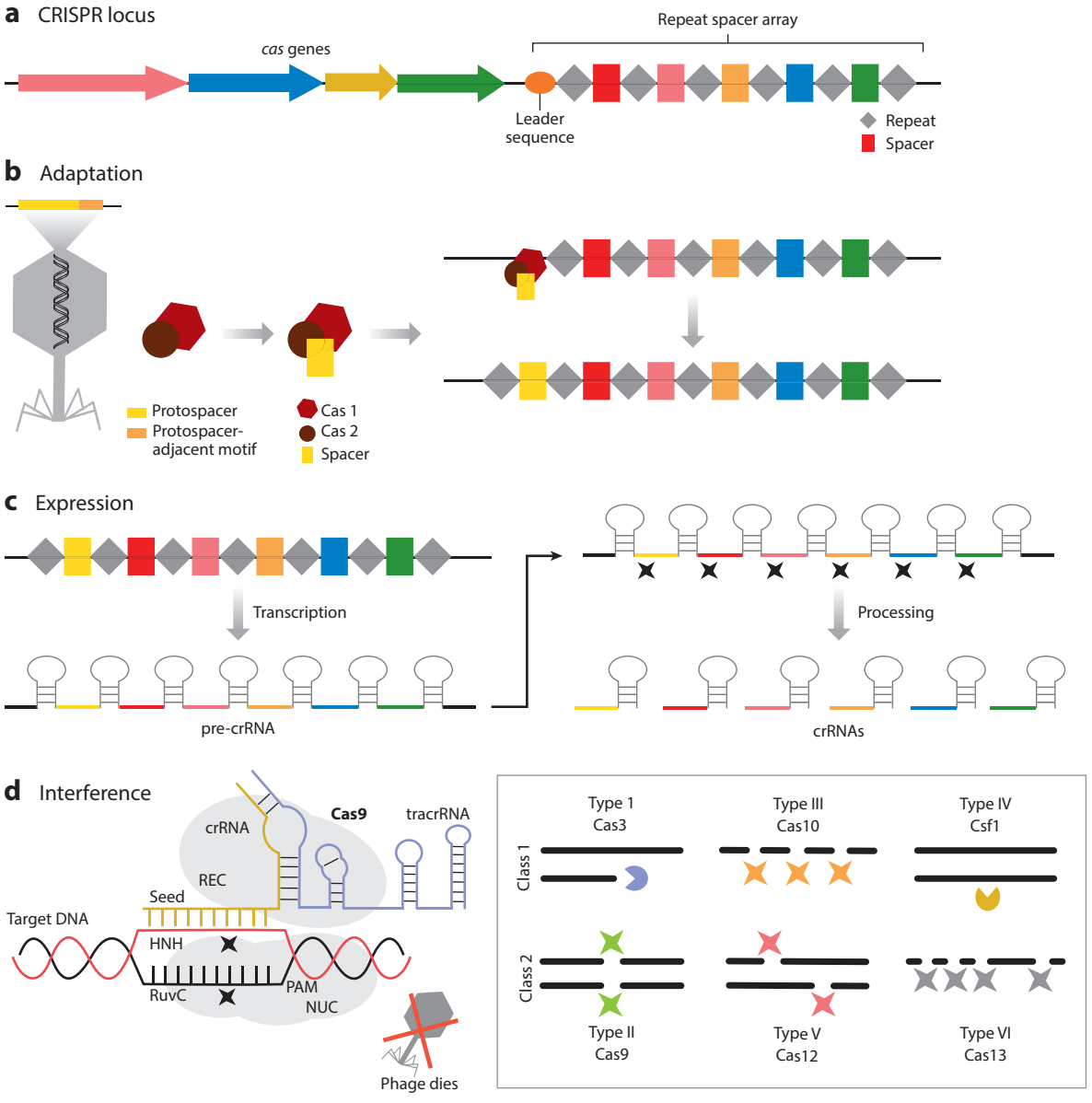

Figure 1

CRISPR (clustered regularly interspaced short palindromic repeat) biology. (*a*) CRISPR locus containing *cas* genes, a leader sequence, and a repeat–spacer array. (*b*) CRISPR adaptation involves the excision of a protospacer from invading DNA and its incorporation as a spacer into the host's repeat–spacer array. (*c*) CRISPR expression involves the transcription and processing of crRNAs (CRISPR RNAs). (*d*) CRISPR interference is achieved when Cas9 bound to the crRNA::tracrRNA complex recognizes the PAM in phage DNA, leading to double-strand break and death of the phage. The inset depicts the different cleavage types of each CRISPR system.

crRNA abundance depends on proximity to the leader, with the most proximal being the most abundant (Zoephel & Randau 2013). Having the newest spacers incorporated after the leader makes sense, as it is more likely for a host to be infected by a more recent phage as opposed to an earlier exposure. Transcription produces one long precrRNA (precursor crRNA) that is processed into mature crRNAs by cleaving within the repeat region (**Figure 1*c***) (Deltcheva et al. 2011). A crRNA is composed of the latter end of a spacer and the beginning of the repeat sequence. Each has a defined length for the system (Crawley et al. 2018). Therefore, it is the crRNA that provides the target for the CRISPR system. In Type II systems, the tracrRNA (*trans*-activating crRNA) is essential to the processing of precrRNA and binding to the effector protein Cas9 (Deltcheva et al. 2011). Cas9 recognizes bound crRNA–tracrRNA complexes and undergoes processing. From here, Cas9 remains bound to the mature crRNA::tracrRNA for the final stage of CRISPR biology.

Interference

The process of targeting nucleic acids is termed interference. This stage is directed by the effector protein or complex. The effector complex is formed, recognizes foreign nucleic acids, and generates nucleic acid cleavage. In Type IIs (**Figure 1*d***), the only required components are the Cas9 bound to the crRNA:tracrRNA duplex (Barrangou 2015). Much like during adaptation, DNA is scanned for a PAM site (Sternberg et al. 2014). Cas9 binds to the noncomplementary strand of the PAM and then checks for sequence similarity between the spacer in the crRNA and the target DNA. If no mismatches are identified in the seed region, then Cas9 enacts an exact double-strand break through the use of two nickases (**Figure 1*d***) (Sternberg et al. 2014). Targeted DNA includes phages, plasmids, and chromosomal DNA. Target and cleavage are characteristic of each CRISPR type (**Figure 1**, inset) (Barrangou & Horvath 2017). Type I uses an exonuclease that cleaves and degrades one strand of DNA. Type IIIs target mRNA and cleave in a ruler mechanism. Type Vs use nickases like Type IIs do; however, the result is staggered nicking of the DNA strand. Type VIs introduce a cut in mRNA and then nonspecifically continue to process its target. Finally, the exact cleavage mechanism of Type IV remains unknown.

CRISPR AS A GENOME-EDITING TOOL

Although CRISPR is natively an immune defense system, its basic biology has made it easily adaptable as a genome-editing tool. Although most of the CRISPR systems may be co-opted in this manner, it is the Type II system that has gained the most popularity. One of the earliest systems discovered, the Type II system attracted researchers because of its single effector protein Cas9. Unlike the other known systems at the time, Cas9 was the only protein needed to identify, target, and cleave target DNA sequences. Not only was Cas9 the only protein needed, it was discovered in 2012 that Cas9 was programmable. By providing a synthesized single-guide RNA (sgRNA), researchers could provide Cas9 with specific targets of interest. The sgRNA mimics the native crRNA::tracrRNA, and the only change needed to alter the target is to the spacer region (Jinek et al. 2012). The ability to deliver only a single protein and RNA made exogenous use of the Type II system remarkably simple. **Figure 2** depicts how Cas9 can be used as a genome-editing tool. Programmable sgRNAs provide an edge to CRISPR over other genetic editing techniques. One advantage is that sgRNA synthesis is provided by numerous companies at a relatively low cost (Wang 2015). Changing a target is no longer a cumbersome process. Additionally, it is possible to multiplex sgRNAs, enabling simultaneous editing of targets in a single cell (Cong et al. 2013, Mali et al. 2013). The only requirement for Cas9 targeting outside of the delivered components is the

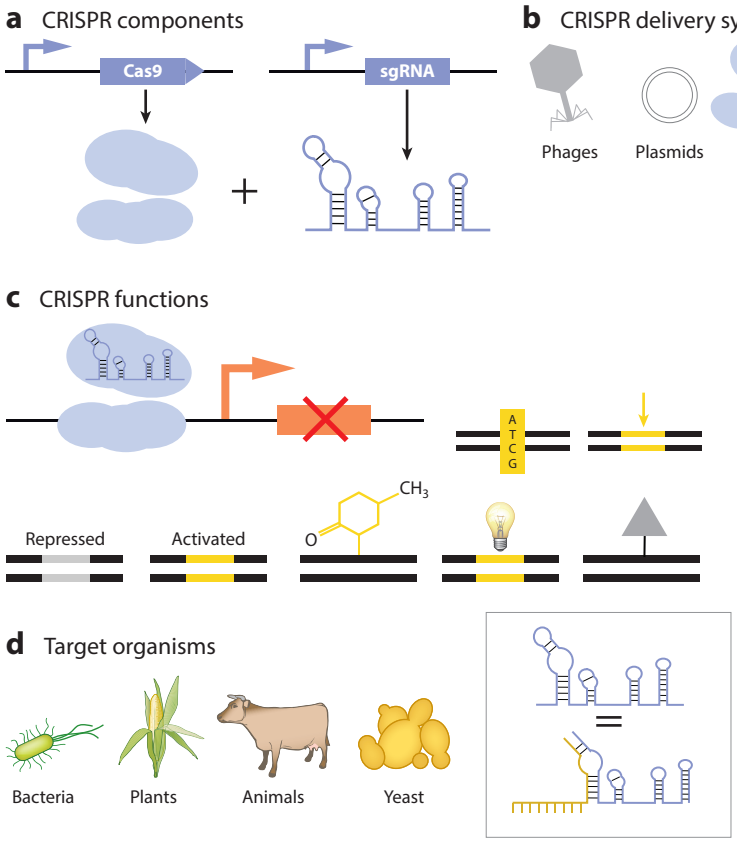

Figure 2

CRISPR (clustered regularly interspaced short palindromic repeats) editing. (*a*) CRISPR components [Cas9 and single-guide RNA (sgRNA)] needed for editing in target organisms. (*b*) CRISPR delivery can be achieved through phages, plasmids, or ribonucleoproteins (RNPs). (*c*) CRISPR can be utilized to cause knockouts, single nucleotide polymorphisms, knockins, repression, activation, epigenetic modification, imaging, and recruitment. (*d*) CRISPR effectiveness has been demonstrated in various target organisms such as bacteria, plants, animals, and yeast. The inset shows the replacement of the crRNA::tracrRNA complex with a single oligonucleotide sgRNA for genome editing.

presence of a PAM in the target region. Once all the components are designed, several delivery options are available: plasmid, phage, or ribonucleoprotein (RNP) delivery. Once delivered, Cas9 technology can be used in numerous ways. The canonical use is to introduce a blunt double-strand break, leaving the native machinery responsible for repairing the breaks (Barrangou & Doudna 2016). One method a cell could use is nonhomologous end-joining (NHEJ). NHEJ fixes double-strand breaks but often at the expense of an added or removed base, creating an INDEL (insertion–deletion) mutation in the target gene. Homologous recombination is an alternative pathway available to the cell. Cells use their own copy of the targeted gene to replace the cut copy. Alternatively, researchers can provide a template to introduce their own desired changes. This can lead to the deletion of a gene, introduction of a gene, or repair of a damaged copy of a gene (Gaj et al. 2013).

Beyond the canonical cleavage event, there are many alternative uses for CRISPR. Although still using the same cleavage scheme, Cas9 can be applied in several unique ways. For instance, self-targeting in bacteria can be used as selection markers or to find rare mutations in a population

(Selle et al. 2015). It is also possible to use CRISPR to alter microbiota composition by targeting undesirable species (Gomaa et al. 2014). Researchers have also altered Cas9 itself to allow for further applications. Nickase Cas9 (nCas9) introduces a single-strand nick as opposed to a double-strand break and was developed for more controlled knockout applications to reduce the risk of off-target effects (Doudna & Charpentier 2014, Tsai et al. 2014). Deactivated Cas9 (dCas9) does not introduce a break because of the inactivation of the two nickase domains (RuvC and HNH) but uses Cas9's homing and binding abilities for a variety of uses (Barrangou & Doudna 2016). dCas9 becomes a transcriptional regulator when combined with activator or repressor domains (Gilbert et al. 2014, Larson et al. 2013); when used in this manner the technology is termed CRISPR activation (CRISPRa) or CRISPR interference (CRISPRi), respectively. Along the same lines, dCas9 can be used for imaging, epigenetic modification, recruitment, and more (Chen et al. 2013, Hilton et al. 2015). Currently, many efforts are underway to fuse dCas9 to various effector domains (e.g., acetyl- or methyl-transferases for epigenetics; deaminases for base editing; or recombinases, gyrases, or helicases to alter DNA locally).

Despite its many advantages, the technology still has limits. Protein size, protein efficiency, and PAM presence can all affect aspects of the overall impact of genetic editing, including delivery, off-target effects, and lack of viable targets (Barrangou & Doudna 2016). As most research applications currently use either SpyCas9 or SauCas9, new Cas9s are being mined and characterized to overcome current difficulties. These new Cas9s—including the new nanoCas9s—differ in efficiency, PAM sequences, and size (Crawley et al. 2018). A second approach is the mining of other CRISPR systems. Cas12 (formerly Cpf1) of Type V is gaining in popularity with its staggered cut double-strand break. Cas3 of Type I, which is an exonuclease, is being utilized as an antimicrobial. With more types being added, it is expected that we are not yet at the saturation level of CRISPR applications.

CRISPR IN AGRICULTURE

The Plant Toolbox

Facing the looming food crisis, plant scientists have been working to improve crops to feed the world's population in the future. Specifically, they have been attempting to breed and develop crops with increased yield, disease and pest resistance, and stress tolerance. Unfortunately, this has been an extremely slow, cumbersome, and expensive process. Traditional breeding in plants for these traits may take 7 to 12 years (Acquaah 2009), and only one trait can be improved at a time. Although results can be achieved with traditional methods, this timeline does not allow for necessary measurable gains by the 2050 deadline. Researchers have thus begun looking at genome-editing technologies as an alternative to traditional breeding. The main technologies utilized in plant genome editing to date have been ZFNs, TALENs, and, most recently, CRISPR. A detailed review comparing the three technologies in plants was completed by Bortesi & Fischer (2015). Of the three, CRISPR is the leading approach for the reasons highlighted above: programmability, efficiency, and specificity, as well as accessibility, ease of use, and multiplexing. Both ZFNs and TALENs are proteins and require new proteins to be designed for each target, a lengthy and costly process compared with the CRISPR sgRNA design, which enables easy and quick reprogramming (Khan et al. 2018). The ability to multiplex with multiple sgRNAs gives CRISPR a further edge over ZFNs and TALENs, as this allows for faster development of desired traits. Additionally, because new sgRNAs are easily and cheaply designed, it is possible to develop high-throughput screens with sgRNAs (S.J. Liu et al. 2017). An additional benefit of CRISPR is that it is not limited by DNA methylation states as ZFNs and TALENs are (Hsu et al. 2013). One

concern with CRISPR is the presence of off-target effects, especially with highly repetitive genomes such as those found in plants; however, researchers have already begun to investigate methods to overcome this, such as using better guide-design tools and chemically altered Cas9s or sgRNAs (Armin et al. 2017). These reasons are why, functionally, CRISPR should be a promising enabler for plant genome editing. Practically, CRISPR has already shown great promise. Multiple groups have determined that CRISPR is effective within the first generation. Other studies have shown homozygous germline alterations that remain stable in later generations (Bortesi & Fischer 2015). Compellingly, innovative delivery and use of CRISPR could allow for nontransgenic crops and non-GMO crops (see below).

Implementation in Crops

CRISPR was first shown to be a viable tool for plant genome engineering in 2013. Jiang et al. (2013) showed proof of concept in *Arabidopsis*, tobacco, sorghum, and rice. Proof of concept was also demonstrated in wheat (Shan et al. 2013, Upadhyay et al. 2013). Beyond ability, groups have already begun using CRISPR to generate crops with the desired traits discussed above. Stress tolerance has been addressed by DuPont Pioneer (now Corteva Agriscience) with its engineered drought-resistant maize (Shi et al. 2017). Cassava has been developed that has increased protection from cassava brown streak disease, which is in the area of disease-resistant crops. And to increase yields, flowering times in soybeans have been altered (Yupeng et al. 2018).

Traditional fruits and vegetables have also been enhanced by CRISPR. Tomatoes have been edited by CRISPR (Brooks et al. 2014). Both tomatoes and potatoes have had multiple targets and traits engineered. Pathenocarpy has been a specific target among the many in tomatoes, an industry-relevant issue specifically with regard to heat-stress conditions (Karkute et al. 2017). Powdery mildew disease resistance is another industry concern that has been addressed (Nekrasov et al. 2017). Potatoes have been edited for a waxy phenotype (Andersson et al. 2017). Fruit engineering, still in the early stages, has begun in strawberries and apples (Gomez et al. 2018, Nishitani et al. 2016).

Path to Market

Along with researchers, companies have also joined the CRISPR journey. Most of the international agricultural businesses have already begun incorporating CRISPR into their pipelines. Last year Syngenta announced its acquisition of CRISPR IP (intellectual property) to begin its use in several of their crops. This list includes many of the crops in which editing has already been successful, such as corn, wheat, and rice, but also new plants such as sunflowers (Maurer 2017). Recently, a group was able to increase grain yield in rice (Miao et al. 2018). This leaves the path open for companies to target similar genes to overcome food shortages. Another group has developed mushrooms with decreased browning that the USDA has decided will not be regulated (Waltz 2016). This sets up the framework for companies' regulatory groups as they develop new products (see further discussion below). One crop is already available in the market—nonbrowning apples (Waltz 2018). Even more companies have begun making commitments to release crops in the future. DuPont is one such company; it announced the release of waxy corn by 2020 (Bomgardner 2017) and is building up an IP portfolio that is open in principle to the agricultural sector, even including competitors such as Bayer and BASF.

CRISPR IN THE ANIMAL KINGDOM

The Livestock Toolbox

In addition to crops, researchers have also been addressing livestock improvement as well. Unlike crop engineering, livestock engineering has been relatively limited in scope and scale. A recent review goes into detail about the history of genetic editing in livestock (Telugu et al. 2017). In short, traditional manipulation techniques such as selective breeding, random trans-genesis, and stem cells were not sufficiently effective to produce reliable results. The advent of genetic editing tools, such as CRISPR, has begun to change this in the animal kingdom. Impressively, groups have already established knockdown and knockin capabilities in livestock. Easy delivery, specificity, and reliable methodology have once again allowed CRISPR to outshine other technologies.

Commercial Implementation

CRISPR implementation in the animal kingdom has occurred mainly in three species: cattle, pigs, and chicken. These account for most of the husbandry business. Cattle have been the focus of many genetic editing approaches, most notably using TALENs for hornless cows (Carlson et al. 2016). However, CRISPR repurposed beyond the canonical knockout is leaving its mark on the field. Using nCas9—an altered Cas9 that cleaves only a single DNA strand—increased resistance to tuberculosis has been introduced into cattle (Gao et al. 2017). Most recently a CRISPR knockin was used to produce only male offspring (Rosenblum 2018). The rationale for this is that males grow faster and bigger than females. In another species, researchers have created lean pigs. Leaner pigs are at a lower risk of mortality, as pigs struggling to regulate temperature and fat deposits have a detrimental effect on pig production. Editing pigs to be leaner will increase pig production and save farmers money on swine going to market (Zheng et al. 2017). Porcine reproductive and respiratory syndrome virus (PRRSV)-resistant pigs have been developed and will soon become commercialized (Van Eenennaam 2018). Additionally, porcine endogenous retroviruses (PERVs) have been removed from pigs, which has a potential impact on the human health industry (Yang et al. 2015). Last, chickens, or more specifically chicken eggs, have been edited. A knockout in chickens has removed a protein from egg whites that is known to cause some allergic reactions (Oishi et al. 2016). The next big target in the animal kingdom will be aquaculture. However, genetic engineering in fish is already facing many regulatory hurdles (Ledford 2015). It has yet to be determined what regulations will be enacted and how this may affect regulation of other genetically engineered livestock. To date, proof of concept has been demonstrated in zebrafish, catfish, and salmon (Edvardsen et al. 2014, J. Liu et al. 2017, Khalil et al. 2017).

CRISPR IN MICROBES

Back to the Future

Ironically, with its success in multicellular organisms, researchers have now come full circle and are looking at new ways to implement CRISPR in microbes. CRISPR has many useful advantages in the microbial industry, both natural and engineered. The CRISPR locus itself allows for strain typing, which is a method that fingerprints each strain, allowing for identification in proprietary blends in food fermentation and probiotic products (Barrangou & Horvath 2012). CRISPR naturally provides immunity against bacteriophages. However, this can be harnessed to specifically vaccinate organisms from bacterial viruses widely encountered in large fermentations to decrease

waste in the food-manufacturing process (Selle & Barrangou 2015). From a genetic engineering standpoint, all the advantages outlined above are applicable to microbes (**Figure 2**). Engineering can be exogenous or endogenous depending on the presence and functionality of a CRISPR-Cas system and the intended use. Finally, it is possible to implement CRISPR for the targeted killing of microbes. This may take the form of self-targeting, in which CRISPR acts as a programmable and specific antimicrobial agent. Self-targeting would be used as a selection marker for the screening of specific alterations or rare natural mutations that occur in mixed bacterial populations (Selle et al. 2015). An antimicrobial agent would be used in mixed populations to remove undesirable organisms from culture blends (Beisel et al. 2014, Gomaa et al. 2014).

Industrial Applications

Even though microbes are the original source of CRISPR, applications in microbiology are still being newly discovered and implemented. One main use to date in bacteria has been typing, specifically of industrially relevant lactobacilli and streptococci as well as foodborne pathogens such as *Escherichia coli* and *Salmonella* (Barrangou & Horvath 2012). In these applications, researchers use the sequence variability within repeat–spacer arrays to distinguish strains. Strains can be completely identical in the rest of their genomes but still see differences in the CRISPR systems. This allows companies to specifically track their strains. Other work has focused on CRISPR's genetic engineering feasibility. CRISPR has been combined with recombineering and used as a selection marker in the probiotic *Lactobacillus reuteri* (Oh & van Pijkeren 2014). Other bacteria of interest in which CRISPR has been effective include *Bacillus subtilis*, *Clostridium*, *Corynebacterium glutamicum*, and *E. coli* (Altenbuchner 2016, Cleto et al. 2016, Jiang et al. 2015, Nagaraju et al. 2016). CRISPR applications in other microbes such as yeasts and fungi have also begun. Proof of concept has been shown in *Candida albicans*, *Saccharomyces cerevisiae*, and *Ustilago maydis* (Biot-Pelletier & Martin 2016, Schuster et al. 2016, Vyas et al. 2015). One study utilized wine yeasts and targeted urea production to limit the potential for carcinogen production in the fermentation process (Vigentini et al. 2017). From a genomic standpoint, fungi have been notoriously difficult to work with and manipulate. But recent proof of concept studies in *Aspergillus*, *Myceliophthora thermophila*, and *Penicillium chrysogenum* show promise for the future (Nødvig et al. 2015, Pohl et al. 2016, Q. Liu et al. 2017).

Company Stakes

A clear indicator of the growing interest in CRISPR in the microbial field is the increasing investment by companies. Several companies have been begun incorporating CRISPR to enhance their microbial products. These companies are using various systems and techniques to improve food products, study the food-chain microbiome, and utilize canonical genome engineering as an alternative to antibiotics. DuPont has been using CRISPR for typing and phage protection in dairy strains for more than a decade (Barrangou et al. 2007). AgBiome recently launched a spin-off company called LifeEdit, whose purpose is to study microbes, specifically soil microbes, and their CRISPR systems to improve agricultural business (Teater 2018). Novozymes is using CRISPR to edit fungi (Nødvig et al. 2018). Locus Biosciences is taking a unique approach by repurposing CRISPR as an antimicrobial agent with the Type I exonuclease Cas3 against *Clostridium difficile*, *Pseudomonas aeruginosa*, and *Enterobacteriaceae* (Teater 2018). These are just a few of the many companies currently investing in CRISPR in the microbiological arena.

WELCOME TO THE CRISPR WORLD

With the promise of new research avenues, faster product development, and solutions to global issues, both industry and academia are embracing CRISPR technology. Studies have shown that CRISPR is effective in plants, animals, yeast, fungi, and bacteria (**Figure 3**). It is no longer a matter of if CRISPR products will be developed but when. Some products, such as nonbrowning apples and faster-growing salmon, have already been released in certain markets (Van Eenennaam 2018, Waltz 2018). Other products, e.g., CRISPR-modified pork and crops such as corn and soybeans to name a few, have begun commercial development research and within two years will be ready. Because of their many advantages, CRISPR technologies have allowed agriculture to generate next-generation products much faster than government regulatory policies can be put in place. Because

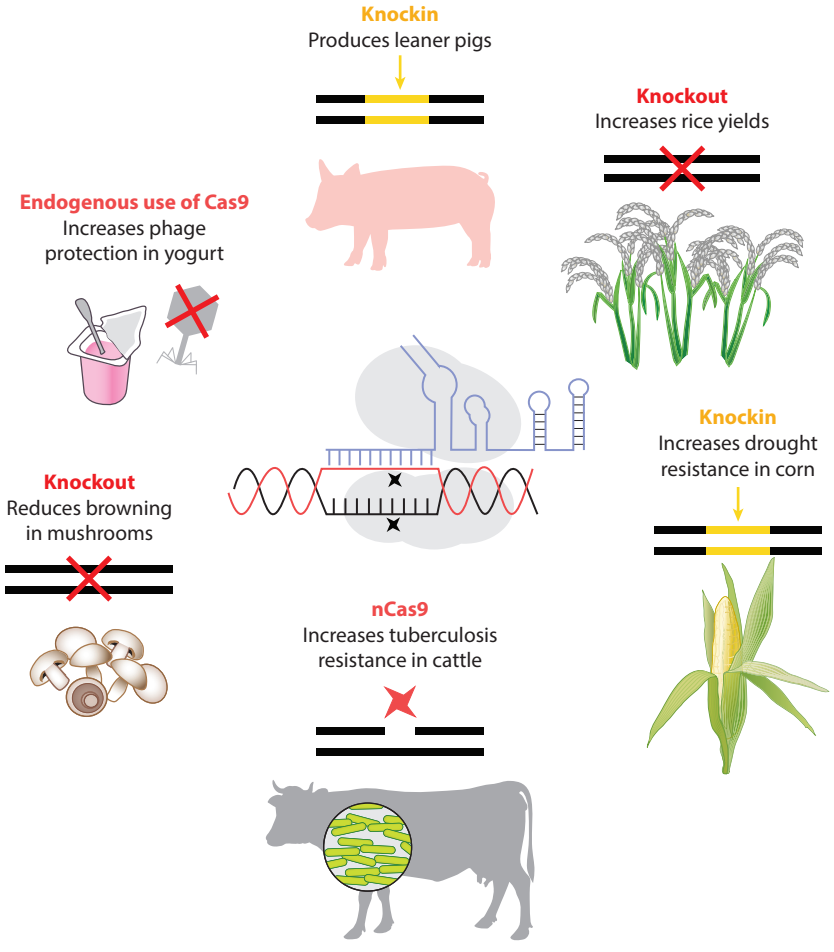

Figure 3

The CRISPR (clustered regularly interspaced short palindromic repeats) farm. CRISPR-Cas9 technology has been utilized in many ways to edit multiple target organisms. Starting from the top of the figure and moving clockwise, there has been a knockin to produce leaner pigs, a knockout to increase rice yields, a knockin to increase drought resistance in corn, nCas9 to increase tuberculosis resistance in cattle, a knockout to reduce browning in mushrooms, and, finally, endogenous use of Cas9 to increase phage protection in yogurt.

of the many CRISPR products entering, or close to entering, the marketplace, a heavy emphasis has been placed on regulation in the CRISPR food arena (Van Eenennaam 2018, Waltz 2018, Wolt et al. 2016). Integral to the conversation is CRISPR's status, or nonstatus, as a GMO. As it currently stands, GMOs are most often defined on the basis of an alteration to an organism that results in the presence of foreign DNA from another species, yielding a transgenic organism. Defined as such, CRISPR technically can readily create non-GMO products. This can be achieved through either CRISPR delivery or novel CRISPR applications. In terms of delivery, the use of RNPs allows DNA-free use of CRISPR editing by solely providing a Cas9 protein loaded with a guide RNA, which is used to cut DNA and prompt the natural repair mechanisms to generate mutations naturally. In terms of application, utilizing CRISPR as a screening tool to identify natural rare mutants in the population allows for new product development without any editing at all. It has become abundantly clear that regulatory policies currently cannot keep up with the CRISPR revolution.

In response to the CRISPR craze, both the United States and the European Union are set to make policy decisions on gene-edited crops and animals this year considering the new technological advances. There are two general approaches for GMO regulation (Araki & Ishii 2015). The first regulation route is product based. In this situation, the end product is under scrutiny. If no foreign DNA is present in the final product, it is not subjected to regulation. The USDA announced earlier this year that this was the route it would use. It stated that if the crop could have been produced naturally, the USDA will not regulate the product, independent of methodology (USDA 2018a). This opens the door for any genetically engineered crop, whether it was developed through CRISPR, ZFNs, TALENs, or other means. The second regulatory route is process based, which is commonly used in the EU. In this situation, the methodology of how a new product is developed is under scrutiny. This approach has also been adopted by the FDA in regard to genetically engineered animals (Van Eenennaam 2018). Methodology-based regulation is considered more conservative than product-based regulation. An alternative regulatory system has recently been proposed in Norway. This process would engage a tiered system. It is hoped that this will make understanding regulations easier and allow products to be regulated on the basis of how much was changed instead of a blanket regulation. This looks to be particularly promising for aquaculture (Fletcher 2017). Recently, the European Court of Justice ruled that edited crops would fall under the existing GMO framework, creating a regulatory burden for commercialization and a challenging route for global companies to manage product approval and regulation differently across regions (Stokstad 2018). Overall, companies favor the first regulation route in which the decision is product based, as illustrated by the recent USDA ruling on nonbrowning white button mushrooms and apples and flax with enhanced omega-3 oil (Waltz 2018). This allows easier entry for agriculture businesses to commercialize crops into the marketplace as millions of dollars are trimmed from the process, and the commercialization window is reduced by years. Some countries have begun incorporating a more blended approach, especially when agricultural trade among countries with different regulatory processes is considered (Araki & Ishii 2015, Ramessar et al. 2008).

As CRISPR has entered the world stage, warnings against potential pitfalls about launching CRISPR agricultural products have arisen. Many interested parties are concerned about a potential fallout reminiscent of the public resistance to GMOs. To avoid this, calls have been made to engage the public in the discussion. A recent study in China has shown that a minority supports the notion of CRISPR crops (Cui & Shoemaker 2018). The authors cite that most were hesitant to support CRISPR-edited foods because of a general lack of understanding of the methodology itself as well as a desire to better understand the risks involved. Interestingly, support is greater for human genetic editing for life-ending diseases than for CRISPR crops. A separate study also revealed modest support for CRISPR when applied to life-threatening conditions (McCaughey et al. 2016). It appears to several researchers that consumers wish to better understand the

risk–benefit analysis for CRISPR foods (Cui & Shoemaker 2018, Funk & Kennedy 2016, Ishii & Araki 2016). It has also been determined that consumers are more interested in benefits they can directly measure (such as increased omega-3s) than those that are indirectly measured (such as benefits to the farmers that may lower costs) (Lucht 2015). Complicating the situation is a lack of trust in scientists when it comes to genetically engineered foods (Cui & Shoemaker 2018, Ishii & Araki 2016, Lucht 2015), despite several studies and reports by esteemed academic organizations that these products do not pose a safety threat to human health. Opinion on CRISPR-edited food is also affected by the media, which many feel is negative (Malyska et al. 2016). To combat these issues, the idea of labeling CRISPR-edited foods has been raised. Many cite the need to increase public trust as a reason to accept labeling. It has been shown that when GMO foods are labeled, consumers tend to trust that food company more (Davis 2018). A study in Switzerland has also revealed that when GMO products are labeled and offered alongside non-GMO products, they are more likely to be purchased than when GMO products are offered alone (Lucht 2015). Overall the trends reveal that consumers desire and likely need a selection of product options and a greater understanding of where their food comes from. Those against the labeling of CRISPR foods cite economic issues involved with adding the labels. There is also fear that this will cause consumers to not purchase the products (McFadden 2017). Another concern is the feasibility of creating labels. As the products could be produced through natural means and not genetic editing intervention, there is concern that it will be impossible to validate labels (Araki & Ishii 2015).

Although there are many differing and conflicting opinions on how to best handle and regulate CRISPR products in the food supply chain, it is clear that the public's opinion will play a major role. With that in mind, it is imperative that researchers, companies, and governments work together to educate and disseminate information to the public so they can make informed decisions on their food choices.

CONCLUSIONS

We are living in a CRISPR world and the future is now. From farm to fork, CRISPR is influencing the food supply chain at every level: from starter cultures to crop and livestock improvement. CRISPR is just one example of how microbes are constantly shaping our world. Advances through microbiome and metagenomic studies are beginning to reveal just how much potential lies ahead. This is especially true in the food industry, with bacteria and yeasts widely used in fermentations and manufacturing of many foods and beverages. This also includes the ability to exploit several CRISPR-based technologies in microbiomes that span the farm (e.g., soil microbiome, livestock microbiome, feed microbiome), the manufacturing facilities (e.g., fermentation tanks, processing lines, food safety control points, packaging environments), and the consumer (e.g., oral and gut microbiomes), as along with CRISPR, microbes are farm to fork. Research is addressing how soil microorganisms affect crops and their yield. The microbiomes of livestock affect their health and growth. Finally, human gut health is largely impacted by its microbiome(s), which is often influenced by diet. It is clear that we are far from exhausting the capabilities of CRISPR in the food supply chain and have much further to go in the utilization of CRISPR-based technologies for the manufacturing of healthier and more sustainable food products.

DISCLOSURE STATEMENT

R.B. is an inventor on several patents related to various uses of CRISPR-based technologies and a shareholder of Dow–DuPont, Caribou Biosciences, Intellia Therapeutics, Locus Biosciences, and Inari, companies with business interests in CRISPR-based applications.

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LITERATURE CITED

Acquaah G. 2009. *Principles of Plant Genetics and Breeding*. Hoboken, NJ: Wiley-Blackwell

Altenbuchner J. 2016. Editing of the *Bacillus subtilis* genome by the CRISPR-Cas9 System. *Appl. Environ. Microbiol.* 82:5421–27

Andersson AF, Banfield JF. 2008. Virus population dynamics and acquired virus resistance in natural microbial communities. *Science* 320:1047–50

Andersson M, Turesson H, Nicolia A, Fält A-S, Samuelsson M, Hofvander P. 2017. Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR-Cas9 expression in protoplasts. *Plant Cell Rep*. 36:117–28

Araki M, Ishii T. 2015. Towards social acceptance of plant breeding by genome editing. *Trends Plant Sci*. 20:145–49

Armin S, Felix W, Jacqueline B, Holger P, David E. 2017. Towards CRISPR/Cas crops: bringing together genomics and genome editing. *New Phytol*. 216:682–98

Barrangou R. 2015. Diversity of CRISPR-Cas immune systems and molecular machines. *Genome Biol*. 16:247

Barrangou R, Coûté-Monvoisin A-C, Stahl B, Chavichvily I, Damange F, et al. 2013. Genomic impact of CRISPR immunization against bacteriophages. *Biochem. Soc. Trans.* 41:1383–91

Barrangou R, Doudna JA. 2016. Applications of CRISPR technologies in research and beyond. *Nat. Biotechnol.* 34:933–41

Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, et al. 2007. CRISPR provides acquired resistance against viruses in prokaryotes. *Science* 315:1709–12

Barrangou R, Horvath P. 2012. CRISPR: new horizons in phage resistance and strain identification. *Annu. Rev. Food Sci. Technol.* 3:143–62

Barrangou R, Horvath P. 2017. A decade of discovery: CRISPR functions and applications. *Nat. Microbiol.* 2:17092

Beisel CL, Gomaa AA, Barrangou R. 2014. A CRISPR design for next-generation antimicrobials. *Genome Biol*. 15:516

Biot-Pelletier D, Martin VJJ. 2016. Seamless site-directed mutagenesis of the *Saccharomyces cerevisiae* genome using CRISPR-Cas9. *J. Biol. Eng.* 10:6

Bomgardner MM. 2017. A new toolbox for better crops. *Chem. Eng. News* 95:30–34

Bortesi L, Fischer R. 2015. The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnol. Adv.* 33:41–52

Brooks C, Nekrasov V, Lippman ZB, Van Eck J. 2014. Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. *Plant Physiol*. 166:1292–97

Brouns SJJ, Jore MM, Lundgren M, Westra ER, Slijkhuis RJH, et al. 2008. Small CRISPR RNAs guide antiviral defense in prokaryotes. *Science* 321:960–64

Carlson DF, Lancto CA, Zang B, Kim E-S, Walton M, et al. 2016. Production of hornless dairy cattle from genome-edited cell lines. *Nat. Biotechnol.* 34:479–81

Carte J, Christopher RT, Smith JT, Olson S, Barrangou R, et al. 2014. The three major types of CRISPR-Cas systems function independently in CRISPR RNA biogenesis in *Streptococcus thermophilus*. *Mol. Microbiol*. 93:98–112

Chen B, Gilbert LA, Cimini BA, Schnitzbauer J, Zhang W, et al. 2013. Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. *Cell* 155:1479–91

Cleto S, Jensen JVK, Wendisch VF, Lu TK. 2016. *Corynebacterium glutamicum* metabolic engineering with CRISPR interference (CRISPRi). *ACS Synth. Biol.* 5:375–85

Cong L, Ran FA, Cox D, Lin S, Barretto R, et al. 2013. Multiplex genome engineering using CRISPR/Cas systems. *Science* 339(6121):819–23

Crawley AB, Henriksen ED, Stout E, Brandt K, Barrangou R. 2018. Characterizing the activity of abundant, diverse and active CRISPR-Cas systems in lactobacilli. *Sci. Rep.* 8:11544

Cui K, Shoemaker SP. 2018. Public perception of genetically-modified (GM) food: a Nationwide Chinese Consumer Study. *npj Sci. Food* 2:10

Davis V. 2018. GMO labeling makes public more likely to trust food companies. *Science* 362

Deltcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, et al. 2011. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature* 471:602–7

Deveau H, Barrangou R, Garneau JE, Labonté J, Fremaux C, et al. 2008. Phage response to CRISPR-encoded resistance in *Streptococcus thermophilus*. *J. Bacteriol*. 190:1390–400

Doudna JA, Charpentier E. 2014. The new frontier of genome engineering with CRISPR-Cas9. *Science* 346(6213):1258096

Edvardsen RB, Leininger S, Kleppe L, Skaftnesmo KO, Wargelius A. 2014. Targeted mutagenesis in Atlantic salmon (*Salmo salar* L.) using the CRISPR/Cas9 system induces complete knockout individuals in the F0 generation. *PLOS ONE* 9:e108622

Fletcher R. 2017. Norwegian legislative debate offers hope for gene editing in aquaculture. *The Fish Site*, Dec. 6. **https://thefishsite.com/articles/norwegian-legislative-debate-offers-hope-for-gene-editing-in-aquaculture**

Funk C, Kennedy B. 2016. *The New Food Fights: U.S. Public Divides Over Food Science*. Washington, DC: Pew Research Center

Gaj T, Gersbach CA, Barbas CF. 2013. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol*. 31:397–405

Gao Y, Wu H, Wang Y, Liu X, Chen L, et al. 2017. Single Cas9 nickase induced generation of NRAMP1 knockin cattle with reduced off-target effects. *Genome Biol*. 18:13

Gilbert LA, Horlbeck MA, Adamson B, Villalta JE, Chen Y, et al. 2014. Genome-scale CRISPR-mediated control of gene repression and activation. *Cell* 159:647–61

Gomaa AA, Klumpe HE, Luo ML, Selle K, Barrangou R, Beisel CL. 2014. Programmable removal of bacterial strains by use of genome-targeting CRISPR-Cas systems. *mBio* 5:e00928-13

Gomez MA, Lin ZD, Moll T, Luebbert C, Chauhan RD, et al. 2018. Simultaneous CRISPR/Cas9-mediated editing of cassava eIF4E isoforms nCBP-1 and nCBP-2 reduces cassava brown streak disease symptom severity and incidence. *Plant Biotechnol. J*. In press

Hale CR, Zhao P, Olson S, Duff MO, Graveley BR, et al. 2009. RNA-guided RNA cleavage by a CRISPR RNA-Cas protein complex. *Cell* 139:945–56

Hilton IB, D'Ippolito AM, Vockley CM, Thakore PI, Crawford GE, et al. 2015. Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. *Nat. Biotechnol.* 33:510

Horvath P, Barrangou R. 2010. CRISPR/Cas, the immune system of Bacteria and Archaea. *Science* 327:167–70

Horvath P, Romero DA, Coûté-Monvoisin A-C, Richards M, Deveau H, et al. 2008. Diversity, activity, and evolution of CRISPR loci in *Streptococcus thermophilus*. *J. Bacteriol*. 190:1401–12

Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, et al. 2013. DNA targeting specificity of RNA-guided Cas9 nucleases. *Nat. Biotechnol.* 31:827–32

Ishii T, Araki M. 2016. Consumer acceptance of food crops developed by genome editing. *Plant Cell Rep*. 35:1507–18

Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A. 1987. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. *J. Bacteriol.* 169:5429–33

Jiang W, Zhou H, Bi H, Fromm M, Yang B, Weeks DP. 2013. Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice. *Nucleic Acids Res*. 41:e188

Jiang Y, Chen B, Duan C, Sun B, Yang J, Yang S. 2015. Multigene editing in the *Escherichia coli* genome via the CRISPR-Cas9 system. *Appl. Environ. Microbiol.* 81:2506–14

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. 2012. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science* 337(6096):816–21

Karkute SG, Singh AK, Gupta OP, Singh PM, Singh B. 2017. CRISPR/Cas9 mediated genome engineering for improvement of horticultural crops. *Front. Plant Sci.* 8:1635

Khalil K, Elayat M, Khalifa E, Daghash S, Elaswad A, et al. 2017. Generation of myostatin gene-edited channel catfish (*Ictalurus punctatus*) via zygote injection of CRISPR/Cas9 system. *Sci. Rep.* 7:7301

Khan MHU, Khan SU, Muhammad A, Hu L, Yang Y, Fan C. 2018. Induced mutation and epigenetics modification in plants for crop improvement by targeting CRISPR/Cas9 technology. *J. Cell. Physiol.* 233:4578–94

Koonin EV, Makarova KS, Zhang F. 2017. Diversity, classification and evolution of CRISPR-Cas systems. *Curr. Opin. Microbiol.* 37:67–78

LaManna CM, Barrangou R. 2018. Enabling the rise of a CRISPR world. *CRISPR J.* 1:205–8

Larson MH, Gilbert LA, Wang X, Lim WA, Weissman JS, Qi LS. 2013. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. *Nat. Protoc.* 8:2180

Ledford H. 2015. Salmon is first transgenic animal to win US approval for food. *Nature*. **http://doi.org/10.1038/nature.2015.18838**

Liu J, Zhou Y, Qi X, Chen J, Chen W, et al. 2017. CRISPR/Cas9 in zebrafish: an efficient combination for human genetic diseases modeling. *Hum. Genet.* 136:1–12

Liu Q, Gao R, Li J, Lin L, Zhao J, et al. 2017. Development of a genome-editing CRISPR/Cas9 system in thermophilic fungal *Myceliophthora* species and its application to hyper-cellulase production strain engineering. *Biotechnol. Biofuels* 10:1

Liu SJ, Horlbeck MA, Cho SW, Birk HS, Malatesta M, et al. 2017. CRISPRi-based genome-scale identification of functional long noncoding RNA loci in human cells. *Science* 355(6320):aah7111

Lucht JM. 2015. Public acceptance of plant biotechnology and GM crops. *Viruses* 7:4254–81

Ma X, Mau M, Sharbel TF. 2018. Genome editing for global food security. *Trends Biotechnol.* 36:123–27

Makarova KS, Aravind L, Wolf YI, Koonin EV. 2011a. Unification of Cas protein families and a simple scenario for the origin and evolution of CRISPR-Cas systems. *Biol. Direct* 6:38

Makarova KS, Haft DH, Barrangou R, Brouns SJJ, Charpentier E, et al. 2011b. Evolution and classification of the CRISPR–Cas systems. *Nat. Rev. Microbiol.* 9:467–77

Makarova KS, Wolf YI, Alkhnbashi OS, Costa F, Shah SA, et al. 2015. An updated evolutionary classification of CRISPR–Cas systems. *Nat. Rev. Microbiol.* 13:722–36

Mali P, Yang L, Esvelt KM, Aach J, Guell M, et al. 2013. RNA-guided human genome engineering via Cas9. *Science* 339:823–26

Malyska A, Bolla R, Twardowski T. 2016. The role of public opinion in shaping trajectories of agricultural biotechnology. *Trends Biotechnol.* 34:530–34

Marraffini LA, Sontheimer EJ. 2008. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. *Science* 322:1843–45

Marraffini LA, Sontheimer EJ. 2010. Self versus non-self discrimination during CRISPR RNA-directed immunity. *Nature* 463:568

Maurer A. 2017. *Syngenta acquires non-exclusive license to use CRISPR-Cas9 gene-editing for agriculture applications*. Press Release, Novemb. 3. **https://www.ncbiotech.org/news/syngenta-acquires-non-exclusive-license-use-crispr-cas9-gene-editing-agriculture-applications**

McCaughey T, Sanfilippo PG, Gooden GE, Budden DM, Fan L, et al. 2016. A global social media survey of attitudes to human genome editing. *Cell Stem Cell* 18:569–72

McFadden BR. 2017. The unknowns and possible implications of mandatory labeling. *Trends Biotechnol.* 35:1–3

Miao C, Xiao L, Hua K, Zou C, Zhao Y, et al. 2018. Mutations in a subfamily of abscisic acid receptor genes promote rice growth and productivity. *PNAS* 115(23):6058–63

Nagaraju S, Davies NK, Walker DJF, Köpke M, Simpson SD. 2016. Genome editing of *Clostridium autoethanogenum* using CRISPR/Cas9. *Biotechnol. Biofuels* 9:219

Nekrasov V, Wang C, Win J, Lanz C, Weigel D, Kamoun S. 2017. Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. *Sci. Rep.* 7:482

Nishitani C, Hirai N, Komori S, Wada M, Okada K, et al. 2016. Efficient genome editing in apple using a CRISPR/Cas9 system. *Sci. Rep.* 6:31481

Nødvig CS, Hoof JB, Kogle ME, Jarczynska ZD, Lehmbeck J, et al. 2018. Efficient oligo nucleotide mediated CRISPR-Cas9 gene editing in Aspergilli. *Fungal Genet. Biol.* 115:78–89

Nødvig CS, Nielsen JB, Kogle ME, Mortensen UH. 2015. A CRISPR-Cas9 system for genetic engineering of filamentous fungi. *PLOS ONE* 10:e0133085

Oh J-H, van Pijkeren J-P. 2014. CRISPR–Cas9-assisted recombineering in *Lactobacillus reuteri*. *Nucleic Acids Res.* 42:e131

Oishi I, Yoshii K, Miyahara D, Kagami H, Tagami T. 2016. Targeted mutagenesis in chicken using CRISPR/Cas9 system. *Sci. Rep.* 6:23980

Pennisi E. 2013. The CRISPR craze. *Science* 341:833–36

Pohl C, Kiel JAKW, Driessen AJM, Bovenberg RAL, Nygård Y. 2016. CRISPR/Cas9 based genome editing of *Penicillium chrysogenum*. *ACS Synth. Biol.* 5:754–64

Ramessar K, Capell T, Twyman RM, Quemada H, Christou P. 2008. Trace and traceability—a call for regulatory harmony. *Nat. Biotechnol.* 26:975–78

Ray DK, Mueller ND, West PC, Foley JA. 2013. Yield trends are insufficient to double global crop production by 2050. *PLOS ONE* 8:e66428

Rosenblum A. 2018. Meet the woman using CRISPR to breed all-male "terminator cattle." *MIT Technol. Rev.* **https://www.technologyreview.com/s/609699/meet-the-woman-using-crispr-to-breed-all-male-terminator-cattle/**

Schuster M, Schweizer G, Reissmann S, Kahmann R. 2016. Genome editing in *Ustilago maydis* using the CRISPR–Cas system. *Fungal Genet. Biol.* 89:3–9

Selle K, Barrangou R. 2015. CRISPR-based technologies and the future of food science. *J. Food Sci.* 80:R2367–72

Selle K, Klaenhammer TR, Barrangou R. 2015. CRISPR-based screening of genomic island excision events in bacteria. *PNAS* 112:8076–81

Shan Q, Wang Y, Li J, Zhang Y, Chen K, et al. 2013. Targeted genome modification of crop plants using a CRISPR-Cas system. *Nat. Biotechnol.* 31:686–88

Shi J, Guo H, Wang H, Lafitte HR, Archibald RL, et al. 2017. ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotechnol. J.* 15:207–16

Sternberg SH, Redding S, Jinek M, Greene EC, Doudna JA. 2014. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. *Nature* 507:62–67

Stokstad E. 2018. European court ruling raises hurdles for CRISPR crops. *Science*. **https://doi.org/10.1126/science.aau8986**

Teater B. 2018. *Five company success stories highlighted at CED Conference*. Press Release, Febr. 18. **https://www.ncbiotech.org/news/five-company-success-stories-highlighted-ced-conference**

Telugu BP, Park KE, Park CH. 2017. Genome editing and genetic engineering in livestock for advancing agricultural and biomedical applications. *Mamm. Genome* 28:338–47

Tilman D, Balzer C, Hill J, Befort BL. 2011. Global food demand and the sustainable intensification of agriculture. *PNAS* 108:20260–64

Tsai SQ, Wyvekens N, Khayter C, Foden JA, Thapar V, et al. 2014. Dimeric CRISPR RNA-guided FokI nucleases for highly specific genome editing. *Nat. Biotechnol.* 32:569

Tyson GW, Banfield JF. 2008. Rapidly evolving CRISPRs implicated in acquired resistance of microorganisms to viruses. *Environ. Microbiol.* 10:200–07

Upadhyay SK, Kumar J, Alok A, Tuli R. 2013. RNA-guided genome editing for target gene mutations in wheat. *Genes Genomes Genet.* 3:2233–38

USDA (US Dep. Agric.). 2018a. *Secretary Perdue issues USDA statement on plant breeding innovation*. Press Release 0070.18, March 28. **https://www.usda.gov/media/press-releases/2018/03/28/secretary-perdue-issues-usda-statement-plant-breeding-innovation**

USDA (US Dep. Agric.). 2018b. *Agricultural projections to 2027*. Rep. OCE-2018–1, Interag. Agric. Proj. Comm., Washington, DC

Van Eenennaam AL. 2018. The importance of a novel product risk-based trigger for gene-editing regulation in food animal species. *CRISPR J.* 1:101–06

Vigentini I, Gebbia M, Belotti A, Foschino R, Roth FP. 2017. CRISPR/Cas9 system as a valuable genome editing tool for wine yeasts with application to decrease urea production. *Front. Microbiol.* 8:2194

Vyas VK, Barrasa MI, Fink GR. 2015. A *Candida albicans* CRISPR system permits genetic engineering of essential genes and gene families. *Sci. Adv.* 1(3):e1500248

Waltz E. 2016. Gene-edited CRISPR mushroom escapes US regulation. *Nature* 532:293

Waltz E. 2018. With a free pass, CRISPR-edited plants reach market in record time. *Nat. Biotechnol.* 36:6–7

Wang B. 2015. Disruptive CRISPR gene therapy is 150 times cheaper than zinc fingers and CRISPR is faster and more precise. *Next Big Future*. **https://www.nextbigfuture.com/2015/06/disruptive-crispr-gene-therapy-is-150.html**

Wolt JD, Yang B, Wang K, Spalding MH. 2016. Regulatory aspects of genome-edited crops. *In Vitro Cell. Dev. Biol. Plant* 52:349–53

Yang L, Güell M, Niu D, George H, Lesha E, et al. 2015. Genome-wide inactivation of porcine endogenous retroviruses (PERVs). *Science* 350(6264):1101–4

Yupeng C, Li C, Xiujie L, Chen G, Shi S, et al. 2018. CRISPR/Cas9-mediated targeted mutagenesis of GmFT2a delays flowering time in soya bean. *Plant Biotechnol. J.* 16:176–85

Zheng Q, Lin J, Huang J, Zhang H, Zhang R, et al. 2017. Reconstitution of UCP1 using CRISPR/Cas9 in the white adipose tissue of pigs decreases fat deposition and improves thermogenic capacity. *PNAS* 114:E9474–82

Zoephel J, Randau L. 2013. RNA-Seq analyses reveal CRISPR RNA processing and regulation patterns. *Biochem. Soc. Trans.* 41:1459–63