ISB NEWS REPORT
AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY

September 2016

PLANT RESEARCH NEWS

CONTENTS

Prospects for Enhancing Plant and Algal Biofuel Production by Expanding the Wavelength Range for Photosynthesis

Donald A. Bryant

Introduction—the problem

According to the Population Division of the United Nations, the current global population of 7.3 billion is estimated to rise to about 9.7 billion by 2050 and to increase to about 11.2 billion (range, 9.5 to 13.3 billion, depending on the model assumptions) by the end of this century[1]. Much of this increase will occur in Africa, where the population is expected to increase by 3- to 5-fold, from the current 1.2 billion to between 3.4 to 5.6 billion. The U. S. population is expected to increase steadily from the current value of 322 million to about 450 million[2]. Farmers Feeding the World estimates that the average US farmer currently produces enough food to feed ~155 persons[3], and the United Nations further estimates that global agriculture will need to produce 70 percent more food 40 years from now. This would mean that the average U. S. farmer will need to produce sufficient food to feed 264 people, and obviously substantially more than this number by 2100. Estimates suggest that only ~10% of the necessary agricultural gains will come from increases in the acreage devoted to food production. Obviously, increasing both acreage and production per unit area for agriculture in developing countries, especially in Africa and Asia, will be extremely important in meeting these challenges. However, much of the responsibility for the requisite increases in food production will likely fall on maximizing production in developed countries—squeezing ever more production from the arable acreage currently in use—or possibly from even substantially less area. These numbers indicate that improving crop production will be a major challenge in the coming years. Climate change could obviously exert additional, severe deleterious effects on agriculture.

At the same time that more food will be required, there will be a parallel and increasing demand for energy, including biofuels, and biomass, which will continue to compete for food crops for acreage. World energy consumption is roughly predicted to double by 2050 and to triple by 2100 from current values. Renewable energy sources are expected to double by 2050, and the International Energy Agency estimates that biofuels will increase from only 2% of global transport fuels today to ~27% in 2050[4]. These dire predictions illustrate the importance of increasing agricultural productivity.

One part of the solution: expand the wavelength range for photosynthesis

Agricultural food production and biofuels production share an obvious common denominator: both depend directly on oxygenic photosynthesis, which couples energy from the sun to the production of biomass. Thus, any improvements in the efficiency of

VirginiaTech
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PUBLISHED BY

Information Systems for Biotechnology

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photosynthesis could lead to improvements in plant productivity and potentially to biofuel production. One way to improve the efficiency of photosynthesis is to expand the wavelength range used for photosynthesis[5-7]. The wavelength range for oxygenic photosynthesis is roughly the same as the sensitivity of the human eye and is driven by wavelengths between 400 and 700 nm, so-called photosynthetically active radiation (PAR) (see Fig. 1). On an energy basis, PAR accounts for ~43% of the full solar spectrum reaching Earth's surface[6,7]. Expanding the wavelength range for photosynthesis to 750 nm would increase the number of photons available for photosynthesis by 19%, and expanding the wavelength range to 800 nm would nearly double this increase (Fig. 1).

Competition for light among phototrophs has been an inescapable consequence of photosynthesis since the first bacterium evolved this capability. This has been a classic "Cain *versus* Abel" conflict. As soon as the first photosynthetic cell divided, those two cells were then directly competing for an essential resource, sunlight, because they contained identical sets of photosynthetic proteins and pigments. Whichever cell was physically on top would have had a clear advantage in absorbing the incident sunlight, which provided a selective advantage to any organism that could modify its pigmentation. It is increasingly obvious that chlorophyll (Chl) *a* was probably the first Chl to be widely and successfully used for photosynthesis, as it still is today in plants, algae, and cyanobacteria. Chlorophyllide (Chlide) *a*, the immediate biosynthetic precursor of Chl *a*, is the "hub compound" of Chl biosynthesis[8]. Pathways leading to nine of the fourteen major Chls, including Chls *b*, *d*, and *f* as well as bacteriochlorophylls (BChl) *a*, *c*, *d*, *e* and *f*, that occur in plants and bacteria, share Chlide *a* as the central precursor. The other five Chls, [3, 8]-divinyl-Chl *a*, [3, 8]-divinyl-Chl *b*, 8-OH-Chl *a*, BChl *g*, and BChl *b* are synthesized from a precursor of Chlide *a*, [3, 8]-divinyl protochlorophyllide[8]. Compared to Chl *a*, seven Chls (Chl *d* and Chl *f*; BChl *a*, *b*, *c*, *d*, and *g*) extend light harvesting into the far-red and near-infrared regions of the solar spectrum (see Fig. 1). Five Chls (Chl *b*, [3, 8]-divinyl-Chl *a*, [3, 8]-divinyl-Chl *b*, BChl *e*, and BChl *f*) enhance light absorption in mostly aquatic environments that become highly enriched for green and blue wavelengths with depth (see Fig. 1). Although BChl *f* has not yet been shown to occur naturally, it can be produced by a single mutation in organisms that synthesize BChl *e*, and it functions similarly to BChl *e* [9].

Far-red absorbing Chls: Chl *d* and Chl *f*

Cyanobacteria perform oxygen-evolving photosynthesis in essentially the same way that higher plants and eukaryotic algae do. They use three light-dependent, multi-protein complexes to do this: Photosystem (PS) I[10], PS II[11], and light-harvesting antenna structures known as phycobilisomes[12]. Cyanobacterial PS I and PS II complexes bind 96 and 35 Chl *a* molecules, respectively, in addition to 22 and 11 photoprotective β-carotene molecules, respectively[10,11]. For many years it was believed that cyanobacteria only used Chl *a* for photosynthesis, but this view changed with the discovery of *Prochloron* spp., *Prochlorothrix hollandica*, and *Prochlorococcus* spp., all of which synthesize Chl *b* (or [3,

8]-divinyl-Chl *b*) in addition to Chl *a* (*Prochlorococcus* spp. also synthesize [3, 8]-divinyl-Chl *a* instead of Chl *a*)[13]. These organisms have specialized membrane-associated antenna proteins that can bind Chl *b* accessory pigments together with Chl *a*. As noted above, these pigments enhance absorption of blue light (Fig. 1).

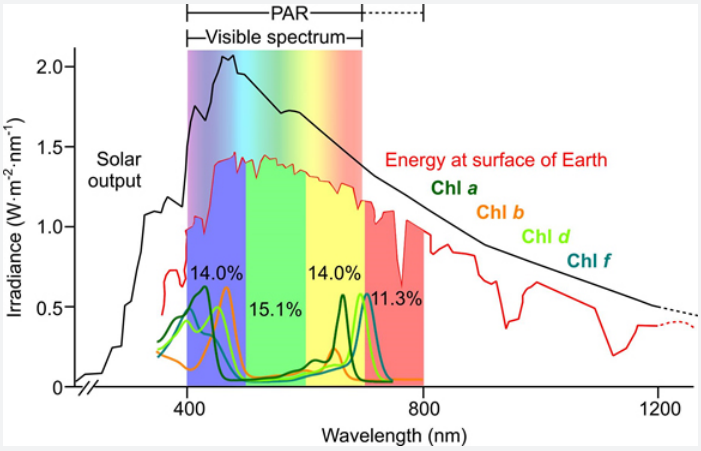


Figure 1. Solar output spectrum (black line) and the solar energy output spectrum at the surface of Earth (red line). The visible spectrum (400 to 700 nm), which roughly corresponds to photosynthetically active radiation (PAR), is shown in the background and by the bar at the top. The percentage of total output energy for 100-nm intervals between 400 and 800 nm are indicated by the violet, green, yellow, and red shading, respectively. Absorption spectra (in methanol) for Chl a (dark green line), Chl b (orange line), Chl d (light green line) and Chl f (teal line) are also shown. The long-wavelength absorption maxima of Chls are typically further red-shifted when the Chls are bound to proteins in the photosynthetic apparatus of cells. Anoxygenic photosynthetic bacteria producing BChl a or BChl b are also able to use light from 800 to more than 1000 nm. Phototrophic bacteria do not use wavelengths of light longer than about 1100 nm because of the strong absorption of light between 1100 and 1200 nm by water. As shown by the dotted line, increasing photosynthetic light utilization of plants and algae into the region between 700 and 800 nm would result in a very substantial increase in light energy (~26%) available for photosynthesis.

The discovery of the unusual cyanobacterium, *Acaryochloris marina*, in 1996 is of particular note, because this cyanobacterium is the first organism identified that synthesizes Chl *d*, which serves as both a major antenna pigment as well as forming P740, the primary electron donor in PS I[14,15]. Compared to Chl *a*, which absorbs maximally at about 665 nm in methanol, Chl *d* absorbs maximally at 695 nm (Fig. 1)[16]. This red-shifted Chl allows *Acaryochloris* spp. to grow in specialized environments that are enriched in far-red light (e.g., on the underside of the fronds of red algae, in mats under layers of Chl *a*-containing cyanobacteria, or in association with primitive marine animals, ascidians, which harbor Chl *a*-containing cyanobacteria on their dorsal side and Chl *d*-containing cyanobacteria on their ventral side).

The cyanobacterium, *Halomicronema hongdechloris*, which occurs in stromatolites in Sharks Bay, was very recently found to synthesize a new Chl, Chl *f* [17]. Further studies have shown that many terrestrial cyanobacteria can synthesize Chl *a*, Chl *d*, and Chl *f* [18-20]. The absorption maximum of Chl *f* is 707 nm in methanol, which is even more red-shifted than that of Chl *d* (Fig. 1)[16,17]. Cyanobacteria that synthesize Chl *f* also live in environments that are strongly enriched in far-red light: in microbial mats under layers of cyanobacteria, in the shade of plants, in soil crusts, in beach rock and other endolithic environments, and in very dense cyanobacterial blooms. These environments are enriched in far-red light because of absorbance filtering of light by Chl *a* or because of the physical properties of light (far-red and near-infrared light penetrate soil more deeply because they are less effectively scattered than light with shorter wavelengths)[20].

Far-red light photoacclimation (FaRLiP)

Cyanobacteria that synthesize Chl *f* only do so when they are grown in light enriched in far-red light (i.e., wavelengths longer than 700 nm). When exposed to far-red light, these organisms synthesize Chl *f* and a much small amount of Chl *d* (~1%) and extensively alter the core components of the three major photosynthetic complexes[18]. This process, known as **Fa**r-red **Li**ght **P**hotoacclimation (FaRLiP), is regulated by three proteins, RfpA, RfpB, and RfpC, which are essential for growth in far-red light[21]. RfpA is a sensor kinase that is also a phytochrome-like photoreceptor[18,21]. RfpA senses the ratio of red and far-red light, and it is predicted that the activity of its histidine kinase domain is dependent on the conformational state of the photoreceptor domain. RfpA probably transfers a phosphate group to a CheY-

like response regulator, which in turn transfers the phosphate to RfpB, a transcriptional activator with two CheY-like, phospho-receiver domains. In its active conformation, RfpB activates the transcription of a highly conserved cluster of seventeen genes, the FaRLiP gene cluster. This cluster encodes six core subunits of PS I, five core subunits of PS II, five core subunits of the phycobilisome, and chlorophyll *f* synthase[18,19]. These proteins are paralogs of genes encoded elsewhere in the genome that are expressed when cells are grown in white light or red light, but whose expression is generally lower in cells grown in far-red light. The modifications to the three major photosynthetic complexes includes the replacement of about 10% of the Chl *a* molecules by Chl *f* and the production of phycobiliproteins absorbing beyond 700 nm, which together allow cells to grow in far-red light[18,19]. As measured by oxygen evolution rate with far-red actinic light, the photosynthetic capacity of photo-acclimated cells is 40% greater than that of cells grown in white light[18].

Chlorophyll *f* synthase

The genes encoding the enzyme(s) responsible for the synthesis of Chl *d* have not yet been identified, but very recently, the enzyme that converts Chl *a* into Chl *f* (or Chlide a into Chlide *f*) has been identified[22]. One of the seventeen genes of the FaRLiP gene cluster, *psbA4*, encodes Chl *f* synthase. Null mutants in the *psbA4* gene are unable to synthesize Chl *f*, and expression of *psbA4* in a cyanobacterium that normally does not synthesize Chl *a* leads to synthesis of Chl *f* [22]. Chl *f* is a photooxidoreductase that is a distant paralog of the PsbA subunit in the core of the PS II reaction center. PsbA binds three Chl *a* molecules, one pheophytin *a* molecule (Chl *a* lacking the central Mg atom), one plastoquinone molecule, and one β–carotene. Most importantly, PsbA ligates the $Mn_4Ca_1O_5$ cluster that is the catalyst for water oxidation in PS II[11]. ChlF lacks the ligands for the $Mn_4Ca_1O_5$ cluster but has the amino acid residues for binding all of the other components[22]. It has been proposed that ChlF functions as a homodimer, oxidizing Chl *a* rather than water to produce Chl *f*. The oxidation of two water molecules to produce dioxygen, and the oxidation of Chl *a* to produce Chl *f*, are both four-electron oxidations. ChlF presumably requires four photons to oxidize Chl *a* as does PS II. Phylogenetic analyses suggest that ChlF diverged from the PsbA lineage prior to the invention of water oxidation by cyanobacteria. The implication of this result is that the oxidation of Chl *a* to enhance photosynthesis by organisms in far-red light, through gene duplication and divergence, may have given rise to water-oxidizing PS II[22].

Prospects for improving the production yields for crop plants and algal biofuels

As noted above, the introduction of the *chlF* gene into a cyanobacterium that is only able to synthesize Chl *a* naturally resulted in the synthesis of Chl *f*. The good news is that the introduction of a single gene was sufficient to detect the synthesis of Chl *f* in *Synechococcus* sp. PCC 7002. However, the bad news is that the amount of Chl *f* synthesized was very small (only ~0.06% of the total Chl)—easily detected by modern biochemical methods against a zero background but insufficient to assess whether Chl *f* is functionally bound to photosynthetic complexes. To achieve levels of Chl *f* similar to those in FaRLiP strains will require a roughly 100-fold increase from this initial level. Prospects for increasing the Chl *f* synthase activity in *Synechococcus* sp. PCC 7002 are excellent. Mutations in *chlF* have already been constructed, and one produces 8-fold greater levels of Chl *f*, which is a very substantial increase. Codon-optimized *chlF* genes have been synthesized and are currently being tested. It should not take very long to determine whether the Chl *f* that is produced is functionally associated with PS I and/or PS II complexes in cells. Because FaRLiP strains produce paralogous core subunits of PS I and PS II in far-red light, it is possible that it will be necessary to introduce these genes into *Synechococcus* sp. as well. The good news again is that the number of genes is rather small—six in the case of PS I, and five in the case of PS II. Interestingly, one FaRLiP strain, *Synechococcus* sp. PCC 7335, apparently replaces mostly its PS I complexes with paralogous complexes, but it retains its Chl *a*-containing PS II complexes when cells are grown in far-red light[22,23].

Once the factors affecting the synthesis and

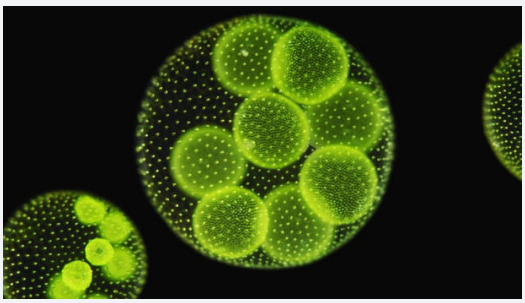
functionality of Chl *f* have been optimized in *Synechococcus* sp. PCC 7002, one can begin to think about producing Chl *f* in plants and algae. It will be necessary to produce the requisite genes in chloroplasts, because ChlF will require either Chl *a* or Chlide a to produce Chl *f*. Again, it might be necessary to co-express the genes encoding the core subunits of PS I and/or PS II together with Chl *f*.

Considering that modern agriculture is land-limited to some extent, crop plants are now being grown at very high densities that produce just the type of filtering of light by Chl *a* (and Chl *b*) that causes far-red light enrichment. At typical plant densities in use today, the lowest blades of corn plants certainly experience light that is highly enriched in far-red light and would benefit from the light harvesting between 700 and 800 nm. Chl *f* would also provide benefits for light harvesting in the early morning, the late afternoon, and due to seasonal changes in the angle of the sun that cause enrichment in far-red light.

In much the same way, algae (and cyanobacteria) grown for biofuel production would also benefit from the ability to absorb far-red light. Although far-red and near-infrared light are attenuated by passage through water, the relatively short pathlengths encountered in shallow ponds or other commercial growth systems used for large-scale cultivation of these organisms would not interfere significantly with light penetration by far-red light. In dense cultures with high Chl *a* contents, organisms with far-red absorbing Chls (either Chl *d* or *f*) could significantly enhance light harvesting in the culture overall. The fact that *Acaryochloris* spp. perform oxygenic photosynthesis while primarily using Chl *d* as the photoactive pigment demonstrates that far-red light is energetically sufficient to support oxygenic photosynthesis, and the same must be true for Chl *f*, since oxygen evolution is supported by far-red light in FaRLiP organisms. However, it is not yet clear whether simply replacing "some" Chl *a* by Chl *f* would still allow efficient photosynthesis. Randomly inserted Chl *f* molecules could trap excitation energy and not efficiently support the photochemical reactions. This problem could be the reason that core proteins of PS I and PS II are replaced during acclimation to far-red light. Further research will be required to determine if this is the case.

Conclusions

The identification of the gene encoding Chl *f* synthase and its successful expression in a cyanobacterium sets the stage for similar experiments in plants and algae. Chl *f*, possibly together with specialized core photosystem proteins, could extend the wavelength range of PAR by up to 100 nm, which would substantially increase the number of photons available for photosynthesis in situations where light filtering by Chl *a* occurs, and could substantially increase light absorption over the course of a growing season for crop plants as well as algae and cyanobacteria used for biofuels production.



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Engineered Gene Drives: After Speedy Development, Time to Downshift?

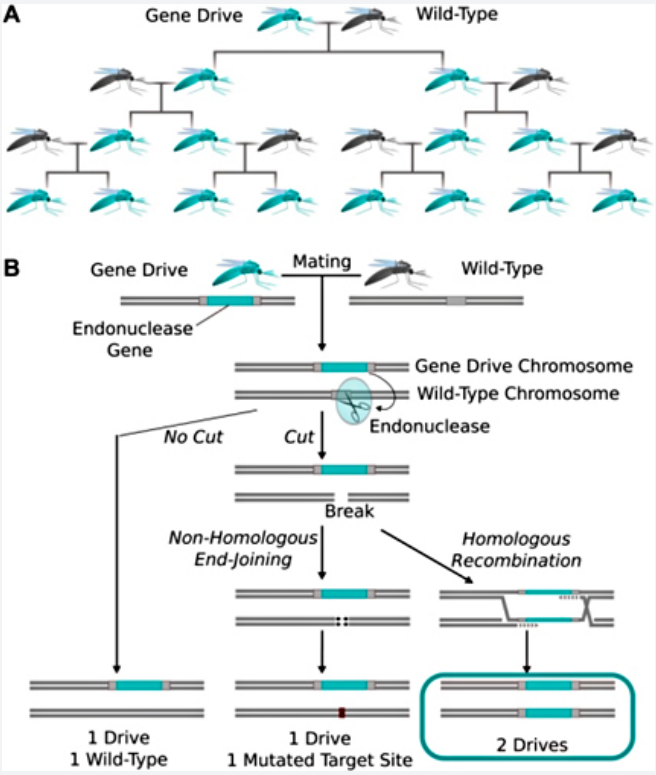
Phill Jones

For more than 50 years, scientists have studied the phenomenon of gene drives. In species that sexually reproduce, chromosomes carry two versions of a gene, representing genes inherited from the mother and father. The chance that an offspring will carry either the maternal or paternal version of a typical gene is 50 percent. A gene drive is not a typical gene; a gene drive is inherited by nearly all offspring. These "selfish genetic elements" eventually spread to all members of a population even if they create a disadvantage to individual organisms.

The P element of fruit flies is an example of a naturally-occurring gene drive. Researchers did not detect the P element transposon before 1950. Today, the P element occurs in almost all fruit flies examined around the world.

Another type of gene drive is the group of endonuclease genes that are duplicated into chromosomes that lack the endonuclease genes. A natural homing endonuclease cleaves the corresponding locus of a chromosome that does not carry the endonuclease gene, stimulating the cell to repair the chromosome using homologous recombination to copy the endonuclease gene into the damaged chromosome. Eggs and sperm derived from cells subjected to a homing endonuclease carry the endonuclease gene.

In 2003, Austin Burt, a biologist at Imperial College (UK), suggested the use of a homing endonuclease gene to force genetic changes into a wild-type population. At the time, a predictable and precise application of a gene drive to alter anything other than the natural site for a homing endonuclease gene did not exist. Nine years later, Jennifer Doudna, a biochemist at the University of California, Berkeley, and Emmanuelle Charpentier, currently at the Max Planck Institute for Infection Biology, Berlin, described technology that can be used to edit a genome in a precise, targeted manner. The CRISPR/Cas9 system enabled researchers to create engineered gene drives to distribute certain genetic alterations through a population.



The spread of endonuclease gene drives. (A) The mating of an organism that carries an endonuclease gene drive (blue) with a wild-type organism (grey) produces offspring that carry the gene drive. (B) The preferential inheritance of the gene drive occurs, because the endonuclease cleaves the homologous wild-type chromosome, which is repaired using homologous recombination with the gene drive chromosome as a repair template.

Source: Esvelt et al. (2014); Open-i service of the National Library of Medicine (https://openi.nlm.nih.gov/detailedresult.php?img=PMC4117217_elife03401f001&req=4).

Engineered Gene Drives: In Theory

Engineered gene drives have limitations. Many generations are required before an engineered gene drive can become established within a population. The amount of time depends upon many factors, such as generation time, the number of gene drive-altered individuals that are released into a natural population, the efficiency of the gene drive, and potential negative effects of the gene drive on the health of an individual.

A gene drive that negatively impacts fitness may have to be reintroduced into the population. Most significantly, gene drive technology requires a species that reproduces mainly by sexual reproduction. As a practical matter, crops grown from established seed stocks would not be candidates for gene drives. Similarly, gene drive technology could not be used with livestock in controlled breeding programs.

So, how might gene drive technology be used? In the area of agriculture, a gene drive could reverse herbicide resistance in a weed population, or restore vulnerability of insect pests to *Bt* toxins. Gene drives could eliminate certain diseases by targeting the insect vectors that spread the diseases, by genetically altering organisms that cause diseases, or by genetically altering animals, such as rats, that are reservoirs for disease-causing organisms. The technology also could be used to exterminate invasive species that destroy native species.

Engineered Gene Drives: In Practice

Engineered gene drives are not just a theory; researchers have proved that the technology works. During December 2014, Valentino M. Gantz and Ethan Bier discovered the results of their gene drive experiment on fruit flies, which they had performed in their lab at the University of California, San Diego. Using a technique based upon the CRISPR/Cas9 genome editing system, they introduced a mutation that disabled wild-type copies of a pigmentation gene. The loss-of-function allele was transmitted to the next generation with 97% efficiency. According to a report in *Nature*, Bier and Gantz contacted Anthony James, a molecular biologist at the University of California (Irvine), told James about their results, and wondered if a gene drive might work in mosquitoes.

James and his colleagues engineered a CRISPR/Cas9-mediated gene drive system for *Anopheles stephensi* mosquitoes, the Asian malaria vector. They inserted two genes that confer resistance to the malaria parasite, *Plasmodium falciparum*. After outcrossing GE mosquitoes with wild-type mosquitoes, the researchers found that the anti-*Plasmodium falciparum* effector genes were transmitted to progeny with greater than 99% efficiency.

A group based at Imperial College London developed another type of CRISPR/Cas9-based gene drive for mosquitoes. In this case, the target was the mosquito species that transmits malaria in sub-Saharan Africa, *Anopheles gambiae*. They inserted genes to disrupt egg production in females as a tactic for reducing mosquito populations. Transmission rates to progeny exceeded 90%.

An early report of engineered gene drives demonstrated the technology in wild and laboratory strains of *Saccharomyces cerevisiae*. Kevin M. Esvelt, George M. Church, and their colleagues at Harvard University's Wyss Institute also developed biosafety measures to reverse changes in a trait produced by their gene drive system. The engineered gene drive, while persisting in cells, would be rendered inactive.

"Gene drive technology has great potential to solve global problems, such as malaria, for which we have no solutions today," Wyss Institute Founding Director Donald Ingber said in a press release. "But the field needs to proactively develop safeguard mechanisms and reversibility capabilities to ensure the safety of this new technology and enable its enormous potential for doing good."

Engineered Gene Drives: Applying Brakes to Further Development

During June, the National Academies of Sciences, Engineering and Medicine released a report summarizing a one-year study on gene drives. "There is insufficient evidence available at this time to support the release of gene-drive modified organisms into the environment," the NAS stated in the report. "However, the potential benefits of gene drives for basic and applied research are significant and justify proceeding with laboratory research and highly-controlled field trials."

Urging a slow development of gene drives, the NAS Committee on Gene Drive Research in Non-Human Organisms warned about possible unintended consequences of the technology. For example, the application of gene drive technology could affect non-target species by horizontal gene transfer. Engineered gene drives designed to eliminate a species, such as mosquitoes, could produce an unanticipated effect on the environment. "The fundamental issue at the crux of ecological consequences of releasing a gene-drive

modified organism," the NAS committee said, "is the fact that species do not exist in an ecological vacuum."

According to the committee, it is too early to consider an environmental release of a gene drive-modified organism. "There are considerable gaps in knowledge regarding the implications of gene drives for an organism's fitness, gene flow in and among populations, and the dispersal of individuals, and how factors such as mating behavior, population sub-structure, and generation time might influence a gene drive's effectiveness," they wrote. "Addressing knowledge gaps about gene drives will require the convergence of multiple fields of study including molecular biology, genome editing, population genetics, evolutionary biology, and ecology."

In his 2003 proposal for using site-specific selfish genes to control natural populations, Austin Burt also urged caution. "[W]ide-ranging discussions are needed on the criteria for deciding whether to eradicate or genetically engineer an entire species," Burt said. "Clearly, the technology described here is not to be used lightly."

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Public Symposium on Regulation of Plant-Incorporated Protectants

For Release: August 24, 2016

EPA's Biopesticides and Pollution Prevention Division will hold a public symposium on Thursday, September 29, from 9:00 a.m. to 5:00 p.m. on the data that support the registration of plant-incorporated protectants (PIPs). The free symposium will be held in The EPA Office of Pesticide Programs' first floor conference center at One Potomac Yard South, 2777 South Crystal Drive, Arlington, VA 22202, and can be attended in person or through a live webcast.

A plant-incorporated protectant is a type of biopesticide that is produced and used in a living plant, or in its produce. These products are, therefore, distinct from topically applied conventional pesticides. PIPs can, under certain circumstances, be regulated by USDA, FDA and EPA, with each agency having specific responsibilities.

The symposium will provide a forum for PIP developers, the agricultural sector and the general public to receive information firsthand on the scope of the scientific review process that determines the safety of PIPs and on the pesticide registration process as a whole. The majority of PIPs registered in the past 20-plus years use insecticidal traits of bacterial proteins to enhance the plant's resistance to insect herbivores. EPA, FDA and USDA representatives will give an overview of the regulatory system that applies to biotechnology in the United States in the context of the Coordinated Framework for Regulation of Biotechnology.

Participants will have multiple opportunities to interact directly with the regulators and ask questions. Presentation materials will be available at regulations.gov in Docket No. EPA-HQ-OPP-2016-0427 following the meeting (https://www.regulations.gov/document?D=EPA-HQ-OPP-2016-0427-0001).

The event is open to the public, but registration is required to participate in person or electronically. We encourage registering early, no later than Monday, September 26, for in-person attendance since space is limited. Sign-in for registered in-person attendees will begin at 8:30 a.m. on September 29 at Potomac Yard South.

For more information on PIPs, see:
https://www.epa.gov/regulation-biotechnology-under-tsca-and-fifra/overview-plant-incorporated-protectants

Contact the EPA to ask a question, provide feedback, or report a problem:
https://www.epa.gov/pesticides/forms/contact-us-about-pesticides