How Can Animal Agriculture Benefit from Genetic Engineering?

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Introduction

A goal of agriculture is to obtain the maximum production from the least input. Genetics set the base for all animal function, and hence maximum production. Given the correct environment, the full genetic potential of an animal can be expressed. Increased production or a change in phenotype (what an animal looks like) can be achieved over time as animals are selected for that trait. Production agriculture has been very efficient at making genetic progress by selection of those animals with the highest genetic or phenotypic merit. For this type of selection to be successful there must be variation already present within the genome. Random variation is introduced with the production of every sperm and egg. If the variation that is desired is not already present then the technology is now available to introduce that genetic variation in a precise manner at a specific location. Below I will use an analogy to explain what genetic engineering means, provide examples of applications, discuss some assumed difficulties and conclude with adoption of the technologies.

An Analogy

Contained within the genome are the blueprints for life. The genome of pigs and cattle is about 3,000,000,000 bases (letters) long. Directions for making everything in the cell is specified there; from making the smallest protein, e.g. thyrotropin releasing hormone at 3 amino acids, to the largest protein, e.g. titin at 27,000 amino acids. Since the pig and cattle genome is becoming well characterized, we have a good idea of the blueprints of life for these species. With the new genetic editing technology, it is now possible to introduce any genetic modification, which is compatible with life, into these blueprints. To better comprehend what types of changes are possible one might think of how a self-replicating factory would function (1).

For a factory to replicate itself, it must make the tools to assemble the machines that make and assemble the tools and machines to replicate the factory (including a new copy of the blueprints). Pause, and let that statement sink in. The factory takes inputs (steel, energy, other raw components) much the same way a cell requires nutrients to grow and replicate. The factory then takes the raw components and fashions them into 3-dimensional structural subunits. The subunits are parts for tools, parts of machines, parts of walls, parts of insulation, nails, and wires with insulation, and on and on. The cell does the same thing, i.e. it makes all the structural parts of the cell. Since structure confers function, the assembly of 20 different amino acids can provide for a wide range of structures (secondary modifications add to these differences). The blueprints are also there that direct the factory to divide into two, as the cell divides at cellular division.

The above description fits quite well with stem cells, i.e. those cells that have the ability to form all the cells in the body. However, in many cases the blueprints tell some of those factories that split to specialize. For example, a skin epithelial cell has all the blueprints for every cell in the body, but only uses the pages of those blueprints to direct it to be an epithelial cell. Other cells

require other pages (read "genes") for them to have the correct phenotype. So, for example, a different set of genes is turned on in white blood cells so that the white blood cell functions as a white blood cell (a subtype of white blood cells is a macrophage, and each subtype of white blood cell uses a different set of genes/pages). Those genes that are "turned on" in a particular cell type are generally clustered together on the chromosome so that the region of a chromosome is expressed. This chromosomal region might be thought of as a chapter in a book. Another example would be the cells that secrete milk. These cells use chapters that contain pages that code for the production of milk proteins (obviously, other cell types do not need to produce milk proteins). Note that these chapters are only used during lactation! Each cell type has its unique repertoire of genes that is used to provide the 3-dimensional structures for that particular cell type and cell function.

Back to the factory comparison. If one of the tools that needs to be made is a hammer, then it can be made as a single piece or as two or more pieces that will need to be assembled. Since we have the blueprints and editing capability, we can make the handle of the hammer longer, or shorter, fatter or thinner, curved or straight. As long as the handle will fit with the head of the hammer and the hammer is still functional (driving and pulling nails), then the factory will use the new hammer handle. In addition, if the blueprints are changed, then the newly replicated factory will use the new hammer handle, because its synthesis is directed by the edited blueprints.

Animal Genetic Engineering

Genetic engineering can add genes, can get rid of genes and can modify genes.

Addition (transgene)

While there have been many genetic modifications made to pigs, most have been for medicine as in many cases the pig is an excellent model of the human condition (2-4). At the University of Missouri we have made over 50 different modifications for studying things like cystic fibrosis, heart disease, cancer and organ transplantation. For the past 14 years we have the only National Institutes of Health funded swine resource center, the National Swine Resource and Research Center (http://NSRRC.missouri.edu). Some of our genetic modifications have agriculture significance. One example is our pigs that produce their own omega 3 fatty acids (5). Here we introduced a new page into the blueprints; in this case a gene called a fatty acid desaturase from a roundworm. That new page (gene) not only directed the conversion of omega 6 fatty acids to omega 3 fatty acids, but the page was replicated in the daughter cells, i.e. passed on to offspring, like every other page of the blueprints.

The addition of a page (gene) to the blueprints is relatively easy. Although, in the past, there was very little control over where the page was inserted in the book of blueprints. Sometimes multiple copies of the same page were inserted at a random location, and sometime copies were inserted at more than one place in the book. As described above, only certain chapters "are read" in each cell type; and so if it inserts into a chapter that is not read often, then the gene will not be expressed. For example, if the page is inserted next to a kappa casein gene it will be more likely to be turned on only in the mammary cells during lactation. More recently, the application of gene editors to genetic engineering have revolutionized the way genetic engineering is completed as the location of insertion can be directed to a specific genomic site.

Subtraction (knockout)

The first gene knockout in a domestic animal was in a pig (6) to create organs for transplant to humans (xenotransplantation). The gene of interest adds certain sugars to the surface of a cell. In this case, those sugars result in a pig cell or organ being immediately rejected after transfer to a primate. We used a technique called homologous recombination to change a particular page (gene) so that the sequence of letters did not make any sense. This genetic change was performed in pig cells growing in a dish. The efficiency was about 1 in 1,000,000 cells. That single cell was identified, expanded, and then used for somatic cell nuclear transfer to create a pig with that particular genetic modification. This resulting pig did not put the sugar on the cell surface of the pig cell, and organs from these pigs were not immediately rejected after transfer to a baboon. The efficiencies of making this genetic modification were quite low. A recently developed gene editing technology called CRISPR/Cas9 has dramatically improved the ability to knockout a gene. Our very first attempt at using this technology involved injecting the components into freshly fertilized eggs. We then performed embryo transfer and produced 4 piglets. All 4 had edits in the gene (in this case *CD163*). We just went from 1 in 1,000,000 to 4 of 4. This new technology can not only cut a gene and disrupt the sequence to knockout a gene, it is also be used to swap out paragraphs, sentences and even single letters of the genome.

Modification

Sometimes you may want to change a specific part of a protein. The example that I want to discuss here is for porcine reproductive and respiratory syndrome (PRRS). The protein that is responsible for the PRRS virus infecting the pig is called CD163. This protein has a number of repeat domains similar to beads on a string. One of those beads (number 5 of 9) has been implicated as being responsible for the PRRS virus infecting the pig. Therefore, we took a sequence from another species that is known to not result in infection and swapped it with domain 5. In essence, we changed one of the beads on the string.

Application

Genetic engineering offers a powerful tool to study basic mechanisms of biology in general and reproductive biology specifically. Below I will outline a few of the more recent applications of the technology.

Pigs

Porcine Reproductive and Respiratory Syndrome Virus is estimated to cost producers in North American and Europe $6,000,000 each day (not including Asia). Vaccination programs have not been effective at controlling the disease. To both discover the route of entry of the virus and to provide a solution we knocked out *CD163* and then in collaboration with Dr. Bob Rowland at Kansas State University showed that the pigs were not susceptible to infection (7). We then showed that when we swapped out domain 5 with another domain that the pigs were resistant to European strains of the PRRS virus, but were not resistant to the North American strains (8). We then showed that knocking out *CD163* in a gilt could protect susceptible fetuses from infection (unpublished).

African Swine Fever Virus has no treatment or cure. It is spreading from Africa into southern Eastern Europe. The group at the Roslin Institute in the UK has identified a variant of a gene in Warthogs that they think confers resistance to disease symptoms. The gene, *RELA*, differs from

RELA in domestic pigs in that it results in changing 3 amino acids in the sequence. The group at Roslin successfully edited the domestic pig genome so that the Warthog version is made in the domestic pig (9). It remains to be determined if these pigs are indeed resistant.
Certain corona and influenza viruses need to be uncoated before they can infect the cell. A specific protease (TMPRSS2) is thought to be responsible for that uncoating. We knocked out that gene (10), and Juergen Richt at Kansas State University will soon begin trials to determine the role of TMPRSS2 in virus uncoating and infection in the pig.

Boars are being developed that might serve as surrogates to host germ cells from a genetically superior boar (11). Here a gene (*NANOS2*) responsible for development of the sperm (germ cells) has been knocked out so that the animals do not produce any sperm. Germ cells from a genetically superior boar will be transferred into a number of these animals so that more sperm can be produced from a single genotype.

Cattle
One of the causes of bovine respiratory disease is *Mannheimia haemolytica*. *Mannheimia haemolytica* produces leukotoxin. The leukotoxin binds to amino acids number 5-17 of CD18 (a string of amino acids that sticks out of the cell). Non-ruminant CD18 contains a glycine at amino acid number 18. The glycine residue results in cleavage of this string of amino acids. Rather than a glycine, ruminants have a glutamine at this position and this string of amino acids is not cleaved. Thus, the leukotoxin binds to this string of amino acids on the surface of the cell and is toxic to the cell. This change of a single amino acid may result in cattle that are not sensitive to leukotoxin (12) and be less likely to express bovine respiratory disease.
Similar to changing a single amino acid, another group used gene editing and moved the gene conferring polled in Celtic cattle to Holstein (13).

Possibilities
Biology is very diverse. Every species has its own unique characteristics. Those characteristics are determined by the genetic code (blueprints). The technology is now available to make any genetic change imaginable (as long as the genetic change is compatible with life). Would you like to make pigs that can digest grass? The gene for cellulase from bacteria might be introduced into pigs. Do you want to make pigs resistant to many parasites? Chitinase degrades the cell walls of fungi and the exoskeletal components of some worms and the gene from bacteria might be introduced into pigs. Do you know the gene(s) responsible for heat tolerance in cattle (*Bos Indicus*)? Those genes or variants could be introduced into *Bos Taurus*. Virtually any genetic sequence from any form of life can be moved from one species to another. Virtually any 3-dimensional structure can be created to deal with whatever problem might exist in any species. One of our goals is to make animals that manage themselves. These animals would have minimal input, e.g. no vaccinations, thrive on low quality inexpensive foodstuffs, etc., and produce a high quality product.

Assumed Difficulties
One question that I am often asked is, ‘isn’t every gene necessary for life?’ To many the surprising answer is ‘no’. We have knocked out a number of genes in the pig, and unless you know where to look and what to look for you would never know. Genes that we have knockout out in pigs with no apparent phenotype include: *GGTA1* (6), *CMAH* (14), *CD163* (7), *TMPRSS2*

(10), and *SIGLEC1* (15). In contrast to this list of genes some knockouts result in infertility, others are embryonic lethal, others are lethal to the animal after they are born (CFTR- Cystic fibrosis causing gene (16, 17), *RAG2* and *IL2RG*- important for immune cell function (18, 19)). Other genetic modifications result in animals that have other disease symptoms (*DMD*- Duchene's muscular dystrophy (unpublished), *PAH*- phenylketonuria (unpublished), *APC*- intestinal polypus (unpublished)). Each genetic modification needs to be evaluated independently to determine if it will result in problems for the animal. While some adverse effects can be predicted it will not be known if there are any deleterious effects until the animal is made.

Adoption

When might genetically edited animals be permitted to enter the food supply? That question will be answered by the Food and Drug Administration (FDA). The genetic edits will also need to be accepted by the public. Agriculture cannot sell this technology on economic principles. Adoption of these technologies will need to be based on things that the public cares about. These include sustainability, food security, animal welfare and the psychological costs to producers. Agriculture cannot continue to supply inputs (fuel, feed, labor, etc.) into animals that underperform or die and still be sustainable. Animals that contract diseases that diminish production are a threat to our food security. Animal welfare is a big issue and genetic edits that can be made to improve the welfare of animals will be more likely to be accepted by the public. The public should be able to see the welfare benefits to animals that do not get sick or need to be dehorned. Finally, the public needs to see farms being run by people instead of faceless corporations. When a barn comes down with PRRS it has tremendous psychological, emotional and interpersonal costs to those families affected. These are people that care about the welfare of their animals. They also have real lives and need to balance the bank account. Devastating losses like these affect if people can pay the bills or declare bankruptcy, or afford to send the kids to college. You can imagine sitting around the kitchen table and the stress on family and marriages when trying to make these decisions.

The FDA currently regulates any intentional genetic change in an animal as a new drug. In contrast, there is no regulation of natural mutations that exist or are derived de novo. For example, in a 3 billion letter genome there are estimated to be 30 errors introduced with every cell division (20). The FDA does not regulate these changes in the genome. However, if a change in a single base is introduced intentionally then the FDA regulates the animal as a new drug. This policy appears to be counterintuitive as random mutations are not regulated and a precise edit results in regulation.

Apogee

The growth and development of animals is controlled and limited by their genetics. The genetic engineering technology is sufficiently developed to create almost any imaginable genetic combination. Biology is already quite diverse and solutions to many biological problems are available somewhere in nature. The germane questions are: "What is the problem?, What might the solution look like?, and Will the public accept it?

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