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Review Article

Genome Editing in Large Animals

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ABSTRACT

Genome editing in large animals has tremendous practical applications, from more accurate models for medical research through improved animal welfare and production efficiency. Although genetic modification in large animals has a 30-year history, until recently technical issues limited its utility. The original methods—pronuclear injection and integrating viruses—were plagued with problems associated with low efficiency, silencing, poor regulation of gene expression, and variability associated with random integration. With the advent of site-specific nucleases such as TAL effector-like nucleases and clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9, precision editing became possible. When used on their own, these can be used to truncate or knockout genes through nonhomologous end joining with relatively high efficiency. When used with a template containing desired gene edits, these can be used to allow insertion of any desired changes to the genome through homologous recombination with substantially lower efficiency. Consideration must be given to the issues of marker sets and off-target effects. Somatic cell nuclear transfer is most commonly used to create animals from gene-edited cells, but direct zygote injection and use of spermatogonial stem cells are alternatives under development. In developing gene editing projects, priority must be given to understanding the potential for off-target or unexpected effects of planned edits, which have been common in the past. Because of the increasing technical sophistication with which it can be accomplished, genome editing is poised to revolutionize large animal genetics, but attention must be paid to the underlying biology to maximize benefit.

1. Introduction

Genetic modification in rodents has been routine now for 35 years [1], and first attempts to transfer the technology to large animals began shortly thereafter. Unfortunately, technical and efficiency limitations precluded the practical use of genetic modification in large animals, with few exceptions.

However, genome editing in large animals would be of tremendous utility to medical research, to medicine, and to agriculture. In medical research, the drawbacks of using rodents to model humans are well established [1]. Because of their small size, their short life cycle, their very different diet and dietary priorities, and details of their physiology, mice make poor models for reproductive physiology, pulmonary problems, metabolic regulation, and many other fields of inquiry. They are an improvement on cell culture, but for many important health problems, improved preclinical models would be of benefit to research. A clear early example of this is the cystic fibrosis transmembrane conductance regulator (CFTR) knockout pig, which is far more clinically similar to humans than a CFTR knockout in mice [2]. Gene editing in large animals also has the potential to aid human medicine directly, from creation of humanized protein drugs [3] to creation of humanized transplant organs (xenografts) [4,5].

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In addition, there is potential for gene editing in livestock to improve both animal welfare and production efficiency in agricultural applications. From feed conversion and other performance traits, to disease resistance, to improved nutrition, to improving fit to environment, precision gene editing is likely to be an essential tool in improvement of our large animal stock.

2. Historical Methods for Large Animal Engineering

Pronuclear injection, developed in 1980, was the first method of genetically modifying animals and in the intervening decades has been the most common [6]. In pronuclear injection, DNA containing a desired gene expression construct is injected into a single-cell fertilized egg. Despite somewhat low efficiency, it integrates randomly into the DNA of the fertilized egg, which is implanted in a recipient mother, and offspring are checked for presence and expression of the new gene. Because the DNA contained in these constructs was essentially never purely that of the host species, animals produced by this method are termed transgenic.

Pronuclear injection has numerous practical drawbacks. Because integration is random and because the constructs are usually integrated in multiple copies as concatemers, expression levels were difficult to control. Moreover, because the maximum size of the constructs is somewhat limited, promoter elements, which had to be included, were necessarily abbreviated, and genes were almost always introduced in their fully spliced forms. Regulation of the genes was thus usually rudimentary. Transgenes also had a tendency to be silenced over multiple generations. For research purposes in rodents, the ease of creating transgenics outweighed these concerns.

In large animals, however, the problem was aggravated by efficiency and mechanics of reproduction. In mice, the efficiency of transgene introduction was about 5%–10%. With a gestation period of 3 weeks and a litter size of 6–10 animals depending on strain, five recipient moms were likely to give you a few founders in just a few weeks. In cattle, however, for unclear reasons, efficiency of transgene introduction was closer to 3%, but with only 18% of blastocysts yielding live calves, this dropped the effective rate to a fraction of a percent. With one calf per mother, this meant hundreds of recipients were needed to ensure successful creation of a founder [7,8]. In some species, for hormonal or other reasons, the method is essentially impossible [9]. The introduction of relatively routine somatic cell nuclear transfer (SCNT) about 15 years ago reduced the efficiency problem because cells could be checked for correct integration before creation of animals, but it did not solve all the other problems with using transgenes [10]. Notwithstanding all these problems, transgenic sheep, pigs, goats, cattle, and others were created, but most had low practical utility [1].

One approach successfully used by several groups to avoid the problem with low transgene integration efficiency was use of integrating viruses. Integrating viruses retain all the problems of random integration associated with pronuclear injection, and because of their smaller cargo size, the problems with promoter strength and specificity are usually worse. In addition, because of the viral elements included, progressive silencing over time worsened with viral integration methods [11].

3. Modern Genome Editing Methods

The fundamentally novel technology that has made the impending revolution in gene editing possible is the ability to precisely target specific areas of the genome. This eliminates essentially all the issues associated with transgenic animals because native promoter elements and splicing can be used for correct gene regulation, and the variability and gene silencing associated with random integration is eliminated. Instead of a largely random effect, gene editing can now be well controlled.

Gene editing in large animals is primarily different than gene editing in laboratory animals in that the higher expense and longer gestation time in large animals necessitate a lower tolerance for error. In mice, one can tolerate high randomness of results because litters are large and gestation times are 3 weeks. In horses or cattle, each embryo must be assured to carry correct edits before gestation is initiated.

There are two relatively new technologies that allow targeting of specific nucleotides: TAL effector-like nucleases (TALENs) and clustered regularly interspaced short palindromic repeat (CRISPR), each with multiple related technologies. TAL effector-like nucleases, and the related technologies of zinc finger nucleases (ZFNs) and MegaTAL, use modular protein-based sequence recognition, whereas CRISPR uses RNA-guided sequence recognition. Although variations on these technologies are likely to develop over time, the core technologies are unlikely to change.

Transcription activator–like (TAL) effectors are a class of enzyme first discovered in the plant pathogen Xanthomonas about 10 years ago [12], with the code for DNA binding specificity worked out in 2009 [13], and engineered to add a nuclease function for genome editing in 2010 [14]. The combination, TALEN, consists of a modular array of TAL recognition sequences fused to a FokI nuclease [15]. These are inserted in pairs, one for each strand, and work as a dimer to create double-stranded breaks in specific DNA sequences.

There are variants on this; for instance, MegaTAL uses a combination of TAL arrays with a nuclease that has site-specific cleavage, meganuclease, increasing overall specificity of the combination [16]. MegaTAL as a technology is still in development, currently with high cost and complexity; they may be a turnkey solution in a few years, but for now are probably best left to those focusing on method development. Zinc finger nucleases are an older solution, with 20 years of history, and share the use of the FokI nuclease and the need for dimers, but use a different protein-DNA recognition mechanism [17]. In our experience, ZFNs are more cumbersome to use, with no compensatory advantages, as compared to TALEN. Both TALEN and ZFN can be created in individual laboratories, but multiple commercial sources exist for each.

The other major method of making targeted cuts in the genome, CRISPR/Cas9, is also derived from bacteria and archaea in which they are part of a viral defense system [18]. It consists of CRISPR which binds a guide RNA and an