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Targeted agricultural production by genetically modified farm animals (#4)

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Short Abstract

The era of genetic modification of farm animals (transgenics) started in 1985 when the first transgenic rabbits, pigs and sheep were reported after microinjection of foreign DNA into early fertilized oocytes, i.e. zygotes. This technology was gradually being replaced by cell-based gene transfer methods after the breakthrough in somatic cell nuclear transfer (SCNT) by the birth of "Dolly", the first cloned mammal. This cell-based transgenesis could overcome critical limitations of the microinjection technology, such as low efficiency, only additive gene transfer possible, random integration and a frequent incidence of mosaicism. Selection at the cellular level and the use of highly selected and defined donor cells improved the production of genetically modified livestock significantly. Using these technologies, a whole range of useful application models has been shown for various livestock species covering both, agricultural and biomedical traits and has turned out to be important for basic research. However, cell mediated transgenesis was still hampered by the inability to produce animals with targeted genetic modifications. This was at least partly due to the fact that in farm animals, in contrast to laboratory species (mouse and rat), true pluripotent stem cells have not yet been reported. This prevented from the necessary selection steps, as primary cells as used in SCNT only have a limited lifespan and are not compatible with the high selection needed for targeted mutations.

This situation has changed during the last decade after informative genomic maps of the major farm animal species had become available and the recent advent of genome editing technologies based on the use of DNA nucleases, including Zinc finger nucleases (ZFN), Transcription Activator-like Effector Nucleases (TALEN) and the CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats) system, which allow precise modifications of the genome. They mediate targeted genetic alterations by enhancing the DNA mutation rate via induction of double-strand breaks at a specific predetermined genomic site. Compared to conventional homologous recombination-based gene targeting, DNA nucleases can increase the targeting rate 10,000-fold, and gene disruption via mutagenic DNA repair is stimulated in a similar frequency.

In animals all three nucleases can be applied either via cytoplasmic microinjection into early fertilized eggs (zygotes) or after transfection into donor cells that are subsequently used in somatic cloning. In the very short time period of their first description, numerous research groups have described the successful production of genetically modified cattle, pigs and sheep with a whole range of eventually useful genetic modifications, both, for agricultural application and biomedical purposes.

Genetically modified farm animals with agriculturally important traits have focused on growth and development, mainly by targeting the MSTN gene, disease resistance (f.ex. PRRS, BSE, tuberculosis), production of hornless cattle, lactation (amount and composition of milk), wool production in sheep, enhancing reproduction success, environmental improvements, dietetic improvements and sex sorting.

With regard to growth and development, the GDF8 gene (growth differentiation factor 8 or myostatin, i.e. MSTN) has been successfully targeted in cattle, sheep, pigs and even dogs. GDF 8 is critically involved in the regulation of muscle growth. A non-functional myostatin gene is known to cause muscular hypertrophy as known in certain cattle breeds such as Belgian Blue and Piedmontese. Animals with a bi-allelic mutation of the MSTN gene showed enhanced growth and development. The phenotypic effects of the MSTN knockout were particularly impressive in breeds that usually do not provide much meat such as Nelore cattle and Merino sheep that usually are maintained for wool production. This approach could render meat production more effective with regard to cost reduction, environmental impact and feed conversion. Another agriculturally important application of DNA nucleases could the production of hornless cattle via gene editing of the bovine Polled locus, either by targeting the Celtic mutation or a 200 bp duplication in the Holstein Friesian genome.

Thus, for the first time, it has become feasible to overcome the limitations of classical breeding and selection concepts which are rather slow, due to the fact that economically important genetic gain occurs over rather long periods of time. The new technologies based on genome editing, represent a more direct approach and make new phenotypes possible within a single generation. Thus, the advent of genome editing in combination with homologous recombination protocols provides a completely new option for improved efficiency and precision in breeding that was previously unprecedented.

Notes