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Genetic engineering as a tool for the generation of mouse models to understand disease phenotypes and gene function

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The usage of mouse models has been vital for biomedical research over the last decades, yet the generation of these models has been extremely difficult and labor-intensive. The identification and generation of nucleases able to introduce site-specific DNA double-strand breaks, particularly the CRIPSR/Cas system, is a major breakthrough for this field as the endogenous DNA repair machinery can be hijacked to specifically introduce genome modifications at these sites. This allows for the time-efficient and cost-efficient generation of mouse models by delivery of designer nucleases together with donor DNA into fertilized oocytes.

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Introduction

Mouse models have become an integral part of biomedical research as they allow for the analysis of gene function *in vivo* and provide opportunities to study cellular function and reactivity under homeostatic conditions, as well as pathophysiological relevant perturbations. In addition, over the last years ample examples have emerged of how mouse models can be used as pre-clinical models to understand disease processes up to the point that the disease-causing genetic alteration can be introduced to phenocopy the human disease.

The introduction of these genetic modifications has been a cumbersome, labor-intensive and time-consuming process, yet the advent of techniques to introduce site-specific alterations in the murine genome, including zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR/Cas system and its relatives, have enabled researchers over the last years to implement technical improvements to expedite the generation of mouse models. This has increased interest in this field and significantly improved turnaround times from the idea to create a mouse model to the initial experiments validating the underlying hypothesis.

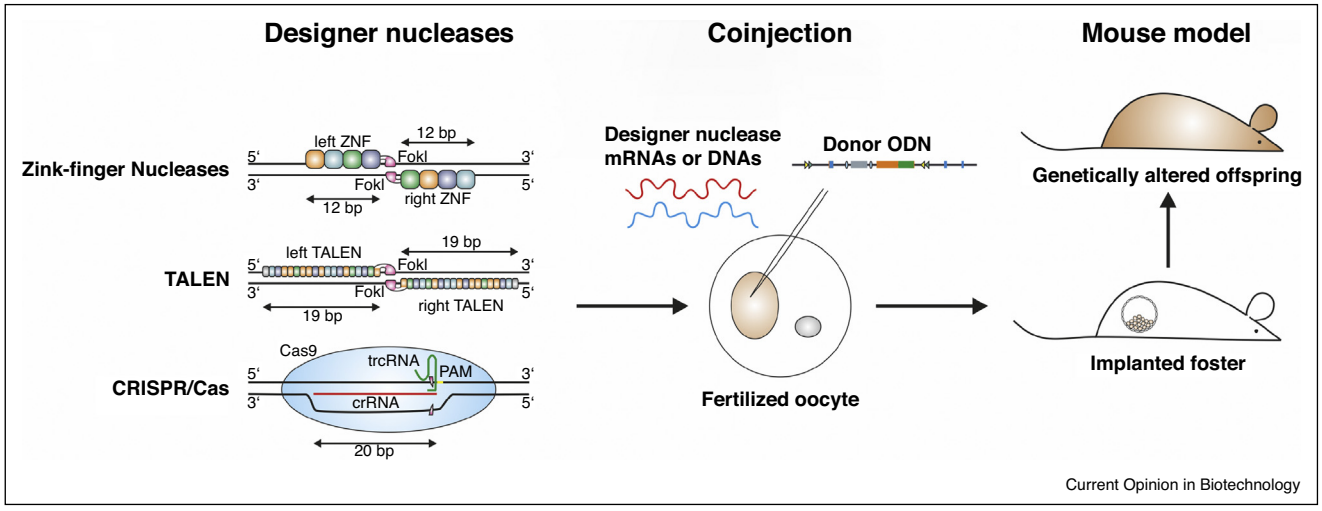
In this review, we will discuss the currently available technologies for genetic engineering, their recent applications, and scientific advances further improving these technologies. We want to highlight how genetic engineering can facilitate biomedical research and expedite the increase in knowledge necessary to understand even complex genetic diseases. Ultimately, once the potential caveats, for example, off-target effects, have been better documented and guided prevention strategies have been implemented, genetic engineering might also be an avenue that has the potential to be applied in humans as a curative effort for genetic diseases.

Emergence of novel techniques for genetic engineering

Traditionally, mouse models have been generated via two complementary approaches: trough injection of plasmid DNA carrying the desired transgene or modified bacterial artificial chromosomes into fertilized mouse oocytes resulting in random integration of these DNA stretches into the murine genome and the expression of the transgene in the founder animals carrying one or multiple copies of the injected DNA, or through the replacement of the endogenous gene locus by genetically altered DNA sequences via homologous recombination. This was mainly carried out in embryonic stem (ES) cells by transfecting ES cells *in vitro* and subsequently selecting ES cell clones carrying the altered DNA sequence. These cells were afterwards injected into murine blastocysts and mice carrying cells with the altered allele identified by coat chimerism and founder animals derived by further breeding steps.

In the last few years this laborious approach has been simplified with our ability to introduce DNA double-strand breaks (DSBs) very specifically at given loci in the genome by genetic engineering through the emergence of multiple genome engineering tools. The most

Figure 1



Approach to generate mouse models by genetic engineering: designer nucleases like zinc-finger nucleases, TALENs or CRIPSR/Cas9 are coinjected with donor DNA into fertilized oozytes. This will induce a site-specific DNA double-strand-break, which will be repaired by homologous integration of the door DNA carrying the desired genomic modification. After implantation into foster animals, these will give rise to offspring carrying the desired genetic modification.

well-known and established tools consist of ZFNs, meganucleases, TALENs and the CRISPR/Cas system. While these site-specific nucleases vary in their structure and mode of target recognition, all of them have been used successfully to target genes in the genome of cell lines [1], primary somatic cells [2,3], embryonic stem cells [4], and early embryos of various model organisms [4,5]. For a detailed review of the individual nucleases and their functional and structural properties, we refer to recent reviews on this topic [6–11]. DSBs induced by all of these nucleases can be repaired via two alternative routes: non-homologous end joining (NHEJ) or homology-directed recombination (HDR). NHEJ is an error-prone mechanism and often results in point mutations, small deletions or insertion and — when targeted correctly — can be used to delete the expression of a target gene or disrupt regulatory elements. Alternatively, by adding donor DNAs with homologous sequences to the targeted region, artificial or modified sequences can be inserted at the targeted site by HDR [12], which will result in a full repair of the DSB with only the inserted sequence reflective of the genomic engineering (Figure 1). Most commonly desired modifications are the insertion of loxP sites, recombinases, reporter molecules and transcriptional regulators.

To generate genetically engineered mice, these modifications are mainly induced by injecting the DNA or RNA encoding for the nuclease or the protein itself together with the donor DNA into fertilized oocytes. The injected oocytes are subsequently implanted back into pseudo-pregnant foster mothers which will give birth to genetically altered animals. Under optimal conditions these animals could already be used for experimental evaluation of the effect of the induced genetic alteration.

Current technological improvements for the efficient generation of mouse models using genetic engineering

Recent efforts have focused on improving and solving the main issues genome engineering still encounters using site-specific nucleases. These include, firstly the improvement of the integration rate of larger constructs by HDR into the genome, secondly the efficient delivery of the required components into the target cell, and finally the reduction of off-target effects.

Rather fast after the first description in cell lines, it was reported that TALENs and the CRISPR/Cas system could be used to induce DSBs, which if repaired by NHEJ can result in the disruption of gene expression [13,14]. In addition, efficient editing of regions so far inaccessible for gene targeting like the Y chromosome was first reported for the injection of TALENs [15] and has since then been replicated using the CRISPR/Cas9 system [16]. It was also reported that the CRISPR/Cas system can be used to simultaneously target multiple genes in close proximity [14] demonstrating the almost unlimited potential of the technique to disrupt expression of genes via NHEJ. This suggested that designer nucleases could be used for direct manipulation of embryos without the time consuming factor of generating modified ES cells for later injection.