ingenious targeting laboratory

Custom rat models for tissue-specific gene knockout

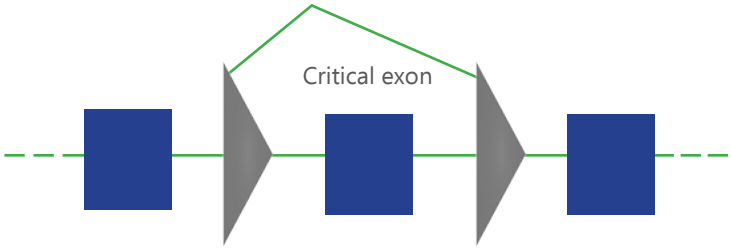
New methods of genetic engineering in rats are changing everything when it comes to choosing the right animal model for your research.

The ability to make targeted genetic modifications in an animal model enables experiments that would otherwise be impossible. Technology to introduce DNA sequence changes at specific sites was pioneered in mice starting in the 1980s and led to the current dominance of mice as a model organism in laboratory research. Before such techniques became widespread researchers would choose an animal model based on how well its natural biology was suited to a particular study. Rats might be chosen for one project and mice for another depending on the relative strengths of each. As an example, in 1980 neuroscience researchers were four times more likely to use rats in their research compared with mice. Over time that number shifted dramatically because mice could be genetically modified in ways that rats could not, and now rats and mice are used with equal frequency in neuroscience studies. New methods for tailoring the biology of mice to meet the needs of specific research projects changed everything.

Conditional knockout in rats using the Cre-lox system

Conditional knockout rat line

2 loxP sequences are inserted at specific sites in target gene

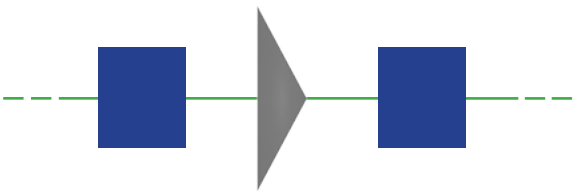


Gene initially functions normally

Cross with Cre-expressing line

Tissue-specific gene knockout

Cre recombinase deletes crucial part of gene



Gene function disrupted

Now specific mutations can be introduced into the rat genome almost as freely as in mice.

For decades when it came to targeting specific genes the greatest difference between rats and mice was the lack of reliable methods for culturing embryonic stem cells (ESCs) from rats. The availability of robust mouse ESCs has been the key to introducing complex genetic modifications. To summarize the method, DNA encoding a mutant gene sequence can be introduced into a population of ESCs and in a portion of the cells the mutation will be permanently integrated into the genome. It's straightforward to identify which cells have the desired mutation and these modified cells are injected into an early-stage mouse embryo which is carried by a surrogate mother. The pup, which is partly derived from mutant ESCs, is called a chimera, and may be able to pass on the mutation to its offspring. Stem cells from rats now enable a very similar process to be performed, with one significant change: the rat stem cells in question are not ESCs but rather spermatogonial stem cells (SSCs). These cells can be genetically modified in a similar fashion but rather than injecting them into embryos they are transplanted into the testes of adult male rats. The genetically modified SSCs give rise to sperm carrying the mutation and thereby pass it on to the next generation. This exciting development greatly expands the scope of genetic modifications that can be introduced into rats.

Tissue-specific gene knockout can now be achieved in rats, after being used for decades in mice.

The standard method for achieving tissue-specific gene knockout is the Cre-lox recombination system which has been validated by hundreds of mouse models and thousands of studies. Cre-lox recombination is a powerful system that can be used to disrupt the function of almost any gene, and the disruption can be targeted to different tissues. This flexibility is possible because two different animal lines, with distinct genetic modifications, are crossed together. In mice there are hundreds of lines available that express Cre recombinase in specific tissues. This enzyme recognizes small genetic sequences known as lox sites which can be inserted into the genome to create conditional knockout animals. The combination of Cre expression and a conditional knockout line produces tissue-specific gene knockout.

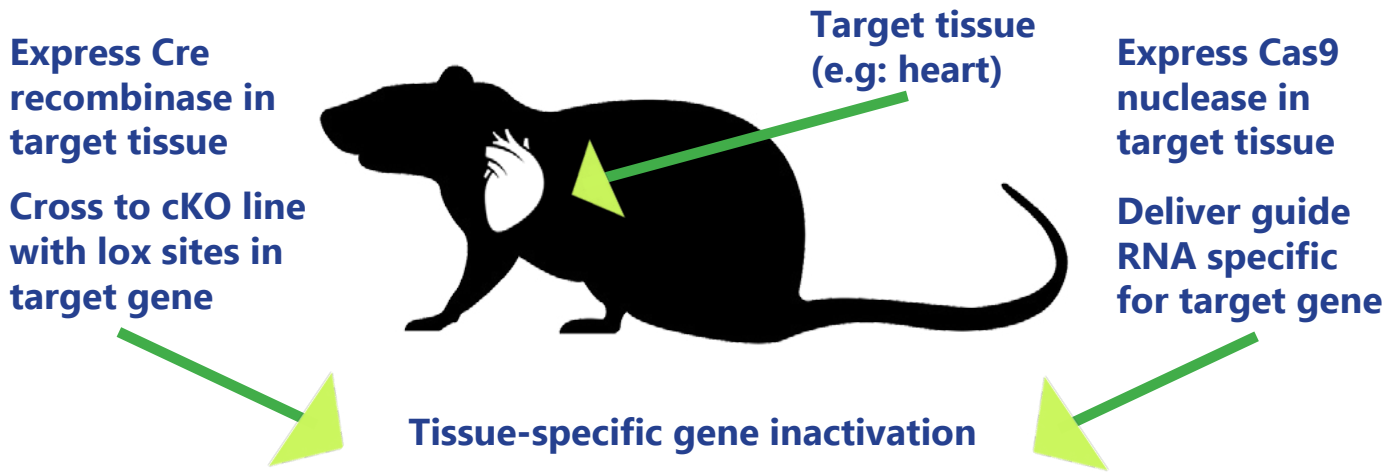
One essential requirement for creating conditional gene knockout animals is the ability to place lox sites at precise locations within a gene. Gene editing in spermatogonial stem cells now enables this kind of precise genetic editing in the rat. Tissue-specific gene knockout using Cre-lox can make experiments possible that could not be performed any other way, as was demonstrated in the case of the TDP43 (Tardbp) gene in mice. Mice with the gene completely knocked out are not able to survive until birth, so the function of the gene in the adult nervous system cannot be studied using those mice. A tissue-specific conditional knockout strategy was used, employing the Cre-lox system, to specifically disrupt the gene in motor neurons. This tissue-specific disruption, which could also be used in rats, allowed for study of the gene's role in the nervous system of adult mice.

Multiple strategies are possible to achieve tissue-specific gene knockout rats.

The CRISPR-Cas9 system is now widely used to generate full-body gene knockouts in a variety of species. However, this system can also be adapted for use in a tissue-specific manner, resulting in conditional gene knockout. This strategy is as versatile as using the Cre-lox system because multiple elements must be brought together to cause gene knock-out. In the case of the CRISPR-Cas9 system the elements are the Cas9 nuclease enzyme and a guide RNA to specify which gene will be disrupted. Tissue-specific gene knockout using CRISPR-Cas9 has been demonstrated in rats by the knockout of two target genes in dopaminergic neurons. In this case the new method of targeted genetic modification using spermatogonial stem cells was used to create a new line for Cas9 expression. The guide RNAs were delivered using a virus, enabling control over the timing and location of gene inactivation.

Further uses of both the Cre-lox and the CRISPR-Cas systems promise to open up more experimental possibilities by enabling even more elaborate genetic designs in model organisms in general and in the rat specifically.

Tissue-specific gene knockout: Two strategies



Two proven strategies for conditional gene knockout in rat: both the Cre-lox and CRISPR-Cas9 systems can now be used to disrupt genes of interest, with the effects limited to specific tissues. Each of these conditional knockout systems provides great flexibility because knockout depends on multiple components working together. In the case of Cre-lox, a Cre-expressing rat line is crossed with a conditional knockout line for the gene of interest. For CRISPR-Cas9, knockout re-quires both the Cas9 enzyme as well as a guide RNA, and the guide RNA can be delivered by virus or other means. Newly-developed methods for culturing spermatogonial stem cells from rats enable precise genetic modifications which were, until now, either very difficult or impossible.

References

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