Mus musculus

Science, Genetics

The house mouse (Mus musculus) is a small rodent, a mouse, one of the most numerous species of the genus Mus. The house mouse has been domesticated as the pet or fancy mouse, and as the laboratory mouse which is one of the most important model organisms in biology and medicine. It is by far the most commonly used genetically altered laboratory mammal. Mouse and man The sequence of the mouse genome was published in 2002. " The mouse and human genomes are very similar," says Dr Jackson. " There are a relatively small number of rearrangements, and the gene content is pretty much the same. Some gene families have expanded in the mouse, such as those involved in scent recognition, innate immunity to pathogens [humans and mice being exposed to different pathogens], and in reproduction. People have tended to comment on the differences between mice and humans, but there are so many similarities. If you find a mutation in a mouse gene, you almost always find a human disease with similar effects." Some mouse models of human disease, such as for obesity, cancer or immune system defects, have arisen spontaneously. Many other models - notably for cystic fibrosis - have been generated as knockouts. Even so, there are still thousands of diseases, and thousands of genes, that researchers have not tackled as yet. With the genome sequenced, allied to high-throughput technology, the concept of testing what every gene does, and of fully untangling how genes contribute to disease, has become a realistic goal. Laboratory mice Mice are the most commonly used mammalian research model with hundreds of established inbred, outbred, and transgenic strains. They are common experimental animals in biology and psychology, primarily because they are mammals, are relatively easy to

maintain and handle, reproduce quickly, and share a high degree of homology with humans. The mouse genome has been sequenced, and many mouse genes have human homologues. In addition to being small, relatively inexpensive, and easily maintained, several generations of mice can be observed in a relatively short period of time as mice reproduce very quickly. Most laboratory mice are hybrids of different subspecies, most commonly of Mus musculus domesticus and Mus musculus musculus. Laboratory mice can have a variety of coat colours, including agouti, black and albino. Many (but not all) laboratory strains are inbred, so as to make them genetically almost identical. The mouse in the lab In France, Lucien Cuénot was the first - in 1902 - to demonstrate Mendelian ratios for the inheritance of coat colour characters in mice. In Harvard, William Castle began his research in the same year, buying mice from a local mouse fancier who had quickly turned her hobby into a business. Together with his student Clarence Little, Castle produced a series of seminal papers on coat-colour genetics. Little is probably best known for his development of 'lab mice' - inbred mouse strains that are still used today. " Inbred strains have been very important for mouse genetics," says Dr Jackson. " You need a uniform genetic background against which you can compare new variations. There are several hundred inbred strains, each with a different background, although only perhaps a dozen are used commonly by the research community." Little's first inbred mouse, DBA (dilute brown non-agouti), was developed in 1909; his most famous strain, C57BL/6, in 1921. C57BL/6 was the strain whose genome was sequenced and published in 2002. Clarence Little's contribution to the field was not finished. In 1929, backed by two car barons, Edsel Ford (Henry's

son) and Roscoe Jackson (head of the Hudson Motorcar Company), he set up the Jackson Laboratory in Maine, USA. The lab is a world-renowned centre for mouse genetics, and has been a major influence on keeping mice at the forefront of mammalian biology. For several decades, researchers focused on finding mutants and variants, and mapping the genes involved. As well as coat colour, other easily identifiable traits were examined - such as ear shape, and tail length and shape. In the 1960s and 1970s, biochemical markers were developed, allowing researchers to look at protein variants. This led to a large increase in the number of known mutations and genes, but the true power of mouse genetics was not unleashed until 1977, when the first mouse gene was isolated. As if the above were not enough, the mouse also has a string of unique technological advantages. Gene transfer technology is highly advanced, so transgenic mice can be created carrying any foreign gene of interest. Also, the mouse is the only vertebrate species in which pre-selected genes can be deliberately mutated in a precise manner (see Knockout mice). This means it is possible to create exact replicas of the genetic defects that cause diseases in humans. For some reason, certain complex diseases are difficult to replicate in the mouse and in such cases the rat is often a suitable alternative. Transgenes and knockout mice Armed with molecular biology techniques, researchers can isolate, examine and modify DNA, teasing apart its role in the body. In the early 1980s, 'transgenic mice' became all the rage, after it was shown that DNA injected into mouse eggs could be incorporated into the genome. The DNA could, for example, be a 'reporter gene' under the control of a promoter from a normal mouse gene. The protein made by the reporter gene is therefore produced in the same

place, and at the same stage of development, as the normal gene. The next innovation in mouse genetics came with the development of knockout mice - mice lacking a specific gene - in the late 1980s. " The crucial things here were embryonic stem cells," says Dr Jackson. " Matt Kaufman and [Sir] Martin Evans (Cambridge), and Gail Martin (San Francisco) grew cells from an early embryo. Evans, Liz Robertson, Alan Clark and Allan Bradley [now Director of the Wellcome Trust Sanger Institute] went on to show that those cells could contribute to a new embryo, grow into an adult mouse, and be part of the germline of the adult. So the idea was, if you can modify a gene in the embryonic stem cells, the modification could be passed onto future generations of mice." The first knockout mice lacked the HPRT gene (hypoxanthine guanine phosphoribosyl transferase gene), mutations in which, in humans, cause a mental retardation disorder called Lesch-Nyhan syndrome. These knockout mice were produced by identifying a random mutation, but Oliver Smithers in the USA had been developing a way of swapping new, modified DNA directly into the genome - a technique called homologous recombination. When his 'gene targeting' technique was ported to embryonic stem cells, the production of knockout mice became far more efficient. " There must be 3500-4000 genes that have been knocked out so far, and tens of thousands of papers have been written about them," says Dr Jackson. " People are now doing more sophisticated knockouts, where the gene is removed only in certain types of cells types, or the gene is turned on or off when a drug is added. Another approach is to 'knock-in' a gene - you can replace the existing gene with another gene, such as a reporter gene. " This is the 'gene-up' approach to mouse genetics," he adds. " You say 'this is

the gene I'm interested in; let's see what it does'. So you knock it out, see what happens. The other approach is 'phenotype down', where you find a mutation that is interesting and find the gene responsible." Opportunities in Transgenic Technology Interest in immunodeficient animals has mushroomed in recent years with advances in molecular biology and genetics that allow researchers to manipulate the genome of mice to eliminate or add genes and even replace selected genes. Science can actually engineer laboratory mice to meet specific research needs and protocols. Creating new, genetically engineered animal research models involves two transgenic techniques-microinjection of cloned genes randomly inserted into the host DNA and gene targeting or homologous recombination between cloned DNA and one of the identical copies of the sequence normally present in the chromosome. The more complete names of these two transgenic techniques are: - classical pronuclear microinjection: introduction of foreign DNA into embryonic pronuclei resulting in random integration and expression and - embryonic stem (ES) cell-mediated gene targeting: introduction of genetically modified ES cells into recipient embryos resulting in the ablation (knockout) or modification of a specific genetic expression. Transgenic animals are designed to exhibit either a gain of function (expression of a novel cell-surface receptor) or a loss of function (knockout of a cellular function). Classical pronuclear microinjection techniques have been used for 15 years to create mouse models, which express unique phenotypes. The major flaw in the pronuclear microinjection models has been the random nature of transgene integration locus and copy number. Expression patterns may vary significantly in a series of lines

expressing identical transgenes. Modifiers of expression such as age, sex and health status further confound the process, increasing potential for variability. By using ES cell gene knockout technology, an investigator can produce an animal model in which expression (or the lack of expression) is highly predictable. A clone of cultured ES cells is selected in which a specific DNA sequence in the mouse genome has been modified (usually inactivated). Transformation of cultured cells with foreign DNA is relatively simple and most commonly is achieved using a procedure called electroporation. All transgenic models, whether targeted or untargeted, still may present unpredictable expression patterns due to incomplete knockout of the targeted gene, redundancy within the genome or unanticipated genetic interactions, such as down-regulation of other genes. Despite some unpredictability questions, transgenic knockout technology can produce research animals that are " custom designed" to meet the specific needs of an investigator's experimental protocol. Knockout technology, or homologous recombination, is also a valuable tool for determining functions of specific genes. Dr. Mario R. Capecchi of the Institutional of Utah School of Medicine, one of the pioneers of gene targeting, explains the concept of targeted gene replacement: If we suspected a particular gene were involved in brain development, we could generate mouse embryos in which the normal gene was " knocked out"-that is, completely inactivated. If this inactivation caused newborn mice to have a malformed cerebellum, we would know that the gene in question was essential to forming that part of the brain. Examples of transgenic models for human diseases - Models for AIDS study : The HIV-1 virus is known to have two major receptors in human

cells CD4 and CCR5. Transgenic rabbits expressing the human CD4 gene were used, the virus replicated in rabbit cells but was unable to generate any disease. Therefore, mouse models were generated. It appeared that transgenic rats expressing all the HIV-1 genes except gag and pol showed pathogeny having many similarities to human AIDS. - Models for aging : Aging is a complex phenomenon which has only been partially described. Defects in genomes appear to be a major cause of aging. A growing number of trangenic models are being used to study aging. Mice in which the XPD gene has been knocked out are more sensitive to oxidative DNA damage. This sensitivity was increased further when the XPA gene was also knonked out. These models reflect some of the aging syndromes in human. - Models for Cancer : Transgenic mice are used to generate models for cancer study. The first oncomouse expressed c-myc gene in the mammary gland. This was sufficient to trigger the formation of mammary tumors. Further studies made it pissible to identify additional genes involve in mammary cancer. Genes whose expression is amplified in mammary tumors have an oncogenic effect when used as transgenes. Crossing mice harboring different oncogenes and having knocked out genes has made it possible to determine the cooperative actions of some of these genes. - The Future : The future of the mouse in genome analysis and as a model organism seems virtually unlimited. Whole genome sequence and gene preditction programs make it quite feasible to knock out or genetically modify every mouse gene. Almost certainly, ES cells will play a key role in future mouse genomics because they can be manipulated in culture. More phenotype screens that can detect mutations prior to making live mice will be needed to increase the number and types of

mutations that can be detected in the ES cells themselves. While other model organisms, such as Drosophilia, yeast, worms and zebrafish may be easier to manipulate and allow analysis that require hundreds or thousands of animals, the mouse is likely to continue to be the premier mammalian model for understanding human inherited diseases. The Knockout Mouse A knockout mouse is a genetically engineered mouse in which one or more genes have been turned off through a gene knockout. They are important animal models for studying the role of genes which have been sequenced. Mice are currently the most closely related laboratory animal species to humans for which the knockout technique can easily be applied. The first knockout mouse was created by Mario R, Capecchi, Martin Evans and Oliver Smilthies in 1989. Use Knocking out the acitivity of a gene provides information about what that gene normally does. Humans share many genes with mice. Consequently, observing the characterisitcs of knockout mice gives researchers information that can be used to better understand how a similar gene may contribute to disease in humans. Knockout mice being used as a model for obesity. Areas of research in which knockout mice have been useful include: - cancer - obesity - heart disease - diabetes - arthritis - anxiety - aging - Parkinson's disease. Limitations of the use of Knockout mice - While knockout mice technology represents a valuable research tool, its use may be limited. For example: - About 15% of gene knockouts are developmentally lethal, which means that the genetically altered embryos cannot grow into adult mice. This problem is often overcome through the use of conditional mutations. The lack of adult mice limits studies to embryonic development and often makes it more difficult to determine a gene's

function in relation to human health. - Knocking out a gene may fail to produce an observable change in a mouse or may even produce different characteristics from those observed in humans in which the same gene is inactivated. E. g mutations in the p53 gene are associated with more than half of human cancers and often lead to tumors in a particular set of tissues. However, when the p53 gene is knocked out in mice, the animals develop tumors in a different array of tissues. Immunodeficient Rodents Immunodeficient rodents are indispensable research models for biomedical investigators for studies in oncology, immunology, and infectious diseases. Today, biomedical researchers use a number of naturally occurring and transgenic strains of immunodeficient mice and rats to study the immune system, rejection of tissue transplants, infections, cancer and tumor growth. With the development of " knockout" immunodeficient mice, in which genes affecting the immune system are inactivated in the research animal, new fields of research are being opened to precisely study the role of selected components of the immune system. The recent flurry of advances in designing research animals including models with multiple immunocompromised functions or genetic deficiencies began 20 years after the discovery of mice with a single, naturally occurring immunodeficiency. In the early days of immune function research, observers noted that all animals have the physiological ability to " self-discriminate." That is, the body can discriminate between its own cells and those of another animal-even one of the same species-and then launch an immune response against foreign cells or substances. Early researchers also noted that blood cells called lymphocytes appeared to play a key role in the immune response. Like other

blood cells, lymphocytes differentiate from pluripotent stem cells in bone marrow. Lymphocytes that continue their maturation in bone marrow develop into B cells, while those that migrate to the thymus and complete maturation there become T cells. Mature B cells and T cells are most concentrated in lymph nodes, the spleen, and other lymphatic organs where the lymphocytes are most likely to encounter antigens-foreign substances that evoke the production of antibodies and cytotoxic cellular responses. Biomedical research The widespread use of the mouse for biomedical research is largely due to the development of many genetic and genomic tools. One of the landmarks in mouse genetics was the isolation of pluripotent mouse embryonic stem (ES) 14 cells from mouse blastocysts (Evans and Kaufman 1981) and the subsequent demonstration that cultured ES cells can transmit through the mouse germline when reintroduced into host blastocysts (Bradley, Evans et al. 1984). Importantly, cultured ES cells maintain their pluripotency after modification of their genome which allows these modifications to be established in mice. Initially, the targets for modification were random or limited to a couple of mouse genes whose disruption could be selected by drugs, such as the Hprt gene on the hemizygous X chromosome (Kuehn, Bradley et al. 1987; Thomas and Capecchi 1987). A more general technology was needed to allow the disruption of the genes that could not be selected in vitro (Goldstein 2001). Then came the second important breakthrough. Several groups independently demonstrated that targeted mutations could be introduced into ES cells by homologous recombination (Zijlstra, Li et al. 1989; Koller, Marrack et al. 1990; McMahon and Bradley 1990; Schwartzberg, Robertson

et al. 1990). This technique allowed the precise disruption of any of the 23, 000 mouse genes. This pioneering work has established a new era in mouse genetics. Precisely engineered loss- or gain-of-function mutations can be established in the mouse through in vitro manipulation of ES cells. These approaches, together with the transgenic technique of zygote injection, are all classified as reverse genetics. Interestingly, the laboratory mouse is the first multi-cellular animal model organism in which gene targeting by homologous recombination became possible. Reverse genetics has become the main approach to identify gene function in mouse. This situation is partly due to the ease of genetic manipulation of mouse ES cells. Another important reason is that the cost of mouse breeding makes forward genetic screens a lot more expensive using mice compared to other model organisms. Embryonic stem cells Teratomas- tumors composed of various tissues foreign to their site of origin. can be formed by transplanting pieces of embryos to extra uterine sites. Teratocarcinoma- undifferentiated malignant stem cells, metastasize, lethal can be made by transplanting day 6-7 mouse embryos under the kidney capsule resulting tumors can be passaged and cultured to yield embryonic carcinoma cells, a small dense cell with a well defined nucleus, frequent mitotic figures. EC cell lines - variety of stages of differentiation - variable capacity to differentiate - exponential growth required to prevent differentiation - often feeder cells are required to prevent differentiation - differentiation can be induced by aggregation, plate on non-tissue culture plastic, cells inherently sticky - differentiation can be induced by drugs, RA or DMSO. ES cells, goal to create- a normal pleuripotent cell line -isolate stem cells from normal embryo without passing

through a tumor stage. -when reintroduced into the embryo ES cells can generate high grade chimeras. -essential to grow on feeder cells (STO fibroblasts or MEFs) Method to generate ES cells: transfer intact blastocyst into culture, grow to stage of early post implantation embryo, dissociate embryonic from extra embryonic portion, continue culture of ICM. LIF/DIA required to maintain ES cells in an undifferentiated state.