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## Mice as experimental organisms.

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# Mice as Experimental Organisms

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**The mouse is extensively used as a model system to reproduce human diseases in a mammalian system, and to consequently allow to examine the molecular and pathogenic features of the disease in a context that is as close as possible to the human being. The use of the mouse has been particularly useful in three large areas of experimental pathogenesis: (1) classic monogenic diseases, (2) cancer development and progression and (3) developmental genetics and congenital pathologies. In each of these areas, significant progress has been made in recent years. Here, after a short introduction to the biology of the mouse and its history as a model organism, we summarise the current status of genetic manipulation of the mouse genome, provide an overview of current knowledge and illustrate some recent advancements.**

## The Mouse as a Model

The house mouse *Mus musculus* plays a prominent role in the field of functional genetics since the rebirth of the subject at the beginning of the twentieth century. Owing to its long history of domestication as a pet, mice with various coat colour and other visible features were selected and bred over the centuries. New traits appeared suddenly in captive-bred animals, often due to single gene mutations. Early animal geneticists appreciated the importance of the genetic resource becoming available and these animals were quickly used to extend the validity of Mendel's laws to mammals. **See also:** [Evolution during Domestication; Mutations and New Variation: Overview](#)

Mice represent the model of choice for genetic studies in mammals. There are a number of compelling reasons for this: that they can be hosted in small rooms due to their size, have a short generation time (8–9 weeks), breed readily in

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## Introductory article

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captivity and are highly prolific (8–12 pups each pregnancy). Importantly, due to the high conservation of their genomes, results from the mouse can often be translated to humans, not without exceptions. After centuries of selection, easy-to-handle normal and mutant strains are widespread and represent an extraordinary resource for genetic studies. **See also:** [Experimental Organisms Used in Genetics](#)

## Life cycle and fertilisation

Embryonic development of the mouse is completed in 21 days, fertility is reached in 5–6 weeks and the average lifespan is 2 years. Several features of their development, before and after birth, are remarkably similar with that of humans. **See also:** [Reproduction in Mammals: General Overview](#)

In male mice the spermatozoans are produced continuously in large numbers throughout adult life. Female germ-line cells, instead, are generated in a fixed number since birth (approximately 50 000). Primordial female germ cells initiate meiosis, arrest at the prophase of the first meiotic division, and remain suspended for weeks (years in women) until puberty. The female mouse will undergo an oestrous cycle during which 8–10 primary oocytes complete the first meiotic division and initiate the second meiotic division; the oocyte is then ovulated, descends the oviducts and is receptive to fertilisation for a short period of time. **See also:** [Reproduction in Eutherian Mammals](#)

Fertilisation occurs when a sperm cell fuses its cell membrane with the oocyte membrane, giving rise to a binucleated diploid cell, the zygote, which represents the earliest (1 cell) stage of a new individual. **See also:** [Sperm–Egg Interactions: Sperm–Egg Binding in Mammals](#)

Fertilisation often produces more zygotes than those that complete development. In mammals, in particular, zygotes that initiate development not necessarily succeed in the subsequent implantation and will be lost. Several causes have been implicated, one of these is the presence of genetic mutations or chromosomal anomalies in the zygote, that are being selected against by natural selection.

## Pre-implantation development

Mammalian development is divided into two stages, separated by the moment of implantation into the uterus. The

pre-implantation stage lasts 4.5 days, during which the zygote is hosted within the mother's oviduct. The zygotes can be easily collected, kept in culture for hours and used for genetic manipulation, without losing their ability to generate a new individual upon reintroduction into a female oviduct. Once the embryo has undergone implantation, it can no longer be removed from its mother's body. The accessibility of the pre-implantation embryo provides the basis for a number of specialised genetic tools that are used to study mammalian development (see next sections). **See also:** [Mouse Early Development: Molecular Basis](#)

Within the pre-implantation zygote the highly compacted sperm genome into a paternal pronucleus expands to match the size of the maternal pronucleus. In the next 60 hours, the zygote undergoes cleavage, that is, four equal divisions that generate a 16-cell embryo, known as morula. During cleavage, all of the cells in the developing embryo are equivalent and totipotent, a term used to describe a cell that is fully undifferentiated and retains the ability (or potency) to generate each and every cell type of the embryo and of the adult animal. As a proof of this, cleavage stage embryos can be separated into individual cells, each able to generate a full individual upon reimplantation. This process occurs spontaneously in humans, giving rise to genetically identical twins. **See also:** [Cleavage and Gastrulation in Mouse Embryos](#); [Whole Animal Cloning](#)

After the morula stage the first specialisation occurs, the potency of individual cells becomes progressively restricted, while cells continue to divide and increase in number. The outer cells of the morula give rise to the trophoectoderm, which will take part in the formation of the placenta, while the inner cells compact into a clump at one pole of the sphere. These cells, known as inner cell mass (ICM) are now pluripotent, will develop into the fetus but have lost the ability to generate a trophoectoderm. At this stage of development, the embryo is called a blastocyst. Implantation initiates the development of the placenta, a combination of embryonic and maternal tissues that mediates the bi-directional flow of nutrients, oxygen and waste products between the mother and embryo.

## Post-implantation development

Following implantation, a period of rapid organisation, organ formation and growth begins. Cells from the ICM first organise into three cell layers (ectoderm, endoderm and mesoderm) via a morphogenetic phase known as gastrulation. Next, the general body plan and its subdivision are laid out, then the primordia of the brain and spinal cord, heart, sensory organs, cranial and axial skeleton are put into place, and the morphogenesis and differentiation of the various tissues and organs initiates. The fetus continues to grow in size, until birth. More information on the biology of mammalian development in section 'Genetic diseases leading to developmental/congenital malformations'.

## The Mouse Genome and its Manipulation

The haploid genomes of humans and mice contain approximately 3 billion base pairs of deoxyribonucleic acid (DNA), with striking similarities revealed upon completion of their respective sequencing projects. **See also:** [Human Genome Project, HUGO and Future Health Care](#); [Human Genome Project: Importance in Clinical Genetics](#)

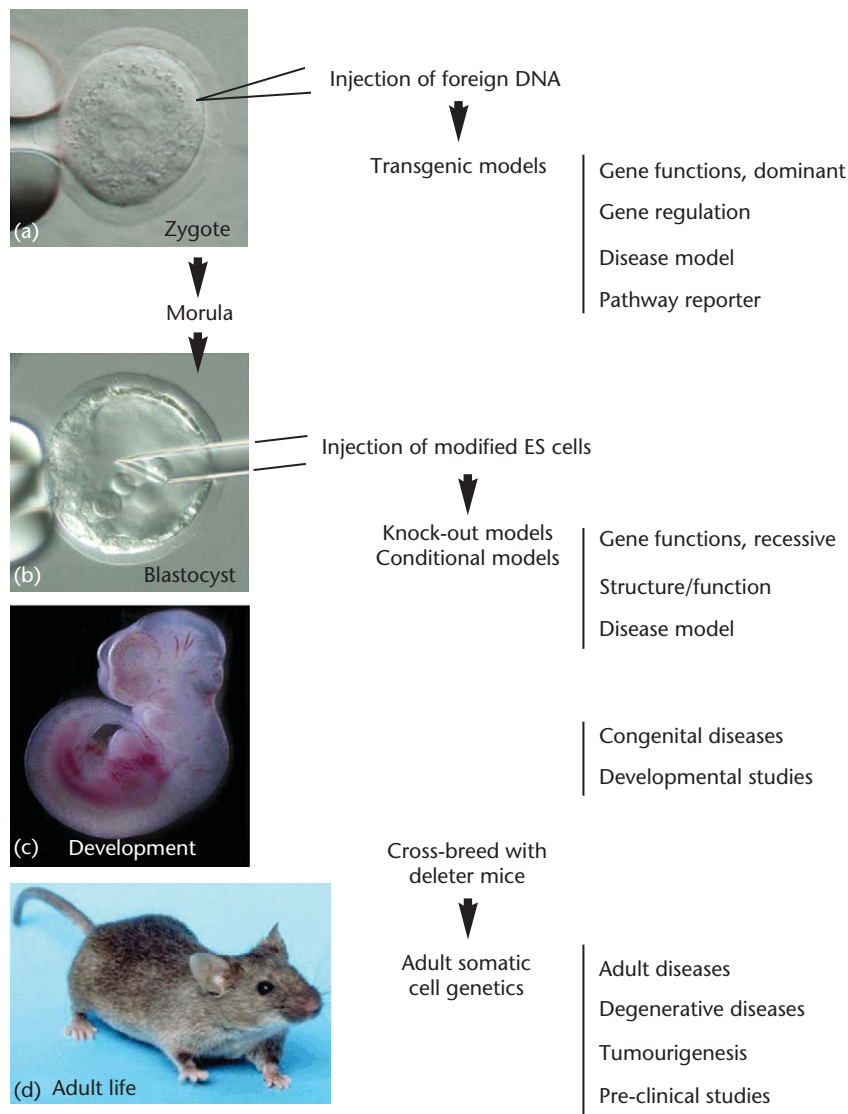
Indeed, most human genes have a corresponding homologue in the mouse genome, named 'hortholog'. Thus, the mouse genome provides a powerful model system for investigating the genetic basis of both simple and complex human traits, especially those related to development and disease. Conversely, most differences consist in species-specific additions to gene families that already existed in the common ancestor, or in noncoding sequences.

The mouse genome is contained within 19 autosomes and two sex chromosomes. Although human and mouse karyotypes do not show any evidence of chromosome banding homologies, genes that are closely linked in one species (syntenic) are usually found to be closely linked in the other (conserved syntenic). By comparing the genetic maps comprising the whole mouse genome with those of the human genome, it becomes evident that syntenic extends across nearly the complete karyotype. **See also:** [Evolution of Genome Organization](#); [Genome Evolution: Overview](#); [Genome Mapping](#); [Genome Organization of Vertebrates](#)

Conserved syntenic has strong evolutionary implications, but just as importantly, it serves as a critical tool for identifying noncoding regions of the genome that are nevertheless conserved, and thus likely to play regulatory roles. **See also:** [Genetic and Physical Map Correlation](#)

The first evidence that mice could represent valuable disease models came from the analysis of spontaneous mutations arising in breeding colonies. This is for example the case of the *leptin* gene, whose mutation is involved in obesity, and of the gene encoding for the *Fas* receptor, involved in auto-immunity. **See also:** [Apoptosis: Inherited Disorders](#); [Human Disease: Mouse Models](#); [Mouse as a Model for Human Diseases](#); [Obesity: Genetics](#)

By far the most useful strategy has however been that known as 'reverse genetics', whereby a gene of interest is studied and characterised *in vivo* via its introduction or modification in the mouse genome. There are three main approaches to manipulate the mouse genome: (1) the generation of transgenic mice, whereby an exogenous sequence, called a transgene, is inserted within the mouse genome; (2) targeted mutagenesis, consisting in the insertion of genetic material at specific locations via a process known as homologous recombination, resulting in the inactivation or modification of the chosen gene; and (3) the two approaches above combined in strategies that only induce the genetic modification in certain cells and at certain times, known as 'conditional mutagenesis'. These



**Figure 1** Manipulating the mouse genome: where, when and why. A scheme to summarise the three principal genetic manipulation and the phenotypic studies that are most commonly performed, in relation with developmental and post-natal stages of the mouse. On the left, a photographic description of the various embryonic stages considered. On the right, the manipulations required to generate the different models and the modified strains that can be obtained: (a) injection of foreign DNA to generate transgenic models, reproduced from Dr. Hirsch, University of Torino, (b) injection of modified ES cells to generate knock-out and conditional models, reproduced from Dr. Hirsch, University of Torino and (c, d) cross-breeding with Cre-expressing animals to carry out somatic cell genetics. For each of these strategies, a short list of common research applications is reported.

approaches are depicted in **Figure 1**, together with some of their most common research applications.

## Generation of transgenic mice

In 1981, Frank Costantini and Elizabeth Lacy published a protocol for inserting foreign DNA into the mouse germline; this drastically changed our approach to mammalian genetics and has so far provided a wealth of knowledge, not obtainable otherwise (Costantini and Lacy, 1981). To generate a transgenic mouse – with a novel DNA sequence integrated into one of its chromosomes – the foreign DNA is injected into the male pronucleus of a one-cell embryo,

and the embryo is then transferred into a pseudo-pregnant female to allow development to proceed. Up to 50% of the mice born from injected embryos will carry multiple copies of the foreign DNA stably integrated into their genomes and will transmit this DNA onto their progeny. **See also:** [Transgenic Animals](#); [Transgenic Mice](#); [Transgenic Mice Production](#)

The recombinant DNA construct has to contain specific sequences in order to be transcribed and translated. In particular, the coding sequence must be placed under the control of suitable eukaryotic regulatory regions, known as promoter and enhancer, followed by a polyadenylation signal. Furthermore, the transgene should contain at least



one intronic region, required to stabilise the transcribed ribonucleic acid (RNA). The main limit of this technology is the random integration of the transgene in the host genome. The chromosomal region of insertion can alter the expression of the transgene either by repressing it, as is the case of heterochromatic, silent genomic regions, or by improperly activating it. Another serious problem is that of insertional mutagenesis, consisting in the inactivation of endogenous genes caused by insertion of the transgene within its coding or regulatory regions.

Transgenic technologies are mainly used to assess the effects of expressing the transgene, and to generate *in vivo* readouts of the activity of regulatory regions or of signalling pathways. Transgenic expression provides indications as to the function of the expressed sequence. According to the regulatory regions used for generating the construct, expression can be ubiquitous or tissue-specific, or it can occur in tissues/cell types, which do not normally express the corresponding protein (ectopic expression). Particularly useful is the ability to control transgene expression so that it can be induced or repressed at will, in order to avoid toxic or even lethal effects of permanent expression and to more closely reproduce the effects of acute gene activation.

Gene function studies are usually performed by expressing the transgene at high levels (overexpression), followed by the analysis of the phenotype of the resulting mice. **See also:** [Transgenic Mice Production](#)

Transgenic mice are also useful to assess the functionality of regulatory regions or the activation of specific signalling pathways. Both approaches are based on the use of a so called 'reporter gene'. This is a sequence encoding for a protein whose expression is readily detected either by fluorescence or via its enzymatic properties. The presence, abundance and cell/developmental stage specificity of the reporter gene expression can thus be easily characterised. This approach has been widely used to test the activity of complex transcriptional regulatory regions, which cannot be faithfully tested in cell culture assays. The 'reporter gene cassette' is placed under the control of the regulatory region(s) under study, and microinjected into mouse zygotes to obtain transgenic mice where the reporter gene activity can be analysed in detail.

If on the other hand the 'reporter gene' is placed under the control of a regulatory region already characterised as being responsive to the activation of a specific signalling pathway/transcriptional program, this approach allows to analyse the pathway activation under specific conditions such as during development or, for example, under pathological stimuli. Thus, the presence and position of reporter-positive cells indicate that pathway activation has occurred and that a specific signal has been used. This approach has been successfully used for the Wnt- $\beta$ -catenin and for the Notch pathways (Maretto *et al.*, 2003; Vilas-Boas *et al.*, 2011).

## Targeted mutagenesis: taking genes away

In addition to the problem of random integration, conventional nuclear injection technology can only add – not

subtract – genetic material. In genetic terms, this means that transgenic mice are only useful for the analysis of dominantly transmitted phenotypes.

By 1988, an independent transgenic technology – known as gene targeting – was developed that allows to disrupt, eliminate or modify any cloned gene, in a 'targeted' way (Mansour *et al.*, 1988). This method is widely used to determine gene function by examining the phenotype of mice/embryos lacking the gene of interest, or expressing an alternative form of it; this approach is often useful to generate a mouse model for human diseases caused by loss of gene function (recessively transmitted phenotypes). **See also:** [Knockout and Knock-in Animals](#); [Mouse Knockouts: Modifying the Mouse Genome by using Embryonic Stem Cells](#); [Mutagenesis: Site-specific](#)

Targeted genome manipulation was achieved by coupling two innovative tools: homologous recombination and embryonic stem cells (ES) (Thomas and Capecchi, 1987; Evans and Kaufman, 1981). The fact that both Mario Capecchi and Martin Evans have won the Nobel Prize in Physiology or Medicine in 2007, together with Oliver Smithies, 'for their discoveries of principles for introducing specific gene modifications in mice by the use of embryonic stem cells' illustrates well the importance of this development.

ES cells are derived from the ICM of a blastocyst and can be manipulated without losing their differentiation potential to be then reintroduced by microinjection into a host blastocyst embryo. The injected blastocyst is implanted in the uterine horns of a foster mother and allowed to develop. The ES cells will contribute to the tissues of the developing embryos including the germ line, leading to the birth of 'chimeric' pups, formed partly by cells of the donor embryo and partly by the injected ES cells.

The ES cells genome can be transmitted to the progeny, therefore any genetic modification obtained in ES cells can be passed on to generate a strain of mutant mice.

However, homologous recombination is a rare event in mammalian cells and requires appropriate selection and screening methods. To obtain ES cell clones genetically modified at a specific locus, a DNA sequence containing the desired mutation can be engineered as to contain regions of homology flanking the gene of interest, then introduced into ES cells. ES cell clones undergone homologous recombination will show integration of the recombinant sequence into the host genome precisely at the site of homology due to the exchange of genetic material, resulting in loss of the wild type gene from the host DNA.

Gene inactivation via gene targeting is currently an essential tool to define gene function through the phenotypic analysis of mutant embryos and mice. Often the results confirm the predicted functions, but even more often these studies reveal unexpected new roles and/or functional *in vivo* redundancy not predicted by previous *in vitro* studies or by sequence/structure-based gene classifications. This has been for example the case of genes encoding for cytokines and interleukins (ILs), soluble peptides involved in the control of immune functions.

*In vitro*, IL-4 promotes the growth and differentiation of many hematopoietic cells *in vitro*, and directs the immunoglobulin (Ig) class switch to IgG1 and IgE. However, surprisingly in the *IL-4* null mice, among the first germ-line targeted mutation to be published (Kühn *et al.*, 1991), the number and differentiation of both B and T cells were unchanged, although the serum levels of IgG1 and IgE were strongly reduced, showing how only some of the *in vitro* properties of IL-4 are critical for the physiology of the immune system *in vivo*. Indeed, functional redundancy appears to be more the rule than the exception in the mammalian genome, providing protection against the adverse consequences of mutations. **See also:** [Cytokines](#)

## The advent of conditional mutagenesis

Gene targeting has tremendously expanded our knowledge and revolutionised our way of thinking about genetics. However, it does not allow the functional analysis of those genes, which also maintain defined functions in the adult organism. Moreover, the lack of a given gene product during development can bring about adaptation phenomena that may obscure the phenotype. The solution to this problem has come with the application of conditional mutagenesis mediated by the Cre recombinase, an enzyme derived from the bacteriophage P1. **See also:** [Cre-lox-Inducible Gene Targeting](#)

Cre recombinase can mediate the excision of a portion of DNA flanked by its recognition sequence, called loxP sites. Thanks to DNA looping, the length of the intervening sequence can be very long, up to 3–5 centi-Morgan. Briefly, a 'conditional allele' is generated by homologous recombination in ES cells by inserting two loxP sites flanking at least one essential exon. These alleles are functional but amenable to Cre-mediated deletion and inactivation. Cre is expressed by transgenesis or by viral transduction, either ubiquitously or in specific tissues and/or inducibly, and deletes the loxP-flanked sequence thus triggering gene inactivation. A great number of Cre transgenic mice have been generated that express the enzyme in specific tissues, cell types or developmental stages, and/or where Cre expression or function can be controlled by exogenous molecules, and most of them are available to the scientific community.

The Cre-lox system can also be used to activate transgenes whose regulatory sequences have been blocked by a 'STOP' cassette flanked by loxP sites: in this case Cre activation will delete the stop sequence, thus activating gene transcription. This approach, widely used in the tumour field (see section 'Tumour models'), has been very useful for cell lineage studies, whereby a fluorescent reporter gene with a lox-stop-lox sequence is introduced in the germ line, and becomes activated upon Cre expression and deletion of the STOP cassette, thus leading to lineage-specific fluorescence positivity.

The Cre-lox system allows the generation of even very large chromosomal deletions or translocations. Owing to the great genomic distances involved, in this case the loxP

sites have to be separately introduced into the ES cells genome, together with suitable markers allowing to select for the rare recombination event. **See also:** [Translocation Breakpoints in Cancer](#)

## New frontiers in the manipulation of the mouse genome

The recent discovery of the RNA interference phenomenon has allowed new approaches to gene inactivation, whereby transgenic mice expressing shRNAs able to target specific genes for degradation can be generated, bypassing the need for the tedious passages involved in the homologous recombination in ES cells and their injection in mouse embryos. **See also:** [RNA Interference \(RNAi\) and MicroRNAs](#)

However promising, technical limitations regarding off-target effects and silencing of the interfering RNA have so far diminished the enthusiasm for this approach. Recently, DNases that can be targeted to specific genomic sequences have been used to mutagenise genes either in the mouse zygote or in somatic cells. **See also:** [Meganucleases and Their Biomedical Applications](#)

## Mouse as a Model for Diseases

The approach to manipulate the expression/sequence of a gene that represents the mouse ortholog of a disease gene in human has been used to unequivocally demonstrate the cause-effect relationship between a known mutation and a disease phenotype. Mouse models of diseases are moreover precious to identify pathogenic molecular mechanisms and, on the long term, to test and validate novel therapeutic strategies. Here, we illustrate a few key examples in different categories of human diseases, both inherited and acquired. **See also:** [Human Disease: Mouse Models; Mouse as a Model for Human Diseases](#)

## Classic monogenic diseases

A full collection of human monogenic diseases, the description of their phenotype and the known genetic mutations in human is contained in the database OMIM (<http://www.ncbi.nlm.nih.gov>). Mouse models for each of these human conditions have been or are being generated. An example that illustrates at the same time the usefulness of this approach and its limits is the generation of a mouse model for cystic fibrosis. Cystic fibrosis is caused by mutations inactivating the *CFTR* gene, which encodes for a Cl<sup>-</sup> ion channel present at the apical membrane of epithelial cells, and is characterised by airways, intestinal and reproductive organs obstruction due to dense secretions and water flux impairment. The intestinal phenotype was perfectly reproduced in *CFTR* null mice, which however did not show any alteration of the respiratory airways or of the reproductive organs due to anatomical and physiological differences between mouse and man. Attempts to

correct the CFTR defect are rapidly advancing thanks to the use of this model (Ostedgaard *et al.*, 2011).

Other two important examples are the *dystrophin* null (MDX) and the  $\alpha$ -*sarcoglycan* null models of muscular dystrophy (MD). Although these mice exhibit a muscular phenotype that does not well recapitulate the degeneration seen in MD patients, they have provided important new knowledge on muscle regeneration and muscle stem cells. Moreover, they have been a key instrument to test the regeneration properties of grafted cells (Galvez *et al.*, 2006; Tedesco *et al.*, 2011; Goehringer *et al.*, 2009) or the efficacy of pharmacological treatments (Minetti *et al.*, 2006; Hori *et al.*, 2011). The mouse line devoid of laminin- $\alpha$ 2, a protein of the basement membrane, has been extensively used as a model of MD to examine the effectiveness of gene therapy approaches or administration of pharmacologic and trophic factors (Kumar *et al.*, 2011).

Mice knock-out for the methyl-DNA-binding protein gene *Mecp2* partly reproduce the neurological and cognitive impairments of children with the Rett Syndrome, a rare but devastating congenital condition caused by mutations in *Mecp2*. Surprisingly, the generation of a new strain of animals in which the *Mecp2* mutation could conditionally be eliminated showed that restoration of the wild type gene even in adult life could revert some of the cognitive and neurological defects (Guy *et al.*, 2007; Gadalla *et al.*, 2011), with striking therapeutic implications.

Many other mouse models for important diseases have been generated with the same general purpose to provide a model to test therapeutic approaches. For instance, lack of collagen-VI leads to a specific form of MD, in model mice and human. Mice have been used to revert the muscular dysfunction and prove the validity of pharmacological therapies (Grumati *et al.*, 2010). Mice defective for laminin-5 display the phenotype of Epidermolysis Bullosa, a severe congenital skin pathology. Transplantation of modified epidermal stem cells, re-expressing laminin-5, has been shown to correct the defect and support the regeneration of a nearly normal skin (Mavilio *et al.*, 2006). Mouse models of Metachromatic Leurodystrophy, a severe storage disease affecting the central nervous system, are paving the way to testing new therapies (Miyake *et al.*, 2010; Lattanzi *et al.*, 2010). It is expected that in the near future models of each monogenic or polygenic disease will be available for testing. **See also:** [Cystic Fibrosis](#); [Duchenne Muscular Dystrophy](#); [Rett Syndrome](#)

## Genetic diseases leading to developmental/congenital malformations

Babies are born with congenital diseases at a low but consistent frequency. Inborn errors and diseases are not usually life-threatening, however they pose serious medical and social problems.

The study of the impact of physical/chemical insults (known as 'teratogens') on development is usually considered the subject of 'teratology'. The mouse embryo is still used for studies analysing the effect of teratogens

applied to pregnant females or cultured whole embryos. Although providing limited scientific information, teratology helps to define parameters and measures to prevent congenital diseases.

Investigating the role of genes in normal development, and in parallel the cellular and tissue dysfunctions that are caused by their mutations, is instead the subject of developmental genetics. Since human embryo cannot be studied for evident ethical reasons, we heavily depend on the mouse embryo for these studies. This is facilitated by the large number of both spontaneous and experimentally generated mutant mouse strains already available (Rosenthal and Brown, 2007). The mouse embryo is moreover amenable to studies of cell lineage and pathway activation via the transgenic expression of fluorescent proteins combined with the Cre-Lox conditional systems (see section 'The advent of conditional mutagenesis'). **See also:** [Human Developmental Molecular Genetics](#)

Developmental genetic focuses on specific organs and structures, and often information derived from one experimental system cannot be directly extended to other systems. The two main considerations, or concerns, guiding developmental geneticists are:

1. To identify a simple system, easily accessible to experimental procedures, and test the role of individual genes. This is the case of the limbs, the tooth, the palate.
2. To identify genes causing human congenital diseases and malformations, and to generate a mouse model harbouring the corresponding mutation. This is the case of genes affecting the skeleton, the limbs, the skin and the neural tube.

Significant advances have been made in several directions: cardio-vascular development and the corresponding heart congenital syndromes (Snider and Conway, 2011); craniofacial development and the corresponding malformations, such as holoprosencephaly and cleft lip/palate (Gritli-Linde, 2008; Schachter and Krauss, 2008); limb development and the corresponding limb malformations (Zeller, 2010); ectoderm development and skin dysplasia (Vanbokhoven *et al.*, 2011); the closure of the neural tube and the neural tube defects such as Spina Bifida (Greene and Copp, 2009); and kidney development (Ly *et al.*, 2011). For each of these conditions, a number of relevant genes have been identified and studies. **See also:** [Craniofacial Abnormalities: Molecular Basis](#); [Craniofacial Defects and Cleft Lip and Palate](#); [Dysmorphic Syndromes](#); [Paediatric Congenital Heart Disease](#); [Skeletal Dysplasias: Genetics](#)

Congenital diseases can result from altered cell migration during development; this is the case of neural crest cell-related conditions (Cordero *et al.*, 2011; Theveneau and Mayor, 2011), specific neurological disease (Liu, 2011; Valiente and Marín, 2010) and hypogonadotropic hypogonadic syndromes (Hardelin and Dodé, 2008). **See also:** [Neural Crest: Origin, Migration and Differentiation](#)



Metabolic disorders represent large set of rare diseases, often recessive, caused by the absence or inactivity of an enzyme, and the consequent accumulation of specific metabolites in tissues and organs. Animal models have been generated and hopefully will soon be used to initiate pre-clinical studies to attempt restoring or bypassing enzymatic functions (i.e. glycogenesis-type Ia – Grinshpun *et al.*, 2010). It is important to note that, in the case of metabolic diseases, the mouse model may not faithfully recapitulate the human condition, since mice and humans are metabolically different. **See also:** [Metabolism: Hereditary Errors](#)

## Tumour models

Although cancer is most often not inherited, it can nevertheless be considered a genetic disease since it is brought about by mutations in key genes with either a pro-oncogenic (oncogenes) or an anti-oncogenic (tumour suppressors) function. **See also:** [Oncogenes](#); [Tumor Suppressor Genes](#)

Typically, mutations in oncogenes generate aberrantly active or overexpressed proteins, whereas loss of function mutations affect the activity or expression of tumour suppressor genes. Thus, the generation of transgenic mice expressing/overexpressing oncogenes, either in their wild type form or mutated, has represented an excellent method to study tumour onset and progression and, later, to test therapeutic treatments. Indeed, the ability of a predicted oncogene to induce tumour onset when overexpressed in transgenic mice is considered the gold standard to classify it as a bona fide oncogene. Moreover, transgenic models have been instrumental to demonstrate that the tumour transformation process requires the interaction between more than one oncogene or between oncogenes and onco-suppressors (the multi-hit theory of tumour transformation, Knudson, 1971), thus confirming data previously obtained *in vitro*. **See also:** [Tumor Formation: Number of Mutations Required](#)

Conversely, gene targeting has allowed to reproduce mutations in onco-suppressor genes. Among the first genes subject to this analysis has been the famous onco-suppressor *TP53*. Its targeted inactivation has allowed not only to demonstrate and dissect its onco-suppressive functions, but also to define its preferential target tissues and its tissue-specific interactions with oncogenes such as for example *c100-myc*. Further studies, introducing specific mutations found in human tumours in the *tp53* allele, have allowed finally to demonstrate their dominant negative role. Again, the ‘status’ of bona fide tumour suppressor is granted only if its ablation leads to tumour development. **See also:** [Cell Cycle Control: Molecular Interaction Map; P53 and Cell Death](#)

Almost infinite combinations of the different approaches used to manipulate the mouse genome are possible, allowing the generation of more and more precise tumourigenesis models. For example, the expression of the transgenic oncogene must be activated by Cre expression/activity induction by deleting a stop sequence (see section

‘The advent of conditional mutagenesis’). The generation of mice expressing the K-Ras oncogene under this Cre-mediated control has allowed to appreciate the tissue specific functions of this oncogene, able to induce tumours only in the lung when activated alone (Guerra *et al.*, 2003). Conversely, sporadic K-Ras activation triggered by adenoviral-mediated delivery of Cre recombinase to lung epithelial cells gave rise to pulmonary adenocarcinomas with 100% penetrance, showing that a sporadic Ras mutation is sufficient to elicit lung tumourigenesis (Meuwissen *et al.*, 2001). Finally, the combination of oncogene expression with the inactivation of genes thought to act downstream of it allows to finely dissect the oncogenic pathway thus identifying potential new therapeutic targets. This approach has for example recently allowed to identify c-Raf as an essential mediator of K-Ras-driven nonsmall cell lung carcinoma (Blasco *et al.*, 2011).

## Large-scale mutagenesis to find new disease genes or modifiers

Spontaneous mutations in mice may result in benign phenotypes such as variable coat colours, or in disorders that have similarities to diseases in man. However, spontaneous mutations occur at very low frequencies. Long-time efforts have been initiated with the general aim to generate an unbiased collection of cells and mice that harbour mutations in each of the approximately 25 000 genes in the mammalian genome. The goal is to saturate the mouse genome with mutations causing loss or gain of function in each gene, with the possibility to later establish a line of animals and examine their phenotypes (Cartwright, 2009). Consortia for large-scale mutagenesis have been initiated in the USA, Europe and Japan, with different strategies and tools:

- Chemical mutagens that induce preferential types of mutations, at a given frequency, randomly in the genome. Erythro-nitroso-urea (ENU) is a potent alkylating agent and induces mainly missense point mutations. ENU is being used to mutagenise the male germ cells and generate from the treated males a progeny that is screened for interesting phenotypes with a panel of morphological, biochemical and behavioural tests. A caveat: this strategy mostly detects dominantly transmitted phenotypes, and exclusively those affecting non-essential developmental processes and organs.
- Gene-trap insertional mutagenesis, to introduce specifically engineered sequences into the genome of mouse ES cells. The sequence usually contains a reporter gene, to facilitate the detection of interesting expression patterns, and a splicing sequence that serves to ‘trap’ an exon of an endogenous gene. The randomly trapped genes in most cases are also ‘null’, therefore the selected ES cells can be used to generate heterozygous (first generation) and homozygous (second generation) mice. Caveat: the selection of the ES cells is still based on gene-driven hypothesis or interesting expression patterns, the



generation of mutant mice is time consuming and the gene inactivation is sometimes incomplete, making the interpretation of phenotypes sometimes difficult.

- Use of transposon-type elements, such as ‘sleeping beauty’ or ‘piggyBac’ to extensively mutagenise the mouse genome, by random insertion of these elements in ES cells (Landrette and Tian, 2011). These approaches, applied to ES cells, have the same advantages and caveats of gene-trap strategies. However, they can also be used for somatic cells genetic, and in this case only few mouse strains need to be generated, that can subsequently be used in a variety of ways.
- *In vivo* use of RNAi libraries targeting all known genes. This approach has the caveat of incomplete loss of gene function and possible off-targets effect that need to be monitored and excluded to avoid serious data misinterpretation. **See also:** [Deconstructing Gene Function through ENU Mutagenesis](#); [Mouse \*N\*-ethyl-\*N\*-nitrosourea \(ENU\) Mutagenesis](#); [Transposons as Natural and Experimental Mutagens](#)

## The Mouse as Valuable *In Vivo* Experimental Tool

Below are reported a few examples of what is made possible by the new technologies that allow to genetically manipulate the mouse genome, not necessarily entailing disease models.

### Genetically expressed fluorophores and imaging

The combination of genetically coded fluorophores that are nontoxic, monomeric and do not require exogenous substrates or cofactors, with new imaging techniques, has allowed to dynamically visualise subcellular biological process, such as photo-activation and photo-conversion (Nowotschin *et al.*, 2009). With the combination of these tools, the visualisation of single cells and their biochemical and genetic properties within embryonic and adult tissues is now feasible. **See also:** [Fluorescence Microscopy](#)

One fascinating example of the application of advanced fluorescent imaging is the possibility to monitor the migration of young neurons during brain development, when combined with intrauterine electroporation through the embryonic cortex (Borrell *et al.*, 2005). This method is used to introduce expression or silencing vectors, combined with the green fluorescent protein for the visualisation. This approach was instrumental to establish several routes and modes of migration and their molecular regulation.

The use of genetically coded fluorophores can be combined with the use of Cre–Lox systems to turn-on or turn-off fluorophore expression in specific cell types. Furthermore, the nucleotide analog BrdU can be injected and used to

monitor cell proliferation and exit from the cell cycle, *in vivo*. In a recent work, Encinas *et al.* (2011) have used these methods to precisely determine how many neurons and of which type are generated by neural stem cells in the post-natal hippocampus, and defined their fate. The results represent a key advancement in the field of stem cells and have a great impact on our knowledge on adult neurogenesis. **See also:** [Cerebral Cortex Development](#); [Neural Stem Cells](#)

Finally, recent work illustrates the possibility to dissect molecular pathways and their regulation directly in embryonic tissues (Grigoryan *et al.*, 2008; Beronja *et al.*, 2010), bypassing the use of genetically modified animals. This takes advantage of the fact that the ectoderm, the embryonic precursor of tissues like skin, hair, glands, cornea, being the most superficial layer in a developing embryo, is easily accessible to vectors delivering exogenous sequences upon a simple intra-uterine injection. The effect of their expression on the morphogenesis and maturation of ectoderm derivatives can then be observed. This experimental strategy is a very promising new avenue towards a deep comprehension of the molecular pathways controlling multi-cellular organisations. **See also:** [Brain: Neurodevelopmental Genetics](#); [Neural Crest: Origin, Migration and Differentiation](#); [Signal Transduction Pathways in Development: Wnts and their Receptors](#)

### Insights into mammalian development

The use of the mouse as a model of mammalian development has opened extraordinary avenues. Today we can investigate the following developmental features, previously established in *Drosophila*, also in mammalian embryos:

- **Body plan:** a set of early positional information (usually as gradients of signals or specific cell-cell contacts) that defines anterior versus posterior, dorsal versus ventral; left versus right, and defines the general identity and number of segmental structures (head, thorax and tail). Mutations in genes controlling the identity of large structures are called ‘homeotic’.
- **Pattern:** the positional organisation of group of cells that, apparently equal, are in fact distinct in their prospective fate. The difference in fate relies on cellular–molecular differences, established in earlier times. Patterns are usually plastic in early stages and became fixed at later stages.
- **Morphogenesis:** the acquisition of specific 3D organisations and shapes from more simple organisation. Examples are neural tube closure, heart looping, extension and fusion of the pharyngeal arches and limb outgrowth. Once initiated, morphogenesis is usually irreversible.
- **Cell communication:** groups of cells establish dialogues during development via diffusible molecules (signalling at distance) and via cell surface/adhesion molecules (signalling by contact). Extensive cell communications occur not only between the ectoderm and the mesoderm

during development, guide morphogenesis and cell migration, but also takes place in the control of homeostasis of adult tissues and cancer formation. **See also:** [Mouse Early Development: Molecular Basis](#)

The typical questions that mouse developmental geneticists can now ask are:

- Which molecules make up a pattern, how is this established and maintained?
- How is the morphogenesis of a specific structure organised and executed? Which precise signals and cell interactions take place?
- How do cells coordinate their proliferation, migration, differentiation and programmed death?
- How do cells migrate, where to, and in response to which directional clues? Migration is typical of neurons during brain development, of neural crest cells during formation of craniofacial skeleton, and primordial germ-line cells during the colonisation of the gonads.
- Is cell fate determined intrinsically or by the social context? Can cell fate be modified? **See also:** [Apoptosis: Inherited Disorders](#); [Mammalian Embryo: Branching Morphogenesis](#); [Neural Crest: Origin, Migration and Differentiation](#); [Neuronal Migration](#); [Signal Transduction: Overview](#)

Results from developmental genetic approaches in the mouse have proven that the general body plan is maintained during development of all Vertebrates, and that to a large extent the same genes and signals are used to establish it. This is not totally surprising, as we now recognise that the human and Vertebrate genomes are highly conserved, especially in the protein coding, micro-RNA and key regulatory elements.

A striking example of a conserved molecule for a conserved process is *Pax6*, a homeodomain gene essential for eye formation from flies to man (Gehring and Ikeyo, 1999). The forced expression of the mammalian *Pax6* in a *Drosophila* embryo can induce the formation of an ectopic eye in the adult fly. Notably, the aberrant eye is of a *Drosophila* type and not a mammalian type. This means that certain genes have conserved a general 'master developer' function across evolution, and that the rest of the genome acts as an 'executor' of the master program, thus defining the details.

A converse example is the *Drosophila engrailed* gene, able to restore normal cerebellum development in mice devoid of the mouse ortholog *En1* (Hanks *et al.*, 1998), even though the fly does not possess any structure in its nervous system resembling a mammalian cerebellum. Similarly, the *distalless* gene of the fly is essential to allow appendages such as mouthparts, antennae and limbs to extend and grow. The *distalless* mouse and human orthologs are essential for normal limb development, and their disruption causes limb congenital malformations (Panganiban and Rubenstein, 2002; Merlo *et al.*, 2002). Intriguingly however, the fly and the Vertebrate limbs have nothing in common except that they protrude out of the

body. This 'master' function has thus been maintained across evolution. **See also:** [Evolutionarily Conserved Noncoding DNA](#); [Genome Evolution: Overview](#); [Hox Genes: Embryonic Development](#); [Mammalian Embryo: Hox Genes](#); [Transcription Factors](#)

Finally, as mentioned in section 'Genetically expressed fluorophores and imaging', the use of fluorophores in the mouse embryo has allowed us to appreciate the importance and precision of cell migration, particularly in the peripheral and central nervous system and of the neural crest. The precise timing and location of the migratory processes imply the existence of signals and signal receptors, guiding the cells to the correct target tissues: a subject of intense research.

## The paradigm of cellular pluripotency and reprogramming approaches

A new frontier in biomedical research is the possibility to force somatic cells to de-differentiate, thus assuming the features of pluripotent, or totipotent, stem cells (induced pluripotent stem (iPS) cells). These cells can potentially be used for a number of important applications such as tissue regeneration and correction of genetic defects. For obvious ethical reason, again the organism of choice to experiment both iPS generation and their therapeutic application is the mouse. Different approaches have been developed to confer pluripotency to adult cells: somatic cell nuclear transfer, parthenogenesis of unfertilised eggs, reprogramming by cell fusion and, more recently, forced expression of key transcription factors and/or the use of small molecules. The team led by Yamanaka in 2006 first identified four transcription factors (Klf4, Sox2, Oct4 and c-Myc) that could reprogram mouse fibroblasts into pluripotent cells upon retroviral transduction (Takahashi and Yamanaka, 2006). These iPS exhibit the morphology and growth properties of ES cells and express ES cell marker genes. iPS can now be generated from human somatic cells using nonintegrating episomal vectors (Yu *et al.*, 2009), taking these cells a step closer to potential clinical use. Pluripotency of iPS cells can be verified by microinjecting these cells into mouse blastocysts and by analysing chimera formation and germline transmission (mouse iPS cells), and teratoma formation (human and mouse iPS cells) (Yamanaka, 2009). Several cell types have been hitherto reprogrammed such as dermal fibroblasts and keratinocytes from hair (Aasen and Belmonte, 2010; Lowry *et al.*, 2008). **See also:** [History and Ethics of Stem Cell Research](#)

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